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Extending grassland age for climate change mitigation and adaptation on clay soils

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Abstract

Permanent grassland soils can act as a sink for carbon and may therefore positively contribute to climate change mitigation and adaptation. We compared young (5–15 years since latest grassland renewal) with old (>20 years since latest grassland renewal) permanent grassland soils in terms of carbon stock, carbon sequestration, drought tolerance and flood resistance. The research was carried out on marine clay soil at 10 dairy farms with young and old permanent grassland. As hypothesized, the carbon stock was larger in old grassland (62 Mg C ha^{-1}) topsoil (0–10 cm) than in young grassland topsoil (51 Mg C ha^{-1}). The carbon sequestration rate was greater in young (on average 3.0 Mg C ha^{-1} $year^{-1}$) compared with old grassland (1.6 Mg C ha^{-1} $year^{-1}$) and determined by initial carbon stock. Regarding potential drought tolerance, we found larger soil moisture and soil organic matter (SOM) contents in old compared with young grassland topsoils. As hypothesized, the old grassland soils were more resistant to heavy rainfall as measured by water infiltration rate and macroporosity (at 20 cm depth) in comparison with the young grassland soils. In contrast to our hypothesis we did not find a difference in rooting between young and old permanent grassland, probably due to large variability in root biomass and root tip density. We conclude that old grasslands at dairy farms on clay soil can contribute more to the ecosystem services climate change mitigation and climate change adaptation than young grasslands. This study shows that under real farm conditions on a clay topsoil, carbon stock increases with grassland age and even after 30 years carbon saturation has not been reached. Further study is warranted to determine by how much extending grassland age can contribute to climate change mitigation and adaptation.

Highlights

- We studied the effect of young versus old grassland on a range of soil properties related to climate change mitigation and adaptation.

- Old, more than young, grassland soils offer potential to mitigate and adapt to climate change.
- This finding gives farmers insight into the merits of extending grassland age on clay soils.
- Soil carbon stock determines carbon sequestration rate more than grassland age.

KEYWORDS

carbon sequestration, dairy, ecosystem services, permanent grassland, soil carbon stock, water infiltration

1 | INTRODUCTION

The concentration of greenhouse gases in the atmosphere, particularly those containing carbon (CO_2 and CH_4), is increasing due to human activities, which contributes to climate change (IPCC, 2013). The effects of climate change are expected to vary across European regions, but all areas experience elevated atmospheric CO_2 concentrations and higher temperatures (Dellar et al., 2018). Also, extreme events, such as heatwaves, heavy rainfall events and severe droughts, are conjectured to become more common across the continent (Kovats et al., 2014).

Global models linking the atmospheric CO_2 concentration to temperature show that a decrease in atmospheric carbon of 3.5–4 Gt year^{-1} will limit the temperature increase to 1.5–2°C by 2050 (Meinshausen et al., 2009; Minasny et al., 2017) (i.e., the threshold beyond which climate change has a significant impact; IPCC, 2013). An annual decrease in the atmospheric CO_2 concentration could temporarily be achieved by annually increasing carbon stocks in the top 30-cm soil horizon by 0.4% (4 per 1,000) (Paustian et al., 2016; Minasny et al., 2017).

Globally, grassland soils are important stores of terrestrial carbon (Gobin et al., 2011; Stockmann et al., 2013; Smith, 2014; Lal et al., 2018; Reinsch et al., 2018b). Measured and modelled rates of carbon sequestration in permanent grassland soils range from 0 to > 8 Mg C ha^{-1} year^{-1}, depending on management practices (Jones & Donnelly, 2004), soil texture, climate and initial carbon stock (Skinner, 2008; Klumpp et al., 2011; Smith, 2014; Conant et al., 2017). Specifically, permanent grassland soils without cultivation can potentially contribute to climate change mitigation (Klumpp & Fornara, 2018) by acting as a carbon sink (Soussana et al., 2007, 2010; Minasny et al., 2017).

On clay soils in the Netherlands, permanent grasslands are renewed (i.e., destroyed by herbicides, ploughed and reseeded) on average once every 10 years (Vellinga et al., 2004; Velthof et al., 2009; Smit & Velthof, 2010). The conversion of grassland to arable land leads to a decrease in soil carbon stock and therefore a net release of CO_2 over a timeframe of several years (Johnston et al., 2009). Single tillage events, such as ploughing of grassland for grass renewal, significantly reduce soil carbon stocks as well (Linsler et al., 2013; Necpálová et al., 2014). Besides a reduction of soil carbon stock, grassland renewal leads to losses of nitrogen via leaching of NO_3^- and emission of the greenhouse gas N_2O (Velthof et al., 2009; Reinsch et al., 2018a).

In addition to climate change mitigation, permanent grassland can potentially play a role in climate change adaptation by increased drought tolerance and flood resistance.

Regarding potential drought tolerance, soil carbon stock is closely related to soil organic matter (SOM) content. An increase in SOM content leads to larger plant available water capacity (Hudson, 1994; Lal, 2020), improved soil structure (Newell-Price et al., 2013; Jensen et al., 2019) and increased water-holding capacity of the soil (Acín-Carrera et al., 2013; Assi et al., 2019). Therefore, permanent grassland with good soil structure is likely to be more resistant to periods of drought than grassland with a weaker soil structure, as is likely in young grassland. In addition, a larger root biomass, which is typical for older grassland (Acharya et al., 2012; Carolan & Fornara, 2016), can make permanent grassland more resistant to periods of drought.

Regarding potential flood resistance, grasslands with a large soil carbon content can also be more resistant to periods of excess rainfall, because an increased soil carbon stock has a positive effect on porosity and water infiltration rate (Dexter et al., 2008; McLenaghen et al., 2017; Lal, 2018). However, permanent grassland soils are often compacted due to machine traffic, livestock treading and natural soil consolidation (Bohner et al., 2017; De Boer et al., 2018, 2020), which has a negative effect on water infiltration rate (Sochorec et al., 2015) and may negate the positive effect.

In an earlier paper we reported the effects of permanent grassland age on grass productivity in relation to chemical soil quality in an on-farm experiment (Iepema et al., 2020). Here we report from the same experiment on the effect of permanent grassland age (young versus old grassland) on (1a) carbon stock and (1b) the rate of carbon sequestration on marine clay topsoil (0–10 cm), which can contribute to climate change mitigation. Additionally, we investigated the effect of permanent grassland age on (2a) root biomass, SOM content and soil structure, which can influence resistance to periods of drought. Also, we investigated the effect of permanent grassland age on (2b) water infiltration, macroporosity and soil penetration resistance, which can have an impact on the resistance to excess rainfall. Resistance to excess rainfall and periods of drought are both aspects of climate change adaptation.

We hypothesize that (1a) topsoil carbon stock is larger in old compared to young grassland and (1b) that the carbon sequestration rate is greatest in the first years after grassland renewal, when soil carbon stock is decreased due to ploughing, followed by a gradual decrease in carbon sequestration rate, as the maximum amount of carbon that can be stored in the soil is gradually reached. Additionally, we hypothesize that (2a) root biomass, SOM content and soil structure quality, which have a positive effect on soil moisture content, are larger in the old grassland topsoil in comparison with the young grassland topsoil and that (2b) soil water infiltration and macroporosity are larger in old grassland topsoil as well.

2 | MATERIALS AND METHODS

2.1 | Field selection

The study was conducted in 2014, on marine clay soil (Haplic Fluvisol; FAO, 2015) at 10 dairy farms in the north of the Netherlands. At each farm, a young (5–15 years since grassland renewal) and an old (> 20 years since grassland renewal) grassland were selected and compared with farm as a block factor (for more information about the selected grasslands, see Supplementary Material, Table S1 and Iepema et al., 2020). Grassland age (years since the latest renewal) was recorded by interviewing the farmers, except for two very old (older than 30 years) grasslands where the age was conservatively set at 30 years because the exact year of renewal was not known. The young grasslands were on average 9 years old; the old grasslands on average 25 years old (Table 1). Other criteria for the selection of the grasslands were: sufficiently fertilized with slurry manure and artificial fertilizer (see level of fertilization in Table S1), no visual soil compaction, no clover seeded and (based on visual estimation in March 2014) having at least 70% cover of the grasses *Lolium perenne* and *Phleum pratense*, which are considered desirable by farmers. All grasslands were used for (intensive) dairy farming, and no high-impact plant diseases or extreme weather events occurred in the years before the measurements took place. These criteria were applied to minimize the differences between the grasslands, other than grassland age. In 2013, the year before the experiment was implemented, the young and the old grasslands received on average 298 and 255 kg available N ha^{-1} from a combination of slurry manure and artificial fertilizer, respectively, and these doses did not differ significantly. During the year of measurement on each grassland, a non-fertilized 5 × 9 m plot was used to determine soil quality parameters, as described below. Adjacent to the non-fertilized plot, three fertilization subplots of 10 × 3 m were installed to determine grass productivity in relation to chemical soil quality. The results of this experiment were published by Iepema et al. (2020) with more details about the selected grasslands.

2.2 | Soil sampling and analysis to measure parameters related to climate change mitigation

On each non-fertilized 5 × 9 m plot, soil samples were taken on 29 or 30 April 2014 and measurements were carried out to determine chemical and physical soil quality. For chemical analysis a field-moist bulk composite sample of the topsoil was collected, comprising 70 randomly taken cores (0–10 cm depth; 2.3 cm diameter), sieved through a 1-cm mesh, homogenized and analysed for SOM, C-total, hot water-extractable carbon (HWC) and soil particle analysis. We focused our sampling on the 0–10-cm soil layer according to the official Dutch fertilization recommendations (www.bemestingsadvies.nl). SOM was determined by loss-on-ignition (Ball, 1964): after drying at 105 ± 5°C, the soil sample was ignited at 550 ± 25°C and corrected for water bound on clay minerals that had not been evaporated at 105 ± 5°C. C-total was measured by incineration of dry material at 1150°C, after which the CO_2 produced was determined by an infra-red detector (LECO Corporation, St. Joseph, Michigan, USA). The carbon percentage of the SOM (C:SOM ratio) was calculated by dividing C-total (g C 100 g^{-1} dry soil) by SOM (%). Soil bulk density was determined in the 5–10-cm layer below the soil surface, in three

TABLE 1 Characteristics of the topsoil (0–10 cm) of young ($n = 10$) and old ($n = 10$) grasslands on marine clay soil. Means are means before transformation; c.v., coefficient of variation (%); *p*-values in bold when < 0.05

		Young grassland		**Old grassland**		
Parameter	**Unit**	**Mean**	**c.v.**	**Mean**	**c.v.**	***p*-value**
Related to climate change mitigation						
Age of the sward	Years since cultivation	9	40	25	15	**<0.001**
Soil organic matter content (SOM)	g kg^{-1}	107	31	133	17	**0.001**
C-total	g C kg^{-1} dry soil	45.2	39	61.0	20	**<0.001**
Soil carbon stock	Mg C ha^{-1}	50.9	31	62.3	15	**0.013**
C:SOM ratio		41.4	10	45.6	5	**0.002**
Hot water-extractable carbon (HWC)	g C kg^{-1} dry soil	2.41	43	3.35	18	**0.002**
HWC as percentage of C-total	%	5.27	10	5.53	5	0.224
C:clay ratio		17	37	25	25	**0.008**
Related to climate change adaptation						
Response to drought						
Soil moisture content	Volume %	28.6	11	31.7	9	**0.007**
Root tip density at 10 cm	Number dm^{-2}	109	14	118	34	0.524
Root tip density at 20 cm	Number dm^{-2}	81	21	65	35	0.185
Proportion of root tips at 10 cm	%	57	12	64	7	**0.002**
Soil bulk density	g cm^{-3}	1.16	9	1.03	8	**<0.001**
Crumbs 0–10 cm	% of total weight	67.3	29	80.3	19	**0.005**
Angular blocky elements 0–10 cm	% of total weight	12.2	117	4.6	175	0.052
Crumbs 10–20 cm	% of total weight	16.2	69	17.2	113	0.894
Angular blocky elements 10–20 cm	% of total weight	45.1	79	50.1	76	0.694
Root biomass 0–10 cm	Mg AFDM ha^{-1}	6.4	27	5.4	36	0.262
Root biomass 0–30 cm	Mg AFDM ha^{-1}	8.5	38	6.6	40	0.180
Response to excess rainfall						
Water infiltration rate	Mm min^{-1}	3.7	175	11.1	75	**0.033**
Macropores at 10 cm	Number dm^{-2}	3.5	67	5.3	48	0.175
Macropores at 20 cm	Number dm^{-2}	1.5	59	3.4	60	**0.013**
Penetration resistance 0–10 cm	MPa (1 cm^2 conus)	0.85	25	0.93	38	0.395
Penetration resistance 11–20 cm	MPa (1 cm^2 conus)	1.30	19	1.46	29	0.200
Penetration resistance 21–30 cm	MPa (1 cm^2 conus)	1.31	17	1.44	17	0.120
Penetration resistance 31–40 cm	MPa (1 cm^2 conus)	1.42	36	1.48	29	0.471

undisturbed ring samples containing 100 cm^3 soil each. Samples were weighed, oven-dried at 70°C, and reweighed to determine bulk density. Carbon stock was calculated on an equivalent mass basis as a product of C-total, soil bulk density and the depth layer as described by Ellert et al. (2007).

To measure the effect of extending grassland age, as a soil management change, on soil active carbon stock, soil HWC was used, because this parameter has been highlighted as a soil quality indicator that is more sensitive to management changes than total carbon stock (Ghani et al., 2003; Jensen et al., 2020). HWC was analysed according to the method of Ghani et al. (2003): field-moist samples of 4 g soil were extracted at room temperature with 30 ml distilled water for 30 min, centrifuged for 20 min, and the supernatant with water-soluble carbon was discarded. Then a further 30 ml distilled water was added to the sediments, shaken for 10 s and left for 16 h in a hot-water bath at 80°C. After centrifugation the supernatant was filtered and extracted carbon was measured. To calculate which part of the C-total was HWC, HWC content per 100 g soil was divided by C-total per 100 g soil.

Clay (<2 μm diameter) content was determined through density fractionation (NEN 5753, 2018). Soil texture was determined by the pipette method and sieving (fractions 2, 16, 50, 63, 125, 180, 250, 355, 500, 1000 and 2000 μm). The C:clay ratio was calculated through dividing C-total by the % clay.

In 2018, at six out of the 10 original farms a second soil sample was taken from both the young and old grasslands for C-total analysis. In contrast to the other four farms, at these six farms the fields had not been renewed, nor had fundamental changes regarding grassland management (fertilisation, grazing or mowing) taken place since 2014. These soil samples were taken in December 2018 at the same spots as in 2014, based on GPS coordinates. Additionally, soil samples for C-total analysis were taken from recently renewed (i.e., 1 to 4 years before sampling date) fields on four out of these six farms (at the other two farms no recently renewed grasslands were available). From both sets of 2018 soil samples a field-moist composite sample of the topsoil comprising 40 cores (0–10 cm depth; 2.3 cm diameter) was collected randomly, sieved through a 1-cm mesh, homogenized and analysed for C-total following the protocol described above. Bulk density was calculated with the following equation:

$$\text{Bulk density}\left(\text{g cm}^{-3}\right) = -0.0557 * \text{C-total}\left(\text{g } 100\,\text{g}^{-1}\text{ soil}\right) + 1.3898 \tag{1}$$

which was based on the correlation (r = −0.85; $p = 0.001$) between C-total and bulk density as measured in all grasslands in 2014 (see Supplementary material, Figure S1). Equation (1) was used to calculate soil carbon stocks in 2018.

2.3 | Soil sampling and analysis to measure parameters related to climate change adaptation

2.3.1 | Indicators for drought resistance

To investigate the resistance to periods of drought we used three indicators: soil structure, rooting and soil moisture content. To assess soil structure we measured bulk density and SOM content and we carried out a visual soil structure analysis. Rooting was assessed by measuring the ash-free root biomass in the 0–10 and 0–30-cm soil layers and by counting root tips at 10 and 20-cm soil depth to determine root tip density. The 0–10-cm soil layer was sampled because this is the depth used in the standard procedure for sampling grasslands in the Netherlands. The 0–30-cm soil layer was sampled because, according to Cougnon et al. (2013, 2017), 95.5% of the root biomass of *Lolium perenne* can be found in this soil layer. Carolan and Fornara (2016) found an increase in root biomass between 0 and 20-cm soil depth with increasing grassland age, so to distinguish possible differences between young and old grassland, we counted root tips at 10 and 20-cm soil depth.

Soil moisture content was determined in the 5–10-cm layer below the soil surface, in three undisturbed ring samples containing 100 cm^3 soil each, which were also used for determining soil bulk density. Samples were weighed, oven-dried at 105°C, and reweighed to determine moisture content using the following equation:

$$\text{Soil moisture content} = \left(1 - \left(\text{DW}_{105}/\text{FW}\right)\right) * 100, \tag{2}$$

where DW_{105} represents the oven-dry weight of the sample (g) and FW the initial fresh soil mass (g).

Visual assessments of soil structure and rooting were conducted in situ, on two 20 × 20 × 10 cm (l × w × d) soil blocks from the 0–10 and the 10–20-cm soil layers. The soil blocks were dug out with a spade and broken in horizontal and vertical directions. Root tip density and macroporosity were assessed by counting root tips and macropores (diameter > 2 mm) on the 20 × 20 cm soil surface at 10 cm and at 20-cm soil depth. Soil structure was assessed by dividing the soil from the soil blocks into soil crumbs, subangular blocky elements and angular blocky elements and weighing the different forms of elements according to Peerlkamp (1959) and Shepherd (2010). Soil structure was analysed for the 0–10 and 10–20-cm soil layers. To measure root biomass, three soil cores (82 mm diameter) were taken randomly per plot from three soil layers (0–10, 10–20 and 20–30-cm depth) using a root auger (Eijkelkamp, The Netherlands). The cores were pooled per soil layer per plot and thoroughly rinsed with water over a sieve with a mesh size of 2 mm. Organic debris was removed by hand and samples were oven-dried at 105°C for 24 h for dry matter measurement. Then, samples were incinerated for 4 h at 600°C to determine the ash content in the root samples. The root biomass was expressed as ash-free dry matter (AFDM) per hectare. The root biomass was analysed per 10-cm soil layer and for all soil layers (0–30 cm) combined.

2.3.2 | Indicators for response to excess rainfall

To investigate the response to excess rainfall we used two indicators: water infiltration and soil penetration resistance. To assess water infiltration we measured the number of macropores, as described above, and also the water infiltration rate. The water infiltration rate into the soil was measured as described in Van Eekeren et al. (2010). In short: for each unfertilized 5 × 9 m subplot, at three randomly chosen spots, a PVC pipe with a diameter of 15 cm was driven into the soil to a depth of 10 cm, after which 300 ml of water was poured into the pipe. The number of minutes it took for the 300 ml water to infiltrate the soil was recorded and calculated as infiltration rate in mm water per minute.

Soil penetration resistance was measured with an electronic penetrometer (Eijkelkamp) with a cone diameter of 1 cm^2 and an apex angle of 60°. Penetration resistance was recorded per cm soil depth at 10 randomly chosen points per plot, at least 50 cm inside of the plot borders (Glyn Bengough et al., 2000; De Boer et al., 2018), and expressed as the average of 10 penetrations for the 0–10, 11–20, 21–30 and 31–40-cm soil layers.

2.4 | Calculations and statistical analysis

The data were not normally distributed for four parameters: root tip density at 10 cm, angular blocky elements at 0–10 cm, penetration resistance at 11–20 cm and penetration resistance at 31–40 cm. Therefore, these soil parameters were log-transformed before further analysis. Analysis of variance (ANOVA) was performed to test for significance of the differences between young and old grasslands, using Genstat software (18th edition, VSN International, UK). Each of the 10 farms comprised a young and an old grassland; the factor "farm" was therefore used as block factor in the ANOVA structure. Pearson correlations and their significance were calculated for all possible parameter pairs using the *psych* package in R 3.6.2. Significance for these correlation analyses was detected using the false discovery rate for multiple comparisons as described by Benjamini and Hochberg (1995). The false discovery rate was set at 5%. A regression analysis was performed to investigate the relation between grassland age (years since renewal) and carbon stock of the topsoil using the packages *nls2* and *propagate* in R 3.6.2. With a linear model we tested the effect of young or old grassland and the initial C stock on C stock increase as measured between 2014 and 2018, using the package *doBy* in R 3.6.2.

3 | RESULTS

3.1 | Parameters related to climate change mitigation

As hypothesized, soil organic matter content and soil carbon stock of the topsoil (0–10 cm) were significantly ($p < 0.001$) larger in old than in young grassland. The topsoil (0–10 cm) of old grassland contained on average 13.3% SOM, 61 g C kg^{-1} dry soil and a carbon stock of 62 Mg C ha^{-1}, whereas young grassland topsoil contained 10.7% SOM, 45 g C kg^{-1} dry soil and 51 Mg C ha^{-1} carbon stock (Table 1). The hot water-extractable carbon (HWC) content, C:SOM ratio and C:clay ratio were also significantly larger in old than in young grassland topsoil (Table 1). HWC as a percentage of C-total, was not significantly different between old and young grassland soils. The ranges (coefficients of variation) in SOM content, C-total, C:SOM ratio, HWC and C stock were larger in young than in old grassland (Table 1). All topsoil parameters related to climate change mitigation correlated significantly positively with eachother and also with the age of the sward (years since renewal) (Table 2).

At most farms, the old grassland topsoil contained more carbon than the young grassland topsoil (Table 3), with the exception of farms no. 3 and 9. At these two farms, the soil carbon stock in the young grassland was larger than at the other farms (Table 3; farms 3 and 9: > 70 Mg C ha^{-1}; the other farms: < 60 Mg C ha^{-1}).

In the grasslands that were resampled in 2018 (i.e., the young and old grasslands at farms 2, 3, 4, 5, 7 and 8) we

TABLE 2 Pearson's correlations (r) between soil parameters related to climate change mitigation for all ($n = 20$) grasslands. Green squares indicate positive correlations, r > 0.5; all correlations were significant ($p < 0.05$) correlations; parameters are described in Table 1

	SOM	C-total	C:SOM ratio	HWC	C-stock	C:Clay ratio
C-total	**0.99**					
C:SOM ratio	**0.82**	**0.88**				
Hot water-extractable carbon	**0.97**	**0.98**	**0.90**			
C-stock	**0.96**	**0.97**	**0.86**	**0.95**		
C:Clay ratio	**0.61**	**0.68**	**0.84**	**0.70**	**0.63**	
Age of the sward	**0.51**	**0.54**	**0.61**	**0.58**	**0.47**	**0.58**

TABLE 3 Topsoil (0–10 cm) carbon stock (Mg C ha^{-1}) at the farm level in young and old grasslands, differences between young and old grasslands, difference in age between young and old grasslands and calculated topsoil carbon stock increase or decrease per year. Numbers are farm numbers, sorted from large to small topsoil C-stock difference per year

Farm number	Soil C-stock young grassland (Mg C ha^{-1})	Soil C-stock old grassland (Mg C ha^{-1})	Soil C-stock difference (Mg C ha^{-1})	Difference in age between young and old grassland (years)	Soil C-stock difference per year
4	58.7	69.3	10.6	3	3.53
2	49.7	72.4	22.6	15	1.51
6	28.5	51.2	22.6	16	1.41
8	45.9	69.4	23.6	18	1.31
10	44.5	63.3	18.8	19	0.99
5	43.1	50.0	6.9	9	0.76
7	37.5	50.4	12.9	20	0.64
1	46.6	57.1	10.5	21	0.50
3	75.5	73.4	-2.0	18	-0.11
9	78.9	66.5	-12.3	24	-0.51
Average	*50.9*	*62.3*	*11.4*	*16.3*	*1.00*

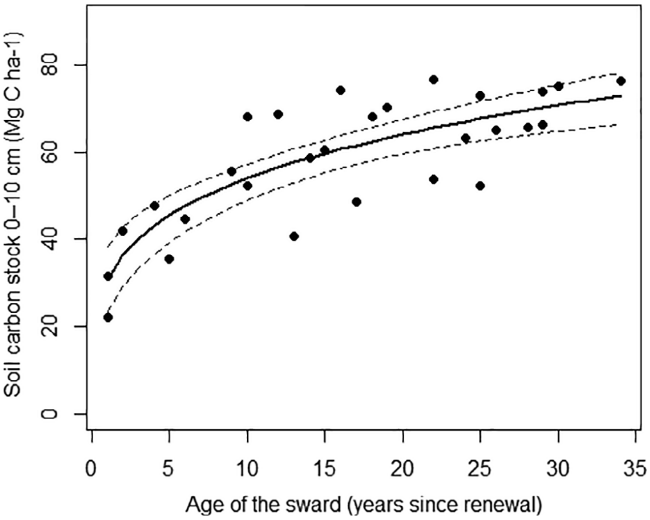

FIGURE 1 Carbon stock in the 0–10-cm soil layer (measured in 2014 and 2018) as a function of grassland age (years since renewal). Dotted lines, 95% confidence interval; the black line shows the model $y = 30.7 * x^{0.27}$; $R^2 = 0.67$

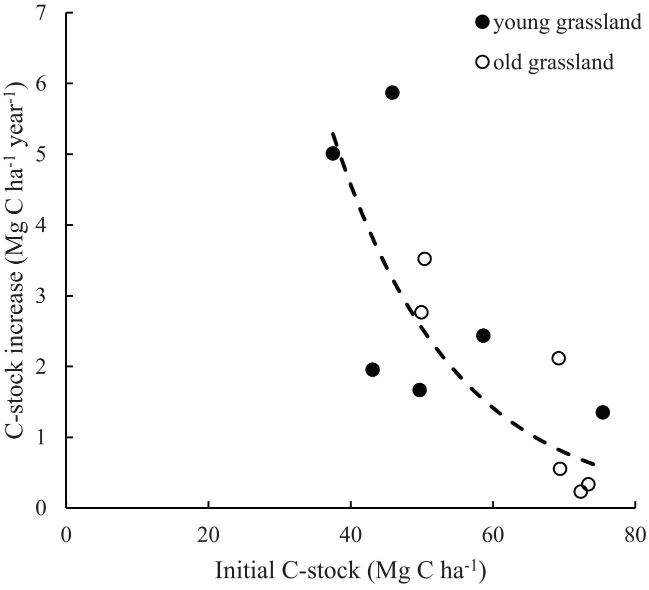

FIGURE 2 Carbon stock increase between 2014 and 2018 at six young and six old grasslands in relation to initial carbon stock in 2014 in the 0–10-cm soil layer. Closed dots represent young grasslands; open dots old grassland

found an increase in C-total in all fields, and also in farm 3 (data not shown) compared to the sampling in 2014. Based on Equation (1) in the Materials and Methods section, soil bulk density and carbon stock were calculated for 2014 and 2018. In the young grasslands, the topsoil carbon stock increased in these 4 years on average by 3.0 Mg C ha^{-1} year^{-1}, whereas in the old grasslands the increase was on average 1.6 Mg C ha^{-1} year^{-1}. By combining the observations from 2014 and 2018, we found a significant positive curvilinear relation ($r = 0.82$; $p = 0.001$; Figure 1) between the age of the sward and carbon stock in the 0–10-cm soil layer. In the first years after renewal, carbon stock increased relatively fast and after approximately 10 years the line curved to a flatter response (Figure 1).

The smaller initial carbon stock was the reason for the larger carbon sequestration rate we found in the young grasslands. A linear regression model showed that

TABLE 4 Pearson's correlations (r) between soil parameters related to climate change adaptation potential in response to drought for all ($n = 20$) grasslands. Green squares indicate positive ($r > 0.5$) and orange squares negative ($r < -0.5$) correlations; numbers in bold indicate significant ($p < 0.05$) correlations; parameters are described in Table 1

	Soil moisture content	Root tip density at 10 cm	Root tip density at 20 cm	Percentage root tips at 10 cm	Soil bulk density	SOM, %	Crumbs, % 0–10 cm	Angular bl. Elements, % 0–10 cm	Crumb, % 10–20 cm	Angular bl. Elements, % 10–20 cm	Root biomass at 0–10 cm	Root biomass at 0–30 cm
Root tip density at 10 cm	0.55											
Root tip density at 20 cm	−0.08	0.48										
Percentage root tips at 10 cm	**0.61**	0.40	**−0.61**									
Soil bulk density	**−0.93**	−0.52	0.20	**−0.69**								
SOM, %	**0.82**	0.47	−0.21	**0.65**	**−0.84**							
Crumbs, % 0–10 cm	**0.56**	0.49	−0.12	**0.57**	−0.49	0.37						
Angular blocky elements, % 0–10 cm	**−0.65**	−0.46	0.25	**−0.70**	**0.55**	−0.52	**−0.81**					
Crumbs, % 10–20 cm	0.27	0.40	0.13	0.23	−0.31	0.03	0.52	−0.36				
Angular blocky elements, % 10–20 cm	−0.26	−0.48	−0.12	−0.33	0.26	−0.01	**−0.58**	0.50	**−0.83**			
Root biomass at 0–10 cm	0.11	0.32	**0.70**	−0.40	0.05	0.03	0.09	0.13	−0.04	−0.08		
Root biomass at 0–30 cm	−0.01	0.19	**0.61**	−0.43	0.19	−0.10	0.17	0.06	−0.04	−0.10	**0.91**	
Age of sward	0.52	0.15	−0.39	**0.56**	**−0.64**	0.51	0.40	−0.39	−0.04	0.05	−0.21	−0.27

the carbon stock increase in 4 years measured at the six old and six young grasslands was strongly dependent on the initial carbon stock but was not significantly affected by grassland age. There was a negative significant curvilinear correlation between initial carbon stock (as measured in 2014) and carbon stock increase between 2014 and 2018 (r = −0.76; *p* = 0.01; Figure 2).

3.2 | Parameters related to climate change adaptation

3.2.1 | Indicators for potential drought resistance

Old grassland topsoil had a significant larger soil moisture content (31.7 vol%) than young grassland topsoil (28.6 vol%; Table 1). There was no significant difference in root tip density at 10 or 20 cm between young and old grassland, but in old grassland we found a greater percentage of root tips from the total number of root tips at 10 cm (64%) than in young grassland (57%; *p* = 0.002; Table 1). Soil bulk density was significantly lower in old grassland topsoil (1.03 g cm^{-3}) than in young grassland topsoil (1.16 g cm^{-3}; Table 1). Old grasslands contained significantly more crumbs in the topsoil (0–10 cm) than young grasslands. Root biomass did not significantly differ between young and old grasslands for either the 0–10 or 0–30-cm layers (Table 1).

The soil moisture content was significantly positively correlated with the percentage of root tips at 10 cm, SOM and percentage of crumbs in the 0–10-cm soil layer and negatively with soil bulk density and percentage of angular blocky elements in the 0–10-cm soil layer (Table 4). Root tip density at 20 cm soil depth was significantly positively correlated with root biomass in the 0–10-cm soil layer and also with root biomass in the 0–30-cm soil layer. The percentage of root tips at 10 cm was significantly positively correlated with SOM percentage and percentage of crumbs in the 0–10-cm soil layer and significantly negatively with soil bulk density and percentage of angular blocky elements at 10 cm.

3.2.2 | Indicators for response to excess rainfall

For old grassland we found a significantly larger water infiltration rate compared with young grassland. Also, macropore density at 20 cm soil depth was significantly larger in old grassland than in young grassland. Soil penetration resistance did not significantly differ between young and old grassland soils (Table 1). We did not find significant correlations between the parameters that were used to express the resistance to excess rainfall, except for unexpected positive correlations between the average penetration resistance between 0 and 10 cm and the water infiltration rate (r = 0.74; *p* = 0.001) and between the average penetration resistance between 10 and 20 cm and the water infiltration rate (r = 0.80; *p* = 0.003), but these correlations were determined by an outlier in the water infiltration rate.

4 | DISCUSSION

4.1 | Parameters related to climate change mitigation

In accordance with hypothesis 1a, the carbon stock in the topsoil (0–10 cm) was significantly larger in old (on average 62 Mg C ha^{-1}) than in young grassland (51 Mg C ha^{-1}; Table 1). Other studies on managed grassland in clay soils in Europe reported smaller (Watson et al., 2007) or much larger (Hopkins et al., 2009; Necpálová et al., 2014; Carolan & Fornara, 2016) soil carbon stocks (see Supplementary Material, Table S2). These differences can be partly explained by the soil layer sampled. In the present study and in the study in sandy soils by Poeplau et al., (2018), carbon stock was measured in the topsoil (0–10 cm), whereas other studies measured carbon stock in deeper soil layers (0–15, 0–23, 0–30 cm; Table S2). On a sandy soil in the Netherlands, Hoogsteen (2020) found that, of the total SOM in the 0–30-cm layer, 53% was in the 0–10-cm layer. It would be interesting to also measure carbon stock in deeper soil layers of our grasslands (10–30 cm or even 30–60 cm) as suggested by Hoogsteen et al. (2020).

The labile fraction of soil carbon, represented by HWC, was strongly correlated with SOM and C-total (Table 2). HWC as a percentage of C-total was not affected by grassland age. This indicates that HWC represented a fixed portion of C-total and was not related to management changes, which was also found by Jensen et al. (2019). Because the management change in our study (i.e., extending grassland age) was more than 5 years ago, HWC probably did not act as a sensitive indicator because an equilibrium between labile and stabilized carbon had already been established (Haynes, 2005).

In the old grassland soils the variation in parameters related to climate change mitigation (SOM, C-total, C:SOM ratio and HWC) was smaller than in the young grassland soils (Table 1). This can be an indication that in old grassland the soils were closer to carbon saturation (Six et al., 2002; Stewart et al., 2007; Feng et al., 2013). This is corroborated by the C:clay ratio (Dexter et al., 2008), which was significantly larger in old than in young grassland soils.

We found a significant negative correlation between initial carbon stock and carbon stock increase ($r = -0.76$, $p = 0.01$; Figure 2). This is consistent with our hypothesis 1b that in the first years after grassland renewal, when soil carbon stock is smaller due to ploughing, the carbon sequestration rate is initially large and then slows down in subsequent years until nearing the maximum amount of carbon that can be sequestered.

Our calculated annual rates of C sequestration are generally larger when comparing soil C-total measured in 2014 and 2018 than those reported in other recently published field studies in Europe (Table S2). This can partly be explained by sampling depth, because in contrast to the increased carbon sequestration in the topsoil (0–10 cm), carbon stock can decrease over time in deeper soil layers (10–30 cm and 30–60 cm) (Watson et al., 2007; Don et al., 2009; Hoogsteen et al., 2020). Other factors, next to sampling depth and grassland age, affecting the rate of carbon sequestration are grass productivity (Jones & Donnelly, 2004; Conant et al., 2017), fertilization (van den Pol - van Dasselaar & Lantinga, 1995), grazing and cutting management (Smith, 2008; van den Pol - van Dasselaar, 2017; Hewins et al., 2018), botanical composition (Cong et al., 2014; Hoogsteen et al., 2020) and soil texture (Hassink, 1997; Mestdagh et al., 2006).

4.2 | Parameters related to climate change adaptation

4.2.1 | Indicators for potential drought resistance

In line with our hypothesis 2a, potential drought resistance, as indicated by SOM content and soil structure, was significantly larger for old grasslands than for young grasslands. Rooting, the third parameter for indicating potential drought resistance, was not significantly different between young and old grassland, as discussed below.

The soil moisture content was significantly larger in old grassland topsoil compared with young grassland topsoil. Because soil moisture content was measured only once, this can be seen as a snapshot, not as a reliable indicator for plant water availability. However, we found a strong positive correlation ($r = 0.82$, $p < 0.01$; Table 4) between SOM content and soil moisture content. Other studies also reported strong effects of SOM content on plant available water capacity (Ankenbauer & Loheide, 2017), although some studies only report small effects (Minasny & McBratney, 2018); see Lal (2020) for an overview of studies on SOM content and plant available water capacity. We have no explanation for the statistically significant increase in the C:SOM ratio from young to old grassland topsoil. The increase might be associated with greater humification of the ageing SOM. In future research the chemical nature of soil organic matter should be worth measuring.

As hypothesized, we found improved soil structure in old compared with young grassland soils (Table 1), which can have a positive effect on plant available water (Acín-Carrera et al., 2013). Root tip density in the soil at 10 cm and at 20 cm was, in contrast with our expectations, not significantly different between young and old grassland. From the total number of root tips the proportion of root tips at 10 cm soil depth was significantly larger in old (64%) compared with young (57%) grassland (Table 1). We also found significant correlations between the proportion of root tips and soil moisture, SOM content and % crumbs in the 0–10-cm soil layer (positive correlations) and between soil bulk density and % angular blocky elements in the 0–10-cm soil layer (negative correlations). It might be that in old grassland, moisture and nutrients (SOM) are more concentrated in the 0–10-cm soil layer, causing a larger proportion of root tips at 10 cm, which also results in better soil structure in this soil layer. Conversely, better soil structure in old grassland topsoil causes a larger proportion of root tips at 10 cm in comparison with young grassland where the soil structure of the 0–10-cm soil layer is worse (percentage of crumbs is smaller; Table 1).

The percentage of crumbs in the topsoil (0–10 cm) layer was significantly positively correlated ($r = 0.56$, $p = 0.05$; Table 4) and the percentage of angular blocky elements significantly negatively correlated ($r = -0.65$, $p = 0.02$; Table 4) with soil moisture content. This is comparable with the results in clay soils reported by Sonneveld et al. (2014).

In contrast to our hypothesis, we did not find a difference in root biomass between young and old grassland. This is comparable with the results of Necpálová et al. (2014) on soils with a clay loam texture; that is, no significant differences in root biomass between 7-year-old permanent and renovated grassland in the 0–15 and 15–30-cm soil layers. In a few other field studies, root biomass tended to increase with grassland age (Whitehead et al., 1990; Acharya et al., 2012; Chen et al., 2016). However, in these studies grassland age did not exceed 15 years.

Rooting is influenced by grass species, grass cultivars and grassland management (Deru et al., 2014; Hoekstra et al., 2019; Hoogsteen et al., 2020). Root biomass in the 0–30-cm layer in the present study was on average 7.6 Mg AFDM ha^{-1} with a range from 4.3 to 15.7 Mg AFDM ha^{-1}. So, the reason that we did not find a difference in rooting between young and old grassland in our study might be that the variation in root biomass between the different grasslands was too large due to the limited number of cores measured per plot or caused by other factors than the age of the grassland. We tried to prevent the latter by the selection criteria for the different grasslands, as described in the Material and Methods section.

4.2.2 | Indicators for response to excess rainfall

As hypothesized, the old grassland soils showed the potential for greater resistance to heavy rainfall in comparison with the young grassland soils, as indicated by the larger water infiltration rate (11.1 mm water min^{-1} in old vs. 3.7 mm water min^{-1} in young grasslands), a larger percentage of crumbs in topsoil and more macropores at 20 cm soil depth (Table 1). Macropores at this soil layer increase infiltration capacity under subsoil (Jarvis et al., 2017). These results are comparable with the results of a study in New Zealand where the physical regeneration of the topsoil was monitored over a 4-year period after establishment of permanent pasture where macroporosity and water infiltration rate significantly increased from year one to year four (McLenaghen et al., 2017).

Penetration resistance was not significantly different between young and old grassland. The topsoil of both young and old permanent grasslands in the present study was not compacted, because the penetration resistance did not exceed the threshold of 1.5 Mpa (Table 1), which defines a soil as compacted (Carter & Kunelius, 1998).

5 | CONCLUSIONS

Our comparison of the effect of young versus old grassland on clay soil on the soil ecosystem services of climate change mitigation and adaptation in response to periods of drought and heavy rainfall leads to the following conclusions, in line with our hypotheses, unless stated otherwise.

- Old grassland topsoil contained more carbon compared with young grassland topsoil.
- The carbon sequestration rate of young grassland topsoil was larger than that of old grassland topsoil.
- In all resampled grasslands, carbon stock was increased after 4 years.
- Initial carbon stock appeared to determine the carbon sequestration rate.
- Old grassland soils appeared more resistant to periods of drought than young grassland soils, as expressed by soil structure, soil moisture and SOM content. However, in contrast with our hypothesis, root biomass and root tip density were not different between young and old grassland.
- Old grassland soils were more resistant to heavy rainfall in comparison with young grassland soils, as expressed by water infiltration rate, soil structure and macroporosity. None of the grasslands showed severe soil compaction.
- Extending grassland age can positively contribute to climate change mitigation and adaptation, but by how much warrants further study.

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AUTHOR CONTRIBUTIONS

Goaitske Iepema: Conceptualization; data curation; funding acquisition; investigation; writing-original draft; writing-review & editing. **Nyncke Hoekstra:** Formal analysis; supervision; writing-review & editing. **Ron de Goede:** Conceptualization; supervision; writing-review & editing. **Jaap Bloem:** Conceptualization; funding acquisition; investigation; writing-review & editing. **Lijbert Brussaard:** Conceptualization; supervision; writing-review & editing. **Nick van Eekeren:** Conceptualization; funding acquisition; investigation; methodology; supervision; writing-review & editing.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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