Supplementary information for

Higher concentrations of microplastics in runoff from biosolid-amended croplands than manure-amended croplands

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Supplementary Note 1: Extraction method discussion

Although extraction from more complex matrices such as soil or biosolids appears to be more challenging, MP extraction from liquid samples has its own difficulties. Most of the studies either used the method previously proposed by Masura et al.,[1] or simply used sieving and filtration [2,3]. Although the method proposed by Masura et al.,[1] and filtration methods could be useful for large MP fragments, extracting very fine MP such as small fragments or fibers may be challenging with such methods and more accurate procedures are needed. The main advantage of the method used in the present study is that drying, digestion, and density separation occurred in the same glass jar that was used for sampling. Moreover, during the drying phase, small MP are typically attached to the walls of the glassware which could not be released by rinsing. To overcome this challenge, a critical step of sonication was added to the procedure after the drying phase. The same approach was considered for the solid samples. The fact that both organic digestion and density separation steps were occurring inside the same centrifuge tube, as well as using sonication for allowing all the attached MP to be recovered, increased the accuracy of the method. However, in most of the other reported methods [1,4–7], one of the recurring disadvantages is that by transferring the samples between glassware, a portion of the fine MP may be lost, even by multiple rinsing of the glassware.

Some studies reported high recovery rates of more than 90% for MP extraction from liquid or dry samples [1,4,5]. However, most of the studies used a limited number of plastic types for method validation, while we used a variety of plastic types that have the potential of being found in the environment. One of the plastic types that is usually omitted in those procedures is teflon which has a density of more than 2 g/cm^3, which is higher than the saturated $ZnBr_2$ solution's density. Therefore, although by considering denser particles in our study, we expected the recovery rate to decline, rates of 88% for runoff samples and 90% for solid samples appear to be realistic values for our modified method.

Table S1. The concentration of MPs in different soil layers of the plots

Plot type	Soil layer	Average MP concentration (particle/g) dry weight
Plot 1 (biosolid)	Top layer (0-5 cm)	3.27 ± 0.95
	Bottom layer (5-15 cm)	0.20 ± 0.2
Plot 2 (control)	Top layer (0-5 cm)	1.00 ± 0.2
	Bottom layer (5-15 cm)	0.27 ± 0.12
Plot 3 (manure)	Top layer (0-5 cm)	0.80 ± 0.00
	Bottom layer (5-15 cm)	0.20 ± 0.20
Plot 4 (biosolid)	Top layer (0-5 cm)	2.00 ± 0.35
	Bottom layer (5-15 cm)	0.07 ± 0.12
Plot 5 (control)	Top layer (0-5 cm)	0.87 ± 0.23
	Bottom layer (5-15 cm)	0.13 ± 0.1
Plot 6 (manure)	Top layer (0-5 cm)	1.33 ± 0.42
	Bottom layer (5-15 cm)	0.07 ± 0.12

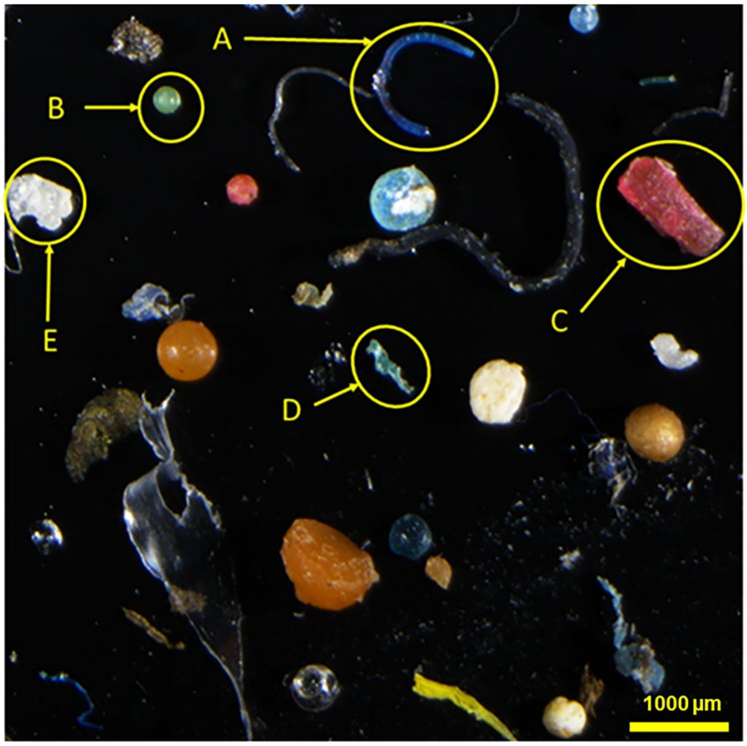

Figure S1. Different shapes of MP were extracted from biosolids. A) fiber, B) bead, C) fragment, D) film and E) foam

Table S2. Volume of the runoff collected in each tank

		Volume of the collected runoff (L)				
Amendment	**Tank number**	**21-Jul**	**27-Jul**	**30-Jul**	**17-Aug**	**10-Sep**
Biosolid	1	109.05	75.22	140.15	NA[1]	96.34
Control	2	131.64	92.77	225.25	57.32	82.58
Manure	3	115.35	72.70	141.41	51.56	82.58
Biosolid	4	136.68	94.03	225.25	57.07	82.58
Control	5	117.88	75.22	153.91	57.07	82.58
Manure	6	75.12	79.00	NA	NA	NA

[1] The volume of collected runoff was not enough to sample and it is mentioned as NA

Table S3. Amount of rainfall occurred in the sampling dates

Sampling date	Rainfall (cm)
21-Jul	4.38
27-Jul	3.27
30-Jul	1.66
17-Aug	2.30
10-Sep	5.07

Table S4. Average, maximum, and minimum amounts of rainfall in the sampling location, from 2010 to 2020

Year	Average amount of annual rainfall (cm)	Maximum amount of rainfall (cm)	Minimum amount of rainfall (cm)
2010	0.84	4.43	0.02
2011	0.76	4.86	0.02
2012	0.75	7.5	0.02
2013	0.92	8.52	0.02
2014	0.93	8.34	0.02
2015	1.34	11.48	0.02
2016	1.08	8.76	0.02
2017	0.95	9.02	0.02
2018	0.83	5.08	0.02
2019	0.86	6.33	0.02
2020	0.64	5.06	0.02
Average	0.9		

Table S5. Main effects and interaction effect

Type III tests of fixed effects				
Effect	**Num DF[1]**	**Den DF[2]**	**F Value**	**Pr > F**
Treatment	2	4.47	3.45	0.1241
Time	4	11.81	1.50	0.2649
Treatment × time	8	11.33	2.80	0.0562

[1]Num DF is the numerator degrees of freedom.
[2]Den DF are the denominator degrees of freedom.

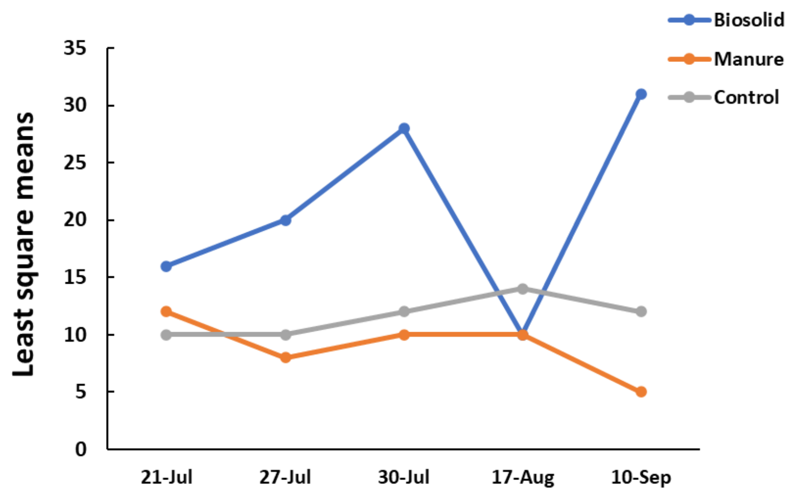

Figure S2. Least square means of MP concentrations at different sampling points

Table S6. Comparison of least square means at each time points

Simple effect comparisons of treatment × time least squares means by time										
Sampling date	**Plot type**	**In comparison with**	**Estimate**	**Standard Error**	**DF**[1]	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
21-Jul	**Biosolid**	**Control**	6.00	7.43	10.82	0.81	0.43	0.05	-10.38	22.389
21-Jul	**Biosolid**	**Manure**	4.00	7.43	10.82	0.54	0.60	0.05	-12.38	20.38
21-Jul	**Control**	**Manure**	-2.00	7.43	10.82	-0.27	0.79	0.05	-18.38	14.38
27-Jul	**Biosolid**	**Control**	10.00	7.43	10.82	1.35	0.20	0.05	-6.38	26.38
27-Jul	**Biosolid**	**Manure**	12.00	7.43	10.82	1.61	0.13	0.05	-4.38	28.38
27-Jul	**Control**	**Manure**	2.00	7.43	10.82	0.27	0.79	0.05	-14.38	18.38
30-Jul	**Biosolid**	**Control**	16.00	7.43	10.82	2.15	0.05	0.05	-0.38	32.38
30-Jul	**Biosolid**	**Manure**	18.00	7.43	10.82	2.42	0.03	0.05	1.61	34.38
30-Jul	**Control**	**Manure**	2.00	7.43	10.82	0.27	0.79	0.05	-14.38	18.38
17-Aug	**Biosolid**	**Control**	-4.00	7.43	10.82	-0.54	0.60	0.05	-20.38	12.38
17-Aug	**Biosolid**	**Manure**	0.00	7.43	10.82	0.00	1.00	0.05	-16.38	16.38
17-Aug	**Control**	**Manure**	4.00	7.43	10.82	0.54	0.60	0.05	-12.38	20.38
10-Sep	**Biosolid**	**Control**	19.00	7.43	10.82	2.56	0.02	0.05	2.61	35.38
10-Sep	**Biosolid**	**Manure**	26.00	7.43	10.82	3.50	0.00	0.05	9.61	42.38
10-Sep	**Control**	**Manure**	7.00	7.43	10.82	0.94	0.36	0.05	-9.38	23.38

[1] Degrees of freedom

Table S7. Analysis of Variance for the top soil layer concentrations of MP

Type III tests of fixed effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatment	2	3	5.62	0.0966

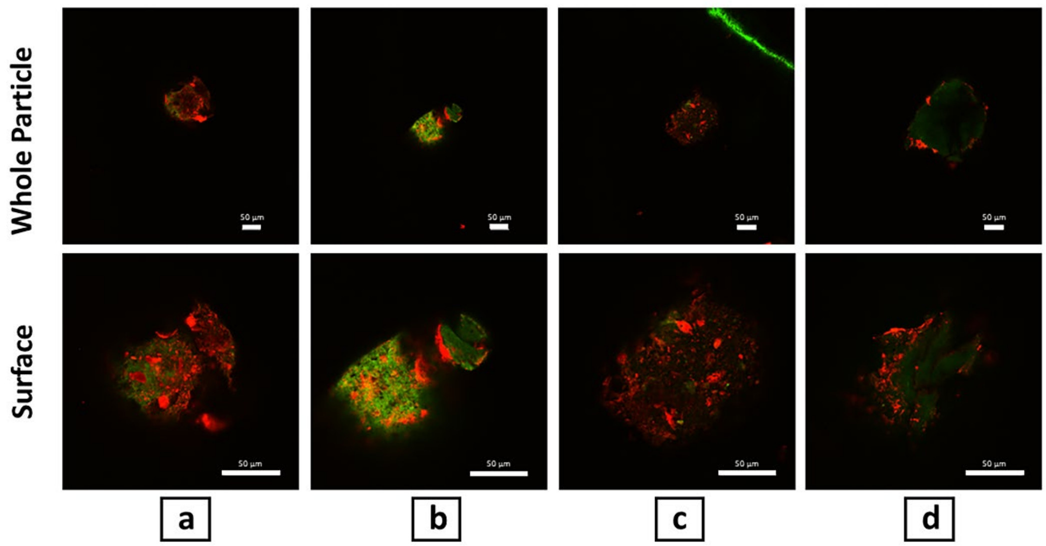

Figure S3. Confocal images from the beads that have been stained by propidium iodide

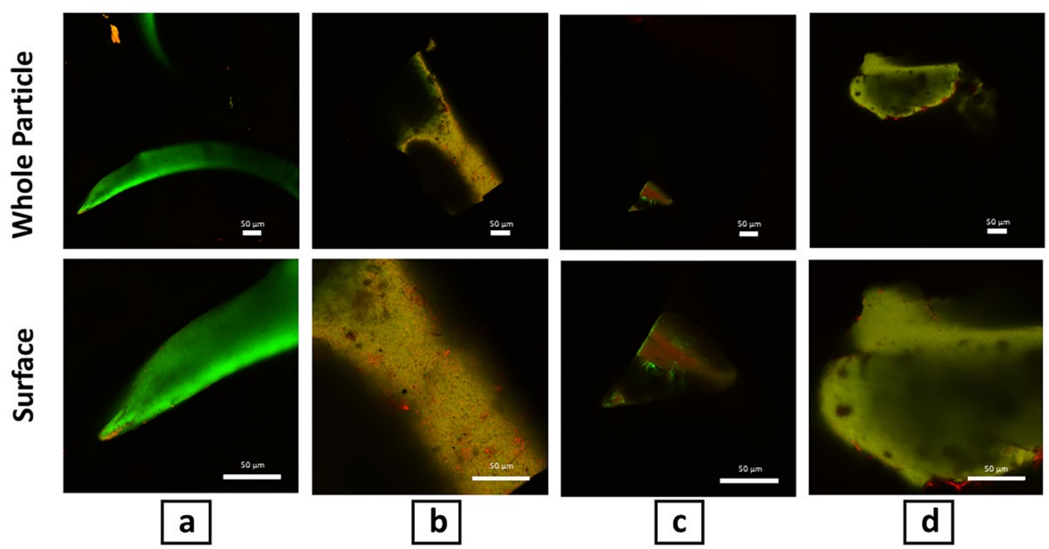

Figure S4. Confocal images from fiber (a), film (b), and fragments (c and d)

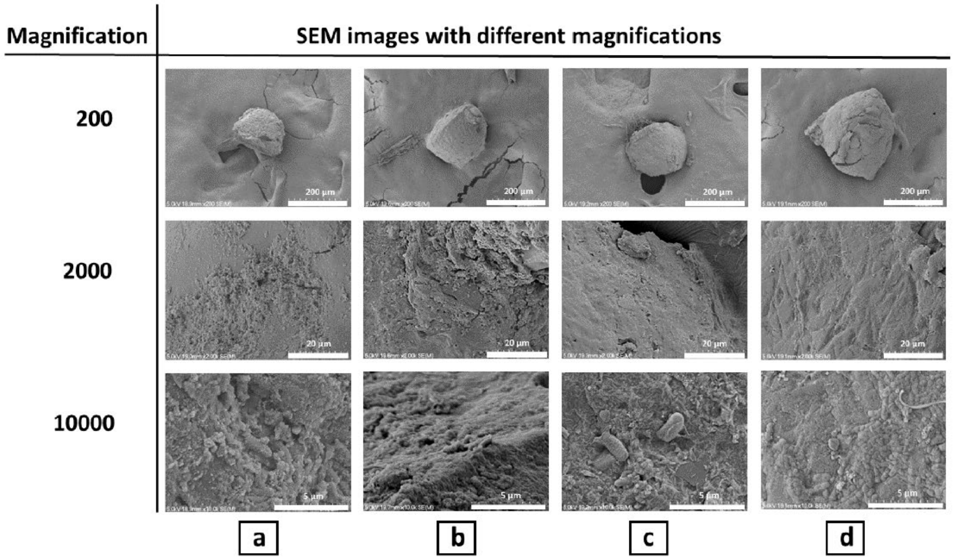

Figure S5. SEM images from the beads using three magnifications

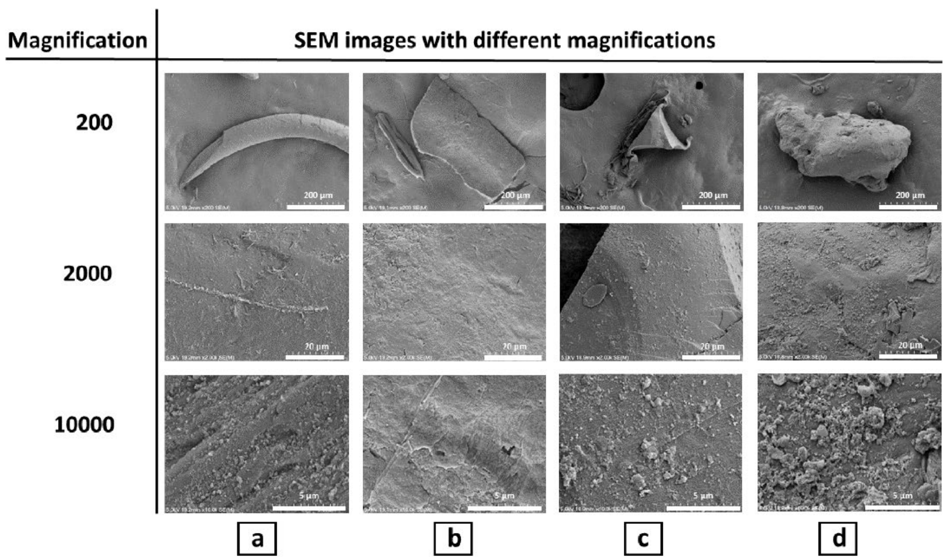

Figure S6. SEM images from fiber (a), film (b), and fragments (c and d)

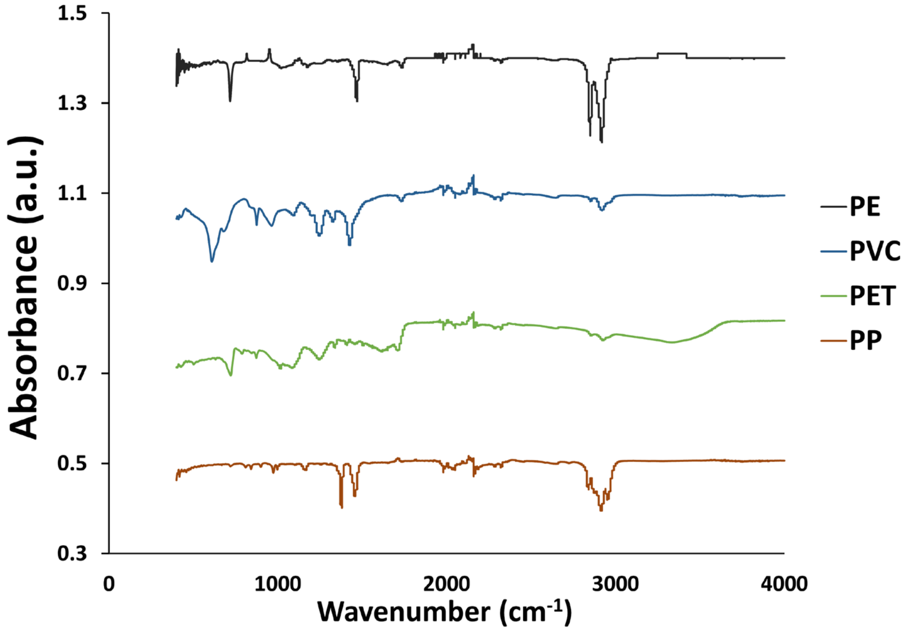

Figure S7. Spectra for four most detected MPs

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Table S8. Worldwide concentrations of MP in stormwater runoff according to the studies reviewed

MP concentration (particle/ L)	Runoff type	Location	Reference
1.1 to 24.6	Urban stormwater runoff	Watersheds in San Francisco Ba	Werbowski et al., (2021)[8]
230	Highway runoff	Sundsvall, Sweden	Lange et al., (2022) [9]
66 to 191	Urban runoff	Semiarid region, Tijuana, Mexico	Piñon-Colin et al., (2020)[10]
Up to 704	Urban runoff	Vaughan, Ontario, Canada	Smyth et al., (2021)[11]
02.75 to 19.04	Urban runoff from lands with commercial purposes	Hongshan district, Wuhan China	Sang et al., (2021)[12]
15.4	Stormwater runoff	Northwestern Lake Ontario, Canada	Grbić et al., (2020)[13]
0.9	Agricultural runoff	Northwestern Lake Ontario, Canada	Grbić et al., (2020)[13]
0.9 and 4.0	Road runoff	Inlet and outlet of a stormwater floating treatment wetland on Queensland's Gold Coast, Australia	Ziajahromi et al., (2020)[14]
29	Suburban stormwater runoff	The outlet of the suburban catchment in one of the suburbs of Paris, France	Treilles et al., (2021)[15]
0.4 to 36.48	Urban stormwater	Stormwater drains in Hong Kong	Zhang et al., (2022)[16]

Table S9. Soil characteristics of two soil layers (top and bottom) from plot 1 after biosolid application

Parameter	0-5 cm	5-15 cm
Electrical Conductivity (ds/m)	1.09	0.58
Nitrate (mg/kg)	88.70	45.60
% Organic matter	6.30	3.50
Ca (mg/kg)	3670.00	3196.00
Mg (mg/kg)	479.00	558.00
Na (mg/kg)	34.00	24.00
K (mg/kg)	348.00	256.00
pH	5.8	5.0

Table S10. Soil characteristics of two soil layers (top and bottom) from plot 2 which was the control

Parameter	0-5 cm	5-15 cm
Electrical Conductivity (ds/m)	0.3	0.15
Nitrate (mg/kg)	23.30	2.50
% Organic matter	7.50	3.50
Ca (mg/kg)	3470	3349
Mg (mg/kg)	529.00	570.00
Na (mg/kg)	9.00	12.00
K (mg/kg)	420.00	302.00
pH	6.70	6.10

Table S11. Soil characteristics of two soil layers (top and bottom) from plot after manure application

Parameter	0-5 cm	5-15 cm
Electrical Conductivity (ds/m)	0.68	0.33
Nitrate (mg/kg)	36.40	13.50
% Organic matter	6.10	3.90
Ca (mg/kg)	3009	3537
Mg (mg/kg)	540.00	549.00
Na (mg/kg)	80.00	65.00
K (mg/kg)	887.00	450.00
pH	7.00	6.40

Table S12. Soil characteristics of two soil layers (top and bottom) from plot 4 after biosolid application

Parameter	0-5 cm	5-15 cm
Electrical Conductivity (ds/m)	1.02	0.74
Nitrate (mg/kg)	82.50	54.50
% Organic matter	6.10	3.40
Ca (mg/kg)	3634	3670
Mg (mg/kg)	453.00	429.00
Na (mg/kg)	29.00	18.00
K (mg/kg)	437.00	365.00
pH	5.80	6.60

Table S13. Soil characteristics of two soil layers (top and bottom) from plot 5 which was the control

Parameter	0-5 cm	5-15 cm
Electrical Conductivity (ds/m)	0.24	0.23
Nitrate (mg/kg)	5.30	3.50
% Organic matter	5.40	3.30
Ca (mg/kg)	3793	3417
Mg (mg/kg)	432.00	507.00
Na (mg/kg)	7.00	11.00
K (mg/kg)	367.00	287.00
pH	7.80	6.80

Table S14. Soil characteristics of two soil layers (top and bottom) from plot 6 after manure application

Parameter	0-5 cm	5-15 cm
Electrical Conductivity (ds/m)	0.89	0.49
Nitrate (mg/kg)	66.70	14.00
% Organic matter	9.90	4.40
Ca (mg/kg)	2390	3442
Mg (mg/kg)	718.00	544.00
Na (mg/kg)	293.00	83.00
K (mg/kg)	2218.00	665.00
pH	7.40	7.30

Table S15. Characteristics of biosolids with two replicates

Parameter	Sample 1	Sample 2
%Ammonium nitrogen (total)	0.33	0.34
%Organic nitrogen	0.90	0.97
%Total Kjeldahl nitrogen (TKN)	1.23	1.31
%Phosphorus (as P_2O_5)	1.10	1.05
%Potassium (as K_2O)	0.05	0.05
%Sulfur (total)	0.35	0.34
%Calcium (total)	0.91	0.87
%Magnesium (total)	0.10	0.09
%Sodium (total)	0.04	0.04
Copper (total) (mg/kg)	166	169
Iron (total) (mg/kg)	3440	3330
Manganese (total) (mg/kg)	155	155
Zinc (total) (mg/kg)	159	161
%Moisture	77.80	77.80
%Total solids	22.20	22.20
%Total salts	1.43	1.39
pH	8.8	8.9

Table S16. Characteristics of manure with two replicates

Parameter	Sample 1	Sample 2
%Ammonium nitrogen (total)	0.15	0.26
%Organic nitrogen	0.69	0.52
%Total Kjeldahl nitrogen (TKN)	0.84	0.78
%Phosphorus (as P_2O_5)	1.05	1.17
%Potassium (as K_2O)	1.04	1.11
%Sulfur (total)	0.22	0.24
%Calcium (total)	1.20	1.34
%Magnesium (total)	0.36	0.39
%Sodium (total)	0.14	0.18
Copper (total) (mg/kg)	24	24
Iron (total) (mg/kg)	7160	6530
Manganese (total) (mg/kg)	360	302
Zinc (total) (mg/kg)	104	108
%Moisture	26.00	28.50
%Total solids	74.00	71.50
%Total salts	2.89	3.28
pH	9.1	9.2

Supplementary References

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