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No-Till Mitigates SOC Losses after Grassland Renovation and Conversion to Silage Maize

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Abstract: Many studies recommend no-till (NT) to increase soil organic carbon (SOC) in the topsoil (<30 cm) of arable land to counterbalance greenhouse gas emissions. Its potential use to mitigate SOC losses during conversion and renovation of grassland ecosystems in the top meter soil is yet to be determined. The SOC dynamics of a 10-year-old grassland converted to silage maize (CM) and renovated and seeded (GR) using either conventional tillage (CT) or NT were compared to an undisturbed grassland control (GC) for 7 years, across three fixed soil depth increments (0–30, 30–60, 60–90 cm). The annual C inputs (C_{input}) from crop residues were further analyzed. The systems were either non-fertilized (N0) or fertilized with mineral N (N1) according to a demand of 180 and 380 kg N ha^{-1} year^{-1} in the silage maize and grassland systems, respectively. For the 7-year period, the renovated grassland using NT ensured maintenance of the initial SOC in the topsoil, while a conversion toward arable cropping resulted in SOC losses, regardless of the tillage method. The use of NT during conversion significantly reduced these losses from 2.5 Mg ha^{-1} year^{-1} to 1.8 Mg ha^{-1} year^{-1}, for a 28% reduction compared to CT. In the subsoil (30–90 cm), SOC remained stable and was not affected by the cropping systems nor by the tillage method. Reduced annual C_{input} was found as the main factor affecting SOC losses after grassland removal, regardless of the tillage method. Our findings highlight the potential of NT to mitigate annual SOC losses after grassland conversion if annual C_{input} remains high.

Keywords: land use change; conservation tillage; climate change mitigation; soil carbon sequestration; soil fertility

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1. Introduction

The conversion and renovation of grasslands using conventional tillage (CT) often result in high soil organic carbon (SOC) losses in agricultural ecosystems [1]. Conventional tillage promotes both disruption of soil aggregates, exposing aggregate-protected SOC to microbial activity, and enhanced conditions for faster SOC mineralization and erosion [2]. These losses can be up to two-thirds of the initial SOC levels within a few decades after conversion to arable land use and up to a quarter within the first years after a single renovation event, particularly in the topsoil [3–5]. With these losses, soil fertility is threatened, and high amounts of carbon dioxide (CO_2) and other greenhouse gases (GHGs) of greater warming potential are released, contributing to global warming [6,7].

To counter this problem, ceasing the use of CT and replacing it with less intensive tillage methods, such as reduced (RT) and no-till (NT) during conversion and renovation, has been recommended as a suitable method not only to reduce losses but also to conserve SOC and soil fertility [8,9]. Despite these claims, animated debates are arising concerning the efficacy of NT over CT in promoting SOC sequestration in arable systems. Several studies suggest that replacing CT with NT could increase SOC stocks by at least 0.3 Mg C ha^{-1} $year^{-1}$ in the topsoil (<30 cm) or by 4 to 10% across temperate and tropical climates [10,11]. Other studies argue that this increase is limited to the top 10 cm soil, and thus, these apparent benefits disappear when larger soil depths are taken into account, resulting in a simple redistribution or stratification of SOC and soil mass without real gains in SOC [12–15].

While contrasting evidence exists for arable land, research investigating the use of NT for SOC conservation during grassland conversion and renovation for the top and subsoil is still very limited and non-conclusive. Research conducted under drought conditions or low SOC levels found that NT can conserve SOC after grassland conversion at the same level as an unplowed adjacent grassland site, at 30 cm soil depth [9,16]. Nonetheless, other research works combining results from different sites reported similar SOC losses using both NT and CT after conversion of natural sites to arable land when the subsoil was considered at 60 cm soil depth [12,13].

Subsoil SOC can contribute one-third to more than half of the top-meter SOC stocks [17,18]. It can be affected by grassland conversion through changes in vegetation, e.g., when perennials are displaced by annual crops with lower C inputs (C_{input}), particularly from belowground [19–21]. Perennials can mobilize high C_{input} through the soil profile by growing deep taproots and by high rhizodeposition production, favoring an increase in SOC [22–26]. Increasing subsoil SOC has been observed in the conversion of arable land to grassland, whereas the opposite has been reported in grassland conversions to arable or to other land uses [5,20,27]. Subsoil SOC can also be affected by the tillage method, as this affects both crop residue incorporation and root growth patterns. While CT incorporates crop residues deeper in the soil and supports root penetration, favoring C_{input} in the subsoil, NT accumulates these on or near the soil surface, limiting C_{input} only to the topsoil [12,28–30]. Many studies have been limited to determining SOC changes in the topsoil where plowing takes place [15,31]. This approach neglects the SOC changes occurring in the subsoil, creating high uncertainties regarding the efficacy of replacing CT with NT as a method to mitigate SOC losses following grassland removal [8,15,32]. It is thus necessary to assess the effects of grassland conversion and renovation using different tillage methods on SOC across the top-meter profile, as both the top and subsoil SOC can turn into important sink or source of CO_2 in different ways depending on the management applied [12,19,22].

In this study, we investigated the effects of the tillage method on SOC dynamics after conversion to silage maize and after renovation of a 10-year-old permanent grassland for the 0–90 cm soil depth over a 7-year period. We hypothesized that (1) NT can reduce SOC losses after grassland conversion to arable land; (2) the conversion and renovation of permanent grassland negatively affects the subsoil SOC; and (3) SOC changes are negatively affected by the reduction in annual soil C_{input} in the unplowed systems.

2. Materials and Methods

2.1. Study Site

The long-term experiment was conducted at the research farm "Hohenschulen" of the Christian-Albrechts-University of Kiel in northwest Germany (54°19′07″ N; 9°59′32″ E; 30 m a.s.l.). The climate is maritime, with a mean annual temperature of 9.4 °C and a mean annual precipitation of 759 mm (1991–2020). The dominant soil type is stagnic Luvisol (according to the FAO Guidelines for soil description, 2006) with a sandy loam to loam texture (Table 1).

Before the study, a 10-year-old permanent grassland experiment organized in a split-plot design was located on the field between 2005 and 2014, comparing different cutting frequencies (3 vs. 5 cuts $year^{-1}$) and N rates (0 vs. 360 kg N ha^{-1} $year^{-1}$) in 6 × 6 m plots. The seeded grassland consisted of a multi-species mixture of grasses and legumes, including *Lolium perenne*, *Festuca pratensis*, *Poa pratensis*, *Phleum pratense*, *Dactylis glomerata*, *Trifolium repens and Medicago sativa*. By 2014, the botanical composition of the swards was dominated by *Medicago sativa* and *Trifolium repens* from the legume species and by *Dactilys glomerata* and *Lolium perenne* from the grass species in the 3- and 5-cut treatments, respectively [23,34]. Despite the differences in botanical composition, no significant differences in SOC stocks were found between the treatments at different soil depths prior to initiation of the experiment (Supplementary Table S1).

Table 1. Soil characteristics at the study site. Adopted from Zink et al. 2010 [33].

Layer (cm)	Texture (%)			pH	Soil Type	Horizon
	Sand	Silt	Clay	in CaCl2		
0–28	54	30	16	6.2	Highly loamy sand	Ap
28–45	50	31	19	6.5	Medium sandy loam	Btg1
45–70	49	33	18	6.5	Medium sandy loam	Btg2
>70	55	31	14	-	Highly loamy sand	Bg

In spring 2015, three cropping systems were introduced into the grassland sward randomly. Randomly, plots of 6 × 6 m were either converted to continuous silage maize Zea Mays L. (CM), using either CT or NT (named CT-CM and NT-CM, respectively), or renovated and re-seeded with *Lolium perenne* and *Trifolium repens* using NT (NT-GR). The undisturbed permanent grassland served as the control treatment (GC). Further, the systems were either non-fertilized (N0) or fertilized (N1) with calcium ammonium nitrate (CAN) according to an expected demand in the silage maize and grassland systems of 180 and 380 kg N ha^{-1} year^{-1}, respectively. The number of replicates per treatment used after 2015 is provided in Appendix A (Table A1).

During conversion and renovation, all plots except for the GC were sprayed with glyphosate-containing herbicides (Round-Up Ultra and Round-Up Powerflex). Plots with the CT treatment were subsequently rotovated (10 cm depth), moldboard plowed (25–30 cm depth) and treated with rotary harrow for seedbed preparation. The plots with the NT treatment received no soil cultivation. Seeding in the CM systems was performed with the maize variety Ronaldinio (KWS), using a direct drilling seeder (Horsch, Germany), at a rate of 10.5 seeds m^{-2}, a row distance of 0.75 m and a sowing depth of 5 cm. In the GR treatment, a 5:1 seed mixture of *Lolium perenne* to *Trifolium repens* was sown using a direct seeder (Vredo, Netherlands) at a rate of 40 kg ha^{-1}, once, in spring 2015. Nutrients in addition to N (P, K, Mg and S) were supplied in sufficient amounts at the beginning of the growing season to avoid nutrient deficiencies. Further details about the management can be found in Struck et al. (2019) [35].

2.2. Measurements

2.2.1. Soil Sampling and Carbon Analysis

Soil sampling was performed annually from 2014 (the year prior to conversion) to 2021 at the end of each growing season in fixed soil-depth increments: 0–30, 30–60 and 60–90 cm. To do so, samples from each plot were collected using a hydraulic-driven soil auger (inner Ø 2 cm). Afterward, soil samples were oven dried at 30 °C and sieved to pass a 2 mm mesh size for further analyses. The C and N contents (%) were determined using a CN elemental analyzer (Vario Max CN, Elementar) (ISO). No carbonates were detected within the soil samples after testing with HCl. Thus, the estimated total C was equal to SOC. The soil sample results from 2018 had to be discarded due to technical problems.

Soil bulk density measurements were conducted for the years 2014, 2015, 2016 and 2020 in all the treatments in the topsoil (0–30 cm). To do so, four soil cores from randomly chosen plots were collected using a steel cylinder (Ø = 5.1 cm; L = 5 cm), according to DIN ISO 11272 (HBU, 1998). No significant differences in soil bulk densities were found between the treatments and the years. Thus, a mean soil bulk density of 1.6 g cm^{-3} was adopted in all treatments in the 0–30 cm soil depth. For the 30–60 and 60–90 cm soil depths, soil bulk densities of 1.77 and 1.70 g cm^{-3} were used as reported in Zink et al. (2010) [33] for the study site. These values were adopted, as no significant differences between tillage treatments were reported in the subsoil at the study site by Hartmann et al. (2012) [36]. The SOC stocks calculations for each soil depth were performed following Equation (1):

$$\text{SOC}\left[\text{Mg C ha}^{-1}\right] = \text{C content }[\%] \times \text{Soil Bulk Density}\left[\text{g cm}^{-3}\right] \times \text{Soil Depth }[\text{cm}] \quad (1)$$

2.2.2. Plant biomass sampling and C input calculations

The annual crop yields (Y_P) in the CM systems were obtained using a two-row plot harvester (Kemper 1200, Haldrup, Logstor, Denmark) at the silage maturity stage, at a stubble height of 25 cm. After harvesting, the stubbles were left on the soil surface in the NT system, whereas in the CT system, these remained on the field until their incorporation via tillage during spring. The grassland systems were harvested with a cutting frequency of 4 cuts year^{-1} (May, July, August and October), using a forage plot harvester (Haldrup, Logstor, Denmark) at a cutting height of 5 cm. Dry matter yields were determined from fresh matter subsamples that were oven dried at 58 °C to estimate their humidity content. The mean Y_P measured for the period 2015–2021 are provided in Supplementary Table S2.

To obtain yield-based allocation coefficients, total above- (AGB) and belowground biomass (BGB) measurements conducted during and after the growing seasons (March–October and November–March, respectively) by Struck (2018) [37] in the years 2015 and 2016 were used. During the growing season, AGB samples were taken at each grassland cutting and maize harvest from an area of 0.25 and 1 m^2 in 4 plots, at a cutting height of 5 and 25 cm, respectively, and separated into harvestable biomass and stubbles. In the same plots, BGB was measured using the ingrowth core method [38], starting from mid-March and following the grassland cutting intervals (every 6 weeks). Roots were washed from the soil using a hydro-pneumatic elutriation system [39] and collected using a mesh size of 0.63 mm. After drying the samples, the ash-corrected dry matter and carbon contents were determined for both AGB and BGB samples. Harvest (HI) and stubble indices (SI) were calculated by the ratio of Y_P and stubbles to total AGB, respectively. The R:S was determined by the ratio between the annual sums of the ash-corrected BGB and AGB. To determine the BGB produced after the growing season, the same method was applied as explained above, using the cumulative biomass of three sampling dates (November, February and March). A detailed description of the measurements is available in Struck (2018) [37].

The annual C_{input} were estimated in each cropping system by using the measured Y_P and yield-based allocation coefficients to calculate the C_{input} produced above- (AG) and belowground (BG) during the growing season, and by adding fixed inputs to account for the residues produced AG and BG in the grassland systems after the growing season (AW–AG_{input} and AW–BG_{input}, respectively). For the AG residues produced during the growing season, stubbles were calculated using the estimated SI in the CM systems, whereas in the grassland systems, the harvest residues and litter deposition were calculated by using a SI equal to 15% on a DM basis, as proposed by Bolinder et al. 2007 [40]. The BG residues produced in the same season were calculated using the mean ash-corrected R:S ratios measured in the first two years. For the fixed inputs in the grassland systems, the BGB measured after the growing season in the first two experimental years was used. Since AGB was not measured in this period, we assumed these inputs using the AGB measured after the growing season from a similar grassland experiment located at a nearby experimental farm [26,41]. To account for the extra inputs derived from rhizodeposition, an additional 50% of the total BGB produced during and after the growing season was added to the BG C_{input} calculations, as suggested by Pausch and Kuzyakov (2018) [42]. Ash-corrected dry matter values were used for the C_{input} calculations, as these are preferred to total dry matter [40]. Carbon content of 48% was calculated on the ash-corrected AG and BG plant material. The steps and equations used to calculate the annual soil C_{input} in each cropping system using the Y_P, yield-based allocation coefficients and fixed inputs are provided in Supplementary Tables S3 and S4.

2.3. Statistical Analysis

To determine their effects on SOC dynamics, the factors cropping system, N rate and soil depth) were tested for their interaction with time (year) using analysis of covariance (ANCOVA) in a linear mixed-effects model. To do so, the nlme package [43] was used in R statistical software [44]. Normal distribution and heteroscedasticity of the data with respect to the different treatments were assumed in the statistical models after graphical residual analysis. Two separate ANCOVAs were performed: one considering soil depths and another summing the SOC stocks across the fixed depth increments (0–90 cm). When the study factors significantly interacted with time, separate regressions were estimated and tested for whether the ΔSOC statistically differed from zero. Additionally, a Tukey contrast was used for mean ΔSOC comparisons using the multcomp package [45]. All the tested effects were considered significant with a p-value < 0.05.

The relationships between the ΔSOC and the corresponding annual soil C_{input} were fitted to a linear and a non-linear asymptotic regression model in the 0–30 cm soil depth increment, using the observations of the unplowed systems (GC, NT-GR and NT-CM). Both approaches estimate the SOC sequestration efficiency and the limits to soil C sequestration [46,47].

3. Results

3.1. Effects of Grassland Conversion and Renovation on Soil Organic Carbon Dynamics

A significant interaction between the factors soil depth, cropping system and time was found after grassland conversion and renovation in this study (p < 0.0001, Supplementary Table S5). Similarly, across soil depths (0–90 cm), cropping system significantly interacted with time (p < 0.0001, Supplementary Table S6). No interaction was found between N rate and time. Thus, the ΔSOC of the cropping systems were pooled over the N rates.

At the 0–30 cm depth, significant SOC losses over time were observed in CM for both tillage methods (p < 0.0001, Figure 1a). By using NT during conversion, SOC losses were reduced from −2.5 to −1.8 Mg C ha^{-1} year^{-1}, for a 28% reduction in annual SOC losses in comparison to the CT treatment. By the end of the 7-year period, the SOC levels in NT-CM and CT-CM were 14 and 19 Mg ha^{-1} lower than the levels measured before conversion, for a reduction in 18 and 26%, respectively. In contrast, SOC remained unchanged in the topsoil of NT-GR, similar to the GC. In the subsoil (30–60 and 60–90 cm), no changes in SOC were observed after conversion or renovation by using any of the tillage methods. Across the soil depth increments (0–90 cm, Figure 1b), the ΔSOC were significantly different to zero in both NT-CM and CT-CM (p < 0.0001), with SOC losses of −1.58 and −2.63 Mg C ha^{-1} year^{-1}, respectively. In contrast, in the grassland systems, SOC remained stable, with non-significant ΔSOC.

The mean comparisons of ΔSOC between the cropping systems revealed significant differences overall between the grassland and maize systems, and between tillage methods within the maize systems; the grassland systems did not differ in ΔSOC (Figure 2a,b). These differences occurred mainly in the topsoil, whereas in the subsoil, no significant differences between cropping systems were observed.

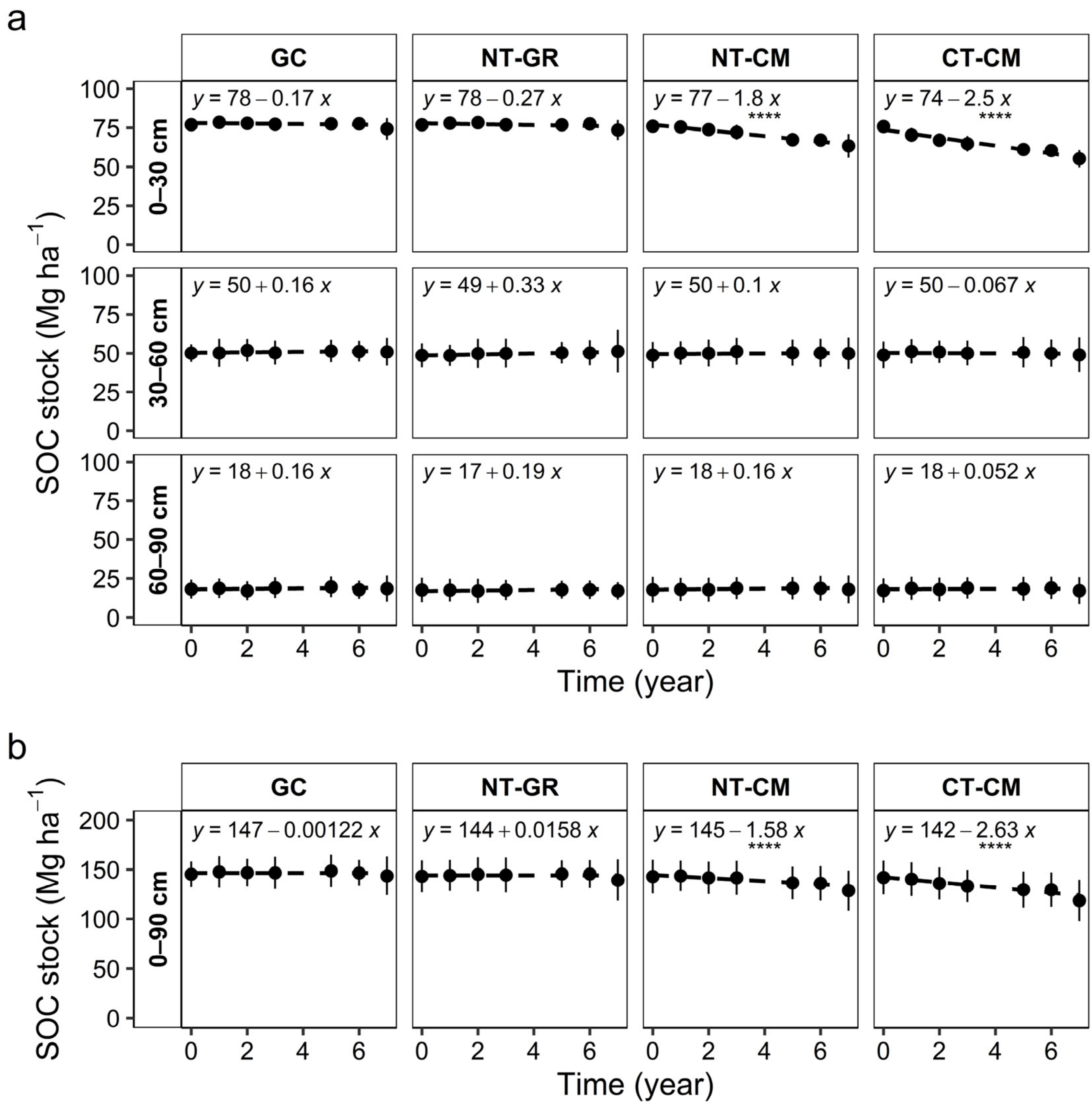

Figure 1. Mean temporal development of soil organic carbon (SOC) stocks (±S.D.) in the different cropping systems, (**a**) for the different soil depth increments (0–30, 30–60 and 60–90 cm) and (**b**) across the fixed soil depth increments (0–90 cm) for the period 2014–2021, pooled over the two N rates. Cropping systems with **** indicate ΔSOC significantly different from zero at $p < 0.0001$. Estimates represent the number of replicates for each treatment, as described in Table A1. GC is undisturbed grassland control, NT-GR is no-till grassland renovation, NT-CM is no-till continuous silage maize, CT-CM is conventionally tilled continuous silage maize; N0 is non-fertilized and N1 is fertilized with CAN according to an expected demand of 180 and 380 kg N ha^{-1} $year^{-1}$ in the silage maize and grassland systems, respectively.

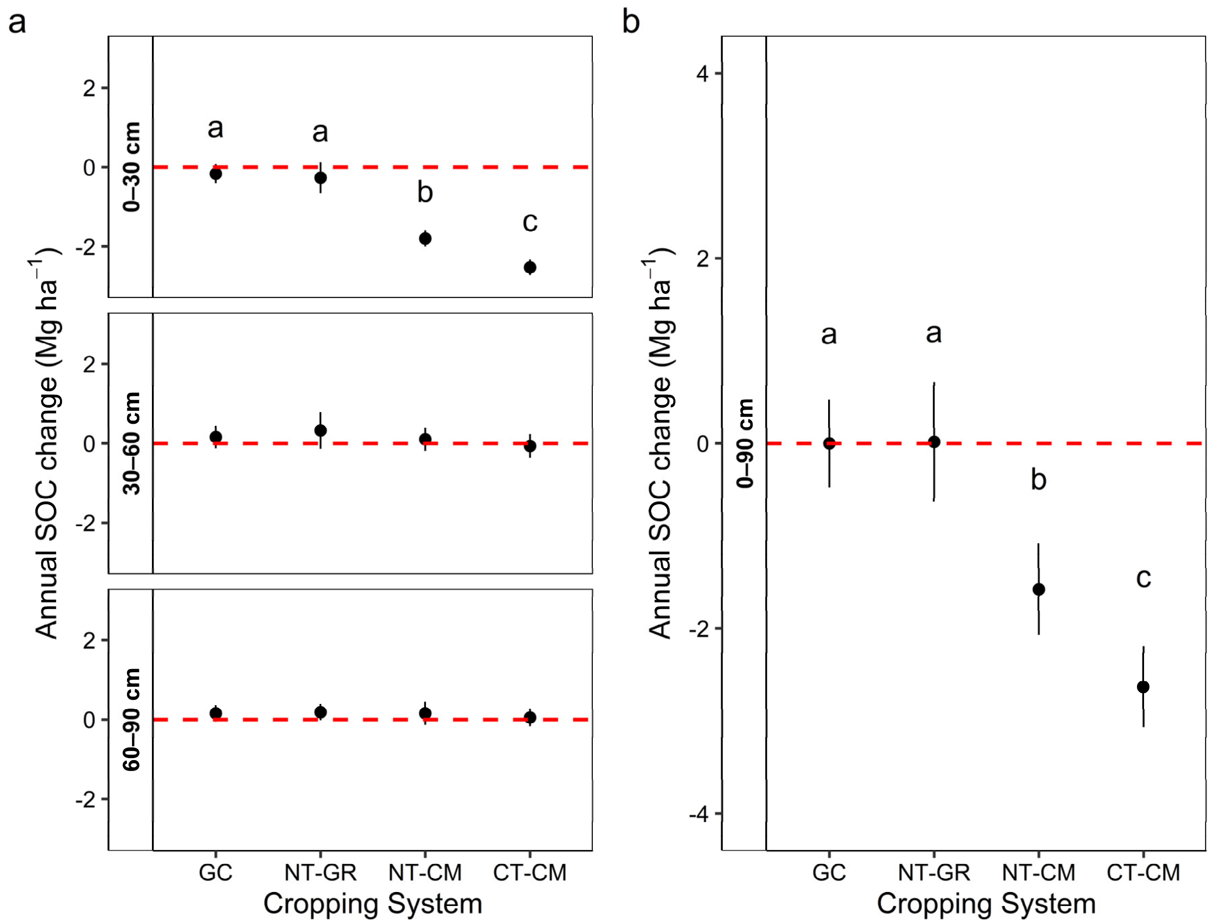

Figure 2. Mean annual SOC change rates (±95% confidence intervals) in the different cropping systems (**a**) for the different soil depth increments (0–30, 30–60 and 60–90 cm) and (**b**) across the soil depths increments (0–90 cm) during the period 2014–2021, pooled over the two N rates. Different letters indicate significantly different mean ΔSOC between cropping systems. Treatment descriptions are provided in Figure 1.

3.2. Effects of Soil C Inputs on Topsoil SOC Dynamics

The total annual C_{input} decreased by 73% (from 6.7 to 1.8) after conversion to silage maize, whereas after grassland renovation, the total C_{input} was comparable to the GC (6.7 compared to 6.3) (Figure 3). Lower C_{input} after conversion occurred mainly due to a 70% reduction in BG C_{input} (from 5.5 to 1.5) compared to a 33% reduction in AG C_{input} (from 1.2 to 0.8) across the N rates (Figure 3a,b) due to the altered root-to-shoot ratios of the following crop. After renovation, BG C_{input} decreased only by 7% (from 5.5 to 5.1), whereas AG C_{input} remained unchanged (1.2 Mg C ha^{-1} year^{-1}). Differences between the annual C_{input} of the two tillage methods in the CM systems were marginal.

In all four systems, N fertilization increased AG C_{input}, while it reduced BG C_{input} except for CT-CM. The grassland systems decreased their annual C_{input} from 8.1 to 5.2 (35%) and from 7.5 to 5 Mg ha^{-1} (33%) in GC and NT-GR, respectively. The CM systems, on the other hand, did not increase their annual C_{input} with N fertilization in the NT-CM, whereas in the CT-CM, a 0.4 Mg ha^{-1} increase was observed.

The relationship between ΔSOC with annual soil C_{input} for the unplowed systems (GC, NT-GR and NT-CM) showed both a significant linear and non-linear relationship in the topsoil (Figure 4). A better fit was obtained using a non-linear asymptotic function compared to the linear function due to a lower score in the Akaike information criteria (AIC). Annual SOC losses (−ΔSOC) were reduced by increasing the annual C_{input}, up to ~5 Mg ha^{-1}. Additional

C_{input} did not produce higher ΔSOC, resulting in SOC equilibrium at 1.6% C content. A reduction in the annual C_{input} in the grasslands at N1 was not different to the ΔSOC at N0. In the maize systems, both N rates produced a similar annual C_{input} and mean negative ΔSOC (SOC losses). Due to the absence of ΔSOC, any effects of the C_{input} across the unplowed systems in the subsoil were discarded.

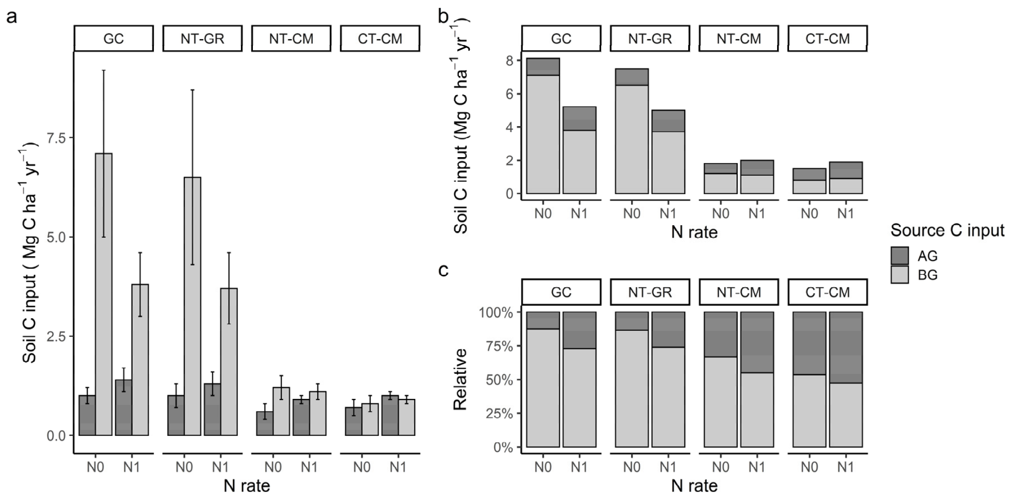

Figure 3. Mean annual C inputs from above-(AG) and belowground (BG) crop residues (±S.D.) (**a**,**b**) and relative values (**c**) estimated for the period 2015–2021 in the different cropping systems and N rates using annual yields and yield-based allocation coefficients, for the topsoil. Treatment descriptions are provided in Figure 1.

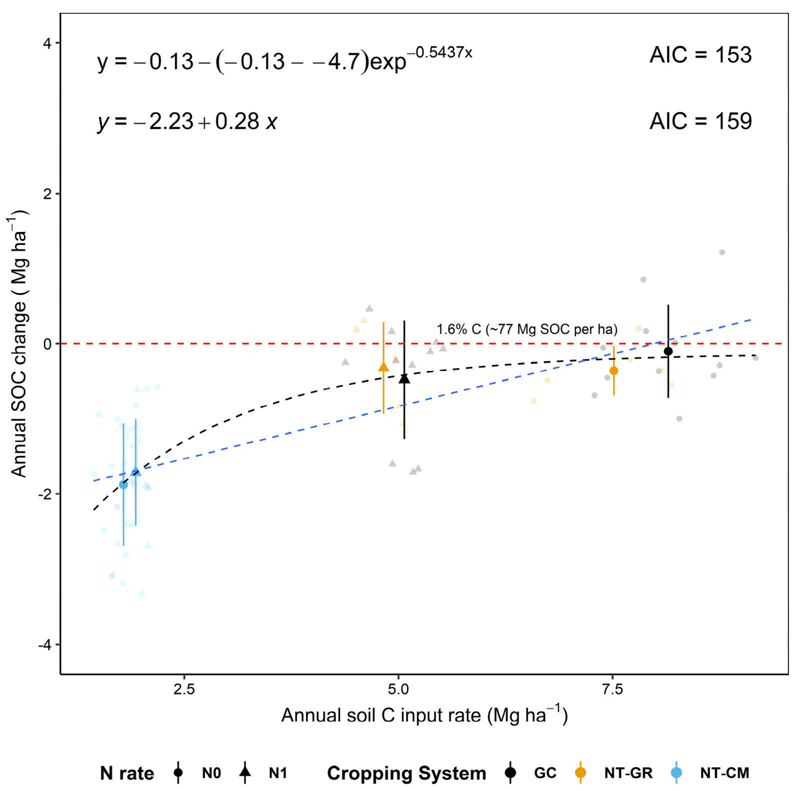

Figure 4. Soil organic carbon change rates (ΔSOC) expressed as a function of annual soil C input rates for the unplowed systems (GC, NT-GR and NT-CM). Observations indicate the replicates for the different cropping systems and N rates. Treatment descriptions are provided in Figure 1.

4. Discussion

4.1. No-Till (NT) Effects on Topsoil SOC Dynamics after Grassland Conversion and Renovation

In this study, it was hypothesized that NT can reduce SOC losses after grassland conversion to arable land and maintain SOC stocks when used for grassland renovation. In agreement with the initial hypothesis, annual SOC losses were significantly reduced by almost 30% when NT was used instead of CT, despite the substantial losses observed (Figure 1).

These results present both similarities and differences with those of previous research works in different site conditions. While our results show SOC losses using both tillage methods, a 3-year study by Franzluebbers and Stuedemann (2008) [16] found SOC losses in CT but not in NT during grassland conversion. Moreover, the annual SOC losses in the CT were higher in our study with -2.5 compared to -1.2 Mg ha^{-1} in their study, whereas the absolute difference between CT and NT in our study was only 0.7 Mg ha^{-1} after seven years of land use change to arable land. These results were obtained at a site with a similar soil texture as in the present study but with much higher precipitation (1250 vs. 847 mm year^{-1}) and much lower initial SOC stocks (~35 vs. ~74 Mg ha^{-1}). In another study, no SOC losses were found 6 years after grassland was converted to continuous maize using NT, at a site with high initial SOC stocks (~91 Mg ha^{-1}) but with a different soil texture (silt loam vs. sandy loam) combined with an extended drought period [9].

Environmental differences can be crucial in this context, as the water potential in the soil affects the turnover rates of SOC. Periods of drought can bring microbial activity to its minimum, while wet conditions increase it [5], regardless of the tillage method used. Moreover, these rates can be proportional to the initial SOC content, the soil C balance and the amount of silt and clay protecting the SOC [30]. Soils high in SOC are often rich in labile carbon fractions and are thus more susceptible to high decay rates during the first years of grassland conversion compared to soils with low initial SOC and higher proportions of inert SOC. This could potentially explain the differences between the studies. Accordingly, the turnover of SOC in the soil depends on many factors, making mechanistic dynamic models necessary in order to predict potential losses after management changes. A previously applied soil carbon model calibrated with soil carbon and yield data from the presented experimental site confirmed the decay of SOC regardless of the tillage method used [28].

Contrary to the results observed for grassland conversion, using NT during grassland renovation (NT-GR) conserved SOC stocks and showed no differences in the 0–30 cm soil depth compared to the GC (Figures 1 and 2). Research works using NT for SOC conservation during grassland renovation are scarce. The existing studies are inconclusive regarding the advantages of NT over CT during grassland renovation. For instance, Linsler et al. (2013) [48] reported no differences in SOC stocks between grassland that was renovated using CT and the adjacent undisturbed grassland system sampled 5 years after renovation. Similarly, no changes in SOC stocks over time in either NT or CT during grassland renovation in the 0–30 cm soil depth were observed, sampled 3 years after renovation [49]. In contrast, a 25% reduction in SOC stocks was observed after 2.5 years with a single use of CT for grassland renovation compared to the adjacent undisturbed grassland in a study located in a temperate wet region [3]. Our study did not include a grassland renovation treatment using CT; however, high decay rates in the first year after grassland renovation using CT were found close to our experimental site at similar soil conditions in a long-term grassland renovation trial [50]. Thus, we can assume that replacing CT with NT for renovation can significantly decrease the risks of high carbon losses in the initial phase of management and, consequently, the risk of high nitrogen emissions in the environment [6]. By comparing it with GC, it can be deduced that SOC balance was not affected by the grassland renovation measure using NT in the mid-term.

4.2. Land Use Change and Tillage Effects on the Subsoil SOC Dynamics

It was hypothesized that due to changes in vegetation and residue distribution caused by tillage treatments, the conversion and renovation of permanent grassland negatively affect subsoil SOC. Contrary to what was expected, in this study, subsoil SOC was not affected by the grassland conversion or renovation nor by the tillage method over the measured period (Figures 1 and 2). The same results were obtained by Jarvis et al. (2017) [51], who observed significant effects of different ley durations, varying from 1 to 5 years, in 6-year rotations on topsoil SOC stocks but no effects on the subsoil SOC, after 54 years of establishment. Similarly, Syswerda et al. (2011) [52] found no differences in subsoil SOC comparing annual cropping systems using different tillage methods with perennial crops and successional systems, after 12 years of establishment. Meanwhile, Luo et al. (2010) observed similar SOC losses between NT and CT after conversion of natural to arable land in the subsoil [12].

Despite the higher C_{input} usually observed in soils under both permanent grassland and the grassland phase of a ley-arable rotation compared to arable soils [20,26,53], the absence of SOC changes in this and other studies supports the existence of factors other than management and annual C_{input} that exert a great influence on the subsoil SOC dynamics. The absence of SOC changes observed over time might be attributed to the influence of site-specific conditions associated with soil texture, structure and pedological processes that affect SOC in the soil profile [18,54,55]. The amount of soil mineral-binding particles that protect SOC from microbial degradation and the existence of anoxic conditions that inhibit microbial activity can increase the turnover time of SOC in the subsoil [56]. Likewise, the presence of high soil bulk densities impeding root penetration due to both anoxic conditions and high soil compaction might also limit the influence of C_{input} on the subsoil. For instance, changes in subsoil SOC stocks for a loamy site but not for a clayey site were reported after using similar rotations and N fertilization rates at different sites [57]. Similarly, after observing the subsoil SOC dynamics of 24 sites, Poeplau and Don (2013) [55] found the processes affecting subsoil SOC varied between the sites. At our site, the presence of a stagnic subsoil (Table 1) could have impeded both the decomposition of SOC and the root growth of both the grassland and maize systems equally, keeping SOC relatively stable over time. In addition to this, the nature of C compounds in the subsoil is highly recalcitrant, dominated mostly by slow and passive pools of mean residence time that range from several hundreds to thousands of years [18]. This means that any change from vegetation and thereby the amount of fresh C_{input} entering the soil will have a faster impact on the topsoil than on the subsoil, as the decoupling of C_{input} with the SOC cycling increases with depth [58]. This makes the subsoil SOC less sensitive to land use change or to management than the topsoil SOC [55,58]. Assuming the introduction of deep rooting crop species into shallow-rooted systems or inversely regulating subsoil SOC differently through the different belowground C_{input} sources, it would probably require a longer time to observe changes occurring due to grassland conversion than the 7-year period of this study. Based on these observations, the potential site-specific effects on subsoil SOC turnover time and on translocation of freshly added C_{input} would require to be analyzed in addition to the treatment effects to draw definite conclusions [18,55,58].

4.3. Plant-Derived C Inputs Affect Annual SOC Changes (ΔSOC)

It was hypothesized that SOC changes are negatively affected by the reduction in annual soil C_{input} in the unplowed systems. Both significant linear and non-linear asymptotic relationships between ΔSOC and annual C_{input} were found across the unplowed systems, with higher SOC losses (negative ΔSOC) related to lower annual C_{input} obtained in the maize systems after conversion and with SOC conservation in the grassland systems related to similar annual C_{input} (Figures 3 and 4).

The use of NT during conversion and renovation offers the opportunity to compare both the reduction and the maintenance of C_{input} to the levels existing before a management change. According to studies, the equilibrium (i.e., steady state) levels of SOC are determined by the balance between the inputs and losses of organic C [59]. This means that a reduction in C_{input} to soil could lead to SOC losses even when decomposition rates remain unaltered due to the absence of tillage [60]. In support of this, the SOC equilibrium maintained in the grassland systems was interrupted mainly as a result of a reduction in BG C_{input}, which represented 70 to 90% of the total inputs in the grassland systems (Figure 3c). As opposed to conversion, SOC levels after grassland renovation were maintained due to similar C_{input}. These results are consistent with those found in a 3-year study where the conversion of grasslands to arable land using NT led to a reduction in BG C_{input} of 43% in the top meter and to the degradation of the labile C pools, which is used as an early indicator for the deterioration in soil quality [21]. Another study comparing NT continuous wheat and NT wheat–fallow systems with a native undisturbed grassland also observed 17 and 22% lower SOC levels at the top 30 cm in the annual systems compared to the undisturbed grassland, respectively, due to the 90% lower BG C_{input} [61]. This suggests that, with the use of NT alone to minimize the turnover rates, it is unlikely that SOC can be conserved to the levels seen prior to grassland conversion unless the C_{input} remains unchanged or minimally reduced. On the other hand, to increase the SOC stocks in cropland soils under NT, it would be necessary to increase the C_{input} either by increasing the cropping frequency from single to double cropping [12,62] or by rotating annual crops with crop species high in belowground net primary productivity, such as perennial forages [28,30,63]. This confirms the expected importance of maintaining C_{input} levels to conserve SOC stocks after grassland conversion.

5. Conclusions

In this study, the measurement of all relevant sources of C inputs to the soil allowed a deeper insight on SOC regarding the impact of land use change and tillage method during grassland conversion and renovation for the top-meter soil. While no-till (NT) could not conserve SOC after grassland conversion, it strongly reduced its losses by 28% compared to conventional tillage (CT) and conserved SOC during renovation under the conditions of the study site. Subsoil SOC was not affected by the tillage method, which is likely to be attributed to the lack of carbon inputs to the subsoils, as both grass and maize systems expand their rooting systems predominately in the topsoil. Overall, the much lower soil carbon inputs in the maize (1.8) system compared to the grassland (6.7 Mg C ha^{-1}) system was the reason for the observed SOC losses under NT-CM. Since topsoil SOC followed an asymptotic relationship with increasing annual C inputs, a SOC equilibrium was indicated. This strengthens the need to maintain annual C input high in order to conserve SOC even when no-till approaches are implemented. Our results indicate that NT is a suitable option for mitigating SOC losses when a grassland conversion and renovation measure seems unavoidable.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12081204/s1, Table S1: Analysis of variance (ANOVA) evaluating the effects of cutting frequency and N rate on the soil organic carbon (SOC) stocks at different soil depths (0–30, 30–60 and 60–90 cm) in the 10-year-old grass sward in year 2014. Table S2: Mean annual yields (Y_P) with standard deviations (S.D.) measured for the period 2015–2021. Table S3: Yield-based allocation coefficients and fixed inputs used to calculate the annual soil C inputs (C_{input}) from plants during and after the growing season, respectively. Table S4: Steps and equations used to calculate annual soil C inputs (C_{input}) in each cropping system, using the annual yields (Y_P) from Table S2 and the yield-based allocation coefficients and fixed inputs from Table S3. Table S5: Analysis of covariance (ANCOVA) evaluating the interaction between cropping system, N rate and soil depth with time on soil organic carbon (SOC) stocks after conversion and renovation of 10-year-old permanent grassland. Table S6: Analysis of covariance (ANCOVA) evaluating the interaction between cropping system and N rate with time across soil depth increments (0–90 cm) on soil organic carbon (SOC) stocks after conversion and renovation of 10-year-old permanent grassland. Table S7: Analysis of covariance (ANCOVA) evaluating the influence of the covariate annual soil

C_{input} on annual SOC changes (ΔSOC) at the 0–30 cm soil depth after conversion and renovation of 10-year-old permanent grassland in the unplowed system (GC, NT-GR and NT-CM).

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Appendix A

Table A1. Number of replicates for the different cropping systems and N rates used in the current study, after their introduction into the 10-year-old permanent grassland in 2015. Treatment descriptions are provided in the text.

Cropping Systems from 2015	N Rate	Permanent Grassland (2005–2014)				Treatment Replicates
		Cutting Frequency × N Rates				
		3 × 0	5 × 0	3 × 360	5 × 360	
GC	N0	3	3	3	3	12
	N1	3	3	3	3	12
NT-GR	N0	3	3	3	3	12
	N1	3	3	3	3	12
NT-CM	N0	3	3	7	7	20
	N1	2	2	6	6	16
CT-CM	N0	3	3	6	6	18
	N1	3	3	6	6	18
Total number of replicates		23	23	37	37	120

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