

Short- and long-term impacts of anaerobic digestate spreading on earthworms in cropped soils

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Supplementary Materials

S.M.1 Treatments in the microcosm ecotoxicity tests, series #1 (2019) and #2 (2020)

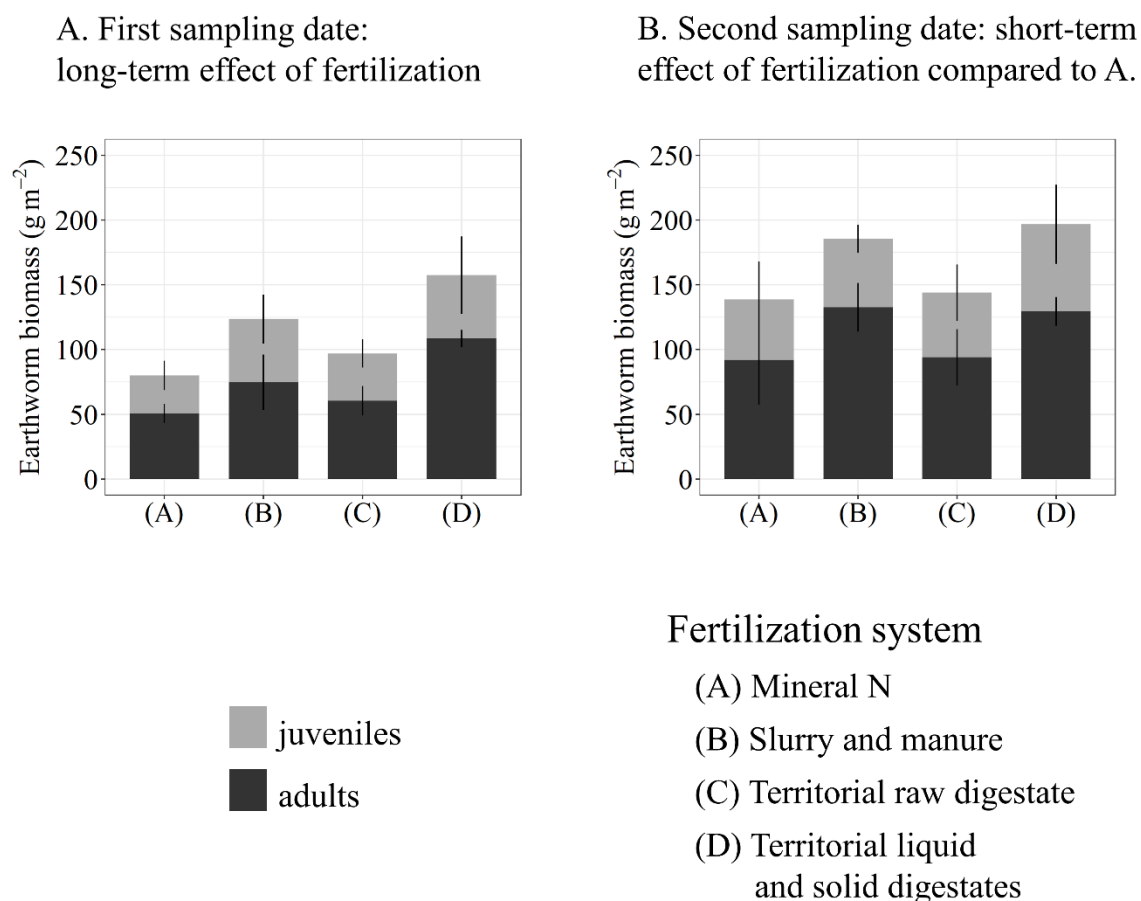
Supplementary Table S1 – Description of the treatments in the microcosm ecotoxicity tests, series #1 (2019)

Series	Species	Organic products	Dose (mass of fresh organic products): (mass of fresh soil)	soil N _{amm} content (mgN _{amm} kgDS ⁻¹)
#1	<i>L. terrestris</i>	Territorial digestate	2.5%	79
			5%	159
			10%	317
			20%	634
		Territorial digestate, aerated	20%	370
		Cattle slurry	10%	199
			20%	398
		Control - no products		0
	<i>A. caliginosa</i>	Territorial digestate	2.5%	79
			5%	159
			10%	317
			20%	634
		Territorial digestate, aerated	20%	370
		Cattle slurry	10%	199
			20%	398
		Control - no products		0

Supplementary Table S2 – Description of treatments in the microcosm ecotoxicity tests, series #2 (2020).

Series	Species	Organic products	Dose (mass of fresh organic products): (mass of fresh soil)	soil N _{amm} content (mgN _{amm} kgDS ⁻¹)
#2	<i>L. terrestris</i>	Territorial digestate	5%	120
			10%	240
			15%	360
			20%	480
		Territorial digestate, aerated	5%	95
			10%	191
			15%	286
			20%	382
		Digestate from cover crops	2.5%	105
			5%	209
			10%	418
			15%	627
			20%	836
		Cattle slurry	5%	49
			10%	98
			20%	196
		Ammonia solution (NH ₃)	equivalent to territorial digestate 5%	109
			equivalent to territorial digestate 9%	217
			equivalent to territorial digestate 18%	437
			equivalent to territorial digestate 27%	723
			equivalent to territorial digestate 36%	874
		Ammonium solution (NH ₄ Cl)	equivalent to territorial digestate 5%	109
			equivalent to territorial digestate 9%	217
			equivalent to territorial digestate 18%	437
			equivalent to territorial digestate 36%	874
		Control - no products		0

S.M.2 Biomass of earthworms in the field



Supplementary Fig. S1- Biomass of earthworms in the field before and after the spreading of organic products. Error bars indicate one standard error. Considering the two dates together, the date significantly influenced total and adult earthworm biomass (Scheirer-Ray-Hare tests, p -values < 0.05). A) Long-term effect of repeated organic product applications: first sampling date, in plots differentiated for 2 years, 7 days before spreading. Adult and total biomasses were not found impacted by treatment (Kruskal-Wallis test, p -value > 0.05). B) Second sampling date, 14 days after spreading. Comparison with subfigure 1.A show the impact of fertilization after 2 weeks on soil earthworm populations. Adult and total biomasses were not found impacted by treatment (Kruskal-Wallis test, p -value > 0.05).

S.M.3 Weight change in microcosm ecotoxicity tests, series #1 (2019) and #2 (2020)

Supplementary Table S3 – Weight change in the microcosm ecotoxicity tests in series #1 (2019) after 15 days of the experiment. Weight change is expressed as a percentage of the initial body mass. A positive value indicates weight gain, and a negative value indicates weight loss. The number of living earthworms after 15 days is indicated in parentheses (7 earthworms initially).

Series	Species	Organic products	Dose (mass of fresh organic products): (mass of fresh soil)	Weight change in % \pm standard error (sample size)
#1	<i>L. terrestris</i>	Territorial digestate	2.5%	5.8 \pm 4.3 (7)
			5%	3.4 \pm 3.6 (7)
			10%	(0)
			20%	(0)
		Territorial digestate, aerated	20%	(0)
		Cattle slurry	10%	-0.5 \pm 4.3 (7)
			20%	(0)
		Control - no products		5.6 \pm 3.6 (7)
	<i>A. caliginosa</i>	Territorial digestate	2.5%	-17.1 \pm 2.9 (7)
			5%	-7.3 \pm 2.0 (6)
			10%	-14.6 \pm 7.5 (3)
			20%	(0)
		Territorial digestate, aerated	20%	(0)
		Cattle slurry	10%	-34.7 \pm 10.3 (4)
			20%	(0)
		Control - no products		-14.2 \pm 7.5 (7)

Supplementary Table S4 – Weight change in microcosm ecotoxicity tests in series #2 (2020) after 15 days of the experiment. Weight change is expressed as a percentage of the initial body mass. A positive value indicates weight gain, and a negative value indicates weight loss. The number of living earthworms after 15 days is indicated in parentheses (7 earthworms initially).

Series	Species	Organic products	Dose (mass of fresh organic products): (mass of fresh soil)	Weight change in % ± standard error (sample size)
#2	<i>L. terrestris</i>	Territorial digestate	5%	1.6 ± 5.1 (7)
			10%	3.5 ± 2.9 (7)
			15%	-5.3 (1)
			20%	-14.8 (1)
		Territorial digestate, aerated	5%	-4.5 ± 2.2 (7)
			10%	2.5 ± 4.9 (7)
			15%	-9.1 ± 2.5 (7)
			20%	-4.3 ± 2.8 (3)
		Digestate from cover crops	2.5%	-1.4 ± 1.7 (7)
			5%	-2.1 ± 2.2 (7)
			10%	-0.6 ± 4.2 (7)
			15%	(0)
			20%	(0)
		Cattle slurry	5%	3.2 ± 1.9 (7)
			10%	5.6 ± 1.3 (7)
			20%	8.1 ± 4.9 (7)
		Ammonia solution (NH ₃)	equivalent to territorial digestate 5%	-3.3 ± 2.7 (7)
			equivalent to territorial digestate 9%	-5.0 ± 4.3 (7)
			equivalent to territorial digestate 18%	-3.9 ± 1.7 (7)
			equivalent to territorial digestate 27%	-8.6 ± 1.9 (2)
			equivalent to territorial digestate 36%	(0)
		Ammonium solution (NH ₄ Cl)	equivalent to territorial digestate 5%	-2.4 ± 3.8 (7)
			equivalent to territorial digestate 9%	0.5 ± 2.7 (7)
			equivalent to territorial digestate 18%	6.1 ± 2.9 (7)
			equivalent to territorial digestate 36%	3.6 ± 3.2 (7)
		Control - no products		3.9 ± 2.2 (7)

S.M.4 Weight change in macrocosm experiments

Supplementary Table S5 – Weight change in the macrocosm tests after 28 days of the experiment. Weight change is expressed as a percentage of the initial body mass. A positive value indicates weight gain, and a negative value indicates weight loss.

Treatment	Weight change as % \pm standard error	
	<i>L. terrestris</i>	<i>A. caliginosa</i>
(C) Control, 40 t.ha ⁻¹ of water spreading	-17.7 \pm 7.1	-15.9 \pm 2.0
(LD40) 40 t.ha ⁻¹ of liquid territorial digestate spreading	-9.7 \pm 6.0	-9.4 \pm 2.4
(S40) 40 t.ha ⁻¹ of slurry spreading	-4.3 \pm 7.6	-5.0 \pm 5.2
(LD80) 80 t.ha ⁻¹ of liquid territorial digestate spreading	1.5 \pm 2.8	-10.7 \pm 3.5
(ALD40) 40 t.ha ⁻¹ of aerated liquid territorial digestate spreading	-6.2 \pm 1.1	-7.9 \pm 1.6
(LD40-H+) 40 t.ha ⁻¹ of liquid digestate spreading and a higher soil humidity of 80% WHC	-11.9 \pm 11.1	-6.9 \pm 2.8