ANAEROBIC DIGESTION OF ANIMAL MANURE – IMPLICATIONS FOR CROP YIELDS AND SOIL BIOTA IN ORGANIC FARMING

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Abstract. *Anaerobic digestion of farmyard manures may help farmers to produce bioenergy instead of using fossil fuels, support cycling of nutrients and reduce greenhouse gas emission. However, compared to pristine slurry, digested slurry has a reduced content of organic carbon which may impact the soil biota negatively due to substrate shortage. Our knowledge on these processes and their influence on soil quality is scarce. Hence, a field experiment with two organic cropping systems (grass-clover ley and arable system; at two slurry-application levels) was established in 2011, to study how application of digestates affects crop yields, soil characteristics and soil biota (earthworms, springtails, microbiota). The grass-clover system showed comparable yield levels over 3 years when digested slurry was compared to untreated slurry. Digested slurries had no influence on soil nutrient concentrations or on soil organic matter levels over the first 2 years. Application of high levels of manure increased the mortality of both surface-dwelling and soil-living earthworms just after application, but the long-term effect of manure application seemed more positive, especially at low application levels. Springtails and microorganisms seemed only little affected by application of digested slurry.*

Key words: *Collembola, bioenergy, grass-clover, digestate.*

INTRODUCTION

Agriculture is criticized for emitting high amounts of greenhouse gas, both as methane (CH_4) and nitrous oxide (N_2O), through the use of fossil energy and animal husbandry. By anaerobic digestion (AD) of farmyard manures, farmers may produce biogas to replace fossil fuels and reduce the emission of methane produced during storage of slurries. AD may also ease the handling of the manure and reduce viability of animal pathogens and weed seeds [1]. However, the process needs stable temperatures of at least 30°C, so the construction and maintenance of small-scale biogas plants for Nordic conditions requires technically skilled farmers. AD alters the physicochemical composition of the manure, reducing the proportion of easily degradable C in the digested manure. This may negatively affect soil organic matter pools and the soil biota that live on the organic C. Organic nitrogen (N) is mineralised during AD, thus enhancing the proportion of mineral N in the digestate compared with untreated manure [2]. The higher concentration of ammonium, constituting most of the mineral N, may be toxic to soil fauna, such as earthworms [3]. On the other hand, when used as fertilizer, greater availability of N applied in the digestate may increase root and shoot residues due to increased plant growth. This may compensate for the organic C lost during digestion, and support the growth of soil fauna and microorganisms. The effects of AD on the quality of animal manure, and derived effects on crop yields and biota, are studied in the project "SoilEffects" at Tingvoll, NW Norway. Results from 2011-2014 are presented, to evaluate whether AD affects manure, crop yields and quality or soil fauna and microbiota.

MATERIALS AND METHODS

Anaerobic digestion of manure

A biogas plant was established in 2010 at Tingvoll Research Farm, owned by the Norwegian Centre for Ecological Agriculture, to treat the slurry from 25 organically managed dairy cows. In spring 2011, an associated field experiment was initiated to study long-term effects of AD treated slurry on soil quality characteristics and crop yields [4]. Because a stable digestion process was first reached in autumn 2011, both the conventional dairy cow slurry and digestate applied in 2011 was obtained from Bioforsk, Soil and Environment Division (Ås), where the digestate was produced in a 6-m^3 batch digester. In later seasons, digested and non-digested slurries were sampled during winter and stored in 1-m^3 plastic containers at Tingvoll. The farm has a loose-housing cow house, from which slurries flow to a collection pit. Until a pump was installed to homogenize the slurry during flow, only the most liquid part of the slurry went into the digester. Hence, during 2012-13, the physicochemical differences between digested and non-digested slurry were less than those normally found in such comparisons. When better homogenisation was achieved, the content of dry matter (DM) and the proportion of mineral N became closer to those obtained in other AD studies [2] (Table 1).

Field experiment and treatments

Non-digested and digested slurry were applied at two levels in two cropping systems: 1) arable crops (without legumes) with annual ploughing and 2) perennial grass-clover ley (established in 2009). The crops in the arable system were oats in 2011, annual ryegrass in 2012 and spring wheat (harvested, before complete ripeness) in 2013. In 2014, the whole field was ploughed and a new grass-clover ley was established, with green fodder (oats, peas and vetches) as a cover crop. Low (L) and high (H) application levels of digestate (DL, DH) and non-digested slurry (NDL, NDH) contained total N levels of 85 and 170 kg ha^{-1} yr^{-1} , respectively, to arable crops, and 110 (L) and 220 (H) kg ha^{-1} to the perennial ley. This comprised about 25 and 50 tonnes ha^{-1} of slurry in low and high treatments in the arable system (one application), and 20 + 10 and 40 + 20 tonnes ha^{-1} (in early spring + after 1st cut) in the grass system. A control treatment with no slurry was included in both cropping systems. Addition of water to the control was considered unnecessary, due to the high precipitation rates at the Tingvoll area (average of 126 mm per month, April-September, 1995-2014). Each cropping system had four replicate blocks. Within each block, the five treatments (control, NDL, NDH, DL, DH) were randomly distributed on experimental plots sized 3×8 m. The slurry was diluted with water to < 5% DM and applied by hand, using 10-liter cans. In arable crops, the manure was applied after ploughing and harrowing, and raked into the soil in 2011 and 2012. In 2013, the manure was applied on arable plots without ploughing, and incorporated with a horizontal rotavator. In 2014, no manure was applied, and the after-effect of 3 seasons of manure application on crop yields was recorded. The experimental soil is a loamy sand, with low status of ammonium acetate-lactate (AL) extractable phosphorus (P) (< 4 mg 100 g^{-1} dry soil) and very high status (>120 mg 100 g^{-1} dry soil) of nitric acid soluble potassium (K) (Table 2).

Measurements and statistical analyses

For measurement of the chemical composition of the slurries, representative samples were analysed for contents of ash, DM and for total concentrations of N, P, K, Mg, Ca, S and ammonium-N. In Table 1, the proportion of mineral N (NH_4-N/tot-N×100) is shown. Cold (22ºC) and hot (80ºC) water-extractable organic C [5] in manures and soil were measured using a sequential procedure. For measurements of soil pH (H_2O) and nutrient concentrations (P-AL, K-AL, Mg-AL, Ca-AL and K-HNO_3), bulked samples of 10 augerings (diameter 2 cm, 0-20 cm depth) per experimental plot were taken in spring 2011 before the start of the experiment, and in spring 2013 before manure application. The precise location of each soil augering was recorded. P-AL values in six of the 20 soil samples in the arable system were below the detection limit (< 2 mg 100 g^{-1}) in 2011. These were set to 1.5 mg 100 g^{-1} to allow for statistical analysis.

Earthworms and springtails (*Collembola*) were sampled from the grass system to study the effect of manure application over a short and an extended time span. For measurements of diversity and density, 8-liter soil cubes were sampled for earthworms and 100 cm^3 cylinders (0-3.8 cm depth) were used for springtails. Earthworms were sampled on April 13th 2011, May 4th 2012, May 8th 2013 and September 25th 2013, and sorted out by hand [4]. Springtails were sampled on April 28th 2011, and April 26th, May 3rd and June 14th in 2012 and extracted using a drying procedure [9]. The acute toxic effect of manure application on earthworms was studied in spring 2013, by recording dead earthworms on the soil surface in 1×1 m frames just after manure application. One count was made per experimental plot, with the frame placed in a fixed position in all plots. Soil microbial diversity and activity were measured in both crop systems shortly after application of slurries, by phospholipid fatty acid (PLFA) profiling (in 2011 and 2013) and soil respiration (in 2011) [4] .

For measurement of crop yields, subplots sized 1.2×7 m were harvested on the ley plots, the fresh weights were recorded and samples for botanical composition and DM content were collected. In arable plots, the same method was used for ryegrass and wheat. For oats, sheaves were made, their fresh weights recorded, and then they were dried and threshed to measure the amount of straw and grains. Statistical analyses were performed using Minitab 16 and SAS Statistical Software. A general linear model was used to test the effect of treatments on yield levels, and Tukey t-test at the 5% level to compare the mean values of treatments. For comparisons of soil analyses in 2011 and 2013, paired t-tests were used. For the PLFA data, the relative abundance (molar %) of the individual fatty acids was calculated, log transformed and submitted to principal component analysis using Unscrambler 7.6.

RESULTS AND DISCUSSION

Manure chemistry

AD led to a slight reduction in DM content, a small increase in the proportion of mineral N and an increase in pH by up to 0.5 units (Table 1). The content of cold and hot water-extractable organic C (CWC, HWC) was comparable in both ND and D. There was a tendency that digested slurry had less total WC, especially in slurry with low DM content (2012 and 2013 samples). During manure handling and application, it was frequently noticed that digested manure flowed more easily (due to its lower viscosity) and infiltrated more rapidly into the soil than did non-digested manure. The digested manure had less odour, but it had a stronger tendency to foam. Its colour was less brown, and more greenish than that of the undigested manure.

Table 1

Chemical composition of digested (D) and non-digested (ND) slurry; average values with min-max values for DM. x = missing value. CWC, HWC = cold and hot water-extractable organic C, D/ND (%) = (CWC+HWC in D)/(CWC+HWC in ND)×100.

Year, slurry type, (no. of samples)	DM %	pH	Total-N kg Mg^{-1}	Nmin % of tot-N	P	K	Mg	Ca	S	Ash %	CWC | HWC kg Mg^{-1} %D/ND
					------------- kg Mg^{-1} ------------						
2011, ND (6)	6.5 5.1-8.4	7.6	2.7	63	0.50	3.1	0.45	0.83	x	x	3.6 | 2.6
2011, D (4)	4.6 2.6-6.4	8.1	2.8	71	0.46	3.1	0.40	0.67	x	x	2.1 | 3.8 94
2012, ND (5)	3.9 3.0-5.2	7.8	2.2	61	0.39	2.5	0.36	0.83	x	x	1.7 | 2.1
2012, D (6)	2.7 1.5-4.5	7.9	1.6	59	0.33	1.6	0.29	0.64	x	x	0.9 | 0.8 45
2013, ND (3)	4.8 4.0-5.8	7.3	2.4	60	0.43	2.8	0.39	0.92	0.24	1.0	2.2 | 2.2
2013, D (4)	3.1 1.9-4.2	7.5	2.1	67	0.33	2.6	0.31	0.80	0.17	0.8	1.1 | 1.1 49
2014, ND (3)	5.4 5.2-5.9	7.5	2.6	61	0.55	3.5	0.45	1.1	0.27	1.2	3.5 | 3.8
2014, D (3)	5.0 4.5-5.3	8.0	3.1	69	0.53	3.3	0.43	1.1	0.24	1.1	2.2 | 2.5 64

Yields

In the grass system, the yield levels were increased greatly by manure application (Fig. 1), with no difference between the effect of digested manure and untreated manure. Over time, the yields declined in the control treatment, while in the other treatments they increased slightly until 2014, when the lack of manure application resulted in a marked yield decline in all treatments (Fig. 1). Although differences between yield levels in the four manure treatments were only found in some cases, there were significant differences in botanical composition between treatments with digested and undigested slurries. Grass species increased, at the expense of clover and weeds, with increasing levels of manure. Digested manure reduced the clover content to a greater extent than did the non-digested slurry (data not shown).

In the arable system, the apparent yield increment caused by manuring was not statistically significant in any year, although a tendency to enhancement ($P = 0.095$) was observed in 2013 on most of the manured plots (Fig. 2). The lack of yield enhancement was surprising, as other growth characteristics appeared to respond positively to manuring. Clear differences in straw length (significant) and plant colour were visible during the growing season of 2011 (data not shown). Yield levels in 2012 were generally very low due to poor establishment of fodder rape, which had to be replaced by a late-sown ryegrass crop.

In 2013, a horizontal rotary harrow was used for combined tillage and incorporation of manure, and the yield effects were then larger than before. Furthermore, the yield effect of digested manure was clearly better than that of undigested manure in this year. Comparisons of the mean spring wheat yield of both digested manure treatments with the mean of the untreated manure treatments and the control, showed close to significant differences (P = 0.056), with respective mean yields of 3.9(a), 3.5(ab) and 3.0(b) tonnes DM ha^{-1}. A yield increase in spring cereals with rapid slurry incorporation was found in a German study [6], in which no significant yield differences between digested and non-digested slurry were otherwise found. In 2014, the green fodder yield levels in the arable system were comparable to wheat yields obtained in 2013, and slightly lower than those achieved in the grass system in 2014.

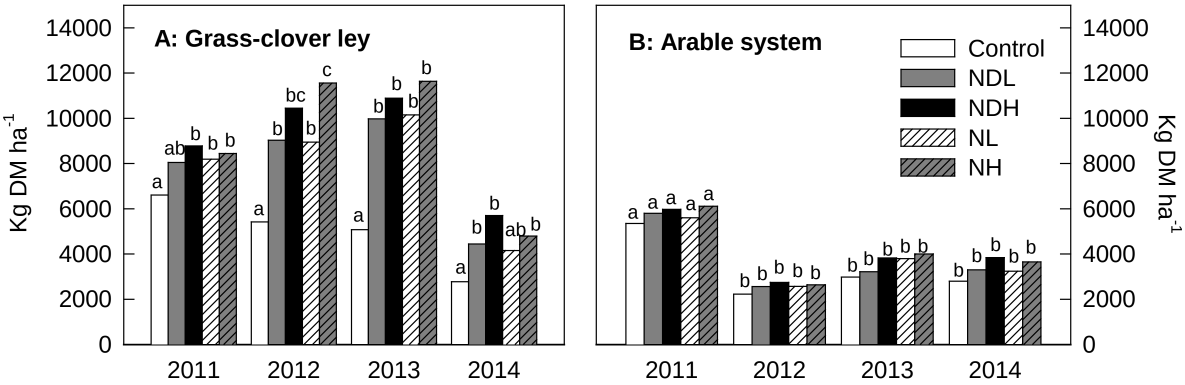

Figure 1. **Crop yields in (A) grass-clover ley and in (B) an arable system, either without manure (Control), or with low (L) and high (H) applications of digested (D) and non-digested (ND) slurries during 2011-2013. In 2014, after-effects with no further manuring were measured in green fodder in both systems.**

Soil chemistry

Some changes in soil chemistry were found, e.g. P-AL increased in most manured treatments and soil organic C was reduced in treatments with annual soil tillage [7]. This shows that the sampling technique was accurate enough to reveal possible effects caused by manure composition on relevant soil characteristics. A time span of two years is probably too short to reveal possible effects due to differences in manure chemistry caused by anaerobic digestion.

Soil fauna

Five earthworm species were found in the entire experiment and these were studied in detail in the grass system. The species *Aporrectodea caliginosa* was most common, but *Octolasion cyaneum* and *Lumbricus terrestris* were also found. In general, the density of earthworms was stable, at about 150 individuals m^{-2} in the control treatment (spring 2011 to autumn 2013). In early spring 2013 there was an exception, with densities down to 50 m^{-2}. In the manured treatments DL, DH and NDL, the mean values were slightly higher in 2013 than in 2011, in both spring and autumn. The values were about 150 individuals m^{-2} in spring, and 180-240 in autumn. With high application levels of NDH, the earthworm density declined to about 90 in spring and 120 in autumn. Hence, modest levels of manure application, independent of type, seem to increase earthworm density over a time span of one or more growing seasons. Following application of slurry in 2013, an average of 19 dead worms m^{-2} on the surface was found in the NDH treatment, and 11 in DH. In the low manure application treatments, the corresponding values for NDL and DL were 4 and 2 dead worms m^{-2}. Accordingly, at high levels, non-digested manure seemed to affect earthworms more negatively than digested manure; this was opposite to our expectations, based on their relative ammonium concentrations.

Springtails constitute a diverse fauna group which is not well described in Norwegian agricultural soils. Across treatments, 8000-40000 individuals m^{-2} were recorded on the four sampling dates [9]. We found 42 species in the experimental field [8], with one species, *Onychiurus edinensis*, not previously observed in Norway and one species, *Oligaphorura ursi*, rarely found in agricultural soil. The most common species was *Parisotoma notabilis*, followed by three species of *Mesaphorura*, two species of *Protaphorura* and *Isotomurus graminis*. A high number of *P. notabilis* has also been found in pastures in Iceland, forest habitats in Norway, and in agricultural soil in Denmark and Sweden [8].

Collembolan species may be grouped into "epigeic" (in soil surface) and "endogeic" (below soil surface), on basis of presence or absence of eye organs and colour intensity. Following this approach, we found that the epigeic species may be more vulnerable to manure application than the endogeic species (data not shown). A significant drop in springtail density was seen some days after manure application.

Soil microbial community and activity

We assumed that addition of the various slurries would have a direct effect on the diversity and activity of the soil microbial community, by supplementing the soil native C pool with readily available organic C, depending on the level and type of slurry. This could not be confirmed by analysis of hot- and cold-water extractable C (CWC, HWC) in the soil in 2011 (data not shown) where availability of C could not be correlated with slurry applications. Measurements of accumulated soil respiration supported this assumption, showing increased levels (10-15%) in treatments with high addition levels of slurry (Fig. 2A). The measurements of available C in soil also revealed that the grass-clover ley contained relatively more native available C than the soil in the arable system. This difference in soil C was not reflected in the microbial biomass, as indicated by soil total PLFA content, which was unaffected by treatments (data not shown). On the other hand, the microbial community composition was strongly influenced by the experimental conditions, as revealed in a principal component analysis of the PLFA profiles (Fig. 2B). The most marked separation/grouping of data points is along the PC1 axis (grass-clover to the left and arable system to the right) showing that the main explanation for differences in community structure was caused by the two cropping systems. Besides the cropping system, field variation in native soil characteristics may also have contributed, as the grass system had higher levels of soil organic matter. The average content of the total organic matter in the arable system was 6%, and in the grass system 11%.

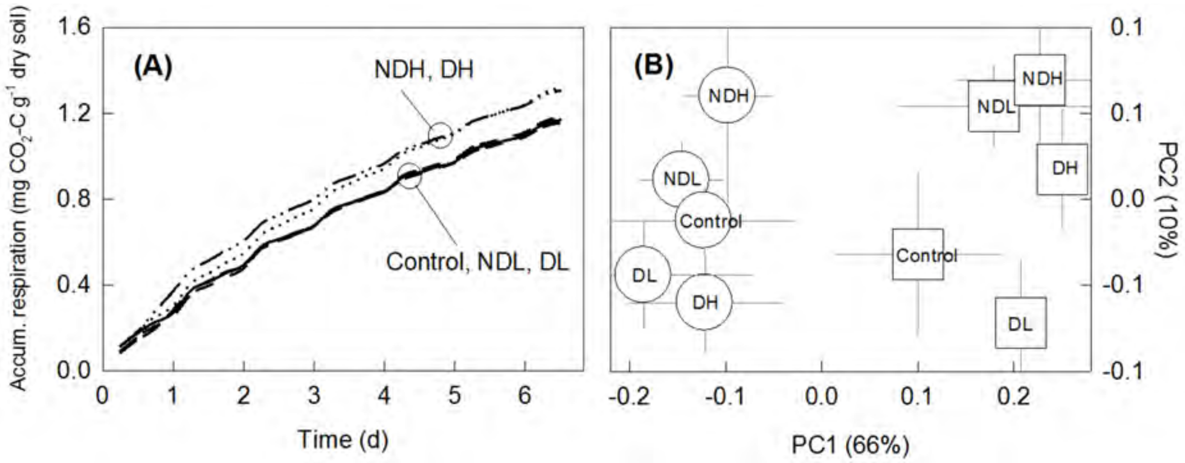

Figure 2. **Microbial (A) accumulated respiration and (B) PLFA profiles (principal component analysis of molar % of individual fatty acids) in soil sampled in field plots without manure (Control), or with low (L) and high (H) applications of digested (D) and non-digested (ND) slurries in spring 2011. Circular and square markers indicate grass-clover and arable systems, respectively, in plot B.**

Although, the PC2 accounts for a minor part (10%) of the explained variation, the non-digested and digested slurries were grouped at opposite ends of this axis. This was observed in both cropping systems, indicating that the digested and non-digested types of slurry had different impacts on the microbiota. Since five days elapsed from application of slurries until soil sampling in 2011, we assume that microbial biomass supplied together with the slurries was decimated by the time of sampling, and did not contribute directly to the PLFA analysis. Microbial diversity and activity are responsible for important ecosystem services, especially in organic farming systems – e.g. degradation power to mobilize nutrients to crops, plant-beneficial microorganisms which can oppose plant pests, and improvement of soil structure. Within the present experimental framework, the soil microbial community seemed to be impacted much more by the cropping system and soil organic matter than by the type of manure. In this respect, manuring with anaerobically digested slurries seems a suitable and safe alternative to untreated cow slurry.

CONCLUSIONS

Anaerobic digestion of dairy cow slurry produces a digestate with slightly lower DM content, but with somewhat higher pH (typically from 7.5 to 8.0) and a proportion of ammonium-N in relation to total N. We found digested slurry to have similar effects on grass-clover ley yields as non-digested, but it reduced clover more than did the non-digested slurry. Our results suggest that a positive yield effect of digested slurry may be achieved in arable crops, provided the slurry is rapidly and well incorporated into the soil, and a crop is rapidly established to utilise the nitrogen. Digested slurry seemed to have less negative effects on earthworms than did non-digested slurry, but in leys earthworms appeared sensitive to the amount of manure applied. Springtails were found in high numbers and with high species diversity and were negatively affected by manure application. Springtails partake in a range of different roles in the turnover of soil organic matter, which is especially important in organic farming systems, and thus they may serve as good indicators of soil quality. Applying digested vs. non-digested slurry affected the microbial community to a much less extent than the differences found between system and the native organic C in the soil. All in all, the use of digestate thus appears a good opportunity to recycle plant nutrients in a system which includes sustainable production of bioenergy. Hence, with respect to soil fertility it appears to be acceptable to recycle plant nutrients combined with production of bioenergy. However, longer time series as well as other types of slurries (anaerobically digested vs. non-digested) and cropping systems needs to be studied in order to establish if the present findings are valid in general.

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