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Influence of biogas digestate on density, biomass and community composition of earthworms

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ABSTRACT

In recent years, the increasing number of biogas plants in operation has also led to a considerable rise in fermentative substrates, which are now widely used as agricultural fertilizers. The impact on earthworm fauna of using biogas digestate as a fertilizer has yet to be sufficiently researched. At two different tests sites, the short-term (four months after fertilization) and longer-term (three-year test period) influence of using fermented residues as a fertilizer was examined on earthworm density, biomass and community composition compared to using traditional fertilizers (cattle and pig slurry, chemical fertilizers as well as an unfertilized control). The crop grown was maize (*Zea mays* L.). Applying biogas digestate and slurry had a positive overall impact at both sites on earthworm density and biomass. Observing different fertilization regimes in the short term, the significantly highest earthworm density was seen where slurry had been applied. In the treatments with digestate and conventional slurry, earthworm biomass differed significantly in comparison with chemical fertilization and the untreated variant. After three years, earthworm biomass in the variants fertilized with conventional slurry and digestate tended to be higher than in the chemical fertilizer and untreated variants. Community composition was strongly influenced by the application of digestate. A decrease in the species *Aporrectodea rosea* was accompanied by an increase in *Aporrectodea caliginosa*. The earthworm population was supported equally positively at both sites by the variants with conventional slurry and digestate.

1. Introduction

In recent years, the promotion of renewable energy generation has resulted in a significant increase in the importance of agricultural biogas production (Weiland, 2010). In addition to biogas, digestate is a by-product of microbial anaerobic digestion (Bauer et al., 2009). The considerable amounts of digestate that accumulate are now being used more widely as secondary fertilizers in agriculture. So far it has not been possible to make any sufficient statements about the influence of using digestate as a fertilizer on soil quality. The properties of this substance are decisively influenced on the one hand by the anaerobic, microbial fermentation process and on the other by the actual substrates used. Apart from a reduction in the amounts of dry matter caused by the decomposition of easily convertible organic matter (Voća et al., 2005), major changes to its properties include a higher NH_4–N concentration (Möller, 2009). In terms of its properties, therefore, this type of fertilizer is considerably different from conventional cattle and pig slurry.

As representatives of macrofauna, earthworms respond very sensitively to various forms of land cultivation, which is why they are used as an important bio-indicator when evaluating soil quality (Paoletti, 1999). Organic as well as chemical fertilizers serve as a food source for earthworms, either directly or indirectly by increasing crop and root residues. Fertilization is thus, also highly important for earthworm activity in addition to tillage, pH value, soil moisture and a site's weather conditions. In many respects, earthworms have important functions in the agricultural ecosystem. For the most part this involves decomposing organic matter as well as forming stable clay-humus complexes and the establishment of a consistent macro-pore system.

Due to the rapid growth of biogas plants there is still a considerable lack of underlying data concerning the influence on earthworm populations of fertilizing soil using digestate. So far there have only been rudimentary attempts at comprehensively studying the impact on earthworms – as a bio-indicator – of fertilizing soil using

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Table 1
Main characteristics of cattle slurry and digestate used in the experiment in Cunnersdorf on a dry weight basis for cattle slurry and digestate respectively (idm = in dry matter).

Cattle slurry		Digestate	
Applied amount[m^3]	86	Applied amount[m^3]	70
Dry matter[%]	9.00	Dry matter[%]	4.90
Total nitrogen[%]	0.38 idm	Total nitrogen[%]	0.42 idm
NH_4–N[%]	0.21 idm	NH_4–N[%]	0.25 idm

Table 2
Main characteristics of pig slurry manure and digestate used in the experiment in Pfahlheim on a fresh weight basis for pig slurry and digestate respectively (ifm = in fresh matter).

Pig slurry		Digestate	
Applied amount[m^3]	30	Applied amount[m^3]	30
pH value	8.3	pH value	8.6
Total nitrogen[%]	0.7 ifm	Total nitrogen[%]	0.8 ifm
NH_4–N[%]	0.5 ifm	NH_4–N[%]	0.5 ifm

digestate (Ernst et al., 2008; Bermejo Domínguez, 2012). In particular, it is still not possible to draw any conclusions about the influence of fertilization with digestate on earthworms after a couple of years. Based on two field trials, this study aims to assess the impact, both in the short term and over a three-year period, on earthworms of applying fermentation residues to soil. The study will also investigate and evaluate the influence of using digestate as a fertilizer compared with using traditional slurry, chemical fertilizers and an untreated control.

2. Materials and methods

2.1. Experimental site

2.1.1. Site 1 Cunnersdorf

The field trial in Cunnersdorf (Saxony, 12° 13'E, 51° 24'N) is located in central Germany at 130–140 m AMSL. The predominant soil texture in the topsoil is a silt loam (90 g kg^{-1} clay, 330 g kg^{-1} sand). In terms of soil typology, the test site can be classified as a *Stagnic Luvisol*. Cunnersdorf has a mean annual temperature of 8.9 °C (1977–2011) and mean annual precipitation of 619 mm (1977–2011).

The experimental setup was a one-year, single-factor strip design with four replicates. The crop grown was maize, and the preceding crop was oat. In addition to an unfertilized control variant and a test element fertilized with mineral nitrogen only, the trial investigated raw slurry and fermentation residues.

Before the experiment was set up, the land was ploughed to a depth of approximately 25 cm. The slurry was spread on individual test elements on 19.03.2009 using a special method to inject it at a soil depth of approximately 10 cm. As the capacity of the tractor's slurry tank was not large enough for an entire test element, each test element was driven over twice in immediate succession. It was possible to place the second portion in precisely the area that remained after application of the first portion. Approximately 86 m^3 of cattle slurry and 70 m^3 of digestate were spread in order to meet nitrogen requirements of about 160 kg ha^{-1} NH_4–N. The contents of the two agricultural fertilizers are shown in Table 1. The digestate originated from a co-fermentation plant with a secondary fermenter. In addition to cattle slurry, the biogas plant mainly ferments maize. Earthworms were caught on 09.04.2009. The maize was drilled five weeks after spreading the slurry (22.04.2009).

2.1.2. Site 2 Pfahlheim

The test site Pfahlheim is located 484 m AMSL in southern Germany in the foothills of the Swabian Jura. The soil type is a Luvisol. Based on the particle size composition, the soil texture in the Ap horizon can be described as a silt loam (244 g kg^{-1} clay, 146 g kg^{-1} sand). On average this site has 840 mm of precipitation each year as well as an annual average temperature of 7.7 °C.

The experiment was set up back in 2007 using a fully randomized, single-factor block design with four replications, and from the very beginning conservation tillage was employed down to a soil depth of 15 cm. The raw slurry and digestate were spread using drag hoses on 23.04.2009. With 30 m^3 each of digestate and pig slurry, 130 kg N were spread per hectare. Next a tooth cultivator was used to carefully incorporate the organic fertilizers into the ground. The amount of chemical fertilizer used was equivalent to 165 kg N per hectare; it was applied by adding two doses of calcium ammonium nitrate (65/100 kg ha^{-1} N). Using the experimental design of the study presented here, the following test elements were examined on 28.05.2009 during the third year of the experiment: 'untreated control variant', 'chemical fertilizer (calcium ammonium nitrate)', 'pig slurry' and 'digestate'.

The digestate used in the study came from a co-fermentation plant. There was no secondary fermenter at the time of sampling. The substrates used in this biogas plant include renewable raw materials such as maize and grass silage as well as cereal grain. Poultry dung is also fed into the biogas plant to be used as an additional fermentation substrate. More information about the properties of the organic fertilizers is listed in Table 2.

2.2. Sampling and analysis

Earthworms were caught approximately four weeks after fertilization (ISO 23611-1 (2006)) using a combination of hand-sorting to a depth of 30 cm and a subsequent extraction of deeper living earthworms by formaldehyde solution. By combining the ethological, active method of formalin extraction with the passive method of hand-sorting, it is possible to significantly increase the effectiveness of catching worms on arable land (Terhivuo, 1982). The total sampling area covered 1 m^2; a metal ring 0.125 m^2 in size was used to demarcate the border of the sampling area. Then a block of soil approximately a spade deep was removed from the demarcated area and searched for earthworms. Afterwards approximately 2 l of a 0.2% formalin solution were poured into the hole in the ground. Any earthworms that emerged were collected, washed in water for approximately 20 min and preserved in jars of ethanol. The test was performed with eight replicates per variant. The sampling areas were selected in such a way that they proportionately contained different aspects of one plot (row of maize and the space between two rows of maize). Species identification followed Sims and Gerard (1985). For each variant the parameters 'abundance' [individuals m^{-2}], 'biomass' [g m^{-2}] and 'species dominance' [%] were defined.

2.3. Statistical analyses

The earthworm abundance and biomass results between earthworm populations in each variant were statistically verified using the distribution-free Mann-Whitney test (U-test) (Kasuya, 2001). Significant values ($p < 0.05$) are represented by different letters.

3. Results

3.1. Site 1 Cunnersdorf

No significant differences in earthworm abundance and biomass were observed in the variants with raw slurry and digestate at the Cunnersdorf site (Table 3). In these two organic fertilizers, significantly higher biomasses were identified in comparison with the remaining variants. Overall abundance (adult and juvenile specimens) was also higher in the test elements with raw slurry and