Work Package 1 Appendices

DC-Agri; field experiments for quality digestate and compost in agriculture

Work Package 1 report: Appendices

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This document contains the appendices to the *DC-Agri* Work Package 1 final report

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APPENDIX 1. Soil quality results

Results from the soil quality sampling undertaken in spring 2013 at each of the seven experimental sites. Results are a mean of samples taken from three replicate plots of each treatment with output from individual site ANOVAs. A separate ANOVA was carried out at each site (data was normally distributed), after which *post-hoc* testing was undertaken to evaluate which treatment means were different from each other using a Duncan's multiple range test (using Genstat version 12; VSN International Ltd, 2010). This test assigns different letters to treatment values which are significantly different from each other at the 5% level ($P<0.05$). In the tables of results treatments which are statistically significantly different are marked with different letters. Note manure-based digestate was only applied at the Scottish sites (Aberdeen & Ayr).

1 Soil physical properties

Shear Strength (kPa)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	10.8	54.0 [b]	7.53	25.4	18.4 [c]	62.6	8.39
Green compost	11.2	47.9 [a]	8.12	25.7	16.2 [ab]	60.8	8.47
Green/food compost	11.1	54.0 [b]	7.14	26.4	18.3 [c]	61.0	8.76
Food-based digestate	11.2	59.8 [c]	7.53	25.1	17.5 [bc]	59.7	9.31
FYM	11.4	43.3 [a]	7.91	25.6	14.6 [a]	61.3	7.19
Slurry	10.7	45.3 [a]	7.28	26.3	16.1 [ab]	56.1	7.45
Manure-based digestate	11.0	54.7 [b]	~	~	~	~	~
P	*0.41*	***<0.001***	*0.53*	*0.97*	***0.009***	*0.30*	*0.14*

Penetrometer resistance (N/cm^2)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	40.6	77.2 [a]	33.9	34.9	61.7	219.5	30.1
Green compost	38.9	85.6 [a]	34.4	35.7	55.1	214.7	36.0
Green/food compost	46.1	74.9 [a]	33.4	31.0	53.0	231.7	35.1
Food-based digestate	45.9	105.2 [b]	35.3	30.1	52.3	211.0	39.2
FYM	39.0	70.2 [a]	29.7	31.2	48.4	201.0	27.2
Slurry	48.2	75.6 [a]	34.8	35.5	46.1	211.3	28.4
Manure-based digestate	42.9	79.8 [a]	~	~	~	~	~
P	*0.06*	***0.017***	*0.39*	*0.20*	*0.17*	*0.45*	*0.12*

Initial infiltration rate (mm/hr)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	220.0	84.0	1528.0	25.9	56.0	80.0	1212.0
Green compost	204.0	104.0	1180.0	13.3	32.0	128.0	1540.0
Green/food compost	276.0	160.0	1662.5	55.3	52.0	176.0	1084.7
Food-based digestate	216.0	64.0	1268.0	5.00	64.0	282.0	1412.0
FYM	240.0	72.0	1176.0	181.7	188.0	316.0	1760.0
Slurry	188.0	148.0	1304.0	280.0	40.0	120.0	1360.0
Manure-based digestate	212.0	60.0	~	~	~	~	~
P	*0.99*	*0.61*	*0.22*	*0.08*	*0.18*	*0.15*	*0.76*

Equilibrium infiltration rate (mm/hr)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	146.7	44.0	419.9	11.6	29.3	64.0	542.7
Green compost	108.0	72.0	396.8	13.4	30.7	129.3	851.5
Green/food compost	256.0	101.3	398.6	33.5	18.7	124.0	638.0
Food-based digestate	122.7	41.3	408.3	2.41	53.3	222.0	736.0
FYM	156.0	50.7	441.3	66.6	133.3	180.0	970.4
Slurry	110.7	50.7	376.7	69.5	40.0	112.0	768.2
Manure-based digestate	114.7	33.3	~	~	~	~	~
P	*0.12*	*0.14*	*0.99*	*0.20*	*0.12*	*0.30*	*0.48*

Bulk density (g/cm^3)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.98	1.23^{bc}	0.63	1.04	1.51^{b}	0.91	1.49
Green compost	0.98	1.16^{a}	0.64	1.02	1.38^{a}	0.86	1.46
Green/food compost	0.96	1.17^{ab}	0.63	1.02	1.46^{ab}	0.78	1.48
Food-based digestate	0.98	1.28^{c}	0.67	1.04	1.48^{ab}	0.97	1.45
FYM	0.98	1.17^{ab}	0.64	0.98	1.37^{a}	0.88	1.40
Slurry	0.95	1.25^{c}	0.64	1.02	1.39^{a}	0.96	1.49
Manure-based digestate	0.97	1.25^{c}	~	~	~	~	~
P	*0.90*	***0.008***	*0.38*	*0.18*	***0.04***	*0.06*	*0.20*

Porosity (%)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	63.0	53.4^{ab}	76.1	60.9	43.0^{a}	65.6	43.8
Green compost	62.9	56.1^{c}	75.7	61.5	48.0^{b}	67.5	44.9
Green/food compost	63.8	55.7^{bc}	76.1	61.4	44.8^{ab}	70.4	44.1
Food-based digestate	62.9	51.6^{a}	74.7	60.7	44.3^{ab}	63.5	45.2
FYM	63.2	55.8^{bc}	75.9	62.9	48.3^{a}	66.6	47.1
Slurry	64.0	52.8^{a}	76.0	61.5	47.4^{a}	63.9	43.6
Manure-based digestate	63.5	52.7^{a}	~	~	~	~	~
P	*0.90*	***0.008***	*0.38*	*0.18*	***0.04***	*0.06*	*0.20*

Stability (% dispersion ratio)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	2.47	3.13	1.75	3.66^{a}	10.29	2.52	8.77
Green compost	2.42	3.35	1.74	7.13^{b}	8.27	2.58	8.13
Green/food compost	2.26	3.35	2.52	6.41^{a}	8.08	2.55	8.95
Food-based digestate	2.29	2.79	2.23	6.33^{a}	7.44	2.59	8.02
FYM	2.55	2.74	1.59	4.93^{a}	7.51	2.88	9.02
Slurry	2.70	3.36	2.07	5.13^{a}	8.36	2.21	8.57
Manure-based digestate	2.33	3.20	~	~	~	~	~
P	*0.88*	*0.45*	*0.31*	***0.039***	*0.10*	*0.58*	*0.78*

Moisture @ field capacity (% v/v)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	42.9	47.3	41.2	59.1	29.0	51.8^{a}	38.0
Green compost	43.0	49.8	40.2	58.9	29.4	55.5^{b}	38.5
Green/food compost	42.9	49.1	40.1	59.5	29.5	50.9^{a}	37.1
Food-based digestate	44.8	47.1	41.0	60.1	29.9	51.8^{a}	36.5
FYM	44.2	50.0	40.8	60.6	31.0	56.8^{b}	38.5
Slurry	42.4	47.6	40.6	61.4	29.7	53.9^{ab}	38.8
Manure-based digestate	44.3	48.7	~	~	~	~	~
P	*0.48*	*0.11*	*0.95*	*0.30*	*0.57*	***0.021***	*0.18*

Moisture @ 2 bar (% v/v)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	20.4	26.7 [ab]	20.3	35.6	14.1	27.9 [a]	25.2
Green compost	20.7	27.7 [ab]	20.3	35.6	13.3	29.3 [a]	25.7
Green/food compost	20.7	25.2 [a]	20.8	35.9	13.6	26.5 [a]	25.4
Food-based digestate	21.3	29.5 [b]	21.7	37.2	14.2	30.5 [ab]	24.7
FYM	20.3	27.4 [ab]	20.0	35.3	14.3	33.6 [b]	25.1
Slurry	19.8	25.4 [a]	20.4	37.5	13.5	30.0 [ab]	26.1
Manure-based digestate	20.9	28.6 [b]	~	~	~	~	~
P	*0.06*	***0.040***	*0.20*	*0.24*	*0.54*	***0.024***	*0.18*

Moisture @ 15 bar (% v/v)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	11.9	16.1 [a]	14.4	29.1	10.4	18.5 [ab]	18.8
Green compost	12.4	18.1 [ab]	14.8	28.8	10.0	19.8 [ab]	19.4
Green/food compost	12.5	16.1 [a]	15.2	28.8	10.5	17.9 [a]	18.8
Food-based digestate	13.0	18.6 [b]	15.4	29.9	10.4	20.8 [b]	18.2
FYM	12.3	18.6 [b]	14.4	28.3	10.4	24.4 [c]	18.7
Slurry	12.3	16.1 [a]	14.8	30.1	9.84	20.9 [b]	18.9
Manure-based digestate	12.7	18.2 [ab]	~	~	~	~	~
P	*0.13*	***0.05***	*0.30*	*0.30*	*0.61*	***0.003***	*0.30*

Available Water Capacity (AWC; %)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	31.0	31.1	26.8	30.1	18.6	33.4	19.2
Green compost	30.7	31.7	25.4	30.1	19.4	35.7	19.1
Green/food compost	30.4	33.0	24.9	30.7	19.0	33.0	18.3
Food-based digestate	31.9	28.5	25.6	30.2	19.5	31.0	18.2
FYM	31.9	31.5	26.4	32.3	20.6	32.4	19.7
Slurry	30.1	31.5	25.8	31.3	19.9	33.0	19.8
Manure-based digestate	31.6	30.5	~	~	~	~	~
P	*0.61*	*0.17*	*0.64*	*0.40*	*0.22*	*0.31*	*0.10*

Easily Available Water Capacity (%)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	22.6	20.5 [ab]	20.9	23.6	14.9	24.0	12.8
Green compost	22.3	22.1 [bc]	19.9	23.3	16.1	26.3	12.8
Green/food compost	22.3	23.9 [c]	19.3	23.5	15.9	24.3	11.6
Food-based digestate	23.6	17.5 [a]	19.2	22.9	15.7	21.2	11.8
FYM	24.0	22.6 [bc]	20.8	25.3	16.7	23.2	13.4
Slurry	22.5	22.1 [bc]	20.2	23.8	16.2	23.9	12.6
Manure-based digestate	23.4	20.1 [ab]	~	~	~	~	~
P	*0.64*	***0.012***	*0.80*	*0.51*	*0.23*	*0.34*	*0.18*

2 Soil biological properties

Microbial biomass C (mg/kg dm)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	360.8	276.5	388.1	718.0	177.6 ^{ab}	791.4	272.8 ^{a}
Green compost	458.2	275.1	294.3	740.4	212.6 ^{ab}	785.9	322.1 ^{ab}
Green/food compost	458.4	312.2	339.3	638.0	171.4 ^{a}	849.3	311.3 ^{a}
Food-based digestate	385.4	303.2	314.8	759.7	167.1 ^{ab}	687.0	306.8 ^{a}
FYM	424.1	323.2	306.1	712.9	312.7 ^{c}	753.2	374.0 ^{c}
Slurry	429.5	359.4	329.6	722.2	234.6 ^{b}	819.4	360.2 ^{bc}
Manure-based digestate	451.1	339.1	~	~	~	~	~
P	*0.25*	*0.39*	*0.20*	*0.51*	***0.002***	*0.23*	***0.007***

Microbial biomass N (mg/kg dm)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	64.4	78.1	145.4	97.4	27.6 ^{a}	162.4	50.3 ^{a}
Green compost	58.4	86.0	165.4	112.9	37.4 ^{b}	142.9	54.7 ^{a}
Green/food compost	60.5	84.8	143.0	92.3	28.1 ^{a}	165.3	55.7 ^{a}
Food-based digestate	64.0	88.5	137.9	108.1	29.5 ^{a}	148.4	54.8 ^{a}
FYM	67.3	91.4	140.5	98.4	45.6 ^{c}	145.9	68.7 ^{b}
Slurry	57.2	88.7	142.3	112.5	36.1 ^{b}	177.2	57.9 ^{a}
Manure-based digestate	58.2	84.3	~	~	~	~	~
P	*0.71*	*0.55*	*0.89*	*0.18*	***<0.001***	*0.18*	***0.035***

Respiration rate (mg CO_2-C/kg/hr)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.77	0.61	1.61	0.45	1.52	2.47	0.66
Green compost	1.04	0.92	1.71	0.40	1.79	2.51	0.64
Green/food compost	0.66	0.54	2.31	0.85	1.80	2.43	0.78
Food-based digestate	0.66	0.72	1.92	0.39	1.45	2.22	0.68
FYM	1.43	1.20	2.34	0.90	1.59	3.04	0.74
Slurry	0.68	0.35	1.90	1.21	1.45	2.33	0.61
Manure-based digestate	1.20	0.95	~	~	~	~	~
P	*0.24*	*0.09*	*0.15*	*0.19*	*0.80*	*0.42*	*0.49*

PMN (mg/kg dm)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	51.6	93.5	115.7 ^{ab}	93.1	30.0 ^{a}	179.5	22.2 ^{a}
Green compost	53.9	112.0	112.3 ^{ab}	89.2	40.1 ^{ab}	198.8	35.0 ^{a}
Green/food compost	52.8	82.4	115.5 ^{ab}	94.5	37.9 ^{ab}	195.1	31.9 ^{a}
Food-based digestate	50.1	118.4	89.8 ^{a}	89.6	30.3 ^{a}	162.2	23.2 ^{a}
FYM	54.2	107.3	143.3 ^{b}	93.0	44.3 ^{b}	216.7	60.6 ^{b}
Slurry	52.7	74.7	140.4 ^{b}	97.7	43.9 ^{b}	223.1	45.2 ^{ab}
Manure-based digestate	47.0	77.8	~	~	~	~	~
P	*0.25*	*0.34*	***0.033***	*0.99*	***0.028***	*0.55*	***0.028***

Earthworm numbers (counts/m^2)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	301	607 ^{b}	33	158 ^{a}	10	222 ^{ab}	119 ^{ab}
Green compost	326	691 ^{b}	53	205 ^{ab}	58	351 ^{abc}	151 ^{bc}
Green/food compost	281	760 ^{b}	53	147 ^{a}	49	420 ^{bc}	86 ^{ab}
Food-based digestate	232	291 ^{a}	58	147 ^{a}	44	173 ^{a}	75 ^{a}
FYM	370	825 ^{b}	44	270. ^{b}	69	553 ^{c}	195 ^{c}
Slurry	242	691 ^{b}	88	244 ^{b}	25	400 ^{bc}	146 ^{abc}
Manure-based digestate	222	583 ^{b}	~	~	~	~	~
P	*0.69*	***0.009***	*0.21*	***0.013***	*0.06*	***0.015***	***0.024***

Earthworm weight (g/m²)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	68.7	272.6	7.59	37.1 ab	2.91	130.3	29.9 ab
Green compost	86.3	286.6	19.0	49.8 abc	8.18	107.2	29.6 ab
Green/food compost	79.9	328.0	18.6	33.5 a	12.4	129.7	15.1 a
Food-based digestate	81.4	181.1	26.6	45.1 ab	18.2	103.1	11.5 a
FYM	92.0	363.3	12.2	74.2 c	18.0	124.0	47.9 b
Slurry	52.0	316.4	34.3	65.4 bc	10.7	127.8	27.3 a
Manure-based digestate	58.9	289.4	~	~	~	~	~
P	*0.89*	*0.09*	*0.16*	***0.044***	*0.67*	*0.86*	***0.015***

3 Soil chemical properties

Soil organic matter (SOM; %)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	6.97	4.26	7.38	6.28	2.31 a	7.27	2.67 a
Green compost	7.10	4.14	7.62	6.05	2.95 b	7.77	3.20 b
Green/food compost	7.82	3.74	7.42	6.09	2.62 ab	7.90	2.89 ab
Food-based digestate	8.05	4.00	7.20	6.10	2.43 a	6.99	2.71 a
FYM	7.35	4.31	7.66	6.57	2.92 b	7.55	3.20 b
Slurry	7.18	3.84	7.59	6.33	2.61 ab	7.29	2.74 a
Manure-based digestate	7.30	3.94	~	~	~	~	~
P	*0.27*	*0.58*	*0.56*	*0.50*	***0.016***	*0.41*	***0.046***
Light fraction organic matter (LFOM; g/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	3.80	5.30	1.67 a	0.92 ab	1.49 a	10.5 bc	3.19
Green compost	6.02	7.85	3.17 b	1.82 c	2.83 c	10.7 bc	4.44
Green/food compost	4.15	5.72	3.01 b	1.53 bc	2.54 bc	12.7 cd	3.86
Food-based digestate	4.38	6.10	1.29 a	0.63 a	1.18 a	4.24 a	2.96
FYM	3.41	5.15	3.10 b	1.04 ab	1.81 ab	16.3 d	4.37
Slurry	4.09	5.84	1.80 a	0.82 ab	1.57 a	7.01 ab	3.57
Manure-based digestate	4.94	5.14	~	~	~	~	~
P	*0.20*	*0.06*	***<0.001***	***0.034***	***0.004***	***0.002***	*0.06*
Dissolved organic carbon (DOC; mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	65.6	113.6	108.2	41.1	25.1	127.2 a	0.57 a
Green compost	47.6	136.7	110.6	65.3	48.0	151.7 ab	6.95 a
Green/food compost	49.0	118.1	98.7	53.1	31.1	160.0 ab	2.70 a
Food-based digestate	56.0	114.7	88.2	48.7	24.6	123.3 a	0.10 a
FYM	45.6	128.2	114.8	51.0	55.9	178.9 b	15.4 b
Slurry	58.1	89.3	103.5	58.6	32.4	135.9 a	2.45 a
Manure-based digestate	43.8	127.3	~	~	~	~	~
P	*0.07*	*0.21*	*0.13*	*0.06*	*0.22*	***0.035***	***0.006***
Cation Exchange Capacity (CEC; meq/100g)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	19.3	11.8	21.7	28.4	5.5 a	17.1 ab	10.4
Green compost	19.5	12.8	21.9	29.4	8.3 b	18.6 bc	11.4
Green/food compost	20.6	11.5	22.0	28.5	5.7 a	18.8 bc	10.8
Food-based digestate	21.4	11.9	22.0	28.6	5.8 a	15.0 a	10.5
FYM	20.4	12.8	23.2	27.6	6.4 a	20.4 c	11.5
Slurry	20.3	10.8	21.8	28.2	6.2 a	16.9 ab	10.3
Manure-based digestate	20.6	11.6	~	~	~	~	~
P	*0.47*	*0.11*	*0.92*	*0.54*	***0.008***	***0.015***	*0.19*

pH

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	5.77	5.73 [a]	8.07	7.30	6.63 [ab]	5.97 [b]	7.43 [a]
Green compost	5.73	6.07 [ab]	8.23	7.40	6.87 [bc]	6.20 [c]	7.43 [a]
Green/food compost	5.67	6.33 [b]	8.03	7.33	6.60 [ab]	6.30 [c]	7.60 [ab]
Food-based digestate	5.63	6.13 [b]	8.03	7.33	6.50 [a]	5.73 [a]	7.83 [b]
FYM	5.70	6.27 [b]	8.03	7.37	7.03 [c]	6.20 [c]	7.93 [b]
Slurry	5.73	6.17 [b]	8.20	7.40	6.97 [c]	6.20 [c]	7.87 [b]
Manure-based digestate	5.77	6.00 [b]	~	~	~	~	~
P	*0.74*	***0.027***	*0.42*	*0.75*	***0.018***	***<0.001***	***0.027***

Extractable P (mg/l)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	53.0	42.3 [a]	23.3	15.3	71.7 [a]	27.3 [a]	26.0 [ab]
Green compost	55.0	43.0 [a]	27.0	15.3	74.3 [a]	30.7 [a]	30.7 [ab]
Green/food compost	61.7	43.7 [a]	25.7	17.3	71.3 [a]	33.7 [a]	31.0 [ab]
Food-based digestate	54.7	44.3 [a]	23.3	17.7	73.3 [a]	29.0 [a]	23.0 [a]
FYM	55.0	58.7 [b]	30.7	17.7	88.7 [b]	49.0 [b]	54.7 [c]
Slurry	53.3	43.0 [a]	28.0	19.0	71.7 [a]	33.7 [a]	34.0 [b]
Manure-based digestate	56.3	40.3 [a]	~	~	~	~	~
P	*0.12*	***0.028***	*0.11*	*0.62*	***0.002***	***0.004***	***<0.001***

Extractable K (mg/l)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	129.3 [a]	142.0 [a]	190.3 [a]	303.3 [a]	91.0 [a]	250.7	269.0 [a]
Green compost	167.7 [bc]	176.7 [ab]	310.0 [bc]	324.0 [ab]	168.0 [c]	337.7	350.0 [b]
Green/food compost	155.3 [ab]	147.7 [a]	262.7 [b]	305.7 [a]	147.0 [bc]	358.0	293.0 [a]
Food-based digestate	138.0 [ab]	238.3 [b]	256.0 [b]	315.3 [ab]	138.0 [b]	324.0	253.7 [a]
FYM	188.0 [c]	394.7 [c]	497.7 [d]	361.0 [c]	282.0 [e]	404.3	408.3 [c]
Slurry	154.0 [ab]	157.0 [a]	354.7 [c]	339.7 [bc]	199.0 [d]	475.0	359.7 [bc]
Manure-based digestate	140.7 [ab]	206.7 [ab]	~	~	~	~	~
P	***0.022***	***<0.001***	***<0.001***	***0.009***	***<0.001***	*0.07*	***<0.001***

Extractable Mg (mg/l)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	69.7	120.7 [a]	49.3 [ab]	282.3	36.0 [a]	64.0 [a]	257.7
Green compost	83.0	139.7 [b]	57.3 [c]	283.3	52.3 [b]	90.0 [bc]	247.3
Green/food compost	67.0	121.7 [a]	56.0 [bc]	266.0	47.7 [b]	80.7 [ab]	213.7
Food-based digestate	60.0	121.7 [a]	46.0 [a]	276.0	40.7 [a]	60.0 [a]	194.3
FYM	74.0	162.3 [c]	69.7 [d]	295.3	79.0 [d]	131.5 [d]	224.0
Slurry	77.3	143.7 [b]	62.7 [c]	301.7	59.7 [c]	102.0 [c]	221.7
Manure-based digestate	67.0	141.3 [b]	~	~	~	~	~
P	*0.34*	***<0.001***	***<0.001***	*0.08*	***<0.001***	***<0.001***	*0.66*

Total N (%)

Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.30 [a]	0.21	0.59	0.36	0.09 [a]	0.44 [ab]	0.15 [a]
Green compost	0.37 [bc]	0.23	0.62	0.37	0.12 [bc]	0.47 [abc]	0.18 [b]
Green/food compost	0.37 [bc]	0.19	0.60	0.37	0.10 [ab]	0.50 [c]	0.15 [a]
Food-based digestate	0.36 [bc]	0.22	0.58	0.38	0.09 [a]	0.44 [a]	0.14 [a]
FYM	0.32 [ab]	0.25	0.61	0.36	0.12 [c]	0.48 [bc]	0.17 [b]
Slurry	0.35 [bc]	0.22	0.61	0.36	0.10 [ab]	0.45 [ab]	0.15 [a]
Manure-based digestate	0.40 [c]	0.22	~	~	~	~	~
P	***0.012***	*0.18*	*0.76*	*0.07*	***0.020***	***0.035***	***0.003***

Extractable S (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	27.6	22.8	31.4	27.5 [a]	7.33 [a]	67.6	9.37 [ab]
Green compost	25.5	23.6	31.7	29.5 [a]	9.83 [bc]	58.4	8.10 [a]
Green/food compost	28.2	25.5	35.0	27.5 [a]	9.37 [bc]	76.5	8.77 [ab]
Food-based digestate	28.2	25.8	32.1	31.1 [ab]	7.97 [ab]	72.5	7.83 [a]
FYM	27.9	28.3	33.2	30.8 [ab]	12.7 [d]	80.2	10.9 [b]
Slurry	29.9	20.9	33.2	34.4 [b]	10.2 [c]	87.2	10.4 [b]
Manure-based digestate	31.3	26.8	~	~	~	~	~
P	*0.45*	*0.12*	*0.57*	***0.023***	***<0.001***	*0.43*	***0.032***
Total Zn (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	41.3	75.6	57.9	90.2 [a]	49.9	65.1	64.2 [a]
Green compost	50.4	78.5	62.1	93.1 [ab]	49.6	68.6	63.9 [a]
Green/food compost	51.0	71.2	58.3	96.0 [b]	45.0	70.0	68.4 [a]
Food-based digestate	53.6	79.5	57.6	93.0 [ab]	43.8	63.5	64.7 [a]
FYM	46.4	79.4	59.5	91.6 [a]	48.7	74.4	77.7 [b]
Slurry	50.8	72.3	60.0	92.5 [a]	46.0	67.8	67.7 [a]
Manure-based digestate	49.4	75.4	~	~	~	~	~
P	*0.35*	*0.18*	*0.76*	***0.038***	*0.90*	*0.06*	***<0.001***
Total Cu (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	10.5	17.1	8.40	12.3	9.43	13.8	13.1 [a]
Green compost	12.9	17.6	10.2	12.9	11.3	14.5	13.5 [a]
Green/food compost	12.2	15.4	8.97	13.2	10.0	14.9	14.2 [a]
Food-based digestate	13.0	16.1	8.40	12.7	9.60	12.9	13.4 [a]
FYM	10.3	17.8	8.73	12.6	10.9	14.4	17.3 [c]
Slurry	13.0	15.9	8.80	13.2	9.90	15.8	15.2 [b]
Manure-based digestate	12.3	17.0	~	~	~	~	~
P	*0.46*	*0.16*	*0.23*	*0.20*	*0.57*	*0.09*	***<0.001***
Ext Cu (mg/l)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	2.20	4.47	0.73	3.60	4.90 [a]	1.93 [ab]	5.63 [a]
Green compost	2.57	4.33	0.70	3.90	5.67 [b]	2.47 [bcd]	6.37 [ab]
Green/food compost	2.03	4.70	0.63	3.90	5.87 [bcd]	2.17 [abc]	6.87 [b]
Food-based digestate	1.73	4.50	0.73	3.10	5.67 [bc]	1.83 [a]	6.43 [ab]
FYM	1.83	4.83	0.73	3.80	6.13 [cd]	2.77 [cd]	8.87 [d]
Slurry	2.70	4.70	0.73	3.67	5.73 [bcd]	2.97 [d]	7.80 [c]
Manure-based digestate	2.33	4.80	~	~	~	~	~
P	*0.59*	*0.59*	*0.95*	*0.10*	***0.002***	***0.007***	***<0.001***
Total Pb (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	18.3	28.6	16.1 [a]	25.0	17.8	24.7	17.9 [a]
Green compost	24.4	29.5	20.2 [b]	27.3	17.2	18.4	20.4 [b]
Green/food compost	27.7	27.2	16.4 [a]	25.4	15.4	19.4	18.1 [a]
Food-based digestate	24.0	29.0	16.0 [a]	26.1	13.3	16.6	17.1 [a]
FYM	18.0	31.6	16.2 [a]	26.3	14.0	15.6	17.6 [a]
Slurry	21.7	27.4	16.7 [a]	25.2	13.7	16.9	17.8 [a]
Manure-based digestate	31.0	29.7	~	~	~	~	~
P	*0.49*	*0.06*	***0.012***	*0.44*	*0.60*	*0.17*	***0.005***

Total Ni (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	13.0	18.3	9.20	31.2	6.70	15.3	21.2
Green compost	12.6	18.8	9.13	31.9	5.00	14.9	20.5
Green/food compost	12.2	18.0	10.9	33.0	6.73	15.1	21.4
Food-based digestate	12.9	17.7	11.1	31.0	5.00	14.5	21.1
FYM	12.2	17.8	10.4	32.3	5.00	15.1	21.2
Slurry	11.8	17.6	9.17	30.7	5.00	14.9	21.1
Manure-based digestate	11.8	18.7	~	~	~	~	~
P	*0.63*	*0.94*	*0.57*	*0.14*	*0.47*	*0.91*	*0.16*
Total Cd (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.20 [a]	0.15	0.64	0.28	0.22	0.09	0.12
Green compost	0.25 [bc]	0.15	0.64	0.30	0.22	0.09	0.14
Green/food compost	0.27 [cd]	0.17	0.63	0.31	0.20	0.13	0.13
Food-based digestate	0.29 [d]	0.18	0.64	0.30	0.19	0.09	0.13
FYM	0.23 [ab]	0.19	0.64	0.30	0.21	0.12	0.12
Slurry	0.25 [bc]	0.16	0.66	0.30	0.20	0.13	0.12
Manure-based digestate	0.25 [bc]	0.17	~	~	~	~	~
P	***0.004***	*0.48*	*0.94*	*0.81*	*0.57*	*0.37*	*0.29*
Total Cr (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	76.0	36.0	23.7	48.1	22.7	32.0	26.3
Green compost	67.4	35.3	23.8	49.6	18.5	30.1	26.8
Green/food compost	61.7	33.8	24.3	50.7	19.0	30.2	27.1
Food-based digestate	59.1	35.8	25.6	53.5	21.4	30.5	27.1
FYM	66.0	35.1	22.3	49.3	20.4	31.7	27.4
Slurry	59.7	34.8	22.5	49.8	18.7	30.8	26.7
Manure-based digestate	63.9	37.1	~	~	~	~	~
P	*0.37*	*0.59*	*0.77*	*0.41*	*0.19*	*0.32*	*0.58*
Total As (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	2.13	4.50	5.73	15.7	4.63	11.8	11.0
Green compost	3.63	4.63	5.80	15.8	5.03	11.9	10.3
Green/food compost	3.60	4.90	5.60	16.0	4.67	11.7	10.9
Food-based digestate	3.90	5.03	5.53	15.4	4.67	11.6	10.7
FYM	3.17	5.30	5.43	15.7	4.63	11.0	10.9
Slurry	2.83	4.53	5.87	15.5	4.67	11.5	10.7
Manure-based digestate	2.87	4.77	~	~	~	~	~
P	*0.25*	*0.17*	*0.77*	*0.88*	*0.90*	*0.79*	*0.06*
Total Hg (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.07	0.10	0.07	0.05	0.04	0.07	0.08
Green compost	0.07	0.09	0.08	0.05	0.04	0.06	0.07
Green/food compost	0.07	0.09	0.08	0.05	0.03	0.08	0.06
Food-based digestate	0.09	0.11	0.07	0.05	0.03	0.06	0.07
FYM	0.06	0.11	0.08	0.05	0.04	0.07	0.06
Slurry	0.10	0.10	0.08	0.05	0.04	0.07	0.06
Manure-based digestate	0.08	0.10	~	~	~	~	~
P	*0.48*	*0.35*	*0.57*	*0.62*	*0.74*	*0.71*	*0.35*

Total Se (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.41 [a]	0.34	0.37	0.37	0.07	0.71	0.13
Green compost	0.48 [ab]	0.32	0.38	0.38	0.08	0.67	0.13
Green/food compost	0.53 [bc]	0.30	0.35	0.39	0.09	0.70	0.13
Food-based digestate	0.60 [c]	0.33	0.38	0.39	0.08	0.69	0.13
FYM	0.47 [ab]	0.34	0.37	0.38	0.11	0.65	0.15
Slurry	0.49 [ab]	0.30	0.40	0.38	0.08	0.71	0.15
Manure-based digestate	0.51 [b]	0.35	~	~	~	~	~
P	***0.016***	*0.71*	*0.80*	*0.29*	*0.28*	*0.59*	*0.10*
Total Mo (mg/kg dm) : limit of detection = 0.5mg/kg							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	1.13	<0.50	<0.50	<0.50	<0.50	0.67	<0.50
Green compost	0.90	<0.50	<0.50	<0.50	<0.50	0.50	<0.50
Green/food compost	0.73	<0.50	<0.50	0.83	<0.50	0.70	<0.50
Food-based digestate	0.73	<0.50	<0.50	1.07	<0.50	0.50	<0.50
FYM	0.87	<0.50	<0.50	0.73	<0.50	0.93	<0.50
Slurry	<0.50	<0.50	<0.50	0.67	<0.50	0.70	<0.50
Manure-based digestate	0.73	<0.50	~	~	~	~	~
P	*0.65*	*1.00*	*1.00*	*0.41*	*1.00*	*0.52*	*1.00*
Total F (mg/kg dm): limit of detection = 0.5 mg/kg							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	37.7	27.5	<0.50	10.5	23.1	28.9	33.3
Green compost	40.2	27.6	<0.50	10.5	24.0	24.5	35.4
Green/food compost	38.1	25.9	<0.50	11.2	23.0	24.7	30.0
Food-based digestate	39.7	29.1	<0.50	8.30	23.2	26.6	27.2
FYM	38.5	26.7	<0.50	10.3	22.8	24.8	19.4
Slurry	39.8	25.3	<0.50	9.73	23.5	27.3	34.3
Manure-based digestate	39.9	26.5	~	~	~	~	~
P	*0.55*	*0.26*	*1.00*	*0.61*	*0.81*	*0.19*	*0.21*
Total Co (mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	nd	8.20	nd	nd	nd	8.33	nd
Green compost	nd	8.73	nd	nd	nd	8.13	nd
Green/food compost	nd	8.47	nd	nd	nd	8.50	nd
Food-based digestate	nd	8.37	nd	nd	nd	8.50	nd
FYM	nd	8.90	nd	nd	nd	8.67	nd
Slurry	nd	8.37	nd	nd	nd	8.63	nd
Manure-based digestate	nd	8.43	~	~	~	~	~
P	*nd*	*0.71*	*nd*	*nd*	*nd*	*0.87*	*nd*
Soluble B (mg/l)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.77	0.77	2.27	2.83	0.50 [a]	0.90	2.20
Green compost	0.83	0.80	1.70	2.77	0.60 [ab]	1.13	2.33
Green/food compost	0.80	0.87	1.70	2.83	0.57 [ab]	1.03	2.17
Food-based digestate	0.80	0.83	1.73	2.70	0.57 [ab]	0.90	2.03
FYM	0.80	0.93	1.70	2.83	0.73 [c]	1.33	2.27
Slurry	0.87	0.77	1.70	2.80	0.63 [bc]	1.03	2.23
Manure-based digestate	0.83	0.80	~	~	~	~	~
P	*0.73*	*0.10*	*0.09*	*0.31*	***0.010***	*0.14*	*0.30*

DEHP – phthalate (mg/kg dm) : limit of detection = 0.05 mg/kg							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Green compost	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Green/food compost	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Food-based digestate	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
FYM	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Slurry	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Manure-based digestate	<0.05	<0.05	~	~	~	~	~
P	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*
Sum of 9 Polycyclic aromatic hydrocarbons (PAHs; mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.200	0.291	0.214	0.160	0.241	0.137	0.293
Green compost	0.331	0.285	0.259	0.179	0.255	0.190	0.397
Green/food compost	0.244	0.315	0.366	0.174	0.230	0.196	0.285
Food-based digestate	0.263	0.459	0.197	0.274	0.426	0.125	0.326
FYM	0.296	0.327	0.203	0.147	0.532	0.195	0.281
Slurry	0.267	0.258	0.215	0.178	0.219	0.126	0.278
Manure-based digestate	0.212	0.285	~	~	~	~	~
P	*0.93*	*0.54*	*0.32*	*0.51*	*0.70*	*0.12*	*0.57*
Sum of 7 polychlorinated biphenyls (PCBs; mg/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	0.0005	0.0003 [a]	0.0005 [a]	0.0005	0.0007	0.0004 [a]	0.0004
Green compost	0.0005	0.0006 [b]	0.0008 [b]	0.0006	0.0009	0.0005 [a]	0.0005
Green/food compost	0.0006	0.0004 [a]	0.0010 [c]	0.0006	0.0008	0.0007 [b]	0.0005
Food-based digestate	0.0006	0.0004 [a]	0.0005 [a]	0.0006	0.0009	0.0003 [a]	0.0003
FYM	0.0004	0.0003 [a]	0.0005 [a]	0.0005	0.0014	0.0004 [a]	0.0003
Slurry	0.0004	0.0003 [a]	0.0005	0.0006	0.0008	0.0004 [a]	0.0004
Manure-based digestate	0.0005	0.0003 [a]	~	~	~	~	~
P	*0.51*	***0.005***	***<0.001***	*0.41*	*0.76*	***<0.001***	*0.34*
Dioxins and Furans (TEQ ng/kg dm)							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	1.229	0.937	0.970	0.851	1.344	0.961	1.320
Green compost	1.544	1.029	1.075	1.011	1.636	1.091	1.257
Green/food compost	1.324	1.064	1.206	0.982	1.719	1.073	1.009
Food-based digestate	1.208	0.949	1.083	0.963	1.437	0.940	1.123
FYM	1.177	0.961	0.988	0.863	1.438	0.960	0.925
Slurry	1.236	0.932	1.157	1.082	1.548	0.941	1.019
Manure-based digestate	1.204	0.970	~	~	~	~	~
P	*0.08*	*0.09*	*0.37*	*0.35*	*0.13*	*0.10*	*0.68*
Total I (mg/kg dm): limit of detection = 5.0 mg/kg; not determined at arable sites							
Treatment	Aberdeen	Ayr	Devizes	Faringdon	Harper Adams	Lampeter	Terrington
Control	nd	<5.00	nd	nd	nd	<5.00	nd
Green compost	nd	<5.00	nd	nd	nd	<5.00	nd
Green/food compost	nd	<5.00	nd	nd	nd	<5.00	nd
Food-based digestate	nd	<5.00	nd	nd	nd	<5.00	nd
FYM	nd	<5.00	nd	nd	nd	<5.00	nd
Slurry	nd	<5.00	nd	nd	nd	<5.00	nd
Manure-based digestate	nd	<5.00	~	~	~	~	~
P	*nd*	*1.00*	*nd*	*nd*	*nd*	*1.00*	*nd*

Appendix 2. Earthworm Studies – Technical Briefing Note

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1 Introduction

One of the key aims of the *DC-Agri* project was to quantify the effects of *repeated* digestate and compost applications on soil quality and fertility, including earthworm numbers. Earthworms have a major influence on soil quality and are "probably, the most important soil macro-animal" (Brady, 1974). They are often referred to as "ecosystem engineers", due to their role in breaking down organic matter, improving soil structure and allowing water/oxygen to move through the soil profile (Blouin *et al*., 2013).

2 Worm populations at *DC-Agri* sites

Earthworm populations were measured in spring 2013 on 3 'blocks' of soil (each 30 x 30 x 25 cm deep) per plot; by counting all adult and immature worms collected within a 5 minute period. The measurements were made between 6 and 8 months after the last digestate application.

2.1 Results

2.2 Site-by-site analysis

- There were treatment differences in earthworm numbers at 4 sites - Ayr, Faringdon, Lampeter and Terrington ($P<0.05$), but not at Aberdeen, Devizes or Harper Adams ($P>0.05$).
- At Ayr, the application of food-based digestate *reduced* earthworm numbers in comparison with all the other treatments ($P<0.05$). Notably, the Ayr site had the greatest number of earthworms.
- 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 treatment at Terrington, but not against the fertiliser only control at these sites ($P<0.05$), see Appendix 2.1.

2.3 Cross-site analysis

- At the grassland sites (Ayr and Lampeter), overall earthworm numbers on the food-based digestate treatments were *lower* than on all the other treatments ($P<0.001$ in cross site ANOVA; Figure 1).
- In contrast, earthworm numbers at the arable sites were similar on all the treatments; although overall earthworm numbers on the FYM treatment were significantly higher than on the control, green/food compost and food-based digestate treatments ($P<0.01$ in cross site ANOVA; Figure 2).

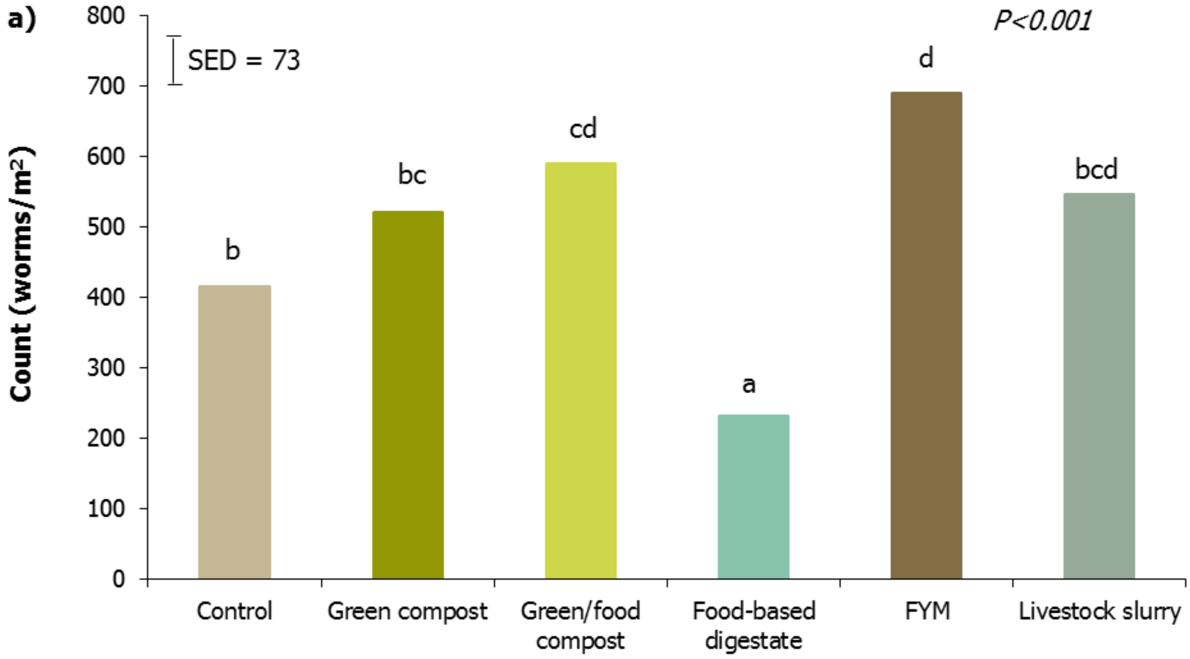

Figure 1. Earthworm numbers (worms/m^2 to 25 cm depth) at the two grassland sites (standard error of difference between means (SED) 73.3). Bars labelled with different letters differ significantly (*P<0.05* in Duncans analysis).

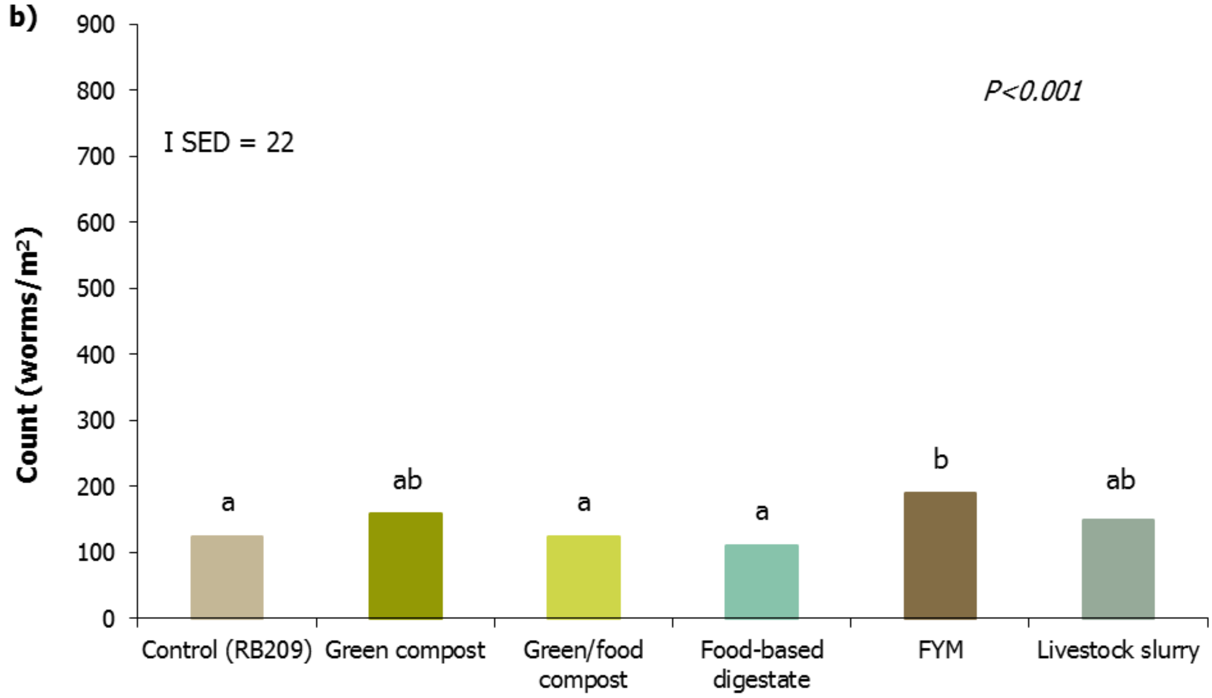

Figure 2. Earthworm numbers (worms/m^2 to 25 cm depth) at the five arable sites (SED = 22.0). Bars labelled with different letters differ significantly (*P<0.05* in Duncans analysis).

Our hypothesis to explain the lower earthworm numbers on the food-based digestate treatments at the grasslands sites is that these sites have the largest initial earthworm populations and that the *majority of the earthworms were residing in close proximity to the soil surface, as their main food-source would be located there, and this is where the digestate would initially infiltrate.*

2.4 Regression analysis

Figure 3 shows the relationship between earthworm numbers on the food-based digestate treatment minus the control, against numbers on the control treatment at each site. The graph indicates a trend across sites that the more earthworms that are initially present, the greater the reduction in earthworm numbers on the food based digestate treatments compared to the control**.** In other words the food-based digestate shows a greater negative impact on earthworm numbers in comparison with the control with increasing control numbers.

Regression lines were put on the graph, but no equation or r^2 was quoted as it was felt that this could be misleading, as the points on the graph were site means and did not take into account the variability across treatment replicates at each site. Any r^2 value calculated could have been an overestimate of the accuracy of the goodness of fit of the line, and it was felt that the equation of the fitted line was not sufficiently reliable to attempt to quantify the effect. The *P* value indicates that there is good evidence of a relationship, but should not be taken as an exact figure.

The trend in earthworm numbers was also evident when the Ayr site (outlier value) was excluded. This general trend existed across all sites, but it was not possible to say whether the rate of reduction (i.e. the slope of the line) was the same for arable and grassland sites as we only had two grassland sites.

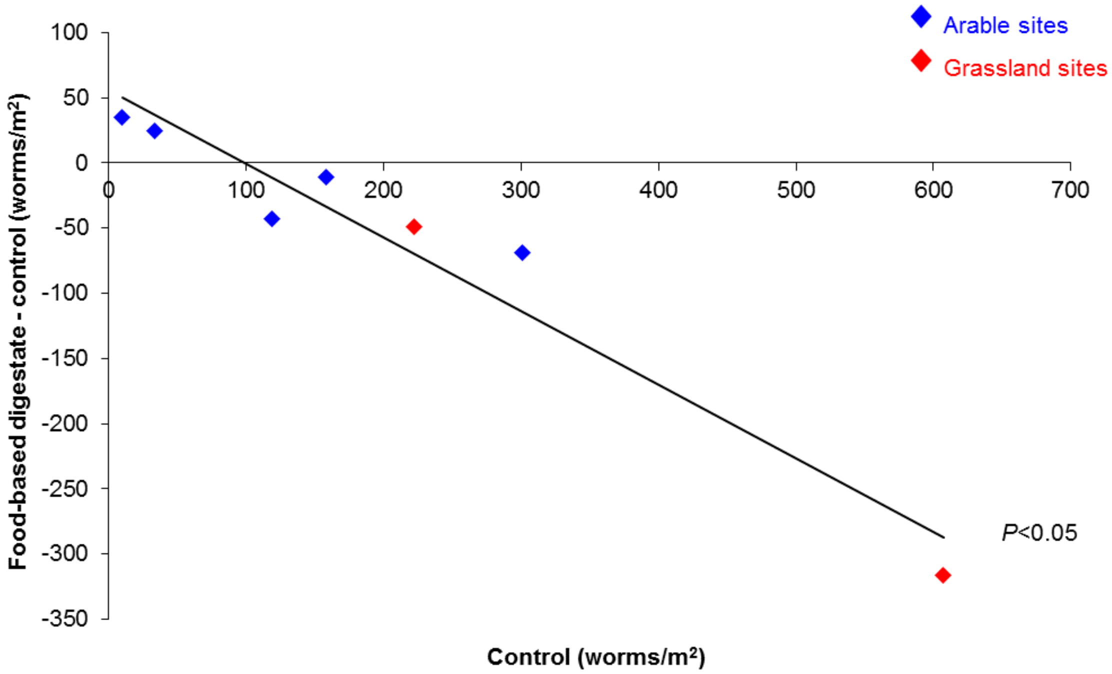

Figure 3. Relationship between earthworm numbers on the control (i.e. site baseline) and food-based digestate treatments.

Similarly, Figure 4 shows the relationship between earthworm numbers on the food-based digestate treatment *minus* the slurry treatment against numbers on the slurry (i.e. comparator) treatment at each site. This also shows the trend that the reduction in numbers of earthworms on the food-based digestate treatment compared to the slurry treatment increased as earthworm numbers increased. This was also evident when the Ayr site was excluded. No equation or r^2 figure was quoted for Figure 4, for the same reasons as explained above in relation to Figure 3. The *P* value indicates that there is good evidence of a relationship, but should not be taken as an exact figure.

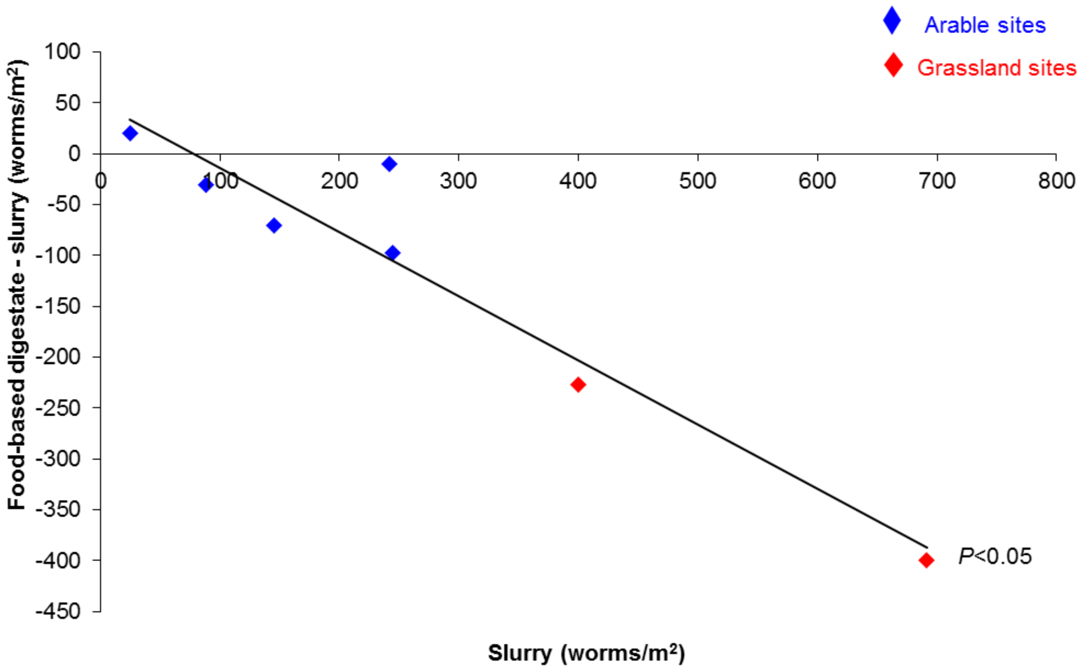

Figure 4. Relationship between earthworm numbers on the slurry (i.e. comparator material) and food-based digestate treatments.

3 Suggested activities to increase understanding of the effect of food-based digestate on worm numbers

- *Undertake a literature review to collate current evidence on the effects of organic material applications on earthworm populations.*
- *Collate available data on the main determinants considered likely to be affecting earthworm populations in a range of food-based digestates i.e.*
    - *Conductivity*
    - *Volatile fatty acids*
    - *Biochemical oxygen demand*
    - *Chemical oxygen demand*
    - *pH* } Most likely
    - *Ammonium-nitrogen* }
- *Continue long-term (and repeated) monitoring of earthworm populations on the DC-Agri Work Package 1 sites (i.e. Ayr, Lampeter, Terrington and Faringdon where earthworm numbers in spring 2013 were >100/m²) to include separation of earthworms into juveniles/adults, and epigeic (i.e. surface dwelling)/endogenic (horizontal burrowing)/anecic (i.e. vertical burrowing) species.*

Note: The Code of Good Agricultural Practice for the Protection of Soil (MAFF, 1998) states "Excessive amounts of fertilisers or manures which contain a high proportion of their nitrogen in the form of ammonium, such as ammonium sulphate and certain animal manures and slurries, may reduce the number of earthworms in soil. You can reduce harmful effects on earthworms *by not applying slurry on wet, poorly drained soils*. However, the long-term effect may be to increase numbers due to the extra food source provided*".*

- *Consider the need to undertake controlled studies to evaluate the effects of contrasting food-based digestate applications on earthworm populations at contrasting grassland sites - to develop management approaches to mitigate negative effects on soil quality and fertility.*

References

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D. and Brun, J.J. (2013). A review of earthworm impact on soil function and ecosystems services. *European Journal of Soil Science*, 64, 161-182.

Brady (1974). *The Nature and Properties of Soils* (8th Edition). Macmillan Publishing Co., Inc.

MAFF (1998). *Code of Good Agricultural Practice for the Protection of Soil*. MAFF Publications.

Appendix 2.1

Table 1. Earthworm counts (number of worms/m^2 to 25 cm depth) at seven sites in spring 2013

Treatment	**Aber (arable)**	**Ayr (grass)**	**Dev (arable)**	**Far (arable)**	**Harper (arable)**	**Lamp (grass)**	**Ter (arable)**
Control	301	**607**^{a}	33	**158**^{a}	10	**222**^{ab}	**119**^{ab}
Green compost	326	**691**^{a}	53	**205**^{ab}	58	**351**^{abc}	**151**^{bc}
Green/food compost	281	**760**^{a}	53	**147**^{a}	49	**420**^{bc}	**86**^{ab}
Food-based digestate	232	**291**^{b}	58	**147**^{a}	44	**173**^{a}	**75**^{a}
Manure-based digestate	222	**583**^{a}	~	~	~	~	~
FYM	370	**825**^{a}	44	**270**^{b}	69	**553**^{c}	**195**^{c}
Slurry	242	**691**^{a}	88	**244**^{b}	25	**400**^{bc}	**146**^{abc}
*P value**	*NS (0.69)*	***<0.01***	*NS (0.21)*	***<0.05***	*NS (0.06)*	***<0.05***	***<0.05***

*Statistical analysis undertaken using ANOVA (data normally distributed).

Note: Brady (1974) report earthworm numbers in arable soils in the range 30-300/m^2, with more than 500/m^2 found in rich grassland soils.

Appendix 3.
Earthworm studies – Literature Review

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Executive summary

Scientific and grey literature on the impact of livestock manures, biosolids, compost and digestate on earthworm populations and biomass were collated from European studies to contextualise earthworm data from the *DC-Agri* field experiments. Notably, no studies were found where the impact of food-based digestate on earthworms had been studied; all published studies had used either manure or crop-based digestate.

In autumn 2010, seven field experimental sites were established in contrasting agroclimatic areas; Aberdeen (arable), Ayr (grass), Devizes (arable), Faringdon (arable), Harper Adams (arable), Lampeter (grass) and Terrington (arable) to quantify the effects of repeated compost and digestate applications, in comparison with farmyard manure and slurry, on soil biological, physical and chemical properties and crop quality. At each site, 18 experimental plots were laid out in a randomised block design (6 treatments, with 3 replicates of each). Organic material applications at a target rate of 250 kg total nitrogen (N)/ha were made in harvest years 2011, 2012 and 2013 at the seven sites. Earthworm populations and biomass were measured in spring 2013 on 3 'blocks' of soil (each 30 x 30 x 25 cm deep) per plot; by counting all adult and immature worms collected within a 5 minute period. The measurements were made at least 6 months after the last (of at least 3) organic material applications.

At the two grassland sites (Ayr and Lampeter), overall earthworm *numbers* on the food-based digestate treatments were *lower* than on all the other treatments, but this was only statistically significant ($P<0.05$) at Ayr. In contrast, earthworm *numbers* at the five arable sites were similar on all the treatments. Effects on earthworm biomass were less consistent. At two further sites (established in 2013) where ammonia emission studies were being undertaken, dead earthworms were also noted shortly (i.e. within 90 minutes) following digestate application and also following cattle slurry additions.

Literature data were used to help elucidate the factors potentially responsible for the observed negative effects of digestate additions on earthworms measured in the *DC-Agri* field experiments. The conclusions of this review are summarised below:

- **Ammonium nitrogen**: Organic materials can be transiently toxic to earthworms as a result of the presence of ammonium/ammonia-N in applied organic materials. The digestates used in the *DC-Agri* experiments had a higher ammonium-N content (mean 3.8 kg/m^3) than the comparator livestock slurries (mean 1.4 kg/m^3). Annual ammonium-N loadings at the sites ranged from 140-235 kg/ha from the food-based digestate compared to 62-145 kg/ha from the livestock slurries; both of which would be subject to ammonia loss by volatilisation post application. By way of context, a typical ammonium nitrate application of 120 kg/ha N would supply 60 kg/ha ammonium-N.
- **pH:** In general, earthworms do not thrive in soils with a pH below 5 and are known to be affected by changes in pH e.g. due to manufactured fertiliser nitrogen applications. The digestate used in the *DC-Agri* experiments had a higher pH (mean 8.5) than the comparator livestock slurry (mean 7.4); the higher digestate pH is likely to result in a higher proportion of the ammonium-N being present as ammonia-N.
- **Electrical conductivity (i.e. salt effects):** High soil electrical conductivity levels can have detrimental effects on earthworms, as a result of exposure to 'salts' (i.e. desiccation). Our data show that food-based digestate typically has a higher conductivity (mean *c.*6,750 µS/cm.) than slurry (mean c.3,600 µS/cm), so could have been a causal factor for the observed reductions in earthworm populations.
- **Volatile fatty acids (VFAs):** We were unable to identify any studies on the effects of VFAs on earthworm populations or biomass. Our data show that food-based digestate

typically has a higher overall VFA content (0.04g Chemical Oxygen Demand-COD/g Volatile Solids-VS) than livestock slurry (0.01g COD/g VS). VFAs will not decrease the pH of food-based digestate, as a result of the buffering capacity provided by the lime (calcium carbonate) content of digestate.

- **Biochemical oxygen demand (BOD):** Organic materials with an elevated BOD will deplete oxygen levels in the soil following land application and can potentially have an adverse effect on earthworm populations. However, our data show that food-based digestate typically has a lower BOD *c.*9000 mg/l than livestock slurry (*c.*14,000 mg/l), and hence is unlikely to be responsible for the observed reductions in earthworm populations.

*Based on data from the scientific literature and the DC-Agri experiments to date it is not possible to identify **unequivocally** the causal factor, or factors, responsible for the observed effects of food-based digestate on earthworm populations/biomass. It is probable that a number of factors **(in particular, ammonium-N, pH and conductivity)** are responsible for the negative effects on earthworm populations.*

Based on the available evidence from the literature review we have summarised (below) the likelihood of food-based digestate properties being responsible for the negative effects observed on earthworm populations.

Food-based digestate properties	Likelihood of effect	Underpinning rationale
Ammonium-N	Probable	Earthworms are known to be sensitive to ammonium (ammonia)-N. Based on the available scientific literature, it is difficult to precisely identify a 'threshold' ammonium-N addition rate, however, applications >100 kg ammonium-N/ha have commonly been related to negative effects on earthworms.
pH	Possible (in conjunction with ammonium-N)	Earthworms are known to be sensitive to ammonium (ammonia)-N; the higher pH of digestate (mean 8.5) compared with cattle slurry (mean 7.4) will result in a greater proportion of ammonium-N being present as ammonia-N.
Electrical conductivity (i.e. salt effects)	Possible	Earthworms are known to be sensitive to exposure to salts (i.e. desiccation effects). Food-based digestate typically has a higher conductivity than cattle slurry.
VFAs	Unknown. We were unable to locate any data in the literature on VFA effects on earthworms.	Food-based digestate typically has a higher VFA content than cattle slurry.
BOD	Unlikely	Food-based digestate typically has a lower BOD than cattle slurry.

There are a number of possible solutions to minimise the risk of negative effects of food-based digestate applications on earthworms, for example, lowering digestate application rates, lowering digestate pH (to influence the ammonium-N/ammonia-N balance), more complete digestion, application timing in relation to earthworm locations within the topsoil, etc. However, without more completely understanding the factors controlling the negative effects of digestate applications on earthworms, it is difficult to confidently identify effective and reliable management solutions.

1 Objective

- To provide a *comprehensive review of UK and international scientific and grey literature* (in temperate climates) on the impact of anaerobic digestates, livestock slurries, biosolids and other similar materials on earthworms (as an indicator of soil biology) in agricultural soils.
- To use information from the literature review to contextualise earthworm responses in the *DC-Agri* soil and crop quality experiments, where a range of contrasting organic materials were applied, on earthworm populations and biomass.

2 Earthworms

2.1 Earthworm species

Earthworms (Order: Oligochaeta) have a major influence on soil quality and are "probably, the most important soil macro-animal" (Brady, 1974). More than 500 worms/m^2 have been noted on rich grassland soils and between 30-300/m^2 in arable soils (Brady, 1974). Earthworm species vary in size from the large *Lumbricus terrestris*, which may be more than 25 cm long and weigh between 2000 and 7000 mg live weight, to small species that are about 2.5 cm long and weigh about 50 mg live weight (Russell, 1973). There are 27 species of earthworm in the UK (Natural England, 2014). Earthworm species can be classified into three broad functional groups – epigeic, endogeic and anecic. These groupings, and the allocation of species to particular groupings, are subject to some uncertainty. Nevertheless, they reflect the risk of direct exposure to potential adverse effects of surface additions, since they broadly correspond to species with a near surface, intermediate and deeper habit range within soils. The three primary groups are:

1. **Endogeic species (shallow dwelling):** These live in and feed on the soil and associated organic matter. They make non-permanent horizontal burrows through the soil, which are sometimes re-used. Endogeic earthworm species include *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea icterica*, *Aporrectodea rosea*, *Murchieona muldali*, *Octolasion cyaneum* and *Octolasion tyrtaeum*.
2. **Anecic species (deep burrowing):** These live in permanent deep vertical burrows connected to the soil surface. They feed on fresh litter on the soil surface, which they drag into their burrows. They also produce casts on the soil surface. Anecic species are the largest species of earthworms in the UK and include *Lumbricus terrestris* and *Aporrectodea longa.*
3. **Epigeic species (litter dwelling):** These generally live near the surface of the soil mainly in leaf litter and produce casts at the soil surface affecting soil surface roughness and the distribution of macropores. They feed on undecomposed litter and its associated micro-flora and ingest relatively little mineral soil matter. Epigeic earthworm species include *Dendrobaena octaedra*, *Dendrobaena attemsi*, *Dendrodrilus rubidus*, *Eiseniella tetraedra*, *Heliodrilus oculatus*, *Lumbricus rubellus*, *Lumbricus castaneus*, *Lumbricus festivus*, *Lumbricus friendi*, *Satchellius mammalis.*

   NB: Worms that live in compost bins or other environments with a ready supply of fresh compostable materials are sometimes classified as a fourth group, but are part of the epigeic group e.g. *Eisenia fetida* and *Eisenia veneta.*

Endogeic (shallow dwelling) earthworms tend to be most abundant in disturbed soils and sites with higher soil pH (arable land, field margins, pastures and amenity grasslands). In contrast, epigeic earthworms (litter dwelling) are more closely associated with woodland habitats and sites with more acid soils (Natural England, 2014). In British arable soils, *A. caliginosa, A. chlorotica* and *A. rosea* are usually numerically dominant; the first two species are also important in short-term grassland leys. Other common but less numerous species in British pastures are *O. cyaneum, L castaneus* and *D. rubidus* (Russell, 1973).

2.2 Factors affecting earthworm numbers

Earthworm numbers (and biomass), as well as the species present in a soil, are dependent on soil properties (e.g. pH or texture), as well as land use and management. For example, ploughing may directly (physically and through exposure to predation) kill and indirectly (through destroying earthworm burrows and lowering food supply) reduce earthworm numbers. In contrast, organic material applications provide additional organic matter and earthworm numbers may increase because of an abundance of food. Typically, livestock manure fertilised grasslands have the greatest number of earthworms, as they provide a continuous food source (Van Vliet *et al.*, 2007).

For earthworms to be abundant, a field must meet several conditions that are also associated with soil quality and agricultural sustainability: slightly acid-neutral pH, litter for food and protection, and soil conditions that are not waterlogged, compacted, droughty, or excessively sandy. Important factors are:

- *Organic matter (food sources):* Higher additions of fresh organic matter are usually associated with greater earthworm populations (Van Vliet *et al.*, 2007).
- *Soil type:* Populations are highest in medium textured soil (USDA, 2001): the soil must be aerated and hence heavy clay or poorly drained soils are unfavourable (Russell, 1973).
- *Depth to a restrictive layer*: Earthworms (particularly endogeic and anecic species) prefer deeper soils; thin soils overlying rock are not usually favourable habitats (Russell, 1973).
- *Soil pH:* In general, earthworms will not thrive in a soil with a pH below 5 (Edwards and Lofty, 1977).
- *Moisture holding capacity and drainage*: Earthworms need moist, but well-aerated soil, with shallow dwellers intolerant of drought and frost (Russell, 1973).
- *Rainfall and temperature:* Climate affects the soil environment and food sources (plant biomass) for earthworms.

2.3 Importance of earthworms to soil quality

The sustainability of UK agricultural production is dependent on the long-term maintenance of soil function and fertility, which are key aspects of soil quality. Soil organic matter levels are intimately linked to soil properties that are important in the maintenance of soil quality and fertility, and sustainable crop production. Organic materials such as livestock manures, biosolids, compost and digestate are recognised as being valuable sources of organic matter and plant available nutrients. These materials are generally considered beneficial to earthworms, as the addition of organic matter is likely to increase available food resources (MAFF, 1998).

The contribution of earthworms to soil processes and structure was first recognised by Darwin (1881). They are often referred to as “ecosystem engineers”, due to their role in breaking down organic matter, improving soil structure and allowing water/oxygen to move through the soil profile (Blouin *et al.*, 2013). In temperate climates, earthworms ingest 2-24% of organic matter inputs (Whalen and Parmelee, 1999; Whalen and Parmelee, 2000) and expend much energy in modifying soil properties, with 74-91% of assimilated carbon respired (Petersen and Luxton, 1982). Earthworms contribute to soil functions by:

- Shredding surface litter, stimulating microbial decomposition and nutrient release;
- Producing casts rich in nitrogen (N), phosphorus (P), potassium (K), and other nutrients. Earthworm casts are higher in organic matter, total and nitrate-nitrogen, available P and K, cation exchange capacity and pH than the surrounding soil (Brady, 1974);
- Improving soil structural stability, air porosity and moisture holding capacity by burrowing and aggregating soil;

- Turning soil over, bringing deeper soil to the surface and burying organic matter. Earthworms transport soil from lower in the soil profile to the surface and mix organic matter into the soil through dragging surface litter into their burrow. Earthworm burrows increase aeration and drainage. This is more important in uncultivated grassland than arable land where soil cultivation will mix soil (Brady, 1974);
- Improving water infiltration by forming channels and promoting soil aggregation. Earthworm channels contribute to the macroporosity of the soil; larger pores enable the drainage of water from the soil surface when the infiltration capacity of the soil matrix has been exceeded, for example, during heavy rainfall events (or during irrigation) (Blouin *et al.,* 2013). This reduces surface run-off and hence reduces the risk of soil erosion; and
- Improving root growth by creating channels lined with nutrients for plant roots to follow (Russell, 1973).

2.4 Organic matter additions

Earthworms need organic matter as a source of food (Brady, 1974) and many authors have noted that the application of livestock manure can increase earthworm abundance and diversity (e.g. Berry and Karlen, 1993; Carpenter-Boggs *et al*., 2000). Most kinds of livestock manure are highly palatable and nutritious food sources for earthworms (Marhan and Scheu, 2005; Lowe and Butt, 2005). Edwards and Lofty (1982) reported that increases in earthworm populations following repeated organic manure applications tended to be more significant in arable than grassland systems. The smaller population effects in grassland are due probably to the large amounts of organic matter already present and under such conditions food is unlikely to be limiting to earthworm populations. In contrast, our experiments showed a greater effect on earthworm populations on grassland sites than on arable sites.

In general, systems that provide the greatest organic matter returns to the soil support the highest earthworm populations (Scullion *et al*., 2002). Earthworm species diversity is often low in conventionally tilled soils (Birkas *et al*., 2004), without any regular organic matter applications. Arable cropping is considered to reduce earthworm abundance, biomass and diversity (e.g. Lapied *et al*., 2009), while grassland pastures will typically support higher earthworm populations. Endogeic (surface dwelling) and anecic species are probably more vulnerable to adverse management, such as frequent tillage and low inputs of organic matter that deprive earthworms of food supply (Edwards and Bohlen, 1996). Reduced (minimal) tillage systems that leave crop residues on the surface will increase potential food supply for these earthworms.

Field 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 compound concentrations), as well as differences in soil characteristics. Note: The Code of Good Agricultural Practice for the Protection of Soil (MAFF, 1998) states *"Excessive amounts of fertilisers or manures which contain a high proportion of their nitrogen in the form of ammonium, such as ammonium sulphate and certain animal manures and slurries, may reduce the number of earthworms in soil. You can reduce harmful effects on earthworms by not applying slurry on wet, poorly drained soils. However, the long-term effect may be to increase numbers due to the extra food source provided".*

2.4.1 Livestock manures

The application of slurry typically increases earthworm populations and biomass more than the use of manufactured fertilisers with a similar total N content due to the higher amounts of organic matter (OM) applied (Edwards and Lofty, 1982); although manufactured fertilisers may contribute indirectly to increases in earthworm populations due to increased amounts of crop residues entering the soil. Equally, the application of farmyard manure (FYM) will usually increase earthworm populations more than the same amount of slurry (due to the higher OM content). An increase in the N content in fertiliser with the same OM content, can also increase earthworm populations. For example, Cotton and Curry (1980b) applied pig slurry with the same OM content (9%) but two different ammonium nitrogen rates, and measured more earthworms and total earthworm biomass following application of slurry with the higher ammonium nitrogen concentration.

Marhan and Scheu (2005) measured changes in the biomass of individual juvenile endogeic earthworms (*O. tyrtaeum)* in soils with different C and N contents resulting from different fertiliser treatments: (1) non-fertilised soil, (2) manufactured NPK fertilised soil, (3) FYM fertilised soil and (4) manufactured NPK + FYM fertilised soil in a microcosm experiment. Over the course of the experiment, earthworm biomass decreased in non-fertilised soil by 48.6%, in manufactured NPK soil by 9.4%, but increased in FYM soil by 19.7% and 42.8% (soil with additional manufactured NPK application). The authors suggested that soil N content and earthworm body mass correlated poorly, but that earthworm body mass was well correlated with soil organic matter content. As a result, they concluded that the organic matter content of fertilisers (organic materials or manufactured fertiliser) was more important than the N content in determining earthworm populations.

Although livestock manures provide food and can increase earthworm numbers and biomass, slurry may be toxic to earthworms as found in the short-term over 7-8 weeks in laboratory experiments (Curry, 1976). Following very heavy applications of slurry (562 m^3/ha; *c.*2250 kg total N/ha; *c.*1450 kg ammonium-N/ha) earthworms were almost totally eliminated, but recovered to their former level after about 1 year. Similarly, direct toxicity from ammonium-based fertilisers (i.e. ammonium nitrate supplying 83-209 kg total N per annum; 44-110 kg ammonium-N/ha) to earthworms has been demonstrated, especially in sandy soils (Hansen and Engelstad, 1999). Earthworms are also known to be affected by changes in pH due to mineral fertiliser applications (Lee, 1985), which is particularly an issue where ammonium-based fertilisers, such as ammonium sulphate, are applied.

Cattle slurry and urine have been shown to be transiently toxic to earthworm populations as a result of ammonia, benzoic acid and sodium sulphide contents over 7-8 weeks in laboratory experiments (Curry, 1976). Cotton and Curry (1980b) also noted a detrimental effect on earthworm numbers in an experiment applying pig slurry at 345 m^3/ha per year in three equal applications within four months (Cotton and Curry, 1980b). The authors attributed the decrease in earthworm numbers to the high copper content of the slurry. In the *DC-Agri* experiments, the copper content of the slurry was higher (mean 153 mg/kg dry matter) than from the digestate (mean 46 mg/kg dry matter), although the latter had a negative effect on earthworm numbers. As a result, it is unlikely that copper was a causal factor in the observed decrease in earthworm populations.

Hansen (1996) reported that many dead earthworms (not quantified) were observed on the soil (sandy loam) surface a few hours after fertilisation of a grass ley with slurry, especially after 'heavy' dressings (*c.*75 m^3/ha applying *c.*170 kg N/ha; *c.*102 kg ammonium-N/ha) of diluted cattle slurry (diluted with water and silage effluent to 200% of original volume), suggesting an immediate toxic effect of the slurry (Hansen, 1996). The negative effect was most pronounced in compacted soil. Unwin and Lewis (1986) carried out a replicated small plot experiment to investigate the effects of large applications of pig slurry (from pigs receiving a diet supplemented with copper sulphate) to grassland on a poorly drained silty clay loam soil. This treatment was compared to two fertiliser only controls that received either manufactured P and K fertiliser, or N, P and K fertiliser. At the highest rate, 5528 m^3/ha (N content not specified) of pig slurry (5% dry matter) was applied over a 4-year period, applying 212 kg Cu/ha and 150 kg Zn/ha. During winter months (when soil was at or above field capacity) 'heavy' applications of pig slurry waterlogged the surface layers (potentially cutting of the oxygen supply to the earthworms), with dead earthworms noted on the soil surface within 1-2 days of application. However, despite the large loadings of Cu and Zn to the soil and the adverse short-term effects of winter slurry pig applications, long-term earthworm numbers were increased by the pig slurry treatment. Edwards and Lofty (1982) also noted an initial detrimental effect on earthworm populations from liquid sewage sludge applications to grassland (at *c.*400 kg N/ha; no data on ammonium-N content) applied in two equal applications (application rate not stated), with dead earthworms appearing on the surface after treatment (number of days post application not stated). The authors indicated that the slurry was toxic to earthworms, but did not expand on this statement. Similarly, Curry (1976) concluded that whilst earthworm populations could be adversely affected by large applications of slurry (cattle slurry at 562 m^3/ha; *c.*2250 kg N/ha; *c.*1450 kg ammonium-N/ha) to grassland (permanent pasture), the effects were usually transitory.

Slurry aeration (to decrease the ammonium-N content) has been proposed as a strategy for, among other things, decreasing the toxicity of slurry to earthworms (Hansen and Engelstad, 1999), but this does not always seem to be effective (Hansen, 1996). For example, Hansen (1996) measured no differences in earthworm populations after fertilisation (85-170 kg total N/ha) with aerated slurry (aerated with a submersible pump 8 times per day; 55-110 kg ammonium-N/ha) and diluted slurry (diluted with water and silage effluent to 200% of original volume; 50-100 kg ammonium-N/ha).

Leroy *et al.* (2007) suggested that not only the quantity, but also the quality of the organic matter applied to the soil, had a significant influence on earthworm populations. They investigated the influence of different types of organic material applications (FYM, cattle slurry, 3 types of compost and manufactured fertiliser N applied in April and October 2005) compared to two unfertilised treatments (cropped v uncropped) on short-term earthworm abundance in an arable sandy loam soil (measurements in spring 2006). To correct for differences in the plant available N content of the different organic amendments, extra mineral N (ammonium nitrate) was applied on organically amended plots where needed to achieve equal levels of plant available N in all treatments. Similarly, P and K fertilisers were also applied to achieve equal minimum levels of plant available phosphate and potash. The results from the study did not support their hypothesis that organic matter type was important, as there were no differences in earthworm populations following the addition of any of the organic material types. However, earthworm populations were higher ($P<0.05$) where organic materials had been applied than on the unfertilised treatments (and the manufactured N treatment).

De Goede *et al.* (2003) looked at the effect of surface broadcast and slit-injected slurry applications on earthworm populations in grassland; earthworms were collected from six cores (0.2 m x 0.2 m x 0.2 m) per field in an across-farm comparison (12 fields at 12 farms; *c.*200 kg N/ha applied as slurry + variable amounts of manufactured N fertiliser) and one core per plot in replicated field experiments (slurry applied at 76 kg N/ha with/without 182 kg manufactured N/ha, and manufactured fertiliser N applied at 76, 182 or 258 kg N/ha) at two sites. In the across-farm experiment (12 fields), total earthworm numbers were higher with slit-injection than surface broadcast applied slurry. For the replicated plot field experiment, there was typically (5 out of 6 measurements) no difference in earthworm numbers between slit injection and surface broadcast slurry. Both studies also indicated contrasting effects of slit injection on epigeic and endogeic species. Endogeic species are at much lower risk of physical damage from slit injection, as they are generally lower down in the soil profile. Epigeic species numbers were decreased following slit injection, whereas endogeic species number were increased. Epigeic species live on the soil surface and negative effects are more likely due to physical damage. De Goede *et al.* (2003) estimated that 30% of earthworms measuring over 10 cm were damaged by slit injection at 20 cm spacing. Application of manufactured fertiliser (at either 182 or 258 kg N/ha) at the field sites had a marked negative effect on the number of earthworms (no information on species given) in autumn ($P<0.05$). The authors do not suggest any reason for the observed negative effect of manufactured N fertiliser (no details on type of fertiliser are given).

Van Vliet and De Goede (2006) suggested that the effect of slurry application methods (slit injected *vs.* broadcast) on earthworms depended on soil moisture and season, highlighting the need for longer-term field experiments (and repeated measurements). Under wet conditions (45-60% soil moisture), the number of earthworms (especially epigeic earthworms) decreased following broadcast slurry application (no rate or N concentration data detailed) to grassland. In contrast, under dry conditions (13-20% soil moisture) the number of earthworms (and percentage of epigeics), decreased after slit injection. Van Vliet and De Goede (2006) suggested that under wet conditions exposure to 'salts' (i.e. high conductivity levels) present in the broadcast slurry may have adversely affected epigeic earthworm abundance, with changes in osmotic conditions in the soil negatively affecting the survival of juvenile epigeic earthworms; although these parameters were not measured (conductivity or salts).

2.4.2 Biosolids

Biosolids are processed solids from municipal wastewater treatment plants, and are applied to agricultural land in many countries. The application of biosolids has been shown to have a positive effect on earthworm populations. For example, Adair *et al.* (2014) grew *Brassica napus* and *Camelina sativa* in a (controlled) pot experiment in soil amended with biosolids (at a rate equivalent to 316 kg N/ha; no information on ammonium-N content given) to assess any effects on earthworm (A. *caliginosa*) growth and survival, and heavy metal accumulation. The experiment showed that there was no difference (P>0.05) in earthworm survival between the control (72%) and biosolids pots (80%). Total earthworm biomass increased over the course of the experiment from a mean of 1.66 to 1.84 g in the biosolids pots, compared with a mean decrease in the control pots from 1.64 to 1.29 g (P<0.01). However, earthworms in the biosolids pots had increased levels of copper. Baker *et al.* (2002) studied the effect of a single dewatered biosolids application (infertile acidic soils) to grazed grassland in Australia (application rates 30, 60 and 120 tonnes/ha; no information on N content given) on earthworm numbers, compared to a fertiliser/lime control (50:50 lime/superphosphate at 400 kg/ha). Seven years after de-watered biosolids had been applied earthworm numbers were low in control plots (7.5 earthworms/m^2), compared to the dewatered biosolids treated plots (*c.*60 earthworms/m^2); there were no significant differences in earthworm numbers between the three rates of dewatered biosolids. Artuso *et al.* (2011) investigated the effects of biosolids additions on earthworms (*Eisenia fetida*) in laboratory tests. Biosolids from five different sewage works were assessed at rates equivalent to 0, 2 (*c.*76 kg N/ha), 5 (*c.*190 kg N /ha), 10 (*c.*380 kg N/ha) and 20 (*c.*760 kg N/ha) t/ha. Biosolids applied at 2 (*c.*76 kg N/ha) and 5 t/ha (*c.*190 kg N /ha) did not cause any mortality of adult earthworms, whereas applications at 10 (*c.*380 kg N/ha) and 20 t/ha (*c.*760 kg N/ha) did. Also, at the 5, 10 and 20 t/ha application rates (≥ 190 kg N/ha) significantly fewer juvenile worms were present relative to unamended controls. However, Artuso *et al.* (2011) noted that the mortality of earthworms observed at high biosolids rates needed to be interpreted carefully since it may be related (at least in part) to the artificial conditions of the experiment and not toxicity *per se* of the materials tested. They concluded that generally, the negative effects were not related to biosolids heavy metal concentrations and that more probable causes were ammonia production, lack of oxygen or a decrease in pH, however, Artuso *et al.* (2011) did not measure these parameters. The size of the negative effects was related to increased biosolids addition rates.

Butt (1999) carried out experiments, using thermally dried sludge granules (total N = 32.2 kg/tonne; 94% dry matter), to investigate effects on a variety of earthworm species *(E. fetida, A. chlorotica, A. longa, L. terrestris and O. cyaneum*) in laboratory and field studies. Laboratory results from small pot experiments (rates up to *c.*3000 kg N/ha; no information on ammonium-N content given) suggested negative effects on earthworms might result from field application of granules. However, further field experimentation (0, 130, 258 or 515 kg N/ha; no information on ammonium-N content given) showed that this was not the case. After 9 months, earthworm population size and species composition in permanent grassland was not affected (P>0.05) by thermally dried granule application, at rates up to 515 kg N/ha, compared with the untreated control. Butt (1999) suggested the adverse effects observed in the pot experiments were almost certainly caused by the non-natural conditions; and most probably very high sludge granule application rates. For example, the 'enclosed' nature of pot experiments and the raised temperatures may have led to a build-up of ammonia, to which earthworms are particularly sensitive (Edwards, 1988). Also, a build-up of soluble salts may have caused problems, as demonstrated for containerised plants by Cox (1995).

Overall the data show that biosolids can be a valuable source of organic matter for earthworms (Barrera *et al*., 2001; Artuso *et al*., 2011), however, (very) high rates of biosolids applications have also been shown to cause direct mortality of earthworms which were most probably due to ammonia production, lack of oxygen or a decrease in pH (Artuso *et al*., 2011). Some species differences in responses to biosolids applications at higher rates were also noted, emphasising the need to understand the effect of organic material applications on species composition.

2.4.3 Compost

Leroy *et al.* (2007) studied the effects of green/food compost (i.e. composted: vegetables, fruit and garden waste) application at three different rates (0, 22.5 t/ha - *c*.321 kg N/ha) annually or 45 t/ha (*c*.643 kg N/ha) applied every other year with or without cattle slurry (*c*.44 t/ha; 163 kg N/ha. *c*.60% RAN; *c*.100 kg ammonium-N/ha) in combination with manufactured N fertiliser (ammonium nitrate at either 0, 100 or 200 kg N/ha) additions. Earthworms were sampled on two occasions (in spring and autumn) from a 0.2 m x 0.2 m x 0.2 m soil sample. The highest earthworm numbers and biomass ($P<0.05$) were measured on the annual compost (22.5 t/ha) treatment in spring. Almost no earthworms were found in autumn, due to a very dry summer. The addition of slurry had no effect ($P>0.05$) on earthworm numbers. The applications of compost at 45 t/ha (applied every other year) did not increase earthworm number and Leroy *et al.* (2007) suggested that yearly doses of compost stimulated earthworms more than larger less frequent applications. D'Hose *et al.* (2014) also looked at the effect of compost on earthworm numbers and soil quality in a field-scale experiment. Plots received 0, 100 or 200 kg N/ha as ammonium nitrate, and 0 or 50 t/ha of compost (applying 200-250 kg N/ha, no information on feedstock) applied annually between 2004 and 2010. Earthworm numbers were measured in autumn 2009 and spring/autumn 2010; on two of the three occasions (autumn 2009 and autumn 2010), earthworm numbers were increased following the repeated compost additions.

2.4.4 Digestate

Anaerobic digestate is a natural product which results from the controlled biological decomposition of biodegradable materials in the absence of oxygen. Suitable input materials include domestic and commercial food 'wastes', animal manures and purpose-grown energy crops. Digestates are an alternative to manufactured fertilisers and by using them, farmers and growers can improve the sustainability of their cropping systems, whilst saving money on purchased fertiliser. As digestate is a relatively new material, there was only limited information on its impact on earthworm population and biomass following land application.

Digestate has lower dry matter and organic matter contents, and higher ammonium-N and pH values than slurry (Clements *et al*., 2012). It has been suggested that because easily available carbohydrates are converted to methane and removed during digestion, less energy and organic matter will be available to earthworms than from a soil incorporated green manure crop (Frøseth *et al*., 2014). Also, ammonium (0.3-1.5 kg ammonium-N) and sulphide that can be toxic to earthworms (Curry, 1976) are formed during anaerobic digestion.

Ernst *et al*. (2008) used a microcosm experiment (sandy loam soil) to assess the C and N turnover from digestate (derived from feedstocks comprising cattle slurry, grass silage and maize – 10:1:16) and cattle slurry, in the presence of two anecic (deep burrowing) (*L. terrestris* and *A. longa*) and one endogeic (shallow dwelling) earthworm (*A. caliginosa*) species. Both digestate and slurry (applying *c*.250 kg N/ha) were mixed with the sandy loam soil to a 5 cm depth. For both treatments, the mass of the anecic earthworm species increased, but the mass of the endogeic species decreased (significantly more in the digestate treatment). The authors suggested that reduced amounts of readily available nutrients and less decomposable organic matter in the digestate treatment caused a reduction in microbial activity and decline in *A. caliginosa* relative to the slurry treatment. The differential species response to digestate application could cause a reduction in earthworm abundance, but an increase in individual earthworm mass, and highlights the need to measure species specific responses to organic material application.

Frøseth *et al*. (2014) studied the effects of crop (green manure)-based digestate on barley yields, N recovery, soil structure and earthworm populations in four field experiments, with different soil properties (6-35% clay in topsoil, 1.39-4.90% total C, 0.11-0.40% total N) and climatic conditions. Prior to sowing the barley crop, the green manure crop was: 1) mulched (x 3); 2) removed (x2) + mulched (x1); 3) removed; 4) removed and digestate (110 kg N/ha) applied prior to sowing; 5) digestate (110 kg N/ha) applied prior to sowing (no green manure crop); or 6) inorganic fertiliser N applied. Earthworms were sampled in 2009 (after the last green manure cut) and in 2010 (after barley harvest) from two 0.2 m x 0.2 m x 0.2 m samples per plot. A positive effect on earthworm numbers and biomass was seen in 2009 (after the last cut) where the green manure was left on the surface compared to when it was removed. The digestate application (110 kg total N; 60 kg/ha ammonium-N) had no effect on earthworm numbers or biomass when similar treatments were compared (i.e. mulch removed, with/without digestate).

Clements *et al*. (2012) compared the effects of: 1) dairy cattle slurry from an organic farm (72 kg N/ha; 39 m^3/ha applied in spring and autumn) and 2) digested slurry (72 kg N/ha and 36 kg/ha ammonium-N; 34 m^3/ha applied in spring and autumn) in comparison with an untreated control on earthworm populations in ley grassland. Earthworms were sampled one week prior to organic material application, one week after organic material application and six weeks after application from an area of 160 cm^2 to a depth of 15 cm. One week after application the slurry treated plots had more *($P<0.05$)* earthworms than the untreated control plots, with earthworm numbers in the digestate plots the same as in the untreated control. Six weeks after application there were no differences in earthworm numbers between any of the treatments, indicating that slurry effects on earthworm populations were only short-term. However, due to the small plot size the authors suggest that some of the results may have arisen from the migration of earthworms into the plots.

Bermejo *et al.* (2010) measured the impact of 'wet' digestate (feedstock: cattle manure, grass and maize silage and millet – applied at 26 t/ha; 8% dry matter) and 'dry' digestate (feedstock: maize silage – applied at 22 t/ha; 17% dry matter) on earthworm populations in comparison with manufactured fertiliser (calcium ammonium nitrate), slurry (28 t/ha; 10% dry matter) and FYM (17 t/ha; 29% dry matter) applications (all applied at 120 kg N/ha; no information on ammonium-N given). One month after application (at 120 kg N/ha) the highest number of earthworms (139 individuals/m^2) was measured in the FYM plots. In comparison, the 'wet' digestate treatment had 93 earthworms/m^2 and the 'dry' digestate had 101 earthworms/m^2. Bermejo *et al.* (2010) suggested that this could have been because the FYM had a higher C:N ratio than the other materials. Aira *et al*. (2006) has also noted that fertilisers with a high C:N ratio have a strong positive influence on earthworm populations.

Owojori *et al*. (2014) showed that salinity, as measured by electrical conductivity, was not a perfect predictor of toxicity to the earthworm *E. fetida*. The authors varied soil salinity (0-125 mM/kg of dry soil), using a range of salts; sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), or magnesium chloride ($MgCl_2$), sodium nitrate ($NaNO_3$), sodium bicarbonate ($NaHCO_3$), sodium phosphate (Na_2HPO_4) or sodium sulphate (Na_2SO_4). Results showed that the cations (sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+})) in association with chloride had similar toxicity, whilst the anions (bicarbonate (HCO_3^{2-}), chloride (Cl^-), hydrogen phosphate (HPO_4^-), sulphate (SO_4^{2-}), nitrate (NO_3^-)) had differential toxicities to the earthworm *E. fetida*, with NO_3 the most toxic, which was not related to electrical conductivity *per se*.

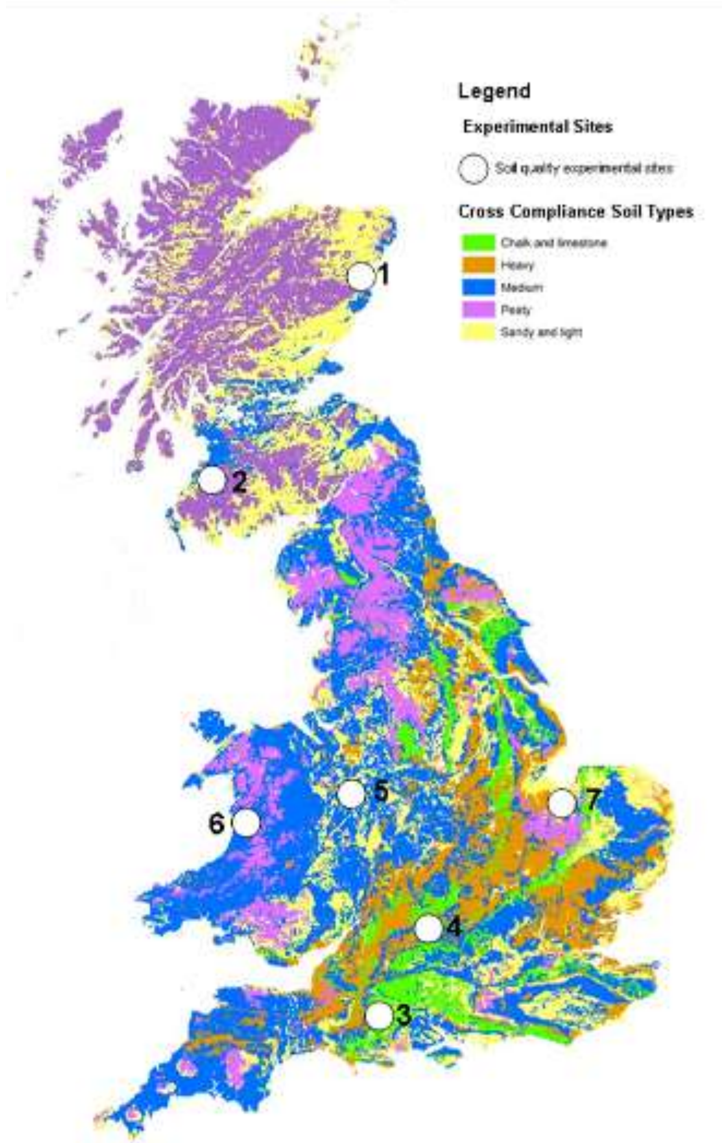
3 DC-*Agri* soil quality sites

In autumn 2010, seven sites were established in contrasting agroclimatic areas; Aberdeen, Ayr, Devizes, Faringdon, Harper Adams, Lampeter and Terrington to quantify the effects of repeated compost and digestate applications, in comparison with farmyard manure and slurry (as comparator materials), on soil bio-physical and physico-chemical properties and crop quality (Table 1 and Figure 1).

Table 1. Characteristics and cropping at the soil and crop quality experimental platforms (3 cropping years)

Site		Soil textural group		Annual rainfall (mm)	Cropping rotation[+]		
		Cross-compliance soil group[1]	% clay		2010-11	2011-12	2012-13
1	Aberdeen	Sandy/light	16	790	SB	WB	WOSR
2	Ayr	Medium	19	1,190	G	G	G
3	Devizes	Chalk	20	850	Lin	WW	WW
4	Faringdon	Heavy	62	830	WW	WW	WC
5	Harper Adams	Sandy/light	11	690	POT	SB	WW
6	Lampeter	Medium	26	980	G	G	G
7	Terrington	Medium (heavy)	28	630	WW	WW	WOSR

[+] SB = spring barley; WB = winter barley; WOSR = winter oilseed rape; SW = spring wheat; WW = winter wheat; G = grassland; POT = potatoes; Lin = linseed; WC = whole crop oats/peas.
[1]EA (2008)



Experimental sites
1: Aberdeen
2: Ayr
3: Devizes
4: Faringdon
5: Harper Adams
6: Lampeter
7: Terrington

Figure 1. Location of experimental sites

3.1 Treatments

At each site, 18 experimental plots were laid out in a randomised block design (6 treatments, with 3 replicates of each), Table 2. Organic material applications (broadcast) at target rates of 250 kg total nitrogen (N)/ha were made in harvest years 2011, 2012 and 2013 at the seven sites.

Table 2. Organic material treatment details

Treatment No.	Treatment details
1	Control (no organic material application)
2	Green compost at 250 kg total N/ha
3	Green/food compost at 250 kg total N/ha
4	Food-based digestate at 250 kg total N/ha
5	Farmyard manure at 250 kg total N/ha
6	Slurry at 250 kg total N/ha

Applications of manufactured fertiliser (nitrogen, phosphate, potash and sulphur) were also made where necessary, based on crop requirements, after accounting for the nutrients supplied by the organic materials (Defra, 2010; SRUC, 2013). This ensured (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 organic matter inputs was from the applied organic materials).

3.2 Properties of applied organic materials at DC-*Agri* sites

A comparison of the characteristics of the food-based digestate and livestock slurries applied at the DC-*Agri* sites is shown in Table 3. Mean data were summarised as there were no notable differences in food-based digestate characteristics between any of the seven sites; as evidenced by the digestate maximum and minimum analysis values in Table 3 being similar. Notably, the same source of food-based digestate was applied at Ayr and Aberdeen in each of the three applications. Slurry was included in the experiments as a comparator material, as it is widely used in agriculture and is similar to digestate in terms of physical properties.

Table 3. Properties of food-based digestate and livestock slurry applied to *DC-Agri* sites

Determinand	***Units****	**Digestate**			**Slurry**		
		Mean	**Max**	**Min**	**Mean**	**Max**	**Min**
Dry matter	%	2.2	3.1	1.4	4.6	6.7	1.4
pH		8.5	8.8	8.4	7.4	7.9	6.8
Total-N	kg/m^3	4.67	5.21	3.40	2.67	3.23	1.84
Readily Available Nitrogen – RAN (Ammonium-N)	kg/m^3	3.78	4.47	2.71	1.44	2.14	0.94
RAN % total N	%	81	86	80	54	66	51
Total Phosphate (P_2O_5)	kg/m^3	0.61	0.93	0.33	0.69	0.89	0.35
Total Potash (K_2O)	kg/m^3	1.96	2.09	1.40	2.29	3.04	1.23
Extractable Potash (K_2O)	kg/m^3	1.70	2.12	1.27	1.94	2.71	1.13
Total Sulphur (SO_3)	kg/m^3	0.36	0.70	0.22	0.78	1.84	0.32
Total Magnesium (MgO)	kg/m^3	0.07	0.12	0.03	0.49	0.73	0.17
Total Calcium	kg/m^3	0.69	1.09	0.33	1.42	4.51	0.36
Organic Matter	% dm	58.2	61.2	51.1	65.5	74.7	54.2
Lignin-C	% dm	6.74	10.24	2.64	6.80	9.91	4.45
Lignin-C as % of OC	%	21	30	8	18	25	11
Total Zinc	mg/kg dm	136	164	101	258	601	151
Total Copper	mg/kg dm	45.5	58.8	28.8	153	483	80.2
Total Cadmium	mg/kg dm	0.49	0.68	0.39	0.34	0.74	0.20
Total Lead	mg/kg dm	7.14	11.67	5.45	9.13	32.1	2.70
Total Nickel	mg/kg dm	42.5	77.2	10.3	8.10	14.6	4.26
Total Chromium	mg/kg dm	7.82	11.09	6.20	3.90	6.55	1.74
Total Mercury	mg/kg dm	2.32	2.89	1.60	1.45	3.69	0.79
Total Arsenic	mg/kg dm	1.11	1.60	0.85	1.22	2.85	0.44
Total Selenium	mg/kg dm	8.64	22.54	1.87	1.25	2.41	0.81
Total Molybdenum	mg/kg dm	9.73	16.46	5.37	4.96	12.00	1.99
Total Fluoride	mg/kg dm	448	577	320	289	739	158

* % = percent; kg/m^3 = kilograms per cubic metre; % dm = percent dry matter; mg/kg dm = milligrams per kilogram dry matter.

Dry matter, organic matter, total major nutrient (i.e. phosphate, potash, sulphur and calcium) and heavy metal contents were broadly similar in both organic materials. The most notable differences were that the food-based digestate had a higher pH (mean 8.5) than livestock slurry (mean 7.4), a higher ammonium-N content (mean 3.8 kg/m^3) than livestock slurry (mean 1.4 kg/m^3) and a higher ammonium-N as a % of total N content (81%) compared with livestock slurry (54%).

Table 4 gives the total ammonium loadings from the organic material applications (plus the inorganic fertiliser ammonium-N) at the *DC-Agri* experimental sites over the three year research period. The application of food-based digestate supplied the most ammonium-N at 415-710 kg/ha which equated to an annual application of 140-235 kg/ha, with the highest loadings at the two Scottish sites (Ayr and Aberdeen). This compared to a total load of between 185-445 kg/ha ammonium-N (or 62-145 kg/ha/yr) from the livestock slurry. Some of the ammonium would most likely be lost to the air via ammonia volatilisation, but even taking potential losses into account, the loadings from the digestate applications would be at the upper end of typical manufactured fertiliser N application rates. Notably, De Goede *et al.* (2003) showed that ammonium nitrate fertiliser N application at rate of 182 and 258 kg N/ha (i.e. 91 and 129 kg/ha ammonium-N), had a marked negative effect on earthworm numbers, however, Leroy *et al.* (2007) and D'Hose *et al.* (2014) reported no effects of ammonium nitrate fertiliser application at rates up to 200 kg/ha N (i.e. 100 kg/ha ammonium-N). In the *DC Agri* experiments, the control plots had manufactured fertiliser N rates applied annually in the range 80-240 kg/ha total N over the season (as ammonium nitrate fertiliser), with individual application rates not exceeding 120 kg/ha total N (i.e. 60 kg/ha ammonium-N). Total ammonium-N loadings on the fertiliser controls over the 3 year period ranged between 180-285 kg/ha (Table 4) or 60-95 kg/ha/yr (note fertiliser N was usually split-applied as 2 or 3 different applications).

Table 4. Total ammonium-N loadings from the organic material (plus fertiliser NH_4-N in brackets) applications over the 3 year DC-*Agri* experimental programme

Cumulative ammonium load over three years (**from organic materials only**) (kg/ha)							
	Ayr	**Lampeter**	**Aberdeen**	**Devizes**	**Faringdon**	**Harper**	**Terrington**
Control	**0** (250)	**0** (180)	**0** (245)	**0** (270)	**0** (245)	**0** (240)	**0** (285)
Cattle FYM	**43** (272)	**8** (156)	**29** (252)	**7** (257)	**38** (260)	**48** (253)	**26** (281)
Cattle slurry	**184** (374)	**243** (356)	**416** (546)	**406** (574)	**259** (401)	**329** (488)	**445** (571)
Compost (G)	**19** (269)	**3** (183)	**5** (250)	**7** (272)	**24** (264)	**7** (242)	**5** (280)
Compost (G/F)	**30** (271)	**55** (219)	**25** (255)	**2** (258)	**2** (233)	**5** (231)	**3** (269)
Digestate	**671** (745)	**445** (532)	**711** (801)	**416** (589)	**470** (595)	**474** (616)	**441** (596)

4 Earthworm populations at *DC-Agri* sites

Earthworm populations were measured in spring 2013 on 3 'blocks' of soil (each 30 x 30 x 25 cm deep) per plot from the central plot area, excluding the outside 1 m of each plot, by counting all adult and immature worms collected within a 5 minute period. The measurements were made at least 6 months after the last (of at least 3) digestate application.

4.1 Results

4.1.1 Site-by-site analysis

- There were treatment differences in earthworm *numbers* at 4 sites - Ayr, Faringdon, Lampeter and Terrington ($P<0.05$), but not at Aberdeen, Devizes or Harper Adams ($P>0.05$).
- At Ayr, the application of food-based digestate *reduced* earthworm *numbers* in comparison with all the other treatments ($P<0.01$). Notably, the Ayr site overall had the greatest number of earthworms.
- 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 treatment at Terrington ($P<0.05$), Table 5. – *See Appendix 3.1 for earthworm biomass data where there was differences (P<0.05) at the Faringdon and Terrington sites*.

By way of context, Brady (1974) reported earthworm numbers in arable soils in the range 30-300/m^2, with more than 500/m^2 found in 'rich' grassland soils.

Table 5. Earthworm counts (number of worms/m^2 to 25 cm depth) at seven sites in spring 2013

Treatment	Aber (arable)	Ayr (grass)	Dev (arable)	Far (arable)	Harper (arable)	Lamp (grass)	Ter (arable)
Control	301	**607^{a}**	33	**158^{a}**	10	**222^{ab}**	**119^{ab}**
Green compost	326	**691^{a}**	53	**205^{ab}**	58	**351^{abc}**	**151^{bc}**
Green/food compost	281	**760^{a}**	53	**147^{a}**	49	**420^{bc}**	**86^{ab}**
Food-based digestate	232	**291^{b}**	58	**147^{a}**	44	**173^{a}**	**75^{a}**
Manure-based digestate	222	**583^{a}**	~	~	~	~	~
FYM	370	**825^{a}**	44	**270^{b}**	69	**553^{c}**	**195^{c}**
Slurry	242	**691^{a}**	88	**244^{b}**	25	**400^{bc}**	**146**^{abc}
*P value**	*NS (0.69)*	***<0.01***	*NS (0.21)*	***<0.05***	*NS (0.06)*	***<0.05***	***<0.05***

*Statistical analysis undertaken using ANOVA (data normally distributed).

4.1.2 Cross-site analysis

- At the grassland land-use sites (Ayr and Lampeter), overall earthworm *numbers* on the food-based digestate treatments were *lower* than on all the other treatments (*P<0.001*; Figure 2).

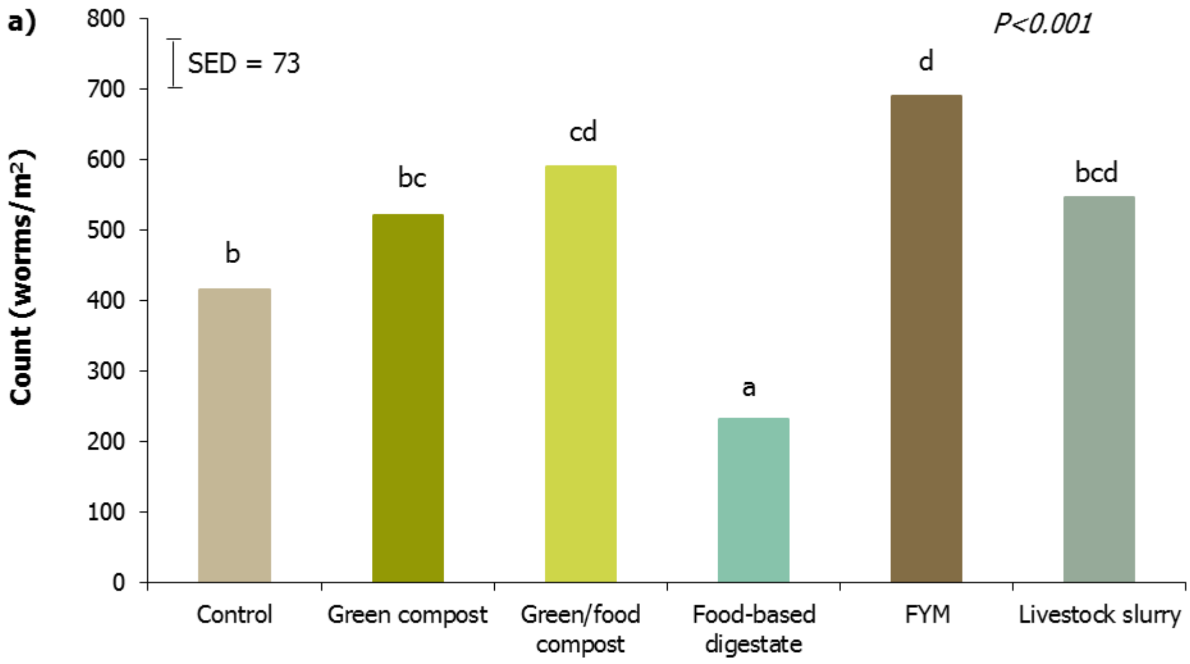


Figure 2. Earthworm numbers (worms/m^2 to 25 cm depth) at the two grassland sites (Standard error of difference between means (SED) 73.3). Bars labelled with different letters differ significantly (*P<0.05*).

- In contrast, earthworm *numbers* at the arable land use sites were similar on all the treatments; although overall earthworm numbers on the FYM treatment were higher than on the control, green/food compost and food-based digestate treatments (*P<0.01;* Figure 3).
- Cross-site regression analyses based on block-by-block treatment comparisons are summarised in Appendix 3.2.

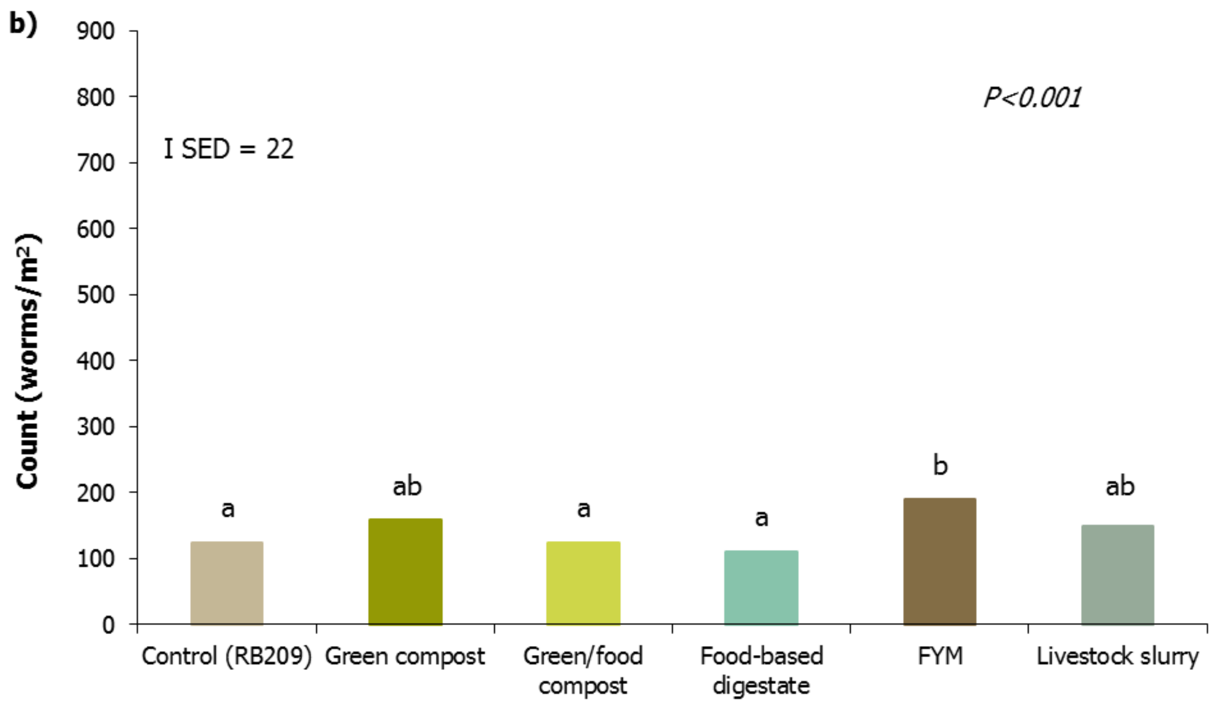


Figure 3. Earthworm numbers (worms/m^2 to 25 cm depth) at the five arable sites (SED = 22.0). Bars labelled with different letters differ significantly (*P<0.05*).

4.1.3 Mean live weight per earthworm

- Reductions in earthworm biomass in response to digestate additions were less consistent than those for abundance. Mean live weight data were calculated as an indicator of shifts in population composition which may influence earthworm numbers.
- A cross-site (all seven sites) analysis of average live weight per earthworm showed that earthworms from the digestate treatment (*c.*0.42 g) were heavier (*P<0.01*) than from the other treatments (*c.*0.30 g average of all other treatments).

The earthworm population abundance to biomass ratio is affected by the balance of adult to juveniles in a population, and the proportions of different species that vary in their normal size range. In the context of this report, an increase in individual earthworm biomass may be due to a population shift towards a greater relative abundance of adults, an increase in the proportion of larger species within the population or a combination of these factors. Given the six-month gap between treatment and sampling and the short life cycle of smaller species, a species shift is considered most likely. The negative effect of liquid organic material applications is liable to be more pronounced for smaller species and also juveniles due to their greater surface area to mass ratio. This emphasises the importance of species identification to determine which species or group (endogeic, anecic or epigeic) is most affected by digestate applications.

4.2 Soil properties at the DC-*Agri* sites

The baseline soil characteristics at each of the DC-*Agri* sites are summarised in Table 6 and soil properties in spring 2013 in Table 7. Baseline soil characteristics and cropping are likely to be important influences on the overall number of earthworms measured at each site, which ranged from *c.*50 earthworms/m^2 at Harper Adams (arable) and Devizes, to >600 earthworms/m^2 at Ayr (grassland). Typically, earthworm numbers are greater in grassland (permanent pasture and leys) than in tillage land that is disturbed annually (for seed bed 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.

Table 6. Baseline soil characteristics at the DC-*Agri* sites established in 2010

Determinand	Units *	Aber (arable)	Ayr (grass)	Dev (arable)	Far (arable)	Harper (arable)	Lamp (grass)	Ter (arable)
pH	-	5.8	5.2	8.0	7.1	5.7	5.3	8.0
Sand	%	58	52	16	10	76	33	10
Silt	%	26	29	64	28	13	41	62
Clay	%	16	19	20	62	11	26	28
Texture Classification	-	Sandy loam	Sandy clay loam	Silty clay loam	Clay	Sandy loam	Clay loam	Silty clay loam
Extractable Phosphorus [ADAS Index]	mg/l	55 [4]	42 [3]	18 [2]	32 [3]	71 [5]	24 [2]	26 [3]
Extractable Potassium [ADAS Index]	mg/l	116 [1]	132 [2-]	273 [3]	268 [3]	86 [1]	86 [1]	283 [3]
Extractable Magnesium [ADAS Index]	mg/l	83 [2]	174 [3]	50 [1]	262 [5]	58 [2]	55 [2]	166 [3]
Extractable Sulphate (SO_4)	mg/l	10	49	8	15	6	11	29
Total Nitrogen	% dm	0.35	0.22	0.7	0.35	0.18	0.49	0.14
Organic Matter	% dm	8.28	4.02	8.39	5.8	3.37	7.76	2.79

* % = percentage; mg/l = milligrams/litre; % dm = percentage dry matter

Table 7. Soil characteristics at the DC-*Agri* sites in spring 2013: Control (cont), food-based digestate (FBD) and livestock slurry (LS) treatments

Determinand	Units*	Aberdeen (arable)			Ayr (grass)			Devizes (arable)			Faringdon (arable)			Harper (arable)			Lampeter (grass)			Terrington (arable)		
		Cont	FBD	LS	Cont	FBD	LS	Cont	FBD	LS	Cont	FBD	LS	Cont	FBD	LS	Cont	FBD	LS	Cont	FBD	LS
pH	-	5.8	5.6	5.7	5.7	6.1	6.2	8.1	8.0	8.2	7.3	7.3	7.4	6.6	6.5	7.0	6.0	5.7	6.2	7.4	7.8	7.9
Extractable Phosphorus [ADAS Index]	mg/l	53 [4]	55 [4]	53 [4]	42 [3]	44 [3]	43 [3]	23 [2]	23 [2]	28 [3]	15 [1]	18 [2]	19 [2]	72 [5]	73 [5]	72 [5]	27 [3]	29 [3]	34 [3]	26 [3]	23 [2]	34 [3]
Extractable Potassium [ADAS Index]	mg/l	129 [2-]	138 [2-]	154 [2-]	142 [2-]	238 [2+]	157 [2-]	190 [2+]	256 [3]	355 [3]	303 [3]	315 [3]	340 [3]	91 [1]	138 [2-]	199 [2+]	251 [3]	324 [3]	475 [4]	269 [3]	254 [3]	360 [3]
Extractable Magnesium [ADAS Index]	mg/l	70 [2]	60 [2]	77 [2]	121 [3]	122 [3]	144 [3]	57 [2]	46 [1]	63 [2]	282 [5]	276 [5]	302 [5]	36 [1]	41 [1]	60 [2]	64 [2]	60 [2]	102 [3]	258 [5]	194 [4]	222 [4]
Extractable Sulphate (SO_4)	mg/l	28	28	30	23	26	21	31	32	33	28	31	34	7	8	10	68	72	87	9	8	8
Total Nitrogen	% dm	0.30	0.36	0.35	0.21	0.22	0.22	0.59	0.58	0.61	0.36	0.38	0.36	0.09	0.09	0.10	0.44	0.44	0.45	0.15	0.14	0.15
Organic Matter	% dm	6.97	8.05	7.18	4.26	4.00	3.84	7.38	7.20	7.59	6.28	6.10	6.33	2.31	2.43	2.61	7.27	6.99	7.29	2.67	2.71	2.74
Biomass N	mg/kg	64.4	64.0	57.2	78.1	88.5	88.7	145	138	142	97.4	108	113	27.6	29.5	36.1	162	148	177	50.3	54.8	57.9
Respiration	Mg CO_2-C /kg/hr	0.77	0.66	0.68	0.61	0.72	0.35	1.61	1.92	1.90	0.45	0.39	1.21	1.52	1.45	1.45	2.47	2.22	2.33	0.66	0.68	0.61

* % = percentage; mg/l = milligrams/litre; % dm = percentage dry matter; mg/kg = milligram/kilogram; mg Co2-C/kg hr = milligrams of carbon dioxide per kilogram/hour

5 Qualitative effects of organic materials on earthworms at grassland ammonia emission sites

As part of the wider DC-*Agri* programme, three new (grassland) experimental sites were established in September 2013 (Table 8) to investigate shallow-injection as a potential method of reducing ammonia emissions from food-based digestate (*c.*30 m^3/ha; 160 kg/ha ammonium-N) and cattle slurry (30 m^3/ha; 45 kg/ha ammonium-N) applications to grassland (in addition to measuring emissions from surface broadcast and trailing shoe applications). *Note*: this was a lower application rate (*c.*30 m^3/ha; 160 kg N/ha ammonium-N) than used on average at the seven soil quality sites (*c.*50 m^3/ha; *c.*190 kg/ha ammonium-N) where earthworm population responses were measured.

Table 8. Baseline soil characteristics at the DC-*Agri* grassland ammonia emission sites established in 2013

Determinand	Units*	Aberaeron	Beith	Newark
pH	-	5.3	6.0	6.8
Sand	%	25	30	22
Silt	%	46	39	42
Clay	%	29	31	36
Texture Classification	-	Clay loam	Clay loam	Clay
Extractable Phosphorus [ADAS Index]	mg/l	19 [2]	44 [3]	11 [1]
Extractable Potassium [ADAS Index]	mg/l	87 [1]	134 [2-]	262 [2+]
Extractable Magnesium [ADAS Index]	mg/l	38 [1]	207 [4]	407 [6]
Extractable Sulphate	mg/l	49	42	24
Total Nitrogen	% dm	0.39	0.62	0.25
Organic Matter[1]	% dm	6.95	11.5	4.20

* % = percentage; mg/l = milligrams/litre; % dm = percentage dry matter

At the Beith (Scotland) site, it was noted that there were dead earthworms (most probably shallow dwelling endogeic species) on the surface immediately (within 90 minutes) following the autumn 2013 digestate application, see Plate 1. No dead earthworms were observed on the cattle slurry treatment. *Note:* earthworm assessments were not scheduled at Beith so no measurements (count or biomass) were taken.

Plate 1. Earthworms at Beith experimental site.

Following the organic material applications at the Aberaeron site (Wales), dead earthworms were also observed immediately after application. At the third experimental site (Newark, England), no earthworms were observed on the surface following any of the treatments. Notably, it was very dry when the organic materials were applied at Newark and as a result earthworm were likely to be deeper in the soil.

At Aberaeron, dead earthworm numbers were measured from three quadrats per plot (from all application techniques – i.e. shallow injection, broadcast or trailing shoe). The highest number of dead earthworms were measured on the food-based digestate treatments, followed by the cattle slurry treatments, with no dead earthworms on the surface of the untreated control (Table 9).

Table 9. Mean number of earthworms on the soil surface (earthworms/m^2) following shallow-injected, broadcast and trailing shoe digestate and slurry applications

Site	Untreated control	Food-based digestate	Cattle slurry
Aberaeron	0	8	2
Newark	Nil	Nil	Nil

The organic materials applied at the three experimental sites were analysed for additional parameters to identify any determinands that might be causal factors for the observed earthworm deaths (Table 10).

Table 10. Mean organic material analysis results from the three experimental sites and 'typical' food-based digestate and cattle slurry properties

Determinand	Units*	Food-based digestate+	Cattle slurry+	'Typical' food-based digestate	'Typical' cattle slurry
Dry matter	%	4.2	7.4	6.0[a]	4.0[d]
Ammonium-N	kg/m^3	5.4	1.5	4.0[a]	1.2[d]
pH		8.5	8.0	8.4[b]	7.2[e]
Electrical conductivity	µS/cm	4,700	3,300	6,750[b]	3,600[b]
Biochemical oxygen demand	mg/l	10,900	15,800	9,300[b]	14,350[b]
Chemical oxygen demand	mg/l	n.d.	n.d.	39,250[b]	55,350[b]
Total zinc	mg/kg dm	n.d.	n.d.	167[c]	196[f]
Total copper	mg/kg dm	n.d.	n.d.	62[c]	137[f]
Volatile fatty acids (VFAs)	g COD/g VS	n.d.	n.d.	0.04[b]	0.01[b]

n.d. = not determined

* % = percentage; kg/m^3 = kilograms per cubic meters; µS/cm = micro siemens per centimetre; mg/l = milligrams per litre; mg/kg dm = milligrams per kilogram dry matter; g COD/g VS = grams chemical oxygen demand per gram volatile solids

\+ average analysis from Aberaeron, Beith and Newark

[a] values taken from WRAP (2012)

[b] values taken from Taylor *et al.* (2011)

[c] values taken from Nicholson *et al.* (2013)

[d] values taken from Defra (2010)

[e] values taken from Chambers *et al.* (2005)

[f] values taken from Nicholson *et al.* (2010)

Notably, the food-based digestates had a higher ammonium-N content, pH and electrical conductivity than cattle slurry (as used on the experimental sites and 'typical' value means). Measured values for most other parameters were similar.

Results from the DC-*Agri* grassland ammonia experiments indicate that food-based digestate (and to a lesser extent slurry) can have a negative effect on earthworms, with dead earthworms reported shortly (within 90 minutes) after application (at *c.*30 m^3/ha; *c.*160 kg N/ha ammonium-N). Longer term reductions in earthworm populations following food-based digestate (*c.*50 m^3/ha, applying *c.*250 kg N/ha and *c.*190 kg/ha ammonium-N) application (compared to untreated control, compost, slurry and FYM) were also measured at the DC-*Agri* grassland soil quality sites, with the most marked effects observed at the Ayr grassland site which had one of the highest ammonium-N loadings.

In the DC-*Agri* grassland ammonia experiments, more dead earthworms were noted following digestate application (suggesting that this was more toxic to earthworms) than following slurry application. Both the measured pH (digestate pH 8.5; slurry pH 7.4), ammonium-N content (digestate 3.8 kg/m^3; slurry 1.4 kg/m^3) and ammonium-N loadings (digestate 160 kg/ha ammonium-N; cattle slurry 45 kg/ha ammonium-N) were higher from digestate than the slurry (comparator material); these properties may have contributed to the observed earthworm deaths. As food-based digestate has a high concentration of ammonium-N as well as a high pH, a large proportion of the ammonium-N will be converted to liquid/gaseous ammonia, which is known to have an adverse effect on earthworms. Earthworm exposure to ammonia will depend on the diffusion of ammonia gas from the digestate into the soil, which will in turn depend on soil physical properties (air-filled pore space, connectivity of pores and soil water content/matric potential) as well as the location of the earthworms within the soil profile.

Unwin and Lewis (1986) suggested that heavy applications of pig slurry during winter months (when soil was at or above field capacity) might result in waterlogging, which could potentially cut of the supply of oxygen to the earthworms. However, in our experiments the observed earthworm deaths were unlikely to be due simply to the amount of material applied (i.e. waterlogging and oxygen starvation), as if this were the case the adverse effect would be expected to greater from the slurry applications than from the digestate, as the former was applied at a higher rate (*c.*80 *vs.* 50 m^3/ha).

In the DC-*Agri* grassland ammonia experiments, food-based digestate had a higher conductivity than slurry and this may have been a causal factor in the increased earthworm deaths noted following digestate application (compared to slurry). Van Vliet and De Goede (2006) noted that in wet conditions (soil moisture 45-60%) the number of earthworms (particularly epigeic earthworms decreased following broadcast slurry applications. They suggested that under wet conditions exposure to 'salts' (i.e. high conductivity) in slurry may have adversely affected epigeic earthworm abundance, with changes in osmotic conditions in the soil negatively affecting the survival of epigeic earthworms, although these parameters were not measured (conductivity or salts).

6 Conclusions

Scientific and grey literature data on the impact of livestock manures, biosolids, compost and digestate on earthworm populations and biomass were collated from European studies. Notably, no references were found in the literature where the impact of food-based digestate on earthworm populations had been studied; all published studies have used either manure or crop-based digestate. Each of the parameters considered to be potentially responsible for the observed negative effects of digestate on earthworms are summarised below:

- **Ammonium nitrogen**: Organic materials can be transiently toxic to earthworms, as a result of the presence of ammonium/ammonia-N in the applied organic materials. The digestate used in *DC-Agri* experiments had a higher ammonium N content (mean 3.8 kg/m^3) than the comparator livestock slurries (mean 1.4 kg/m^3). Annual ammonium-N loadings ranged from 140-235 kg/ha from the food-based digestate compared to 62-145 kg/ha from the livestock slurries; both of which would be subject to ammonia volatilisation loss post application. Earthworm exposure to gaseous ammonia will depend on the diffusion of ammonia gas from the digestate into the soil, which will in turn depend on soil physical properties (as well as the location of the earthworms within the soil profile. By way of context, a typical ammonium nitrate application of 120 kg/ha N would supply 60 kg/ha ammonium-N.

- **pH:** In general, earthworms will not thrive in soils with a pH below 5 and are known to be affected by changes in pH e.g. due to manufactured fertiliser N applications. The digestate used in the *DC-Agri* experiments had a higher pH (mean 8.5) than the comparator livestock slurry (mean 7.4); the 'high' digestate pH is likely to result in a higher proportion of the ammonium-N in the digestate being present as ammonia-N.
- **Electrical conductivity (i.e. salt effects):** High soil electrical conductivity levels can have detrimental effects on earthworms, as a result of exposure to 'salts' (i.e. desiccation). Our data show that food-based digestate typically has a higher conductivity (mean *c.*6,750 µS/cm) than slurry (mean *c.*3,600 µS/cm) and so could have been an important causal factor for the observed reductions in earthworm populations following digestate application.
- **Volatile fatty acids (VFAs):** There were no reported studies on the effects of VFAs from food-based digestate (or indeed on other organic materials) on earthworm populations or biomass. Our data show that food-based digestate typically has a higher overall VFA content (0.04 g COD/g VS) than livestock slurry (0.01 g COD/g VS).

- **Biochemical oxygen demand (BOD):** This is a measure of the total amount of oxygen required for bacteria to degrade the organic components in the organic material. When there is an abundance of bacteria they will use oxygen in order to breakdown the organic material reducing the amount of dissolved oxygen available to earthworms (and other soil fauna). Our data show that food-based digestate typically has a lower BOD (*c.*9000 mg/l) than livestock slurry (*c.*14,000 mg/l), and hence is unlikely to be responsible for the observed reductions in earthworm populations following digestate application.
- **Chemical oxygen demand (COD):** This measures the amount of oxygen required to chemically oxidize (organic and inorganic) components of the organic material. COD values are always higher than BOD values, as COD includes all organic and inorganic substances, whereas BOD measures only more easily biodegradable organic substances. Our data show that food-based digestate (*c.*40,000 mg/l) typically has a lower COD than livestock slurry (*c.*55,000 mg/l), and hence is unlikely to be responsible for the observed reductions in earthworm populations following digestate application.

*Based on data from the scientific literature and the DC-Agri experiments to date it is not possible to identify **unequivocally** the causal factor, or factors, responsible for the observed effects of food-based digestate on earthworm populations/biomass. It is probable that a number of factors **(in particular, ammonium-N, pH and conductivity)** could be responsible for the negative effects on earthworm populations.*

We have summarised (below) the likelihood of food-based digestate properties being responsible for the negative effects observed on earthworm populations.

Food-based digestate properties	Likelihood of effect	Underpinning rationale
Ammonium-N	Probable	Earthworms are known to be sensitive to ammonium (ammonia)-N. Food-based digestate applied at the maximum rate permitted in NVZs (i.e. 250 kg/ha total N) will supply around 200 kg/ha ammonium-N, which is approximately double the amount of ammonium-N supplied by a similar cattle slurry application (*c.*110 kg/ha ammonium-N), and *c.*3-fold greater than supplied by a typical manufactured fertiliser (ammonium nitrate) application (*c.*60 kg/ha ammonium-N). Based on the available scientific literature, it is difficult to precisely identify a 'threshold' ammonium-N addition rate, however, applications >100 kg ammonium-N/ha have commonly been related to negative effects on earthworms.
pH	Possible (in conjunction with ammonium-N)	Earthworms are known to be sensitive to ammonium (ammonia)-N; the higher pH of digestate (mean 8.5) compared with cattle slurry (mean 7.4) will result in a greater proportion of ammonium-N being present as ammonia-N.
Electrical conductivity (i.e. salt effects)	Possible	Earthworms are known to be sensitive to exposure to salts (i.e. desiccation effects). Food-based digestate typically has a higher conductivity than cattle slurry.

VFAs	Unknown. We were unable to locate any data in the literature on VFA effects on earthworms	Food-based digestate typically has a higher VFA content than cattle slurry.
BOD	Unlikely	Food-based digestate typically has a lower BOD than cattle slurry.
COD	Unlikely	Food-based digestate typically has a lower BOD than cattle slurry.

There are a number of possible solutions to minimise the risk of negative effects of food-based digestate applications on earthworms, for example, lowering digestate application rates, lowering digestate pH (to influence the ammonium-N/ammonia-N balance), more complete digestion, application timing in relation to earthworm location within the topsoil etc. However, without more completely understanding the factors controlling the negative effects of digestate applications on earthworms, it is difficult to confidently identify effective and reliable management solutions.

References

Adair, K.L., Wratten, S., Barnes, A.M., Waterhouse, B.R., Smith, M., Lear, G., Weber, P., Pizey, M. and Boyer, S. (2014). Effects of biosolids on biodiesel crop yield and below ground communities *Ecological Engineering*, 68, 270-278.

Aira, M., Monroy, F. and Domínguez, J. (2006). C to N ratio strongly affects population structure of *Eisenia fetida* in vermicomposting systems. *European Journal of Soil Biology*, 43, S127-S131.

Artuso N., Kennedy, T.F., Connery, J., Grant, J. and Schmidt, O. (2011). Effects of biosolids at varying rates on earthworms (*Eisenia fetida*) and springtails (*Folsomia candida*). *Applied and Environmental Soil Science*, 2011.

Baker, G., Michalk, D., Whitby, W. and O'Grady, S. (2002). Influence of sewage waste on the abundance of earthworms in pastures in south-eastern Australia. *European Journal of Soil Biology*, 38, 233-237.

Barrera, I., Andrés, P., and Alcãniz, J.M. (2001). Sewage sludge application on soil: effects on two earthworm species. *Water, Air and Soil Pollution*, 129, 319–332.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D. and Brun, J.J. (2013). A review of earthworm impact on soil function and ecosystems services. *European Journal of Soil Science*, 64, 161-182.

Bermejo, G., Ellmer, F. and Krück, S. (2010). Use of dry and wet digestates from biogas plants as fertiliser in plant production. 14th RAMIRAN Conference, Lisbon, Portugal.

Berry, E.C. and Karlen, D.L. (1993). Comparison of alternative farming systems. II. Earthworm population density and species diversity. *American Journal of Alternative Agriculture*, 8, 21-26.

Birkás, M., Jolánkai, M., Gyuricza, C., Percze, A. (2004). Tillage effects on compaction, earthworms and other soil quality indicators in Hungary, *Soil & Tillage Research*, 78, 185-196.

Brady, N.C. (1974). *The Nature and Properties of Soils* (8th Edition). Macmillan Publishing Co., Inc.

Butt, K.R. (1999). Effects of thermally dried sewage granules on earthworms and vegetation during pot and field trials *Bioresource Technology*, 67, 149-154.

Carpenter-Boggs, L., Kennedy, A.C. and Reganold, J.P (2000). Organic and Biodynamic Management: Effects on Soil Biology. *Soil Science Society of America*, 64, 1651-1659.

Chambers, B.J., Gibbs P.A., Nicholson, F.A. and Edwards, A.D. (2005). *Manure ANalysis DatabasE (MANDE)*. Final report for Defra project NT2006

Clements, L.J., Salter, A.M., Banks, C.J. and Poppy, G.M. (2012). The usability of digestate in organic farming. *Water Science and Technology*. 1864-1870.

Cotton, D.C.F and Curry, J.P. (1980a). The effects of pig and pig slurry fertilisers on earthworms (Oligochaeta, Lumbricidae) in grasslands managed for silage production. *Pedobiologia,* 20, 181-188.

Cotton, D.C.F and Curry, J.P. (1980b). The response of earthworm populations (Oligochaeta, Lumbricidae) to high applications of pig slurry. *Pedobiologia*, 20, 189-196.

Cox, D.A. (1995). Pelletized sewage sludge as a fertilizer for containerized plants: plant growth and nitrogen leaching losses. *Journal of Plant Nutrition*, 18, 2783-2795.

Curry, J.P. (1976). Some effects of animal manures on earthworms in grassland. *Pedobiologia*, 16, 425-438.

Darwin (1881). *The Formation of Vegetable Mould Through the Action of Worms.* J Murray, London.

Defra (2010). *The Fertiliser Manual (RB209).* Department of Environment, Food and Rural Affairs. The Stationery Office, Norwich, UK.

De Goede, R.G.M., Brussaard, L. and Akkermans, A.D.L. (2003). On-farm impact of cattle slurry manure management on biological soil quality. *NJAS - Wageningen Journal of Life Sciences*, 51 (1-2), 103-133.

D'Hose, T., Cougnon, M., De Vliegher, A., Vandecasteele, B., Viaene, Cornelis, W., Van Bockstaele, E. and Reheul, D. (2014). The positive relationship between soil quality and crop production: A case study on the effect of farm compost application. *Applied Soil Ecology*, 75, 189-198

EA (2008). *Think Soils: Soil Assessment to Avoid Erosion and Runoff*. Environment Agency 2008, Bristol, UK.

Edwards, C.A. (1988). Breakdown of animal, vegetable, and industrial organic wastes by earthworms. *Agriculture Ecosystems and Environment*, 24, 21-31.

Edwards, C.A. and Bohlen, P.J. (1996). *Biology and Ecology of Earthworms* (3rd Edition). Boca Raton.

Edwards, C.A. and Lofty, R. (1977). *The Biology of Earthworms* (2nd Edition). Chapman and Hall, London, UK.

Edwards, C.A. and Lofty, R. (1982). Nitrogenous fertiliser and earthworm populations in agricultural soils. *Soil Biology and Biochemistry*, 14, 515-521.

Ernst, G., Müller, A., Göhler, H. and Emmerling, C. (2008). C and N turnover of fermented residues from biogas plants in soil in the presence of three different earthworm species (*Lumbricus terrestris, Aporrectodea longa, Aporrectodea caliginosa*). *Soil Biology and Biochemistry*, 40, 1413-1420.

Frøseth, R.B., Bakken, A.K., Bleken, M.A., Riley, H., Pommeresche, R., Thorup-Kristensen, K. and Hansen S. (2014). Effects of green manure herbage management and its digestate from biogas production on barley yield, N recovery, soil structure and earthworm populations. *European Journal of Agronomy*, 52, 90-102.

Hansen, S. (1996). Effects of manure treatment and soil compaction on plant production of a dairy farm system converting to organic farming practice. *Agriculture, Ecosystem and Environment*, 56, 173-186.

Hansen, S. and Engelstad, F. (1999). Earthworm populations in a cool and wet district as affected by tractor traffic and fertilisation. *Applied Soil Ecology*, 13, 237-250.

Kinney, C.A., Campbell, B.R., Thompson, R., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L. and Hay A.G. (2012). Earthworm bioassays and seedling emergence for monitoring toxicity, aging and bioaccumulation of anthropogenic waste indicator compounds in biosolids–amended soil. *Science of the Total Environment,* 433, 507-515.

Lapied, E. Nahmani, J. and Rousseau, G.X. (2009). Influence of texture and amendments on soil properties and earthworm communities. *Applied Soil Ecology,* 43, 241-249.

Lee, K.E. (1985). *Earthworms their Ecology and Relationships with Soils and Land Use*. Academic press.

Leroy, B.L.M., Bommele, L., Reheul, D., Moens, M. and De Neve, S. (2007). The application of vegetable, fruit and garden waste (VFG) compost in addition to cattle slurry in silage maize monoculture: Effects on soil fauna and yield. *European Journal of Soil Biology*, 43, 91-100.

Leroy, B.L.M., Schmidt, O., Van den Bossche, A., Reheul, D. and Moens, M. (2008). Earthworm population dynamics as influenced by the quality of exogenous organic matter. *Pedobiologia*, 52, 139-150.

Lowe, C.N. and Butt, K.R. (2005). Culture techniques for soil dwelling earthworms: a review. *Pedobiologia,* 49, 401-413.

MAFF (1998). *Code of Good Agricultural Practice for the Protection of Soil*. MAFF Publications.

Marhan, S. and Scheu, S. (2005). The influence of mineral and organic fertilisers on the growth of the endogeic earthworm Octolasion tyrtaeum (Savigny). *Pedobiologia*, 49, 239-249.

Natural England (2014). *Earthworms in England: Distribution, Abundance and Habitats*. Natural England Commissioned Report NECR145.

Nicholson, F.A., Rollett, A.J. and Chambers, B.J. (2010). *Agricultural Soil Heavy Metal Inventory for 2008.* Final report three for Defra project SP0569.

Nicholson, F.A., Brettell, N.R., Chambers, B.J. and Stevenson, S. (2013). *An Examination of the Limits for Potentially Toxic Elements (PTEs) in Anaerobic Digestates.* Final report for WRAP project OMK002-013.

Owojori, J.O. and Reinecke, A.J. (2014). Differences in ionic properties of salts affect saline toxicity to the earthworm *Eisenia fetida Applied Soil Ecology*. In Press.

Petersen, H and Luxton M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 39, 287-388.

Russell, E.W. (1973). *Soil Conditions and Plant Growth* (10th Edition). Longman Group Limited. London and New York.

Scullion, J., Neale, S., Philipps, L. (2002). Comparisons of earthworm populations and cast properties in conventional and organic arable rotations. *Soil Use and Management*, 22, 181-190.

SRUC (2013) Technical Note TN651. *Nitrogen Recommendations for Cereals, Oilseed Rape and Potatoes.*

Taylor, M.J., Rollett, A.J. and Chambers B.J. (2011). *Compost and Anaerobic Digestate Quality for Welsh Agriculture*. Final report for WRAP project OAV032-004.

Unwin, R.J. and Lewis, S. (1986). The effect upon earthworm populations for very large applications of pig slurry to grassland. *Agricultural Wastes*, 16, 67-73.

USDA (2001). Agricultural management effects on earthworm populations. Soil Quality. Agronomy Technical Note No 11. Soil Quality Institute.

Van Vliet, P.C.J., Can der Stelt, B., Rietberg, P.I., De Goede, R.G.M. (2007). Effects of organic matter content on earthworms and nitrogen mineralization in grassland soils. *European Journal of Soil Biology*, 43, S222-S229.

Van Vliet, P.C.J. and De Goede, R.G.M. (2006). Effects of slurry application methods on soil faunal communities in permanent grassland. *European Journal of Soil Biology*, 42, S348–S353.

Whalen, J.K. and Parmerlee, R.W. (1999). Quantification of nitrogen assimilation efficiencies and their use to estimate organic matter consumption by the earthworms *Aporrectodea tuberculata* (Eisen) and *Lumbricus terrestris* L. *Applied Soil Ecology*, 13, 199-208.

Whalen, J.K. and Parmerlee, R.W. (2000). Earthworm secondary production and N flux in agroecosystems: a comparison of two approaches. *Oecologia*, 124, 561-573.

WRAP (2012). *Using Quality Anaerobic Digestate to Benefit Crops*. Available from: http://www.wrap.org.uk/content/using-quality-digestate-benefit-crops-0

Appendix 3.1

Table 1. Earthworm biomass (g/m^2 to 25 cm depth) at seven sites in spring 2013

Treatment	Aber	Ayr	Dev	Far	Harper	Lamp	Ter
Control	68.7	273	7.60	**37.1**[ab]	2.91	130	**29.9**[ab]
Green compost	86.3	287	19.0	**49.8**[abc]	8.20	107	**29.6**[ab]
Green/food compost	79.9	328	18.6	**33.5**[a]	12.4	130	**15.1**[a]
Food-based digestate	81.4	181	26.6	**45.1**[ab]	18.2	103	**11.5**[a]
Manure-based digestate	58.9	289					
Farmyard manure	92.0	363	12.2	**74.2**[c]	18.0	124	**47.9**[b]
Livestock slurry	52.0	316	34.3	**65.4**[bc]	10.7	128	**27.3**[a]
*P value**	*NS (0.89)*	*NS (0.09)*	*NS (0.16)*	***0.04***	*NS (0.67)*	*NS (0.86)*	***0.02***

*Statistical analysis undertaken using ANOVA (data normally distributed).

Appendix 3.2

Regression analysis

Figure 1 shows the relationship between earthworm numbers on the food-based digestate treatment minus the control, against numbers on the control treatment at each site. The graph indicates a trend across sites that the more earthworms that were present, the greater the reduction in earthworm numbers on the food based digestate treatments compared to the control. In other words the food-based digestate showed a greater and greater negative impact on earthworm numbers in comparison with the control with increasing control numbers.

Regression lines were put on the graph, but no equation or r^2 was quoted as it was felt that this could be misleading, as the points on the graph were site means and did not take into account the variability across treatment replicates at each site. Any r^2 value calculated could have been an overestimate of the accuracy of the goodness of fit of the line, and it was felt that the equation of the fitted line was not sufficiently reliable to attempt to quantify the effect.

The P value indicated that there was good evidence of a relationship, but should not be taken as an exact figure.

The trend was also evident when the Ayr site was excluded. This general trend existed across all sites, but it was not possible to say whether the rate of reduction (i.e. the slope of the line) was the same for arable and grassland sites as we only had two grassland sites.

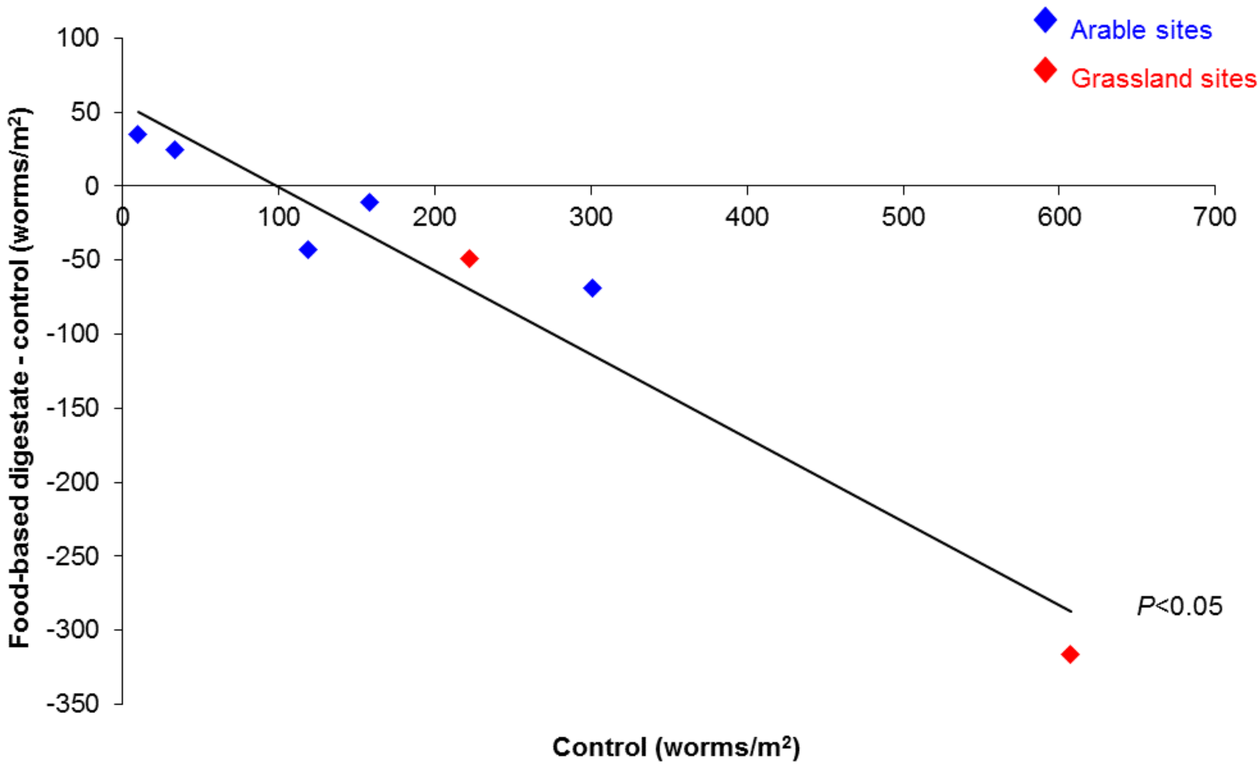


Figure 1. Relationship between earthworm numbers on the control (i.e. site baseline) and food-based digestate treatments; regression based on block-by-block treatment comparisons

Similarly, Figure 2 shows the relationship between earthworm numbers on the food-based digestate treatment *minus* the slurry treatment against numbers on the slurry (i.e. comparator) treatment at each site. This also shows the trend that the reduction in numbers of earthworms on the food-based digestate treatment compared to the slurry treatment increased as earthworm numbers increased. This was also evident when the Ayr site was excluded. No equation or r^2 figure was quoted for Figure 2, for the same reasons as explained above in relation to Figure 1. The P value indicates that there was good evidence of a relationship, but should not be taken as an exact figure.

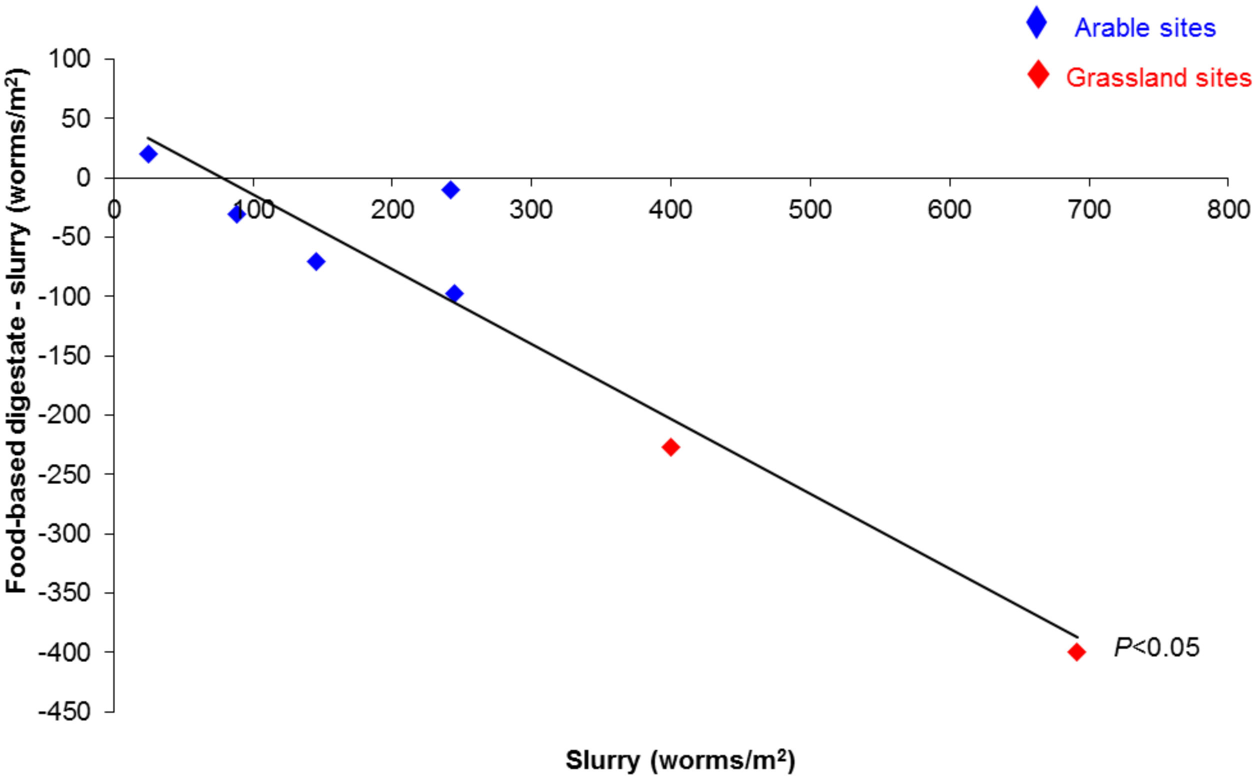


Figure 2. Relationship between earthworm numbers on the slurry (i.e. comparator material) and food-based digestate treatments; regression based on block-by-block treatment comparisons.

APPENDIX 4. Earthworm studies: Phase I Measurements at Existing *DC-Agri* Sites Autumn 2014

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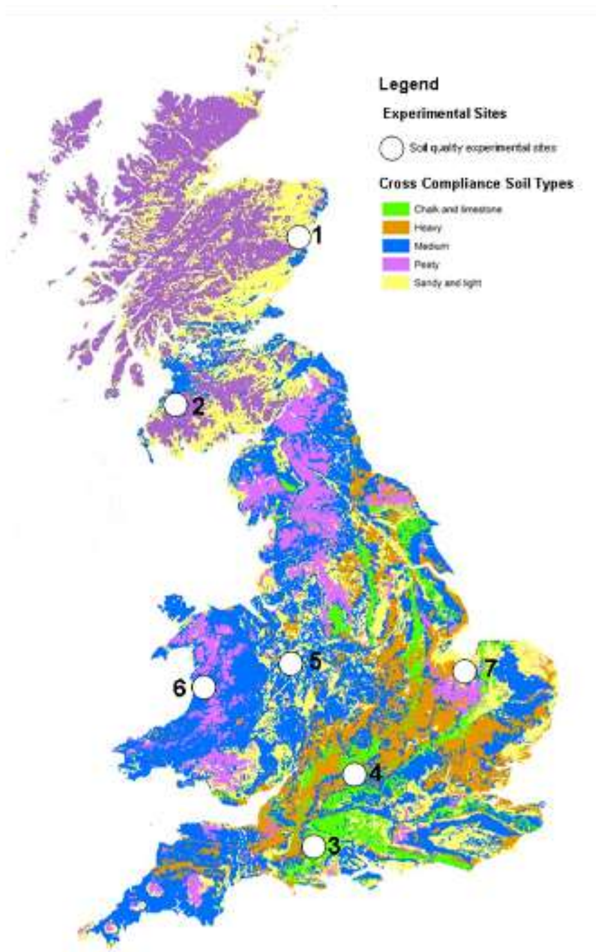
1 Background

In autumn 2010, seven sites were established in contrasting agroclimatic areas; Aberdeen, Ayr, Devizes, Faringdon, Harper Adams, Lampeter and Terrington to quantify the effects of repeated compost and digestate applications, in comparison with farmyard manure and slurry (as comparator materials), on soil bio-physical and physico-chemical properties and crop quality (Table 1 and Figure 1).

Table 1. Characteristics and cropping at the soil and crop quality experimental platforms (3 cropping years)

Site		Soil textural group		Annual rainfall (mm)	Cropping rotation+		
		Cross-compliance soil group[1]	% clay		2010-11	2011-12	2012-13
1	Aberdeen	Sandy/light	16	790	SB	WB	WOSR
2	Ayr	Medium	19	1,190	G	G	G
3	Devizes	Chalk	20	850	Lin	WW	WW
4	Faringdon	Heavy	62	830	WW	WW	WC
5	Harper Adams	Sandy/light	11	690	POT	SB	WW
6	Lampeter	Medium	26	980	G	G	G
7	Terrington	Medium (heavy)	28	630	WW	WW	WOSR

+ SB = spring barley; WB = winter barley; WOSR = winter oilseed rape; SW = spring wheat; WW = winter wheat; G = grassland; POT = potatoes; Lin = linseed; WC = whole crop oats/peas.
[1]EA (2008)



Experimental sites
1: Aberdeen
2: Ayr
3: Devizes
4: Faringdon
5: Harper Adams
6: Lampeter
7: Terrington

Figure 1. Location of experimental sites

1.1 Treatments

At each site, 18 experimental plots were laid out in a randomised block design (6 treatments, with 3 replicates of each), Table 2. Organic material applications (broadcast) at a target rate of 250 kg total nitrogen (N)/ha were made in harvest years 2011, 2012 and 2013 at the seven sites.

Table 2. Organic material treatment details (target application rates)

Treatment No.	Treatment details
1	Control (no organic material application)
2	Green compost at 250 kg total N/ha
3	Green/food compost at 250 kg total N/ha
4	Food-based digestate at 250 kg total N/ha
5	Farmyard manure at 250 kg total N/ha
6	Slurry at 250 kg total N/ha

Applications of manufactured fertiliser (nitrogen, phosphate, potash and sulphur) were also made where necessary, based on crop requirements, after accounting for the nitrogen supplied by the organic materials (Defra, 2010; SRUC, 2013). This ensured (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 organic matter inputs was from the applied organic materials).

1.2 Previous sampling

Earthworm populations were measured in spring 2013 on 3 'blocks' of soil (each 30 x 30 x 25 cm deep) per plot; by counting all adult and immature worms collected within a 5 minute period. The measurements were made between 6 and 8 months after the last digestate application (March/April 2013). The results were analysed using conventional analysis of variance for each individual site separately then across all grassland (2 sites) and arable (5 sites), using Genstat version 12.

1.2.1 Site-by-site analysis

- There were treatment differences in earthworm numbers at 4 sites - Ayr, Faringdon, Lampeter and Terrington (*P<0.05*), but not at Aberdeen, Devizes or Harper Adams (*P=0.69, 0.21 & 0.06, respectively*).
- At Ayr, the application of food-based digestate *reduced* earthworm numbers in comparison with all the other treatments (*P<0.05*). Notably, the Ayr site had the greatest number of earthworms.
- 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 treatment at Terrington (*P<0.05*).

1.2.2 Cross-site analysis

- At the grassland sites (Ayr and Lampeter), overall earthworm numbers on the food-based digestate treatments were *lower* than on all the other treatments (*P<0.001*).
- In contrast, earthworm numbers at the arable sites were similar on all the treatments; although overall earthworm numbers on the FYM treatment were higher than on the control, green/food compost and food-based digestate treatments (*P<0.01*).

2 Re-sampling existing *DC-agri* sites

To determine any longer-term effects of organic material applications on earthworm populations/biomass and communities, earthworm numbers (adults and juveniles), biomass and species were assessed in autumn 2014. The measurements were undertaken at four of the existing sites, where a *significant effect* (*P*<0.05) of food-based digestate application on earthworm numbers/biomass was previously observed when compared to the fertiliser control or other organic materials (i.e. Ayr, Faringdon, Lampeter and Terrington).

2.1 Methodology

All earthworm measurements were undertaken following ADAS Standard Operating Procedures (ADAS SOP ECO/018: Destructive Sampling for Earthworms in the Field), which is based on well-established methodologies. Additionally, earthworm identification was undertaken by qualified experienced staff using an earthworm identification key

Endogeic (shallow dwelling) and epigeic (litter dwelling) earthworm populations were measured on 3 'blocks' of soil (each 30 x 30 x 25 cm deep) per plot from the central 2 m x 2 m plot area (i.e. excluding the perimeter plot area) by counting all adult and immature earthworms collected within a *c.*5 minute period. In accordance with the standard operating procedures, the 5 minute sampling time was increased if the soil was wet or compact (*c.*10 minutes) to ensure the soil was broken up effectively. Anecic (deep burrowing) earthworm species were subsequently extracted from the area immediately *below* the three extracted soil 'blocks', using the mustard method (Scullion *et al.*, 2014; Pelosi *et al.* 2014; Clements *et al.*, 2012). The 3 blocks were located in the central area of each plot, to minimise possible earthworm migration effects from adjacent plots.

Earthworms from each individual soil 'block', plus the extracted deep burrowing species, were returned to the ADAS laboratory in separate plastic containers (*i.e. six containers per plot*) lined with sufficiently damp material (e.g. moss) to provide an environment conducive to the maintenance of earthworm condition (i.e. not too wet or too dry). Earthworms were then sorted as follows (the treatments within each block were assessed in randomised order to ensure there was no bias in the assessments):

1. Adult and juvenile earthworm counts;
2. Adult earthworms counts split into three functional groups (anecic, epigeic and endogeic);
3. Adult earthworm biomass in each functional group;
4. Juvenile earthworm biomass (this was not broken down by species due to immature earthworm development preventing a more accurate breakdown);
5. Adult earthworm species and counts;
6. Adult species biomass;
7. One representative of each identified species was stored in ethanol and one frozen (at -20°C) for further identification (e.g. DNA extraction) or verification if required.

The results of the total earthworm numbers/biomass (adults and juveniles) and broad species groupings (i.e. Endogeic, Epigeic and Anecic) was explored using conventional analysis of variance (ANOVA) and comparison of P-values. A separate ANOVA was carried out at each site, after which *post-hoc* testing was undertaken to evaluate which treatment means were different from each other using a Duncan's multiple range test (using Genstat version 12; VSN International Ltd, 2010). This test assigns different letters to treatment values which are significantly different from each other at the 5% level (*P*<0.05). In the tables of results, treatments which are statistically significantly different are marked with different letters. The individual species data did not have a normal distribution, so these results were analysed using the non-parametric Friedman's test using Genstat version 12.

2.2 Results

2.2.1 Ayr

Table 3. Ayr: mean number of earthworms/m^2

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	614	31	23	1	6	584
Green compost	593	58	26	11	21	535
Green/food compost	654	42	35	0	7	612
Food based digestate	491	59	43	11	5	432
FYM	741	20	11	4	5	721
Slurry	735	49	30	10	9	686
P value[1]	NS (0.13)	NS (0.29)	NS (0.61)	NS (0.12)	NS (0.44)	NS (0.08)

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05)

[a,b,c,d] Different letters within a column indicate significant differences between treatments at 0.05 level

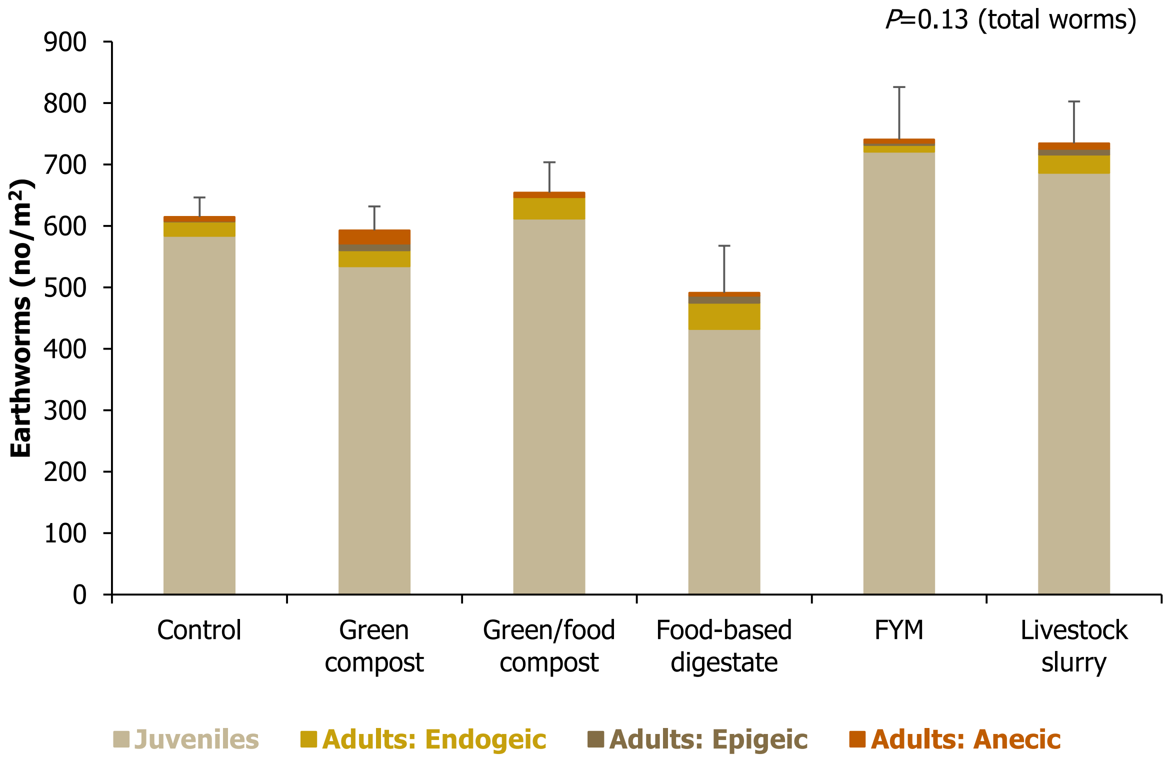


Figure 2. Ayr: mean number of earthworms/m^2

Table 4. Ayr: mean weight of earthworms g/m^2

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	295^b	28	6	0	22	267^b
Green compost	343^b	53	7	6	40	290^b
Green/food compost	308^b	15	7	0	8	293^b
Food based digestate	207^a	30	14	10	5	177^a
FYM	289^b	15	2	1	12	274^b
Slurry	336^b	25	7	6	13	311^b
P value[1]	0.04	NS (0.28)	NS (0.39)	NS (0.21)	NS (0.26)	0.01

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05)

[a,b,c,d] Different letters within a column indicate significant differences between treatments at 0.05 level

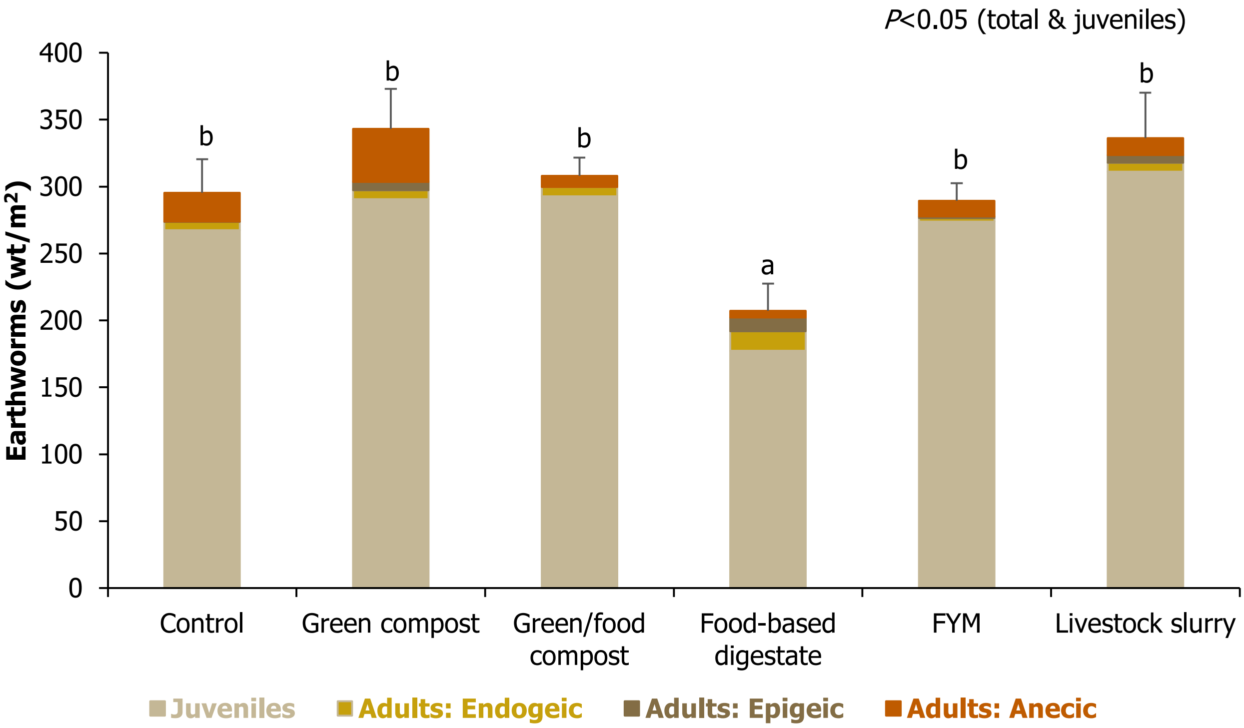


Figure 3. Ayr: weight of earthworms wt/m^2

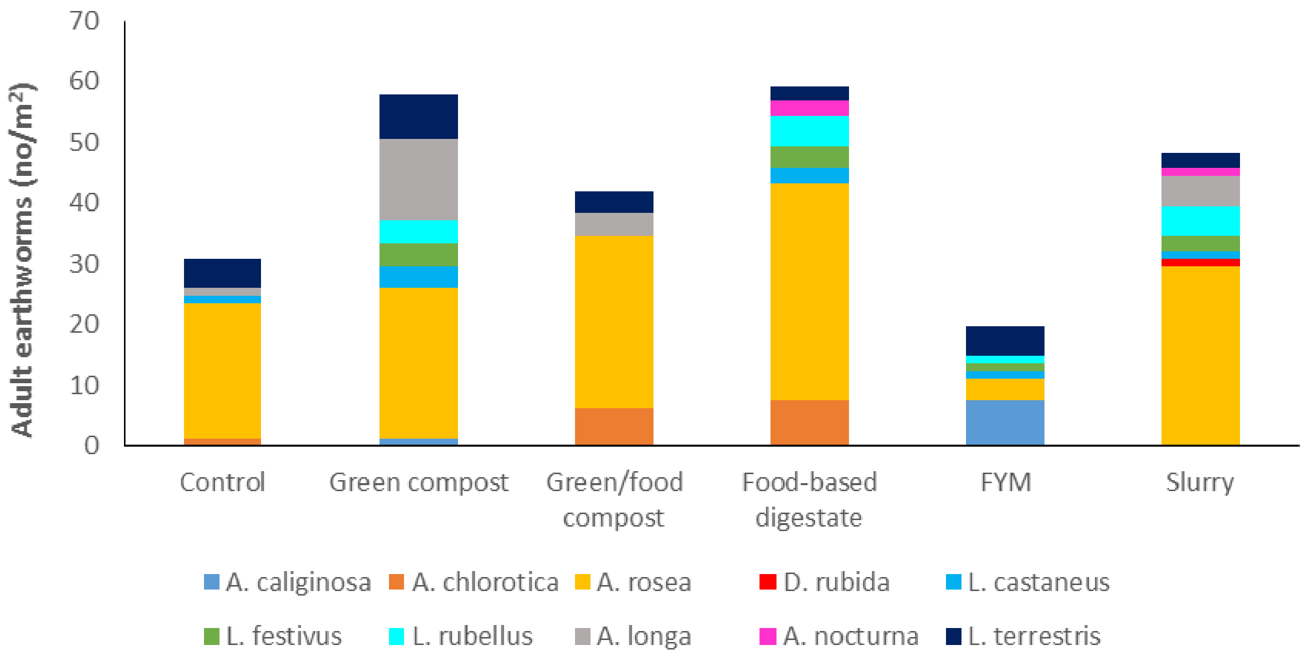


Figure 4. Ayr: species composition of adult earthworms (numbers)

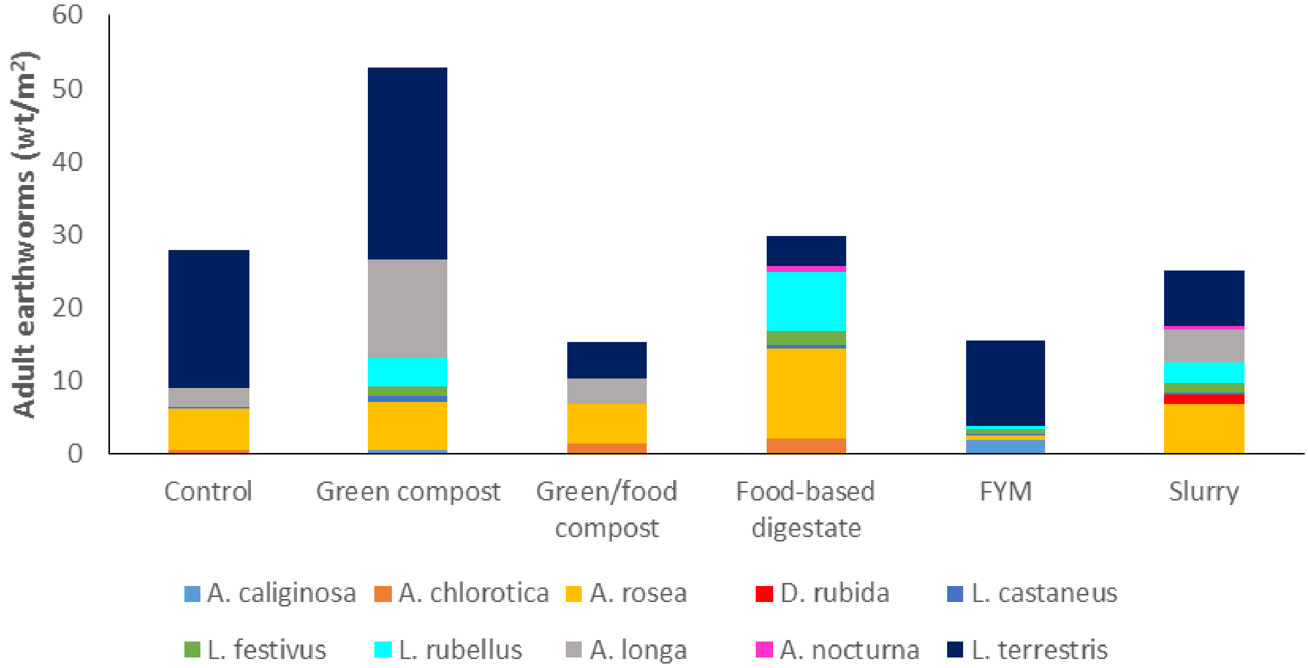


Figure 5. Ayr: species composition of adult earthworms (weight)

Table 5. Ayr: Adult earthworm species (no/m^2)

Treatment	*Endogeic earthworms*				*Epigeic earthworms*					*Anecic earthworms*			
	A. caliginosa	***A. chlorotica***	***A. rosea***	***Total***	***D. rubida***	***L. castaneus***	***L. festivus***	***L. rubellus***	***Total***	***A. longa***	***A. nocturna***	***L. terrestris***	***Total***
Control	0	1	22	**23**	0	1	0	0	**1**	1	0	5	**6**
Green compost	1	0	25	**26**	0	4	4	4	**11**	14	0	7	**21**
Green/food compost	0	6	28	**35**	0	0	0	0	**0**	4	0	4	**7**
Food-based digestate	0	7	36	**43**	0	2	4	5	**11**	0	2	2	**5**
FYM	7	0	4	**11**	0	1	1	1	**4**	0	0	5	**5**
Slurry	0	0	30	**30**	1	1	2	5	**10**	5	1	2	**9**

No significant treatment effects for any of the species as determined using the Friedman's test; $P>0.1$.

Table 6. Ayr: Adult earthworm species (wt/m^2)

Treatment	*Endogeic earthworms*				*Epigeic earthworms*					*Anecic earthworms*			
	A. caliginosa	***A. chlorotica***	***A. rosea***	***Total***	***D. rubida***	***L. castaneus***	***L. festivus***	***L. rubellus***	***Total***	***A. longa***	***A. nocturna***	***L. terrestris***	***Total***
Control	0	0	6	**6**	0	0	0	0	**0**	3	0	19	**22**
Green compost	0	0	6	**7**	0	1	1	4	**6**	14	0	26	**40**
Green/food compost	0	1	6	**7**	0	0	0	0	**0**	3	0	5	**8**
Food-based digestate	0	2	12	**14**	0	0	2	8	**10**	0	1	4	**5**
FYM	2	0	1	**2**	0	0	1	0	**1**	0	0	12	**12**
Slurry	0	0	7	**7**	1	0	1	3	**6**	5	0	8	**13**

No significant treatment effects for any of the species as determined using the Friedman's test; $P>0.1$.

2.2.2 Faringdon

Table 7. Faringdon: mean number of earthworms/m²

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	264	79	64	14	1	185[ab]
Green compost	265	88	81	6	0	178[ab]
Green/food compost	285	72	65	6	0	214[abc]
Food-based digestate	237	72	53	17	1	165[a]
FYM	347	81	69	12	0	265[c]
Slurry	335	94	88	6	0	241[bc]
P value[1]	NS (0.13)	NS (0.92)	NS (0.55)	NS (0.34)	ND	0.04

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05); ND = not determined due to insufficient data

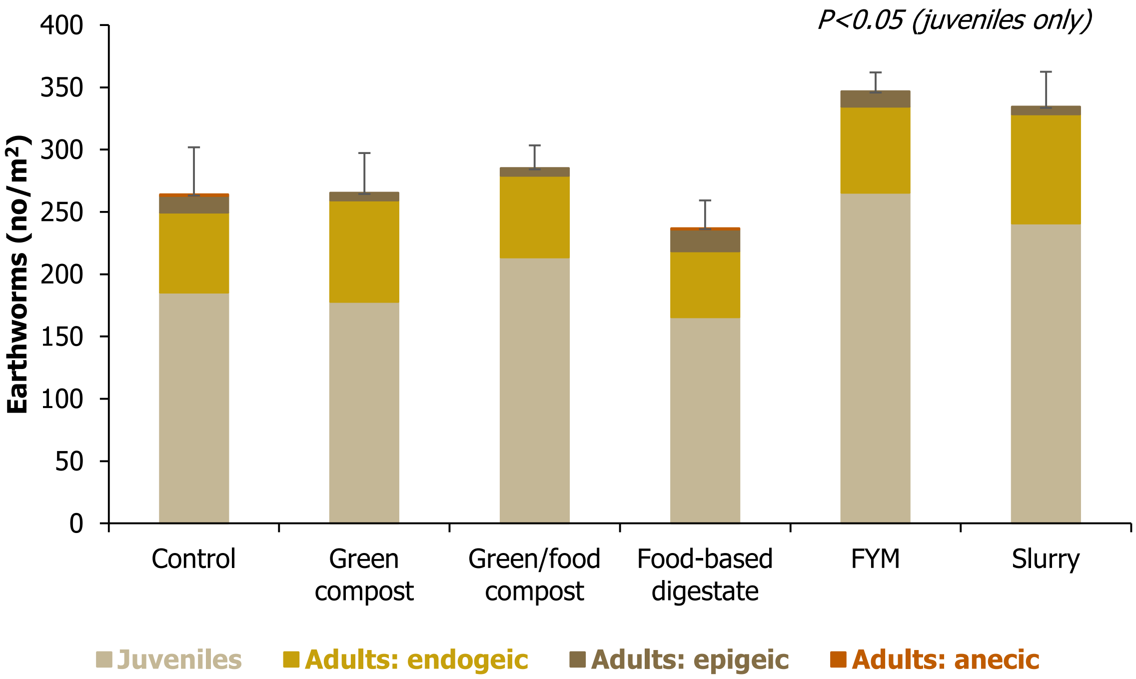


Figure 6. Faringdon: mean number of earthworms/m²

Table 8. Faringdon: mean weight of earthworms g/m²

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	56	33	22	8	3	22.4[a]
Green compost	57	31	27	4	0	26.1[ab]
Green/food compost	57	25	21	4	0	31.6[abc]
Food-based digestate	64	30	16	11	3	33.2[abc]
FYM	78	30	23	7	0	47.5[c]
Slurry	69	29	26	3	0	40.0[bc]
P value[1]	NS (0.67)	NS (0.97)	NS (0.68)	NS (0.41)	ND	0.04

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05); ND = not determined due to insufficient data
[a,b,c,d] Different letters within a column indicate significant differences between treatments at 0.05 level

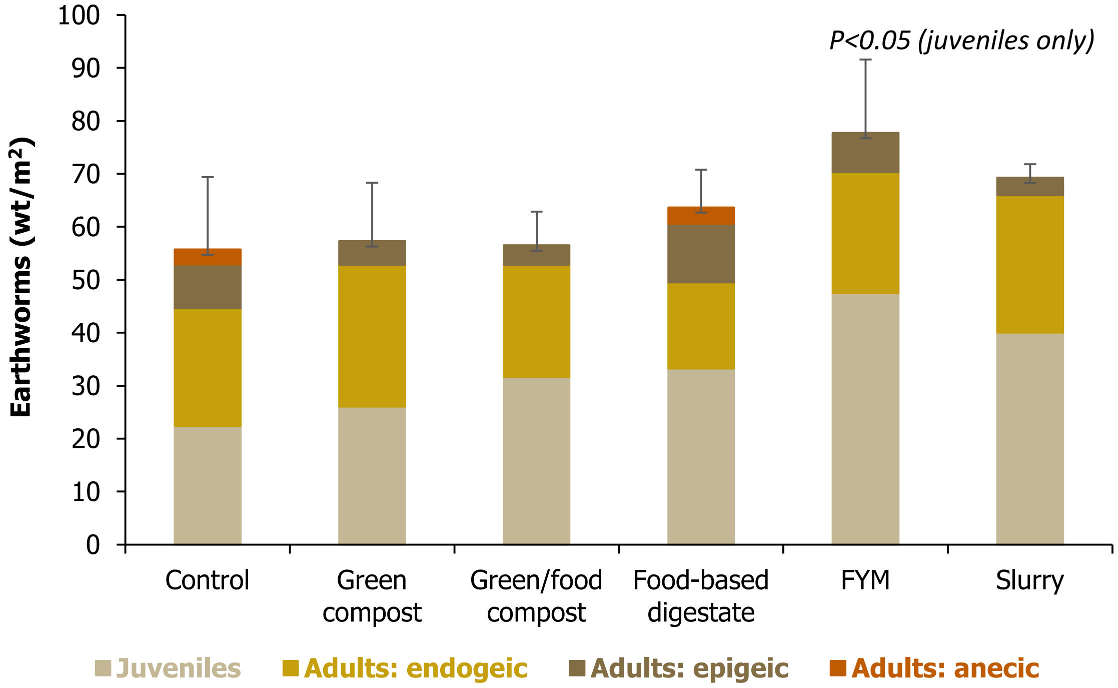


Figure 7. Faringdon: mean weight of earthworms g/m^2

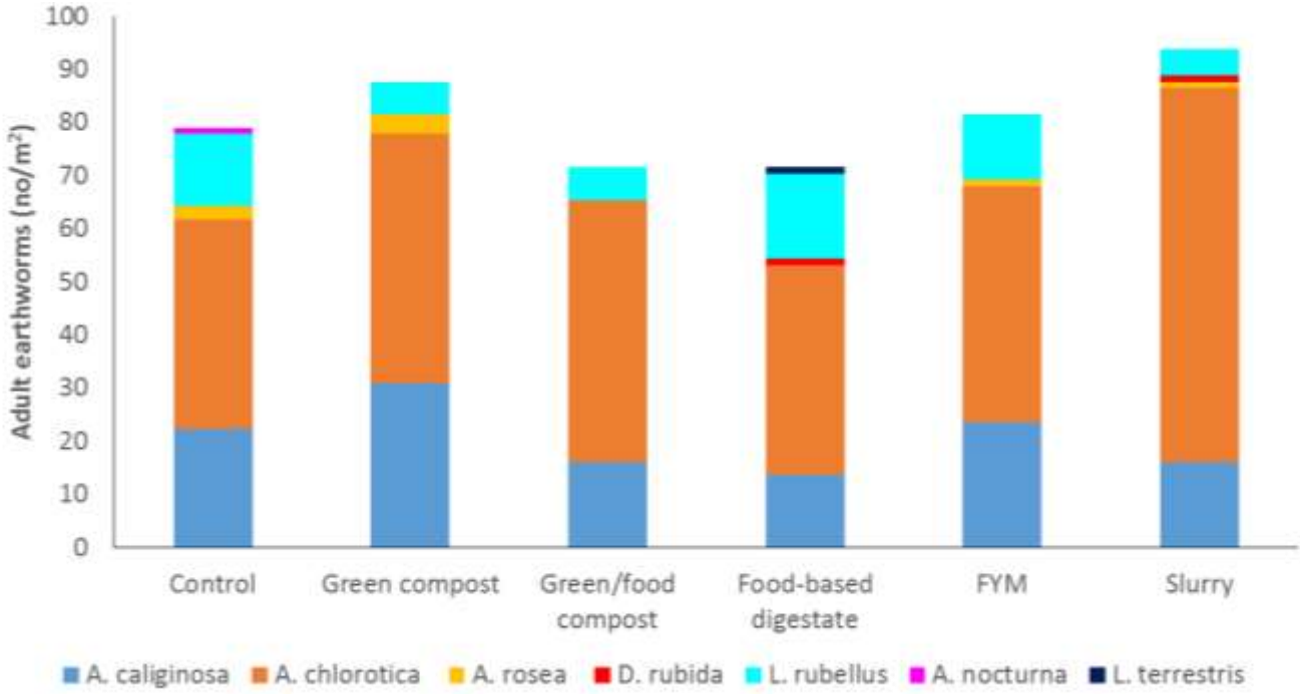


Figure 8. Faringdon: species composition of adult earthworms (no)

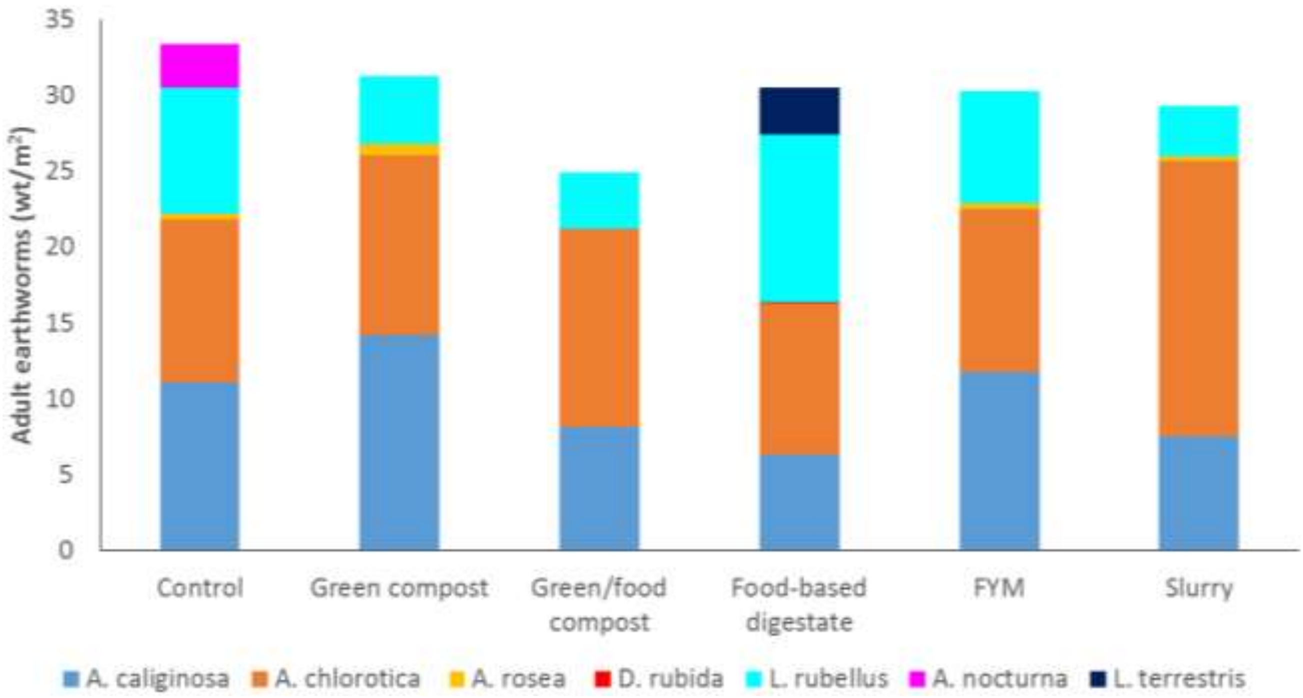


Figure 9. Faringdon: species composition of adult earthworms (wt)

Table 9. Faringdon: Adult earthworm species (no/m^2)

Treatment	***Endogeic earthworms***				***Epigeic earthworms***			***Anecic earthworms***		
	A. caliginosa	***A. chlorotica***	***A. rosea***	**Total**	***D. rubida***	***L. rubellus***	***Total***	***A. nocturna***	***L. terrestris***	***Total***
Control	22	40	2	**64**	0	14	**14**	1	0	**1**
Green compost	31	47	4	**81**	0	6	**6**	0	0	**0**
Green/food compost	16	49	0	**65**	0	6	**6**	0	0	**0**
Food-based digestate	14	40	0	**53**	1	16	**17**	0	1	**1**
FYM	23	44	1	**69**	0	12	**12**	0	0	**0**
Slurry	16	70	1	**88**	1	5	**6**	0	0	**0**

No significant treatment effects for any of the species as determined using the Friedman's test; *P*>0.1.

Table 10. Faringdon: Adult earthworm species (wt/m^2)

Treatment	***Endogeic earthworms***				***Epigeic earthworms***			***Anecic earthworms***		
	A. caliginosa	***A. chlorotica***	***A. rosea***	***Total***	***D. rubida***	***L. rubellus***	***Total***	***A. nocturna***	***L. terrestris***	***Total***
Control	11	11	0.42	**22**	0	8	**8**	3	0	**3**
Green compost	14	12	0.81	**27**	0	4	**4**	0	0	**0**
Green/food compost	8	13	0	**21**	0	4	**4**	0	0	**0**
Food-based digestate	6	10	0	**16**	0.03	11	**11**	0	3	**3**
FYM	12	11	0.34	**23**	0	7	**7**	0	0	**0**
Slurry	8	18	0.27	**26**	0.05	3	**3**	0	0	**0**

No significant treatment effects for any of the species as determined using the Friedman's test; *P*>0.1.

2.2.3 Lampeter

Table 11. Lampeter: mean number of earthworms/m²

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	167	42	35	2	5	125
Green compost	199	52	46	2	4	147
Green/food compost	256	69	65	0	4	186
Food-based digestate	222	42	33	1	7	180
FYM	207	70	68	0	2	137
Slurry	190	46	35	4	7	144
P value[1]	NS (0.73)	NS (0.44)	NS (0.25)	ND	NS (0.87)	NS (0.69)

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05)

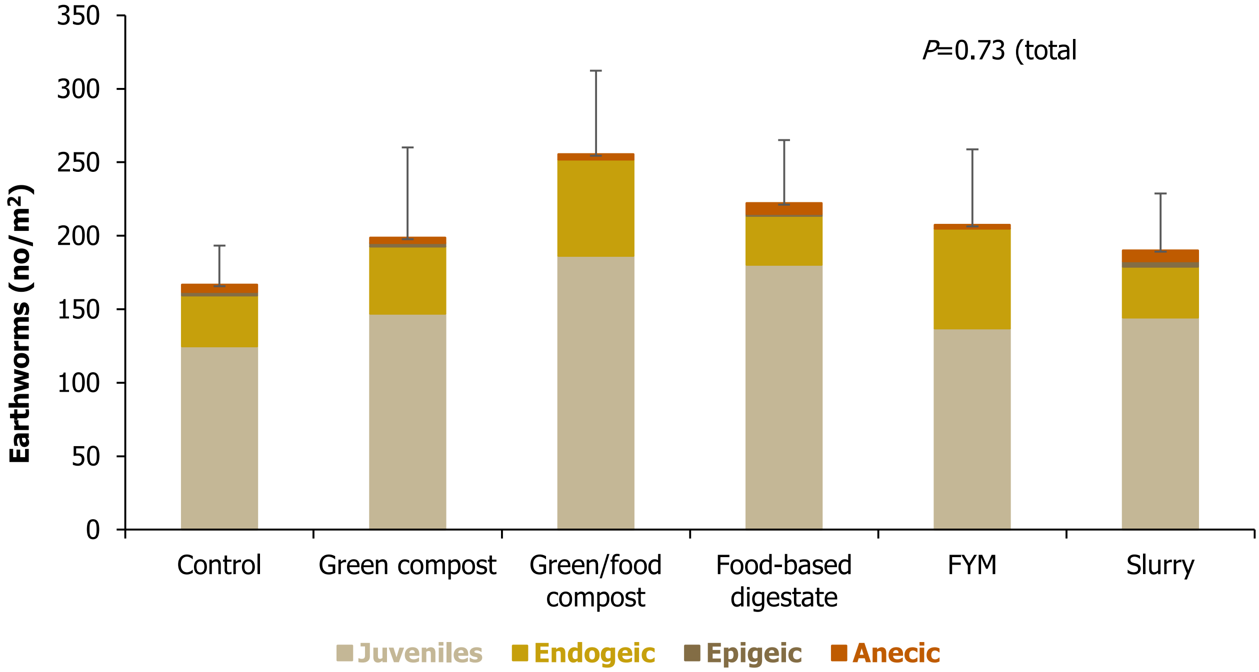


Figure 10. Lampeter: mean number of earthworms/m²

Table 12. Lampeter: mean weight of earthworms g/m^2

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	104	30	17	3	11	74
Green compost	93	36	27	1	8	57
Green/food compost	131	43	34	0	9	88
Food-based digestate	136	36	13	1	22	100
FYM	96	39	32	0	7	57
Slurry	117	35	15	1	18	82
P value[1]	NS (0.74)	NS (0.98)	NS (0.34)	ND	NS (0.81)	NS (0.32)

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05); ND = not determined due to insufficient data

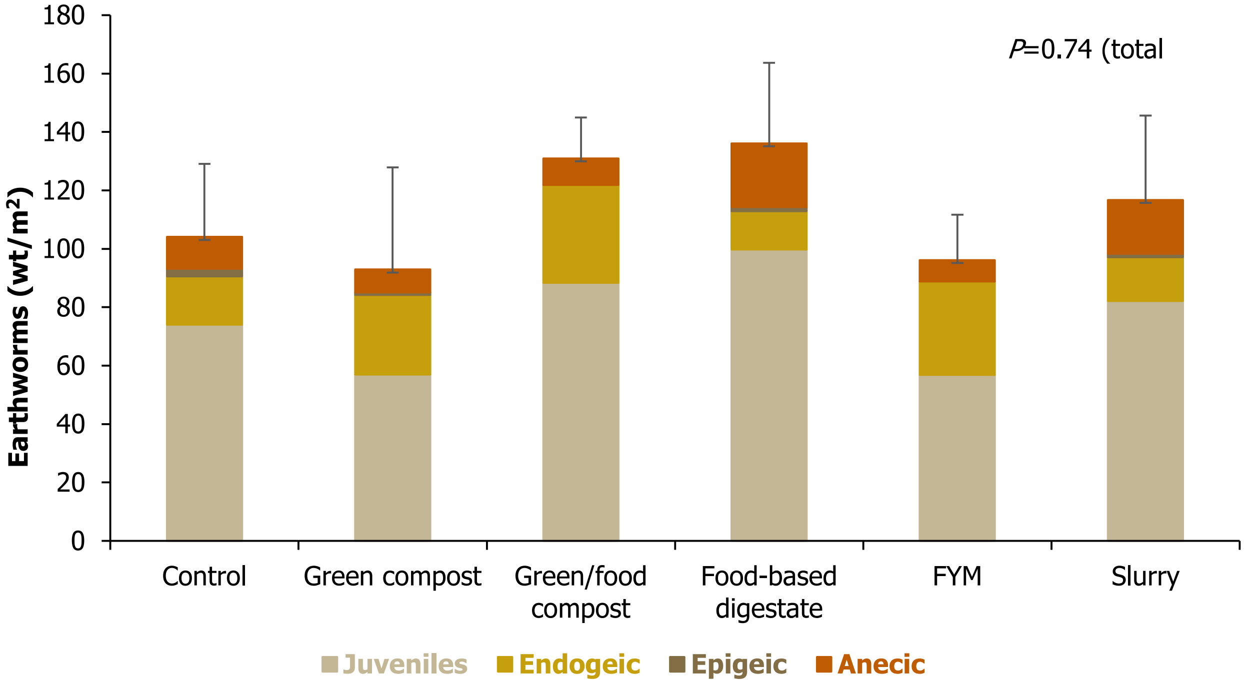


Figure 11. Lampeter: mean weight of earthworms g/m^2

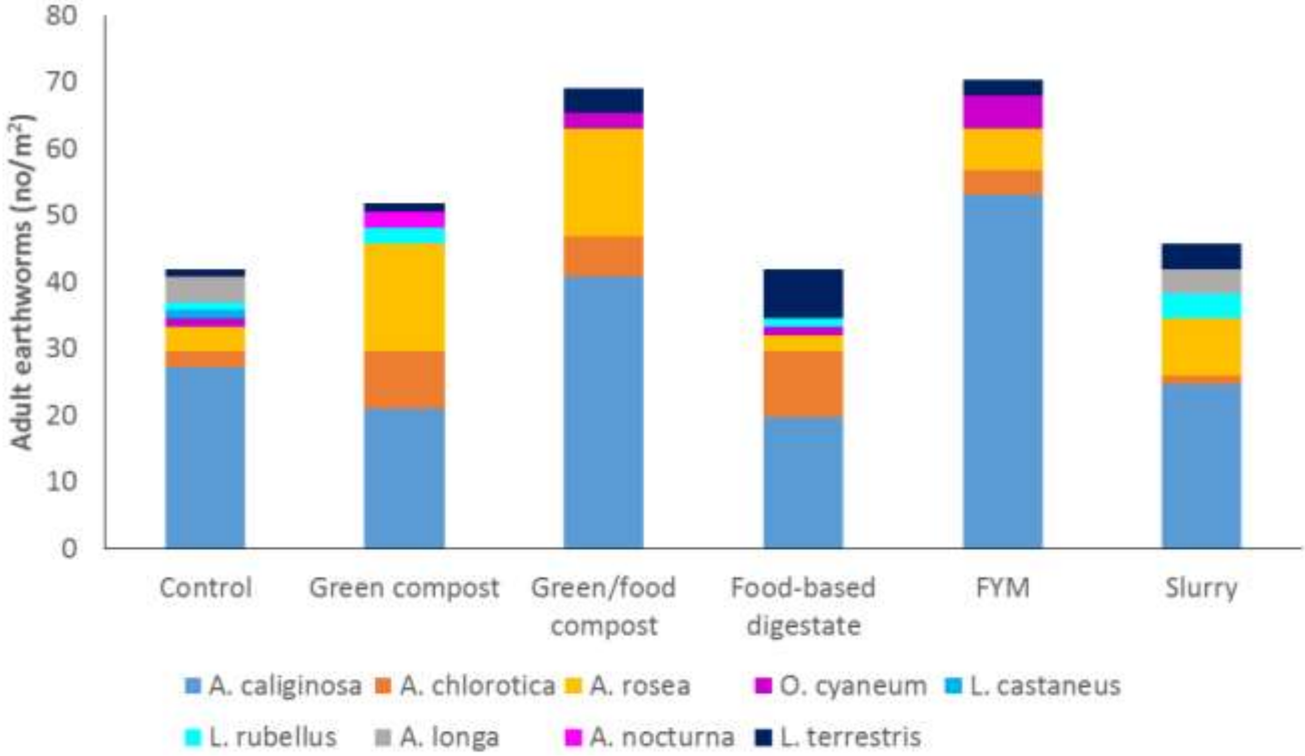


Figure 12: Lampeter: species composition of adult earthworms (no)

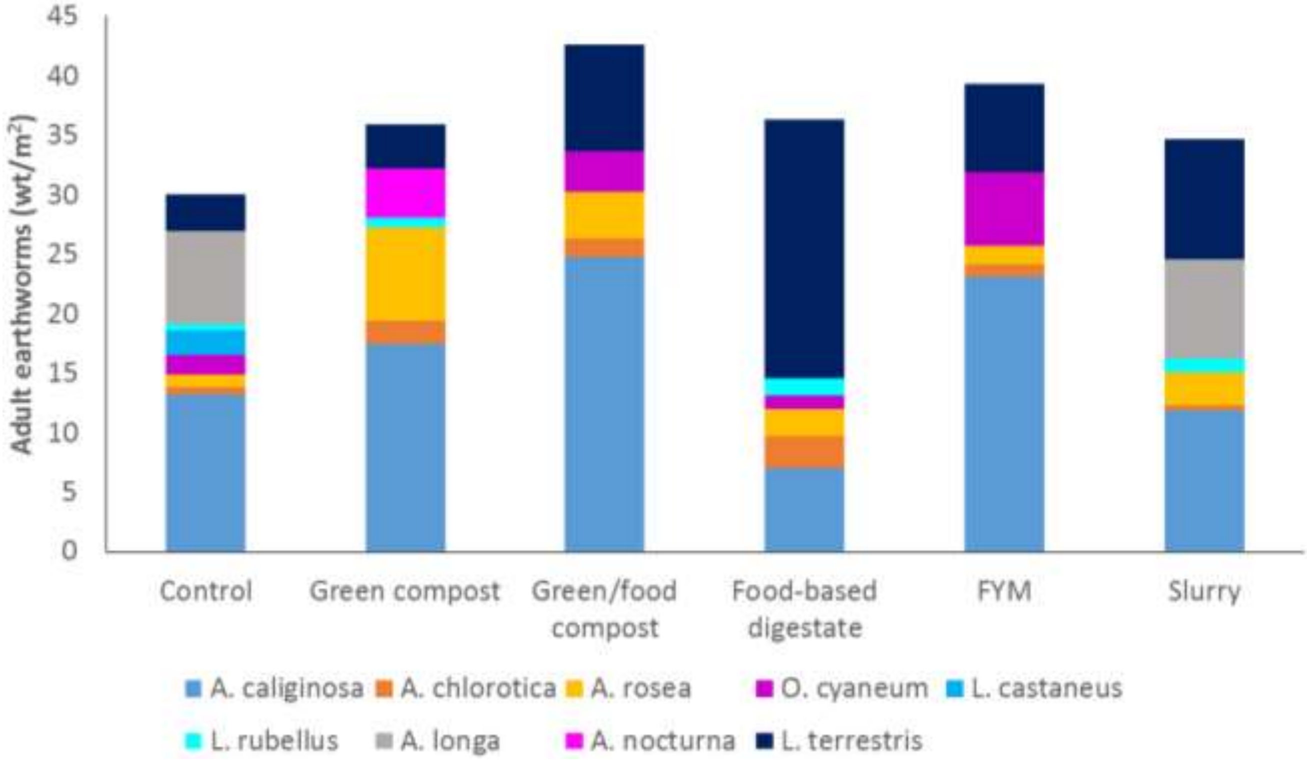


Figure 13: Lampeter: species composition of adult earthworms (wt)

Table 13. Lampeter: Adult earthworm species (no/m^2)

Treatment	***Endogeic earthworms***					***Epigeic earthworms***			***Anecic earthworms***			
	A. caliginosa	***A. chlorotica***	***A. rosea***	***O. cyaneum***	***Total***	***L. castaneus***	***L. rubellus***	***Total***	***A. longa***	***A. nocturna***	***L. terrestris***	***Total***
Control	27	2	4	1	**35**	1	1	**2**	4	0	1	**5**
Green compost	21	9	16	0	**46**	0	2	**2**	0	2	1	**4**
Green/food compost	41	6	16	2	**65**	0	0	**0**	0	0	4	**4**
Food-based digestate	20	10	2	1	**33**	0	1	**1**	0	0	7	**7**
FYM	53	4	6	5	**68**	0	0	**0**	0	0	2	**2**
Slurry	25	1	9	0	**35**	0	4	**4**	4	0	4	**7**

No significant treatment effects for any of the species as determined using the Friedman's test; $P>0.1$.

Table 14. Lampeter: Adult earthworm species (wt/m^2)

Treatment	***Endogeic earthworms***					***Epigeic earthworms***			***Anecic earthworms***			
	A. caliginosa	***A. chlorotica***	***A. rosea***	***O. cyaneum***	***Total***	***L. castaneus***	***L. rubellus***	***Total***	***A. longa***	***A. nocturna***	***L. terrestris***	***Total***
Control	13	0.59	1	2	**17**	2	0.57	**3**	8	0	3	**11**
Green compost	17	2	8	0	**27**	0	0.80	**1**	0	4	4	**8**
Green/food compost	25	2	4	3	**34**	0	0	**0**	0	0	9	**9**
Food-based digestate	7	3	2	1	**13**	0	1	**1**	0	0	22	**22**
FYM	23	1	2	6	**32**	0	0	**0**	0	0	7	**7**
Slurry	12	0.37	3	0	**15**	0	1	**1**	8	0	10	**18**

No significant treatment effects for any of the species as determined using the Friedman's test; $P>0.1$.

2.2.4 Terrington

Table 15. Terrington: mean number of earthworms/m^2

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	121	27	26	0	1	94
Green compost	193	79	78	0	1	114
Green/food compost	101	26	25	0	1	75
Food-based digestate	148	60	60	0	0	88
FYM	200	47	43	0	4	153
Slurry	219	79	74	1	4	140
P value[1]	NS (0.10)	NS (0.10)	NS (0.11)	ND	NS (0.43)	NS (0.35)

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05); ND = not determined due to insufficient data

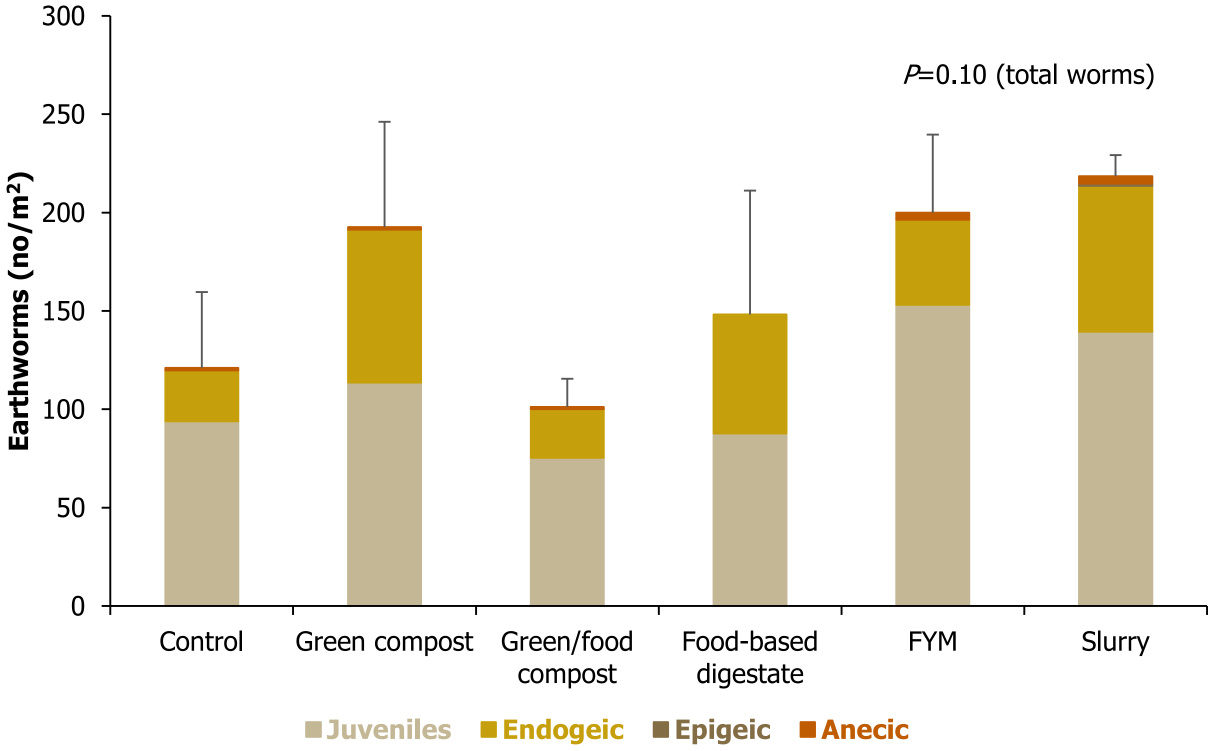


Figure 14. Terrington: mean number of earthworms/m^2

Table 16. Terrington: mean weight of earthworms g/m^2

Treatment	Total earthworms	Adult earthworms				Juvenile earthworms
		Total	Endogeic	Epigeic	Anecic	
Control	21.1	8.09	6.54	0	1.54	13.1
Green compost	42.3	23.1	20.8	0	2.31	19.3
Green/food compost	20.4	8.68	6.81	0	1.87	11.7
Food-based digestate	30.6	14.8	14.8	0	0.00	15.8
FYM	47.8	18.7	11.7	0	7.03	29.1
Slurry	48.2	25.8	20.1	0.29	5.44	22.4
P value[1]	NS (0.10)	NS (0.06)	NS (0.10)	ND	NS (0.54)	NS (0.28)

[1]Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level; NS = not significant (*P*>0.05); ND = not determined due to insufficient data

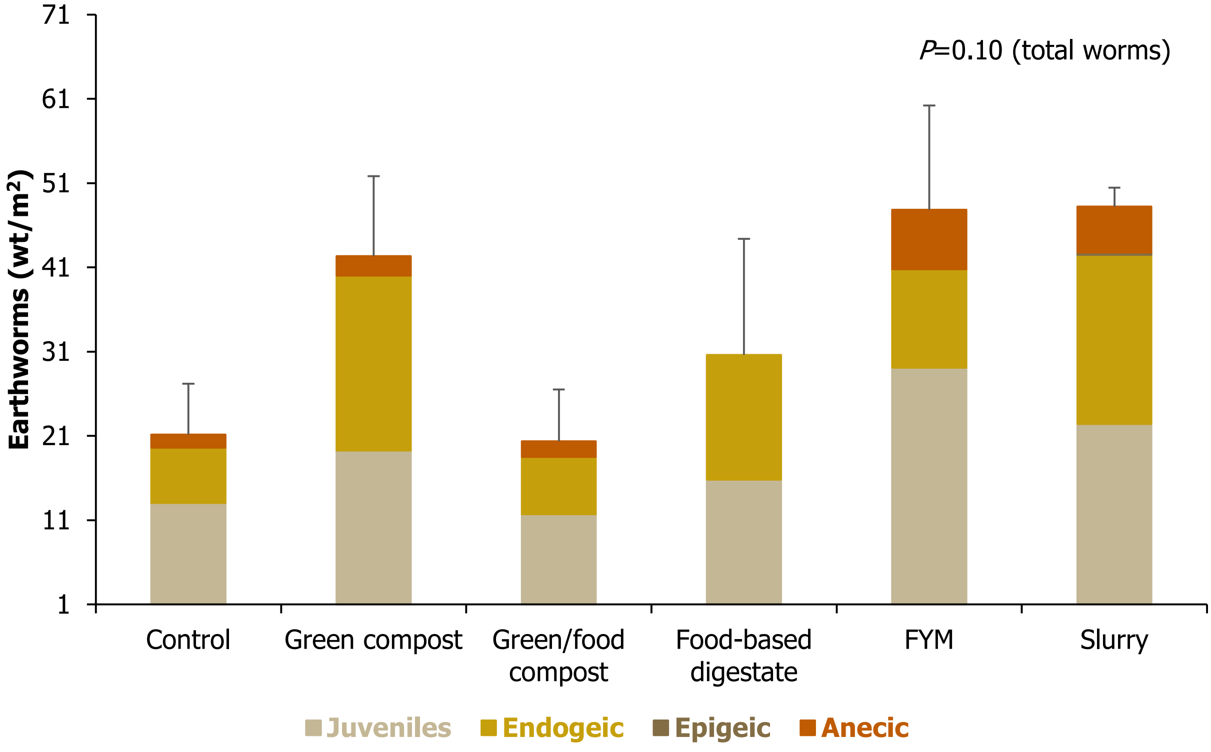


Figure 15. Terrington: mean weight of earthworms g/m^2

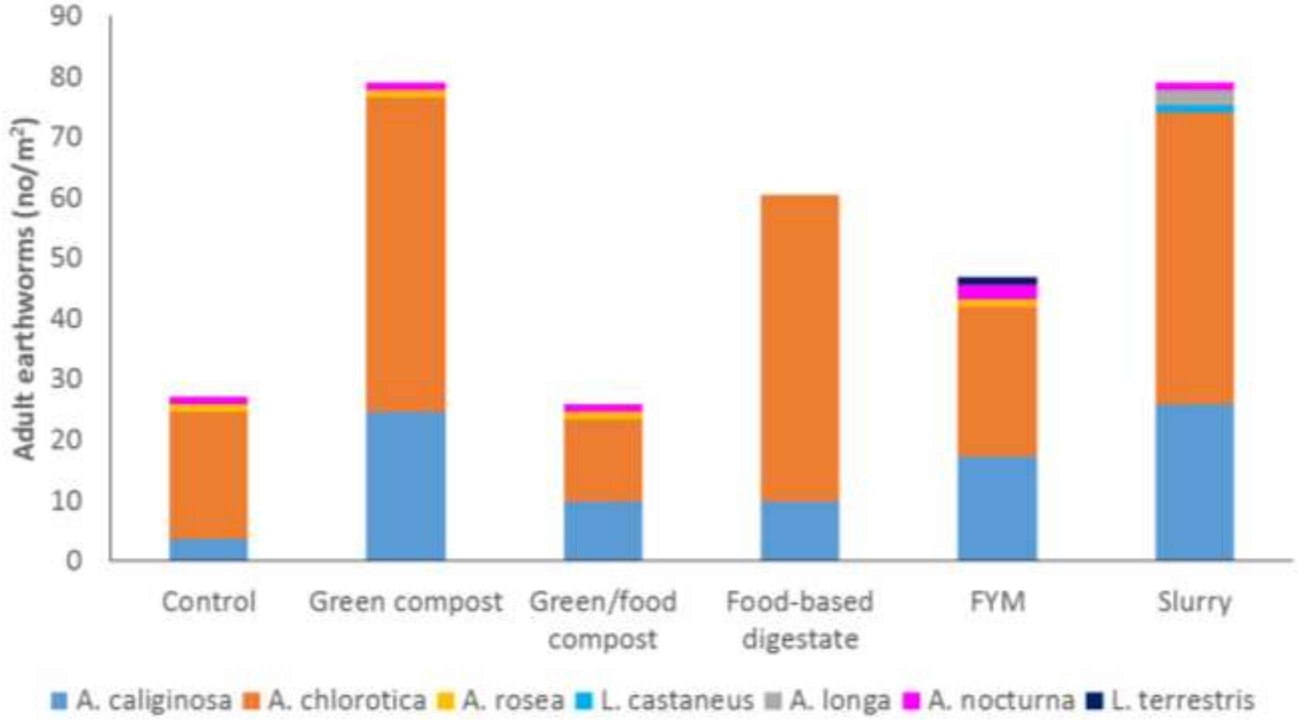


Figure 16. Terrington: species composition of adult earthworms (no)

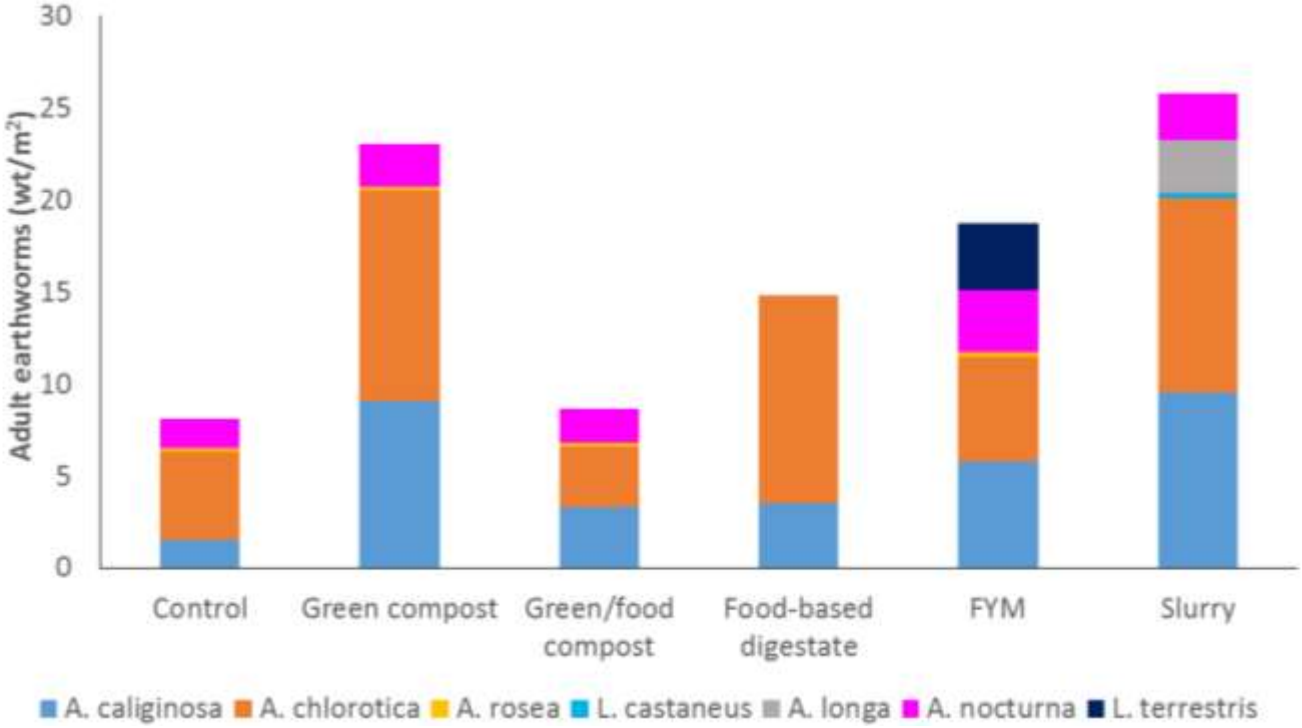


Figure 17. Terrington: species composition of adult earthworms (wt)

Table 17. Terrington: Adult earthworm species (no/m^2)

Treatment	*Endogeic earthworms*				*Epigeic earthworms*		*Anecic earthworms*			
	A. caliginosa	***A. chlorotica***	***A. rosea***	***Total***	***L. castaneus***	***Total***	***A. longa***	***A. nocturna***	***L. terrestris***	***Total***
Control	4	21	1	**26**	0	**0**	0	1	0	**1**
Green compost	25	52	1	**78**	0	**0**	0	1	0	**1**
Green/food compost	10	14	1	**25**	0	**0**	0	1	0	**1**
Food-based digestate	10	51	0	**60**	0	**0**	0	0	0	**0**
FYM	17	25	1	**43**	0	**0**	0	2	1	**4**
Slurry	26	48	0	**74**	1	**1**	2	1	0	**4**

No significant treatment effects for any of the species as determined using the Friedman's test; $P>0.1$.

Table 18. Terrington: Adult earthworm species (no/m^2)

Treatment	*Endogeic*				*Epigeic*		*Anecic*			
	A. caliginosa	***A. chlorotica***	***A. rosea***	***Total***	***L. castaneus***	***Total***	***A. longa***	***A. nocturna***	***L. terrestris***	***Total***
Control	2	5	0.26	**7**	0	**0**	0	2	0	**2**
Green compost	9	11	0.27	**21**	0	**0**	0	2	0	**2**
Green/food compost	3	3	0.21	**7**	0	**0**	0	2	0	**2**
Food-based digestate	4	11	0.00	**15**	0	**0**	0	0	0	**0**
FYM	6	6	0.27	**12**	0	**0**	0	3	4	**7**
Slurry	10	11	0.00	**20**	0.29	**0.29**	3	3	0	**5**

No significant treatment effects for any of the species as determined using the Friedman's test; $P>0.1$.

2.3 Key results and conclusions

At the Ayr site, there was no statistically significant effect (P=0.13) of the organic material additions (last applied in autumn 2012) on total earthworm numbers in autumn 2014, although earthworm numbers were lower on the food-based digestate treatment (Figure 2). However, total earthworm biomass remained lower where food-based digestate had been applied (P=0.04 for biomass only), relative to the fertiliser control two years after the last food-based digestate application; largely as a result of a reduction in the biomass of juvenile earthworms (P=0.01; Figure 3).

Similarly, at the Faringdon arable site, earthworm numbers were numerically lower where food digestate had been applied (Figure 6), although there was no statistical difference between the food-based digestate and the fertiliser control (only between the food-based digestate and the livestock manures for both number and biomass, P<0.05 by Duncan's analysis). Here, earthworm numbers and biomass were highest on the FYM treatment due to an increase in the juvenile population of shallow and surface dwelling species (P=0.06 for numbers and P=0.05 for biomass in the conventional ANOVA of the juvenile population).

At the other grassland site (Lampeter) there were no differences in earthworm numbers or biomass (Figures 10 & 11) as a result of the organic material treatments. Similarly there was no difference in earthworm numbers or biomass on the arable soil at Terrington (Figures 14 & 15).

There were no statistical differences (P>0.05, where there was sufficient data to enable statistical analysis) between the numbers or biomass of different functional groups (i.e. Endogeic, Epigeic and Anecic) due to the organic material additions at any of the sites. Moreover, there were no obvious or consistent differences in the species composition, with no statistical differences (P>0.1 using Friedman's non-parametric test) between the numbers or biomass of different earthworm species as a result of the organic material additions at any of the sites.

APPENDIX 5. Earthworm studies: Phase II laboratory studies

Project code: OMK001-001 (WRAP)/WR1212 (Defra)

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Executive summary

Field studies exploring the effect of repeated digestate and compost additions on crop and soil quality showed that there were differences in earthworm numbers following application of food-based digestate for three consecutive seasons in comparison with other treatments at four out of seven experimental sites in spring 2013. Earthworm numbers were lower following food-based digestate application relative to the fertiliser control at one site (Ayr) and relative to the other organic materials at the other 3 sites. This effect was still apparent at one of the sites (the grassland at Ayr) 18 months later, where numbers of surface and shallow dwelling juvenile earthworms were lower on the food-based digestate treatment. A literature review highlighted a number of possible factors that could have been responsible for the observed results i.e. ammonium-N, pH, electrical conductivity (EC), biochemical oxygen demand (BOD) and volatile fatty acid (VFA) concentration. A programme of laboratory experiments was therefore undertaken to confirm whether or not these factor(s) were responsible for the effects seen on earthworms in the field experiments following applications of food-based digestate. The laboratory experiments included detailed characterisation of different food-based digestates and cattle slurries, contact tests and pot experiments.

Contact tests showed that there was very little effect of conductivity (assessed using potassium and sodium chloride solutions) on earthworm mortality and health (i.e. sub-lethal effects on behaviour and weight). The BOD of a range of digestates was also lower than cattle slurry. These results ruled out conductivity and BOD as causal factors for the effects on earthworms following food-based digestate applications in the field experiments. Contact tests showed that VFAs (assessed using acetic acid and propionic acid) did cause an increase in earthworm mortality and deterioration in health, but only at concentrations in excess of 4500 mg/l. Additional laboratory pot studies using digestate (containing 1000 mg/l acetic acid) increased earthworm mortality, however, amending this digestate with increasing concentrations of acetic acid (2500-5000 mg/l) had no further effect on earthworm weight or mortality, indicating that the initial mortality was not due to the digestate's acetic acid content, but rather some other property of the digestate. In the light of these results and the fact that digestate VFA concentrations are typically considerably lower than 5000 mg/l acetic acid equivalents, VFAs were also ruled out as a causal factor for the effects on earthworms seen in the field.

Ammonium-N was found to have a significant effect on earthworm survival and health, both in the contact tests and pot studies. However, there was only a marginal effect of pH, with mortality and health in the contact tests slightly improved at the lower pHs (i.e. when a greater proportion of the applied N was in the ammonium-N form, rather than the ammonia form). In the pot studies, the pH of the digestate did not have a consistent statistical effect on the earthworms although there was a numeric decrease in mortality and increase in weight gain at the lower pH. This suggested the form of ammonium-N (i.e. whether it is predominantly as ammonium at the lower pH, or ammonia at the higher pH) might not be an important factor. Statistical analysis of the results from the pot experiments showed that the total ammonium-N loading most strongly explained the negative effects observed (which were a function of both the ammonium-N concentration and the application rate). Therefore any guidance should focus on ammonium-N loadings as a potential mitigation measure to ensure food-based digestate applications do not unduly effect earthworm populations.

The laboratory experiments undertaken were valuable in understanding the causal factors and the effects of food-based digestate on earthworm mortality and health. However, due to the worst-case nature of the pot studies and particularly contact tests and the fact they do not accurately simulate conditions in the field, it is not possible to derive a maximum ammonium-N loading.

1 Introduction

In autumn 2010, seven field experimental sites were established in contrasting agroclimatic zones at Aberdeen, Ayr, Devizes, Faringdon, Harper Adams, Lampeter and Terrington (Table 1). The sites aimed to quantify the effects of repeated compost and digestate applications, in comparison with farmyard manure and livestock slurry (as comparator materials), on soil biological, physical and chemical properties and crop quality. A fertiliser-only control treatment that received manufactured fertiliser additions as recommended in Defra's "Fertiliser Manual (RB209)" in England and Wales, and as recommended in "SRUC Technical Notes" in Scotland was also included. The organic material treatments received additional manufactured fertiliser N additions to help ensure, as far as practically possible, that crop yields and residue returns were the same in all treatments.

Table 1. Characteristics and cropping at the soil and crop quality experimental platforms

Site		Soil textural group		Annual rainfall (mm)	Cropping rotation+		
		Cross-compliance soil group	% clay		2010-11	2011-12	2012-13
1	Aberdeen	Sandy/light	16	790	SB	WB	WOSR
2	Ayr	Medium	19	1,190	G	G	G
3	Devizes	Chalk	20	850	Lin	WW	WW
4	Faringdon	Heavy	62	830	WW	WW	WC
5	Harper Adams	Sandy/light	11	690	POT	SB	WW
6	Lampeter	Medium	26	980	G	G	G
7	Terrington	Medium (heavy)	28	630	WW	WW	WOSR

+ SB = spring barley; WB = winter barley; WOSR = winter oilseed rape; WW = winter wheat; G = grassland; POT = potatoes; Lin = linseed; WC = whole crop oats/peas.

2 Previous earthworm assessment results

As part of a wide range of scheduled soil quality assessments, earthworm populations (numbers and biomass) were measured in spring 2013 at the seven sites. The measurements were made at least six months after the last (of at least three) organic material applications and found:

- There were treatment differences in earthworm numbers at 4 sites - Ayr, Faringdon, Lampeter and Terrington ($P<0.05$), but not at Aberdeen, Devizes or Harper Adams ($P>0.05$).
- At Ayr, earthworm *numbers* were lower on the food-based digestate treatment in comparison with all the other treatments ($P<0.01$). Notably, Ayr had the greatest number of earthworms of all the sites.
- Earthworm *numbers* were also *lower* on the food-based digestate treatment in comparison with the FYM and slurry treatments at Faringdon; the FYM, slurry and green/food compost treatments at Lampeter; and the FYM treatment at Terrington ($P<0.05$). Additionally, there was a lower ($P<0.05$) earthworm biomass on the food-based digestate treatments at Faringdon in comparison to the FYM and slurry treatments and at Terrington in comparison to the FYM treatment.

Additional qualitative evidence of an impact of food-based digestate application on earthworm populations was observed at three additional (grassland) experimental sites established in September 2013 to investigate shallow-injection as a potential method of reducing ammonia emissions from food-based digestate applications to grassland (Beith, Aberaeron and Newark). Note: this was a lower application rate (c.30 m^3/ha; 160 kg N/ha ammonium-N) than used on average at the seven soil quality sites (c.50 m^3/ha; c.190 kg/ha ammonium-N) where earthworm population responses were measured.

At the Beith (Scotland) site, it was noted that there were dead earthworms (most probably shallow dwelling endogeic species) on the surface immediately (within 90 minutes) following the autumn 2013 digestate application. No dead earthworms were observed on the cattle slurry treatment. *Note:* earthworm assessments were not scheduled at Beith so no measurements (count or biomass) were taken. Following the organic material applications at the Aberaeron site (Wales), dead earthworms were also observed immediately after application. At the third experimental site (Newark, England), no earthworms were observed on the surface following any of the treatments. Notably, it was very dry when the organic materials were applied at Newark and as a result earthworm were likely to be deeper in the soil. At Aberaeron, dead earthworm numbers were measured from three quadrats per plot (from all application techniques – i.e. shallow injection, broadcast or trailing shoe). The highest number of dead earthworms were measured on the food-based digestate treatments (8/m^2), followed by the cattle slurry treatments (2/m^2), with no dead earthworms on the surface of the untreated control.

Following these results, a review of the scientific and grey literature was undertaken to investigate the impact of livestock manures, biosolids, compost and digestate on earthworm populations and biomass; to contextualise the earthworm data from the *DC-Agri* field experiments. Additionally, the review examined the wider *DC-Agri* data to determine if there were any factors which may have been responsible for the observed effects on earthworms.

Based on data from the scientific literature and the *DC-Agri* experiments it was not possible to identify unequivocally the causal factor, or factors, responsible for the lower earthworm populations/biomass on the food-based digestate treatments compared with the other treatments (including the fertiliser-only control). The review identified that a number of factors (in particular, ammonium-N, pH and EC) could have been responsible for the lower earthworm populations on the food-based digestate treatments (Table 2).

Table 2. Likelihood of food-based digestate properties being responsible for effects observed on earthworm populations

Food-based digestate properties	Likelihood of effect	Underpinning rationale
Ammonium-N	Probable	Earthworms are known to be sensitive to ammonium (ammonia)-N; either in liquid and/or gaseous forms. Based on the available scientific literature, it is difficult to precisely identify a 'threshold' ammonium-N addition rate, however, applications >100 kg ammonium-N/ha have commonly been related to negative effects on earthworms.
pH	Possible (in conjunction with ammonium-N)	Earthworms are known to be sensitive to ammonium (ammonia)-N; either in liquid and/or gaseous forms. The higher pH of food-based digestate compared with cattle slurry will result in a greater proportion of ammonium-N being present as ammonia-N.
Electrical conductivity (EC) (i.e. salt effects)	Possible	Earthworms are known to be sensitive to exposure to salts (i.e. desiccation effects). Food-based digestate typically has a higher conductivity than cattle slurry.
Volatile Fatty Acids (VFAs)	Unknown	Food-based digestate typically has a higher VFA content than cattle slurry.
Biochemical Oxygen Demand (BOD)	Unlikely	Earthworms are known to be sensitive to anaerobic/waterlogged soil conditions (i.e. oxygen depleted environments). Food-based digestate typically has a lower BOD than cattle slurry.

To assess any longer-term effects of organic material applications on earthworm populations/biomass and communities, earthworm numbers (adults and juveniles), biomass and species were measured in autumn 2014 (two years after the last organic material applications and eighteen months since the previous assessments). The measurements were undertaken at four of the existing sites, where a *significant effect* ($P<0.05$) of food-based digestate application on earthworm numbers/biomass was previously noted in comparison to the fertiliser control and other organic material treatments (i.e. Ayr, Faringdon, Lampeter and Terrington). Relative to the fertiliser only control treatment, earthworm biomass was still reduced where food-based digestate had been applied at Ayr ($P<0.05$) and there was a numerical (but not statistically significant) reduction in earthworm numbers. The differences were confined to the surface and shallow dwelling juvenile earthworms. There was also evidence of a similar effect at Faringdon ($P<0.05$), but only for earthworm numbers. The previously observed differences in earthworm populations across the organic material treatments at Lampeter and Terrington were no longer apparent.

Based on the results from the original sampling, the literature review and the re-sampling, a programme of laboratory experiments was undertaken to determine what effect food-based digestate has on earthworm survival and which digestate properties are causing the effect on earthworms. These laboratory tests included detailed characterisation of different food-based digestates and cattle slurries, screening assessments and pot experiments.

3 Screening assessments

The screening assessments used short-term contact tests to identify acute effects, and assess and score early sub-lethal effects (e.g. behavioural responses) on earthworms. This involved exposing earthworms to test substances (e.g. food-based digestates and laboratory solutions) in order to identify which substances within digestates were potentially toxic to earthworms in soil. However, contact tests were unsuitable for assessing BOD, so a detailed laboratory analysis of food-based digestate and cattle slurry BOD was also undertaken (in addition to other determinands).

3.1 Laboratory analysis of organic materials

Due to there being limited publically available data on the chemical properties of food-based digestates (e.g. EC, BOD and VFAs), ten digestates and ten cattle slurries with varying chemical characteristics (i.e. ammonium, pH, EC, VFAs and BOD) were analysed. Where possible the digestates (and cattle slurry) were selected based on chemical analysis data provided by the AD operator, and analysis undertaken previously as part of *DC-Agri* or based on the type of feedstock processed (i.e. five largely processing municipal food waste and five processing commercial/industrial food waste). Once collected by ADAS, all the organic materials were stored at *c.*3°C to minimise any changes in their chemical properties before being submitted for laboratory analysis (within 2 weeks of sampling). Samples were not frozen in order to prevent changes in composition (particularly nitrogen) that can occur when frozen samples are defrosted.

The twenty organic materials were analysed for EC, pH, ammonium-N, BOD and VFAs, Table 3. In addition, each sample was analysed for BOD on a daily basis over a five day period (*note*: a standard BOD test is the cumulative oxygen demand over a five day period). This was to determine if there is any difference in the oxygen requirement immediately (and shortly) after application for food-based digestate and cattle slurry.

Table 3. Food-based digestate and cattle slurry: conductivity, pH, ammonium-N, biochemical oxygen demand and volatile fatty acids mean, minimum and maximum concentrations (n=10)

Determinand	Unit+	Food-based digestate			Cattle slurry		
		Mean	Minimum	Maximum	Mean	Minimum	Maximum
Conductivity (@20°C)	µS/cm	5,480	3,920	6,520	2,910	1,000	4,420
pH		7.6	7.3	7.9	7.1	6.9	7.2
Ammonium-N	kg/m^3	5.29	2.89	8.32	1.77	0.37	2.43
Biochemical oxygen demand	mg/l	7,510	5,580	10,250	13,290	3,780	28,580
Biochemical oxygen demand	mg/l (day 1)	20	< 2	80	570	40	1,380
	mg/l (day 2)	920	260	1,640	4,960	520	10,250
	mg/l (day 3)	2,390	1,040	4,130	5,600	620	11,760
	mg/l (day 4)	3,520	1,120	6,560	6,020	680	12,680
	mg/l (day 5)	4,160	1,180	8,080	7,070	750	14,080
Volatile fatty acids							
N-caproic acid	mg/l	< 20	< 20	< 20	< 20	< 20	< 20
Acetic acid	mg/l	2,682*	712	9,380	4,380	1,670	6,270
Propionic acid	mg/l	260	60	800	1,350	600	2,440
Iso-butyric acid	mg/l	110	30	350	130	50	200
N-butyric acid	mg/l	90	10	330	720	240	1330
Iso-valeric acid	mg/l	< 25	< 25	< 25	80	< 25	120
N-valeric acid	mg/l	< 25	< 25	< 25	80	30	140
Iso-caproic acid	mg/l	< 20	< 20	< 20	< 20	< 20	< 20
Acetic acid equivalents	mg/l	3,030	790	10,490	6,150	2,400	8,540

+ µS/cm = microsiemens per centimetre; kg/m^3 = kilograms per cubic metre; mg/l = milligrams per litre

* a single sample is skewing the mean, the median value of 1,070 mg/l (not shown) maybe more representative. The maximum VFA values for food-based digestate were all from the same site.

Analysis of the organic material samples confirmed that ammonium-N and pH concentrations were greater in food-based digestate than cattle slurry, although the pH was lower than in the majority of other digestate samples tested (i.e. as part of the wider *DC-Agri* programme and other WRAP funded projects, where the pH of food-based digestate was *c.*8.5). EC levels were also higher in food-based digestate than in cattle slurry, whereas concentrations of all the VFAs were greater in cattle slurry than food-based digestate. However, there was more variation in VFA concentrations between the different food-based digestate samples, this is most likely a reflection of the variety of different AD feedstocks, processes and range of mean average retention times which impact on the stability and final composition of the digestates. The BOD of the food-based digestates tested were below those of the cattle slurries. Additionally, the daily BOD measurements for the cattle slurry samples were also higher than those of the food-based digestate samples, confirming that the BOD of digestate is no more immediate than that of cattle slurry.

3.2 Laboratory solution contact tests

Contact tests were an effective method to relatively quickly identify factors that could be relevant and to rule out factors which were unlikely to be affecting earthworm populations. However, they represented an extreme, worst-case situation in terms of exposure risk. The earthworms were in direct contact with the factor with no option of avoidance, the stress was maintained for 48 hours with no reduction in stress (due to dilution, volatilisation etc.); as such the results should be interpreted with these factors in mind.

3.2.1 Methodology

Standard laboratory prepared liquids (i.e. the chemical in question mixed with deionised water) with five different levels of the factor under investigation (based on the findings from the literature review) were investigated to determine their effect on earthworm survival, viz:

- Conductivity (i.e. potassium chloride and, separately, sodium chloride solution);
- Ammonium-N (i.e. ammonium sulphate solution) at pH 6.0;
- Ammonium-N (i.e. ammonium sulphate solution) at pH 9.0;
- VFAs (i.e. acetic acid and, separately, propionic acid solution); and

  Note: these VFAs were selected in conjunction with the project management group as they were present in food-based digestate and cattle slurry at the highest concentrations and were therefore thought most likely to potentially have an effect on earthworm survival.

- VFA and ammonium-N interactions (i.e. acetic acid and ammonium sulphate combined at varying concentration levels).

The methodology described below was adapted from OECD (1984), and comprised:

- A single earthworm was used for each replicate of the contact test.
  Note: It was not appropriate to use more than one earthworm because the death of one earthworm might have adverse effects on others in the same container.
- Juvenile *Lumbricus terrestris* was used for the contact tests.
  Note: *Lumbricus rubellus* was originally chosen as the test species based on the results from the re-sampling exercise at the field experiments, where *L. rubellus* was found at all the sites, plus the fact it is an opportunistic, epigeic species. However, it was not possible to source suitable *L. rubellus* for these experiments so juvenile *L. terrestris* was selected, in agreement with the project management group and their technical expert. Juvenile *L. terrestris* was selected as they were also a common species identified in the re-sampling exercise, plus juvenile *L. terrestris* have a similar surface area to volume ratio as *L. rubellus*, making them a suitable test species for these experiments.
- Batches of 200 earthworms were ordered throughout the testing period as required from the same supplier. This was to ensure tests undertaken later in the process gave the same results as those at the outset and so that results were not affected by the juvenile earthworms growing (i.e. their surface area to volume ratio changing) over the experimental period.
  Note: prior to the experiments all earthworms were stored in optimal conditions (i.e. earthworms were typically kept in groups of 100-125 in a 2 part compost to 1 part straw mix which was kept damp and stored at 5°C in the dark to ensure they were healthy ahead of the contact tests.
- Earthworms used in the experiments were selected to be as uniform in size as possible (based on visual observations) and any earthworms in sub-optimal condition were rejected

prior to experimentation. Earthworms were kept on moist filter paper for three hours to void their gut contents, washed, dried and weighed before being placed in the test dish.

- A standard 90mm petri dish was lined with medium grade filter paper between 80 to 85g/m^2 (approximately 0.2mm thick), cut to cover the bottom of the dish, but with no overlaps.
- Each petri dish was 'sealed' with a lid (tightly fitting but not sealed with tape) to prevent the loss of volatile compounds (e.g. ammonium and VFAs).
  Note: preliminary experiments were undertaken to confirm that earthworms were able to survive for at least 48 hours in the sealed petri dishes.
- There were ten replicates of each treatment and ten untreated controls (i.e. filter paper moistened with deionised water).
  Note: the OECD guidelines state that the mortality in the controls should not exceed 10%. If mortality levels exceeded 10% the test was repeated.
- The tests were undertaken at 20°C, in the dark and for a period of 48 hours.
- The earthworms were monitored continuously throughout the first hour, then assessed again after approximately 12, 18, 24, 36 and 48 hours.
- The effects on the earthworms were scored from 0 (i.e. no reaction) through to 5 (i.e. death), with sub-lethal effects on a scale between these end-points (Table 4).

Table 4: Scoring earthworm response

Score	Observation	Notes
1	No reaction	
2	Coiling or writhing	Avoidance response
3	Mucus production	Mitigation response
4	Blooding or swelling	Sub-lethal response
5	Death	No response to mechanical stimulus

- At the beginning and end of the test, or upon their death, the fresh weight of the earthworm was recorded.

3.2.2 Treatments

The list of test treatments and the different concentrations of laboratory liquids investigated are shown in Tables 5 & 6 below. The concentrations selected were based on typical and maximum concentrations measured in food-based digestates and cattle slurries (Table 3). The highest concentrations were designed to be higher than the maximum concentrations measured in the organic materials to act as a worst case assessment. For the ammonium test, the solutions were buffered to pH 6 and 9, whereas for the interaction test, the pH's varied depending on the concentration of acetic acid and ranged from pH 3 for acetic acid alone to pH 9 for ammonium-N alone, with the mixtures ranging from pH 4 (at 5000 mg/l acetic acid + 2 kg/m^3 ammonium-N) to pH 8 (at 500 mg/l acetic acid + 4 kg/m^3 Ammonium-N).

Table 5. Concentrations of laboratory liquids

Factor	Ammonium-N: ammonium sulphate (kg/m^3)		EC: potassium chloride (µS/cm)	EC: sodium chloride (µS/cm)	VFAs: acetic acid (mg/l)	Additional VFAs: acetic acid (mg/l)	VFAs: propionic acid (mg/l)
	pH 6	pH 9					
1	2	2	2,000	-	500	1,000	-
2	4	4	5,000	-	5,000	1,500	5,000
3	6	6	7,500	-	10,000	2,500	-
4	8	8	10,000	10,000	20,000	3,500	-
5	12	12	12,000	-	50,000	4,500	-

Table 6. Concentrations of acetic acid and ammonium-N laboratory liquids interaction tests

Factor	Acetic acid (mg/l)	Ammonium-N (kg/m^3)
1	500	-
2	2,500	-
3	5,000	-
4	-	2
5	-	3
6	-	4
7	500	2
8	500	3
9	500	4
10	2,500	2
11	2,500	3
12	2,500	4
13	5,000	2
14	5,000	3
15	5,000	4

3.2.3 Statistics

The change in earthworm fresh weight was assessed by analysis of variance (ANOVA) using GenStat (version 12.1, VSN International) with the assumption that initial starting weights were similar across the treatments. Where significant treatment differences in earthworm weight were measured, Duncan's multiple range test was used to compare the means from the ANOVA (different letters indicate a significant difference between treatments). The results from Duncan's test were shown on the tables of means where appropriate (i.e. where $P<0.05$).

The analysis of the score data was complicated by the fact that the scoring system was not on a linear scale i.e. the difference between a score of 1 and 2 was not the same as between 2 and 3, 3 and 4, or 4 and 5. This could result in tables of means which did not accurately reflect what was happening, particularly if there was a large range of scores within a treatment. For example, if half of the earthworms had a score of 1 (no reaction), and half had a score of 5 (death), this would give rise to a mean score of 3 (mucus production) despite the fact that no earthworms actually scored 3.

However, tables of mean scores have been presented in this report to provide a quick overview of the data (indicating the severity of the response), but should be analysed conscious of the comment above.

The score data was rearranged so that the proportion of worms within particular scoring categories could be analysed i.e.:

- Proportion of earthworms unaffected (score of 0)
- Proportion with sub-lethal responses (score 2-4) or
- Proportion dead (score of 5).

This changed the scores into binomial data, i.e. for each of the 3 categories, each earthworm either scored a 1 if it was in that category or 0 if it wasn't. For each category, the proportion of earthworms was compared using a generalised linear model (logistic regression model) in GenStat (version 12.1, VSN International). For each category, the analysis produced an output similar to a normal ANOVA, which tested whether there was significant differences in the percentage of earthworms affected for each of the treatments.

3.2.4 Results

Electrical conductivity

The EC assessments, using potassium chloride, had no effect on earthworm survival, Table 7.

Table 7. Earthworm response to increasing EC (potassium chloride); results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs
Control	1.0	1.0	1.3	1.3	1.3	1.3
2,000 µS/cm	1.0	1.0	1.0	1.0	1.0	1.0
5,000 µS/cm	1.0	1.0	1.0	1.0	1.0	1.0
7,500 µS/cm	1.0	1.0	1.3	1.3	1.3	1.3
10,000 µS/cm	1.0	1.0	1.0	1.0	1.0	1.0
12,000 µS/cm	1.0	1.4	1.0	1.0	1.0	1.4

Only five earthworms, of the sixty used as part of this test, showed any response (one of which was a control), the remainder showed no response to all conductivity levels. Of those that did respond, one earthworm died after forty-eight hours having shown no stress beforehand (at 12,000 µS/cm), two produced mucus after twelve hours, but showed no other response before or after (at 12,000 µS/cm) and two earthworms (one control and one 7,500 µS/cm) exhibited blooding (i.e. blood appeared on the filter papers) from eighteen hours onwards but showed no other responses. For full results see Appendix I.

Unlike the earthworm behavioural responses, the EC test solution did effect earthworm weight, Table 8.

Table 8. Earthworm response to increasing conductivity (potassium chloride); changes in earthworm weight.

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)[1]	Weight change (%)[*]	No. of earthworms alive at 48hrs
Control	1.53	1.32	0.215[ab]	-15	10 (out of 10)
2,000 µS/cm	1.29	1.22	0.075[a]	-6	10 (out of 10)
5,000 µS/cm	1.39	1.11	0.279[b]	-20	10 (out of 10)
7,500 µS/cm	1.49	1.10	0.388[bc]	-26	10 (out of 10)
10,000 µS/cm	1.48	1.02	0.464[c]	-32	10 (out of 10)
12,000 µS/cm	1.14	0.75	0.375[bc]	-34	9 (out of 10)
P value[+]	-	-	< 0.001		-

[*] Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

[+] Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

As per the results in Table 8 above, conductivity did reduce earthworm fresh weight, particularly at higher conductivity levels. It is likely this was a result of water loss from the earthworms, due to the osmotic potential of the high conductivity solutions. The significant reduction in earthworm weight however, is a very different response to that seen in the field experiments i.e. earthworm mortality. Additionally, in the contact tests the earthworms were placed on a filter paper soaked in high conductivity solution, they could not avoid the solution, whereas in the field environment earthworms would have the potential to avoid the liquid or even ameliorate the effects through mucus production (something which is more difficult in the extreme environment of the contact tests). Given these factors, it was thought unlikely that conductivity was the primary cause of the earthworm mortality observed in the field experiments.

Conductivity assessments using sodium chloride showed a similar effect to those observed with potassium chloride, Table 9, i.e. no effect on earthworm survival, suggesting that there were no salt effects on earthworms at the levels in food-based digestate. For full results see Appendix 4.1.

Table 9. Earthworm response to conductivity (sodium chloride); results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death**.**

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs
Control	1.0	1.0	1.0	1.0	1.0	1.4
10,000 µS/cm	1.0	1.0	1.0	1.0	1.0	1.0

As per the conductivity assessments with potassium chloride, there was an effect of sodium chloride on earthworm weight compared with the control at the concentration applied, Table 10. However, similarly to the potassium chloride contact test, this is thought likely to be an osmotic effect, the effect of which was likely enhanced due to the contact test methodology. Hence, salinity/conductivity is unlikely to be the primary cause of the earthworm mortality.

Table 10. Earthworm response to increasing conductivity (sodium chloride); changes in earthworm weight.

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)	Weight change (%)*	No. of earthworms alive at 48hrs
Control	1.34	0.89	0.446[a]	-33	10 (out of 10)
10,000 µS/cm	1.65	1.11	0.534[b]	-33	9 (out of 10)
P value+	-	-	0.013		-

* Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

+ Data analysed by analysis of variance (ANOVA), P value of <0.05 is significant at the 5% level; P value of <0.01 is significant at the 1% level and P value of <0.001 is significant at the 0.1% level.

[a,b,c,d] Different letters within a column indicate significant differences between treatments (P<0.05)

VFAs

The VFA acetic acid had a negative effect on earthworm survival at concentrations of 4,500 mg/l and above within 1 hour of the start of the test, Table 11.

Table 11. Earthworm response to increasing concentrations of acetic acid; results are an average of the individual response scores, with 1 = no reaction & 5 = death.

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs
Control	1.0	1.0	1.0	1.0	1.2	1.2
500 mg/l	1.0	1.0	1.0	1.2	1.0	1.0
1,000 mg/l	1.0	1.0	1.3	1.4	1.4	1.4
1,500 mg/l	1.0	1.0	1.0	1.0	1.0	1.0
2,500 mg/l	1.0	1.3	1.0	1.0	1.0	1.0
3,500 mg/l	1.0	1.0	1.0	1.0	1.6	1.3
4,500 mg/l	3.0	1.0	1.5	1.6	1.7	1.8
5,000 mg/l	2.7	1.4	2.0	2.4	2.7	3.0
10,000 mg/l	4.4	5.0	5.0	5.0	5.0	5.0
20,000 mg/l	5.0	5.0	5.0	5.0	5.0	5.0
50,000 mg/l	5.0	5.0	5.0	5.0	5.0	5.0

The control and lowest concentration of acetic acid (500 mg/l) had no effect on earthworm survival, but at 4,500 mg/l acetic acid and above there was a clear effect on earthworm survival within the first hour. At 5,000 mg/l of acetic acid six earthworms died, but the remainder exhibited blooding, thinning and disfigurement. At 10,000 mg/l of acetic acid and above there was rapid coiling or writhing followed immediately by death, only two earthworms lived beyond the first hour and they died within the first twelve hours.

The effect of acetic acid on earthworm weight was less clear, Table 12.

Table 12. Earthworm response to increasing acetic acid concentrations; changes in earthworm weight.

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)*	Weight change (%)	No. of earthworms alive at 48hrs
Control	1.54	1.21	0.322[a]	-21	19 (out of 20)
500 mg/l	1.39	1.09	0.300[a]	-23[b]	20 (out of 20)
1,000 mg/l	1.59	1.13	0.488[b]	-31	9 (out of 10)
1,500 mg/l	1.92	1.37	0.545[b]	-32	10 (out of 10)
2,500 mg/l	1.35	0.84	0.505[b]	-39	10 (out of 10)
3,500 mg/l	1.63	1.13	0.496[b]	-34	10 (out of 10)
4,500 mg/l	1.89	1.38	0.530[b]	-29	9 (out of 10)
5,000 mg/l	1.57	1.17	0.436[ab]	-25	11(out of 20)
10,000 mg/l	1.31	1.18	-	-	0 (out of 10)
20,000 mg/l	1.37	1.24	-	-	0 (out of 10)
50,000 mg/l	1.45	1.05	-	-	0 (out of 10)
P value+	-	-	<0.001		-

Note: at some concentrations there were 20 replicates rather than 10 as these concentrations were tested twice as part of the additional acetic acid contact tests.

* Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

+ Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

a,b,c,d Different letters within a column indicate significant differences between treatments (*P*<0.05)

The earthworm behavioural response data was converted into binomial data (see Section 3.2.3 for more details) i.e. for each of the 3 categories (unaffected, sub-lethal response and dead), each earthworm either scored a 1 if it was in that category or 0 if it wasn't. For each category, the proportion of earthworms was compared using a generalised linear model (logistic regression model) in GenStat (version 12.1, VSN International) which tested whether there was significant differences in the percentage of earthworms affected for each of the treatments and produced means for each treatment.

Table 13. Earthworm response to increasing acetic acid concentrations (% unaffected, showing a sub-lethal response or dead).

Time period	Response	Rate (mg/l)											*P*-value+
		Control	500	1000	1500	2500	3500	4500	5000	10000	20000	50000	
After 1 hour	% unaffected	100	100	100	100	100	100	0	0	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	100	95	20	0	0	<0.001
	% dead	0	0	0	0	0	0	0	5	80	100	100	<0.001
After 12 hours	% unaffected	100	100	100	100	90	100	100	90	0	0	0	<0.001
	% sub-lethal	0	0	0	0	10	0	0	0	0	0	0	0.865
	% dead	0	0	0	0	0	0	0	10	100	100	100	<0.001
After 18 hours	% unaffected	100	100	90	100	100	100	80	75	0	0	0	<0.001
	% sub-lethal	0	0	10	0	0	0	20	5	0	0	0	0.293
	% dead	0	0	0	0	0	0	0	20	100	100	100	<0.001
After 24 hours	% unaffected	100	95	90	100	100	100	80	60	0	0	0	<0.001
	% sub-lethal	0	5	0	0	0	0	20	20	0	0	0	0.062
	% dead	0	0	10	0	0	0	0	20	100	100	100	<0.001
After 36 hours	% unaffected	95	100	90	100	100	80	80	55	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	20	10	15	0	0	0	0.096
	% dead	5	0	10	0	0	0	10	30	100	100	100	<0.001
After 48 hours	% unaffected	95	100	90	100	100	90	80	50	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	10	0	5	0	0	0	0.77
	% dead	5	0	10	0	0	0	20	45	100	100	100	<0.001

+ Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

As acetic acid concentrations increased there was a slight increase in sub lethal effects and a significant increase in lethal effects. Displayed graphically, it is clear there was a step change in response from 4,500 mg acetic acid/l, Figure 1. Additionally, the figure highlights that acetic acid appeared to be fatal to earthworms or had no effect i.e. there was very little evidence of sub-lethal effects (except early on in the mid-range concentrations). This combined with the fact that acetic acid had an immediate effect on earthworms (Table 13), so matching the anecdotal field evidence (WP2.3 the application techniques studies), suggested that acetic acid was potentially a factor in the reduction in earthworm numbers seen at the field experimental sites.

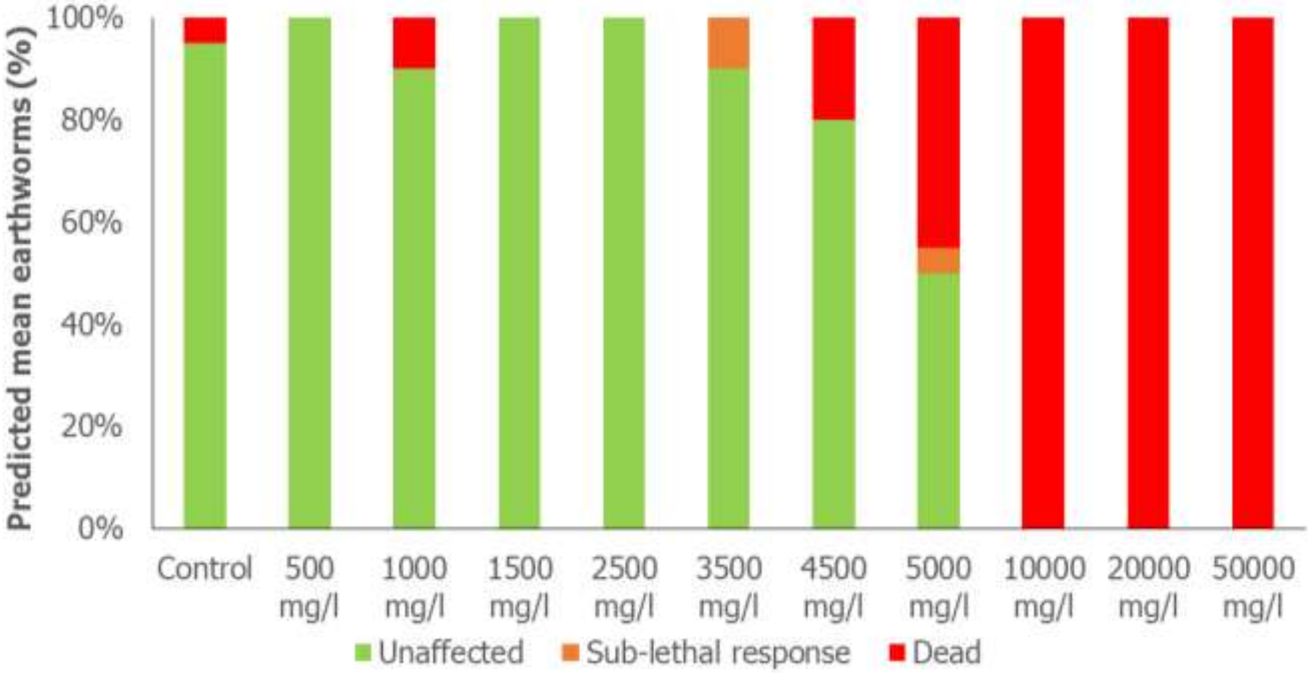

Figure 1. Earthworm response to acetic acid concentrations after 48 hours.

The propionic acid laboratory solution had no effect on earthworm survival, Table 14.

Table 14. Earthworm response to propionic acid; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs
Control	1.0	1.0	1.0	1.0	1.0	1.4
5,000 mg/l	1.0	1.3	1.0	1.0	1.0	1.7

Unlike the earthworm behavioural responses, the propionic acid solutions effected earthworm weight, Table 15.

Table 15. Earthworm response to propionic acid; changes in earthworm weight.

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)	Weight change (%)*	No. of earthworms alive at 48hrs
Control	1.34	0.89	0.448[a]	-33	9 (out of 10)
5,000 mg/l	1.69	1.16	0.518[b]	-32	9 (out of 10)
P value+	-	-	< 0.001		-

* Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

\+ Data analysed by analysis of variance (ANOVA), P value of <0.05 is significant at the 5% level; P value of <0.01 is significant at the 1% level and P value of <0.001 is significant at the 0.1% level.

a,b,c,d Different letters within a column indicate significant differences between treatments (P<0.05)

As per the results in Table 15, propionic acid reduced earthworm weight. The significant reduction in earthworm weight however, was a very different response to that seen in the field experiments, i.e. earthworm mortality, therefore, it is unlikely that propionic acid was the cause of the earthworm mortality seen in the field experiments.

Ammonium-N – pH6

Ammonium-N at pH 6 had a negative effect on earthworm survival at concentrations of 6 kg/m^3 and above and sub lethal effects were observed at 4 kg/m^3 of ammonium-N, Table 16.

Table 16. Earthworm response to increasing ammonium-N concentrations at pH 6; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs
Control	1.0	1.0	1.0	1.0	1.0	1.0
2 kg/m^3	1.0	1.0	1.0	1.0	1.0	1.0
4 kg/m^3	1.6	1.4	1.2	2.0	2.0	2.0
6 kg/m^3	3.2	4.4	4.8	4.8	4.8	4.8
8 kg/m^3	4.8	5.0	5.0	5.0	5.0	5.0
12 kg/m^3	5.0	5.0	5.0	5.0	5.0	5.0

The control and lowest concentration of ammonium-N (2 kg/m^3) had no effect on earthworm survival, with some effects at 4 kg/m^3 of ammonium-N, namely two earthworms died with many others exhibiting avoidance responses (writhing and coiling). At 6 kg/m^3 of ammonium-N only one earthworm survived, the remainder all died; two within an hour, five within twelve hours and two after eighteen hours. All earthworms exhibited avoidance responses, blooding and mucus production, as well as swelling and disfigurement. From, 8 kg/m^3 of ammonium-N and above only one earthworm survived for more than an hour (and died before 12 hours), with lots of rapid avoidance behaviour and mucus production prior to death. For full results see Appendix 4.2.

At the lower ammonium-N concentrations (2 & 4 kg/m^3) where some sub-lethal effects were exhibited, there was a significant effect on earthworm weight, Table 17. Due to the almost complete mortality from 6 kg/m^3 onwards, it was not possible to investigate the effect on earthworm weight at higher ammonium-N concentrations.

Table 17. Earthworm response to increasing ammonium-N concentration at pH 6; changes in earthworm weight**.**

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)	Weight change (%)*	No. of earthworms alive at 48hrs
Control	1.31	1.25	0.06[a]	-4	10 (out of 10)
2 kg/m^3	1.50	1.18	0.32[b]	-22	10 (out of 10)
4 kg/m^3	1.43	0.93	0.51[c]	-36	9 (out of 10)
6 kg/m^3	1.43	0.91		-	1 (out of 10)
8 kg/m^3	1.64	1.27		-	0 (out of 10)
12 kg/m^3	1.70	1.32		-	0 (out of 10)
P value+	-	-	< 0.001		-

Note: at some concentrations there were 20 replicates rather than 10 as these concentrations were tested twice as part of the additional acetic acid contact tests.

* Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

+ Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

a,b,c,d Different letters within a column indicate significant differences between treatments (P<0.05)

The behavioural response (unaffected, sub-lethal and dead categories) results for ammonium-N at pH 6 are shown in Table 18 below.

Table 18. Earthworm response to ammonium-N concentrations at pH 6 (% unaffected, showing a sub-lethal response or dead).

Time period	Response	Rate (kg/m^3)						*p-value*+
		Control	2	4	6	8	12	
After 1 hour	% unaffected	100	100	70	0	0	0	<0.001
	% sub-lethal	0	0	30	90	10	0	<0.001
	% dead	0	0	0	10	90	100	<0.001
After 12 hours	% unaffected	100	100	80	0	0	0	<0.001
	% sub-lethal	0	0	20	30	0	0	0.032
	% dead	0	0	0	70	100	100	<0.001
After 18 hours	% unaffected	100	100	80	0	0	0	<0.001
	% sub-lethal	0	0	20	10	0	0	NS (0.20)
	% dead	0	0	0	90	100	100	<0.001
After 24 hours	% unaffected	100	100	70	0	0	0	<0.001
	% sub-lethal	0	0	10	10	0	0	NS (0.47)
	% dead	0	0	20	90	100	100	<0.001
After 36 hours	% unaffected	100	100	70	0	0	0	<0.001
	% sub-lethal	0	0	10	10	0	0	NS (0.47)
	% dead	0	0	20	90	100	100	<0.001
After 48 hours	% unaffected	100	100	70	0	0	0	<0.001
	% sub-lethal	0	0	10	10	0	0	NS (0.47)
	% dead	0	0	20	90	100	100	<0.001

+ Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

From 4 kg/m^3 and above, there was an increase in sub lethal effects and a significant increase in lethal effects (Table 18). Displayed graphically (Figure 2), it is clear that there was a step change in response at around 4 kg/m^3. Additionally, the figure highlights that after 48 hours of exposure, ammonium-N at pH 6 appeared to be fatal to earthworms or had no effect i.e. there was only little evidence of sub-lethal effects. These results suggested that ammonium-N was a potential factor in the reduction in earthworm numbers seen at the field experimental sites.

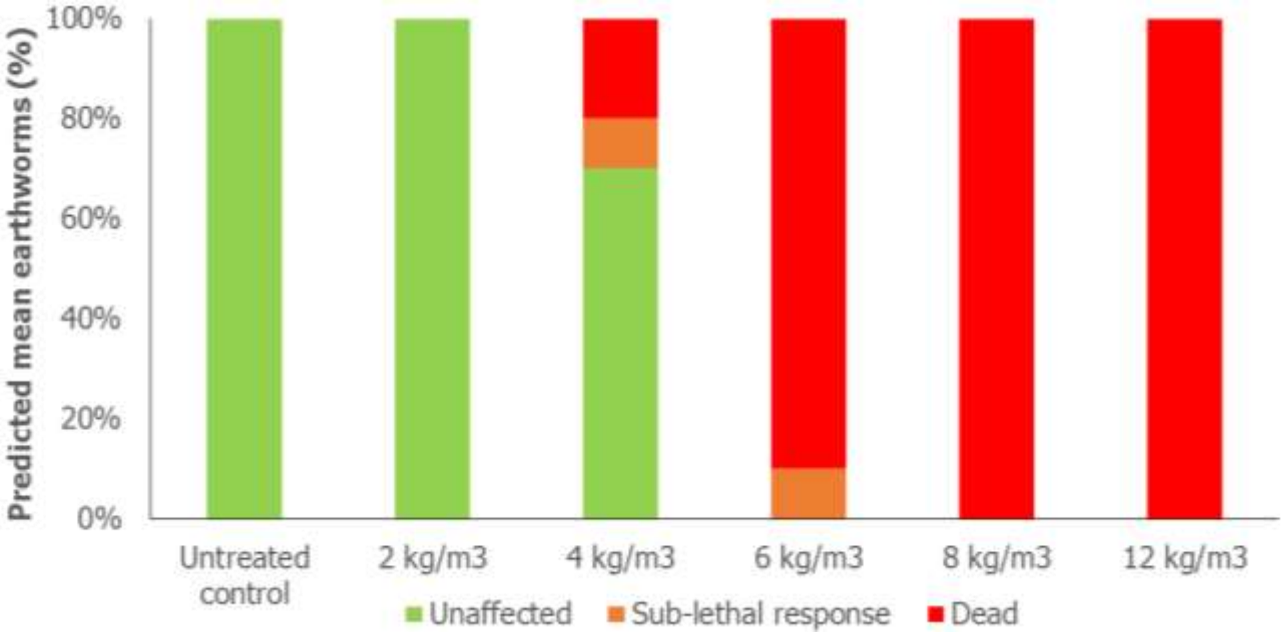

Figure 2. Earthworm response to ammonium-N concentrations at pH 6 after 48 hours.

Ammonium-N – pH9

Ammonium-N at pH 9 had a negative effect on earthworm survival at concentrations of 6 kg/m^3 and above, with sub lethal effects observed from 2 kg/m^3 of ammonium-N and severe sub lethal effects from 4 kg/m^3, Table 19.

Table 19. Earthworm response to increasing ammonium-N concentrations at pH 9; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death**.**

Level	**Score <1hr**	**Score <12hrs**	**Score <18hrs**	**Score <24hrs**	**Score <36hrs**	**Score <48hrs**
Control	1.0	1.0	1.0	1.0	1.0	1.0
2 kg/m^3	1.0	1.0	1.0	1.3	1.8	1.8
4 kg/m^3	2.4	3.0	3.0	3.4	3.4	3.8
6 kg/m^3	5.0	5.0	5.0	5.0	5.0	5.0
8 kg/m^3	5.0	5.0	5.0	5.0	5.0	5.0
12 kg/m^3	5.0	5.0	5.0	5.0	5.0	5.0

The control and lowest concentration of ammonium-N (2 kg/m^3) had no effect on earthworm survival, but from 4 kg/m^3 of ammonium and above there was a severe effect. At 4 kg/m^3 of ammonium-N, seven of the earthworms died having shown avoidance responses (writhing and coiling) as well as mucus production, blooding and swelling. Surprisingly the remaining three earthworms all survived the test period having shown no response at all. From 6 kg/m^3 of ammonium-N and above, all the earthworms died within an hour having exhibited rapid writhing and mucus production within the first five minutes, then movement slowed prior to death. Table 19 also demonstrates that ammonium-N at pH 9 either had no effect on earthworms or an almost immediate effect (within 1 – 12hrs), in line with the anecdotal field evidence. For full results see Appendix 4.3.

At the lower ammonium N concentrations there was a significant reduction in earthworm weight after 48 hours, Table 20. Note: at higher concentration earthworms did not survive for the full 48 hour period so are not included in the table below.

Table 20. Earthworm response to increasing ammonium-N concentrations at pH 9; changes in earthworm weight.

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)	Weight change (%)*	No. of earthworms alive at 48hrs
Control	1.44	1.24	0.20[a]	-13	10 (out of 10)
2 kg/m^3	1.66	1.31	0.36[b]	-21	9 (out of 10)
4 kg/m^3	1.50	1.12	0.45[b]	-29	3 (out of 10)
6 kg/m^3	1.41	1.24			0 (out of 10)
8 kg/m^3	1.49	1.22			0 (out of 10)
12 kg/m^3	1.35	1.13			0 (out of 10)
P value+	-	-	0.017		-

* Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

+ Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

a,b,c,d Different letters within a column indicate significant differences between treatments (P<0.05)

The behavioural responses results for ammonium-N at pH 9 (categorised as unaffected, sub-lethal or dead) are shown in Table 21 below.

Table 21. Earthworm response to ammonium-N solution at pH 9 (% unaffected, showing a sub-lethal response or dead).

Time period	Response	Rate (kg/m^3)						*P*-value+
		Control	2	4	6	8	12	
After 1 hour	% unaffected	100	100	60	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	n.d.
	% dead	0	0	30	100	100	100	<0.001
After 12 hours	% unaffected	100	100	50	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	n.d.
	% dead	0	0	50	100	100	100	<0.001
After 18 hours	% unaffected	100	100	50	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	n.d.
	% dead	0	0	50	100	100	100	<0.001
After 24 hours	% unaffected	100	90	40	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	n.d.
	% dead	0	0	60	100	100	100	<0.001
After 36 hours	% unaffected	100	80	40	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	n.d.
	% dead	0	20	60	100	100	100	<0.001
After 48 hours	% unaffected	100	80	30	0	0	0	<0.001
	% sub-lethal	0	0	0	0	0	0	n.d.
	% dead	0	20	70	100	100	100	<0.001

+ Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

n.d. not determined – no earthworms in this category

From 4 kg/m^3 and above, there was an increase in sub lethal effects and a significant increase in lethal effects. Displayed graphically, it is clear there was a step change in response from around 4 kg/m^3, which was more pronounced at pH9 than at pH6, Figure 3. Additionally, the figure highlights that ammonium-N at pH 9 appeared to be fatal to earthworms or had no effect i.e. there was only little evidence of sub-lethal effects. This combined with the results for ammonium-N at pH 6 suggested ammonium-N was a potential factor in the reduction in earthworm numbers seen at the field experimental sites.

Figure 3. Earthworm response to ammonium-N solution at pH 9 after 48 hours.

A comparison of the ammonium-N results at pH 6 and 9 showed that the only significant differences between pH 6 and pH 9 occurred early on (i.e. during the first 1 – 12 hours), where there was significantly more dead earthworms after 1 hour with pH 9, also the number with scores 2-4 was significantly higher for pH 6 up to 12 hours. With only minimal differences between the effect of ammonium-N at pH 6 and pH 9, it suggested, in the contact test at least, that the pH of the ammonium-N solution was not having a major effect on earthworm mortality.

Table 22. Earthworm response to ammonium-N solution at pH 6 and pH 9 (% unaffected, showing a sub-lethal response or dead).

Time period	Response	Ammonium rate (kg/m³)						*P*-value	
		0	2	4	6	8	12	pH	Rate
After 1 hour	% unaffected: pH 6	100	100	70	0	0	0	0.85	<0.001
	% unaffected: pH 9	100	100	60	0	0	0		
	% sub-lethal: pH 6	0	0	30	90	10	0	<0.001	<0.001
	% sub-lethal: pH 9	0	0	10	0	0	0		
	% dead: pH 6	0	0	0	10	90	100	0.02	<0.001
	% dead: pH 9	0	0	30	100	100	100		
After 12 hours	% unaffected: pH 6	100	100	80	0	0	0	0.58	<0.001
	% unaffected: pH 9	100	100	50	0	0	0		
	% sub-lethal: pH 6	0	0	20	30	0	0	0.008	0.032
	% sub-lethal: pH 9	0	0	0	0	0	0		
	% dead: pH 6	0	0	0	70	10	10	0.14	<0.001
	% dead: pH 9	0	0	50	100	100	100		
After 18 hours	% unaffected: pH 6	100	100	80	0	0	0	0.58	<0.001
	% unaffected: pH 9	100	100	50	0	0	0		
	% sub-lethal: pH 6	0	0	20	10	0	0	0.04	0.198
	% sub-lethal: pH 9	0	0	0	0	0	0		
	% dead: pH 6	0	0	0	90	100	100	0.27	<0.001
	% dead: pH 9	0	0	50	100	100	100		
After 24 hours	% unaffected: pH 6	100	100	70	0	0	0	0.46	<0.001
	% unaffected: pH 9	100	90	40	0	0	0		
	% sub-lethal: pH 6	0	0	10	10	0	0	0.55	0.52
	% sub-lethal: pH 9	0	10	0	0	0	0		
	% dead: pH 6	0	0	20	90	100	100	0.36	<0.001
	% dead: pH 9	0	0	60	100	100	100		
After 36 hours	% unaffected: pH 6	100	100	70	0	0	0	0.35	<0.001
	% unaffected: pH 9	100	80	40	0	0	0		
	% sub-lethal: pH 6	0	0	10	10	0	0	0.09	0.48
	% sub-lethal: pH 9	0	0	0	0	0	0		
	% dead: pH 6	0	0	20	90	100	100	0.20	<0.001
	% dead: pH 9	0	20	60	100	100	100		
After 48 hours	% unaffected: pH 6	100	100	70	0	0	0	0.26	<0.001
	% unaffected: pH 9	100	80	30	0	0	0		
	%sub-lethal: pH 6	0	0	10	10	0	0	0.09	0.48
	% sub-lethal: pH 9	0	0	0	0	0	0		
	% dead: pH 6	0	0	20	90	100	100	0.14	<0.001
	% dead: pH 9	0	20	70	100	100	100		

[+] Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

Ammonium and acetic acid

The preliminary results from the individual laboratory solution contact tests highlighted ammonium-N and acetic acid as the most likely factors effecting earthworm mortality. As such, a set of contact tests were undertaken to investigate if there were any interactions between differing concentrations of ammonium-N and acetic acid (i.e. the effect of both factors combined being more acute than the response to the factors individually). The results are shown below (Table 23).

Table 23. Earthworm response to combinations of acetic acid and ammonium-N; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	**Score <1hr**	**Score <12hrs**	**Score <18hrs**	**Score <24hrs**	**Score <36hrs**	**Score <48hrs**
Control	1.0	1.0	1.0	1.0	1.0	1.0
500 mg/l acetic acid (AA)	1.0	1.0	1.0	1.0	1.0	1.0
2,500 mg/l AA	1.0	1.0	1.0	1.0	1.0	1.0
5,000 mg/l AA	3.0	1.3	1.6	1.9	2.0	2.2
2 kg/m^3 ammonium-N (NH_4)	1.2	1.0	1.0	1.6	1.7	1.4
3 kg/m^3 NH_4	3.0	2.8	2.8	1.4	1.8	2.2
4 kg/m^3 NH_4	3.0	3.4	3.6	2.6	2.2	2.6
500 mg/ AA 2 kg/m^3 NH_4	1.0	1.0	1.0	1.0	1.0	1.0
500 mg/ AA 3 kg/m^3 NH_4	1.0	1.0	1.2	1.0	1.0	1.0
500 mg/ AA 4 kg/m^3 NH_4	3.0	3.0	2.9	2.5	2.9	2.6
2,500 mg/ AA 2 kg/m^3 NH_4	2.8	1.6	1.4	1.0	1.2	1.0
2,500 mg/ AA 3 kg/m^3 NH_4	1.4	1.2	1.2	1.0	1.0	1.0
2,500 mg/ AA 4 kg/m^3 NH_4	3.0	2.4	2.0	1.8	1.4	1.8
5,000 mg/ AA 2 kg/m^3 NH_4	1.8	1.0	1.4	1.3	1.4	1.8
5,000 mg/ AA 3 kg/m^3 NH_4	3.0	1.2	3.6	3.4	3.4	3.8
5,000 mg/ AA 4 kg/m^3 NH_4	3.0	3.2	3.4	4.2	4.6	4.6

It was clear that the combined ammonium-N and acetic acid solutions were affecting earthworms, however, the pattern was less clear than the single factor tests. Ammonium-N appeared to be the dominant factor (particularly at 4 kg/m^3), however at lower concentrations (2 kg/m^3), the addition of acetic acid (at 500 mg/l) appeared to ameliorate the effect. At the highest ammonium-N concentration there was no indication of an increased response with acetic acid after 12 hours, compared to ammonium-N in isolation, although by 48 hours the highest mortality was where both acetic acid and ammonium-N concentrations were the highest. For full results see Appendix 4.4.

There was no consistent effect of the combined ammonium-N, acetic acid solution on earthworm weight. Measured weight loss in the control was similar to that measured at higher concentrations, although at the highest acetic acid and particularly ammonium-N concentrations there was an increase in earthworm mortality which may have affected the calculations, Table 24.

Table 24. Earthworm response to combinations of acetic acid and ammonium-N; changes in earthworm weight.

Level	Weight pre-test (g)	Weight post-test (g)	Weight change (g)	Weight change (%)*	No. of earthworms alive at 48hrs
Control	2.08	1.63	0.45[ab]	-23	10 (out of 10)
500 mg/l AA	2.24	1.79	0.45[ab]	-22	10 (out of 10)
2,500 mg/l AA	2.35	1.92	0.43[ab]	-21	10 (out of 10)
5,000 mg/l AA	2.12	1.53	0.67[c]	-35	7 (out of 10)
2 kg/m^3 NH_4	2.28	1.83	0.48[ab]	-20	9 (out of 10)
3 kg/m^3 NH_4	2.26	1.87	0.43[ab]	-20	7 (out of 10)
4 kg/m^3 NH_4	2.32	1.91	0.52[ab]	-22	6 (out of 10)
500 mg/l AA 2 kg/m^3 NH_4	2.43	2.03	0.40[a]	-17	10 (out of 10)
500 mg/l AA 3 kg/m^3 NH_4	2.70	2.24	0.46[ab]	-17	10 (out of 10)
500 mg/l AA 4 kg/m^3 NH_4	2.49	2.13	0.42[ab]	-16	7 (out of 10)
2,500 mg/l AA 2 kg/m^3 NH_4	2.60	2.15	0.44[ab]	-19	10 (out of 10)
2,500 mg/l AA 3 kg/m^3 NH_4	2.34	1.84	0.50[ab]	-23	10 (out of 10)
2,500 mg/l AA 4 kg/m^3 NH_4	2.54	2.05	0.54[b]	-23	8 (out of 10)
5,000 mg/l AA 2 kg/m^3 NH_4	2.69	2.21	0.49[ab]	-20	8 (out of 10)
5,000 mg/l AA 3 kg/m^3 NH_4	1.81	1.43	0.48[ab]	-23	3 (out of 10)
5,000 mg/l AA 4 kg/m^3 NH_4	1.95	1.64	-	-	1 (out of 10)
P value[+]	-	-	0.008		-

[*] Weight change is only calculated on earthworms that survived the whole experimental period (i.e. 48 hours).

[+] Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

The behavioural response results (unaffected, sub-lethal and dead categories) for the combined ammonium-N and acetic acid tests are shown in Table 25 below.

Table 25. Earthworm response to combined ammonium-N, acetic acid combinations (% unaffected, showing a sub-lethal response or dead).

Time period	Response	Rate of acetic acid (AA) and ammonium-N (NH_4)																*P*-value[+]
		Control	AA (mg/l)			NH_4 (kg/m^3)			AA (mg/l) + NH_4 (kg/m^3)									
			500	2500	5000	2	3	4	500+2	500+3	500+4	2500+2	2500+3	2500+4	5000+2	5000+3	5000+4	
After 1 hour	% unaffected	100	100	100	0	90	0	0	100	100	0	10	80	0	60	0	0	<0.001
	% sub-lethal	0	0	0	100	10	100	100	0	0	100	90	20	100	40	100	100	<0.001
	% dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
After 12 hours	% unaffected	100	100	100	90	100	10	0	100	100	0	70	90	30	100	90	10	<0.001
	% sub-lethal	0	0	0	10	0	90	80	0	0	100	30	10	70	0	10	70	<0.001
	% dead	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	20	NS (0.30)
After 18 hours	% unaffected	100	100	100	80	100	10	0	100	90	10	80	90	60	80	10	10	<0.001
	% sub-lethal	0	0	0	0	200	0	90	70	0	10	90	20	10	30	60	70	<0.001
	% dead	0	0	0	0	0	0	30	0	0	0	0	0	10	0	30	20	NS (0.20)
After 24 hours	% unaffected	100	100	100	70	80	90	50	100	100	50	100	100	70	90	30	0	<0.001
	% sub-lethal	0	0	0	30	20	0	20	0	0	30	0	0	20	10	30	50	0.01
	% dead	0	0	0	0	0	10	30	0	0	20	0	0	10	0	40	50	<0.001
After 36 hours	% unaffected	100	100	100	70	80	80	70	100	100	40	90	100	90	90	40	10	<0.001
	% sub-lethal	0	0	0	20	10	0	0	0	0	30	10	0	0	0	0	0	0.101
	% dead	0	0	0	10	10	20	30	0	0	30	0	0	10	10	60	90	<0.001
After 48 hours	% unaffected	100	100	100	70	90	70	60	100	100	50	100	100	80	80	30	10	<0.001
	% sub-lethal	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0.72 (NS)
	% dead	0	0	0	30	10	30	40	0	0	30	0	0	20	20	70	90	<0.001

[+] Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

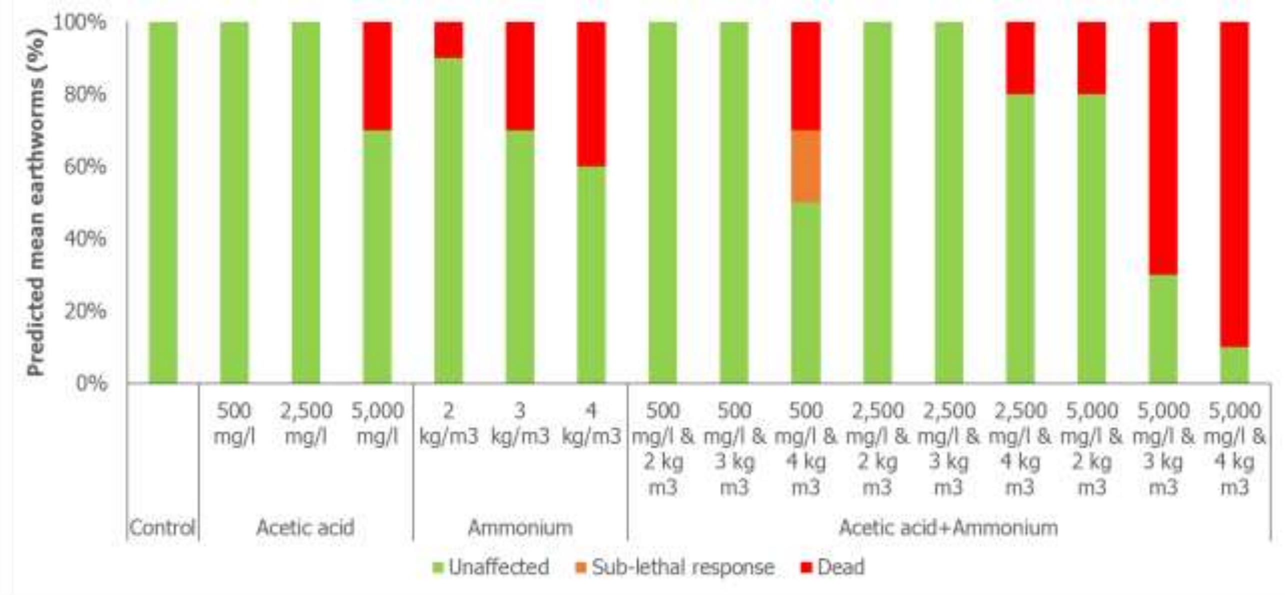

Figure 4. Earthworm response to combined ammonium-N and acetic acid.

The earthworm response results indicated an interaction effect, particularly after the full 48 hours, with a higher percentage of dead earthworms at high concentrations of acetic acid and ammonium-N, than when the factors are tested in isolation. However, there was no immediate interaction effect (i.e. within 1 – 12 hours), suggesting the interaction of ammonium-N and acetic acid was more likely to be a function of the extreme nature of the contact tests (i.e. the earthworms were exposed to the factors for an extended period without the ability to avoid or ameliorate the factors).

In order to look more closely at the effects of acetic acid and ammonium-N the experiment was analysed as a 2 way factorial with 4 levels of acetic acid (0,500,2500,5000) and 4 levels of ammonium-N (0,2,3,4 kg/m3), Table 26. The analysis suggested that both acetic acid and ammonium-N affected earthworm health/mortality, but that ammonium-N had the greater significance. This was particularly seen after 12 hours, where acetic acid had no effect, with all the differences in earthworm response attributed to the rate of ammonium-N application (Table 26).

Table 26. Earthworm response to combined ammonium-N, acetic acid combinations (% unaffected, showing a sub-lethal response or dead).

<table>
<tr><th rowspan="2">Time period</th><th rowspan="2">Response</th><th colspan="4">AA (mg/l)</th><th colspan="2">P-value+</th></tr>
<tr><th>0</th><th>500</th><th>2500</th><th>5000</th><th>AA</th><th>NH4</th></tr>
<tr><td rowspan="15">After 1 hour</td><td colspan="7">Unaffected</td></tr>
<tr><td>0 kg ammonium N/ha</td><td>100</td><td>100</td><td>100</td><td>0</td><td rowspan="4"><0.001</td><td rowspan="4"><0.001</td></tr>
<tr><td>2 kg ammonium N/ha</td><td>90</td><td>100</td><td>10</td><td>60</td></tr>
<tr><td>3 kg ammonium N/ha</td><td>0</td><td>100</td><td>80</td><td>0</td></tr>
<tr><td>4 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="7">Sub-lethal</td></tr>
<tr><td>0 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>100</td><td rowspan="4"><0.001</td><td rowspan="4"><0.001</td></tr>
<tr><td>2 kg ammonium N/ha</td><td>10</td><td>0</td><td>90</td><td>40</td></tr>
<tr><td>3 kg ammonium N/ha</td><td>100</td><td>0</td><td>20</td><td>100</td></tr>
<tr><td>4 kg ammonium N/ha</td><td>100</td><td>100</td><td>100</td><td>100</td></tr>
<tr><td colspan="7">Dead</td></tr>
<tr><td>0 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="4">n.d.</td><td rowspan="4">n.d.</td></tr>
<tr><td>2 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>3 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>4 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="15">After 12 hours</td><td colspan="7">Unaffected</td></tr>
<tr><td>0 kg ammonium N/ha</td><td>100</td><td>100</td><td>100</td><td>90</td><td rowspan="4">0.12</td><td rowspan="4"><0.001</td></tr>
<tr><td>2 kg ammonium N/ha</td><td>100</td><td>100</td><td>70</td><td>100</td></tr>
<tr><td>3 kg ammonium N/ha</td><td>10</td><td>100</td><td>90</td><td>90</td></tr>
<tr><td>4 kg ammonium N/ha</td><td>0</td><td>0</td><td>30</td><td>10</td></tr>
<tr><td colspan="7">Sub-lethal</td></tr>
<tr><td>0 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>10</td><td rowspan="4">0.211</td><td rowspan="4"><0.001</td></tr>
<tr><td>2 kg ammonium N/ha</td><td>0</td><td>0</td><td>30</td><td>0</td></tr>
<tr><td>3 kg ammonium N/ha</td><td>90</td><td>0</td><td>10</td><td>10</td></tr>
<tr><td>4 kg ammonium N/ha</td><td>80</td><td>100</td><td>70</td><td>70</td></tr>
<tr><td colspan="7">Dead</td></tr>
<tr><td>0 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="4">0.13</td><td rowspan="4">0.008</td></tr>
<tr><td>2 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>3 kg ammonium N/ha</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>4 kg ammonium N/ha</td><td>20</td><td>0</td><td>0</td><td>20</td></tr>
</table>

After 18 hours	**Unaffected**						
	0 kg ammonium N/ha	100	100	100	80	<0.001	<0.001
	2 kg ammonium N/ha	100	100	80	80		
	3 kg ammonium N/ha	10	90	90	10		
	4 kg ammonium N/ha	0	10	60	10		
	Sub-lethal						
	0 kg ammonium N/ha	0	0	0	20	0.019	<0.001
	2 kg ammonium N/ha	0	0	20	20		
	3 kg ammonium N/ha	90	10	10	60		
	4 kg ammonium N/ha	70	90	30	70		
	Dead						
	0 kg ammonium N/ha	0	0	0	0	0.037	0.002
	2 kg ammonium N/ha	0	0	0	0		
	3 kg ammonium N/ha	0	0	0	30		
	4 kg ammonium N/ha	30	0	10	20		
After 24 hours	**Unaffected**						
	0 kg ammonium N/ha	100	100	100	70	<0.001	<0.001
	2 kg ammonium N/ha	80	100	100	90		
	3 kg ammonium N/ha	90	100	100	30		
	4 kg ammonium N/ha	50	50	70	0		
	Sub-lethal						
	0 kg ammonium N/ha	0	0	0	30	0.006	0.005
	2 kg ammonium N/ha	20	0	0	10		
	3 kg ammonium N/ha	0	0	0	30		
	4 kg ammonium N/ha	20	30	20	50		
	Dead						
	0 kg ammonium N/ha	0	0	0	0	0.017	<0.001
	2 kg ammonium N/ha	0	0	0	0		
	3 kg ammonium N/ha	10	0	0	40		
	4 kg ammonium N/ha	30	20	10	50		

After 36 hours	**Unaffected**						
	0 kg ammonium N/ha	100	100	100	70	<0.001	<0.001
	2 kg ammonium N/ha	80	100	90	90		
	3 kg ammonium N/ha	80	100	100	40		
	4 kg ammonium N/ha	70	40	90	10		
	Sub-lethal						
	0 kg ammonium N/ha	0	0	0	20	0.659	0.216
	2 kg ammonium N/ha	10	0	10	0		
	3 kg ammonium N/ha	0	0	0	0		
	4 kg ammonium N/ha	0	3	0	0		
	Dead						
	0 kg ammonium N/ha	0	0	0	10	<0.001	<0.001
	2 kg ammonium N/ha	10	0	0	10		
	3 kg ammonium N/ha	20	0	0	60		
	4 kg ammonium N/ha	30	30	10	90		
After 48 hours	**Unaffected**						
	0 kg ammonium N/ha	100	100	100	70	<0.001	<0.001
	2 kg ammonium N/ha	90	100	100	80		
	3 kg ammonium N/ha	70	100	100	30		
	4 kg ammonium N/ha	60	50	80	10		
	Sub-lethal						
	0 kg ammonium N/ha	0	0	0	0	0.132	0.118
	2 kg ammonium N/ha	0	0	0	0		
	3 kg ammonium N/ha	0	0	0	0		
	4 kg ammonium N/ha	0	20	0	0		
	Dead						
	0 kg ammonium N/ha	0	0	0	30	<0.001	<0.001
	2 kg ammonium N/ha	10	0	0	10		
	3 kg ammonium N/ha	30	0	0	70		
	4 kg ammonium N/ha	40	30	20	90		

[+] Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

3.3 Digestate contact tests

Following the same methodology as the laboratory solution contact tests (adapted from OECD, 1984), five separate contact tests were undertaken with five different food-based digestates, each test using different dilutions, Tables 27-31. The full results are detailed in Appendix 4.5.

Table 27. Earthworm response to increasing concentrations of food-based digestate 1; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	**Score <1hr**	**Score <12hrs**	**Score <18hrs**	**Score <24hrs**	**Score <36hrs**	**Score <48hrs**	**Weight start (g)**	**Weight end (g)**
Control	1.0	1.0	1.0	1.0	1.0	1.0	1.99	1.56
10% Digestate 1	1.0	2.6	3.0	3.4	3.8	4.2	1.39	1.10
25% Digestate 1	4.6	5.0	5.0	5.0	5.0	5.0	1.16	1.13
50% Digestate 1	5.0	5.0	5.0	5.0	5.0	5.0	1.35	1.34
75% Digestate 1	5.0	5.0	5.0	5.0	5.0	5.0	1.51	1.50
100% Digestate 1	5.0	5.0	5.0	5.0	5.0	5.0	1.44	1.42

Table 28. Earthworm response to increasing concentrations of food-based digestate 2; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	**Score <1hr**	**Score <12hrs**	**Score <18hrs**	**Score <24hrs**	**Score <36hrs**	**Score <48hrs**	**Weight start (g)**	**Weight end (g)**
Control	1.0	1.0	1.0	1.3	1.4	1.4	2.00	1.51
10% Digestate 2	1.0	2.2	2.2	2.2	2.6	3.8	1.68	1.27
25% Digestate 2	5.0	5.0	5.0	5.0	5.0	5.0	1.45	1.43
50% Digestate 2	5.0	5.0	5.0	5.0	5.0	5.0	1.33	1.32
75% Digestate 2	5.0	5.0	5.0	5.0	5.0	5.0	1.68	1.66
100% Digestate 2	5.0	5.0	5.0	5.0	5.0	5.0	1.40	1.38

Table 29. Earthworm response to increasing concentration of food-based digestate 3; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	**Score <1hr**	**Score <12hrs**	**Score <18hrs**	**Score <24hrs**	**Score <36hrs**	**Score <48hrs**	**Weight start (g)**	**Weight end (g)**
Control	1.0	1.0	1.0	1.0	1.0	1.0	1.57	1.08
10% Digestate 3	1.0	2.6	3.2	3.4	3.8	4.2	1.47	1.13
25% Digestate 3	5.0	5.0	5.0	5.0	5.0	5.0	1.48	1.45
50% Digestate 3	5.0	5.0	5.0	5.0	5.0	5.0	1.52	1.49
75% Digestate 3	5.0	5.0	5.0	5.0	5.0	5.0	1.72	1.62
100% Digestate 3	5.0	5.0	5.0	5.0	5.0	5.0	1.64	1.61

Table 30. Earthworm response to increasing concentrations of food-based digestate 4; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs	Weight start (g)	Weight end (g)
Control	1.0	1.0	1.0	1.0	1.0	1.0	2.23	1.73
10% Digestate 4	1.6	1.6	1.3	1.3	1.6	1.7	2.61	2.15
25% Digestate 4	2.4	3.8	4.2	4.5	4.4	4.4	2.44	2.22
50% Digestate 4	4.7	5.0	5.0	5.0	5.0	5.0	2.58	2.53
75% Digestate 4	5.0	5.0	5.0	5.0	5.0	5.0	2.66	2.64
100% Digestate 4	5.0	5.0	5.0	5.0	5.0	5.0	2.50	2.48

Table 31. Earthworm response to increasing concentrations of food-based digestate 5; results are an average of the individual response scores (n=10), with 1 = no reaction & 5 = death.

Level	Score <1hr	Score <12hrs	Score <18hrs	Score <24hrs	Score <36hrs	Score <48hrs	Weight start (g)	Weight end (g)
Control	1.0	1.0	1.0	1.0	1.0	1.0	2.46	1.99
10% Digestate 5	1.0	1.0	1.4	1.4	1.6	1.6	2.50	2.08
25% Digestate 5	1.2	2.6	3.6	4.2	3.7	3.4	2.53	1.82
50% Digestate 5	3.5	5.0	5.0	5.0	5.0	5.0	2.16	1.57
75% Digestate 5	5.0	5.0	5.0	5.0	5.0	5.0	2.35	1.86
100% Digestate 5	5.0	5.0	5.0	5.0	5.0	5.0	2.38	1.82

Within the 'extreme environment' of the contact tests, the consistently high scores at all but the highest dilution rate clearly demonstrated that food-based digestate had a significant effect on earthworm survival, with three of the digestates causing almost complete mortality even when diluted to 25%. Interestingly, digestates 4 and 5 had less pronounced effects (although they were still fatal at 50% dilution).

Due to the high level of mortality observed, it was not possible to undertake statistical assessment on either the scores or the earthworm weights. However, the data on earthworm mortality was combined with the laboratory analysis data for each parameter and then ranked to assist with identifying the causal agent in the digestate which was affecting earthworm survival, specifically ammonium-N (Table 32), acetic acid (Table 33), propionic acid (Table 34), Iso-butyric acid (Table 35), N-butyric acid (Table 36), pH (Table 37), conductivity (Table 38) and BOD (Table 39). *Note:* the other VFAs were not included as the majority (if not all) of values were below the limit of analytical detection.

Table 32. Earthworm mortality in response to increasing food-based digestate concentrations ranked by ammonium-N concentration.

Ammonium-N (kg/m^3)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0	0	0	0	0	1	1	10
289	0	0	0	0	0	1	10
406	0	0	1	1	1	2	10
535	0	4	5	6	7	8	10
584	0	3	3	3	4	7	10
722	1	6	7	7	7	7	10
832	0	4	5	6	7	8	10
1015	0	1	6	6	6	6	10
1338	9	10	10	10	10	10	10
1444	7	10	10	10	10	10	10
1460	10	10	10	10	10	10	10
2030	3	10	10	10	10	10	10
2080	10	10	10	10	10	10	10
2165	10	10	10	10	10	10	10
2677	10	10	10	10	10	10	10
2887	10	10	10	10	10	10	10
2920	10	10	10	10	10	10	10
3044	10	10	10	10	10	10	10
4016	10	10	10	10	10	10	10
4059	10	10	10	10	10	10	10
4159	10	10	10	10	10	10	10
4380	10	10	10	10	10	10	10
5354	9	10	10	10	10	10	10
5840	10	10	10	10	10	10	10
6238	10	10	10	10	10	10	10
8318	10	10	10	10	10	10	10

Table 33. Earthworm mortality to increasing food-based digestate concentrations ranked by acetic acid concentration.

Acetic acid (mg/l)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0	0	0	0	0	1	1	10
71	0	3	3	3	4	7	10
88	0	4	5	6	7	8	10
107	0	4	5	6	7	8	10
138	0	0	0	0	0	1	10
178	10	10	10	10	10	10	10
219	10	10	10	10	10	10	10
268	9	10	10	10	10	10	10
344	1	6	7	7	7	7	10
356	10	10	10	10	10	10	10
438	10	10	10	10	10	10	10
534	10	10	10	10	10	10	10
536	10	10	10	10	10	10	10
656	10	10	10	10	10	10	10
688	7	10	10	10	10	10	10
712	10	10	10	10	10	10	10
803	10	10	10	10	10	10	10
875	10	10	10	10	10	10	10
938	0	0	1	1	1	2	10
1032	10	10	10	10	10	10	10
1071	9	10	10	10	10	10	10
1376	10	10	10	10	10	10	10
2344	0	1	6	6	6	6	10
4688	3	10	10	10	10	10	10
7031	10	10	10	10	10	10	10
9375	10	10	10	10	10	10	10

Table 34. Earthworm mortality to increasing food-based digestate concentrations ranked by propionic acid concentration.

Propionic acid (mg/l)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0	0	0	0	0	1	1	10
6	0	3	3	3	4	7	10
14	0	0	0	0	0	1	10
15	10	10	10	10	10	10	10
15	0	4	5	6	7	8	10
17	0	4	5	6	7	8	10
29	10	10	10	10	10	10	10
34	1	6	7	7	7	7	10
37	10	10	10	10	10	10	10
42	9	10	10	10	10	10	10
44	10	10	10	10	10	10	10
58	10	10	10	10	10	10	10
68	7	10	10	10	10	10	10
74	10	10	10	10	10	10	10
80	0	0	1	1	1	2	10
84	10	10	10	10	10	10	10
102	10	10	10	10	10	10	10
111	10	10	10	10	10	10	10
126	10	10	10	10	10	10	10
136	10	10	10	10	10	10	10
148	10	10	10	10	10	10	10
168	9	10	10	10	10	10	10
199	0	1	6	6	6	6	10
398	3	10	10	10	10	10	10
597	10	10	10	10	10	10	10
796	10	10	10	10	10	10	10

Table 35. Earthworm mortality to increasing food-based digestate concentrations ranked by Iso-butyric acid concentration.

Iso-butyric acid (mg/l)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0.0	0	0	0	0	1	1	10
2.9	0	3	3	3	4	7	10
5.1	0	0	0	0	0	1	10
5.2	0	4	5	6	7	8	10
7.2	10	10	10	10	10	10	10
7.4	0	4	5	6	7	8	10
13	1	6	7	7	7	7	10
13	10	10	10	10	10	10	10
14	10	10	10	10	10	10	10
18	9	10	10	10	10	10	10
21	10	10	10	10	10	10	10
26	7	10	10	10	10	10	10
26	10	10	10	10	10	10	10
29	10	10	10	10	10	10	10
35	0	0	1	1	1	2	10
37	10	10	10	10	10	10	10
39	10	10	10	10	10	10	10
39	10	10	10	10	10	10	10
51	10	10	10	10	10	10	10
52	10	10	10	10	10	10	10
55	10	10	10	10	10	10	10
74	9	10	10	10	10	10	10
88	0	1	6	6	6	6	10
176	3	10	10	10	10	10	10
264	10	10	10	10	10	10	10
352	10	10	10	10	10	10	10

Table 36. Earthworm mortality to increasing food-based digestate concentrations ranked by N-butyric acid concentration.

N-butyric acid (mg/l)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0	0	0	0	0	1	1	10
1.4	0	3	3	3	4	7	10
1.7	0	4	5	6	7	8	10
3.6	10	10	10	10	10	10	10
4.2	10	10	10	10	10	10	10
4.3	0	4	5	6	7	8	10
5.8	0	0	0	0	0	1	10
7.2	10	10	10	10	10	10	10
8.3	10	10	10	10	10	10	10
10.7	9	10	10	10	10	10	10
10.7	10	10	10	10	10	10	10
12.5	10	10	10	10	10	10	10
14.3	10	10	10	10	10	10	10
14.5	1	6	7	7	7	7	10
16.7	10	10	10	10	10	10	10
21.3	10	10	10	10	10	10	10
28.9	7	10	10	10	10	10	10
31.9	10	10	10	10	10	10	10
32.6	0	0	1	1	1	2	10
42.6	9	10	10	10	10	10	10
43.4	10	10	10	10	10	10	10
58.0	10	10	10	10	10	10	10
82.0	0	1	6	6	6	6	10
163	3	10	10	10	10	10	10
245	10	10	10	10	10	10	10
326	10	10	10	10	10	10	10

Table 37. Earthworm mortality to increasing food-based digestate concentrations ranked by pH.

pH	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
7.00	0	0	0	0	1	1	50
7.17	1	6	7	7	7	7	10
7.19	7	10	10	10	10	10	10
7.21	10	10	10	10	10	10	10
7.24	0	0	0	0	0	1	10
7.40	10	10	10	10	10	10	10
7.69	20	20	20	20	20	20	20
7.70	3	10	10	10	10	10	10
7.87	0	1	6	6	6	6	10
7.89	10	10	10	10	10	10	10
7.93	10	10	10	10	10	10	10
7.94	20	20	21	21	21	22	30
7.95	20	20	20	20	20	20	20
7.97	19	20	20	20	20	20	20
8.02	19	20	20	20	20	20	20
8.03	10	10	10	10	10	10	10
8.05	0	4	5	6	7	8	10
8.06	10	10	10	10	10	10	10
8.10	0	4	5	6	7	8	10
8.15	0	3	3	3	4	7	10

Table 38. Earthworm mortality to increasing food-based digestate concentrations ranked by conductivity level.

Conductivity (µS/cm)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0	50	0	0	0	1	1	10
392	0	0	0	0	0	1	10
474	0	0	1	1	1	2	10
608	0	3	3	3	4	7	10
618	0	4	5	6	7	8	10
652	0	4	5	6	7	8	10
980	1	6	7	7	7	7	10
1185	0	1	6	6	6	6	10
1520	10	10	10	10	10	10	10
1545	9	10	10	10	10	10	10
1630	10	10	10	10	10	10	10
1960	7	10	10	10	10	10	10
2370	3	10	10	10	10	10	10
2940	10	10	10	10	10	10	10
3040	10	10	10	10	10	10	10
3090	10	10	10	10	10	10	10
3260	10	10	10	10	10	10	10
3555	10	10	10	10	10	10	10
3920	10	10	10	10	10	10	10
4560	10	10	10	10	10	10	10
4635	10	10	10	10	10	10	10
4740	10	10	10	10	10	10	10
4890	10	10	10	10	10	10	10
6080	10	10	10	10	10	10	10
6180	9	10	10	10	10	10	10
6520	10	10	10	10	10	10	10

Table 39. Earthworm mortality to increasing food-based digestate concentrations ranked by BOD concentration.

BOD (mg/l)	Number of dead Earthworms						Number of earthworms
	1 hr	12 hr	18 hr	24 hr	36 hr	48 hr	
0	0	0	0	0	1	1	10
558	0	4	5	6	7	8	10
584	0	3	3	3	4	7	10
612	0	4	5	6	7	8	10
980	0	0	0	0	0	1	10
1022	0	0	1	1	1	2	10
1394	9	10	10	10	10	10	10
1460	10	10	10	10	10	10	10
1530	10	10	10	10	10	10	10
2450	1	6	7	7	7	7	10
2556	0	1	6	6	6	6	10
2788	10	10	10	10	10	10	10
2920	10	10	10	10	10	10	10
3060	10	10	10	10	10	10	10
4181	10	10	10	10	10	10	10
4380	10	10	10	10	10	10	10
4590	10	10	10	10	10	10	10
4900	7	10	10	10	10	10	10
5112	3	10	10	10	10	10	10
5575	9	10	10	10	10	10	10
5840	10	10	10	10	10	10	10
6120	10	10	10	10	10	10	10
7350	10	10	10	10	10	10	10
7669	10	10	10	10	10	10	10
9800	10	10	10	10	10	10	10
10225	10	10	10	10	10	10	10

The rankings in Tables 32 – 39 showed that ammonium-N was most likely the causal factor for the severe earthworm responses observed, having the most consistent response in earthworm mortality to increasing concentrations. Conductivity and to a lesser extent BOD also showed reasonable responses, with pH and the various VFAs showing no real correlation with earthworm mortality.

4 Pot studies

Following on from the screening assessments, pot studies were used to investigate the most likely causal factors in a more realistic environment. However, it should be recognised that pot conditions do not simulate those in the field, particularly as earthworms are more limited in their ability to move away from the applied digestate. Also, their density and distribution in the pots will not reflect field conditions, or their exposure to the factors being studied. Despite this, pot studies do provide an environment to assess short-term exposure to food-based digestate in a worst-case environment to identify what factors are effecting earthworm survival/health.

4.1 Methodology

The pot study methodology was based on the standard OECD (1984) guidelines, specifically:

- As in the contact tests, Juvenile *L. terrestris* earthworms were used.
- Pots (*c.*10 cm high, 15 cm diameter) containing 2 kg of free-draining soil with *c.*3% organic matter were maintained at field capacity moisture content (*c.*40% w/w) by periodic water inputs at 10-15°C in a shaded greenhouse subject to a natural day/night cycle (the pot studies were undertaken in March/April so the day/night cycle matched the prevailing conditions).
- To prevent earthworm escape, the top and bottom of the pot were covered by fine metal gauze.
- Seven earthworms of similar size were put into each pot (the combined weight of the earthworms was recorded at the start of the experiment).
    - *Note:* Earthworm densities are somewhat higher than those likely in the field, but have been chosen to ensure high rates of organic material 'exposure' and to allow for the possibility of mortality/escape. Preliminary tests were undertaken which showed that at both application rates there were still small areas of the pots which the digestate did not penetrate, meaning avoidance was possible.
- For each treatment, 16 replicate pots were used allowing four replicates to be sequentially destructively sampled over time (i.e. over four sampling occasions). The time periods were adapted from the standard OECD methodology, due to the 'immediate mortality' identified in earlier field experiments (e.g. WP2.3 the application techniques studies).
- Earthworms were allowed to acclimatise and establish in the soil in the pots for 48 hours before digestate application.
- Two hours, and one, seven and fourteen days after digestate application, four replicate pots per treatment were destructively sampled and earthworm population/biomass and/or mortality were recorded.
- Soil samples were taken as the pots were destructively sampled one day and fourteen days after treatment application, and were analysed for gravimetric moisture content, pH, ammonium-N and nitrate-N concentrations, and VFAs.
- Two separate experiments were undertaken, one investigating ammonium-N (Table 40) and one acetic acid (Table 41).
- Table 42 shows the analysis of the unamended food-based digestate used in the pot experiments.

Table 40. Treatment list for the food-based digestate ammonium-N pot experiment.

Treatment	2 hours (sampled)	1 day (sampled)	7 days (sampled)	14 days (sampled)	Total
Untreated control	4	4	4	4	16
200 kg N/ha fertiliser control	4	4	4	4	16
300 kg N/ha fertiliser control	4	4	4	4	16
Unamended food-based digestate					
pH <7 applied at 50 m^3/ha	4	4	4	4	16
pH ≥7 – <8 applied at 50 m^3/ha	4	4	4	4	16
pH ≥8 applied at 50 m^3/ha	4	4	4	4	16
pH <7 applied at 30 m^3/ha	4	4	4	4	16
pH ≥7 – <8 applied at 30 m^3/ha	4	4	4	4	16
pH ≥8 applied at 30 m^3/ha	4	4	4	4	16
Enhanced ammonium-N food-based digestate					
pH <7 applied at 50 m^3/ha	4	4	4	4	16
pH ≥7 – <8 applied at 50 m^3/ha	4	4	4	4	16
pH ≥8 applied at 50 m^3/ha	4	4	4	4	16
pH <7 applied at 30 m^3/ha	4	4	4	4	16
pH ≥7 – <8 applied at 30 m^3/ha	4	4	4	4	16
pH ≥8 applied at 30 m^3/ha	4	4	4	4	16

Table 41. Treatment list for the food-based digestate acetic acid pot experiment.

Treatment	2 hours (sampled)	1 day (sampled)	7 days (sampled)	14 days (sampled)	Total
Untreated control	4	4	4	4	16
Un-amended Digestate (*c.*1,000 mg/l)	4	4	4	4	16
Digestate + VFA level 1 (*c.*2,500 mg/l)	4	4	4	4	16
Digestate + VFA level 2 (*c.*3,500 mg/l)	4	4	4	4	16
Digestate + VFA level 3 (*c.*5,000 mg/l)	4	4	4	4	16

- As part of the ammonium-N pot studies, two fertiliser controls were used, ammonium nitrate (34.5% nitrogen comprising 50% nitrate and 50% ammonium) was dissolved in distilled water to apply 200 and 300 kg N/ha in the same volume of water as 50m^3/ha digestate (equivalent to 100 and 150 kg ammonium/ha).
- Also as part of the ammonium-N experiment, the pH of the digestate was adjusted using sulphuric acid, the same methodology as used in the contact tests.
- Additionally, two ammonium-N concentrations were investigated; in the unamended treatments the ammonium-N content was unchanged (i.e. 4 kg/m^3) and in the enhanced treatments ammonium sulphate was used to raise the concentration to 6 kg/m^3 (Table 40).
- For the acetic acid pot experiment the acetic acid content of the food-based digestate was enhanced from 1,000 mg/l to 2,500 mg/l, 3,500 mg/l and 5,000 mg/l via the addition of acetic acid solution (Table 41).

Table 42. Analysis of the food-based digestate used in the ammonium-N and acetic acid pot experiments.

Determinand	**Unit**[+]	**Food-based digestate**
Conductivity	µS/cm	6,180
pH		8.1
Ammonium-N	kg/m^3	4.1
Biochemical oxygen demand	mg/l	5,580
N-caproic acid	mg/l	< 20
Acetic acid	mg/l	1,070
Propionic acid	mg/l	170
Iso-butyric acid	mg/l	74
N-butyric acid	mg/l	43
Iso-valeric acid	mg/l	< 25
N-valeric acid	mg/l	< 25
Iso-caproic acid	mg/l	< 20
Acetic acid equivalents	mg/l	1,290

[+] µS/cm = microsiemens per centimetre; kg/m^3 = kilograms per cubic metre; mg/l = milligrams per litre

4.2 Statistics

The change in earthworm weight or earthworm numbers was assessed using analysis of variance (ANOVA) using GenStat (version 12.1, VSN International). To compare between all treatments, a one-way ANOVA was used to determine significant treatments effects. Where significant treatment differences in earthworm weight were measured, Duncan's multiple range test was used to compare the means from the ANOVA. The results from Duncan's test were shown on the tables of means where appropriate (i.e. where $P<0.05$).

In addition, a factorial ANOVA was used to determine significant treatment effects of i) the untreated control compared with the fertiliser controls and digestate (all treatments), ii) ammonium rate (averaged over all pH and application rates), iii) pH (averaged over all ammonium and application rates), iv) application rate (averaged over all pH and ammonium rates) and v) any interactions between factors (e.g. pH and ammonium).

Regression analysis was undertaken on the earthworm weight change, adjusted weight change and number of live earthworms data. The analysis was first performed using 'time' as a factor (i.e. 2 hours, 24 hours, 7 days & 14 days after treatments were applied), then application rate (i.e. 30 & $50m^3$/ha), ammonium concentration (i.e. 0, 2, 3, 4 & 6 kg/m^3), ammonium loading (i.e. 0, 100, 120, 150, 180, 200, 300 kg NH_4/ha) and pH (i.e. pH 6, 6.5, 7.5 & 8.5) were added to the analysis as factors, first separately, then in combination, to establish which of these had most influence (in terms of percentage variance accounted for) on changes in each of the properties (i.e. earthworm weights/numbers). As 'ammonium loading' is a combined effect of both application rate and ammonium concentration, the only combination of factors required was time x ammonium loading x pH. There was no attempt to fit a regression line, with the analysis only looking to establish what factor had the greatest influence on the earthworms, not the nature of the effect (i.e. a linear relationship was not assumed).

4.3 Results

4.3.1 Ammonium-N experiments

After 2 hours, it was clear that the food-based digestate at the higher application rate (50 m^3/ha i.e. the maximum application rate recommended in the code of good agricultural practice, Defra, 2009) was having a significant effect on earthworm survival compared with the controls, Table 43. However, earthworm survival at the lower application rate (even with the enhanced ammonium-N concentration) was not significantly different ($P>0.05$) to the fertiliser and untreated controls, Table 43. There were no consistent statistically significant differences between the different pH treatments, however, the smallest weight gain and lowest numbers of live earthworms were recorded on the highest pH treatments, Table 43. The same patterns were observed across all the time periods (Tables 44-46), as evidenced by the analysis of all time periods (Table 47), which as well as showing that there was a significant effect of sampling time, showed there was also a significant difference between the treatments. For full results see Appendix 4.6.

In addition to the standard ANOVA assessments (described above; Tables 43-47), a series of four-way factorial ANOVA's with four levels of treatment (untreated control, 200 kg N/ha, 300 kg N/ha and digestate), two levels of ammonium concentration (4 or 6 kg/m^3) three levels of pH (6-6.5, 7-7 and 8.5) and two application rates (30 or 50 m^3/ha) were undertaken to identify changes in weight gain (Table 48), adjusted weight gain (Table 49) and live earthworms (Table 50). *Note:* weight gain is the post-test weight minus the pre-test weight; the adjusted weight gain is the post-test weight divided by the number of live earthworms at the end of the test, minus the pre-test weight divided by the number of live earthworms at the beginning of the test. These have also been expressed as a percentage of the pre-test value.

The outputs from the standard ANOVA's and factorial ANOVA's show that food-based digestate does have a negative effect on earthworms. There are inconsistences in the data between time periods and replicates, but for earthworm mortality and health, reducing the quantity of ammonium-N applied did lead to a reduction in the effect of food-based digestate on earthworms.

Table 43. Ammonium-N pot test results after 2 hours

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	18.8	27.8	9.01[e]	48	1.3	48	7[c]	100
200 kg N/ha fertiliser control	17.4	26.5	9.10[e]	52	1.3	52	7[c]	100
300 kg N/ha fertiliser control	19.9	27.7	7.75[de]	39	1.1	39	7[c]	100
Unamended ammonium-N								
pH <7 applied at 50 m^3/ha	18.5	20.3	1.81[abc]	10	1.2	45	5.3[ab]	75
pH ≥7 – <8 applied at 50 m^3/ha	17.3	20.0	2.68[abcd]	15	0.9	37	6.0[abc]	86
pH ≥8 applied at 50 m^3/ha	17.9	18.3	0.37[a]	2	1.1	43	5.0[a]	71
pH <7 applied at 30 m^3/ha	16.5	23.3	6.78[cde]	41	1.1	47	6.8[c]	96
pH ≥7 – <8 applied at 30 m^3/ha	16.1	22.0	5.89[bcde]	36	1.0	42	6.8[c]	96
pH ≥8 applied at 30 m^3/ha	17.1	22.7	5.58[bcde]	32	1.0	43	6.5[bc]	93
Enhanced ammonium-N								
pH <7 applied at 50 m^3/ha	16.6	17.2	0.54[a]	3	0.9	38	5.3[ab]	75
pH ≥7 – <8 applied at 50 m^3/ha	17.3	18.3	0.98[ab]	6	1.0	42	5.3[ab]	75
pH ≥8 applied at 50 m^3/ha	16.9	16.1	-0.82[a]	-5	0.7	30	5.0[a]	71
pH <7 applied at 30 m^3/ha	17.2	24.1	6.92[cde]	40	1.1	46	6.8[c]	96
pH ≥7 – <8 applied at 30 m^3/ha	16.7	23.6	6.83[cde]	41	1.1	47	6.8[c]	96
pH ≥8 applied at 30 m^3/ha	16.2	22.0	5.82[bcde]	35	0.9	40	6.8[c]	96
P Statistic[3]	-	-	< 0.001		0.07		< 0.001	

[1] weight gain = post-test weight minus pre-test weight. [2]adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

3 Data analysed by analysis of variance (ANOVA), *P* value of <0.05 is significant at the 5% level; *P* value of <0.01 is significant at the 1% level and *P* value of <0.001 is significant at the 0.1% level.

[a,b,c,d] Different letters within a column indicate significant differences between treatments (*P*<0.05)

Table 44. Ammonium-N pot test results after 24 hours

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	16.7	24.9	8.18[cd]	49	1.2	49	7.00[c]	100
200 kg N/ha fertiliser control	18.4	25.9	7.48[bcd]	41	1.1	41	7.00[c]	100
300 kg N/ha fertiliser control	18.1	23.9	5.73[bcd]	30	1.0	39	6.50[bc]	93
Unamended ammonium-N								
pH <7 applied at 50 m^3/ha	18.8	22.1	3.29[bc]	18	1.4	52	5.50[b]	79
pH ≥7 – <8 applied at 50 m^3/ha	17.0	20.0	3.01[b]	17	1.0	40	5.75[bc]	82
pH ≥8 applied at 50 m^3/ha	18.2	21.6	3.46[bc]	20	1.0	40	6.00[bc]	86
pH <7 applied at 30 m^3/ha	16.6	25.3	8.66[d]	52	1.2	52	7.00[c]	100
pH ≥7 – <8 applied at 30 m^3/ha	18.1	26.8	8.65[d]	48	1.2	48	7.00[c]	100
pH ≥8 applied at 30 m^3/ha	15.8	22.1	6.33[bcd]	40	0.9	40	7.00[c]	100
Enhanced ammonium-N								
pH <7 applied at 50 m^3/ha	16.5	21.7	5.19[bcd]	31	1.1	48	6.25[bc]	89
pH ≥7 – <8 applied at 50 m^3/ha	16.0	13.7	-2.31[a]	-14	1.2	52	4.00[a]	57
pH ≥8 applied at 50 m^3/ha	15.2	18.6	3.40[bc]	22	1.0	48	5.75[bc]	82
pH <7 applied at 30 m^3/ha	15.8	24.6	8.82[d]	56	1.4	61	6.75[bc]	96
pH ≥7 – <8 applied at 30 m^3/ha	16.6	23.1	6.50[bcd]	40	1.0	44	6.75[bc]	96
pH ≥8 applied at 30 m^3/ha	16.6	21.6	5.01[bcd]	30	1.1	45	6.25[bc]	89
P Statistic	-	-	< 0.001		0.12		< 0.001	

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

Table 45. Ammonium-N pot test results after 7 days.

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	15.5	21.3	5.79^{de}	37	0.83^{abcde}	37	7.0	100
200 kg N/ha fertiliser control	15.2	19.3	4.12^{bcde}	27	0.69^{ab}	32	6.8	96
300 kg N/ha fertiliser control	15.7	20.7	4.98^{cde}	32	0.71^{abc}	32	7.0	100
Unamended ammonium-N								
pH <7 applied at 50 m^3/ha	15.2	20.3	5.13^{cde}	34	1.09^{de}	50	6.3	89
pH ≥7 – <8 applied at 50 m^3/ha	17.4	22.3	4.95^{cde}	28	1.08^{cde}	43	6.3	89
pH ≥8 applied at 50 m^3/ha	16.3	17.9	1.62^{abcd}	10	0.99^{bcde}	42	5.5	79
pH <7 applied at 30 m^3/ha	15.8	21.0	5.21^{cde}	33	1.12^{e}	50	6.3	89
pH ≥7 – <8 applied at 30 m^3/ha	16.0	20.1	4.10^{bcde}	25	0.80^{abcde}	35	6.5	93
pH ≥8 applied at 30 m^3/ha	15.6	21.6	6.08^{de}	39	0.99^{bcde}	44	6.8	96
Enhanced ammonium-N								
pH <7 applied at 50 m^3/ha	16.5	14.9	-1.57^{a}	-9	0.49^{a}	22	5.3	75
pH ≥7 – <8 applied at 50 m^3/ha	15.5	15.6	0.04^{ab}	0	0.75^{abcd}	34	5.3	75
pH ≥8 applied at 50 m^3/ha	15.5	16.4	0.93^{abc}	6	0.79^{cde}	36	5.5	79
pH <7 applied at 30 m^3/ha	16.0	20.2	4.20^{bcde}	26	0.80^{abcde}	35	6.5	93
pH ≥7 – <8 applied at 30 m^3/ha	15.8	22.5	6.73^{e}	43	1.08^{cde}	48	6.8	96
pH ≥8 applied at 30 m^3/ha	15.5	20.1	4.66^{cde}	30	0.88^{bcde}	40	6.5	93
P Statistic	-	-	0.003		0.01	0.09	0.09	

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

Table 46. Ammonium-N pot test results after 14 days.

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	17.9	24.1	6.19[e]	35	0.9	35	7.0	100
200 kg N/ha fertiliser control	18.7	21.7	2.96[cde]	16	0.5	21	6.8	96
300 kg N/ha fertiliser control	18.4	21.1	2.75[cde]	15	0.4	15	7.0	100
Unamended ammonium-N								
pH <7 applied at 50 m^3/ha	17.6	15.7	-1.98[abcde]	-11	0.5	20	5.0	71
pH ≥7 – <8 applied at 50 m^3/ha	17.5	14.4	-3.13[abcd]	-17	0.4	16	5.0	71
pH ≥8 applied at 50 m^3/ha	18.6	10.7	-7.96[a]	-43	0.1	5	3.8	54
pH <7 applied at 30 m^3/ha	17.1	21.2	4.04[cde]	24	0.8	33	6.5	93
pH ≥7 – <8 applied at 30 m^3/ha	19.5	23.9	4.38[de]	22	0.7	27	6.8	96
pH ≥8 applied at 30 m^3/ha	17.9	19.7	1.81[bcde]	11	0.6	24	6.3	89
Enhanced ammonium-N								
pH <7 applied at 50 m^3/ha	19.2	14.4	-4.76[abc]	-25	0.2	6	5.0	71
pH ≥7 – <8 applied at 50 m^3/ha	18.8	17.1	-1.67[abcde]	-9	0.3	11	5.8	82
pH ≥8 applied at 50 m^3/ha	19.2	12.6	-6.51[ab]	-34	0.3	9	4.3	61
pH <7 applied at 30 m^3/ha	18.7	17.1	-1.64[abcde]	-9	0.3	10	5.8	82
pH ≥7 – <8 applied at 30 m^3/ha	19.2	20.8	1.61[bcde]	8	0.5	17	6.5	93
pH ≥8 applied at 30 m^3/ha	18.3	17.3	-1.03[abcde]	-6	0.1	4	5.8	82
P Statistic	-	-	0.01		0.06		NS (0.09)	

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

Table 47. Ammonium-N pot test results averaged across all time periods.

Treatment	Weight gain (g)[1]				Adjusted weight gain (g)[2]				Number of live earthworms			
	2 hours	24 hours	7 days	14 days	2 hours	24 hours	7 days	14 days	2 hours	24 hours	7 days	14 days
Untreated control	9.00	8.18	5.79	6.19	1.29	1.17	0.83	0.88	7.00	7.00	7.00	7.00
200 kg N/ha fertiliser control	9.10	7.48	4.12	2.96	1.30	1.07	0.69	0.54	7.00	7.00	6.75	6.75
300 kg N/ha fertiliser control	7.75	5.73	4.97	2.75	1.11	1.03	0.71	0.39	7.00	6.50	7.00	7.00
Unamended ammonium-N												
pH <7 applied at 50 m^3/ha	1.81	3.29	5.13	-1.98	1.20	1.39	1.09	0.49	5.25	5.50	6.25	5.00
pH ≥7 – <8 applied at 50 m^3/ha	2.68	3.01	4.95	-3.13	.89	0.98	1.08	0.37	6.00	5.75	6.25	5.00
pH ≥8 applied at 50 m^3/ha	0.37	3.45	1.62	-7.96	1.11	1.03	0.99	0.13	5.00	6.00	5.50	3.75
pH <7 applied at 30 m^3/ha	6.78	8.66	5.21	4.04	1.10	1.24	1.12	0.81	6.75	7.00	6.25	6.50
pH ≥7 – <8 applied at 30 m^3/ha	5.88	8.65	4.10	4.38	0.95	1.24	0.80	0.75	6.75	7.00	6.50	6.75
pH ≥8 applied at 30 m^3/ha	5.58	6.33	6.08	1.81	1.05	0.90	0.99	0.59	6.50	7.00	6.75	6.25
Enhanced ammonium-N												
pH <7 applied at 50 m^3/ha	0.54	5.19	-1.57	-4.76	0.90	1.13	0.50	0.15	5.25	6.25	5.25	5.00
pH ≥7 – <8 applied at 50 m^3/ha	0.98	-2.31	0.04	-1.67	1.02	1.18	0.75	0.29	5.25	4.00	5.25	5.75
pH ≥8 applied at 50 m^3/ha	-0.82	3.40	0.93	-6.51	0.74	1.04	0.79	0.25	5.00	5.75	5.50	4.25
pH <7 applied at 30 m^3/ha	6.92	8.81	4.20	-1.64	1.13	1.39	0.80	0.25	6.75	6.75	6.50	5.75
pH ≥7 – <8 applied at 30 m^3/ha	6.83	6.50	6.73	1.61	1.11	1.04	1.08	0.46	6.75	6.75	6.75	6.50
pH ≥8 applied at 30 m^3/ha	5.82	5.01	4.66	-1.03	0.93	1.08	0.88	0.11	6.75	6.75	6.50	5.75
P Statistic (treatment)	< 0.001				< 0.001				0.037			
P Statistic (time)	< 0.001				< 0.001				<0.001			

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments (P<0.05)

Table 48. Factorial ANOVA of ammonium pot experiment –weight gain (g)

Factorial statistics	After 2 hours		After 24 hours		After 7 days		After 14 days		Average	
Time										
2 hours									4.62^{ab}	< 0.001
24 hours									5.43^{a}	
7 days									3.80^{b}	
14 days									-0.33^{c}	
Control vs. 200 N vs. 300 N vs. digestate										
Untreated control	9.01^{a}	< 0.001	8.18	NS (0.12)	5.79	NS (0.35)	6.19^{a}	0.02	7.29^{a}	< 0.001
200 kg N/ha control	9.10^{a}		7.48		4.12		2.96^{ab}		5.91^{a}	
300 kg N/ha control	7.75^{ab}		5.73		4.98		2.75^{ab}		5.30^{a}	
Digestate	3.61^{b}		5.00		3.51		-1.40^{b}		2.68^{b}	
Ammonium rate										
Unamended (4 kg/m^3)	3.85	NS (0.61)	5.56	NS (0.20)	4.52^{a}	0.02	-0.47	NS (0.23)	3.36^{a}	0.01
Enhanced (6 kg/m^3)	3.38		4.43		2.50^{b}		-2.33		1.99^{b}	
pH										
6-6.5	4.01	NS (0.40)	6.49	NS (0.06)	3.24	NS (0.73)	-1.08	NS (0.15)	3.16	NS (0.09)
7-7.5	4.09		3.96		3.96		0.30		3.08	
8.5	2.74		4.55		3.32		-3.42		1.80	
Application rate										
30 m^3/ha	6.30^{a}	< 0.001	7.33^{a}	< 0.001	5.16	< 0.001	1.53^{a}	< 0.001	5.08^{a}	< 0.001
50 m^3/ha	0.93^{b}		2.67^{b}		1.85^{b}		-4.33^{b}		0.28^{b}	

^{a,b,c,d} Different letters within a column indicate significant differences between treatments (*P*<0.05)

Table 49. Factorial ANOVA of ammonium pot experiment – adjusted weight gain (g)

Factorial statistics	After 2 hours		After 24 hours		After 7 days			After 14 days		Average	
Time											
2 hours										1.05[a]	< 0.001
24 hours										1.13[a]	
7 days										0.87[b]	
14 days										0.44[c]	
Control vs. 200 N vs. 300 N vs. digestate											
Untreated control	1.29[a]	0.02	1.17	NS (0.74)	0.83	NS (0.12)		0.88	NS (0.08)	1.01[a]	0.04
200 kg N/ha control	1.30[a]		1.07		0.69			0.54		0.90[ab]	
300 kg N/ha control	1.11[b]		1.03		0.71			0.39		0.81[b]	
Digestate	1.01[b]		1.14		0.90			0.40		0.86[b]	
Ammonium rate											
Unamended (4 kg/m^3)	1.05	NS (0.23)	1.13	NS (0.83)	1.01[a]	0.002		0.54[a]	0.01	0.93[a]	<0.001
Enhanced (6 kg/m^3)	0.97		1.14		0.80[b]			0.25[b]		0.80[b]	
pH											
6-6.5	1.08	NS (0.29)	1.29[a]	0.004	0.88	NS (0.83)		0.43	NS (0.28)	0.92[a]	0.02
7-7.5	0.99		1.11[b]		0.92			0.47		0.87[ab]	
8.5	0.96		1.01[b]		0.91			0.28		0.79[b]	
Application rate											
30 m^3/ha	1.05	NS (0.31)	1.15	NS (0.73)	0.94	NS (0.22)		0.49[a]	0.05	0.91[a]	0.012
50 m^3/ha	0.98		1.12		0.86			0.29[b]		0.81[b]	

[a,b,c,d] Different letters within a column indicate significant differences between treatments (P<0.05)

Table 50. Factorial ANOVA of ammonium pot experiment – number of live earthworms

Factorial statistics	After 2 hours		After 24 hours		After 7 days		After 14 days		Average	
Time										
2 hours									6.20^{ab}	0.037
24 hours									6.30^{a}	
7 days									6.27^{a}	
14 days									5.80^{b}	
Control vs. 200 N vs. 300 N vs. digestate										
Untreated control	7.00^{a}	0.01	7.00^{a}	0.05	7.00^{a}	0.03	7.00	NS (0.07)	7.00^{a}	< 0.001
200 kg N/ha control	7.00^{a}		7.00^{a}		6.75^{ab}		6.75		6.88^{a}	
300 kg N/ha control	7.00^{a}		6.50^{ab}		7.00^{a}		7.00		6.88^{a}	
Digestate	6.00^{b}		6.17^{b}		6.10^{b}		5.52		5.95^{b}	
Ammonium rate										
Unamended (4 kg/m^3)	6.03	NS (0.74)	6.38	NS (0.07)	6.25	NS (0.23)	5.54	NS (0.93)	6.05	NS (0.17)
Enhanced (6 kg/m^3)	5.96		5.96		5.96		5.50		5.84	
pH										
6-6.5	6.00	NS (0.47)	6.38	NS (0.17)	6.06	NS (0.89)	5.56	NS (0.20)	6.00	NS (0.29)
7-7.5	6.19		5.88		6.19		6.00		6.06	
8.5	5.81		6.25		6.06		5.00		5.78	
Application rate										
30 m^3/ha	6.71^{a}	< 0.001	6.79^{a}	< 0.001	6.54^{a}	< 0.001	6.25^{a}	0.002	6.57^{a}	< 0.001
50 m^3/ha	5.29^{b}		5.54^{b}		5.67^{b}		4.79^{b}		5.32^{b}	

[a,b,c,d] Different letters within a column indicate significant differences between treatments (*P*<0.05)

The application rate, ammonium concentration, ammonium loading rate and pH of the various treatments are shown in Table 51. Regression analysis was undertaken on the earthworm weight change, adjusted weight change and live earthworm data using application rate, ammonium concentration, ammonium loading and pH as factors, to investigate which parameter had the greatest effect, Table 52. From this it can be seen that although application rate, ammonium concentration and to a lesser extent pH affected earthworm survival/heath, it was ammonium loading rate (i.e. the quantity of ammonium-N applied to the pots) which had the strongest effect, accounting for 46% of the variation in weight change and 29% of the variation in number of live earthworms. For adjusted weight change ammonium-N loading also had the greatest effect, accounting for 53% of the variation in weight change. Time was also an important factor for this parameter reflecting the changes in total number of earthworms over time. Adding pH to the ammonium-N concentration did increase the accuracy of the prediction, however, it was only very slightly (1% improvement in the variance accounted for), which suggests pH only has a small influence on the effect of ammonium-N loadings on earthworms.

Table 51. Ammonium-N details of the ammonium-N pot experiments

Treatment	**Application rate (m^3/ha)**	**Ammonium concentration (kg/m^3)**	**Ammonium loading (kg NH_4/ha)**	**pH**
Untreated Control	50	0	0	6
Fertiliser Control at 200 kg N/ha	50	2	100	6
Fertiliser Control at 300 kg N/ha	50	3	150	6
Unamended digestate pH <7 applied at 50 m^3/ha	50	4	200	6.5
Unamended digestate pH ≥7 – <8 applied at 50 m^3/ha	50	4	200	7.5
Unamended digestate pH ≥8 applied at 50 m^3/ha	50	4	200	8.5
Unamended digestate pH <7 applied at 30 m^3/ha	30	4	120	6.5
Unamended digestate pH ≥7 – <8 applied at 30 m^3/ha	30	4	120	7.5
Unamended digestate pH ≥8 applied at 30 m^3/ha	30	4	120	8.5
Enhanced digestate pH <7 applied at 50 m^3/ha	50	6	300	6.5
Enhanced digestate pH ≥7 – <8 applied at 50 m^3/ha	50	6	300	7.5
Enhanced digestate pH ≥8 applied at 50 m^3/ha	50	6	300	8.5
Enhanced digestate pH <7 applied at 30 m^3/ha	30	6	180	6.5
Enhanced digestate pH ≥7 – <8 applied at 30 m^3/ha	30	6	180	7.5
Enhanced digestate pH ≥8 applied at 30 m^3/ha	30	6	180	8.5

Table 52. Regression analysis of factors effecting earthworms (% of variance accounted for & *p*-value)

Parameter	Weight change	Adjusted weight change	Live earthworms
Time	18% ($P<0.001$)	45% ($P<0.001$)	2% ($P=0.08$)
Time+Application rate	25% ($P<0.001$)	45% ($P=0.134$)	9% ($P<0.001$)
Time + Ammonium concentration	26% ($P<0.001$)	51% ($P<0.001$)	9% ($P<0.001$)
Time + Ammonium loading	46% ($P<0.001$)	53% ($P<0.01$)	29% ($P<0.001$)
Time + pH	28% ($P<0.001$)	48% ($P<0.001$)	10% ($P=0.07$)
Time + Ammonium loading + pH	47% ($P<0.001$)	54% ($P<0.001$)	29% ($P<0.001$)

Soil samples were taken from the pots after one day and fourteen days and sent for analysis, the results are displayed in Tables 53 and 54. There were no treatment differences other than those that would be expected (e.g. no VFAs were measured due to their high volatility, higher ammonium-N concentrations were measured on pots treated with digestate and higher pH's where the high pH digestate was applied). The ratio of ammonium-N to nitrate-N in the soils gives an indication of the rate of nitrification (an aerobic soil process whereby ammonium-N is converted to nitrate-N). After one day, nitrate concentrations were lower where 50 m^3/ha digestate had been applied, compared to the 30 m^3/ha application and the untreated control (note the fertiliser control's N source was ammonium nitrate, so nitrate levels were elevated at the outset). This suggested an apparent anoxic effect from digestate. However, there was clear evidence of nitrification by day fourteen suggesting the anoxia was only temporary, occurring immediately following digestate application. Given that the BOD of food-based digestate is lower and no more immediate than for cattle slurry, it is highly probable that the same would apply for cattle slurry, as such it is most likely that this short-term anoxia is not the cause of the increased earthworm mortality associated with digestate applications. For full results see Appendix 4.7.

Table 53. Ammonium-N experiment pot test soil analysis results after 24 hours.

Treatment	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-Caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-Butyric acid (mg/l)	N-Butyric acid (mg/l)	Iso-Valeric acid (mg/l)	N-Valeric acid (mg/l)	Iso-Caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	84.0	7.48	0.73	14.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
200 kg N/ha fertiliser control	83.2	7.05	95.2	143	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
300 kg N/ha fertiliser control	83.4	7.33	247	266	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Unamended ammonium-N													
pH <7 applied at 50 m^3/ha	84.2	7.72	402	3.96	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 50 m^3/ha	85.1	8.04	290	13.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 50 m^3/ha	84.2	8.50	337	5.32	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 30 m^3/ha	84.4	7.52	257	17.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 30 m^3/ha	85.1	7.94	214	20.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 30 m^3/ha	85.8	8.02	169	26.5	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced ammonium-N													
pH <7 applied at 50 m^3/ha	84.3	7.52	577	5.24	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 50 m^3/ha	82.9	8.06	407	7.99	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 50 m^3/ha	84.2	8.46	391	14.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 30 m^3/ha	86.6	7.49	342	23.3	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 30 m^3/ha	85.8	7.57	270	25.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 30 m^3/ha	85.3	8.16	277	18.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

Table 54. Ammonium-N experiment pot test soil analysis results after 14 days.

Treatment	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-Caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-Butyric acid (mg/l)	N-Butyric acid (mg/l)	Iso-Valeric acid (mg/l)	N-Valeric acid (mg/l)	Iso-Caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	88.2	7.05	1.24	44.8	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
200 kg N/ha fertiliser control	88.1	6.61	75.3	230	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
300 kg N/ha fertiliser control	88.55	6.65	190	319	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
Unamended ammonium-N													
pH <7 applied at 50 m^3/ha	85.2	6.97	286	96.8	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥7 – <8 applied at 50 m^3/ha	86.8	6.81	198	154	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥8 applied at 50 m^3/ha	86.4	7.16	169	148	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH <7 applied at 30 m^3/ha	88.9	6.65	104	102	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥7 – <8 applied at 30 m^3/ha	88.2	6.61	81.7	147	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥8 applied at 30 m^3/ha	88.7	6.94	110	118	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
Enhanced ammonium-N													
pH <7 applied at 50 m^3/ha	87.0	7.05	486	89.9	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥7 – <8 applied at 50 m^3/ha	87.5	7.03	331	125	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥8 applied at 50 m^3/ha	86.2	7.42	313	97.1	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH <7 applied at 30 m^3/ha	87.3	6.43	379	98.5	<20	<50	58	<12.5	<12.5	<25	<25	<20	82.5
pH ≥7 – <8 applied at 30 m^3/ha	88.1	6.66	209	107	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
pH ≥8 applied at 30 m^3/ha	87.0	6.85	198	99.0	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80

4.3.2 Acetic acid experiment

The results from the acetic acid experiment were less clear cut, there was a statistically significant difference ($p<0.05$) between the control and the food-based digestate treatments, in earthworm weight (with a decrease on the digestate treatments in comparison to the control) and survival (an increase in mortality on the digestate treatments) (Tables 55 – 59). However, the lack of a dose response relationship (i.e. increasing concentrations of acetic acid did not increase weight loss) suggests that acetic acid is unlikely to be responsible for earthworm weight loss and that some other property (e.g. ammonium-N) of the digestate was primarily responsible for the decrease in weight and increase in mortality observed. For full results see Appendix 4.8.

As per the ammonium-N experiments, soil samples were taken after one day and fourteen days and submitted to a laboratory for analysis, Tables 60 and 61. For full results see Appendix 4.9. There were no treatment differences other than those that would be expected (e.g. no VFAs were measured due to their high volatility). As per the ammonium-N pot experiments, the soil results showed an anoxic effect from digestate after one day, however, this effect was not evident after fourteen days, again suggesting it was a short-term effect following digestate application. But again as per the ammonium-N experiments, given the lower and no more immediate BOD of food-based digestate than cattle slurry, it is highly probable that the same would apply for cattle slurry, and therefore this is not likely to be the key factor effecting earthworm mortality.

Table 55. Acetic acid pot test results after 2 hours.

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	18.8	27.8	9.0[b]	48	1.29	48	7.0[c]	100
Un-amended Digestate (*c.*1,000 mg/l)	17.9	18.3	0.4[a]	2	1.11	43	5.0[ab]	71
Digestate + VFA level 1 (*c.*2,500 mg/l)	18.3	16.4	-1.9[a]	-12	1.26	48	4.3[a]	61
Digestate + VFA level 2 (*c.*3,500 mg/l)	18.0	20.3	2.3[a]	14	0.85	33	6.0[abc]	86
Digestate + VFA level 3 (*c.*5,000 mg/l)	17.5	21.2	3.7[ab]	20	0.85	34	6.3[bc]	89
P Statistic	-	-	0.023		NS (0.07)		0.04	

[1] weight gain = post-test weight minus pre-test weight
[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot
[a,b,c,d] Different letters within a column indicate significant differences between treatments (P<0.05)

Table 56. Acetic acid pot test results after 24 hours.

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	16.7	24.9	8.18[c]	49	1.17	49	7.0[c]	100
Un-amended Digestate (*c.*1,000 mg/l)	18.2	21.6	3.46[abc]	20	1.03	40	6.0[abc]	86
Digestate + VFA level 1 (*c.*2,500 mg/l)	16.7	16.2	-0.52[a]	-4	0.98	41	4.8[a]	68
Digestate + VFA level 2 (*c.*3,500 mg/l)	16.3	19.5	3.17[ab]	20	1.16	50	5.6[ab]	80
Digestate + VFA level 3 (*c.*5,000 mg/l)	16.1	22.6	6.45[bc]	40	1.18	51	6.5[bc]	93
P Statistic	-	-	0.009		NS (0.23)		0.01	

[1] weight gain = post-test weight minus pre-test weight
[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot
[a,b,c,d] Different letters within a column indicate significant differences between treatments (P<0.05)

Table 57. Acetic acid pot test results after 7 days.

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	15.5	21.3	5.79	37	0.83	37	7.0	100
Un-amended Digestate (*c.*1,000 mg/l)	16.3	17.9	1.62	10	0.99	42	5.5	79
Digestate + VFA level 1 (*c.*2,500 mg/l)	17.0	19.3	2.27	16	0.81	34	6.0	86
Digestate + VFA level 2 (*c.*3,500 mg/l)	17.6	19.0	1.40	5	1.21	48	5.0	71
Digestate + VFA level 3 (*c.*5,000 mg/l)	19.0	18.8	-0.19	0	1.24	46	4.8	68
P Statistic	-	-	NS (0.60)		NS (0.21)		NS (0.22)	

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

Table 58. Acetic acid pot test results after 14 days.

Treatment	Pre-test weight (g)	Post-test weight (g)	Weight gain[1]		Adjusted weight gain[2]		Number of live earthworms	Percentage live earthworms
			g	%	g	%		
Untreated control	17.9	24.1	6.19[b]	35	0.88[b]	35	7.0	100
Un-amended Digestate (*c.*1,000 mg/l)	18.6	10.7	-7.96[a]	-43	0.12[a]	5	3.8	54
Digestate + VFA level 1 (*c.*2,500 mg/l)	18.2	12.1	-6.14[a]	-32	-0.001[a]	1	4.8	68
Digestate + VFA level 2 (*c.*3,500 mg/l)	16.7	13.9	-2.81[a]	-18	0.20[a]	8	5.3	75
Digestate + VFA level 3 (*c.*5,000 mg/l)	16.6	8.8	-7.87[a]	-48	-0.20[a]	-8	4.0	57
P Statistic	-	-	0.024		0.002		NS (0.28)	

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments ($P<0.05$)

Table 59. Acetic acid pot test results averaged across all time periods.

Treatment	Weight gain (g)[1]				Adjusted weight gain (g)[2]				Number of live earthworms			
	2 hours	**24 hours**	**7 days**	**14 days**	**2 hours**	**24 hours**	**7 days**	**14 days**	**2 hours**	**24 hours**	**7 days**	**14 days**
Untreated control	9.00	8.18	5.79	6.19	1.29	1.17	0.83	0.88	7.00	7.00	7.00	7.00
Un-amended Digestate (*c*.1,000 mg/l)	0.37	3.45	1.62	-7.96	1.11	1.03	0.99	0.13	5.00	6.00	5.50	3.75
Digestate + VFA level 1 (*c*.2,500 mg/l)	-1.94	-0.52	2.27	-6.14	1.26	0.98	0.81	-0.001	4.25	4.75	6.00	4.75
Digestate + VFA level 2 (*c*.3,500 mg/l)	2.30	3.17	1.40	-2.81	0.85	1.16	1.21	0.20	6.00	5.62	5.00	5.25
Digestate + VFA level 3 (*c*.5,000 mg/l)	3.69	6.45	-0.19	-7.87	0.85	1.18	1.24	-0.20	6.25	6.50	4.75	4.00
P Statistic (time	<0.001				<0.001				0.167			
P Statistic (treatment)	<0.001				0.023				0.001			

[1] weight gain = post-test weight minus pre-test weight

[2] adjusted weight gain = post-test weight divided by number live earthworms minus pre-test weight divided by number of earthworms in the pot

[a,b,c,d] Different letters within a column indicate significant differences between treatments (P<0.05)

Table 60. Acetic acid experiment pot test soil analysis results after 24 hours.

Treatment	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-butyric acid (mg/l)	N-butyric acid (mg/l)	Iso-valeric acid (mg/l)	N-valeric acid (mg/l)	Iso-caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	84.0	7.48	0.73	14.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Un-amended Digestate (*c*.1,000 mg/l)	84.2	8.50	337	5.32	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Digestate + VFA level 1 (*c*.2,500 mg/l)	84.2	8.52	345	6.16	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Digestate + VFA level 2 (*c*.3,500 mg/l)	84.1	8.43	276	12.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Digestate + VFA level 3 (*c*.5,000 mg/l)	85.2	8.62	356	5.53	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

Table 61. Acetic acid experiment pot test soil analysis results after 14 days.

Treatment	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-butyric acid (mg/l)	N-butyric acid (mg/l)	Iso-valeric acid (mg/l)	N-valeric acid (mg/l)	Iso-caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	88.2	7.05	1.24	44.8	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
Un-amended Digestate (*c*.1,000 mg/l)	86.4	7.16	169	148	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
Digestate + VFA level 1 (*c*.2,500 mg/l)	86.4	7.44	243	94.4	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
Digestate + VFA level 2 (*c*.3,500 mg/l)	87.3	7.09	151	119	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80
Digestate + VFA level 3 (*c*.5,000 mg/l)	85.8	7.29	200	115	< 20	< 50	< 50	< 12.5	< 12.5	< 25	< 25	< 20	< 80

5 Discussion

The literature review previously undertaken highlighted a number of factors that could have been responsible for the effects on earthworm survival observed in the field experiments (i.e. pH, BOD, VFAs, ammonium-N; conductivity; Table 2). Based on the results of the laboratory experiments, the likely influence of each of these factors is discussed below along with thoughts on potential mitigation methods, where applicable.

5.1 Conductivity

The electrical conductivity of food-based digestate was found to be higher than that of cattle slurry (Table 3). However, the laboratory solution contact tests (using both potassium chloride and sodium chloride) found that conductivity even in the extreme environment of the contact tests (up to 12,000 µS/cm, almost double the highest concentration found in food-based digestate), had no effect on earthworm mortality but did impact on earthworm health (i.e. there was a statistically significant increase in weight loss at higher conductivity). It is likely this was a result of water loss from the earthworms, due to the osmotic potential of the high conductivity solutions.

Given that the laboratory experiments indicated that increased conductivity alone did not increase earthworm mortality, it is clear that the higher conductivity of food-based digestate cannot be the principal cause of the increase in earthworm mortality observed in the field following food-based digestate applications.

5.2 BOD

Earthworms are known to be sensitive to BOD (i.e. they cannot survive in a depleted oxygen environment); however, the BOD of food-based digestate was shown to be lower than for cattle slurry, with no difference in the BOD profile over the five days of the laboratory test (Table 3).

Given the information from the additional laboratory analysis, it is clear that BOD cannot be the cause of the increase in earthworm mortality observed in the field following food-based digestate applications.

5.3 VFAs

The concentration of VFAs in food-based digestate were on average lower than in cattle slurry, however, they were much more variable (Table 3). The variability most likely reflects differences in AD feedstocks and mean average retention times.

Two VFAs (acetic acid and propionic acid) were selected for the laboratory solution contact tests; acetic acid (at pH's ranging from 3.6 at 500 mg/l to 2.4 at 50,000mg/l) had a negative effect on earthworm survival from concentrations of *c.*4,500 mg/l and above while propionic acid (at 5000mg/l and pH 3.1) had no effect on earthworm survival. However, 4,500 mg/l of acetic acid is well in excess of the typical level found in food-based digestates (Table 3).

Ranking the results from the contact tests undertaken with five different food-based digestates, found that VFAs (including acetic acid) had a weak relationship with earthworm survival; but due to the nature of the data it was not possible to undertake statistical assessment of these results.

The results from the acetic acid pot studies showed that the application of food-based digestate did effect earthworm survival (i.e. increased earthworm mortality) and health (i.e. reduced earthworm weight gain), however, increasing the acetic acid concentration of the food-based digestate did not increase the detrimental effects ($P>0.05$ in Duncans Analysis). It is therefore likely that the results seen in the contact tests, were a function of the extreme environment of the contact tests, as well as the elevated acetic acid concentrations.

Based on the results from the laboratory experiments (i.e. no effect of acetic acid in the pot studies, the weak relationship between VFAs and earthworm mortality in the digestate contact tests, and the elevated concentration of acetic acid required in the laboratory solution contact tests to cause mortality), it is clear that VFAs are not the cause of the lower earthworm numbers following food-based digestate applications observed in the field.

5.4 Ammonium-N and pH

Due to the elevated ammonium-N concentration of food-based digestate, particularly in comparison with cattle slurry (Table 3), ammonium-N was highlighted in the literature review as the most likely causal factor, particularly as earthworms are known to be sensitive to ammonium-N/ammonia-N. The interaction between ammonium-N and pH was also considered a possible factor, given the conversion between ammonium and ammonia is pH dependant (along with temperature, moisture etc.).

The laboratory solution contact tests confirmed that ammonium-N did effect earthworm survival and health (weight). There were differences between the earthworm responses at pH 6 and pH 9, with the experiments undertaken at pH 9 showing a greater earthworm response at lower ammonium-N concentrations in comparison with pH 6, however, the differences were not as marked as may have been expected.

Ranking the results from the contact tests using five different food-based digestates based on ammonium-N, showed good correlation with earthworm mortality (Table 32). But due to the nature of the data, it was not possible to undertake statistical assessment of these results.

The ammonium-N pot studies identified that food-based digestate had a negative effect on earthworm survival. Food-based digestate with a higher ammonium-N concentration (i.e. 6 kg NH_4-N/m^3) had a significant effect on earthworm weight (Tables 48 & 49) and a numeric effect on earthworm survival (Tables 50) and the application rate (i.e. 50 m^3/ha compared to 30 m^3/ha) of the applied food-based digestate had a significant effect on both earthworm weight and survival (Tables 48 – 50). The pH of the applied digestate did not have a consistent statistical effect on the earthworms (it was significant for certain factors at some time periods but not significant across all) although there was a numeric decrease in mortality and increase in weight gain at the lower pH.

This suggests the form of ammonium-N (i.e. whether it is predominantly as ammonium at the lower pH, or ammonia at the higher pH) may not be an important factor. The regression analysis undertaken demonstrated that total ammonium-N loading (which is a function of both the ammonium-N concentration and application rate) explained the greatest percentage of the variability in the data. This suggests that controlling ammonium-N loading would be an effective method to ensure food-based digestate applications do not unduly effect the earthworm population.

6 Conclusions

The laboratory experiments considered a number of different factors that were identified in the literature as having the potential to negatively affect earthworms. The results from the organic material analysis, contact tests and pot studies ruled out conductivity, BOD and VFAs as being the cause of the lower earthworm population observed in the field following the application of food-based digestate. But, ammonium-N was found to have a significant effect on earthworm survival and health.

Statistical analysis of the results from the pot studies showed that ammonium-N loadings most strongly explained the negative effects observed. Therefore any guidance/future field experiments should focus on ammonium-N loadings as a potential mitigation measure to ensure food-based digestate applications do not unduly effect earthworm populations.

The laboratory experiments undertaken were important and valuable in understanding the causal factors and the effects of food-based digestate on earthworms. However, due to the worst-case nature of the pot studies (and particularly contact tests) and the fact they do not accurately simulate conditions in the field, it was not possible to derive a maximum ammonium-N loading.

Targeted field experiments would be required to confirm these findings under field conditions in order to derive maximum ammonium-N loadings.

7 References

Defra (2009). Protecting our Water, Soil and Air: a Code of Good Agricultural Practice for Farmers, Growers and Land Managers. Department for Environment, Food and Rural Affairs. ISBN 9780112432845. Stationery Office, Norwich.

OECD (1984). Guideline for Testing of Chemicals, No. 207, Earthworm Acute Toxicity. Organisation for Economic Cooperation Development, Paris, France.

Appendix 4.1. Raw conductivity contact test results

Raw potassium chloride contact test results

Factor	Replicate Number	Score after 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Earthworm (g) pre-test	Earthworm (g) post-test
Control (F0)	1	1	1	1	1	1	1	1.657	1.273
	2	1	1	1	1	1	1	1.349	0.895
	3	1	1	1	1	1	1	1.644	1.539
	4	1	1	1	1	1	1	1.161	0.823
	5	1	1	4	4	4	4	1.475	1.261
	6	1	1	1	1	1	1	1.971	1.659
	7	1	1	1	1	1	1	1.361	1.165
	8	1	1	1	1	1	1	1.495	1.624
	9	1	1	1	1	1	1	1.404	1.254
	10	1	1	1	1	1	1	1.801	1.675
2,000 µS/cm (F1)	1	1	1	1	1	1	1	1.206	1.193
	2	1	1	1	1	1	1	1.023	0.889
	3	1	1	1	1	1	1	1.109	1.094
	4	1	1	1	1	1	1	1.157	1.083
	5	1	1	1	1	1	1	1.502	1.423
	6	1	1	1	1	1	1	1.708	1.518
	7	1	1	1	1	1	1	1.166	0.967
	8	1	1	1	1	1	1	1.453	1.452
	9	1	1	1	1	1	1	1.341	1.084
	10	1	1	1	1	1	1	1.263	1.476
5,000 µS/cm (F2)	1	1	1	1	1	1	1	1.795	1.554
	2	1	1	1	1	1	1	1.809	0.641
	3	1	1	1	1	1	1	1.050	0.755
	4	1	1	1	1	1	1	1.671	1.321
	5	1	1	1	1	1	1	1.946	2.032
	6	1	1	1	1	1	1	1.219	1.116
	7	1	1	1	1	1	1	0.827	0.618
	8	1	1	1	1	1	1	1.283	1.044
	9	1	1	1	1	1	1	0.940	0.768
	10	1	1	1	1	1	1	1.363	1.2658

7,500 µS/cm (F3)	1	1	1	1	1	1	1	1.840	1.333
	2	1	1	1	1	1	1	1.470	1.007
	3	1	1	1	1	1	1	1.477	1.165
	4	1	1	1	1	1	1	1.584	1.061
	5	1	1	4	4	4	4	1.985	1.580
	6	1	1	1	1	1	1	1.489	1.129
	7	1	1	1	1	1	1	1.353	1.009
	8	1	1	1	1	1	1	1.398	0.954
	9	1	1	1	1	1	1	1.133	0.797
	10	1	1	1	1	1	1	1.162	0.981
10,000 µS/cm (F4)	1	1	1	1	1	1	1	1.556	1.042
	2	1	1	1	1	1	1	1.522	0.970
	3	1	1	1	1	1	1	1.481	1.159
	4	1	1	1	1	1	1	1.592	1.044
	5	1	1	1	1	1	1	0.974	0.576
	6	1	1	1	1	1	1	1.518	1.002
	7	1	1	1	1	1	1	0.990	0.622
	8	1	1	1	1	1	1	1.904	1.379
	9	1	1	1	1	1	1	1.935	1.406
	10	1	1	1	1	1	1	1.371	1.004
12,000 µS/cm (F5)	1	1	1	1	1	1	1	1.270	0.713
	2	1	1	1	1	1	5	1.243	0.791
	3	1	1	1	1	1	1	0.811	0.459
	4	1	1	1	1	1	1	0.661	0.503
	5	1	3	1	1	1	1	1.633	1.064
	6	1	3	1	1	1	1	1.657	1.160
	7	1	1	1	1	1	1	0.869	0.450
	8	1	1	1	1	1	1	0.772	0.506
	9	1	1	1	1	1	1	0.872	0.589
	10	1	1	1	1	1	1	1.572	1.302

Notes:

- 100 good earthworms set up to void their gut contents. 60 good earthworms selected for the Conductivity contact test.
- Start of test. Controls no writhing/coiling, didn't seem stressed.
- Start of test. Factor 1 no writhing/coiling, didn't seem stressed.
- Start of test. Factor 2 no writhing/coiling, didn't seem stressed.
- Start of test. Factor 3 no writhing/coiling, didn't seem stressed.
- Start of test. Factor 4 no writhing/coiling, didn't seem stressed.
- Start of test. Factor 5 no writhing/coiling, didn't seem stressed.
- All became slow/still within the one hour assessment period. Didn't seem bothered by any of the chemical at all factors.
- Worms started moving again when the light went on during the assessment period.

Raw sodium chloride contact test results

Factor	Replicate Number	Score after 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Earthworm (g) pre-test	Earthworm (g) post-test
Control (F0)	1	1	1	1	1	1	1	1.435	0.931
	2	1	1	1	1	1	1	1.213	0.784
	3	1	1	1	1	1	1	1.400	0.959
	4	1	1	1	1	1	1	1.813	1.375
	5	1	1	1	1	1	1	1.494	1.052
	6	1	1	1	1	1	1	1.233	0.768
	7	1	1	1	1	1	1	1.189	0.765
	8	1	1	1	1	1	4,5	1.137	0.706
	9	1	1	1	1	1	1	1.244	0.736
	10	1	1	1	1	1	1	1.203	0.824
10,000 µS/cm (F4)	1	1	1	1	1	1	1	2.106	1.506
	2	1	1	1	1	1	1	2.010	1.340
	3	1	1	1	1	1	1	1.425	0.871
	4	1	1	1	1	1	1	1.145	0.597
	5	1	1	1	1	1	1	1.496	0.880
	6	1	1	1	1	1	1	2.009	1.466
	7	1	1	1	1	1	1	1.720	1.215
	8	1	1	1	1	1	1	1.540	1.087
	9	1	1	1	1	1	1	1.677	1.203
	10	1	1	1	1	1	1	1.347	0.969

Notes:

- 60 good earthworms set up to void their gut contents. 30 good earthworms selected for the Single Assessment contact test.
- Start of test. Controls no writhing or coiling, didn't seem stressed. No reaction.
- Start of test. Conductivity Factor 4 no writhing or coiling, similar reaction to the controls although a little bit more active. No reaction.
- The conductivity samples seem a little bit more active than the controls within the 1hr assessment period. At 1hr the controls and conductivity samples had settled and therefore no reaction.

Appendix 4.2. Raw VFA contact test results

Raw acetic acid contact test results

Factor	Replicate Number	Score after 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Earthworm (g) pre-test	Earthworm (g) post-test
Control (F0)	1	1	1	1	1	1	1	1.422	1.331
	2	1	1	1	1	1	1	1.547	1.333
	3	1	1	1	1	1	1	1.215	0.925
	4	1	1	1	1	4,5	-	1.135	0.800
	5	1	1	1	1	1	1	1.710	1.526
	6	1	1	1	1	1	1	1.351	1.917
	7	1	1	1	1	1	1	1.408	0.913
	8	1	1	1	1	1	1	1.324	1.271
	9	1	1	1	1	1	1	1.268	0.937
	10	1	1	1	1	1	1	1.449	1.386
	11	1	1	1	1	1	1	1.994	1.548
	12	1	1	1	1	1	1	1.055	0.676
	13	1	1	1	1	1	1	2.201	1.711
	14	1	1	1	1	1	1	1.566	1.115
	15	1	1	1	1	1	1	1.122	0.624
	16	1	1	1	1	1	1	1.678	1.171
	17	1	1	1	1	1	1	1.184	0.737
	18	1	1	1	1	1	1	2.735	2.041
	19	1	1	1	1	1	1	1.063	0.510
	20	1	1	1	1	1	1	2.278	1.784
500 mg/l (F1)	1	1	1	1	1	1	1	1.895	1.705
	2	1	1	1	1	1	1	1.410	1.309
	3	1	1	1	4	1	1	1.776	1.689
	4	1	1	1	1	1	1	1.423	1.386
	5	1	1	1	1	1	1	1.480	1.423
	6	1	1	1	1	1	1	1.077	0.780
	7	1	1	1	1	1	1	1.724	1.770
	8	1	1	1	1	1	1	0.926	0.818
	9	1	1	1	1	1	1	1.324	1.292
	10	1	1	1	1	1	1	1.158	0.952

	11	1	1	1	1	1	1	1.370	0.868
	12	1	1	1	1	1	1	1.479	0.908
	13	1	1	1	1	1	1	1.104	0.588
	14	1	1	1	1	1	1	1.420	0.834
	15	1	1	1	1	1	1	1.291	0.894
	16	1	1	1	1	1	1	1.761	1.229
	17	1	1	1	1	1	1	1.456	1.056
	18	1	1	1	1	1	1	1.567	1.082
	19	1	1	1	1	1	1	1.085	0.600
	20	1	1	1	1	1	1	1.099	0.637
1,000 mg/l (F2)	1	1	1	1	1	1	1	1.485	0.938
	2	1	1	1	1	1	1	2.392	1.948
	3	1	1	1	1	1	1	2.042	1.570
	4	1	1	1	1	1	1	1.605	1.197
	5	1	1	1	1	1	1	1.100	0.505
	6	1	1	4, 1	4, 5	-	-	0.839	0.621
	7	1	1	1	1	1	1	2.097	1.578
	8	1	1	1	1	1	1	1.171	0.734
	9	1	1	1	1	1	1	1.511	1.046
	10	1	1	1	1	1	1	1.663	1.160
1,500 mg/l (F3)	1	1	1	1	1	1	1	1.236	0.586
	2	1	1	1	1	1	1	3.048	2.491
	3	1	1	1	1	1	1	1.524	0.929
	4	1	1	1	1	1	1	1.386	0.833
	5	1	1	1	1	1	1	1.771	1.163
	6	1	1	1	1	1	1	2.279	1.861
	7	1	1	1	1	1	1	1.043	0.513
	8	1	1	1	1	1	1	2.332	1.739
	9	1	1	1	1	1	1	2.465	2.053
	10	1	1	1	1	1	1	2.068	1.536

2,500 µS/cm (F4)	1	1	1	1	1	1	1	1.117	0.339
	2	1	1	1	1	1	1	1.507	0.921
	3	1	1	1	1	1	1	1.698	1.290
	4	1	1	1	1	1	1	1.414	0.974
	5	1	1	1	1	1	1	1.362	0.854
	6	1	4, 1	1	1	1	1	1.378	0.840
	7	1	1	1	1	1	1	1.044	0.544
	8	1	1	1	1	1	1	0.936	0.549
	9	1	1	1	1	1	1	1.857	1.457
	10	1	1	1	1	1	1	1.190	0.672
3,500 mg/l (F5)	1	1	1	1	1	1	1	1.787	1.265
	2	1	1	1	1	1	1	0.890	0.357
	3	1	1	1	1	1	1	1.817	1.374
	4	1	1	1	1	4, 1	1	1.308	0.906
	5	1	1	1	1	1	1	1.923	1.528
	6	1	1	1	1	4, 1	4, 1	1.628	1.105
	7	1	1	1	1	1	1	2.632	2.045
	8	1	1	1	1	1	1	1.564	1.085
	9	1	1	1	1	1	1	1.651	1.258
	10	1	1	1	1	1	1	1.061	0.382
4,500 mg/l (F6)	1	1, 3	1	1	1	1	1	2.344	1.749
	2	1, 3	1	1	1	1	1	1.581	0.766
	3	1, 3	1	1	1	1	1	1.661	1.058
	4	1, 3	1	4	4	5	-	1.242	0.870
	5	1, 3	1	1	1	1	1	1.953	1.465
	6	1, 3	1	1	1	1	1	1.211	0.784
	7	1, 3	1	1	1	1	1	2.109	1.687
	8	1, 3	1	1	1	1	1	2.492	2.002
	9	1, 3	1	1	1	1	1	2.444	1.961
	10	1, 3	1	3	4	4, 1	5	1.854	1.409

5,000 mg/l (F7)	1	2	1	4,5				1.230	0.877
	2	2	1	1	4	4	5	1.668	1.076
	3	2	1	1	1	1	1	1.185	1.002
	4	2	1	1	1	1	1	2.147	1.879
	5	2,5	-	-	-	-	-	1.480	1.390
	6	2	1	1	4	4	5	1.340	0.730
	7	2	1	1	4	4	5	0.975	0.611
	8	2,3	1	1	1	1	1	1.409	0.988
	9	2	1	1	1	1	1,4	1.062	0.769
	10	2	4,5	-	-	-	-	1.167	1.028
	11	2, 3	1	1	1	1	1	2.153	1.587
	12	2, 3	1	1	1	5	-	1.476	1.118
	13	2, 3	1	5	-	-	-	1.057	0.826
	14	2, 3	1	1	1	1	1	2.230	1.853
	15	2, 3	1	4	4	5	-	1.298	0.917
	16	2, 3	1	1	1	1	1	1.436	0.909
	17	2, 3	1	1	1	1	1	2.679	2.113
	18	2, 3	1	1	1	1	1	1.868	1.324
	19	2, 3	1	1	1	1	1	1.909	1.257
	20	2, 3	1	1	1	1	1	1.684	1.217
10,000 mg/l (F8)	1	2	5	-	-	-	-	1.385	1.179
	2	2,5	-	-	-	-	-	1.020	0.931
	3	2,5	-	-	-	-	-	1.019	0.884
	4	2,5	-	-	-	-	-	1.079	0.981
	5	2,5	-	-	-	-	-	1.577	1.483
	6	2,5	-	-	-	-	-	1.313	1.202
	7	2,5	-	-	-	-	-	1.563	1.379
	8	2	5	-	-	-	-	1.388	1.181
	9	2,5	-	-	-	-	-	1.416	1.270
	10	2,5	-	-	-	-	-	1.381	1.300

20,000 mg/l (F9)	1	2,5	-	-	-	-	-	1.239	1.099
	2	2,5	-	-	-	-	-	1.993	1.778
	3	2,5	-	-	-	-	-	1.592	1.450
	4	2,5	-	-	-	-	-	1.702	1.494
	5	2,5	-	-	-	-	-	1.121	1.087
	6	2,5	-	-	-	-	-	1.370	1.236
	7	2,5	-	-	-	-	-	1.343	1.232
	8	2,5	-	-	-	-	-	1.025	0.953
	9	2,5	-	-	-	-	-	1.171	1.049
	10	2,5	-	-	-	-	-	1.142	1.050
50,000 mg/l (F10)	1	2,5	-	-	-	-	-	1.455	1.261
	2	2,5	-	-	-	-	-	1.256	1.116
	3	2,5	-	-	-	-	-	1.294	1.184
	4	2,5	-	-	-	-	-	1.115	1.067
	5	2,5	-	-	-	-	-	1.225	1.211
	6	2,5	-	-	-	-	-	1.316	1.221
	7	2,5	-	-	-	-	-	0.992	0.871
	8	2,5	-	-	-	-	-	0.985	0.865
	9	2,5	-	-	-	-	-	1.084	1.007
	10	2,5	-	-	-	-	-	0.784	0.673

Notes:

- 260 good earthworms set up to void their gut contents. 130 good earthworms selected for the VFA contact test.
- Start of test. Control earthworms no writhing, acting calmly.
- Start of test. Factor 1 no writhing, acting calmly like the controls although F1:5 immediately voided its gut contents.
- Start of test. Factor 7 writhing around the petri-dish, very active. Voiding their gut contents all over the filter paper.
- Start of test. Factor 8 rapid writhing and coiling. Immediately voiding their gut contents all over the filter paper. Slowed down quickly. Many died within 30 minutes.
- Start of test. Factor 9 rapid writhing and coiling. Didn't void their gut contents maybe because death was quick - within 20 minutes.
- Start of test. Factor 10 rapid writhing and coiling. Didn't void their gut contents maybe because death was quick - within 20 minutes.
- Immediate death for Factor's 9 & 10 No blooding or swelling. Went pale (grey) in colour. Where sticking to the filter paper when trying to remove them for weighing.
- Writhing/coiling did not continue outside the one hour assessment period.
- Remaining worms settled down and became less active. Some started moving again when the light went on during the assessment periods. At times it was quite difficult to tell the difference between a dead and live earthworm.

Raw propionic acid contact test results

Factor	Replicate Number	Score after 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Earthworm (g) pre-test	Earthworm (g) post-test
Control (F0)	1	1	1	1	1	1	1	1.435	0.931
	2	1	1	1	1	1	1	1.213	0.784
	3	1	1	1	1	1	1	1.400	0.959
	4	1	1	1	1	1	1	1.813	1.375
	5	1	1	1	1	1	1	1.494	1.052
	6	1	1	1	1	1	1	1.233	0.768
	7	1	1	1	1	1	1	1.189	0.765
	8	1	1	1	1	1	4, 5	1.137	0.706
	9	1	1	1	1	1	1	1.244	0.736
	10	1	1	1	1	1	1	1.203	0.824
5,000 mg/l (F2)	1	1	1	1	1	1	4, 5	1.401	0.802
	2	1	1	1	1	1	1	1.858	1.333
	3	1	1	1	1	1	1	2.051	1.553
	4	1	1	1	1	1	1	2.038	1.526
	5	1	1	1	1	1	4	1.694	1.207
	6	1	1	1	1	1	1	1.091	0.574
	7	1	1	1	1	1	1	1.924	1.388
	8	1	1	1	1	1	1	1.085	0.580
	9	1	1	1	1	1	1	1.944	1.446
	10	1	4	1	1	1	1	1.775	1.190

Notes:

- 60 good earthworms set up to void their gut contents. 30 good earthworms selected for the Single Assessment contact test.
- Start of test. Controls no writhing or coiling, didn't seem stressed. No reaction.
- Start of test. VFA Factor 2 immediate writhing/coiling and some voiding of their gut contents. Slowed down within 5 - 10 minutes, difficult to tell if some are alive or dead.
- During the 1hr assessment period the VFA samples were very slow and still but towards the end of the 1hr assessment some of the VFA samples had overcome their stillness and were beginning to move from time to time.

Appendix 4.3. Raw ammonium-N contact test results

Raw Ammonium-N pH6 contact test results

Factor	Replicate number	Score after 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	2.106	1.879
	2	1	1	1	1	1	1	1.250	1.310
	3	1	1	1	1	1	1	0.874	0.830
	4	1	1	1	1	1	1	1.672	1.646
	5	1	1	1	1	1	1	1.240	1.224
	6	1	1	1	1	1	1	1.079	1.063
	7	1	1	1	1	1	1	1.518	1.379
	8	1	1	1	1	1	1	1.366	1.377
	9	1	1	1	1	1	1	1.039	0.863
	10	1	1	1	1	1	1	0.967	0.975
2 kg/m^3 (F1)	1	1	1	1	1	1	1	1.301	1.056
	2	1	1	1	1	1	1	1.636	1.279
	3	1	1	1	1	1	1	1.474	1.194
	4	1	1	1	1	1	1	1.155	0.829
	5	1	1	1	1	1	1	1.722	1.303
	6	1	1	1	1	1	1	2.201	1.845
	7	1	1	1	1	1	1	1.738	1.375
	8	1	1	1	1	1	1	1.425	1.085
	9	1	1	1	1	1	1	1.151	0.891
	10	1	1	1	1	1	1	1.236	0.967
4 kg/m^3 (F2)	1	1	1	1	1	1	1	1.658	1.197
	2	1	1	2	1	1	1	1.394	0.965
	3	1	1, 3	1	1	1	1	1.298	0.872
	4	1, 3	1, 3	2	3	3	3	2.171	1.450
	5	1	1	1	1	1	1	1.433	0.946
	6	1	1	1	5	-	-	1.263	0.787
	7	3	1	1	1	1	1	0.938	0.579
	8	1	1	1	1	1	1	1.244	0.721
	9	3	1	1	1	1	1	1.893	1.145
	10	1	1	1	5	-	-	1.009	0.592

6 kg/m^3 (F3)	1	3	3	5	-	-	-	1.062	0.629
	2	3	3	3	3	3	3	1.559	0.862
	3	3	5	-	-	-	-	0.855	0.552
	4	3	5	-	-	-	-	1.555	1.006
	5	3, 5	-	-	-	-	-	1.982	1.562
	6	3	5	-	-	-	-	1.064	0.676
	7	3	3	4, 5	-	-	-	1.629	0.897
	8	3	4, 5	-	-	-	-	1.375	0.834
	9	3	5	-	-	-	-	1.717	1.152
	10	3	5	-	-	-	-	1.496	0.920
8 kg/m^3 (F4)	1	2, 3, 5	-	-	-	-	-	1.690	1.404
	2	2, 3, 5	-	-	-	-	-	1.717	1.435
	3	2, 3, 5	-	-	-	-	-	1.633	1.155
	4	2, 3, 5	-	-	-	-	-	1.465	1.016
	5	2, 3, 5	-	-	-	-	-	1.885	1.462
	6	2,3	5	-	-	-	-	1.307	0.882
	7	2, 3, 5	-	-	-	-	-	1.711	1.433
	8	2, 3, 5	-	-	-	-	-	1.655	1.228
	9	2, 3, 5	-	-	-	-	-	1.243	1.006
	10	2, 3, 5	-	-	-	-	-	2.114	1.680
12 kg/m^3 (F5)	1	3, 5	-	-	-	-	-	2.184	1.677
	2	5	-	-	-	-	-	1.852	1.535
	3	2, 3, 5	-	-	-	-	-	1.742	1.289
	4	3, 5	-	-	-	-	-	1.846	1.396
	5	2, 3, 5	-	-	-	-	-	1.685	1.261
	6	2, 3, 5	-	-	-	-	-	2.301	1.874
	7	3, 5	-	-	-	-	-	1.575	1.204
	8	3, 5	-	-	-	-	-	1.360	1.087
	9	3, 5	-	-	-	-	-	1.178	0.945
	10	3, 5	-	-	-	-	-	1.327	0.942

Notes:

- 100 good earthworms set up to void their gut contents. 60 good earthworms selected for the contact test.
- Start of test. Controls no writhing/coiling, didn't seem stressed.
- 2 kg/m^3: No writhing/coiling, didn't seem stressed.
- 4 kg/m^3: Some writhing and voiding of guts around the filter paper. More rapid than control, but less than 6-12 kg/m^3.
- 6 kg/m^3: Immediate response, fast writhing around the petri dish and immediately voiding their guts around the filter paper. All but one earthworm died during the course of the experiment.
- 8 kg/m^3: Immediate response, fast writhing around the petri dish and immediately voiding their guts around the filter paper. All but one earthworms died during the first hour.
- 12 kg/m^3: Immediate response, fast writhing around the petri dish and immediately voiding their guts around the filter paper. All earthworms died during the first hour.
- Writhing/coiling did not continue outside the first hour.
- Remaining (surviving) earthworms 'settled down' and became less active. Some started moving again when lights went on during the assessments.
- After 48 hrs, the control earthworms were 'hiding' under the filter paper.
- The 4 kg/m^3 earthworms produced some mucus produced throughout the 48 hr period.
- After 1 hr, the 6 kg/m^3 earthworms all produced some mucus, moved very slowly even appearing dead, but all bar one earthworm survived.
- Within 30 minutes the 8 kg/m^3 earthworms all died, some coiled on death.
- Within 30 minutes the 12 kg/m^3 earthworms all died, most coiled on death.

Raw Ammonium-N pH9 contact test results

Factor	Replicate number	Score after 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	1.361	1.205
	2	1	1	1	1	1	1	1.644	1.266
	3	1	1	1	1	1	1	1.860	1.573
	4	1	1	1	1	1	1	1.439	1.120
	5	1	1	1	1	1	1	1.750	1.603
	6	1	1	1	1	1	1	0.914	0.846
	7	1	1	1	1	1	1	1.110	1.131
	8	1	1	1	1	1	1	1.577	1.540
	9	1	1	1	1	1	1	1.323	0.935
	10	1	1	1	1	1	1	1.394	1.174
2 kg/m^3 (F1)	1	1	1	1	4	5	-	1.225	1.126
	2	1	1	1	1	1	1	1.827	1.257
	3	1	1	1	1	1	1	1.388	1.028
	4	1	1	1	1	1	1	1.491	1.135
	5	1	1	1	1	4,5	-	1.274	1.069
	6	1	1	1	1	1	1	1.792	1.381
	7	1	1	1	1	1	1	1.990	1.805
	8	1	1	1	1	1	1	1.685	1.274
	9	1	1	1	1	1	1	2.306	1.728
	10	1	1	1	1	1	1	1.589	1.317
4 kg/m^3 (F2)	1	1	1	1	1	1	4,5	1.297	0.907
	2	1	4,5	-	-	-	-	1.659	1.307
	3	1	1	1	4,5	-	-	1.560	1.105
	4	5	-	-	-	-	-	1.447	1.107
	5	5	-	-	-	-	-	0.956	0.892
	6	3,5	-	-	-	-	-	1.442	1.145
	7	1,3	4,5	-	-	-	-	1.844	1.350
	8	1	1	1	1	1	1	1.436	1.008
	9	1	1	1	1	1	1	1.919	1.425
	10	1	1	1	1	1	1	1.419	0.977

6 kg/m^3 (F3)	1	5	-	-	-	-	-	1.693	1.340
	2	5	-	-	-	-	-	1.662	1.542
	3	5	-	-	-	-	-	1.101	0.922
	4	5	-	-	-	-	-	1.677	1.391
	5	5	-	-	-	-	-	1.311	1.101
	6	5	-	-	-	-	-	1.657	1.629
	7	5	-	-	-	-	-	0.865	0.715
	8	5	-	-	-	-	-	1.275	1.209
	9	3,5	-	-	-	-	-	1.213	1.058
	10	5	-	-	-	-	-	1.666	1.504
8 kg/m^3 (F4)	1	5	-	-	-	-	-	1.010	0.828
	2	5	-	-	-	-	-	1.828	1.561
	3	5	-	-	-	-	-	1.697	1.303
	4	5	-	-	-	-	-	1.459	1.173
	5	5	-	-	-	-	-	1.547	1.302
	6	5	-	-	-	-	-	1.685	1.365
	7	5	-	-	-	-	-	1.086	0.903
	8	5	-	-	-	-	-	1.707	1.380
	9	5	-	-	-	-	-	1.326	1.097
	10	4,5	-	-	-	-	-	1.540	1.258
12 kg/m^3 (F5)	1	5	-	-	-	-	-	1.404	1.501
	2	5	-	-	-	-	-	1.744	1.007
	3	3,4,5	-	-	-	-	-	1.043	1.148
	4	5	-	-	-	-	-	1.072	0.992
	5	5	-	-	-	-	-	1.732	0.984
	6	3,5	-	-	-	-	-	1.031	1.223
	7	5	-	-	-	-	-	1.171	0.942
	8	5	-	-	-	-	-	1.473	0.810
	9	5	-	-	-	-	-	1.240	1.297
	10	5	-	-	-	-	-	1.594	1.440

Notes:

- 100 good earthworms set up to void their gut contents. 60 good earthworms selected for the Ammonium-N Ph9 contact test.
- Start of test. Controls no writhing/coiling, didn't seem stressed.
- Start of test. Factor 1 no writhing/coiling, didn't seem stressed.
- Start of test. Factor 2 some writhing and some voiding of gut contents around the filter paper. More active than control & F1 but not as rapid as F3, F4 & F5.
- Start of test. Factor 3 immediate response. Fast writhing around the petri-dish and some but not all immediately voided their gut contents around the filter paper.
- Start of test. Factor 4 immediate response. Fast writhing around the petri-dish and immediate voiding of gut contents around the filter paper. All but one died within the 1hr assessment period.
- Start of test. Factor 5 immediate response. Fast writhing around the petri-dish and immediate voiding of their gut contents around the filter paper. All died within the 1hr assessment period.
- Writhing/coiling did not continue outside the 1hr assessment period.
- Remaining worms settled down and became less active. Some started moving again when the light went on during the assessment periods.

Appendix 4.4. Raw acetic acid and ammonium-N contact test results

Chemical	Replicate Number	Score at 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	2.421	1.952
	2	1	1	1	1	1	1	1.698	1.201
	3	1	1	1	1	1	1	1.718	1.258
	4	1	1	1	1	1	1	3.429	3.023
	5	1	1	1	1	1	1	1.955	1.446
	6	1	1	1	1	1	1	1.641	1.245
	7	1	1	1	1	1	1	1.710	1.210
	8	1	1	1	1	1	1	2.828	2.459
	9	1	1	1	1	1	1	1.819	1.314
	10	1	1	1	1	1	1	1.608	1.236
500 mg/l acetic acid (AA) (F1)	1	1	1	1	1	1	1	1.856	1.431
	2	1	1	1	1	1	1	1.500	1.070
	3	1	1	1	1	1	1	2.570	2.136
	4	1	1	1	1	1	1	1.752	1.292
	5	1	1	1	1	1	1	2.093	1.610
	6	1	1	1	1	1	1	2.666	2.241
	7	1	1	1	1	1	1	1.944	1.518
	8	1	1	1	1	1	1	2.851	2.474
	9	1	1	1	1	1	1	1.705	1.155
	10	1	1	1	1	1	1	3.473	2.955
2,500mg/l AA (F2)	1	1	1	1	1	1	1	1.916	1.463
	2	1	1	1	1	1	1	1.188	0.597
	3	1	1	1	1	1	1	3.038	2.575
	4	1	1	1	1	1	1	1.496	0.962
	5	1	1	1	1	1	1	2.662	2.356
	6	1	1	1	1	1	1	2.406	2.016
	7	1	1	1	1	1	1	2.102	1.730
	8	1	1	1	1	1	1	2.978	2.532
	9	1	1	1	1	1	1	3.712	3.359
	10	1	1	1	1	1	1	2.039	1.641

5,000 mg/l AA (F3)	1	3	1	4	4	4	5	2.605	2.141
	2	3	1	1	1	1	1	1.806	1.285
	3	3	4	4	4	3, 4, 5	-	1.988	1.674
	4	3	1	1	1	1	1	1.602	1.119
	5	3	1	1	1	1	1	2.325	1.846
	6	3	1	1	1	1	1	2.830	2.359
	7	3	1	1	1	1	1	1.592	0.756
	8	3	1	1	1	1	1	1.761	0.229
	9	3	1	1	4	4	5	1.508	1.035
	10	3	1	1	1	1	1	3.209	2.845
2 kg/m^3 NH_4 (F4)	1	1	1	1	1	1	1	1.548	1.159
	2	1	1	1	4, 1	4	1	2.137	1.634
	3	1	1	1	1	1	1	3.388	2.841
	4	3	1	1	1	1	1	2.618	2.104
	5	1	1	1	1	1	1	2.443	1.975
	6	1	1	1	1	1	1	2.854	2.376
	7	1	1	1	1	1	1	2.014	1.556
	8	1	1	1	4	4, 5	-	1.023	0.744
	9	1	1	1	1	1	1	2.544	2.172
	10	1	1	1	1	1	1	2.252	1.694
3 kg/m^3 NH_4 (F5)	1	3	3	3	1	1	1	2.967	2.540
	2	3	1	1	1	1	1	1.175	0.723
	3	3	3	3	4, 5	-	-	1.546	1.335
	4	3	3	3	1	4, 5	-	1.604	1.364
	5	3	3	3	1	1	1	2.453	1.964
	6	3	3	3	1	1	1	2.096	1.708
	7	3	3	3	1	1	1	2.998	2.592
	8	3	3	3	1	1	4, 5	3.007	2.534
	9	3	3	3	1	1	1	2.747	2.246
	10	3	3	3	1	1	1	1.995	1.659

4 kg/m^3 NH_4 (F6)	1	3	3	5	-	-	-	1.832	1.572
	2	3	3	3	3	1	1	1.947	1.522
	3	3	3	3	1	1	1	3.148	2.556
	4	3	3	3	1	1	3, 4, 5	2.848	2.267
	5	3	3	3	1	1	1	2.111	1.564
	6	3	4, 5	-	-	-	-	1.329	1.218
	7	3	3	3	1	1	1	1.764	1.173
	8	3	3	3	3	1	1	3.127	2.711
	9	3	4, 5	-	-	-	-	2.048	1.964
	10	3	3	3	1	1	1	3.086	2.532
500 mg/l AA & 2 kg/m^3 NH_4 (F7)	1	1	1	1	1	1	1	2.496	2.049
	2	1	1	1	1	1	1	1.283	0.907
	3	1	1	1	1	1	1	1.639	1.292
	4	1	1	1	1	1	1	2.640	2.274
	5	1	1	1	1	1	1	2.896	2.524
	6	1	1	1	1	1	1	2.936	2.549
	7	1	1	1	1	1	1	2.033	1.627
	8	1	1	1	1	1	1	2.441	2.016
	9	1	1	1	1	1	1	2.472	2.070
	10	1	1	1	1	1	1	3.480	3.030
500 mg/l AA & 3 kg/m^3 NH_4 (F8)	1	1	1	1	1	1	1	3.202	2.786
	2	1	1	3	1	1	1	2.287	1.818
	3	1	1	1	1	1	1	3.487	3.043
	4	1	1	1	1	1	1	3.018	2.592
	5	1	1	1	1	1	1	2.123	1.778
	6	1	1	1	1	1	1	3.026	2.519
	7	1	1	1	1	1	1	2.226	1.742
	8	1	1	1	1	1	1	2.600	2.116
	9	1	1	1	1	1	1	2.492	1.982
	10	1	1	1	1	1	1	2.496	1.999

500 mg/l AA & 4 kg/m^3 NH_4 (F9)	1	3	3	3	3	1	1	2.937	2.460
	2	3	3	3	3	3	3	3.462	3.064
	3	3	3	3	1	3	1	2.350	2.021
	4	3	3	1	1	1	1	2.648	2.211
	5	3	3	3	1	1	1	1.990	1.490
	6	3	3	3, 4	4	4	1	2.584	2.206
	7	3	3	3	3, 4, 5	-	-	2.549	2.319
	8	3	3	3	3, 5	-	-	1.594	1.393
	9	3	3	3	1	3, 4, 5	-	2.106	1.861
	10	3	3	3	1	1	3	2.683	2.229
2,500 mg/l AA & 2 kg/m^3 NH_4 (F10)	1	3	3	3	1	1	1	1.779	1.308
	2	1	1	1	1	1	1	4.126	3.777
	3	3	3	3	1	3	1	3.146	2.690
	4	3	1	1	1	1	1	2.782	2.284
	5	3	1	1	1	1	1	2.636	2.215
	6	3	1	1	1	1	1	1.285	0.871
	7	3	1	1	1	1	1	2.226	1.855
	8	3	1	1	1	1	1	3.019	2.565
	9	3	1	1	1	1	1	2.086	1.661
	10	3	3	1	1	1	1	2.910	2.321
2,500 mg/l AA & 3 kg/m^3 NH_4 (F11)	1	3	3	1	1	1	1	2.547	2.086
	2	1	1	1	1	1	1	3.224	2.850
	3	1	1	1	1	1	1	2.063	1.713
	4	1	1	1	1	1	1	2.361	1.941
	5	1	1	1	1	1	1	1.312	0.881
	6	3	1	3	1	1	1	3.245	2.685
	7	1	1	1	1	1	1	1.954	1.443
	8	1	1	1	1	1	1	1.924	1.364
	9	1	1	1	1	1	1	2.762	2.219
	10	1	1	1	1	1	1	2.020	1.184

2,500 mg/l AA & 4 kg/m^3 NH_4 (F12)	1	3	3	3	1	1	1	1.970	1.481
	2	3	3	3	1	1	1	2.751	2.217
	3	3	3	1	1	1	1	2.770	2.310
	4	3	1	1	1	1	4, 5	2.641	2.205
	5	3	3	3	3	1	1	2.611	2.055
	6	3	3	5	-	-	-	2.729	2.538
	7	3	3	1	3	1	1	3.311	2.843
	8	3	1	1	1	1	1	1.637	1.053
	9	3	1	1	1	1	1	2.906	2.387
	10	3	3	1	1	1	1	2.104	1.409
5,000 mg/l AA & 2 kg/m^3 NH_4 (F13)	1	3	1	3	1	1	1	3.014	2.542
	2	3	1	1	1	1	1	4.073	3.656
	3	3	1	1	1	1	1	1.940	1.475
	4	1	1	1	1	1	1	2.365	1.843
	5	1	1	1	1	1	1	3.260	2.847
	6	3	1	3	4	4, 5	-	2.440	2.082
	7	1	1	1	1	1	4, 5	2.814	2.307
	8	1	1	1	1	1	1	1.578	1.148
	9	1	1	1	1	1	1	2.243	1.666
	10	1	1	1	1	1	1	3.137	2.494
5,000 mg/l AA & 3 kg/m^3 NH_4 (F14)	1	3	1	3	1	1	1	1.607	1.151
	2	3	1	5	-	-	-	0.933	0.733
	3	3	1	5	-	-	-	1.355	0.904
	4	3	3	3	3	1	1	2.010	1.527
	5	3	1	1	4	4, 5	-	1.280	0.976
	6	3	1	4, 5	-	-	-	1.594	1.383
	7	3	1	3	1	1	4, 5	2.489	2.030
	8	3	1	3	1	1	1	3.056	2.545
	9	3	1	4	4	4, 5	-	1.754	1.366
	10	3	1	4	5	-	-	2.022	1.729

5,000 mg/l AA & 4 kg/m³ NH_4 (F15)	1	3	3, 4, 5	-	-	-	-	2.019	1.910
	2	3	3	3	3, 4	3, 4, 5	-	2.139	1.848
	3	3	3	3	4, 5	-	-	1.242	0.979
	4	3	3	1	3	1	1	1.763	1.258
	5	3	3	3	3	4, 5	-	2.123	1.643
	6	3	3	3	3	5	-	2.055	1.638
	7	3	3	3, 4	5	-	-	2.404	2.118
	8	3	1	4	5	-	-	1.592	1.346
	9	3	4, 5	-	-	-	-	1.521	1.400
	10	3	3	3	4	4, 5	-	2.670	2.292

Notes:

- Control no response.
- 500mg/l acetic acid no response.
- 2500mg/l acetic acid some moving around only.
- 5000mg/l acetic acid more reactive, no rapid movement though some mucus production along with voiding of guts and writhing around.
- 2kg/m3 NH4 no response.
- 3kg/m3 NH4 some movement and some voiding of guts.
- 4kg/m3 NH4 not rapid writhing along with some voiding of guts.
- 500mg/l AA 2kg/m3 NH4 no response.
- 500mg/l AA 3kg/m3 NH4 some movement but no writhing.
- 500mg/l AA 4kg/m3 NH4 no reaction and hardly any movement.
- 2500mg/l AA 2kg/m3 NH4 no reaction some slight movement.
- 2500mg/l AA 3kg/m3 NH4 some movement no writhing.
- 2500mg/l AA 4kg/m3 NH4 no reaction little movement.
- 5000mg/l AA 2kg/m3 NH4 more movement some writhing but not rapid some voiding of guts.
- 5000mg/l AA 3kg/m3 NH4 more movement some writhing but not rapid some voiding of guts.
- 5000mg/l AA 4kg/m3 NH4 more movement some writhing but not rapid some voiding of guts.

Appendix 4.5. Raw food-based digestate contact test results

Raw digestate 1 contact test results

Chemical	Replicate Number	Score at 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	2.076	1.706
	2	1	1	1	1	1	1	2.115	1.711
	3	1	1	1	1	1	1	1.932	1.525
	4	1	1	1	1	1	1	2.447	2.056
	5	1	1	1	1	1	1	1.971	1.520
	6	1	1	1	1	1	1	1.132	0.681
	7	1	1	1	1	1	1	1.729	1.270
	8	1	1	1	1	1	1	3.396	2.980
	9	1	1	1	1	1	1	2.297	1.857
	10	1	1	1	1	1	1	0.849	0.337
10% food-based digestate 1	1	1	5	-	-	-	-	1.491	1.354
	2	1	5	-	-	-	-	0.449	0.328
	3	1	1	1	5	-	-	1.841	1.554
	4	1	1	1	1	1	1	1.368	0.880
	5	1	5	-	-	-	-	1.157	1.057
	6	1	1	1	1	1	4, 5	1.258	0.773
	7	1	1	4, 5	-	-	-	1.244	1.048
	8	1	1	1	1	1	1	1.719	1.258
	9	1	5	-	-	-	-	1.120	0.991
	10	1	1	1	1	4, 5	-	2.254	1.798
25% food-based digestate 1	1	5	-	-	-	-	-	0.644	0.623
	2	5	-	-	-	-	-	1.096	1.073
	3	5	-	-	-	-	-	1.887	1.865
	4	5	-	-	-	-	-	0.791	0.775
	5	5	-	-	-	-	-	1.712	1.692
	6	5	-	-	-	-	-	0.997	0.981
	7	5	-	-	-	-	-	0.711	0.693
	8	5	-	-	-	-	-	0.979	0.963
	9	1	5	-	-	-	-	1.105	0.973
	10	5	-	-	-	-	-	1.703	1.690

50% food-based digestate 1	1	5	-	-	-	-	-	2.130	2.105
	2	5	-	-	-	-	-	1.011	0.997
	3	5	-	-	-	-	-	1.124	1.112
	4	5	-	-	-	-	-	1.423	1.408
	5	5	-	-	-	-	-	1.453	1.437
	6	5	-	-	-	-	-	1.604	1.590
	7	5	-	-	-	-	-	0.972	0.956
	8	5	-	-	-	-	-	0.960	0.947
	9	5	-	-	-	-	-	1.456	1.442
	10	5	-	-	-	-	-	1.392	1.379
75% food-based digestate 1	1	5	-	-	-	-	-	1.885	1.870
	2	5	-	-	-	-	-	1.781	1.768
	3	5	-	-	-	-	-	1.574	1.555
	4	5	-	-	-	-	-	0.902	0.886
	5	5	-	-	-	-	-	1.573	1.556
	6	5	-	-	-	-	-	1.085	1.071
	7	5	-	-	-	-	-	1.126	1.111
	8	5	-	-	-	-	-	1.464	1.446
	9	5	-	-	-	-	-	1.768	1.746
	10	5	-	-	-	-	-	1.990	1.966
100% food-based digestate 1	1	5	-	-	-	-	-	1.251	1.237
	2	5	-	-	-	-	-	0.330	0.311
	3	5	-	-	-	-	-	1.928	1.904
	4	5	-	-	-	-	-	1.017	1.003
	5	5	-	-	-	-	-	1.368	1.354
	6	5	-	-	-	-	-	2.012	1.997
	7	4, 5	-	-	-	-	-	1.389	1.366
	8	5	-	-	-	-	-	2.295	2.284
	9	5	-	-	-	-	-	1.755	1.738
	10	5	-	-	-	-	-	1.059	1.047

Notes:

- Control no response.
- 10% digestate – active, some voiding of guts. Some writhing, but not rapid.
- 25% digestate – active writhing and coiling, not rapid. Some voiding of guts.
- 50% digestate – very active, faster more frantic writhing and coiling. Some voiding of guts.
- 75% digestate – rapid frantic writhing and coiling, which noticeably slowed down after five minutes. Some voiding of guts.
- 100% digestate – rapid frantic writhing and coiling, which noticeably slowed down after five minutes. Some voiding of guts and mucus production.

Raw digestate 2 contact test results

Chemical	Replicate Number	Score at 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	3.232	2.730
	2	1	1	1	1	1	1	2.446	1.915
	3	1	1	1	1	1	1	2.010	1.559
	4	1	1	1	1	1	1	0.696	0.128
	5	1	1	1	1	1	1	2.197	1.726
	6	1	1	1	1	1	1	1.867	1.460
	7	1	1	1	1	1	1	1.461	0.911
	8	1	1	1	1	1	1	1.927	1.292
	9	1	1	1	4	4, 5	-	1.625	1.341
	10	1	1	1	1	1	1	2.510	2.020
10% food-based digestate 2	1	1	1	1	1	1	1	1.590	1.139
	2	1	1	1	1	1	5	1.929	1.475
	3	1	5	-	-	-	-	1.003	0.892
	4	1	1	1	1	1	1	2.033	1.487
	5	1	4, 5	-	-	-	-	1.876	1.682
	6	1	1	1	1	4, 5	-	2.132	1.731
	7	1	1	1	1	1	1	1.953	1.350
	8	1	1	1	1	1	4, 5	1.338	0.686
	9	1	1	1	1	1	4, 5	1.405	0.887
	10	1	5	-	-	-	-	1.518	1.417
25% food-based digestate 2	1	5	-	-	-	-	-	1.190	1.176
	2	5	-	-	-	-	-	1.721	1.701
	3	5	-	-	-	-	-	1.737	1.717
	4	5	-	-	-	-	-	1.406	1.393
	5	5	-	-	-	-	-	1.241	1.224
	6	5	-	-	-	-	-	1.534	1.521
	7	5	-	-	-	-	-	1.721	1.703
	8	5	-	-	-	-	-	1.797	1.779
	9	5	-	-	-	-	-	1.222	1.205
	10	5	-	-	-	-	-	0.896	0.879

50% food-based digestate 2	1	5	-	-	-	-	-	1.307	1.297
	2	5	-	-	-	-	-	1.010	0.995
	3	5	-	-	-	-	-	0.938	0.921
	4	5	-	-	-	-	-	1.595	1.578
	5	5	-	-	-	-	-	1.938	1.925
	6	5	-	-	-	-	-	1.243	1.228
	7	5	-	-	-	-	-	1.262	1.245
	8	5	-	-	-	-	-	1.937	1.920
	9	5	-	-	-	-	-	1.227	1.211
	10	5	-	-	-	-	-	0.882	0.863
75% food-based digestate 2	1	5	-	-	-	-	-	1.020	1.002
	2	5	-	-	-	-	-	1.577	1.557
	3	5	-	-	-	-	-	1.847	1.829
	4	5	-	-	-	-	-	2.162	2.144
	5	5	-	-	-	-	-	1.553	1.530
	6	5	-	-	-	-	-	2.250	2.233
	7	5	-	-	-	-	-	2.014	1.996
	8	5	-	-	-	-	-	1.553	1.535
	9	5	-	-	-	-	-	1.210	1.187
	10	5	-	-	-	-	-	1.621	1.594
100% food-based digestate 2	1	5	-	-	-	-	-	1.703	1.681
	2	5	-	-	-	-	-	0.901	0.881
	3	5	-	-	-	-	-	1.118	1.103
	4	5	-	-	-	-	-	0.825	0.807
	5	5	-	-	-	-	-	1.705	1.688
	6	5	-	-	-	-	-	2.403	2.385
	7	5	-	-	-	-	-	1.339	1.322
	8	5	-	-	-	-	-	1.330	1.316
	9	5	-	-	-	-	-	0.970	0.951
	10	5	-	-	-	-	-	1.734	1.627

Notes:

- Control no response.
- 10% digestate – active, some voiding of guts. Some writhing, but not rapid.
- 25% digestate – active writhing and coiling, not rapid. Some voiding of guts.
- 50% digestate – very active, faster more frantic writhing and coiling. Some voiding of guts.
- 75% digestate – rapid frantic writhing and coiling, which noticeably slowed down after five minutes. Some voiding of guts.
- 100% digestate – rapid frantic writhing and coiling, which noticeably slowed down after five minutes. Some voiding of guts and mucus production.

Raw digestate 3 contact test results

Chemical	Replicate Number	Score at 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	1.292	0.845
	2	1	1	1	1	1	1	1.129	0.622
	3	1	1	1	1	1	1	1.426	0.914
	4	1	1	1	1	1	1	1.370	0.974
	5	1	1	1	1	1	1	2.361	1.794
	6	1	1	1	1	1	1	2.264	1.722
	7	1	1	1	1	1	1	1.129	0.699
	8	1	1	1	1	1	1	1.388	0.894
	9	1	1	1	1	1	1	1.912	1.406
	10	1	1	1	1	1	1	1.453	0.896
10% food-based digestate 3	1	1	5	-	-	-	-	1.711	1.554
	2	1	1	1	1	4, 5	-	1.697	1.286
	3	1	1	1	1	1	5	1.730	1.094
	4	1	1	1	5	-	-	2.033	1.758
	5	1	5	-	-	-	-	1.063	0.957
	6	1	1	1	1	1	1	1.285	0.751
	7	1	1	3	1	1	1	2.006	1.439
	8	1	5	-	-	-	-	1.273	1.133
	9	1	1	4, 5	-	-	-	1.063	0.697
	10	1	5	-	-	-	-	0.808	0.586
25% food-based digestate 3	1	5	-	-	-	-	-	1.411	1.385
	2	5	-	-	-	-	-	1.772	1.749
	3	5	-	-	-	-	-	1.709	1.688
	4	5	-	-	-	-	-	0.941	0.922
	5	5	-	-	-	-	-	1.310	1.283
	6	5	-	-	-	-	-	1.004	0.978
	7	5	-	-	-	-	-	1.419	1.386
	8	5	-	-	-	-	-	2.169	2.140
	9	5	-	-	-	-	-	1.655	1.622
	10	5	-	-	-	-	-	1.420	1.386

50% food-based digestate 3	1	5	-	-	-	-	-	0.800	0.781
	2	5	-	-	-	-	-	2.229	2.209
	3	5	-	-	-	-	-	1.799	1.778
	4	5	-	-	-	-	-	1.494	1.469
	5	5	-	-	-	-	-	1.359	1.330
	6	5	-	-	-	-	-	1.069	1.042
	7	5	-	-	-	-	-	1.525	1.492
	8	5	-	-	-	-	-	1.374	1.344
	9	5	-	-	-	-	-	1.908	1.874
	10	5	-	-	-	-	-	1.601	1.563
75% food-based digestate 3	1	5	-	-	-	-	-	1.614	1.587
	2	5	-	-	-	-	-	1.776	1.100
	3	5	-	-	-	-	-	1.804	1.727
	4	5	-	-	-	-	-	1.396	1.365
	5	5	-	-	-	-	-	1.856	1.827
	6	5	-	-	-	-	-	1.855	1.824
	7	5	-	-	-	-	-	1.965	1.931
	8	5	-	-	-	-	-	1.521	1.487
	9	5	-	-	-	-	-	1.650	1.614
	10	5	-	-	-	-	-	1.797	1.759
100% food-based digestate 3	1	5	-	-	-	-	-	1.288	1.260
	2	5	-	-	-	-	-	1.694	1.666
	3	5	-	-	-	-	-	1.557	1.530
	4	5	-	-	-	-	-	1.701	1.674
	5	5	-	-	-	-	-	1.828	1.802
	6	5	-	-	-	-	-	1.624	1.594
	7	5	-	-	-	-	-	1.763	1.732
	8	5	-	-	-	-	-	1.552	1.518
	9	5	-	-	-	-	-	1.999	1.964
	10	5	-	-	-	-	-	1.388	1.350

Notes:

- Control no response.
- 10% digestate – active, some voiding of guts. Some writhing, but not rapid.
- 25% digestate – active writhing and coiling, not rapid. Some voiding of guts.
- 50% digestate – very active, faster more frantic writhing and coiling. Some voiding of guts.
- 75% digestate – rapid frantic writhing and coiling, which noticeably slowed down after five minutes. Some voiding of guts.
- 100% digestate – rapid frantic writhing and coiling, which noticeably slowed down after five minutes. Some voiding of guts and mucus production.

Raw digestate 4 contact test results

Chemical	Replicate Number	Score at 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	2.760	2.370
	2	1	1	1	1	1	1	2.357	1.829
	3	1	1	1	1	1	1	1.176	0.786
	4	1	1	1	1	1	1	1.871	1.400
	5	1	1	1	1	1	1	2.286	1.847
	6	1	1	1	1	1	1	3.049	2.426
	7	1	1	1	1	1	1	1.669	1.158
	8	1	1	1	1	1	1	2.368	1.925
	9	1	1	1	1	1	1	2.045	1.495
	10	1	1	1	1	1	1	2.733	2.020
10% food-based digestate 4	1	1	1	1	1	1	1	2.121	1.738
	2	1	1	1	1	1	1	2.528	2.175
	3	3, 4	4	3, 4	4	4	5	2.182	1.695
	4	1	1	1	1	4	4	2.380	1.934
	5	1	1	1	1	1	1	1.590	1.120
	6	1	1	1	1	1	1	2.839	2.425
	7	1	1	1	1	1	1	3.091	2.625
	8	1	1	1	1	1	1	3.024	2.641
	9	1	1	1	1	1	1	2.612	2.110
	10	4	4	1	1	1	1	3.714	3.020
25% food-based digestate 4	1	1	1	1	3	3, 4	3	2.449	2.004
	2	1	1	4, 5	-	-	-	1.560	1.374
	3	3, 5	-	-	-	-	-	1.655	1.641
	4	3	4, 5	-	-	-	-	2.833	2.734
	5	1	3	3	3	1	3	3.698	3.240
	6	3	4, 5	-	-	-	-	2.087	2.007
	7	3	3, 5	-	-	-	-	2.576	2.439
	8	1	3	3	4	3, 4	3	3.436	2.846
	9	3	4, 5	-	-	-	-	1.854	1.769
	10	3	4, 5	-	-	-	-	2.276	2.180

50% food-based digestate 4	1	5	-	-	-	-	-	2.749	2.732
	2	5	-	-	-	-	-	2.503	2.488
	3	5	-	-	-	-	-	1.967	1.953
	4	5	-	-	-	-	-	2.061	2.044
	5	3, 4, 5	-	-	-	-	-	2.726	2.697
	6	3, 4	4, 5	-	-	-	-	2.220	2.101
	7	3, 4	4, 5	-	-	-	-	2.973	2.864
	8	5	-	-	-	-	-	2.763	2.723
	9	5	-	-	-	-	-	2.738	2.727
	10	4	4, 5	-	-	-	-	3.143	2.993
75% food-based digestate 4	1	5	-	-	-	-	-	2.164	2.147
	2	5	-	-	-	-	-	3.122	3.101
	3	5	-	-	-	-	-	3.093	3.075
	4	5	-	-	-	-	-	2.374	2.353
	5	5	-	-	-	-	-	3.983	3.964
	6	5	-	-	-	-	-	1.902	1.891
	7	5	-	-	-	-	-	2.125	2.109
	8	5	-	-	-	-	-	3.937	3.920
	9	5	-	-	-	-	-	2.339	2.323
	10	5	-	-	-	-	-	1.546	1.535
100% food-based digestate 4	1	5	-	-	-	-	-	3.107	3.083
	2	5	-	-	-	-	-	2.378	2.356
	3	5	-	-	-	-	-	2.289	2.271
	4	5	-	-	-	-	-	2.044	2.031
	5	5	-	-	-	-	-	3.207	3.180
	6	5	-	-	-	-	-	3.017	3.001
	7	5	-	-	-	-	-	1.878	1.858
	8	5	-	-	-	-	-	2.797	2.786
	9	5	-	-	-	-	-	2.306	2.295
	10	5	-	-	-	-	-	1.987	1.974

Notes:

- Control no response.
- 10% digestate – some slight coiling and writhing, but did not appear effected.
- 25% digestate – some slight coiling and writhing, but did not appear too effected.
- 50% digestate – coiling, writhing and mucus production.
- 75% digestate – coiling, writhing and mucus production.
- 100% digestate – coiling, writhing and mucus production.

Raw digestate 5 contact test results

Chemical	Replicate Number	Score at 1hr	Score at 12hrs	Score at 18hrs	Score at 24hrs	Score at 36hrs	Score at 48hrs	Worm (g) pre-test	Worm (g) post-test
Control (F0)	1	1	1	1	1	1	1	3.155	2.645
	2	1	1	1	1	1	1	1.695	1.212
	3	1	1	1	1	1	1	2.200	1.709
	4	1	1	1	1	1	1	2.520	2.070
	5	1	1	1	1	1	1	2.542	2.144
	6	1	1	1	1	1	1	3.238	2.831
	7	1	1	1	1	1	1	2.030	1.463
	8	1	1	1	1	1	1	2.473	1.983
	9	1	1	1	1	1	1	2.684	2.309
	10	1	1	1	1	1	1	2.071	1.572
10% food-based digestate 5	1	1	1	1	1	1	1	1.739	1.234
	2	1	1	1	1	1	1	2.946	2.549
	3	1	1	1	1	1	1	2.268	1.813
	4	1	1	1	1	1	1	2.972	2.428
	5	1	1	1	1	3	5	2.538	2.183
	6	1	1	1	1	1	1	2.788	2.423
	7	1	1	4,5	-	-	-	1.881	1.351
	8	1	1	1	1	1	1	3.116	2.742
	9	1	1	1	1	1	1	2.509	2.232
	10	1	1	1	1	1	1	2.210	1.872
25% food-based digestate 5	1	1	3	4,5	-	-	-	2.497	1.434
	2	1	3	4,5	-	-	-	2.428	1.377
	3	1	1	1	4	4	1	2.710	2.202
	4	1	3	3	3	1	1	2.615	2.193
	5	1	3	4,5	-	-	-	2.462	1.433
	6	1	1	1	3,4	1	1	2.716	2.329
	7	1	3	4,5	-	-	-	2.465	1.669
	8	3	4,5	-	-	-	-	1.661	1.047
	9	1	3	4,5	-	-	-	3.387	2.490
	10	1	1	1	1	1	1	2.407	1.982

50% food-based digestate 5	1	3,5	-	-	-	-	-	2.059	1.552
	2	3	4,5	-	-	-	-	2.270	1.511
	3	3	3,5	-	-	-	-	3.033	2.120
	4	4,5	-	-	-	-	-	2.446	1.885
	5	3	4,5	-	-	-	-	1.816	1.353
	6	3	4,5	-	-	-	-	2.234	1.541
	7	3	3,4,5	-	-	-	-	1.742	1.057
	8	5	-	-	-	-	-	1.475	1.223
	9	4	4,5	-	-	-	-	2.159	1.586
	10	1	4,5	-	-	-	-	2.365	1.896
75% food-based digestate 5	1	4,5	-	-	-	-	-	2.577	2.068
	2	5	-	-	-	-	-	2.361	2.014
	3	3,5	-	-	-	-	-	2.573	2.108
	4	5	-	-	-	-	-	1.841	1.539
	5	3,5	-	-	-	-	-	2.965	2.253
	6	3,5	-	-	-	-	-	2.577	2.060
	7	5	-	-	-	-	-	1.894	1.478
	8	5	-	-	-	-	-	2.762	2.189
	9	3,5	-	-	-	-	-	2.113	1.437
	10	3,5	-	-	-	-	-	1.820	1.478
100% food-based digestate 5	1	3,4,5	-	-	-	-	-	3.012	2.353
	2	3,5	-	-	-	-	-	1.515	1.060
	3	5	-	-	-	-	-	1.598	1.315
	4	5	-	-	-	-	-	2.295	1.747
	5	3,4,5	-	-	-	-	-	2.765	2.088
	6	3,4,5	-	-	-	-	-	2.968	2.114
	7	3,5	-	-	-	-	-	2.556	2.062
	8	5	-	-	-	-	-	2.107	1.597
	9	3,5	-	-	-	-	-	1.952	1.513
	10	3,5	-	-	-	-	-	3.065	2.313

Notes:

- Control no response.
- 10% digestate – some initial movement around the petri-dish, but not rapid. Three voided their guts but all soon settled.
- 25% digestate – initial writhing around the petri-dish, not rapid but more active than F1. Five voided their guts, but calmed down within 10 minutes.
- 50% digestate – initial rapid writhing around the petri-dish. A lot of voiding of guts. Still some movement after 10 minutes, but not rapid.
- 75% digestate – rapid writhing, but no coiling. Voiding of guts from most. Some movement after 10 minutes but a lot have slowed down, some mucus.
- 100% digestate – rapid writhing, but no coiling. Voiding of guts from most. Some movement after 10 minutes but a lot have slowed down, some mucus.

Appendix 4.6. Raw ammonium-N experiment pot test results

Raw ammonium-N pot test results – 2 hours

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	19.89	27.63	7	7
	114	2	18.71	28.51	7	7
	114	3	17.45	25.98	7	7
	114	4	19.17	29.12	7	7
Fertiliser control (200 kg N/ha)	114	1	19.19	29.11	7	7
	114	2	16.89	24.10	7	7
	114	3	16.65	26.69	7	7
	114	4	16.99	26.21	7	7
Fertiliser control (300 kg N/ha)	114	1	19.49	29.13	7	7
	114	2	18.49	24.65	7	7
	114	3	20.61	28.52	7	7
	114	4	21.04	28.34	7	7
Unamended digestate pH <7 applied at 50 m^3/ha	114	1	17.83	14.85	7	4
	114	2	19.01	14.29	7	4
	114	3	19.30	27.20	7	6
	114	4	17.81	24.85	7	7
Unamended digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	19.19	22.98	7	7
	114	2	16.36	14.38	7	4
	114	3	16.95	19.80	7	6
	114	4	16.57	22.64	7	7
Unamended digestate pH ≥8 applied at 50 m^3/ha	114	1	18.60	15.59	7	4
	114	2	17.43	17.95	7	5
	114	3	18.14	21.57	7	6
	114	4	17.60	18.14	7	5
Unamended digestate pH <7 applied at 30 m^3/ha	68	1	15.77	22.03	7	7
	68	2	16.81	22.72	7	6
	68	3	15.90	22.57	7	7
	68	4	17.52	25.80	7	7

Unamended digestate pH ≥7 – <8 applied at 30 m³/ha	68	1	15.86	23.23	7	7
	68	2	16.35	21.64	7	7
	68	3	15.50	18.94	7	6
	68	4	16.62	24.06	7	7
Unamended digestate pH ≥8 applied at 30 m³/ha	68	1	19.17	26.85	7	7
	68	2	16.63	17.64	7	5
	68	3	16.91	24.28	7	7
	68	4	15.63	21.88	7	7
Enhanced digestate pH <7 applied at 50 m³/ha	114	1	16.39	12.66	7	4
	114	2	17.20	20.44	7	6
	114	3	16.85	17.87	7	5
	114	4	16.14	17.76	7	6
Enhanced digestate pH ≥7 – <8 applied at 50 m³/ha	114	1	17.08	21.06	7	6
	114	2	18.16	14.43	7	4
	114	3	18.28	19.78	7	6
	114	4	15.57	17.74	7	5
Enhanced digestate pH ≥8 applied at 50 m³/ha	114	1	15.72	18.1	7	6
	114	2	16.33	15.71	7	5
	114	3	18.49	22.26	7	6
	114	4	16.95	8.15	7	3
Enhanced digestate pH <7 applied at 30 m³/ha	68	1	15.85	21.00	7	7
	68	2	16.51	24.81	7	7
	68	3	17.72	27.13	7	7
	68	4	18.66	23.49	7	6
Enhanced digestate pH ≥7 – <8 applied at 30 m³/ha	68	1	15.34	22.75	7	7
	68	2	16.79	21.94	7	6
	68	3	15.99	23.31	7	7
	68	4	18.79	26.22	7	7
Enhanced digestate pH ≥8 applied at 30 m³/ha	68	1	15.04	16.69	7	6
	68	2	17.16	25.23	7	7
	68	3	15.26	21.94	7	7
	68	4	17.15	24.04	7	7

* The two application rates are equivalent to 50 m³/ha (114 ml per pot) and 30 m³/ha (68 ml per pot).

Raw ammonium-N pot test results – 24 hours

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	16.14	24.06	7	7
	114	2	17.82	26.31	7	7
	114	3	16.33	24.76	7	7
	114	4	16.67	24.54	7	7
Fertiliser control (200 kg N/ha)	114	1	17.49	24.44	7	7
	114	2	17.59	24.57	7	7
	114	3	18.28	25.74	7	7
	114	4	20.12	28.66	7	7
Fertiliser control (300 kg N/ha)	114	1	20.10	28.96	7	7
	114	2	15.90	14.65	7	5
	114	3	17.08	24.47	7	7
	114	4	19.48	27.40	7	7
Unamended digestate pH <7 applied at 50 m^3/ha	114	1	22.49	23.62	7	5
	114	2	16.26	21.58	7	7
	114	3	18.56	26.33	7	7
	114	4	17.85	16.79	7	4
Unamended digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	18.21	26.26	7	7
	114	2	16.01	11.21	7	4
	114	3	16.52	20.71	7	6
	114	4	17.23	21.82	7	6
Unamended digestate pH ≥8 applied at 50 m^3/ha	114	1	20.85	20.36	7	5
	114	2	17.15	25.13	7	7
	114	3	16.65	19.65	7	6
	114	4	17.96	21.29	7	6
Unamended digestate pH <7 applied at 30 m^3/ha	68	1	16.83	24.26	7	7
	68	2	15.44	22.70	7	7
	68	3	17.89	28.69	7	7
	68	4	16.30	25.44	7	7
Unamended digestate pH ≥7 – <8 applied at 30 m^3/ha	68	1	17.57	26.67	7	7
	68	2	17.48	26.98	7	7
	68	3	19.59	28.53	7	7
	68	4	17.79	24.86	7	7

Unamended digestate pH ≥8 applied at 30 m^3/ha	68	1	15.69	22.88	7	7
	68	2	16.25	20.81	7	7
	68	3	15.21	22.11	7	7
	68	4	15.88	22.53	7	7
Enhanced digestate pH <7 applied at 50 m^3/ha	114	1	15.39	17.10	7	5
	114	2	18.72	24.70	7	6
	114	3	15.36	22.26	7	7
	114	4	16.44	22.60	7	7
Enhanced digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	16.75	12.76	7	4
	114	2	15.70	16.22	7	5
	114	3	15.46	13.54	7	4
	114	4	15.93	12.08	7	3
Enhanced digestate pH ≥8 applied at 50 m^3/ha	114	1	14.38	15.26	7	5
	114	2	16.70	24.35	7	7
	114	3	15.03	14.71	7	5
	114	4	14.85	20.25	7	6
Enhanced digestate pH <7 applied at 30 m^3/ha	68	1	14.54	23.32	7	7
	68	2	17.55	29.62	7	7
	68	3	15.86	22.03	7	6
	68	4	15.10	23.34	7	7
Enhanced digestate pH ≥7 – <8 applied at 30 m^3/ha	68	1	15.30	23.41	7	7
	68	2	17.25	25.22	7	7
	68	3	16.79	18.78	7	6
	68	4	16.91	24.85	7	7
Enhanced digestate pH ≥8 applied at 30 m^3/ha	68	1	16.86	21.73	7	6
	68	2	16.53	22.88	7	7
	68	3	17.14	25.58	7	7
	68	4	15.81	16.17	7	5

* The two application rates are equivalent to 50 m^3/ha (114 ml per pot) and 30 m^3/ha (68 ml per pot).

Raw ammonium-N pot test results – 7 days

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	16.30	21.83	7	7
	114	2	15.66	22.53	7	7
	114	3	15.19	19.40	7	7
	114	4	14.83	21.36	7	7
Fertiliser control (200 kg N/ha)	114	1	14.61	19.83	7	7
	114	2	14.03	19.01	7	7
	114	3	15.59	16.74	7	6
	114	4	16.65	21.78	7	7
Fertiliser control (300 kg N/ha)	114	1	16.03	21.73	7	7
	114	2	16.47	22.01	7	7
	114	3	15.15	19.62	7	7
	114	4	15.16	19.35	7	7
Unamended digestate pH <7 applied at 50 m^3/ha	114	1	15.71	20.33	7	6
	114	2	16.13	21.48	7	6
	114	3	14.76	18.03	7	6
	114	4	14.25	21.54	7	7
Unamended digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	16.36	22.97	7	7
	114	2	16.08	16.97	7	5
	114	3	18.52	21.20	7	6
	114	4	18.48	28.11	7	7
Unamended digestate pH ≥8 applied at 50 m^3/ha	114	1	18.21	22.44	7	6
	114	2	15.43	22.38	7	7
	114	3	15.01	15.68	7	6
	114	4	16.46	11.10	7	3
Unamended digestate pH <7 applied at 30 m^3/ha	68	1	14.87	17.83	7	5
	68	2	15.61	22.58	7	7
	68	3	16.19	22.71	7	7
	68	4	16.68	21.06	7	6
Unamended digestate pH ≥7 – <8 applied at 30 m^3/ha	68	1	17.35	24.40	7	7
	68	2	15.77	20.27	7	7
	68	3	16.42	18.20	7	6
	68	4	14.60	17.67	7	6

Unamended digestate pH ≥8 applied at 30 m^3/ha	68	1	16.23	24.06	7	7
	68	2	15.84	19.90	7	6
	68	3	14.48	19.90	7	7
	68	4	15.67	22.67	7	7
Enhanced digestate pH <7 applied at 50 m^3/ha	114	1	15.79	15.02	7	5
	114	2	15.90	16.03	7	5
	114	3	15.75	12.63	7	5
	114	4	18.50	15.98	7	6
Enhanced digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	14.57	13.69	7	5
	114	2	14.74	16.48	7	5
	114	3	16.39	17.75	7	6
	114	4	16.38	14.34	7	5
Enhanced digestate pH ≥8 applied at 50 m^3/ha	114	1	15.51	17.81	7	6
	114	2	15.70	9.28	7	3
	114	3	15.04	18.58	7	6
	114	4	15.77	20.06	7	7
Enhanced digestate pH <7 applied at 30 m^3/ha	68	1	15.49	22.74	7	7
	68	2	16.98	23.93	7	7
	68	3	15.44	16.85	7	7
	68	4	15.95	17.13	7	6
Enhanced digestate pH ≥7 – <8 applied at 30 m^3/ha	68	1	16.14	23.11	7	7
	68	2	15.60	19.13	7	6
	68	3	15.40	23.71	7	7
	68	4	15.91	24.03	7	7
Enhanced digestate pH ≥8 applied at 30 m^3/ha	68	1	16.21	22.65	7	7
	68	2	15.02	17.68	7	6
	68	3	15.55	18.09	7	6
	68	4	15.18	22.16	7	7

* The two application rates are equivalent to 50 m^3/ha (114 ml per pot) and 30 m^3/ha (68 ml per pot).

Raw ammonium-N pot test results – 14 days

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	18.18	22.89	7	7
	114	2	17.82	24.41	7	7
	114	3	18.03	23.91	7	7
	114	4	17.75	25.31	7	7
Fertiliser control (200 kg N/ha)	114	1	19.60	24.21	7	7
	114	2	17.09	20.03	7	6
	114	3	18.75	20.84	7	7
	114	4	19.43	21.62	7	7
Fertiliser control (300 kg N/ha)	114	1	19.05	21.98	7	7
	114	2	17.19	19.00	7	7
	114	3	18.15	21.60	7	7
	114	4	19.11	21.92	7	7
Unamended pH <7 applied at 50 m^3/ha	114	1	18.42	18.62	7	6
	114	2	17.41	4.84	7	2
	114	3	15.62	18.03	7	6
	114	4	19.08	21.14	7	6
Unamended digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	17.10	21.38	7	7
	114	2	16.60	14.20	7	4
	114	3	17.54	12.09	7	5
	114	4	18.71	9.76	7	4
Unamended digestate pH ≥8 applied at 50 m^3/ha	114	1	19.78	8.17	7	3
	114	2	18.27	17.97	7	6
	114	3	18.57	16.47	7	6
	114	4	17.84	-	7	0
Unamended digestate pH <7 applied at 30 m^3/ha	68	1	16.69	21.73	7	7
	68	2	17.07	23.49	7	7
	68	3	16.44	23.16	7	7
	68	4	18.33	16.31	7	5
Unamended digestate pH ≥7 – <8 applied at 30 m^3/ha	68	1	19.10	25.23	7	7
	68	2	19.11	20.45	7	6
	68	3	19.88	25.27	7	7
	68	4	19.84	24.49	7	7

Unamended digestate pH ≥8 applied at 30 m^3/ha	68	1	15.26	18.38	7	6
	68	2	18.91	17.78	7	6
	68	3	18.38	19.86	7	6
	68	4	19.11	22.87	7	7
Enhanced digestate pH <7 applied at 50 m^3/ha	114	1	18.50	15.98	7	6
	114	2	19.69	21.36	7	7
	114	3	19.82	11.20	7	4
	114	4	18.75	9.18	7	3
Enhanced digestate pH ≥7 – <8 applied at 50 m^3/ha	114	1	18.23	17.09	7	6
	114	2	19.33	21.74	7	7
	114	3	19.53	12.09	7	4
	114	4	18.04	17.54	7	6
Enhanced digestate pH ≥8 applied at 50 m^3/ha	114	1	19.20	-	7	0
	114	2	19.35	15.31	7	6
	114	3	18.63	19.41	7	6
	114	4	19.45	15.86	7	5
Enhanced digestate pH <7 applied at 30 m^3/ha	68	1	18.15	14.19	7	5
	68	2	19.19	24.25	7	7
	68	3	18.12	17.08	7	6
	68	4	19.42	12.81	7	5
Enhanced digestate pH ≥7 – <8 pH <7 applied at 30 m^3/ha	68	1	19.44	19.84	7	6
	68	2	19.99	23.00	7	7
	68	3	18.83	22.10	7	7
	68	4	18.58	18.33	7	6
Enhanced digestate pH ≥8 pH <7 applied at 30 m^3/ha	68	1	18.12	2.88	7	2
	68	2	15.88	18.63	7	7
	68	3	19.47	22.57	7	7
	68	4	19.81	25.09	7	7

* The two application rates are equivalent to 50 m^3/ha (114 ml per pot) and 30 m^3/ha (68 ml per pot).

Appendix 4.7. Raw soil analysis data from ammonium-N pot test

Raw ammonium-N soil analysis pot test results – 24 hours

Treatment	Rep num.	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-Caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-Butyric acid (mg/l)	N-Butyric acid (mg/l)	Iso-Valeric acid (mg/l)	N-Valeric acid (mg/l)	Iso-Caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	1	84.9	7.54	<0.1	13.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.8	7.52	<0.1	15.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	83.6	7.48	<0.1	14.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	82.6	7.36	0.73	12.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
200 kg N/ha fertiliser control	1	81.8	7.19	92.4	155	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.2	6.78	67.0	115	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	82.0	7.18	166	163	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	83.6	7.04	55.5	140	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
300 kg N/ha fertiliser control	1	83.0	7.39	192	234	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	83.0	7.46	215	261	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	83.7	7.17	325	289	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	83.9	7.31	257	279	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 50 m^3/ha	1	83.3	7.73	318	2.40	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.4	7.72	571	2.84	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.3	7.62	368	9.61	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	82.8	7.80	350	0.97	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 50 m^3/ha	1	85.3	7.78	297	16.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.6	8.14	288	13.2	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	85.2	8.12	336	6.34	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	84.4	8.13	238	18.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 50 m^3/ha	1	84.4	8.53	281	14.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.2	8.44	391	5.10	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	82.8	8.70	300	1.57	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	83.4	8.31	376	0.60	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 30 m^3/ha	1	85.5	7.43	268	14.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.2	7.59	534	0.59	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	84.5	7.52	173	26.4	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	82.3	7.52	51.9	26.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

pH ≥7 – <8 applied at 30 m^3/ha	1	84.1	8.01	216	22.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.4	7.57	140	38.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	81.9	8.22	290	1.83	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.1	7.97	210	20.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 30 m^3/ha	1	86.6	7.78	91.9	39.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.8	7.76	150	26.5	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	85.5	8.29	346	5.97	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	84.2	8.25	87.4	34.4	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 50 m^3/ha	1	82.7	7.75	243	<0.10	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.1	7.45	881	0.82	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	84.9	7.42	670	6.01	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	84.3	7.44	514	8.90	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 50 m^3/ha	1	84.4	7.98	375	11.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	82.3	8.02	301	9.84	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	81.9	8.11	481	<0.10	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	82.8	8.12	470	3.02	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 50 m^3/ha	1	83.6	8.41	318	18.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.6	8.47	421	10.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	84.0	8.60	471	8.81	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	84.4	8.35	352	21.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 30 m^3/ha	1	85.7	7.30	277	28.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.4	8.27	398	19.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	87.6	7.48	531	12.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	86.8	6.92	163	32.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 30 m^3/ha	1	84.1	7.76	266	23.2	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.6	7.55	292	28.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	87.4	7.60	330	17.4	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	87.2	7.38	193	34.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 30 m^3/ha	1	83.7	8.22	258	14.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.3	8.29	331	15.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	85.4	7.94	296	20.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	86.8	8.18	222	24.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

Raw ammonium-N soil analysis pot test results – 14 days

Treatment	Rep num.	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-Caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-Butyric acid (mg/l)	N-Butyric acid (mg/l)	Iso-Valeric acid (mg/l)	N-Valeric acid (mg/l)	Iso-Caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	1	88.9	7.10	1.24	48.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	89.3	6.99	1.44	44.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.6	7.06	1.12	46.5	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.0	7.04	1.17	40.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
200 kg N/ha fertiliser control	1	89.0	6.37	73.3	203	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.7	6.55	48.0	241	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	89.4	6.89	105	268	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.3	6.63	74.7	206	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
300 kg N/ha fertiliser control	1	87.6	6.52	127	268	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	91.0	6.60	98.4	216	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.1	6.88	181	311	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	87.5	6.61	353	479	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 50 m^3/ha	1	84.8	7.20	308	64.3	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	82.7	7.34	366	60.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.1	6.75	212	120	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	85.0	6.60	258	142	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 50 m^3/ha	1	86.9	6.55	138	168	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	87.6	6.98	187	128	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.4	6.80	179	174	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	86.1	6.90	287	146	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 50 m^3/ha	1	88.5	7.39	241	126	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.5	7.07	196	139	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.8	7.21	84.6	169	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	85.6	6.95	154	158	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 30 m^3/ha	1	90.1	6.71	104	85.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	90.4	6.57	119	90.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	87.4	6.75	100	105	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	87.7	6.55	92.2	126	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

pH ≥7 – <8 applied at 30 m^3/ha	1	87.4	6.43	66.2	145	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	88.1	6.63	77.5	140	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.9	6.92	86.1	134	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.3	6.46	96.8	167	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 30 m^3/ha	1	90.0	7.07	88.8	96.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	88.2	6.75	54.6	126	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	87.6	7.13	90.0	115	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.9	6.82	206	136	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 50 m^3/ha	1	87.2	6.95	444	106	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	89.3	6.88	385	103	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	87.7	6.95	457	114	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	83.6	7.43	657	36.4	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 50 m^3/ha	1	88.9	6.80	308	124	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	87.9	7.02	345	128	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	84.8	7.17	333	123	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.3	7.12	336	125	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 50 m^3/ha	1	83.3	8.14	510	4.44	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.5	7.28	278	117	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.1	6.98	197	125	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.0	7.28	268	142	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH <7 applied at 30 m^3/ha	1	87.0	5.96	190	142	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	89.3	6.30	610	76.2	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.3	6.50	292	90.3	<20	<50	82	<12.5	<12.5	<25	<25	<20	90
	4	84.4	6.96	422	85.3	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥7 – <8 applied at 30 m^3/ha	1	86.3	6.38	200	132	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	90.7	6.84	240	73.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.8	6.85	170	99.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.4	6.56	225	122	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
pH ≥8 applied at 30 m^3/ha	1	84.0	7.52	315	60.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.1	6.91	88.7	128	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.2	6.17	125	112	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	89.7	6.78	262	96.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

Appendix 4.8. Raw acetic acid experiment pot test results

Raw acetic acid pot test results – 2 hours

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	19.89	27.63	7	7
	114	2	18.71	28.51	7	7
	114	3	17.45	25.98	7	7
	114	4	19.17	29.12	7	7
Unamended digestate (*c.*1,000 mg/l acetic acid)	114	1	18.60	15.59	7	4
	114	2	17.43	17.95	7	5
	114	3	18.14	21.57	7	6
	114	4	17.60	18.14	7	5
Enhanced digestate (*c.*2,500 mg/l acetic acid)	114	1	19.62	17.89	7	4
	114	2	15.31	9.70	7	3
	114	3	19.53	25.37	7	7
	114	4	18.71	12.44	7	5
Enhanced digestate (*c.*3,500 mg/l acetic acid)	114	1	18.27	26.61	7	7
	114	2	18.71	18.99	7	6
	114	3	15.27	20.17	7	7
	114	4	19.64	15.32	7	4
Enhanced digestate (*c.*5,000 mg/l acetic acid)	114	1	17.00	19.53	7	6
	114	2	18.19	25.12	7	7
	114	3	16.56	14.73	7	5
	114	4	18.26	25.38	7	7

* The application rate is equivalent to 50 m^3/ha (114 ml per pot).

Raw acetic acid pot test results – 24 hours

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	16.14	24.06	7	7
	114	2	17.82	26.31	7	7
	114	3	16.33	24.76	7	7
	114	4	16.67	24.54	7	7
Unamended digestate (*c.*1,000 mg/l acetic acid)	114	1	20.85	20.36	7	5
	114	2	17.15	25.13	7	7
	114	3	16.65	19.65	7	6
	114	4	17.96	21.29	7	6
Enhanced digestate (*c.*2,500 mg/l acetic acid)	114	1	18.42	22.89	7	6
	114	2	16.12	12.13	7	4
	114	3	15.19	16.15	7	5
	114	4	17.07	13.55	7	4
Enhanced digestate (*c.*3,500 mg/l acetic acid)	114	1	15.13	20.35	7	7
	114	2	17.26	14.70	7	4
	114	3	17.04	22.26	7	6
	114	4	15.75	20.55	7	6
Enhanced digestate (*c.*5,000 mg/l acetic acid)	114	1	15.51	23.92	7	7
	114	2	16.92	23.40	7	7
	114	3	15.98	20.89	7	6
	114	4	16.11	22.11	7	6

* The application rate is equivalent to 50 m^3/ha (114 ml per pot).

Raw acetic acid pot test results – 7 days

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	16.30	21.83	7	7
	114	2	15.66	22.53	7	7
	114	3	15.19	19.40	7	7
	114	4	14.83	21.36	7	7
Unamended digestate (*c.*1,000 mg/l acetic acid)	114	1	18.21	22.44	7	6
	114	2	15.43	22.38	7	7
	114	3	15.01	15.68	7	6
	114	4	16.46	11.10	7	3
Enhanced digestate (*c.*2,500 mg/l acetic acid)	114	1	15.76	19.82	7	6
	114	2	14.75	21.65	7	7
	114	3	19.37	18.59	7	5
	114	4	18.31	17.21	7	6
Enhanced digestate (*c.*3,500 mg/l acetic acid)	114	1	16.55	11.09	7	3
	114	2	15.22	12.49	7	4
	114	3	19.33	24.33	7	6
	114	4	19.48	28.28	7	7
Enhanced digestate (*c.*5,000 mg/l acetic acid)	114	1	19.24	26.31	7	7
	114	2	17.77	21.78	7	5
	114	3	19.91	16.22	7	4
	114	4	19.11	10.97	7	3

* The application rate is equivalent to 50 m^3/ha (114 ml per pot).

Raw acetic acid pot test results – 14 days

Chemical	Application rate*	Replicate Number	Worm (g) pre-test	Worm (g) post-test	Number of live worms pre-test	Number of live worms post-test
Untreated control	114	1	18.18	22.89	7	7
	114	2	17.82	24.41	7	7
	114	3	18.03	23.91	7	7
	114	4	17.75	25.31	7	7
Unamended digestate (*c.*1,000 mg/l acetic acid)	114	1	19.78	8.17	7	3
	114	2	18.27	17.97	7	6
	114	3	18.57	16.47	7	6
	114	4	17.84	-	7	0
Enhanced digestate (*c.*2,500 mg/l acetic acid)	114	1	16.15	18.82	7	7
	114	2	18.22	13.32	7	5
	114	3	19.25	16.13	7	7
	114	4	19.20	-	7	0
Enhanced digestate (*c.*3,500 mg/l acetic acid)	114	1	15.64	8.68	7	4
	114	2	17.32	10.67	7	4
	114	3	18.49	22.14	7	7
	114	4	15.34	14.07	7	6
Enhanced digestate (*c.*5,000 mg/l acetic acid)	114	1	15.71	10.25	7	4
	114	2	16.45	6.67	7	3
	114	3	18.40	12.78	7	6
	114	4	16.00	5.37	7	3

* The application rate is equivalent to 50 m^3/ha (114 ml per pot).

Appendix 4.9. Raw soil analysis data from acetic acid pot test

Raw acetic acid soil analysis pot test results – 24 hours

Treatment	Rep num.	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-Caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-Butyric acid (mg/l)	N-Butyric acid (mg/l)	Iso-Valeric acid (mg/l)	N-Valeric acid (mg/l)	Iso-Caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	1	84.9	7.54	<0.10	13.9	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.8	7.52	<0.10	15.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	83.6	7.48	<0.10	14.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	82.6	7.36	0.73	12.1	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Unamended digestate (*c.*1,000 mg/l acetic acid)	1	84.4	8.53	281	14.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.2	8.44	391	5.10	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	82.8	8.70	300	1.57	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	83.4	8.31	376	0.60	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced digestate (*c.*2,500 mg/l acetic acid)	1	82.6	8.63	338	2.06	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.7	8.70	284	10.3	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	85.0	8.46	315	10.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	84.3	8.28	444	1.66	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced digestate (*c.*3,500 mg/l acetic acid)	1	82.2	8.29	298	<0.10	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.7	8.56	211	16.4	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	84.3	8.61	319	4.74	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	85.3	8.24	275	17.6	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced digestate (*c.*5,000 mg/l acetic acid)	1	84.5	8.60	300	13.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.7	8.69	345	4.20	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.1	8.59	438	0.93	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	84.6	8.61	340	3.19	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

Raw acetic acid soil analysis pot test results – 14 days

Treatment	Rep num.	Dry matter (%)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	N-Caproic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Iso-Butyric acid (mg/l)	N-Butyric acid (mg/l)	Iso-Valeric acid (mg/l)	N-Valeric acid (mg/l)	Iso-Caproic acid (mg/l)	Acetic acid eqv. (mg/)
Untreated control	1	88.9	7.10	1.24	48.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	89.3	6.99	1.44	44.0	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.6	7.06	1.12	46.5	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	88.0	7.04	1.17	40.7	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Unamended digestate (*c.*1,000 mg/l acetic acid)	1	88.5	7.39	241	126	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	84.5	7.07	196	139	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.8	7.21	84.6	169	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	85.6	6.95	154	158	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced digestate (*c.*2,500 mg/l acetic acid)	1	89.3	7.15	171	124	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	85.1	7.14	185	116	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	88.1	7.36	164	131	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	83.2	8.11	450	6.61	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced digestate (*c.*3,500 mg/l acetic acid)	1	86.7	7.11	191	124	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	87.2	7.02	188	116	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	89.6	6.92	76.8	110	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	85.8	7.29	149	127	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
Enhanced digestate (*c.*5,000 mg/l acetic acid)	1	84.7	7.60	251	58.8	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	2	86.3	7.47	239	126	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	3	86.8	6.94	147	141	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80
	4	85.5	7.15	162	134	<20	<50	<50	<12.5	<12.5	<25	<25	<20	<80

APPENDIX 6. Crop quality results

Table 1. Nutrient concentrations in harvested plant material (2011)

	Nutrient concentration (% dm)				
Site/treat	**Total N**	**Total P**	**Total K**	**Total Mg**	**Total S**
Aberdeen (SB):					
Control	1.51^{a}	0.19	0.53	0.09	0.14
Green compost	1.57^{ab}	0.12	0.68	0.08	0.14
Green/food compost	1.56^{ab}	0.18	0.85	0.10	0.14
Food-based digestate	1.72^{b}	0.20	0.77	0.11	0.15
FYM	1.57^{ab}	0.19	0.75	0.10	0.14
Livestock slurry	1.74^{b}	0.19	0.71	0.09	0.15
Manure-based digestate	1.74^{b}	0.22	0.49	0.09	0.15
P^{1}	***0.04***	*NS 0.60*	*NS 0.54*	*NS 0.41*	*NS 0.28*
Ayr (grass):					
Control	2.34	0.20	1.92	0.17	0.20
Green compost	2.01	0.20	1.98	0.17	0.20
Green/food compost	2.06	0.21	2.12	0.16	0.16
Food-based digestate	1.94	0.22	2.09	0.16	0.17
FYM	2.30	0.24	2.21	0.16	0.19
Livestock slurry	2.11	0.20	2.27	0.16	0.18
Manure-based digestate	2.01	0.21	2.38	0.16	0.17
P^{1}	*NS 0.09*	*NS 0.13*	*NS 0.10*	*NS 0.75*	*NS 0.25*
Devises (linseed):					
Control	3.11	0.42	0.69	0.29	0.22
Green compost	3.05	0.46	0.74	0.31	0.21
Green/food compost	3.12	0.44	0.74	0.30	0.22
Food-based digestate	3.09	0.43	0.73	0.30	0.22
FYM	3.17	0.40	0.67	0.29	0.22
Livestock slurry	3.04	0.46	0.76	0.31	0.21
P^{1}	*NS 0.26*	*NS 0.30*	*NS 0.51*	*NS 0.22*	*NS 0.40*
Faringdon (WW):					
Control	1.93^{a}	0.22	0.44	0.08	0.13
Green compost	1.92^{a}	0.23	0.45	0.09	0.13
Green/food compost	1.96^{a}	0.23	0.46	0.09	0.12
Food-based digestate	1.92^{a}	0.22	0.45	0.09	0.12
FYM	1.94^{a}	0.21	0.43	0.08	0.12
Livestock slurry	1.80^{b}	0.23	0.46	0.08	0.11
P^{1}	***0.007***	*NS*	*NS*	*NS*	*NS*
Harper Adams (POT):					
Control	1.84	0.19	1.72^{a}	0.09^{a}	0.11
Green compost	1.98	0.18	2.14^{bc}	0.10^{b}	0.10
Green/food compost	1.94	0.20	2.06^{bc}	0.10^{b}	0.11
Food-based digestate	1.78	0.20	1.99^{b}	0.10^{b}	0.10
FYM	1.94	0.22	2.40^{d}	0.11^{b}	0.11
Livestock slurry	2.06	0.19	2.24^{cd}	0.11^{b}	0.13
P^{1}	*NS 0.36*	*NS 0.11*	***<0.001***	***0.007***	*NS 0.60*
Lampeter (grass):					
Control	1.34	0.22	1.09^{a}	0.12	0.13
Green compost	1.46	0.19	1.21^{a}	0.13	0.14
Green/food compost	1.42	0.19	1.06^{a}	0.12	0.12
Food-based digestate	1.51	0.19	1.59^{b}	0.11	0.12
FYM	1.29	0.19	1.40^{a}	0.09	0.14
Livestock slurry	1.62	0.22	2.03^{c}	0.12	0.16

P[1]	*NS 0.44*	*NS 0.20*	***0.001***	*NS 0.07*	*NS 0.15*
Terrington (WW):					
Control	2.86	0.29^{a}	0.43^{a}	0.11	0.17
Green compost	2.62	0.31^{ab}	0.43^{a}	0.12	0.18
Green/food compost	2.71	0.36^{c}	0.49^{c}	0.13	0.17
Food-based digestate	2.23	0.35^{c}	0.50^{c}	0.12	0.16
FYM	2.41	0.32^{b}	0.46^{b}	0.12	0.16
Livestock slurry	2.63	0.31^{ab}	0.44^{ab}	0.11	0.16
P[1]	*NS 0.11*	***<0.001***	***<0.001***	*0.009*	*NS 0.74*

Table 2 Nutrient concentrations in harvested plant material (2012)

	Nutrient concentration (% dm)				
Site/treat	**Total N**	**Total P**	**Total K**	**Total Mg**	**Total S**
Aberdeen (WB):					
Control	2.40^{d}	0.41	0.53	0.11	0.14^{bcd}
Green compost	2.37^{cd}	0.41	0.53	0.11	0.14^{bcd}
Green/food compost	2.39^{cd}	0.42	0.54	0.12	0.15^{cd}
Food-based digestate	2.38^{cd}	0.40	0.53	0.11	0.16^{d}
FYM	2.17^{bc}	0.40	0.55	0.11	0.14^{abc}
Livestock slurry	1.77^{a}	0.39	0.54	0.11	0.12^{a}
Manure-based digestate	1.99^{b}	0.41	0.53	0.11	0.13^{ab}
P^{1}	***0.001***	*NS 0.61*	*NS 0.85*	*NS 0.58*	***0.008***
Ayr (grass):					
Control	3.02	0.36	3.74	0.17	0.21
Green compost	2.76	0.34	3.96	0.16	0.22
Green/food compost	2.60	0.34	3.77	0.16	0.21
Food-based digestate	2.76	0.36	3.79	0.16	0.21
FYM	2.89	0.34	3.84	0.17	0.22
Livestock slurry	2.71	0.32	3.80	0.17	0.21
Manure-based digestate	2.81	0.31	3.72	0.17	0.22
P^{1}	*NS 0.55*	*NS 0.06*	*NS 0.75*	*NS 0.68*	*NS 0.85*
Devizes (WW):					
Control	2.39^{c}	0.30^{a}	0.53	0.09	0.16^{c}
Green compost	2.29^{ab}	0.34^{bc}	0.53	0.09	0.15^{abc}
Green/food compost	2.30^{ab}	0.33^{bc}	0.54	0.09	0.15^{abc}
Food-based digestate	2.30^{ab}	0.32^{ab}	0.53	0.09	0.15^{ab}
FYM	2.35^{bc}	0.35^{c}	0.56	0.09	0.15^{bc}
Livestock slurry	2.25^{a}	0.34^{c}	0.56	0.09	0.15^{a}
P^{1}	***0.03***	***0.01***	*NS 0.09*	*NS 0.06*	***0.05***
Faringdon (WW):					
Control	2.42	0.34	0.59	0.10	0.17
Green compost	2.38	0.33	0.56	0.09	0.15
Green/food compost	2.43	0.36	0.61	0.10	0.16
Food-based digestate	2.36	0.38	0.62	0.11	0.15
FYM	2.39	0.37	0.62	0.10	0.16
Livestock slurry	2.38	0.36	0.61	0.10	0.16
P^{1}	*NS 0.96*	*NS 0.73*	*NS 0.68*	*NS 0.73*	*NS 0.82*
Harper Adams (SB):					
Control	1.76^{a}	0.40^{a}	0.56^{a}	0.11^{a}	0.12^{a}
Green compost	2.25^{c}	0.46^{bc}	0.62^{bc}	0.12^{ab}	0.15^{c}
Green/food compost	2.21^{bc}	0.45^{bc}	0.60^{b}	0.12^{ab}	0.14^{b}
Food-based digestate	2.08^{bc}	0.43^{ab}	0.59^{b}	0.11^{a}	0.13^{b}
FYM	2.20^{bc}	0.47^{c}	0.64^{c}	0.13^{bc}	0.13^{b}
Livestock slurry	2.04^{b}	0.47^{c}	0.64^{c}	0.13^{c}	0.13^{ab}
P^{1}	***0.002***	***0.002***	***0.001***	***0.019***	***0.003***
Lampeter (grass):					
Control	1.35^{a}	0.22^{ab}	1.41^{a}	0.12^{abc}	0.14
Green compost	1.38^{a}	0.21^{a}	1.90^{b}	0.10^{a}	0.15
Green/food compost	1.65^{abc}	0.26^{bc}	2.29^{c}	0.11^{abc}	0.16
Food-based digestate	1.91^{c}	0.27^{c}	2.24^{bc}	0.12^{bc}	0.17
FYM	1.79^{bc}	0.29^{c}	2.28^{c}	0.13^{c}	0.18
Livestock slurry	1.50^{ab}	0.24^{abc}	2.27^{c}	0.10^{ab}	0.16
P^{1}	***0.021***	***0.022***	***0.001***	***0.04***	*NS 0.08*

Terrington (WW):					
Control	2.30[a]	0.33	0.53	0.10	0.14[a]
Green compost	2.39[ab]	0.33	0.54	0.10	0.15[b]
Green/food compost	2.36[ab]	0.34	0.54	0.10	0.14[a]
Food-based digestate	2.30[a]	0.34	0.53	0.10	0.14[a]
FYM	2.44[b]	0.36	0.55	0.11	0.16[b]
Livestock slurry	2.45[a]	0.34	0.55	0.10	0.15[b]
P[1]	***0.05***	*NS 0.31*	*NS 0.85*	*NS 0.40*	***0.001***

1 Statistical analysis was undertaken using ANOVA (data normally distributed). There were three replicates of each treatment; *NS:* No significant difference ($P>0.05$).

Table 3. Nutrient concentrations in harvested plant material, Harvest 2013

	Nutrient concentration (% dm)				
Site/treat	**Total N**	**Total P**	**Total K**	**Total Mg**	**Total S**
Aberdeen (WOSR)					
Control	2.94	0.55	0.83	0.28	0.21
Green compost	2.98	0.59	0.85	0.30	0.22
Green/food compost	2.96	0.57	0.84	0.29	0.23
Food-based digestate	2.98	0.60	0.86	0.30	0.23
FYM	2.99	0.61	0.88	0.30	0.22
Livestock slurry	2.98	0.59	0.89	0.30	0.24
Manure-based digestate	3.00	0.61	0.87	0.30	0.24
***P*[1]**	*NS 0.876*	*<0.005*	*NS 0.062*	*<0.042*	*NS 0.083*
Ayr (grass):					
Control	1.84	0.29	2.87	0.13	0.14
Green compost	1.90	0.29	2.96	0.13	0.13
Green/food compost	1.86	0.29	2.90	0.12	0.12
Food-based digestate	1.36	0.26	2.63	0.10	0.11
FYM	1.79	0.29	2.99	0.12	0.13
Livestock slurry	1.75	0.28	2.79	0.12	0.13
Manure-based digestate	1.64	0.27	2.76	0.11	0.12
***P*[1]**	*<0.020*	*NS 0.564*	*NS 0.446*	*<0.005*	*NS 0.134*
Devizes					
Control	2.43	0.18	0.41	0.08	0.11
Green compost	2.43	0.20	0.42	0.09	0.12
Green/food compost	2.50	0.19	0.41	0.08	0.11
Food-based digestate	2.42	0.20	0.41	0.08	0.11
FYM	2.35	0.21	0.40	0.08	0.11
Livestock slurry	2.40	0.19	0.40	0.08	0.11
***P*[1]**	*NS 0.649*	*NS 0.449*	*NS 0.839*	*NS 0.707*	*NS 0.954*
Faringdon					
Control	1.39	0.16	2.29	0.10	0.13
Green compost	1.17	0.18	2.32	0.09	0.12
Green/food compost	1.27	0.18	2.38	0.09	0.12
Food-based digestate	1.19	0.17	2.25	0.09	0.12
FYM	1.36	0.17	2.40	0.10	0.12
Livestock slurry	1.19	0.17	2.31	0.09	0.11
***P*[1]**	*<0.021*	*NS 0.610*	*NS 0.784*	*NS 0.665*	*NS 0.248*
Harper Adams					
Control	2.28	0.28	0.47	0.09	0.13
Green compost	2.29	0.27	0.48	0.09	0.13
Green/food compost	2.26	0.28	0.49	0.09	0.12
Food-based digestate	2.22	0.31	0.53	0.09	0.13
FYM	2.25	0.31	0.50	0.10	0.13
Livestock slurry	2.23	0.27	0.50	0.09	0.12
***P*[1]**	*NS 0.923*	*NS 0.145*	*NS 0.055*	*<0.039*	*NS 0.414*
Lampeter (grass):					
Control	2.52	0.32	3.10	0.13	0.20
Green compost	2.56	0.35	3.89	0.12	0.20
Green/food compost	2.75	0.37	4.04	0.12	0.21
Food-based digestate	2.69	0.31	3.81	0.12	0.20
FYM	2.52	0.39	3.92	0.13	0.19

Livestock slurry	2.58	0.34	4.06	0.12	0.20
***P*[1]**	*NS 0.799*	*<0.007*	*<0.009*	*NS 0.320*	*NS 0.880*
Terrington (WOSR):					
Control	3.09	0.77	0.85	0.37	0.37
Green compost	3.17	0.60	0.71	0.30	0.30
Green/food compost	3.07	0.60	0.73	0.32	0.33
Food-based digestate	2.77	0.49	0.77	0.32	0.34
FYM	3.14	0.67	0.75	0.33	0.34
Livestock slurry	3.12	0.67	0.76	0.33	0.34
Control	3.29	0.45	0.49	0.21	0.24
***P*[1]**	*<0.004*	*NS 0.676*	*NS 0.383*	*NS 0.383*	*NS 0.503*

[1] Statistical analysis was undertaken using ANOVA (data normally distributed). There were three replicates of each treatment.
NS: No significant difference (*P*>0.05).

Table 4 Oil content (%) of the rape seed at Aberdeen and Terrington in 2013.

Treatment	Aberdeen	Terrington
Control	42.8	43.4
Green compost	42.7	43.0
Green/food compost	42.7	43.3
Food-based digestate	42.8	44.4
FYM	42.3	41.9
Livestock slurry	42.8	43.2
Manure-based digestate	42.9	42.5
***P*[1]**	*NS 0.95*	*NS* 0.07

Table 5 Titanium content of the topsoil and grass at Ayr and Lampeter in 2013.

Treatment	Ayr		Lampeter	
	Soil	Grass	Soil	Grass
Control	407	2.97^{bc}	75	4.27
Green compost	367	2.87^{c}	83	3.93
Green/food compost	463	3.07^{a}	71	4.33
Food-based digestate	507	2.43^{a}	74	3.90
FYM	447	2.47^{abc}	82	4.17
Livestock slurry	537	2.67^{ab}	72.3	3.67
Manure-based digestate	407	2.53^{abc}		
***P*[1]**	NS 0.29	0.05	NS 0.33	NS 0.26

Table 6 Total metal content of the harvested materials

Site/Trt	Total metal concentrations (mg/kg dw)									
	Cu	**Zn**	**Pb**	**Mo**	**Ni**	**Cd**	**Hg**	**As**	**Cr**	**Se**
Aberdeen (WOSR)										
Control	3.97	37.6	0.02	0.60	0.17	0.137	0.010	<0.1	0.07	<0.02
Green Compost	3.93	38.8	0.01	0.68	0.20	0.083	0.009	<0.1	0.12	<0.02
Green/Food Compost	3.93	38.9	0.02	0.62	0.20	0.090	0.024	<0.1	0.15	<0.02
Food-based digestate	4.00	39.3	0.02	0.62	0.30	0.096	0.012	<0.1	0.10	<0.02
FYM	4.20	38.5	0.02	0.69	0.23	0.105	0.009	<0.1	0.08	<0.02
Livestock slurry	4.30	38.8	0.03	0.66	0.20	0.116	0.007	<0.1	0.25	<0.02
Manure-based digestate	4.10	40.0	0.07	0.66	0.17	0.106	0.031	<0.1	0.17	<0.02
***P*[1]**	*NS (0.17)*	*NS (0.42)*	*NS (0.46)*	*NS (0.15)*	*NS (0.13)*	*NS (0.69)*	*NS (0.79)*	*NS (1.0)*	*NS (0.18)*	*NS (1.0)*
Ayr (Grass)										
Control	7.30	27.4^{abc}	0.07	0.71^{a}	0.50	0.016	0.015	<0.1	0.07	<0.02
Green Compost	5.70	27.4^{abc}	0.08	1.17^{c}	0.37	0.014	0.009	<0.1	0.20	<0.02
Green/Food Compost	5.30	27.8^{abc}	0.07	1.04^{bc}	0.40	0.014	0.007	<0.1	0.18	<0.02
Food-based digestate	4.43	22.9^{a}	0.06	0.65^{a}	0.50	0.007	0.011	<0.1	0.12	<0.02
FYM	5.37	29.9^{c}	0.08	0.82^{ab}	0.47	0.015	0.023	<0.1	0.15	<0.02
Livestock slurry	5.43	29.1^{bc}	0.06	0.59^{a}	0.57	0.011	0.009	<0.1	0.07	<0.02
Manure-based digestate	5.13	24.2^{ab}	0.06	0.63^{a}	0.47	0.013	0.018	<0.1	0.13	<0.02
***P*[1]**	*NS (0.19)*	***0.05***	*NS (0.45)*	***0.002***	*NS (0.65)*	*NS (0.50)*	*NS (0.39)*	*NS (1.0)*	*NS (0.39)*	*NS (1.0)*
Devizes (WW)										
Control	3.93^{c}	28.3	0.04	0.31	0.07	0.023	0.007	0.005	0.07	<0.02
Green Compost	3.43^{abc}	27.9	0.11	0.42	0.17	0.030	0.108	0.037	0.07	<0.02
Green/Food Compost	3.53^{bc}	27.5	0.10	0.37	0.07	0.023	0.017	0.037	0.08	<0.02
Food-based digestate	3.50^{bc}	26.9	0.04	0.30	0.05	0.027	0.012	0.005	0.05	<0.02
FYM	2.93^{a}	24.5	0.18	0.31	0.13	0.030	0.048	0.037	1.00	<0.02
Livestock slurry	3.03^{ab}	24.2	0.05	0.30	0.43	0.027	0.017	0.005	0.07	<0.02
***P*[1]**	***0.012***	*NS (0.28)*	*NS (0.41)*	*NS (0.10)*	*NS (0.50)*	*NS (.69)*	*NS (0.19)*	*NS (0.70)*	*NS (0.49)*	*NS (1.0)*
Faringdon (forage oats)										
Control	3.87^{b}	17.9	0.05	0.83	0.97^{b}	0.050	0.012	<0.1	0.050	0.020
Green Compost	3.37^{a}	16.6	0.04	0.91	0.70^{a}	0.030	0.007	<0.1	0.050	0.013
Green/Food Compost	3.53^{ab}	17.3	0.05	0.93	0.70^{a}	0.033	0.005	<0.1	0.067	0.013
Food-based digestate	3.50^{ab}	17.6	0.03	0.86	0.73^{a}	0.030	0.017	<0.1	0.067	0.013

Site/Trt	Total metal concentrations (mg/kg dw)									
	Cu	**Zn**	**Pb**	**Mo**	**Ni**	**Cd**	**Hg**	**As**	**Cr**	**Se**
FYM	3.53[ab]	17.6	0.06	0.84	0.87[a]	0.033	0.007	<0.1	0.133	0.010
Livestock slurry	3.23[a]	15.4	0.04	0.75	0.70[a]	0.027	0.023	<0.1	0.067	0.010
***P*[1]**	***0.034***	*NS (0.44)*	*NS (0.37)*	*NS (0.74)*	***0.012***	*NS (0.08)*	*NS (0.31)*	*NS (1.0)*	*NS (0.08)*	*NS (0.11)*
Harper Adams (WW)										
Control	2.67	29.5	0.02	0.40	0.10	0.037	0.015	<0.1	0.100	0.010
Green Compost	2.33	29.3	0.02	0.65	0.05	0.027	0.012	<0.1	0.167	0.010
Green/Food Compost	2.77	32.6	0.03	0.69	0.12	0.037	0.052	<0.1	0.133	0.017
Food-based digestate	2.87	37.0	0.02	0.50	0.09	0.043	0.028	<0.1	0.200	0.017
FYM	2.50	29.4	0.04	0.61	0.07	0.037	0.112	<0.1	0.200	0.010
Livestock slurry	2.53	31.2	0.02	0.50	0.05	0.037	0.070	<0.1	0.167	0.013
***P*[1]**	*NS (0.23)*	*NS (0.36)*	*NS (0.38)*	*NS (0.60)*	*NS (0.63)*	*NS (0.69)*	*NS (0.68)*	*NS (1.0)*	*NS(0.39)*	*NS (0.63)*
Lampeter (Grass)										
Control	5.40[c]	23.3	0.08	0.44[a]	0.40[bc]	0.009	0.023	<0.1	0.100	0.010
Green Compost	5.07[bc]	25.3	0.09	0.67[a]	0.27[a]	0.007	0.076	<0.1	0.167	0.010
Green/Food Compost	4.67[ab]	26.4	0.07	0.95[a]	0.30[ab]	0.007	0.023	<0.1	0.200	0.010
Food-based digestate	4.37[a]	25.8	0.08	0.42[a]	0.43[c]	0.015	0.052	<0.1	0.150	0.014
FYM	5.07[bc]	28.4	0.09	1.59[b]	0.30[ab]	0.007	0.024	<0.1	0.133	0.013
Livestock slurry	5.60[c]	25.2	0.09	0.47[a]	0.30[ab]	0.008	0.023	<0.1	0.117	0.010
***P*[1]**	***0.003***	*NS (0.30)*	*NS (0.81)*	***0.006***	***0.022***	*NS (0.19)*	*NS (0.14)*	*NS (1.0)*	*NS (0.32)*	*NS (0.61)*
Terrington (WOSR)										
Control	5.13	45.1	0.04	1.07	0.20[a]	0.017	0.010	<0.1	0.233	0.210[b]
Green Compost	5.37	42.8	0.05	1.11	0.23[ab]	0.013	0.010	<0.1	0.233	0.117[a]
Green/Food Compost	4.83	43.2	0.04	1.07	0.33[c]	0.010	0.005	<0.1	0.200	0.107[a]
Food-based digestate	5.97	47.2	0.05	1.18	0.30[bc]	0.013	0.005	<0.1	0.300	0.123[a]
FYM	5.47	41.9	0.04	1.11	0.37[c]	0.010	0.005	<0.1	0.267	0.067[a]
Livestock slurry	5.83	44.9	0.04	0.88	0.37[c]	0.010	0.007	<0.1	0.233	0.087[a]
***P*[1]**	*NS (0.67)*	*NS (0.81)*	*NS (0.54)*	*NS (0.11)*	***0.002***	*NS (0.23)*	*NS (0.66)*	*NS (1.0)*	*NS (0.70)*	***0.032***

[1]Statistical analysis undertaken using ANOVA (data normally distributed). There were three replicates of each treatment; NS: No significant difference ($P>0.05$); different letters indicate significant differences between treatments ($P<0.05$).

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