

# Assessment of Earthworm Services on Litter Mineralisation and Nutrient Release in Annual and Perennial Energy Crops (*Zea mays* vs. *Silphium perfoliatum*)

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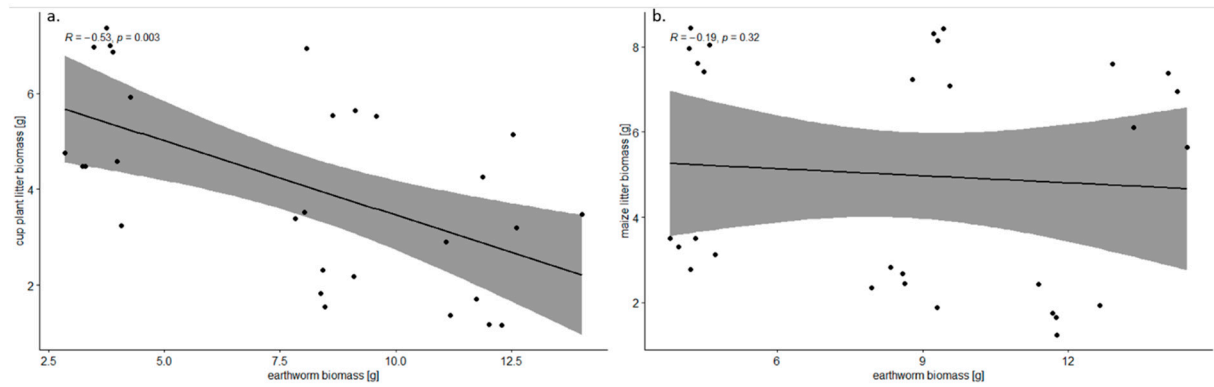
## Supplementary Information

**Table S1.** Initial and final earthworm biomass (raw) [g] (means  $\pm$  SE, n = 5) for cup plant and maize treatments for 4 and 8 weeks of incubation. Primary = primary decomposer *L. terrestris*; Secondary = secondary decomposers *A. caliginosa* and *A. rosea*; Mix = primary and secondary decomposers.

		<u>Cup plant</u>		<u>Maize</u>	
		Initial earthworm biomass (raw) [g]	Final earthworm biomass (raw) [g]	Initial earthworm biomass (raw) [g]	Final earthworm biomass (raw) [g]
4 weeks	Primary	12.95 $\pm$ 0.11	12.42 $\pm$ 0.48	12.68 $\pm$ 0.23	13.80 $\pm$ 0.29
	Mix	7.99 $\pm$ 0.16	8.69 $\pm$ 0.30	8.04 $\pm$ 0.05	9.26 $\pm$ 0.13
	Secondary	3.76 $\pm$ 0.02	3.84 $\pm$ 0.13	4.04 $\pm$ 0.01	4.37 $\pm$ 0.08
8 weeks	Primary	12.48 $\pm$ 0.09	11.81 $\pm$ 0.24	12.11 $\pm$ 0.02	11.84 $\pm$ 0.21
	Mix	8.33 $\pm$ 0.05	8.44 $\pm$ 0.20	8.33 $\pm$ 0.10	8.56 $\pm$ 0.22
	Secondary	3.69 $\pm$ 0.01	3.49 $\pm$ 0.23	3.94 $\pm$ 0.02	4.21 $\pm$ 0.16

**Table S2.** Remaining litter biomass, decomposition rate  $k$  and weekly consumption rate  $C$  [ $\text{g g}^{-1}$ ] (means  $\pm$  SE,  $n = 5$ ) for cup plant and maize treatments for 4 and 8 weeks of incubation. Control = no earthworms (i.e., no consumption rate); Primary = primary decomposer *L. terrestris*; Secondary = secondary decomposers *A. caliginosa* and *A. rosea*; Mix = primary and secondary decomposers. For statistics see Table 3 of the main article and Table S6 of the Supplementary Information.

		<u>Remaining litter biomass [g]</u>		<u>Decomposition rate <math>k</math></u>		<u>Consumption rate <math>C</math> [<math>\text{g g}^{-1}</math>]</u>	
		Cup plant	Maize	Cup plant	Maize	Cup plant	Maize
4 weeks	Control	6.652 $\pm$ 0.253	9.016 $\pm$ 0.233	0.021 $\pm$ 0.001	0.010 $\pm$ 0.001		
	Primary	3.794 $\pm$ 0.405	6.736 $\pm$ 0.374	0.042 $\pm$ 0.004	0.021 $\pm$ 0.002	0.129 $\pm$ 0.012	0.068 $\pm$ 0.008
	Mix	5.430 $\pm$ 0.546	7.838 $\pm$ 0.283	0.029 $\pm$ 0.004	0.015 $\pm$ 0.001	0.126 $\pm$ 0.027	0.064 $\pm$ 0.010
	Secondary	6.822 $\pm$ 0.241	7.896 $\pm$ 0.178	0.020 $\pm$ 0.001	0.015 $\pm$ 0.001	0.139 $\pm$ 0.020	0.127 $\pm$ 0.013
8 weeks	Control	5.264 $\pm$ 0.109	6.048 $\pm$ 0.092	0.015 $\pm$ 0.000	0.012 $\pm$ 0.000		
	Primary	1.353 $\pm$ 0.129	1.796 $\pm$ 0.192	0.039 $\pm$ 0.002	0.034 $\pm$ 0.002	0.102 $\pm$ 0.002	0.097 $\pm$ 0.003
	Mix	2.250 $\pm$ 0.312	2.436 $\pm$ 0.163	0.031 $\pm$ 0.002	0.029 $\pm$ 0.001	0.127 $\pm$ 0.006	0.124 $\pm$ 0.003
	Secondary	4.304 $\pm$ 0.271	3.242 $\pm$ 0.137	0.018 $\pm$ 0.001	0.023 $\pm$ 0.001	0.183 $\pm$ 0.011	0.220 $\pm$ 0.007



**Figure S1.** a.) final cup plant litter biomass [g] and b.) final maize litter biomass [g] the respective final earthworm biomasses (raw) [g] averaged over duration of incubation. Measured biomasses are indicated by dots. Confidence intervals are indicated by the grey area and correlation coefficients derived from Spearman's rank correlation are given in each plot.

**Table S3.** Total litter derived  $C_{litter}$ ,  $N_{litter}$  and  $P_{litter}$  inputs [mg] (means  $\pm$  SE,  $n = 5$ ) of primary decomposers *L. terrestris* (Primary), secondary decomposers *A. caliginosa* and *A. rosea* (Secondary), a mix of both decomposer guilds (Mix) and the Control (without earthworms) in cup plant and maize treatments after 4 and 8 weeks of incubation. Standard error combined according to Higgins et al. (2020). For statistics see Table 3 and Table S6 of the Supplementary Information.

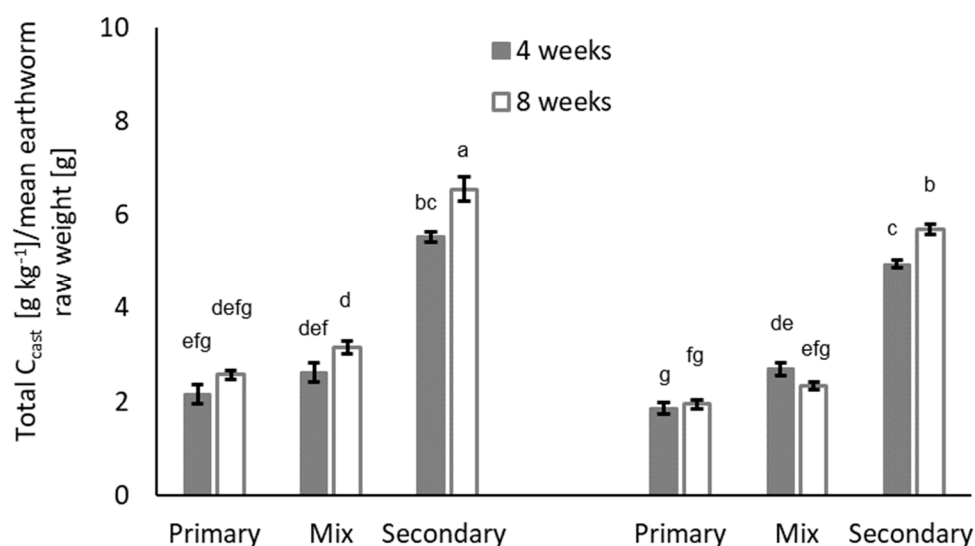
		Cup plant			Maize		
		total $C_{litter}$	total $N_{litter}$	total $P_{litter}$	total $C_{litter}$	total $N_{litter}$	total $P_{litter}$
		[mg]	[mg]	[mg]	[mg]	[mg]	[mg]
4 weeks	Control	2227.0 $\pm$ 738.8	15.1 $\pm$ 26.5	6.12 $\pm$ 5.19	1168.8 $\pm$ 808.0	13.1 $\pm$ 46.8	0.47 $\pm$ 3.48
	Primary	3607.0 $\pm$ 984.1	57.2 $\pm$ 34.5	13.17 $\pm$ 7.50	2883.4 $\pm$ 922.1	111.5 $\pm$ 42.9	12.60 $\pm$ 2.84
	Mix	3088.0 $\pm$ 1274.8	47.7 $\pm$ 36.1	9.90 $\pm$ 8.57	2344.2 $\pm$ 781.8	76.0 $\pm$ 42.9	7.71 $\pm$ 3.05
	Secondary	2428.4 $\pm$ 642.9	28.4 $\pm$ 22.0	4.62 $\pm$ 5.33	1887.8 $\pm$ 619.4	53.8 $\pm$ 36.7	4.67 $\pm$ 2.42
8 weeks	Control	2820.1 $\pm$ 346.7	4.4 $\pm$ 19.1	4.70 $\pm$ 3.71	2427.1 $\pm$ 415.3	41.6 $\pm$ 31.7	3.41 $\pm$ 1.76
	Primary	4479.1 $\pm$ 108.9	81.6 $\pm$ 4.5	18.11 $\pm$ 1.04	4468.9 $\pm$ 491.4	165.4 $\pm$ 27.4	18.73 $\pm$ 1.12
	Mix	4218.3 $\pm$ 692.3	73.4 $\pm$ 23.7	16.54 $\pm$ 5.14	4323.4 $\pm$ 432.1	157.5 $\pm$ 27.5	17.84 $\pm$ 1.09
	Secondary	3276.4 $\pm$ 744.7	38.8 $\pm$ 28.8	8.95 $\pm$ 6.40	3819.9 $\pm$ 473.2	134.4 $\pm$ 30.4	15.35 $\pm$ 1.40

Higgins, J.P.T.; Thomas, J.; Chandler, J.; Cumpston, M.; Li, T.; Page, M.J.; Welch, V.A. Cochrane Handbook for Systematic Reviews of Interventions; version 6.1., 2020. Available online: [www.training.cochrane.org/handbook](http://www.training.cochrane.org/handbook) (accessed on 16 December 2020).

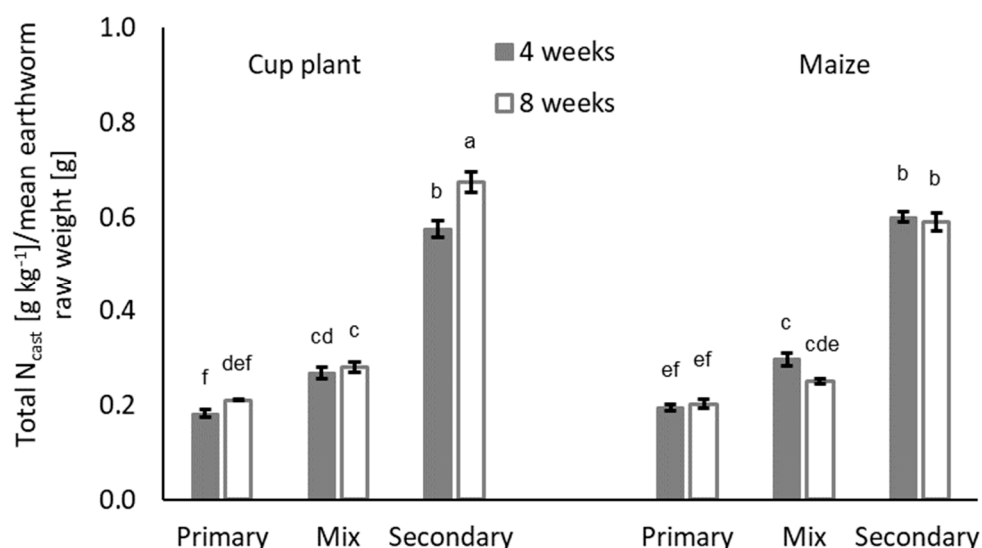
**Table S4.** Total C and N [g kg<sup>-1</sup>] and plant-available CAL-P [CAL-P<sub>cast</sub> 10<sup>-3</sup> g kg<sup>-1</sup>] in casts (means ± SE, n = 5) in cup plant and maize treatments for 4 and 8 weeks of incubation for earthworm treatments Primary = primary decomposer *L. terrestris*; Secondary = secondary decomposers *A. caliginosa* and *A. rosea*; Mix = primary and secondary decomposers. For statistics see Table 3 of the main article and Table S6 of the Supplementary Information.

		<u>Cup plant</u>			<u>Maize</u>		
		Total C <sub>cast</sub> [g kg <sup>-1</sup> ]	Total N <sub>cast</sub> [g kg <sup>-1</sup> ]	CAL-P <sub>cast</sub> [10 <sup>-3</sup> g kg <sup>-1</sup> ]	Total C <sub>cast</sub> [g kg <sup>-1</sup> ]	Total N <sub>cast</sub> [g kg <sup>-1</sup> ]	CAL-P <sub>cast</sub> [10 <sup>-3</sup> g kg <sup>-1</sup> ]
4 weeks	Primary	27.39±2.20	2.31±0.08	81.00±4.62	24.73±1.93	2.57±0.10	69.47±3.39
	Mix	21.83±1.44	2.23±0.06	68.91±2.33	23.32±1.15	2.57±0.12	66.60±2.05
	Secondary	21.07±0.49	2.19±0.05	67.96±1.67	20.86±0.32	2.52±0.06	63.67±0.68
8 weeks	Primary	31.30±1.16	2.56±0.04	97.47±2.55	23.31±0.98	2.42±0.11	61.57±2.15
	Mix	26.42±1.00	2.34±0.07	87.57±5.32	19.71±0.70	2.11±0.05	57.39±1.05
	Secondary	23.42±0.51	2.41±0.02	81.24±1.97	23.17±0.52	2.40±0.06	63.52±1.72

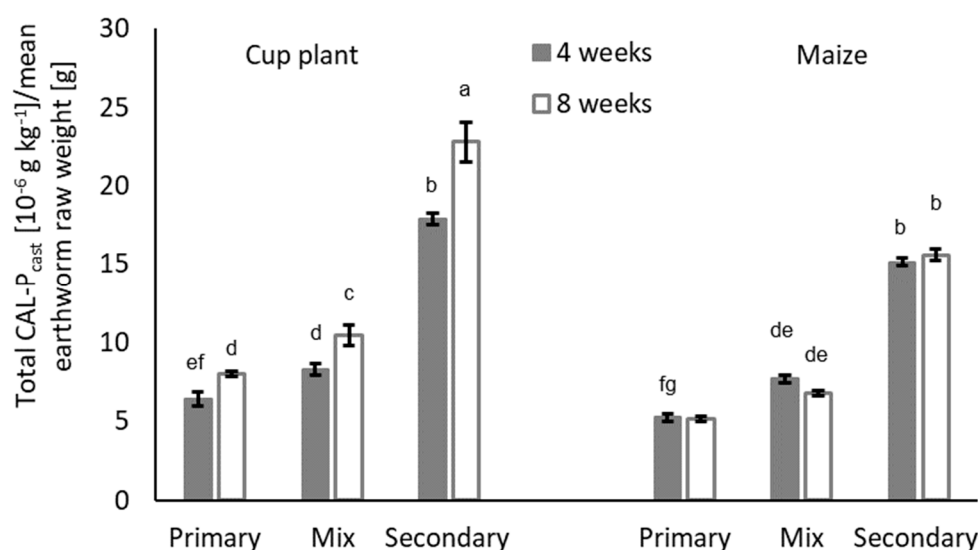
**Figure S2.** Total C [ $\text{g kg}^{-1}$ ] in casts per mean earthworm biomass [g] (means  $\pm$  SE,  $n = 5$ ) in cup plant and maize treatments for 4 and 8 weeks of incubation for earthworm treatments Primary = primary decomposer *L. terrestris*; Secondary = secondary decomposers *A. caliginosa* and *A. rosea*; Mix = primary and secondary decomposers. For statistics see Table S5 of the Supplementary Information.



**Figure S3.** Total N [ $\text{g kg}^{-1}$ ] in casts per mean earthworm biomass [g] (means  $\pm$  SE,  $n = 5$ ) in cup plant and maize treatments for 4 and 8 weeks of incubation for earthworm treatments Primary = primary decomposer *L. terrestris*; Secondary = secondary decomposers *A. caliginosa* and *A. rosea*; Mix = primary and secondary decomposers. For statistics see Table S5 of the Supplementary Information.



**Figure S4.** Total CAL-P [ $10^{-6}$  g  $\text{kg}^{-1}$ ] in casts per mean earthworm biomass [g] (means  $\pm$  SE,  $n = 5$ ) in cup plant and maize treatments for 4 and 8 weeks of incubation for earthworm treatments Primary = primary decomposer *L. terrestris*; Secondary = secondary decomposers *A. caliginosa* and *A. rosea*; Mix = primary and secondary decomposers. For statistics see Table S5 of the Supplementary Information.



**Table S5.** ANOVA results for Total  $C_{\text{cast}}$ , Total  $N_{\text{cast}}$  [ $\text{g kg}^{-1}$ ] and Total  $\text{CAL-P}_{\text{cast}}$  [ $10^{-6}$  g  $\text{kg}^{-1}$ ] in casts per mean earthworm biomass [g] (means  $\pm$  SE,  $n = 5$ ) for the predictor variables Earthworm, Litter, Duration as well as their interactions (Earthworm:Litter, Earthworm:Duration, Litter:Duration), F and p values (F,  $\text{Pr(>F)}$ ) are provided. Estimates for regression coefficients  $\pm$  SE as well as t and p values (t,  $\text{Pr(>|t|)}$ ) are given for specific predictors within treatments.

Predictor	Estimate	SE Estimate	F/t/z value	Pr(>F)/Pr(> t )
<b>Total <math>C_{\text{cast}}</math> [<math>\text{g kg}^{-1}</math>]/mean earthworm raw weight [g]</b>				
Earthworm			155.41	< 0.001***
EarthwormPrimary	-3.1069	0.1854	-16.76	< 0.001***
EartwormMix	-2.4139	0.1845	-13.09	< 0.001***
Litter			7.39	0.009**
LitterCup plant	0.4741	0.1743	2.72	0.009**
Duration			12.84	< 0.001***
Duration8 weeks	0.6248	0.1743	3.58	< 0.001***
Earthworm:Litter			1.40	0.257
EarthwormPrimary:LitterCup plant	-0.2504	0.2162	-1.16	0.252
EarthwormMix:LitterCup plant	-0.3447	0.2130	-1.62	0.112
Earthworm:Duration			7.65	0.001**
EarthwormPrimary:Duration8 weeks	-0.6263	0.2162	-2.90	0.006**
EarthwormMix:Duration8 weeks	-0.7910	0.2130	-3.71	< 0.001***
Litter:Duration			8.04	0.007**
LitterCup plant:Duration8 weeks	0.4981	0.1756	2.84	0.007**
<b>Total <math>N_{\text{cast}}</math> [<math>\text{g kg}^{-1}</math>]/mean earthworm raw weight [g]</b>				
Earthworm			279.36	< 0.001***

EarthwormPrimary	-0.3842	0.0170	-22.63	< 0.001***
EartwormMix	-0.2901	0.0169	-17.17	< 0.001***
Litter			0.01	0.928
LitterCup plant	-0.0015	0.0160	-0.09	0.928
Duration			0.59	0.448
Duration8 weeks	0.0122	0.0160	0.77	0.448
Earthworm:Litter			1.59	0.215
EarthwormPrimary:LitterCup plant	-0.0309	0.0198	-1.56	0.125
EarthwormMix:LitterCup plant	-0.0296	0.0195	-1.52	0.136
Earthworm:Duration			5.01	0.010*
EarthwormPrimary:Duration8 weeks	-0.0243	0.0198	-1.23	0.225
EarthwormMix:Duration8 weeks	-0.0613	0.0195	-3.15	0.003**
Litter:Duration			15.21	< 0.001***
LitterCup plant:Duration8 weeks	0.0627	0.0161	3.90	< 0.001***
<b>Total CAL-P<sub>cast</sub> [10<sup>-6</sup> g kg<sup>-1</sup>]/mean earthworm raw weight [g] (log scale)</b>				
Earthworm			235.38	< 0.001***
EarthwormPrimary	-1.0743	0.0505	-21.29	< 0.001***
EartwormMix	-0.7131	0.0502	-14.21	< 0.001***
Litter			8.05	0.007**
LitterCup plant	0.1346	0.0475	2.84	0.007**
Duration			0.00	0.976
Duration8 weeks	-0.0014	0.0475	-0.03	0.976
Earthworm:Litter			0.81	0.452
EarthwormPrimary:LitterCup plant	0.0509	0.0588	0.87	0.391
EarthwormMix:LitterCup plant	-0.0222	0.0580	-0.38	0.703
Earthworm:Duration			1.02	0.367
EarthwormPrimary:Duration8 weeks	-0.0264	0.0588	-0.45	0.656
EarthwormMix:Duration8 weeks	-0.0814	0.0580	-1.40	0.167
Litter:Duration			31.97	< 0.001***
LitterCup plant:Duration8 weeks	0.2703	0.0478	5.66	< 0.001***

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**Table S6.** ANOVA results for the decomposition rate  $k$  and the consumption rate  $C$  [ $\text{g g}^{-1}$ ], litter derived total  $C$ ,  $N$  and  $P$  [ $\text{mg}$ ] and cast total  $C$  and  $N$  [%] as well as  $CAL\text{-}P$  [ $\text{mg kg}^{-1}$ ]. For the predictor variables Earthworm, Litter, Duration as wells as their interactions (Earthworm:Litter, Earthworm:Duration, Litter:Duration],  $F$  and  $p$  values ( $F$ ,  $\text{Pr}( > F )$ ) are provided. Estimates for regression coefficients  $\pm$  SE as well as  $t$  and  $p$  values ( $t$ ,  $\text{Pr}( > |t| )$ ) are given for specific predictors within treatments.

Predictor	Estimate	SE Estimate	F/t/z value	$\text{Pr}( > F ) / \text{Pr}( >  t  ) / \text{Pr}( >  z  )$	Estimate	SE Estimate	F/t value	$\text{Pr}( > F ) / \text{Pr}( >  t  )$
<b>Decomposition rate <math>k</math> (log scale)</b>					<b>Consumption rate <math>C</math> (log scale)</b>			
Earthworm			23.51	$< 0.001^{***}$			14.09	$< 0.001^{***}$
EarthwormPrimary	0.3224	0.0850	3.80	$< 0.001^{***}$	-0.5548	0.1297	-4.28	$< 0.001^{***}$
EartwormMix	0.0193	0.0845	0.23	0.8205	-0.6262	0.1290	-4.85	$< 0.001^{***}$
EarthwormNone	-0.3878	0.0845	-4.59	$< 0.001^{***}$				
Litter			15.67	$< 0.001^{***}$			2.47	0.1222
LitterCup plant	0.3059	0.0773	3.96	$< 0.001^{***}$	0.1918	0.1219	1.57	0.1222
Duration			34.97	$< 0.001^{***}$			31.40	$< 0.001^{***}$
Duration8 weeks	0.4570	0.0773	5.91	$< 0.001^{***}$	0.6833	0.1219	5.60	$< 0.001^{***}$
Earthworm:Litter			7.94	$< 0.001^{***}$			4.73	0.0132*
EarthwormPrimary:LitterCup plant	0.3905	0.0991	3.94	$< 0.001^{***}$	0.4085	0.1512	2.70	0.0094**
EarthwormMix:LitterCup plant	0.3114	0.0976	3.19	$< 0.01^{**}$	0.3890	0.1490	2.61	0.0119*
EarthwormNone:LitterCup plant	0.4303	0.0976	4.41	$< 0.001^{***}$				
Earthworm:Duration			7.02	$< 0.001^{***}$			3.13	0.0524(*)
EarthwormPrimary:Duration8 weeks	0.0505	0.0991	0.51	0.6123	-0.3458	0.1512	-2.29	0.0265*
EarthwormMix:Duration8 weeks	0.1735	0.0976	1.78	0.0801(*)	-0.0360	0.1490	-0.24	0.8101
EarthwormNone:Duration8 weeks	-0.2612	0.0976	-2.68	0.0094**				
Litter:Duration			63.91	$< 0.001^{***}$			16.11	$< 0.001^{***}$
LitterCup plant:Duration8 weeks	-0.5559	0.0695	-8.00	$< 0.001^{***}$	-0.4930	0.1228	-4.01	$< 0.001^{***}$
<b>Litter-derived total <math>C</math> <math>C_{\text{litter}}</math></b>					<b>Total <math>C</math> in cast <math>C_{\text{cast}}</math></b>			
Earthworm			1283.54	$< 0.001^{***}$			1.67	0.1995
EarthwormPrimary	903.05	49.87	18.11	$< 0.001^{***}$	2.5609	1.5020	1.71	0.0945(*)
EartwormMix	397.61	49.84	7.98	$< 0.001^{***}$	0.4219	1.4945	0.28	0.7789
EarthwormNone	-823.98	49.84	-16.53	$< 0.001^{***}$				

Litter			82.14	< 0.001***			2.05	0.1588
LitterCup plant	412.44	45.51	9.06	< 0.001***	-2.0210	1.4126	-1.43	0.1588
Duration			1571.38	< 0.001***			0.00	0.9560
Duration8 weeks	1803.98	45.51	39.64	< 0.001***	0.0784	1.4126	0.06	0.9560
Earthworm:Litter			160.68	< 0.001***			4.20	0.0207*
EarthwormPrimary:LitterCup plant	368.08	57.65	6.39	< 0.001***	5.0765	1.7514	2.90	0.0056**
EarthwormMix:LitterCup plant	320.82	57.55	5.57	< 0.001***	2.3822	1.7257	1.38	0.1737
EarthwormNone:LitterCup plant	727.51	57.55	12.64	< 0.001***				
Earthworm:Duration			129.99	< 0.001***			0.58	0.5658
EarthwormPrimary:Duration8 weeks	-161.62	57.65	-2.80	0.0051**	-1.1119	1.7514	-0.64	0.5285
EarthwormMix:Duration8 weeks	164.66	57.55	2.86	0.0042**	-1.8396	1.7257	-1.07	0.2917
EarthwormNone:Duration8 weeks	-463.87	57.55	-8.06	< 0.001***				
Litter:Duration			413.15	< 0.001***			10.02	0.0027**
LitterCup plant:Duration8 weeks	-827.87	40.73	-20.33	< 0.001***	4.5051	1.4231	3.17	0.0027**

#### Litter-derived total N $N_{\text{litter}}$ (sqrt scale)

Earthworm			211.825	< 0.001***			0.12	0.8838
EarthwormPrimary	2.5950	0.4304	6.030	< 0.001***	0.0349	0.0908	0.39	0.7021
EarthwormMix	1.0828	0.4300	2.518	0.0118*	-0.0074	0.0903	-0.08	0.9348
EarthwormNone	-3.4228	0.4300	-7.960	< 0.001***				
Litter			34.591	< 0.001***			20.21	< 0.001***
LitterCup plant	-2.3094	0.3927	-5.881	< 0.001***	-0.3838	0.0854	-4.50	< 0.001***
Duration			101.439	< 0.001***			4.17	0.0465*
Duration8 weeks	3.9547	0.3927	10.072	< 0.001***	-0.1743	0.0854	-2.04	0.0465*
Earthworm:Litter			12.899	0.0049**			0.63	0.5363
EarthwormPrimary:LitterCup plant	0.2653	0.4977	0.533	0.5940	0.0966	0.1058	0.91	0.3661
EarthwormMix:LitterCup plant	0.7942	0.4966	1.599	0.1097	0.1064	0.1043	1.02	0.3127
EarthwormNone:LitterCup plant	1.6518	0.4966	3.327	< 0.001***				
Earthworm:Duration			24.618	< 0.001***			2.98	0.0601(*)
EarthwormPrimary:Duration8 weeks	-0.6988	0.4977	-1.404	0.1603	0.0055	0.1058	0.05	0.9587
EarthwormMix:Duration8 weeks	0.1688	0.4966	0.340	0.7338	-0.2188	0.1043	-2.10	0.0410*

#### Total N in cast $N_{\text{cast}}$

EarthwormNone:Duration8 weeks	-2.0428	0.4966	-4.114	< 0.001***				
Litter:Duration			61.458	< 0.001***			26.22	< 0.001***
LitterCup plant:Duration8 weeks	-2.7558	0.3515	-7.840	< 0.001***	0.4403	0.0860	5.12	< 0.001***
<b>Litter-derived total P P<sub>litter</sub> (^2 scale)</b>					<b>CAL-P in cast P<sub>cast</sub> (log scale)</b>			
Earthworm			112.576	< 0.001***			0.99	0.3805
EarthwormPrimary	107.37	15.47	6.941	< 0.001***	0.0511	0.0450	1.14	0.2613
EartwormMix	19.50	15.47	1.261	0.2074	-0.0067	0.0447	-0.15	0.8815
EarthwormNone	-53.79	15.47	-3.478	< 0.001***				
Litter			8.114	0.004**			0.06	0.8156
LitterCup plant	-40.22	14.12	-2.849	0.004**	0.0099	0.0423	0.24	0.8156
Duration			151.750	< 0.001***			1.87	0.1775
Duration8 weeks	173.94	14.12	12.319	< 0.001***	-0.0579	0.0423	-1.37	0.1775
Earthworm:Litter			35.953	< 0.001***			4.13	0.0219*
EarthwormPrimary:LitterCup plant	73.81	17.87	4.130	< 0.001***	0.1507	0.0524	2.87	0.0060**
EarthwormMix:LitterCup plant	74.97	17.86	4.198	< 0.001***	0.0698	0.0517	1.35	0.1831
EarthwormNone:LitterCup plant	101.80	17.86	5.700	< 0.001***				
Earthworm:Duration			169.008	< 0.001***			0.58	0.5653
EarthwormPrimary:Duration8 weeks	37.09	17.87	2.076	0.0379*	-0.0519	0.0524	-0.99	0.3269
EarthwormMix:Duration8 weeks	81.16	17.86	4.544	< 0.001***	-0.0439	0.0517	-0.85	0.3992
EarthwormNone:Duration8 weeks	-138.18	17.86	-7.737	< 0.001***				
Litter:Duration			35.839	< 0.001***			46.55	< 0.001***
LitterCup plant:Duration8 weeks	-75.62	12.63	-5.987	< 0.001***	0.2907	0.0426	6.82	< 0.001***

Significance levels: P<0.001\*\*\*; P<0.01\*\*; P<0.05\*; P<0.1(\*)