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Renovation of grasslands with grass and white clover – Effects on yield and carbon sequestration☆

R. Loges[a], I. Vogeler[a,b,*], C. Kluß[a], M. Hasler[c], F. Taube[b,d]

[a] Grass Forage Science/Organic Agriculture, Christian Albrechts University, Kiel 24118, Germany
[b] Department of Agroecology, Aarhus University, Tjele 8830, Denmark
[c] Lehrfach Variationsstatistik, Christian Albrechts University, Kiel 24118, Germany
[d] Grass Based Dairy Systems, Animal Production Systems Group, Wageningen University, Wageningen 6708, the Netherlands

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ABSTRACT

There is a pressing need to support farmers' decisions on grassland renovation, based on sound scientific evidence regarding its effects on productivity, herbage quality and soil organic carbon stocks. To quantify these effects a long-term experiment with grass/white clover swards was set up at the Lindhof research farm in Northern Germany in 1995. Treatments included control plots of undisturbed grassland as well as 10 grassland renovations starting after 10 (2005) years and repeated on different plots 10 times until 2019, and without and with addition of slurry (equivalent to 240 kg N ha^{-1} yr^{-1}). Grassland renovation resulted in a significant drop in biomass production in the first year after renovation, and the slightly higher yields in the third year after renovation could not compensate for this drop. Yields from the year of renovation to three years afterwards were generally lower, with average reductions over the 4-year periods of 2600 kg DM ha^{-1} for the treatments without slurry and 1500 kg DM ha^{-1} for the slurry treatments. Differences in herbage quality between permanent and renovated grassland were negligible and generally not statistically significant. The soil organic carbon showed a rapid and significant drop in the year of renovation, followed by a gradual increase. Without slurry application, the initial levels of soil organic carbon stocks could not be reached even after a period of 18 years following renovation, and with slurry application, it took about 8–10 years. Deep ploughing to a depth of 30 cm did not increase the SOC stocks compared with the undisturbed permanent grasslands, suggesting that the topsoil has not reached the carbon saturation level. We conclude that maintaining productivity of permanent grassland without renovation measures is a promising way towards yield stability and natural climate solutions.

1. Introduction

Cultivated grasslands are an important low-cost feed base for livestock production systems, especially for so called grassland-based systems with low external inputs (Taube et al., 2013; Vibart et al., 2016). They are also an important part of the cultural landscape in north-western (NW) Europe (Eriksson and Cousins, 2014), comprising about 15% of the land area. Besides these, grasslands are important agro-ecosystems, delivering various ecosystem services, including sequestration of atmospheric carbon (C) in soils and its associated benefits on water and nutrient use efficiencies (Magdoff and Van, 2000), as well as a reduced environmental carbon footprint of milk (Schönbach et al., 2012; Vellinga et al., 2011). Globally, grasslands store about 30% of the soil carbon stocks (Janzen, 2005), and are thus important for the global carbon cycle and for reducing atmospheric carbon dioxide concentrations (Soussana et al., 2010).

Current agricultural intensification and land use change from grasslands to arable farming in temperate regions rely on increasing production via high external inputs of fertilisers and energy. This specialization promotes less diversified and highly specialized production systems, and has led to a decoupling of local nutrient cycles between animal and crop production systems (van der Wiel et al., 2021). Furthermore, benefits from grass and legume-based mixtures on soil C and N levels via soil carbon sequestration and N fixation are being displaced, which has negative effect on the SOC stocks, thereby contributing to climate change. Apart from the large potential C sink of

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* Corresponding author at: Grass Forage Science/Organic Agriculture, Christian Albrechts University, Kiel 24118, Germany.
E-mail address: iris.vogeler@agro.au.dk (I. Vogeler).

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grasslands, greenhouse gas emissions and N-leaching losses from these systems are low, which indicates effective N-cycling in such systems (Schmeer et al., 2014).

To maintain or increase the productivity and nutritive quality of the grasslands, cultivated grasslands are occasionally renovated by reseeding (Glassey et al., 2010; Hopkins et al., 1990; Stevens and Knowles, 2011), which in NW Europe is typically done every 5–10 years (Elias et al., 2023; Schils et al., 2007; Velthof et al., 2010). Numerous studies have been conducted to assess the effect of grassland renovation on yield responses. However, while some studies have shown yield advantages in the first two years of up to 30% (Kayser et al., 2018), other studies have shown either no effects or production losses in the year of sward disturbance and reseeding (Nevens et al., 2002; Velthof et al., 2010). These contrary results can be due to management practices such as cutting, grazing, fertilisation, weed control, over-sowing, cleaning cuts (Taube and Conijn, 2007), the botanical composition of the sward (Søegaard et al., 2007), nitrogen fixation by legumes, the degree of sward and soil disturbance during renovation (Kayser et al., 2018), and the timing of renovation and loss of production in spring (Elsaesser, 2012).

Prior to reseeding grasslands are often ploughed to even out the soil surface and thereby avoid soil contamination and poor silage fermentation, breaking up compacted areas, and having a clean firm seedbed for optimal seed germination and establishment. The ploughing exposes aggregate-protected SOC to microbial activity, and this together with incorporation of a high amount of easy decomposable plant biomass results in the release of high amounts of carbon dioxide and other greenhouse gases (GHGs) into the atmosphere, contributing to global warming (Reinsch et al., 2018; Willems et al., 2011). To mitigate these, at least short-term, SOC losses following grassland renovation, the use of no-till during grassland renovation has also been promoted. De Los Rios et al. (2022) have demonstrated that no-till can conserve SOC stocks during renovation, despite a lower C input from roots after renovation. Other studies have shown that the reduced biomass production after grassland renovation, together with increased soil mineralization rates in the year of ploughing (Drewer et al., 2017) triggers a temporary decline in soil carbon stock, with losses of up to 25% in the topsoil within the first years after renovation (Kayser et al., 2018; Necpálová et al., 2014). The increase in SOC mineralisation after grassland renovation causes a flush of soil organic nitrogen (N) mineralization (Eriksen, 2001). As grassland renovation is generally done in autumn to avoid the loss of forage during the high production period in spring, this flush in mineralisation can increase N losses via leaching and gaseous emissions (Reinsch et al., 2018; Seidel et al., 2009). For this reason, autumn grassland renovation is ruled out in some countries, like the Netherlands (Conijn and Taube, 2004).

Carbon inputs into grassland soils depend on the amounts of crop residues, above (AGB) and belowground biomass (BGB), left in the field. In temperate grasslands, where most of the AGB is removed from the field, the accumulated SOC is mainly derived from the BGB (Chen et al., 2016a). The importance of root derived C for carbon sequestration is further attributable to the much higher mean residence time compared with AGB (Poeplau et al., 2021). Other C inputs in grasslands are through the return of animal excreta, either directly as under grazing or through application solid or liquid manure from external sources. In addition, application of manures can induce indirect C input via an increase in the net primary production due to addition of nutrients (N and P), which increases the amount of crop residues and potential for soil carbon sequestration. However, while application of solid manure to grasslands has been shown to increase soil carbon stocks, the effect of slurry addition on soil carbon is less known. Studies have shown that slurry application can even decrease soil carbon stocks due to its high fraction of rapidly decomposable organic C, as well as through priming effects triggered by the addition of N, P and labile C (Angers et al., 2010; Kuzyakov et al., 2000).

While grassland renovation is likely to reduce SOC stocks in the short-term, the absence of tillage in permanent grasslands means that most of the carbon is stored in the top 15 cm of the soil, due to the high input of above and below ground plant residues (Poeplau, 2021), and an exponential decrease in the root distribution with depth (Crush et al., 2005). Thus, the upper soil layers have often accumulated carbon to their maximum ability or saturation point regarding the bonding of SOC to the fine mineral particles, and have reached a steady-state (Six et al., 2002; Wiesmeier et al., 2014). In contrast, deeper soil layers generally have not reached the saturation point, due to lower inputs from plant residues, often higher clay and fine silt fractions, and infrequent tillage operations, which are generally limited to the top 10–15 cm (McNally et al., 2015, 2017). In a grassland chrono-sequence study on commercial dairy farms in England, Elias et al. (2023) found that topsoils (0–15 cm) of coarse textured grasslands were close to their C saturation irrespective of grassland age, while loamy textured soils reached saturation about 10 years after renovation, and fine-textured topsoils, and subsoils (15–30 cm) of all textures were under saturated. This suggests that deeper tillage can potentially increase the carbon sequestration due to C input into deeper soil layers. In line with this, Pereira et al. (2017) found that deeper tillage to 25 cm decreased the carbon stocks in the top layer (0–5 cm), but this was offset by increases in lower layers, resulting in an overall increase. Similarly, based on data from a literature review, Madigan et al. (2022) suggested that full inversion tillage (FIT) to a depth of 30 cm during grassland renovation could significantly increase carbon sequestration potentials in grassland in Ireland, due to bringing C-deficient subsoil to the surface and burying of carbon-rich topsoil. However, they also highlighted the need for long-term studies to assess potential soil carbon stock developments, changes in emissions of other GHG, especially N_2O, and above- and below-ground dry matter production following FIT and grassland renovation.

The benefits of grassland renovation have not been quantified sufficiently, as was highlighted during the EGF meeting in 2007 (De Vliegher and Carlier, 2007). Only a few studies have compared productivity, herbage quality and SOC between renovated and undisturbed permanent grasslands. Thus, there is a pressing need to support farmers' decisions on grassland renovation. This should be informed by long-term studies, which take account of environmental conditions, such as summer draughts and winter colds during and after renovation, and capture long lasting effects on C stocks.

The objective of our study was to (i) to quantify the effects of grassland renovation on biomass production and quality and (ii) to quantify changes in soil organic carbon stocks. For this, a long-term experiment with a grass/clover sward was set up at the Lindhof research farm in Northern Germany in 1995. Treatments included ploughing and reseeding after 10 (2005) to 24 years (2019), and with and without addition of slurry at a rate equivalent to 240 kg N ha^{-1} yr^{-1}, as well as a control without grassland renovation. The hypothesis were that grassland renovation results in (i) an initial rapid drop in pasture production due an initial increased resource input into belowground biomass followed by an increase above that of a permanent grassland, (ii) an initial rapid drop in SOC due to increased respiration, followed by steady increase in the following years, with a higher increase with the addition of slurry due to both directly inputs via slurry, and indirectly via a higher productivity and belowground biomass allocation, and (iii) long term SOC above that of permanent grassland due to the ploughing which mixes carbon-rich topsoil with C-deficient subsoil and thereby increases the OC accumulation capacity.

2. Materials and methods

2.1. Experimental site and setup

The experiment was carried out at the Lindhof research farm (N 54°27 E 9°57) in Northern Germany. Since 1993 the farm is under organic management. The soil is a loam, classified as an Eutric Luvisol composed of 11% clay, 29% silt and 60% sand in the first 30 cm soil depth. The site has a mean air temperature of 8.7°C, and a mean annual precipitation of 785 mm. The historical management of the experimental field before conversion to permanent grassland was intensive conventional arable cropping with a 5- year crop rotation consisting of silage maize, winter wheat, winter barley, winter oilseed rape and winter wheat until 1993. The fields was fertilised on average with 240 kg N/ha/year, half of it applied as mineral N and half as cattle slurry. Since 1993, the experimental farm is managed according to the guidelines of the German organic grower's association "Bioland" prohibiting the use of chemical fertilizers and pesticides. In 1994, a grass-clover mixture was undersown into the first organically managed cereal crop (winter wheat) and it was subsequently managed as grassland. The seed mixture for establishment in 1994 was identical to the one later used for renovation (30 kg ha^{-1} of a grass-clover commercial seed mixture, with Lolium perenne (70%), Poa pratensis (12%), Phleum pratense (12%) and Trifolium repens (6%). The grass was initially managed in a mixed system with two to three grazing cycles combined with two silage cuts per year. Since 2005, the swards were only cut for silage, with generally four cuts per year, and the experiment with grassland renovation started. One of the treatments, the control, remained under permanent grassland. In the other treatments the grasslands were renovated. For this, the swards were rotovated shortly after the third cut in late August to a depth of 5 cm using a Howard HR30–300 rotavator in order to disconnect the sward from the root system. Depending on weather conditions, approximately a week later, the plots were ploughed to a soil depth of 30 cm using a Brix 16 in. reversible two furrow mouldboard plough equipped with skimmers. The plots were then re-sown. This renovation was done, in separate treatments once yearly between 2005 and 2010, and between 2016 and 2019 (Fig. 1).

Every 4–5 years, the permanent grassland was harrowed with a chain harrow to pulls out thatch, moss and shallow-rooted weeds, and then oversown with the same grass mixture as the renovated treatments, but using only a third of the seed rate (12 kg ha^{-1}). An additional treatment included slurry-based N fertilisation, with either no slurry, or slurry application (using surface-near trailing hoses) at a rate equivalent to 240 kg N ha^{-1} yr^{-1} (referred to as 0_N and 240_N). Applications of cattle slurry were split into dressings that supplied 80 kg N ha^{-1} in April, and 60 kg N ha^{-1} after the first, 60 kg N ha^{-1} after the second and 40 kg N ha^{-1} after the third harvest date. The mean dry matter (DM) content in the applied cattle slurry was 5.7%, and the C and N in DM were 40 and 3.66%, respectively (C:N 10.9). Generally, all treatments received 45 kg P ha^{-1}, 100 kg K ha^{-1}, 24 kg Mg ha^{-1} and 68 kg S ha^{-1} every two years. Based on soil analysis additional fertilisers (P, K, Mg, S) and lime were applied to balance nutritional status of the soil in accordance with the recommendation by VDLUFA (https://www.vdlufa.de/). The treatments, permanent grassland and resown grassland were arranged in a complete randomized plot experiment with three replicates/blocks. The plot size was 6 m x 12 m. The grass was cut four times over the year (20th May, end of June, mid-August, and mid-October) with a Haldrup harvester (Haldrup, Løgstør, Denmark) to a residual height of 5 cm. Measurements for the permanent grassland started in 2005, while for the renovated grassland blocks, measurements started one year before the renovation. The dry matter was determined on subsamples dried at 59 ^{0}C. To determine the nutritional value, the dried subsamples were ground to 1 mm and NIRS (NIR-system 5000 monochromator; FOSS Silver Spring, USA) was used to determine the amounts of N and energy in terms of ME (metabolisable energy; MJ kg^{-1} DM), based on the approach from the Committee for Requirement Standards of the Society of Nutrition Physiology (Physiology, 2008). The soil carbon (0–30 cm) was determined once a year in spring based on a pooled sample from three soil samples per plot, and the soil C measured by dry combustion using a CN-Analyzer Vario Max CN Elementar, Hanau, Germany). For calculation of soil carbon stock, the soil bulk density was measured in spring (10–20 cm), with six samples per plot.

2.2. Statistical analysis

For the statistical analysis the software R (R_Core_Team, 2023) was used. The data evaluation started with the definition of appropriate statistical mixed models (Laird and Ware, 1982; Pinheiro and Bates, 2000). For the evaluation of the permanent grassland, the mixed models included Fertiliser and Year (numeric) as well as their interaction term as fixed factors. Thus, a linear trend is modelled over the years and for each fertiliser level. The block was regarded as a random factor. The residuals were assumed to be normally distributed and to be homoscedastic. These assumptions are based on a graphical residual analysis. Based on this model, a Pseudo Rˆ2 was calculated (Nakagawa and Schielzeth, 2013) and an analysis of covariances (ANCOVA) was conducted (Cochran, 1957). If appropriate, these models were simplified by excluding insignificant effects. For the evaluation of the grassland renovation, the models included Fertilizer and Treatment as well as their interaction term as fixed factors. Here, Treatment is a pseudo factor consisting of the actual factors System (Year of renovation) and Year. This pseudo factor was necessary because the actual factors System and Year were not orthogonal (Schaarschmidt and Vaas, 2009). The block (per System) was regarded as a random factor. The residuals were again assumed to be normally distributed and to be homoscedastic. Based on this model, a Pseudo Rˆ2 was calculated and an analysis of variances (ANOVA) was conducted, followed by appropriate multiple contrast

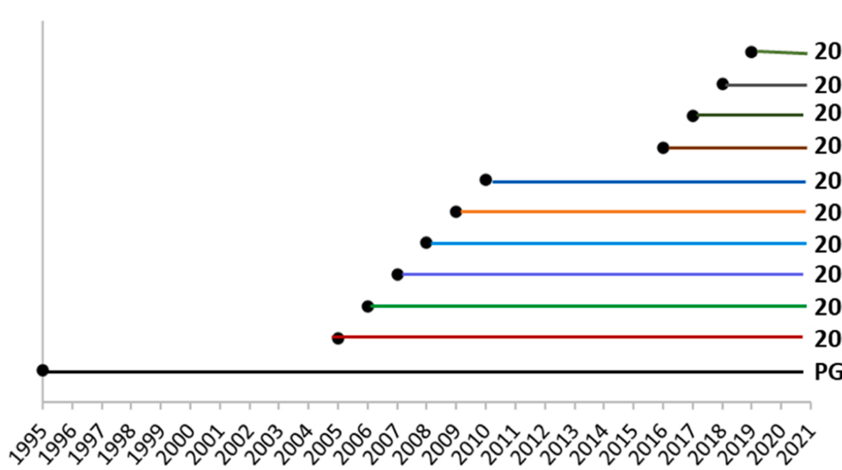

Fig. 1. Experimental setup, showing the duration of the experiment with permanent grassland (PG) and the years of grassland renovations. Each of the treatments was without and with addition of slurry at a rate of 240 kg N ha^{-1}.

tests (Bretz et al., 2016; Hothorn et al., 2008) in order to compare the several systems with the control system (permanent grass land), each for the corresponding year. Results from the ANOVA are provided in the Supplementary Material.

3. Results and discussion

3.1. Grass growth and quality of permanent grassland

The dry matter yield of the four cuts from the permanent grassland are generally, and on average over the 17 years of measurements, highest at the first cut and decline thereafter, due to maximum growth rates in spring during the reproductive development of the grass tillers, followed by lower growth rates during the subsequent vegetative stage (Fig. 2) according to Taube (1990). The addition of slurry increases the DM, with the highest increase, averaged over the years, at Cut3 (+29%), followed by Cut4 (+19%) and Cut1 (10%) and Cut3 (9%). This out-yielding at Cut3 reflects the high nitrogen use efficiency from slurry, with a precipitation in late summer allowing fast release of organic N fractions from the slurry. The overall large inter- and intra-annual fluctuations are driven by temperature, and water and nutrient availability, with common morpho-physiological and drought-related summer depression (Wingler and Hennessy, 2016).

The crude protein (CP) content of the grass increases over the year from Cut1 to Cut4 (Fig. 3), due to changes in the botanical composition, with lower percentages of white clover at Cut 1 of about 15%, and increasing to about 30–40% at Cut3 and Cut4. The difference in CP between 0_N and 240_N is a combination of N fertilisation increasing the N concentration of the ryegrass and decreasing the percentage of protein rich white clover in the sward. On average over the years, the individual cuts have almost the same CP contents, with differences of 1–3%. The ME is generally higher in Cut1 and Cut 4, but not affected by the fertilisation.

Annual DM yields show large inter-annual variability, ranging from 4.9 to 11.5 t ha^{-1} in the 0_N treatment, and from 5.9 to 12.4 t ha^{-1} in the 240_N treatment, with averages of 7.7 ha^{-1} and 9 t ha^{-1} (Fig. 4). Thus, moderate N fertilisation increased the average annual herbage yield by 1.3 t ha^{-1}, equivalent to an agronomic nitrogen use efficiency AE_N (Dobermann, 2005) of 5.5 kg DM kg^{-1} N applied as inorganic plus organic N in the slurry. Calculation on the basis of inorganic N only, as often done, would approximately double this value. However, as the grass yield is a long-term average, it is appropriate to include residual effects of organic N in the calculation of AE_N, as long-term application of organic N through cattle slurry will lead to a steady state condition, in which the annual release of N by mineralisation equals the annual net N-input (ten Berge et al., 2002). Furthermore, the effect of the reduced fraction of white clover, and thus reduced nitrogen fixation rates due to N fertilization need to be considered (Chen et al., 2016b). This would also increase the AE_N of the slurry. The annual yields show no significant reduction with year or grassland age, but a significant difference between the average over years of the 0_N and 240-N ($p = <0.0001$). This shows that deterioration of permanent grassland can be counteracted by harrowing, and oversowing every 4–5 years with new cultivars, which would slowly rejuvenate the permanent grassland.

3.2. Effect of renovation on grass growth and quality

Annual dry matter yields for grassland plots renovated at different years generally show both higher and lower yields compared with the permanent grassland (Fig. 5), with a general decrease following renovation, due to a shorter growing period for the 4th cut, and the need for the grass to develop a dense sward and rooting system during the following winter and spring (Chen et al., 2016b). For the evaluation of grassland renovation on productivity, only the period from one year prior to renovation to three years after renovation was considered for the statistical analysis. This resulted in a total of eight periods for both fertilisation levels (the first renovation done in 2006, and the last in 2018). The statistical analysis shows, as expected, no significant differences between the yields of the permanent grassland and the renovated grasslands in the year prior to the renovation (data not shown). In the year of renovation, only one year out of the eight in the 240_N treatment showed a significant reduction in yield, with no significant differences in the 0_N treatments (Table 1). In the year after renovation, nearly half of the treatments show a significant reduction in yield, ranging from about 1400–2900 kg DM ha^{-1}, compared with the permanent grassland. After two years these yield reductions have disappeared. Three years after renovation annual yields in the 240_N treatments, are in 2 out of 8 years significantly higher, with about 1500 kg DM ha^{-1}. One of the main driver for the lower aboveground biomass yields in the first year after renovation is the necessity to first establish a well-functioning rooting system according to the functional equilibrium theory (Lambers, 1983). The study by Chen et al. (2016b) has shown clearly that the net primary productivity (NPP) is not influenced by the age of grassland, but the fraction of below ground NPP (fBNPP). This indicates that in the first year a high share of assimilated carbon is invested into a new rooting system, whereas in the following years, the fBNPP values were low. Beyond the functional equilibrium effects in the first year after renovation, differences in the response to grassland renovation are likely due to a combination of the prevailing environmental conditions during grass establishment, and differences in the botanical composition (proportion of ryegrass, white clover and herbs). When summed up over the 4-year period (year of renovation up to 3 years after renovation), 0_N treatments had a reduction in yield ranging from 1179 to 5082 kg DM ha^{-1}. In the 240_N treatments, grassland renovation also reduced yields in 6 of the 8 renovation years investigated, ranging from 275 to 4562 ha^{-1}. In the remaining 2 years, yields were slightly higher compared with the permanent grassland, with about 450 kg ha^{-1} over the 4-year period. These results indicate that grassland renovation does not increase dry matter yields, at least for a site with a sandy loam, and good management as done on the Lindhof research farm, with oversowing to maintain a desirable botanical sward composition, avoiding under and over-grazing, preventing soil compaction, and adequate

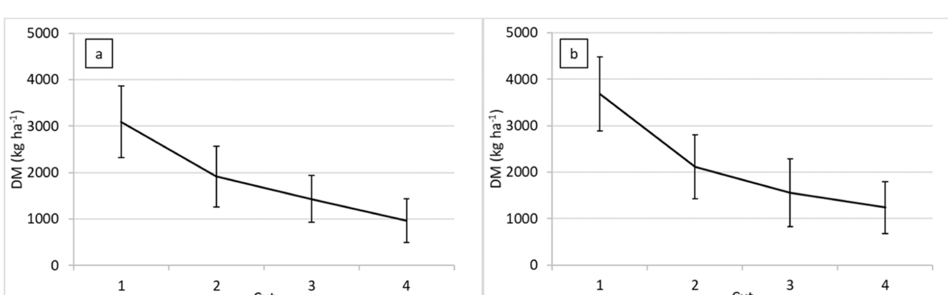

Fig. 2. Dry matter (DM) yields for the four cuts (taken on the 20th May, end of June, mid August, and mid October) under permanent grassland, showing the average and standard deviation over 17 different years (2005–2021), and either (a) without or (b) application of cattle slurry equivalent to 240 kg N ha^{-1}.

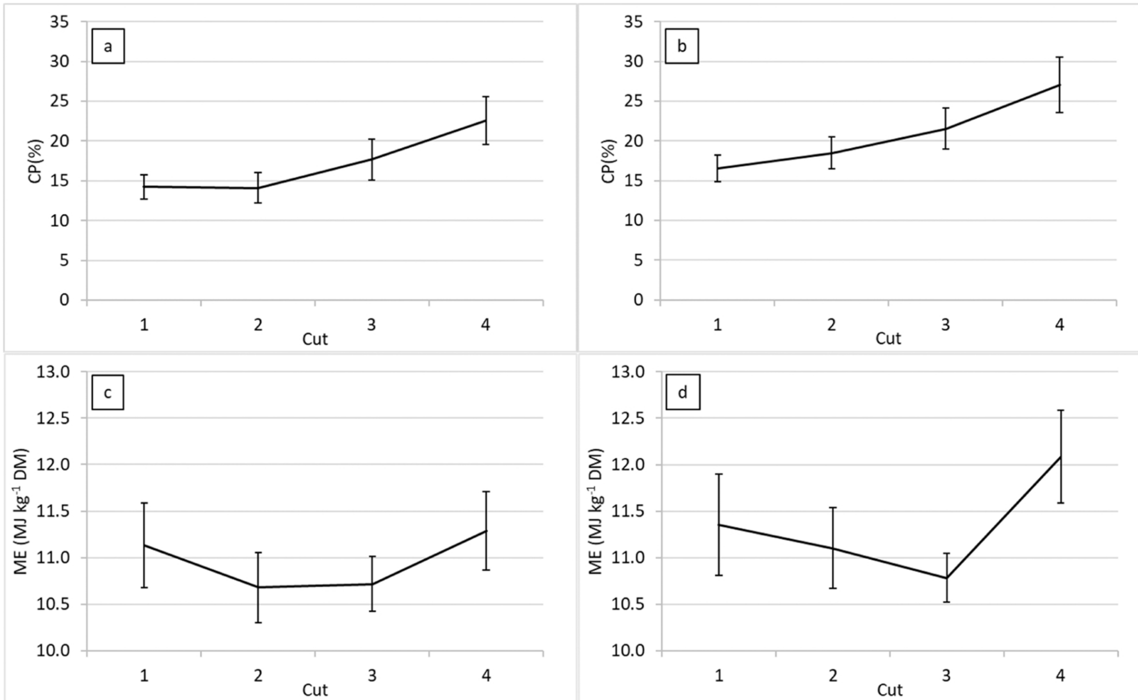

Fig. 3. Crude Protein (CP) and metabolisable energy (ME) for the dry matter of the four cuts (taken on the 20th May, end of June, mid August, and mid October) under permanent grassland, showing the average and standard deviation over 17 different years (2005–2021), and either (a, c) without or (b,d) application of cattle slurry equivalent to 240 kg N ha^{-1}.

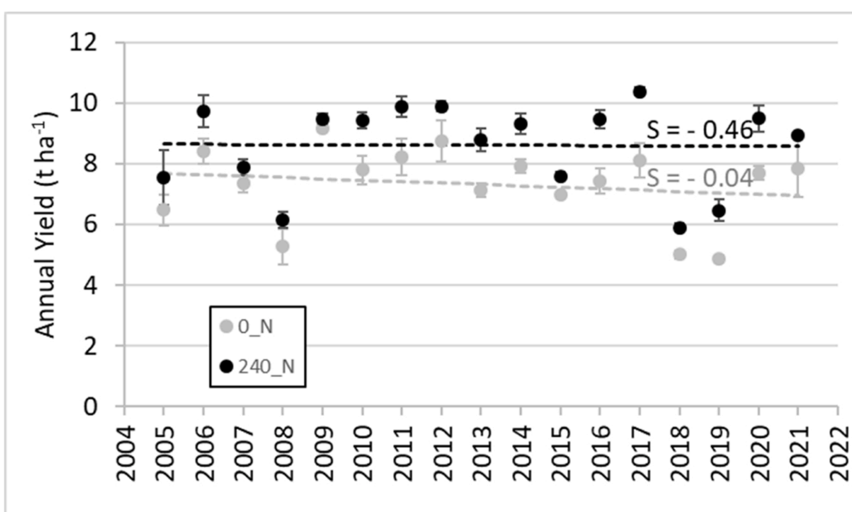

Fig. 4. Annual dry matter yields for permanent grassland for 17 different years, either without (0_N) or with (240_N) application of cattle slurry equivalent to 240 kg N ha^{-1}, with S = slope of the mixed model.

fertilisation to prevent grassland deterioration.

In line with this, Conijn and Taube (2004) also found no benefits of grassland renovation on productivity over a period of three years after renovation, based on various studies across North and West Europe. Other field and on-farm studies from Europe have also not shown any long-term positive yield effects following grassland renovation (Iepema et al., 2020; Nevens and Reheul, 2003; Velthof et al., 2010), and especially in low input systems, several species common in permanent swards can yield more than ryegrass swards (Hopkins et al., 1990).

Measurements of the sward composition, done in 2010 and 2011 on permanent grassland, 2-year and 5-year-old swards and done for both fertiliser treatments (Biegemann; 2013) show that grassland renovation increased the fraction of ryegrass, and with a significantly higher proportion in the 2-year old sward of 77% at 240_N compared to 72% at 0_N ($p < 0.05$). With increasing grassland age the proportion of ryegrass decreased significantly to about 50% (0_N) and 60% (240_N), and is replaced by other species (herbs, mainly dandelion (Taraxacum offizinale) and ribswort plantain (Plantago lanceolata). Grassland renovation also increased the proportion of white clover, with averages over the two years of 24% (0_N) and 14% (240_N) in PG; 43% (0_N) and 18% in the 2-year old swards; and 31% (0_N) and 13% (240_N) in the 5-year old swards. Biegemann (2013) also estimated the N fixation of the various treatments by including clover-free subplots in each of the treatments, and using a difference between the N yield in the above

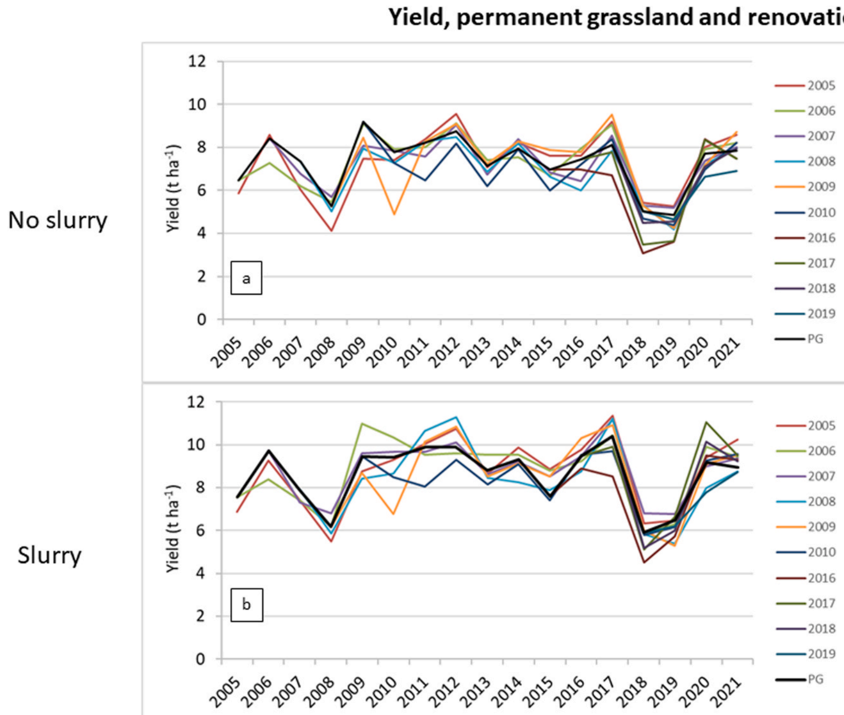

Fig. 5. Annual dry matter yields for permanent grassland and grassland renovated in different years either a) without or b) with application of cattle slurry equivalent to 240 kg N ha^{-1}. Measurements for the treatments started one year before the grassland renovation.

Table 1
Estimated difference in annual dry matter yield (DM diff; kg ha^{-1}) between permanent grassland and renovated grassland either without with application of cattle slurry equivalent to 240 kg N ha^{-1}, based on a mixed model. YR = year of renovation, YaR = year after renovation. Significant differences are indicated by * = p<0.05, ** = p<0.01, *** p<0.001.

		No slurry (0_N)		With slurry (240_N)	
YR	YaR	DM diff	p	DM diff	p
2006	0	-1125	0.240*	-1356	0.038
2007	0	-560	1.000	-602	1.000
2008	0	-260	1.000	-318	1.000
2009	0	-748	0.969	-779	0.944
2010	0	-507	1.000	-957	0.607
2016	0	-468	1.000	-570	1.000
2017	0	-333	1.000	-492	1.000
2018	0	-534	1.000	-706	0.988
2006	1	-1134	0.226	-481	1.000
2007	1	434	1.000	655	0.997
2008	1	-1225	0.115	-1063	0.620
2009	1	-2906	< 0.001***	-2667	< 0.001***
2010	1	-1747	< 0.001***	-1828	< 0.001***
2016	1	-1415	0.022*	-1854	< 0.001***
2017	1	-1515	0.008**	-777	0.946
2018	1	-320	1.000	-493	1.000
2006	2	182	1.000	17	1.000
2007	2	-1107	0.271	151	1.000
2008	2	-531	1.000	-775	0.991
2009	2	52	1.000	243	1.000
2010	2	-564	1.000	-591	1.000
2016	2	-1942	< 0.001***	-1399	0.026*
2017	2	-1213	0.127	171	1.000
2018	2	-596	1.000	631	0.999
2006	3	-89	1.000	1523	0.008**
2007	3	54	1.000	242	1.000
2008	3	50	1.000	720	0.998
2009	3	354	1.000	961	0.597
2010	3	-948	0.628	-652	0.997
2016	3	-1257	0.089	-739	0.974
2017	3	634	0.999	1529	0.007**
2018	3	92	1.000	293	1.000

ground biomass of the grass/clover sward and the pure grass sward. According to this calculation, N fixation in the PG was about twice as high under 0_N (193 kg N ha^{-1}) compared to 240_N (88 kg N ha^{-1}), which is in line with results from Trott et al. (2004). N fixation also increased with the increased proportion of white clover in the swards and thus the renovation of the grassland. The additional N fixation above that of PG was 127 kg N ha^{-1} (0_N) and 81 kg N ha^{-1} (240_N) in the 2-year old swards, and 31 kg N ha^{-1} (0_N) and 45 kg N ha^{-1} (240_N) in the 5-year old swards.

Regarding the forage quality parameters, the CP content generally decreased in the first year following grassland renovation, although differences were not always significant (data are provided in the Supplement Material). The energy value (ME) shows mixed results, with initial decreases and subsequent increases following renovation (Table S2), with a trend of higher ME with years. Statistical differences occur, however in only a few years, indicating that the hypothesis of better forage quality following grassland renovation due to the improved sward composition was not confirmed by our results. Thus, despite the more valuable sward composition (with higher proportions of ryegrass and white clover), the herbage quality was not improved through grassland renovation.

For a more generalised evaluation of the effect of grassland renovation on productivity, these annual yields, CP and ME were normalised using the permanent grassland as the baseline, and these were then averaged (Fig. 6a). Annual DM yields show an initial dip, with a drop of about 15% in the 0_N treatment, and a drop of about 10% in the 240_N treatment, followed by a steady increase, with difference between the fertiliser treatments. In the 0_N treatment, annual yields remain below those of the permanent grassland. In contrast, with in the 240_N treatment annual yields are by up to 3.3% higher after 2–3 years following renovation, and thereafter even out to those of the permanent grassland. This drop in annual yield in the first year is due to the need to partition more C into the newly establishing rooting system (Chen et al., 2016b; Loges et al., 2018). Especially in low N input systems, a large part of the N uptake is used for the growth of the root system, in line with the functional equilibrium theory (Lambers, 1983), which partly explains

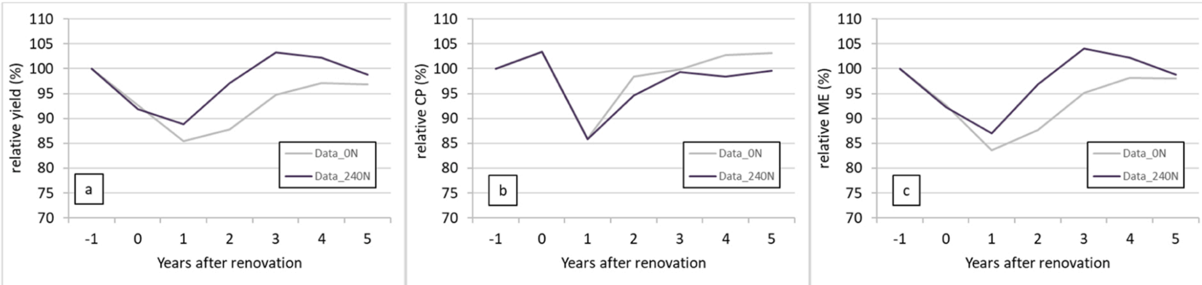

Fig. 6. average relative a) annual dry matter yields, b) crude protein (CP) and c) metabolisable energy (ME) for grassland renovated in different years using the permanent grassland as the baseline, either without (Data_0N) or with application of cattle slurry equivalent to 240 kg N ha^{-1} (Data_240N).

the higher drop observed in the treatment without slurry.

The relative CP yield also drops after grassland renovation and then slowly increases to reach a similar value as the permanent grassland 5 years after renovation (Fig. 6b). In contrast, in the in the 0_N treatment, the ME yield increases after grassland renovation above that of the permanent grassland after three years, and then drops again (Fig. 6). This indicates that renovation only has a minor effect on grass quality in terms of ME and protein concentration, but a significant effect on CP and ME yields following the trend of DMY.

3.3. Soil organic carbon of permanent grassland

The soil organic carbon content (%) in the upper 30 cm of the soil profile for the permanent grassland increases significantly from 1.56% to 1.63% over the period of 18 years with the application of slurry, and significantly decreases without slurry from 1.58% to 1.49% (Fig. 7). These data represent SOC development of undisturbed grassland in the time period 10–28 years after the conversion from arable into the permanent grassland, and show that the topsoil (30 cm) has not reached the C saturation level yet. This is in line with the model developed by Hassink and Whitmore (1997), which gives a C saturation value of 2.5% SOC for a soil with 11% clay. However climatic conditions, mineralogy, surface area of the mineral particles have also been shown to influence the C stabilisation capacity of soils (Beare et al., 2014; McNally et al., 2017).

The SOC amounts in the upper 30 cm increase significantly from 69.3 to 72.9 t ha^{-1} in the 240_N treatment, and decrease significantly from 70.7 to 69.2 t ha^{-1} in the 0_N treatment. The increase in the 240_N treatment can be due to both increased biomass inputs and direct input from the slurry. With an annual input of 240 kg N ha^{-1}, a C:N of 10.9, and a long-term C recovery from cattle slurry of 11% (Jensen et al., 2022), the C sequestration potential of cattle slurry would equate to 0.29 t ha^{-1}, which is slightly higher than the observed rate of 0.21 t ha^{-1}. Thus, the increase in SOC could be entirely due to the manure C inputs, provided sufficient N application via the slurry. From several long-term grassland studies in Germany and the Netherlands, Poeplau et al. (2018) found that to sequester 1 kg SOC ha^{-1}, a similar amount of N (1.15 kg ha^{-1}) was needed.

In line with our study, Jensen et al. (2022) found in a long-term crop rotation experiment with a proportion of 2/3 grassland that SOC stocks increased linearly over a period of 13 years, with no indication of reaching a steady state. A global metadata analysis by Conant et al. (2001) shows a similar average C sequestration rate of grasslands from inorganic fertilisers of 0.29 t C ha^{-1} yr^{-1}. A later global data synthesis by Conant et al. (2017) confirmed that improved grassland management can increase soil C stocks, but the authors emphasize that the response depends not only on management but also on climate, soil and vegetation characteristics. Furthermore, non-CO_2 GHGs need to be considered, as grassland productivity can alter methane emissions via livestock management, and fertiliser applications gaseous N emissions. This latter was shown in the sub-study of the current experiment, done by Reinsch et al. (2018), in which they measured nitrous oxide emissions (N_2O) over a period of two years. They found that application of slurry increased annual N_2O emissions in PG, and grassland renovation amplifying the emissions.

3.4. Effect of grassland renovation on soil organic carbon

For the renovated grasslands, the SOC content drops considerably directly after autumn renovation, for both fertiliser treatments, followed by a slow gradual increase (Fig. 8a). On average over the various years of grassland renovation the SOC drops by about 0.15% (10% relative change in SOC) or 7 t ha^{-1} under both treatments. For the 0_N treatment the initial SOC was not attained even 16 years after renovation, in line with the drop in SOC under the permanent grassland. In contrast, for the 240_N, the initial SOC was attained after about 8–10 years after renovation, and thereafter increased to above the initial value, similar to the fertilised undisturbed permanent grassland. In a poorly drained clay

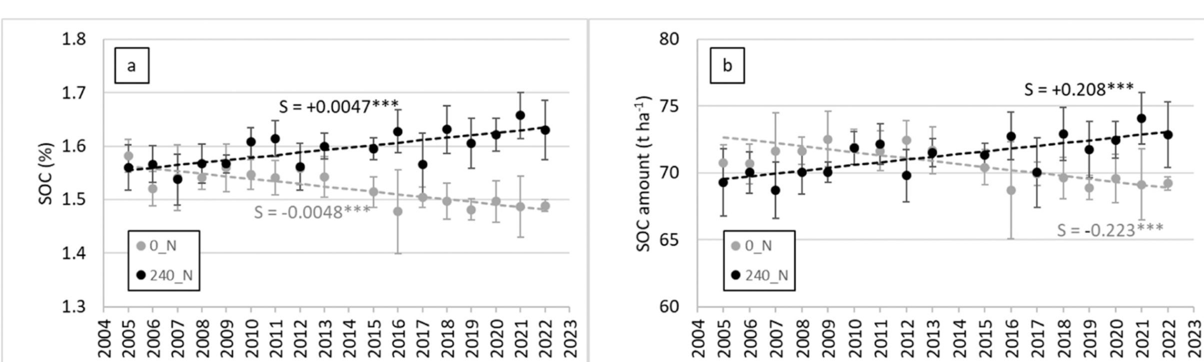

Fig. 7. a) Soil Organic carbon content (SOC, 0–30 cm) and b) SOC amount for permanent grassland for 17 different years, either without or (0_N) or with (240_N) application of cattle slurry equivalent to 240 kg N ha^{-1}, averaged over three blocks, with standard deviation. Slopes (S) were tested to be significantly different from zero, significance level: ***p < 0.001.

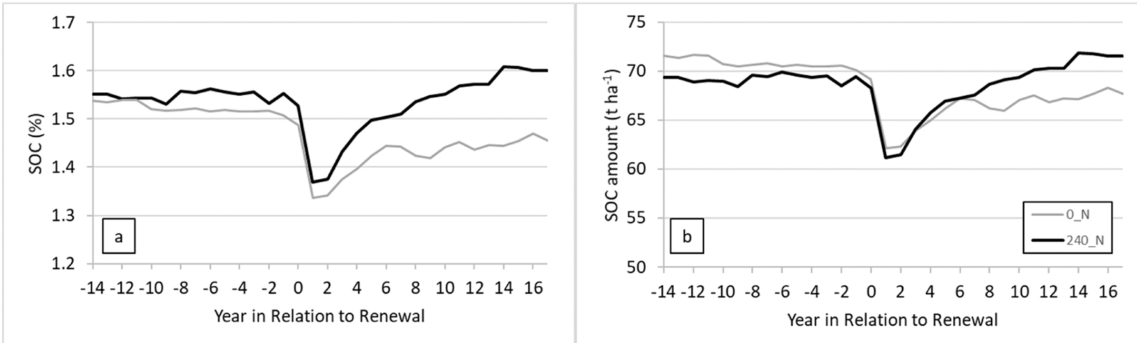

Fig. 8. a) Average dynamics in Soil Organic carbon (SOC, 0–30 cm) and b) SOC amounts after renovation with application of cattle slurry equivalent to 240 kg N ha^{-1}.

loam soil in Ireland, Necpálová et al. (2014) found an even higher reduction in SOC of 32.2 t ha^{-1} (0–30 cm) following grassland renovation, with levels not reaching the initial values during the study period of 2.5 years.

Measurements of the bulk density only showed significant differences ($p = 0.0316$) for +/- slurry, with a higher bulk density without slurry (1.55 g cm^{-3}) compared to slurry treatments (1.49 g cm^{-3}). Average changes of SOC amounts in relation to year after renovation show a significant reduction after the year of renovation with a subsequent gradual increase (Fig. 8b). Application of slurry (240_N) resulted in higher SOC amounts for the renovated grasslands, and also a slightly higher increase following the initial drop after renovation.

This indicates that to recover carbon stocks in tillage-based renovated grasslands it takes about a decade, and fertiliser input is required. Ploughing to a depth of 30 cm, which would move newly accumulated C from the BGB and slurry in the topsoil into deeper layers (10–30 cm), did not increase the SOC stocks compared with the permanent grasslands, even after a period of 18 years following renovation, and with the application of additional C via slurry. This is in line with the topsoil not having reached the C saturation level.

Statistical analysis shows, as expected, no significant differences in the SOC stocks between the permanent grassland and the renovated ones in the year of renovation, apart from the renovation year 2017 (Table 2). For this analysis, only data from 4 years after renovation were used (9 renovation years), as the number of observations decreased with increasing years after renovation. In the two years following renovation, all renovated grasslands had significantly lower carbon stock than the undisturbed permanent grassland. This difference in SOC stocks continued for the following two years (3 and 4 years after renovation), although some of the differences were not significant.

Table 2
Estimated difference in Soil Organic Carbon stock (SOC diff; t ha^{-1}) between permanent grassland and renovated grassland either without with application of cattle slurry equivalent to 240 kg N ha^{-1}., based on a linear mixed model. YR = year of renovation, YaR = year after renovation, Significant differences are indicated by * = $p<0.05$, ** = $p<0.01$, *** $p<0.001$.

		No slurry (0_N)		With slurry (240_N)	
YR	YaR	SOC diff	p	SOC diff	p
2005	0	-1.06	1	0.63	1.000
2006	0	0.04	1	-3.65	0.593
2007	0	-1.40	1	1.11	1.000
2008	0	-2.45	0.998	-3.55	0.660
2009	0	-4.01	0.368	-3.21	0.852
2010	0	-0.83	1	-2.37	0.999
2016	0	1.88	1	-1.95	1.000
2017	0	-8.25	< 0.001***	-5.26	0.029*
2018	0	0.49	1	-4.24	0.257
2005	1	-5.83	0.007**	-8.23	< 0.001***
2006	1	-7.88	< 0.001***	-8.92	< 0.001***
2007	1	-8.97	< 0.001***	-10.46	< 0.001***
2008	1	-13.62	< 0.001***	-9.47	< 0.001***
2009	1	-11.98	< 0.001***	-10.81	< 0.001***
2010	1	-7.02	< 0.001***	-11.78	< 0.001***
2016	1	-5.79	0.008**	-6.71	< 0.001***
2017	1	-9.76	< 0.001***	-10.99	< 0.001***
2018	1	-7.41	< 0.001***	-9.75	< 0.001***
2005	2	-6.06	0.004**	-5.84	0.007**
2006	2	-8.17	< 0.001***	-10.48	< 0.001***
2007	2	-8.90	< 0.001***	-7.69	< 0.001***
2008	2	-10.54	< 0.001***	-10.22	< 0.001***
2009	2	-11.44	< 0.001***	-12.90	< 0.001***
2010	2	-7.95	< 0.001***	-7.29	< 0.001***
2016	2	-8.15	< 0.001***	-12.14	< 0.001***
2017	2	-8.54	< 0.001***	-8.45	< 0.001***
2018	2	-8.52	< 0.001***	-13.03	< 0.001***
2005	3	-4.47	0.167	-3.09	0.901
2006	3	-8.01	0.006**	-7.64	0.013*
2007	3	-7.89	< 0.001***	-7.09	0.001**
2008	3	-9.61	< 0.001***	-10.23	< 0.001***
2009	3	-8.09	< 0.001***	-5.98	0.004**
2010	3	-6.16	0.003**	-8.00	< 0.001***
2016	3	-6.43	0.001**	-9.26	< 0.001***
2017	3	-8.23	< 0.001***	-6.84	< 0.001***
2018	3	-5.92	0.005**	-10.95	< 0.001***
2005	4	-4.89	0.070	-2.77	0.978
2006	4	-5.18	0.036*	-6.31	0.002**
2007	4	-4.59	0.133	-4.58	0.134
2008	4	-8.86	< 0.001***	-5.74	0.009**
2009	4	-6.74	< 0.001***	-6.41	0.001**
2016	4	-7.17	< 0.001***	-7.59	< 0.001***
2017	4	-5.04	0.049*	-6.57	< 0.001***
2018	4	-6.26	0.002**	-7.90	< 0.001***

4. Conclusions

The current study highlights that under good management grassland renovation does not increase productivity but results in a rapid decline in SOC stocks. For the stocks to build up again takes at least a decade and requires application of slurry. The deep ploughing during grassland renovation also did not make up for the decline in SOC stocks, as the topsoil had not reached the carbon saturation level, even 28 years after conversion from cropland. The significant increase in SOC stocks in the permanent undisturbed grassland with slurry application shows the potential role of well managed and utilised grasslands for mitigating climate change through build-up of soil carbon, a promising way towards natural climate solutions. Further studies are, however, warranted to derive information relevant to different environmental conditions and farm management practices. The effect of non-CO_2 GHGs also needs to be considered when developing sustainable grassland systems.

Good management and utilisation of grasslands should be the main strategy for sustainable grassland systems, thereby reducing the need for full grassland renovation. Management should focus on maintaining a desirable botanical sward composition, with functional diversity by utilising breeding progress via oversowing of new varieties, avoiding under and over-grazing, preventing soil compaction, adequate fertilisation to prevent grassland deterioration, and in sandy soils irrigation. Frequent harrowing to pull out thatch and moss, breaking up compacted areas, and evening out the soil surface, is another measure for regenerating grasslands.

Overall, it can be concluded that circularity of nutrients in dairy or beef production systems can be convincingly attained on well managed low input grassland as it ensures a high production level and a sink for soil organic carbon and nitrogen, and thus can contribute to natural climate solutions.

CRediT authorship contribution statement

Iris Vogeler: Writing – review & editing, Writing – original draft, Validation, Formal analysis. **Ralf Loges:** Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Christof Kluß:** Validation, Formal analysis, Data curation. **Friedhelm Taube:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Mario Hasler:** Validation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2024.106076.

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