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Anaerobic digestion of dairy cattle slurry—long-term effects on crop yields and chemical soil characteristics

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Abstract Renewable energy in the form of biogas can be produced by anaerobic digestion (AD) of animal manure. However, there is still a lack of knowledge on the long-term effects of AD-treated manure on soil characteristics and crop productivity, compared with untreated manure. A field experiment was established in a perennial grass-clover ley in 2011 to study the effects on important soil and crop characteristics when the slurry from a herd of organically managed dairy cows is anaerobically digested. While the rate of manure application affected soil concentrations of extractable nutrients and pH, these variables were unaffected by AD. Soil organic matter (SOM) concentrations decreased in all plots and faster on the plots with high intrinsic SOM. The decrease was similar with application of untreated (non-digested) slurry (US) and anaerobically digested slurry (ADS), and it was not affected by application rates. The general decline may be explained by the initial high SOM content, the long-term effect of drainage, and higher temperatures with climatic change. US and ADS gave similar yields of grass-clover ley (2 cuts/year) and green fodder, on average 0.79 and 0.40 kg DM m^{-2}, respectively. Clover yield was similar in manured treatments and the non-fertilized control. With respect to crop yields and chemical soil characteristics, long-term (10 years) effects of AD in an organic dairy cow farming system seem to be minor. The benefits of extracting energy from the slurry did not compromise grassland productivity or soil quality in the long term.

Highlights
- Long-term application of anaerobically digested (ADS) and non-digested slurry (US)
- US and ADS effects on SOM, soil fertility, and crop yield were similar
- Long-term application of US and ADS did not maintain the SOM levels
- Extracting energy from the slurry did not compromise grassland productivity

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Introduction

Organic farming systems strive to recycle nutrients and organic matter and restrict the use of non-renewable resources. This is in line with the four basic principles of ecology, health, fairness, and care as expressed by the International Federation of Organic Agriculture Movements (IFOAM 2023). Whereas nutrient cycling has received much attention, and nutrient management has advanced with significant research efforts, little has been done to make organic farming systems less dependent on fossil energy. Anaerobic digestion (AD) of organic residues is an alternative, where farmers locally can produce renewable energy to replace fossil fuels. By modelling different scenarios for a 1000 ha combined organic dairy and cash crop farm, Pugesgaard et al. (2014) concluded that the production of biogas holds the possibility for organic farms to achieve a positive energy balance and provide self-sufficiency with nitrogen (N) fertilizer. For animal husbandry farmers, further benefits of AD include easier handling of the manure, since malodour is reduced (Tafdrup 1995) and rapid soil infiltration is facilitated by decreased manure viscosity (Möller and Müller 2012; Sommer et al. 2003). Further, the amounts of animal pathogens and viable weed seeds are significantly reduced, even under mesophilic AD conditions (35 °C) (Johansen et al. 2013a).

Some major differences between non-digested and digested slurry are a slightly increased pH, an increased proportion of mineral N, and a decrease in the content of carbon (C) (Möller and Müller 2012). This may increase gaseous emissions of N after spreading in the field. During digestion, a major part of easily degradable C is transferred into methane (CH_4) and carbon dioxide (CO_2), reducing the amount of C returned to soil compared to non-digested slurry (US). A higher content of cold (CWC) and hot water extractable carbon (HWC) was found in US compared to digested slurry (Johansen et al. 2013b). Application of organic materials to soil may lead to decomposition, not only of the applied organic matter, but also of the intrinsic soil organic matter (SOM), a phenomenon known as positive "priming effect" (Bol et al. 2003). The higher mineral N in the digested slurry could lead to a stronger priming effect, especially in soils with lower levels of plant-available N as may be found in organically managed soils (Berry et al. 2006). When positive priming occurs, the addition of organic materials to soil may not necessarily result in a net C storage.

The application of AD slurry, instead of US, may further alter the biogeochemical conditions in the soil, with potential consequences for nutrient availability and crop production. Because of the enhanced availability of N as ammonium (NH_4^+) in the digested slurry compared with raw slurry, a higher N utilization and thereby higher yields are expected (e.g. Tafdrup 1995). In grass-clover leys, this may benefit the growth of the grass component, reducing the proportion of clover in the total yield (Steinshamn 2001). This needs further investigation as the clover-to-grass ratio may influence the nutritional quality of the livestock diet (Fraser et al. 2004; Johansen et al. 2018) and a decrease of clover in the canopy is negative for organic farming systems relying on legume N fixation. Studies found that N fertilization tends to decrease N_2-fixation due to higher grass-to-clover ratios and higher clover N uptake from the soil. The type of fertilizer and mineral N source (NH_4 or NO_3) has a direct impact on the magnitude of this effect (Kristensen et al. 2022). Nevertheless, few studies have found significant effects of increased mineral N in the digested slurry on ley yields in field experiments, likely due to loss of N before plant uptake (Möller and Müller 2012).

Since AD will change the characteristics of the slurries (Möller and Müller 2012), changes in soil characteristics upon long-term application of US versus slurry treated by AD (ADS) could be expected. Hence, in the present study, the effects on soil characteristics and crop yields as a response to long-term application (10 years) of ADS versus US were investigated. The field investigation was undertaken at an organic dairy cow farm. The field experiment was established in 2011 and named "SoilEffects", to study changes in the farm fertilizer management, with a broader aim to assess whether organic farmers could apply AD of animal manures without negatively affecting soil characteristics or crop yields. The hypotheses were as follows:

1. Long-term application of ADS or US will increase the concentrations of SOM, ammonium acetate-lactate (AL) extractable nutrients, and pH in soil, proportionally to the application rate.

2. The expected increase in soil concentrations of AL-extractable nutrients and pH will not be affected by AD of the slurry, compared to no digestion.

3. Crop yields will be higher in ADS than in US treatments, due to the higher concentration of mineral N.

Material and methods

Experimental site

The experiment was established in 2011 at Tingvoll research farm (62°54′N,8°11′E) in North-Western Norway, owned by the Norwegian Centre for Organic Agriculture (NORSØK). This area has a temperate, humid climate (Fig. 1). Tingvoll research farm has about 23 ha of cultivated land, which is managed by a tenant with a herd of 22 organically managed dairy cows (+ heifers and calves). Areas for field experiments are included in the farmer's field management.

At the research farm, a biogas reactor was established in 2010 for AD of the slurry from the dairy cow herd. To study the effects of AD of the slurry on soil characteristics and crop yields, a long-term field experiment was established in a field with low to medium concentrations of extractable phosphorus (P). The average initial concentration of ammonium acetate-lactate (AL)-extractable P in the topsoil (0–20 cm) was 25.9 ± 6.3 mg kg^{-1}, measured across

all experimental plots ($n = 40$) in a screening in 2010 (Løes et al. 2013).

The experiment was designed to compare the effects on soil characteristics and crop yields of digested versus non-digested slurry at two rates of application, with a control receiving no manure. The manure application rates were planned to mimic an organic system self-sufficient in animal feed (low rate) and a conventional system (high rate). Over time, the purchase of concentrates has increased in Norwegian milk production in general, as well as on Tingvoll research farm. This has led to a situation where the high rate of manure application now mimics the farm management at Tingvoll research farm, with a purchase of concentrate mixtures to cover about 40% of the cows' total net energy intake, whereas the low rate may still mimic an organic dairy farm self-sufficient in animal feed. Before localizing the experiment on the field, a thorough soil mapping was carried out to ensure the most even soil conditions possible on the site. The soil texture on the experimental site is a loamy sand with a range of 4.8–14% soil organic matter (SOM) in the topsoil (0–20 cm) (Løes et al. 2013).

Experimental design, crops, and fertilizer

The experiment (Table 1) was established in 2011 in a 2nd year grass-clover ley. Untreated (non-digested) slurry (US) or anaerobically digested slurry (ADS) at low or high rates, normalized by the amount of total
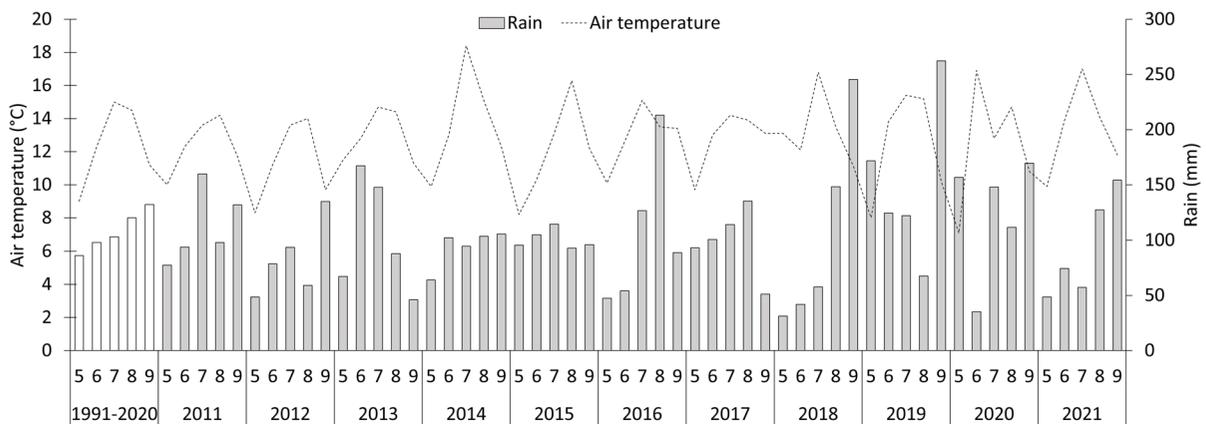

Fig. 1 Monthly averages of air temperature (2 m above ground) and monthly precipitation during May (5)–September (9) for experimental years 2011–2021 compared with normal values for Tingvoll 1991–2020 (DNMI.no). Values recorded at Tingvoll farm since March 1995, obtained from https://lmt.nibio.no/

Table 1 Overview of the long-term field experiment "SoilEffects", with information about origin of the applied manure, crops grown in each experimental year, and important dates of experimental activity

Year	Manure origin	Crop	Details	Manure date (s)	Harvest date(s)
Arable system					
2011[*]	NMBU	Oats	Harvest of grain and straw	May-11	Sep-8
2012[*]	Tingvoll farm	Ryegrass	Late sown after failure of fodder rape	May-22	Aug-23, Sep-25
2013[*]	Tingvoll farm	Spring wheat	Harvest as green fodder	May-22	Jul-30
2014	No manure	Green fodder	Oats, peas, and vetches	No manure	Jul-21
Grass system					
2011[*,δ]	NMBU	2nd year ley	-------	May-4, Jun-22	Jun-20, Aug-23
2012[*,δ]	Tingvoll farm	3rd year ley	-------	Apr-30, Jun-27	Jun-21, Aug-27
2013[*,δ]	Tingvoll farm	4th year ley	-------	May-14, Jun-26	Jun-19, Sep-5
2014[δ]	No manure	Green fodder	Oats, peas, and vetches	No manure	Jul-21
Grass system and Arable system converted to grass in 2014					
2015[*]	Tingvoll farm	1st year ley	-------	Apr 17, Jun-25	Jun-11, Sep-3
2016	Tingvoll farm	2nd year ley	Marble application mixed with slurry	May 10, Jun-16	Jun-09, Aug-4
2017	Tingvoll farm	3rd year ley	-------	May 2, Jun -16	Jun-15, Aug-10
2018[δ]	Tingvoll farm	4th year ley	Struvite application, 40 kg P ha^{-1}	April 24-25, Jun-21	Jun-06, Aug-28
2019	No manure	Green fodder	Oats, peas, and vetches	No manure	Aug-01
2020	No manure	1st year ley	-------	No manure	Jun-19, Sep-2
2021[*,δ]	Tingvoll farm	2nd year ley	-------	May 3–4, Jun-22	Jun-16, Aug 11–12

[*]Analyses of botanical composition; [δ]Analyses of plant nutrients

N applied, were compared with a non-fertilized control in replicated blocks. The treatments were randomly organized inside individual blocks. Initially, two cropping systems were compared, each with 5 treatments and 4 replicates over altogether 8 blocks. The "Arable crops" system had annual ploughing and annual crops, whereas the "Grass system" had perennial grass-clover ley, re-established with cereals as a cover crop, to be harvested as green fodder each 5th year. After 3 seasons (2011–2013), the Arable system was converted to perennial ley because of significant challenges in weed control. Hence, from 2014, data for 8 replicate plots of each treatment, 4 per system, are available. Each experimental plot measures 3 m × 8 m. Further details about seed mixtures, varieties, etc. are provided somewhere else (Rittl et al. Submitted).

US and ADS were compared at *low* (*L*) and *high* (*H*) application rates, with total rates of approximately 85 (L) and 170 (H) kg total N ha^{-1} year^{-1} applied to arable crops (2011–2013) and 110 (L) and 220 (H) kg total N ha^{-1} year^{-1} applied to grass-clover ley (Table 1). The amounts of manure equal about 30 (L) and 60 (H) Mg of slurry ha^{-1} applied to grass-clover

ley and 25 (L) and 50 (H) Mg to annual crops in the Arable system (2011–2013). The slurry was diluted by tap water to assure equal amounts of liquid applied to plots receiving US or ADS and was applied by hand using 10-l cans. In grass-clover leys, 2/3 of the manure was applied in early spring and 1/3 after the first cut. Every production year, the ley was cut twice. In arable crops, the manure was applied after ploughing and harrowing and rapidly incorporated. In 2011 and 2012, a hand rake was used for this purpose, whereas in 2013, it was done by a tractor with a horizontal rotavator. In grass-clover leys, the slurry was not incorporated. The crop rotation was similar to the rotation used at the farm and comprised a cereal cover crop (oats, peas, and vetches, grown as green fodder), which was used to reduce weed infestation in re-established grass-clover ley after ploughing every 4th year.

In 2011, the biogas plant at Tingvoll farm did not yet produce digestate, and US and ADS from cattle were purchased from the Norwegian University of Life Sciences (NMBU). After 2018, the biogas plant was out of function, and slurry applied in 2021 was stored from 2018. In years of re-establishing the ley (2014, 2019), the yield was recorded by only one

cut and no manure was applied, allowing for a registration of residual effects of manure application. In 2020, fresh manure was not available, and 2nd year residual effects were recorded in the 1st year ley.

Some initial characteristics of soil varied between the Arable and Grass systems plots (Løes et al. 2013). Hence, the effect of slurry application was studied for each set of plots in each year where soil analytical data were available between 2011 and 2021 (see section below). In the growing season of 2016, the experiment was used for testing the application of finely ground marble mixed into the slurry to possibly affect the yield levels by more efficient uptake of N (Løes et al. 2017), and in the growing season of 2018, it was used to test the effect of application of struvite (Rittl et al. 2019) on soil and crop characteristics (Table 1). In both cases, the same two blocks in the Arable system and two blocks of the Grass system were used for application of the additional input in spring, whereas the other blocks (two per system) received the similar amount of application of marble (2016) and struvite (2018) after final harvest. This management was assumed to equalize the conditions at the experimental plots.

Chemical analyses of slurry

For measurement of the chemical composition of the slurry, representative samples were taken, frozen, and analyzed by Eurofins (initially Kristianstad, Sweden, later Eurofins Germany) for dry matter (DM), pH (H_2O), total nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulphur (S), and the concentration of ammonium-N. The proportion of mineral N (NH_4-N*100/tot-N) was calculated. The number of samples subject to chemical analyses, and which characteristics were analyzed, differed between years.

Dry matter yields, botanical composition, and measurement of plant nutrient concentrations

Yields were recorded annually with two harvests of ley and ryegrass (Table 1) and one for cereals and green fodder in the five treatments (control, USL, USH, ADSL, ADSH) in each system. Harvest plots were cut with a power scythe with a 120-cm cutter bar in the middle of each experimental plot, with a size of 1.2 m × ca. 7.0 m. The plot size and fresh

weight of the crop canopy were recorded, and the harvested plot size was used to calculate yields per ha. A representative sample of the harvest plot crop was weighed and dried to stable weight at 60 °C to calculate the dry matter (DM) yield. Representative samples for botanical composition, and for plant analyses, were also taken in selected years (Table 1). In the Arable system, the oats crop in 2011 was dried after measuring the fresh weight and separated into grains and straw, whereas the wheat in 2013 was not separated in these fractions. For botanical analysis, representative samples (about 500 g) of the harvested plant canopy were sorted by hand into grass, clover, and weeds, and dry matter yields of each fraction were recorded. In the Arable system, the amounts of weeds were sorted out and recorded in 2011–2013. In 2015 and 2021 (Table 1), botanical composition was performed only in the plots of the Grass system ($n= 20$). Weeds were included in the total yield and nutrient uptake. In some years, samples of plant material were sent for analysis of their nutrient concentrations; here, we only present data for N, P, and K, which were used to calculate nutrient budgets (see below).

Soil sampling and chemical analyses

For analysis of extractable nutrients, pH, and SOM, topsoil samples (0–20 cm) were taken by hand-augering as composite samples from five fixed locations, evenly distributed from each side of each experimental plot, in total 10 augerings per plot, with an inner auger diameter of 1.8 cm. Samples were taken in spring 2011, 2013, 2016, and 2018 before manure application, and in 2018 and 2021 after the final harvest. Soil samples were sent to Eurofins, Sweden, and later Eurofins, Germany, for soil analyses.

SOM and nutrient budget calculations

Loss on ignition (%) was used as a proxy for SOM concentrations (%), with no corrections since the content of clay is low (on average, 9% of the topsoil for Arable system plots and 7% for grass system plots; Løes et al. 2013). Norwegian soils have a low likelihood of carbonates due to their mineralogy, so no corrections for carbonates were required. Loss on ignition (LOI) was measured as percentage weight loss of 2 mm sieved dry soil samples (about 10 g) after ignition at 550 °C for 4 h in a laboratory

furnace. LOI correlated closely with the soil's total carbon concentration, which was measured at 0–20 cm and 20–40 cm depth on all plots in 2010 and at 0–20 cm on all plots in 2011 (120 samples). Analysis of total C was performed by the "dry combustion" method described in Nelson and Sommers (1982). Ca. 200 mg of crushed soil was weighed in for analysis in a Leco CHN 1000 instrument. This instrument oxidizes carbon to CO_2 at 1050 °C and measures this gas by means of an infrared light cell. LOI values were in the range of 2–15%, while those of total C were in the range of 1–9%. The regression equation shown in Fig. 2 suggests that LOI values of 6% and 12% correspond to total C values of 3.2 % and 6.6%, respectively (Løes et al. 2013).

Nutrient budgets for nitrogen (N), phosphorus (P), and potassium (K) were calculated as the total of N, P, and K applied with slurry (slurry rate (kg ha^{-1}) × nutrient concentration in the slurry (g kg^{-1})) minus NPK removed by yields (yield (kg ha^{-1}) × nutrient concentration in the yield (g kg^{-1})). For the Grass system plots, we used data from 2011 to 2021, while for the Arable system plots, we calculated budgets for 2015–2021 only. The N input was the same in all years where manure was applied, as the N concentrations in the US and ADS were used to calculate the amount of manure to be applied. For P and K, input amounts were calculated by multiplying the amount of manure by the nutrient concentration in the manure in each respective year. The nutrient outputs were calculated by multiplying the dry yield in each cut for each plot by their respective nutrient concentrations,
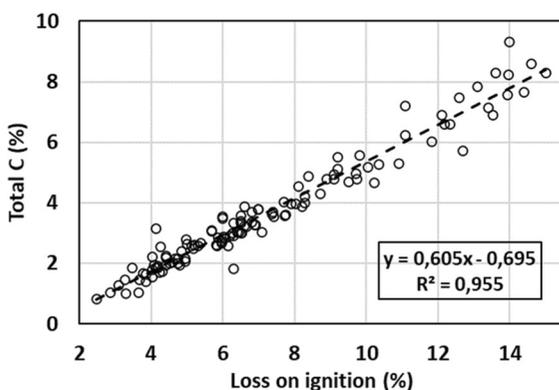
when available. When chemical analyses were not available (2015, 2016, 2017, 2019, 2020), we used data from leys of similar age analyzed in other years. The amount of N and P applied with the struvite was not included in the budget as there was only one single application equivalent to 40 kg P ha^{-1} and 18 kg N ha^{-1} to all plots in 2018.

Between 2011 and 2013, crop samples were sent to Bioforsk for determination of total N and macronutrients (P, K, Ca, S, Mg) using NIRS. In 2014, samples of green fodder were sent to Eurofins to analyses of macronutrients (P, K, S, Mg). In 2018, grass-clover ley samples were sent to Actlabs and analyzed for total N and macronutrients (P, K, Ca, S, Mg). In 2021, twenty composite grass samples, merged by equal amounts of dry, homogenized plant material from each replicate plot (n= 4) in each treatment ×system (Grass or Arable), were sent to Nemko Norlab for determination of total N and P, K, Ca, S and Mg. Total N was determined using the modified Kjeldahl method (Christensen and Fulmer 1927). The concentrations of other elements were determined using ICP-MS (Internal method based on NS-EN ISO 17294-2: 2016), after chemical digestion of the sample by a nitric acid/hydrogen peroxide solution at 120 °C for 30 min to bring ions into aquatic solution.

Statistical analysis

Statistical analyses were performed using Minitab Statistical Software (version 22.1). We used a general linear model (GLM) to test whether the chemical composition of the slurry was affected by the AD process, with slurry type (ADS and US) as the fixed factor and year as covariant. We conducted a simple ANOVA to assess the changes of SOM over time for each treatment, with year as the fixed factor. We used regression analyses to analyze if the changes in the soil characteristics of the treatments (pH, LOI, AL-extractable nutrients) differed from the control from 2011 to 2018. For that, we determined whether the changes in the soil parameters over time depended on the treatment. To do so, we assessed if the interaction between treatment and year was significant. The effect of treatments on soil parameter values was considered significant when the p-value of the differences between the regression coefficients of the treatments was 0.05 or less. The soil data of 2021 was not included in the regression analyses, because

Fig. 2 Soil total carbon concentrations plotted against loss on ignition, values for topsoil samples (0–20 cm) from the SoilEffects experimental field, 2010

of the two consecutive years without manure application. We used a simple ANOVA to assess the effect of treatments on total DM yield in each year, DM yield in each ley year (1st, 2nd, 3rd, or 4th year ley) and clover yield. Differences between mean values were considered statistically significant at $p < 0.05$ and mentioned as tendencies at $0.05 \leq p < 0.1$, applying the Tukey t-test.

Results

Slurry characteristics and the effect of AD on slurry composition

The chemical composition of US applied in the field experiment changed slightly over time (Table 2). The content of DM increased after installing an additional agitator pump in the slurry floating channel in the autumn of 2014. In 2021, the US and ADS applied were stored since 2018, and the US presented a high proportion DM (%), while the ADS had a high total N concentration (g kg DM^{-1}). With ADS, the proportion of DM did not increase over time, although ADS produced in 2015 had enhanced levels of DM. On average, the DM % in the slurry was reduced from 5.0 to 3.5% by AD.

A slight increase in pH (p=0.001) was found in ADS, with an average value of 7.7, as compared with 7.4 in US (Table 2). The mineral nutrient concentrations (g kg^{-1} DM) were mostly higher in ADS than in the US, indicating a loss of DM in the ADS. In ADS, a higher proportion of the N was found as NH$_4$-N compared to US (Table 2).

During manure handling and application, it was repeatedly noticed that digested slurry flowed more easily (lower viscosity) and infiltrated more rapidly into the soil than non-digested slurry. The smell was different and less strong, but the digested manure had a stronger tendency of foaming. The colour was brown towards greenish, whereas non-digested slurry was darker brown.

Soil nutrients, pH, and organic matter

Because of differences in the initial soil characteristics between the Arable and Grass system plots in 2011, changes over time are presented separately. Soil pH, and the concentrations of most AL-extractable

Table 2 Average values of the chemical composition of non-digested slurry (US) and anaerobically digested slurry (ADS) in 2011–2021 for samples taken during storage and application. ADS and US were received from Tingvoll farm, except for 2011, when ADS was purchased from the Norwegian University of Life Sciences (NMBU). ADS applied in 2021 was stored from 2018. Asterisks (*) indicate statistically significant differences between mean values of the chemical composition of ADS and US

Chemical composition	US							ADS							Mean Values	
	2011	2012	2013	2015	2016	2018	2021	2011	2012	2013	2015	2016	2018	2021	US	ADS
Dry matter (%)	5.3	3.7	4.8	5.4	5.7	5.1	8.1	4.6	3.2	2.0	4.8	4.2	3.0	2.2	5.3	3.4*
Tot-N (g kg DM^{-1})	51.3	61.9	50.1	47.5	52.0	46.0	42.0	60.9	65.5	88.9	61.8	52.9	57.4	143.2	50.0	71.0*
NH$_4$-N (g kg DM^{-1})	34.1	39.6	30.5	29.0	33.4	27.5	-	-	40.2	59.2	42.5	32.0	42.2	-	31.7	42.1*
NH$_4$-N (% of total N)	59.5	64.3	60.7	61.1	60.3	59.0	-	71.0	61.0	66.7	68.0	60.7	73.6	-	60.6	65.2
pH	7.6	7.6	7.3	7.5	7.3	7.3	-	8.1	7.7	7.7	7.9	7.6	7.5	-	7.4	7.7*
P (g kg DM^{-1})	10.1	11.3	9.0	10.1	7.9	9.3	-	10.0	13.1	9.5	10.8	8.6	11.0	-	9.4	10.9*
Ca (g kg DM^{-1})	19.8	23.5	19.2	20.9	58.3	19.0	-	14.6	26.5	29.2	23.1	74.3	31.4	-	30.6	35.5
Mg (g kg DM^{-1})	9.1	10.9	8.3	8.3	7.9	6.9	-	8.7	11.8	11.0	8.9	8.8	11.4	-	8.5	10.3*
S (g kg DM^{-1})	-	7.7	5.0	5.1	4.7	4.9	-	-	7.2	6.2	4.9	4.1	5.6	-	5.1	5.5
K (g kg DM^{-1})	51.9	76.7	59.7	65.0	63.5	65.5	-	67.4	74.3	115.3	67.1	75.1	96.2	-	63.6	81.4*

nutrients, showed a clear decrease from 2018 to 2021 (Fig. 3). No manure was applied in 2019 or 2020. For K-AL, the concentrations in 2021 came close to the value of the control soil.

The long-term effects of manure application can be seen between 2011 and 2018, when manure was applied in all years except 2014 (Table 1). Soil pH and the concentrations of AL-extractable nutrients then increased with increasing rates of manure in the plots of both systems (Fig. 3).

The SOM as assessed by loss on ignition (LOI) varied considerably across the experimental field and is generally higher in the Grass than in the Arable system plots. In 2011, significant differences in LOI were found between experimental plots in the Grass system, but not between manure treatments in any system (Løes et al. 2013). As shown in Fig. 3, the SOM concentrations decreased from 2011 to 2021 in all treatments and the control. Across treatments that received manure, the average values were 11.5% in 2011 vs. 9.2% in 2021 for the Grass system plots and 6.5% in 2011 vs. 5.3% in 2021 for the Arable system plots. In the control, the average value was 10.8% in 2011 vs. 8.2% in 2021 in the Grass system plots and 6.7% in 2011 vs. 5.3% in 2021 in the Arable system plots (Fig. 3). Even with high manure application, and independent of whether the slurry was digested or not, the SOM did not increase (Fig. 3). On average, the decline in SOM was 1.3% for both systems. However, in plots where the initial SOM concentration was higher, a higher decline was found (Fig. 4). It should be noted here that no manure was applied to any plot during 2019 and 2020.

Yields and botanical composition from 2011 to 2021

In the initial years (2011–2013), DM yields were generally much higher in the Grass system than in the Arable system, but from 2015, the yields became comparable as all plots were converted to grass-clover ley in 2014, and from 2019, the yields became quite similar in the two systems (Fig. 5). In the Arable system, weeds posed a significant problem which reduced yield levels during 2011–2013. The low application of manure and short growing season for the arable crops as compared with perennial ley also explains the yield differences in these years. The significantly lower yields in years of re-establishing the ley (2014, 2019) also illustrate that the yield of

a cereal crop, even when harvested as green fodder at maximum dry matter production, cannot compare with the dry matter production from two cuts of ley.

On the average, the proportion of weeds was higher in the control, 15%, than in the treatments, where the average weed proportion varied from 10 (ADSL) to 12% (ADSH and USL). The proportion of weeds was the smallest in the first year of the ley (5%) and highest in the 3rd year of the ley (16%). The most important weeds in the Arable system were corn spurrey (*Spergula arvensis* L.), couch grass (*Elytrigia repens* L.), and annual meadow grass (*Poa annua* L.). Docks (*Rumex obtusifolius* ssp.) and dandelion (*Taraxacum officinalis* L.) were major weeds in the Grass system plots, and large plants of docks were removed by hand.

No significant differences were obtained between the yields in fertilized treatments in 2011 (oats), 2012 (ryegrass), or 2013 (spring wheat) in the Arable system (Supplementary Information, Table 2). The average DM production across all years and treatments was 0.79 kg m^{-2}.

The total DM yields of grass-clover ley were significantly lower in the non-fertilized control than in fertilized treatments across all years. Across all years of ley, and summarized for cuts 1 and 2, ADSH and USH gave higher yields than ADSL and USL (Fig. 6).

The relationship between total DM yield and ley age (production year) differed significantly between treatments ($p<0.001$) (Fig. 7). The total DM yield in the control decreased by 35% in the 4th year compared to the 1st year ley. There was no significant effect of the manure rate (see table in Fig. 7). In older leys, the effect of manure rate was more evident, with ADSH producing the highest yields in 3rd and 4th year ley, but the difference between ADSH and USH was not statistically significant. The decrease in ley yield over time was much lower for the fertilized treatments than for the control, and across fertilized treatments, the decline in ley yield from years 2 to 4 was not statistically significant.

The proportions of grass and clover were significantly affected by the application rate ($p=0.029$; see Supplementary Material Fig. 4), but not by AD ($p=0.497$). With increasing manure rate, the proportion of grass increased, and the proportion of clover decreased significantly. The proportion of clover (32%) was highest in the control, although it did not significantly differ from the low application rate

Fig. 3 Changes in soil pH, organic matter (LOI), and AL-extractable nutrients in a long-term field experiment established in 2011 with an unfertilized control compared with application of untreated slurry (US) and anaerobically digested slurry (ADS) at two rates low (L) and high (H), in two systems, grass and arable. The Arable system was converted to a Grass system in 2014. Inserted tables indicate the *p*-values for the regression coefficients compared to the reference values (control) for samples taken in spring of 2011, 2013, 2016, and 2018
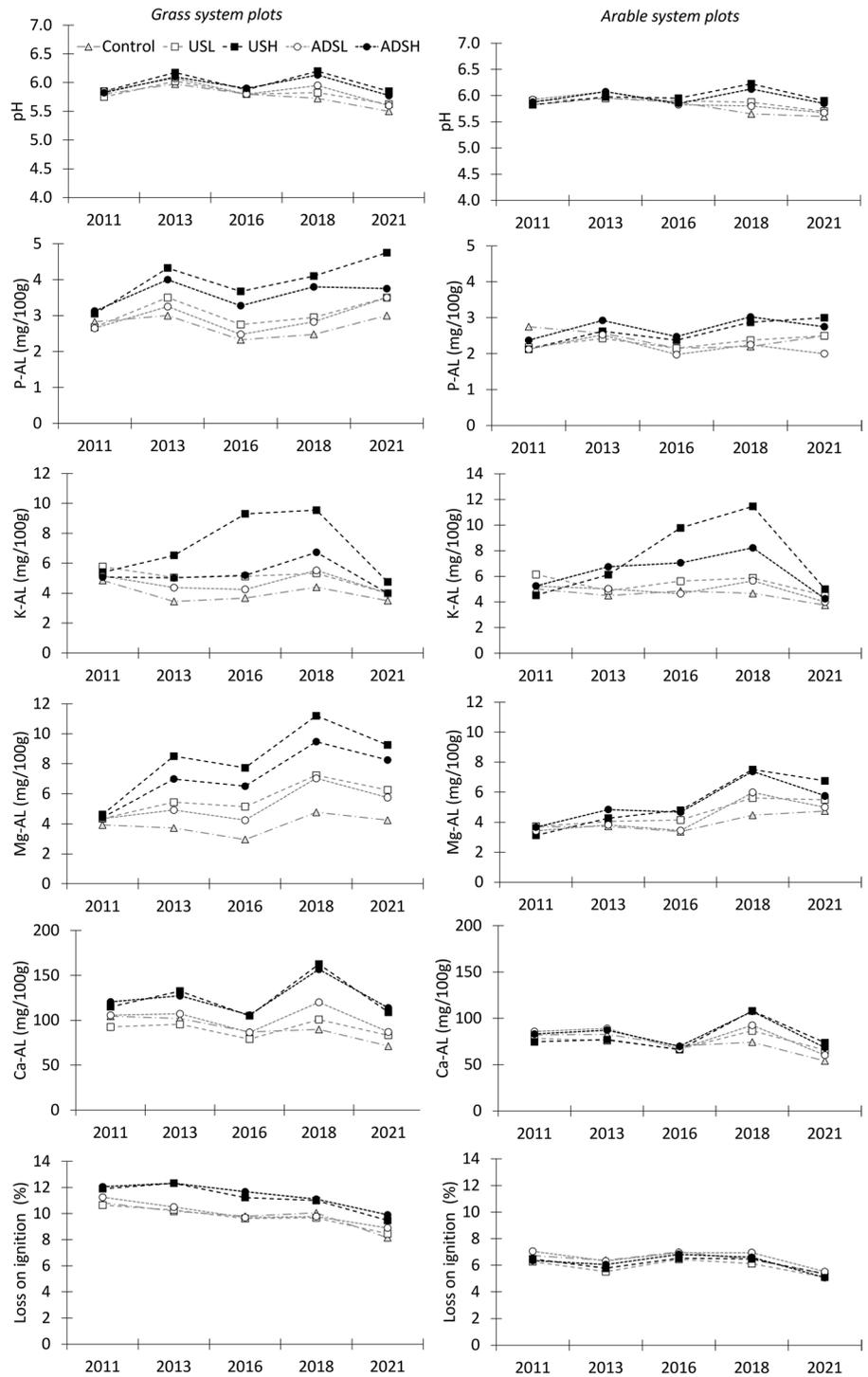
Grass system plots

Treat.	pH	P-AL	K-AL	Mg-AL	Ca-AL	LOI
USL	0.492	0.279	0.971	0.128	0.370	0.900
USH	0.050	**0.011**	**0.002**	**0.000**	**0.025**	0.863
ADSL	0.480	0.378	0.702	0.201	0.283	0.727
ADSH	0.067	0.069	0.241	**0.007**	0.098	0.609

Arable system plots

Treat.	pH	P-AL	K-AL	Mg-AL	Ca-AL	LOI
USL	0.229	0.128	0.843	0.330	0.279	0.697
USH	**0.000**	**0.011**	**0.000**	**0.004**	**0.010**	0.622
ADSL	0.851	0.244	0.724	0.193	0.519	0.780
ADSH	**0.008**	**0.028**	**0.010**	**0.029**	0.063	0.392
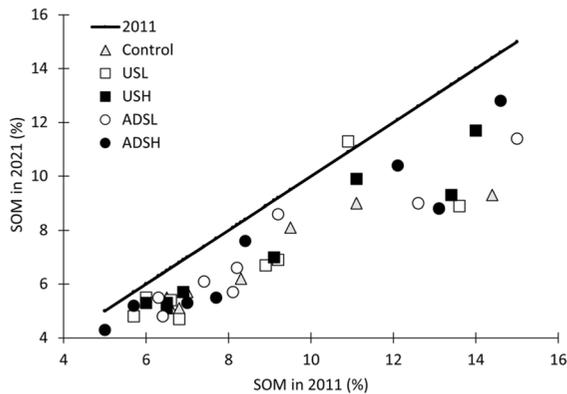
Fig. 4 Topsoil (0–20 cm) SOM concentrations (LOI, %) in the treatment plots in 2011 (line) and in 2021 (symbols)

treatments. On average, the ADSL treatment had the greatest proportion of clover, and USH the lowest across all years and cuts. Clover yield (kg m^{-2}) was significantly higher in the 2nd than the 1st year, with a significant sharp decline in the 4th year (Fig. 8). The yield was higher in the first than in the second cut; however, this difference disappeared in the 4th year leys, when the clover yields were comparable in both cuts. Within the same production year (ley age), the mean clover yields (kg DM m^{-2}) of the fertilized treatments did not differ significantly from the control (Fig. 8b).

Nutrient budgets

The average nutrient budgets, recorded as nitrogen (N), phosphorus (P), and potassium (K) applied with manure minus N, P, and K removed by the harvested crop (divided by 11 years for the Grass system and 7 years for the Arable system), were negative for K for almost all treatments (Fig. 9). The only exceptions were the treatments with high application of slurry and digestate in the Arable system. For P, the deficits were much smaller, and the treatments with high application showed a slight surplus of 10 kg of P ha^{-1} year^{-1}. For N and P, the input values do not include the struvite applied to all plots (including the control) in 2018, equaling to 18 kg N ha^{-1} and 40 kg P ha^{-1}. The total P deficits across the whole study period ranged from 2.8 kg P ha^{-1} for ADSL in the Arable system to 86 kg P ha^{-1} for the control in the Grass system. Hence, the application of 40 kg P ha^{-1} was clearly significant as compared with the accumulated P deficits.

For N, deficits were found for the control and the treatments with low application rates, whereas there were surpluses for the treatments with high application rates. The average value of topsoil N across the experimental site in 2010 was 0.16% of soil DM in the Arable system and 0.32% in the Grass system plots (Løes et al. 2013). The N deficit in the controls, where the N fixation was likely low because legumes
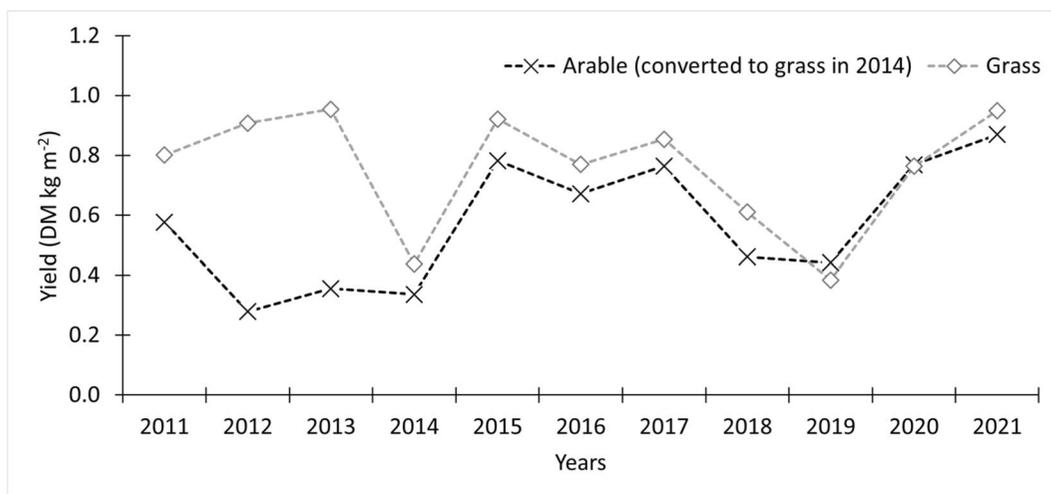

Fig. 5 Total mean DM yields across treatments in a field experiment established in 2011, divided in an Arable and a Grass system. The Arable field was converted to Grass in 2014. In the Grass system, perennial ley is re-established every 5th year with cereals as a cover crop, harvested for green fodder
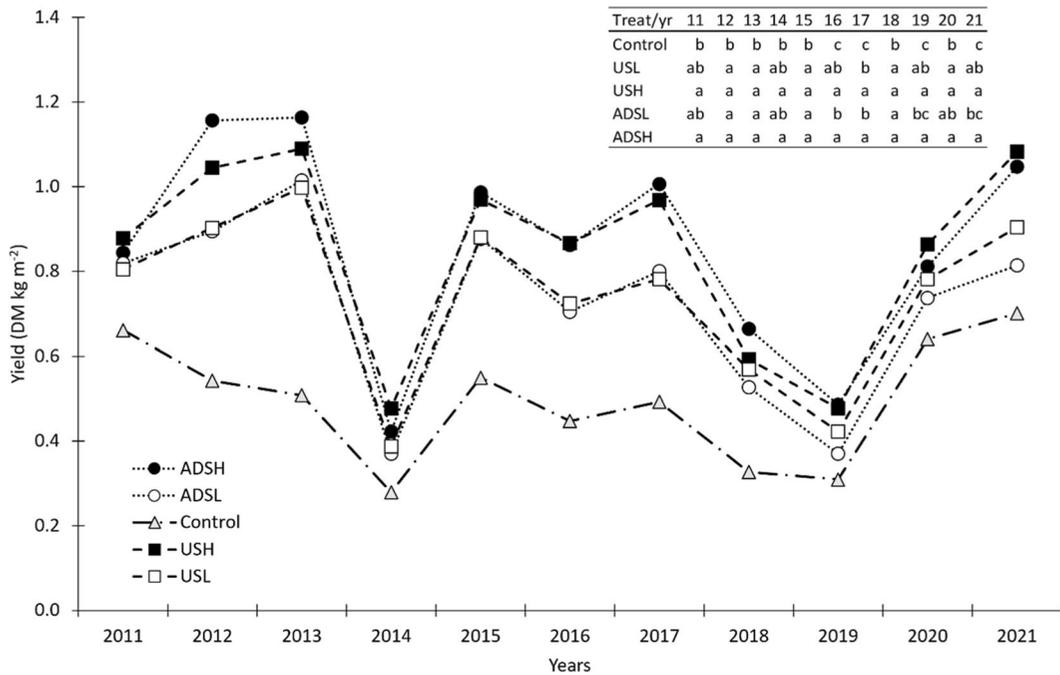
Fig. 6 Summarized mean DM yields (sum of 1st and 2nd cuts) of grass-clover ley in a field experiment 2011–2021 where an unfertilized control is compared with application of non-digested slurry (US) and anaerobically digested dairy cattle slurry (ADS) in two rates low (L) and high (H). Yields from nine growing seasons in the Grass system, six growing seasons in the Arable System, and 2 re-establishment years (2014 and 2019) are included in the values. Inserted table: Means within year with different letters are significantly different (*P*<0.05)

Fig. 7 Total mean DM yields (sum of 1st and 2nd cuts) of grass-clover ley in each production year in a field experiment established in 2011 where an unfertilized control is compared with application of non-digested slurry (US) and anaerobically digested slurry (ADS) in two rates low (L) and high (H). Yields from 9 growing seasons in the Grass system and 6 growing seasons in the Arable system (converted to grass in 2014) are included in the values. Inserted table: Means within production year (ley age) with different letters are significantly different (*P*<0.05). Lines between the dots were added to facilitate the visualization of the results
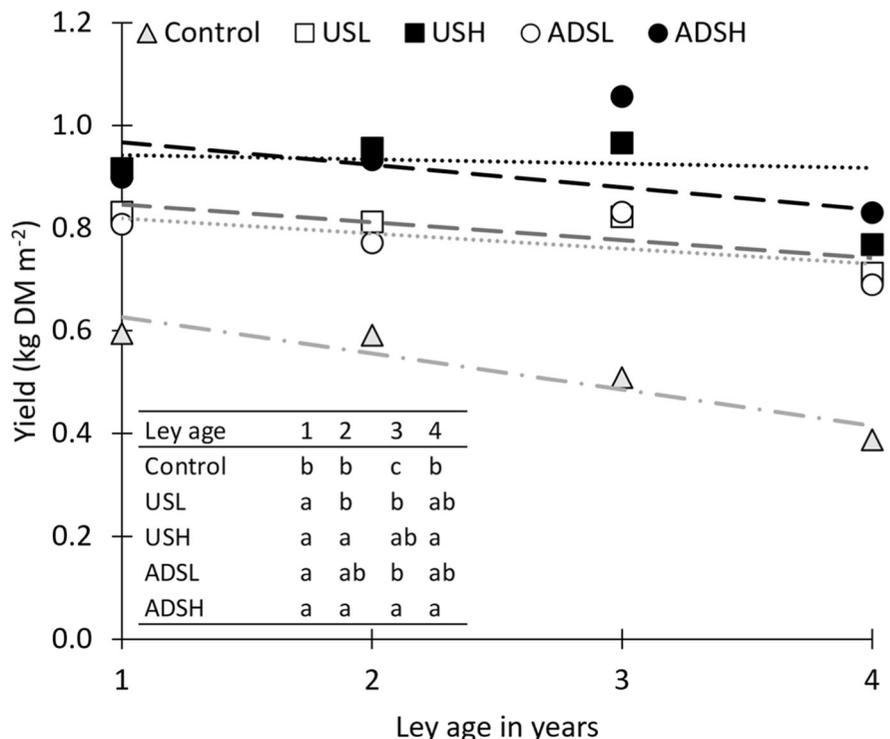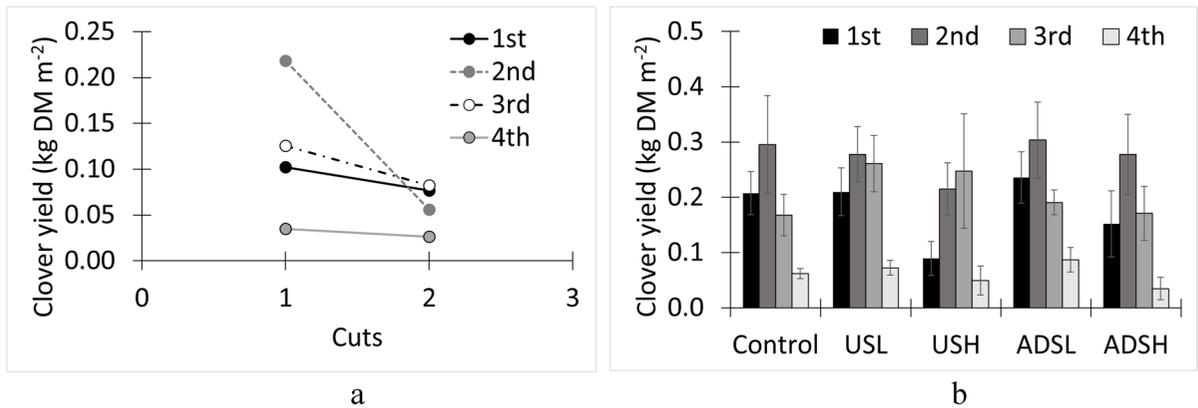
Fig. 8 **a** Clover yield in the 1st and 2nd cuts in ley of increasing age. **b** Clover yield across the treatments and control in each ley of increasing age. Yields from nine growing seasons in the Grass system and six growing seasons in the Arable system are included in the values. Error bars indicate the standard errors

Fig. 9 Nutrient (NPK) budgets (inputs minus outputs, kg ha^{-1} year^{-1}) for fertilized treatments with low (L) and high (H) applications of (AD) digested and (U) non-digested dairy cow slurry (S) compared with an unfertilized control (CON). Average values for observations 2011–2021 for the Grass system and 2015–2021 for the Arable system
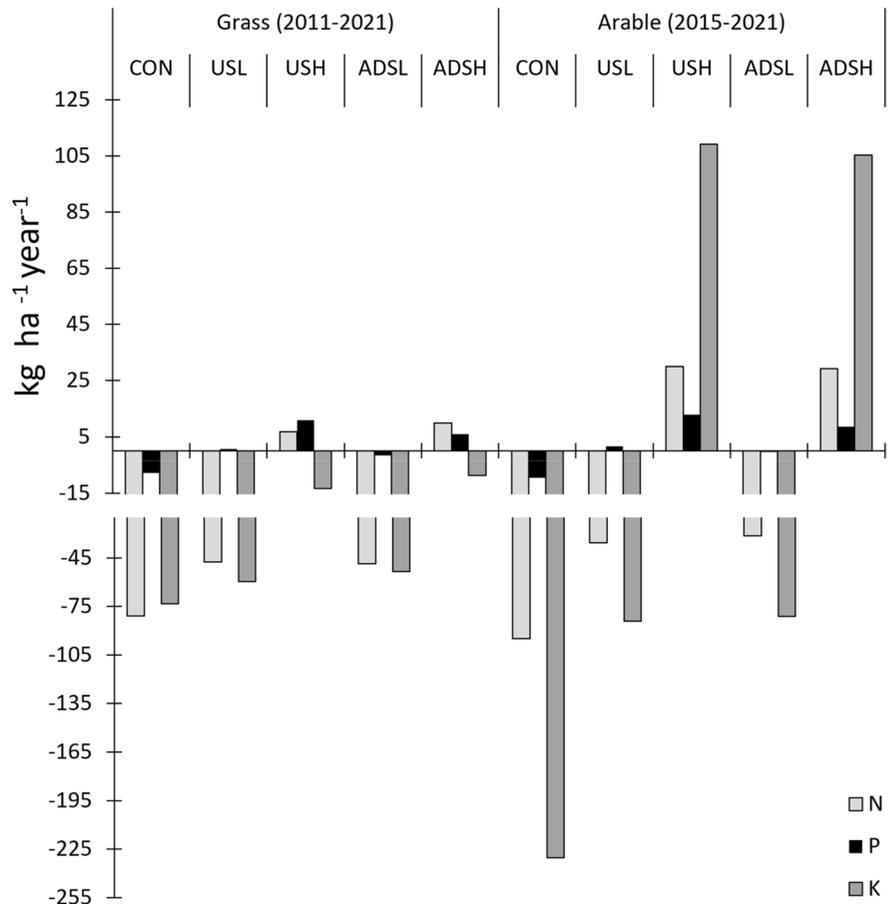
need nutrients for this process, was not much different in the two systems, despite initially lower total N values in the soil in the Arable system plots. The proportion of clover in the canopy was higher in the low-rate than in the high-rate treatments, especially for digested slurry (Supplementary Material, Fig. 1).

Discussion

Effect of AD in the slurry

The measured parameters regarding pH, ammonium to N total, changes in dry matter content, and P and K concentrations are in the range of data reported in the literature (Möller and Müller 2012); therefore, the differences between US and ADS can be considered as representative.

General decline of soil organic matter

The decline in SOM in all the ADS and US treatments contrasts with our expectations that long-term application of ADS and US would increase the SOM concentrations. Indeed, the content of dry matter in the US was higher than in the ADS, and US had a higher content of both cold (CWC) and hot water extractable carbon (HWC) than ADS (Johansen et al. 2015; Pommeresche and Løes 2017). However, in our field study, there was no effect of AD, nor of the manure application rate, on SOM concentrations. Similar results regarding SOM were found by Möller (2009) and by Barłóg et al. (2020) after 4 years of treatment with digested (cattle slurry with maize silage and haylage) vs. non-digested cattle slurry. Thomsen et al. (2013) suggested that in a longer-term perspective, the retention of plant-derived C in soil is similar whether the initial turnover of plant biomass occurs in the soil, in the digestive tract of ruminants, in an anaerobic reactor, or in a combination of the latter two. Our results seem to confirm this suggestion, since no difference in the SOM storage potential was found between ADS and US. Even compared with the unfertilized control, there was no indication of higher SOM concentrations after 10 years in the fertilized treatments.

The lack of difference in SOM storage between the fertilized treatments and non-fertilized control suggests that other factors than slurry quality and quantity influence SOM accumulation. The addition of readily decomposable organic matter in slurry to the soil has been shown to increase the activity of soil microorganisms, resulting in a positive priming effect, i.e. increased decomposition of intrinsic SOM (Bol et al. 2003). These authors observed a strong positive priming effect of slurry in a lab study with soil rich in intrinsic SOM. Using ^{13}C-labelled ADS, Coban et al. (2015) estimated that 73% of the SOM-derived CO_2 release was from increased mineralization of the native soil matter after slurry amendment. When positive priming occurs, even high application rates of ADS and US to soils may not result in SOM accumulation. In our study, the application of ADS and US seemed to induce a loss of organic matter in the soil, especially in plots with initial high native organic matter content. However, there was also observed a decline on SOM in the control plots, so other factors have likely contributed.

The more evident loss in the soil with high SOM could also have been promoted by decomposition of grass-clover residues in the sward and root system, since the yield levels in some years were higher in the Grass system (Fig. 5). In a laboratory experiment, grass-clover residues applied to soil significantly increased microbial biomass when compared with adding untreated or anaerobically digested cattle slurry, leading to 10 times higher loss of C as CO_2 emissions (Johansen et al. 2013b).

Further, SOM translocation to deeper soil layers, tillage, drainage, and climate change may also have contributed to an overall decomposition of intrinsic and applied SOM. US and especially ADS (due to a reduction in dry matter content and viscosity) may have percolated through the upper 0–20 cm of soil and accumulated in deeper layers. Barłóg et al. (2020) observed a slight (but non-significant) increase in the soil organic C stocks in the 30–60 cm soil layer depth, in a 4-year field experiment with the application of slurry and digestate (feedstock was slurry and plant materials), compared with a non-amended control and a mineral (NPK) fertilizer treatment. They speculated that with a longer time, more organic C would have been transported to deeper layers. In the present experiment, ploughing of the perennial ley may have increased SOM decomposition by break-down of soil aggregates and exposing SOM to decomposer organisms, as shown by the significant increases in yields in the unfertilized control after re-establishment of

the ley. Another possible contributing factor was the effect of drainage. The field, a forest and peatland, was turned into an agriculture land in 1970, and the drainage was systematically renovated in 1991. The main drainage is located diagonally across the experimental field, and suction drains connected to the main drainage line affect almost all the experimental plots, decreasing SOM. Nevertheless, SOM concentrations seem to have stabilized since 2009 (Supplementary Material, Fig. 2). Furthermore, changing climatic conditions may also have contributed to an overall decline in the SOM concentration (Riley and Bakkegard 2006). Over the last 30 years in Tingvoll, the average air temperature during the growing season increased from about 10 to about 11 °C (Supplementary Material, Fig. 3), which may have led to higher SOM turnover rates.

Soil fertility and yield levels

Long-term soil fertility, as indicated by AL-extractable nutrients, was positively affected by manure application rate, but not by AD of slurry. It may seem surprising that the highest concentrations of AL-extractable soil nutrients were found for a high rate of US in both systems when, on average, AD slurry had somewhat higher concentrations of P, K, and Mg (Table 1). However, ADS also had higher concentrations of total N, and since the total N concentration was used to determine the actual application of slurry, somewhat less P, K, and Mg were applied with ADS. The amount of nutrients in ADS as compared with US (Table 1) was + 30% for total N (when US = 100), but lower for K (10%), Mg (15%), and P (18%).

Regular application of animal manure to agricultural soil will increase the soil fertility, as shown here not only by higher concentrations of soil nutrients but also by higher yields in years when no manure was applied in the previous season(s). In this study, residual effects were planned to be studied in the years of re-establishing the ley, but since no manure was available in 2020, the differences in yield levels of the 1st year ley in 2020 were mainly due to previous fertilization. In 2021, with manure application, the yield levels were again higher, and the effect of manure rate was more evident. Higher yields in the control treatment in 2020 and 2021 than in previous years is a surprising result, but the increase from 2019 to 2020 may be due to soil tillage in 2019 when the ley

was re-established, causing mineralization of SOM. A comparable increase from re-establishment to the 1st year of ley was seen in the control treatment from 2014 to 2015. An increase in yield even in the control without manure application is expected as grass-clover leys are characterized by a particular nutrient cycle. Grass-clover ley nutrient cycle involves a build-up during the ley phase and a rapid decomposition after ley termination. The build-up of SOM is mainly concentrated in the upper layer due to roots and leaves and stubble deposits, while the mineralization of the nutrients increases after grass-clover ley ploughing.

The residual effect of previously applied manure was visible, leading to higher yields than the control. Yet, the yields did not differ between US vs. ADS. As expected, higher yields with higher application rates were observed for both types of manure. The residual effect of the manure is due to the different mineralization rates of organic compounds present in the manure making nutrients available for several years (Hansen et al. 2005). AD increases the proportion of mineral N in the manure and hence may be prone to losses of N if the slurry is not mixed into field soil. However, in the present study, the proportion of mineral N in the US was also quite high. In a study with digested and non-digested slurry from Tingvoll farm, Kvande and Løes (2014) recorded higher yields with ADS than with US. The fertilizers were applied in pots with barley as a test crop, with soil from an area adjacent to the experimental site described in this paper. Fertilizers were then immediately covered with 5 cm soil. This demonstrates the importance of incorporating the fertilizer in soil for efficient utilization of N. In our field study, slurry and digestate were manually applied by cans to the sward with no incorporation, and this may have led to N losses. Higher losses of N_2O from ADS than US were in fact observed in the Arable system in 2012 (Serikstad et al. 2013), indicating that slightly more mineral N in ADS was lost before plants could utilize it.

In the present study, ADS and US produced comparable yields and higher yields with higher application rates. The yields across the age of ley followed a normal pattern, with higher yields in the 2nd and 3rd ley year than in the 1st year after re-establishment and somewhat lower yields in the 4th year. The proportion of clover was highest in the 2nd ley year and then declined. The need for re-establishing clover and

productive grasses, and not least, to control perennial weeds are the most important reasons for ploughing the ley. Some large plants of docks (*Rumex obtusifolius* ssp.) were removed by hand in the field experiment, but this did not affect the general growth pattern, and the decline in clover with ley age was clearly demonstrated. The proportion of weeds increased until the 3rd production year of ley in the treatments with the application of non-digested slurry and digested slurry. The similarity in the proportion of clover between the ADS and US treatments in our study differs from the study by Walsh et al. (2018), who found more clover in soil treated with US than ADS. The application of N fertilizers, including cattle slurry, stimulates grass growth, which is a stronger competitor for nutrients than clover (Steinshamn 2001). Consequently, the clover content, and sometimes the clover yields, decrease with fertilization. However, differences in the average yield of clover between treatments and control were not found. A high proportion of clover is positive, since the presence of clover in grass swards and silage is advantageous for ruminant diets (Johansen et al. 2018).

Nutrient budgets

Higher manure application rate led to a surplus of N, while a deficit was observed in the control and the treatments with low application rates. N fixation in legumes is known to decline with higher N supply from fertilizers (e.g. Nesheim and Øyen 1994). In that study, the proportion of clover, and also the N fixation, increased with lower manure rate. Higher was the concentration of N in the herbage (grass+clover), higher was the N output (Nesheim and Øyen 1994). In the present study, in the high-rate treatments with less clover, not all N input was converted to aboveground plant material.

Even in the treatments with low application rate, the total P deficit was minimal, 18 kg P ha^{-1}. The concentrations of AL-extractable P were medium to low on the experimental site, and the low deficit (applied minus removed in yields) may indicate that the soil was not able to deliver much P except from what was available in the manure. For K, there was a deficit in all treatments in the Grass system and in the treatments with low application rate in the Arable system. This is likely due to that the experimental soil has a higher capacity for delivering K to the

crop plants than it has for P. Initial concentrations of AL-extractable K on the experimental field were low (Løes et al. 2013), but the reserves of acid-soluble K were medium-high to very high. The higher surplus of K in treatments with high application rate in the Arable system plots, which have received the same fertilization as the Grass system plots since 2015, was not an artefact of the different time periods included in the data series (11 years in the Grass system vs. 7 years in the Arable system). The exclusion of 2011–2014 from the Grass system data series did not notably change the values of the average K budgets of those treatments. Hence, the reason for somewhat larger K surplus in high-rate fertilized treatments in the Arable system is likely somewhat lower yields in that system during 2015–2018.

K is a nutrient element of particular importance on grassland. In intensive dairy farming, farm-level surpluses of K may arise. Organic farming, however, is characterized by limitations in the input of nutrient sources and quantities. Øgaard and Hansen (2010) reported a K deficit on most of the 26 organic grassland farms that were investigated, which they attributed to the generally low K supply. A deficiency of K may negatively impact the yields and reduce the ability of legumes to fixate N on the long term (Øgaard and Hansen 2010). Yet, in field experiments on low K soil, Øgaard and Hansen (2010) did not observe a significant effect of K fertilization on yield, even when K concentration in herbage without K application was low. They attributed the lack of response of K input to the low amounts of crop-available N (Øgaard and Hansen 2010). In the present study, long-term application of high rates of manure showed a surplus in N. Even if soil concentrations of K are much higher than for P at the experimental site, the results of this study show that the current manure application and crop production mines K from the soil.

Conclusions

The present study confirmed that anaerobic digestion reduces the content of organic matter in dairy cow slurry. However, long-term application of ADS vs. US to soil had similar effects on SOM, soil fertility, and crop yields. This may be due to the lack of incorporation of the applied digestate in the soil. Soil pH and nutrient concentrations increased with manure

application and did not vary between the two types of slurry. Organic fertilizers did not increase SOM, and a decline in SOM was observed in all treatments; this may be because of drainage, initial high native organic matter content, and higher temperatures with changing climatic conditions.

This result highlights that in Nordic countries, where soils may be naturally rich in SOM especially in humid areas, it may be a challenge to even maintain the content of organic matter in soil, especially with the foreseen climate changes. The reduction in SOM, even in this farming system, which could have conserved and possibly increased SOM, is an important result which deserves further study.

Increasing application rates of ADS and US gave higher yields, showing that overall growth was N limited on the site. An increase of external inputs may increase the overall productivity of the farming system on this site. Yields were higher in the 2nd and 3rd ley year than in the 1st year after re-establishment and in the 4th year of ley. Higher rates of manure reduced the proportion of clover, especially with ADS.

The most important result of the present study is that the benefits of extracting energy from the animal manure by anaerobic digestion did not compromise grassland productivity and soil fertility in an organically managed dairy production system. To achieve higher crop yields with an increased proportion of mineral N in the slurry, it is likely required to incorporate it into the soil rather than just adding it on top of the soil.

Author contributions Tatiana F. Rittl wrote the main manuscript text and prepared figures and tables. Reidun Pommeresche, Anders Johansen, Håvard Steinshamn, and Hugh Riley revised and edited the manuscript. Anne-Kristin Løes established the field experiment, revised and edited the manuscript, and discussed all results and interpretations thoroughly with the main author.

Declarations

Competing interests The authors declare no competing interests.

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