Assessing the effects of no-till on SOC dynamics throughout the soil profile after grassland renovation and conversion to silage maize

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Abstract. Land-use change (LUC) and conventional tillage (CT) result in the reduction of the high soil organic carbon (SOC) stocks stored in grassland ecosystems during their conversion and renovation, contributing to global warming. While plenty

10 studies show the use of no-tillage (NT) as a promising option to increase the topsoil SOC stocks of arable lands, its potential to conserve SOC during grassland conversion and renovation events has been poorly investigated. Further, the effects of LUC and tillage methods on the SOC dynamics have been limited to the topsoil by most studies, thus overlooking their impact on the subsoil where significant amounts of SOC are stored, and changes in vegetation and residue distribution can negatively affect these. In this study, a 10-year-old grassland was converted to continuous silage maize (CM) using NT (NT-CM) and CT

15 (CT-CM), and renovated using NT (NT-GR), while some part remained undisturbed as a control (GC). The systems were either non-fertilized (N0) or fertilized according to a demand of 180 and 380 kg N ha^{-1} yr^{-1} (N1) in the silage maize and grassland systems, respectively. SOC stocks were measured annually and annual SOC changes (ΔSOC, in Mg C ha^{-1} yr^{-1}) were calculated for different soil layers (0-30, 30-60 and 60-90 cm) and across the whole profile (0-90 cm) over a 6-year period (2014-2020). Annual soil carbon inputs (C_i) via plant residues were quantified and related to ΔSOC. Results showed that

20 cropping systems significantly affected SOC dynamics over time. At 0-30 cm, SOC stocks were significantly reduced after conversion using both tillage methods, however, 44 % lower annual losses were obtained in NT-CM (-1.5 Mg C ha^{-1} yr^{-1}) compared to CT-CM (-2.7 Mg C ha^{-1} yr^{-1}). Conversely, SOC stocks remained unchanged after NT-GR same as the GC. In the subsoil, SOC stocks increased under GC (1.1 Mg C ha^{-1} yr^{-1}) and remained unchanged in the other systems. Across the whole profile, SOC stocks increased in GC, remained stable in NT-GR, and decreased in NT-CM and CT-CM with mean annual

25 change rates of 1.3, -0.1, -1.9 and -3.4 Mg C ha^{-1} yr^{-1}, respectively. The differences in ΔSOC between the unploughed systems (NT-GR, NT-CM and GC) were strongly related to the annual soil C_i from plant residues in the topsoil. Our findings highlight the great potential of NT to slow down the annual SOC losses after grassland conversion or renovation, and that C sequestration can occur in the subsoil of permanent grasslands when the topsoil C is already saturated. This strengthens the need to consider the SOC changes occurring in the whole profile after a LUC event.

1 Introduction

30 Land-use change (LUC) and conventional tillage (CT) by ploughing often result in the reduction of the high soil organic carbon (SOC) stocks stored in grassland ecosystems. This reduction of SOC stocks can be up to two thirds within a few decades after conversion to arable land use, and up to a quarter within the first years after a single renovation event (Guo and Gifford, 2002; Necpálová et al., 2014; Reinsch et al., 2018a; Kayser et al., 2018). With these losses, soil fertility is reduced and great amounts

35 of carbon dioxide (CO_2) and other greenhouse gases of greater warming potential (e.g., nitrous oxide) are released, contributing to the anthropogenic-related global warming (Velthof et al., 2010; Reinsch et al., 2018b). Reducing or even ceasing the use of CT and replacing it with less intensive soil management, like reduced (RT) and no-tillage (NT) during conversion and renovation are proposed as avenues not only to reduce SOC losses but also to conserve them (Diabate et al., 2018; Kern and Johnson, 1993; Thorn et al., 2011).

40 Some studies suggest that replacing CT with NT can increase SOC stocks by at least 0.3 Mg ha^{-1} yr^{-1} in the top 30 cm soil layer of arable land (West and Marland, 2002; West and Post, 2002). According to IPCC (2019), this replacement can increase SOC stocks by 10 % annually across temperate and tropical wet climates (Ogle et al., 2019b). While some evidence of its benefits is available for changing tillage methods in established arable lands, little research has been done in observing the use of NT for SOC conservation during grassland conversion and renovation, thus, potentially limiting its application when the

45 destruction of grasslands seems unavoidable (Guo and Gifford, 2002). Few findings from limited locations suggest that SOC can be conserved after conversion and renovation, at the same level as an unploughed adjacent grassland site (Franzluebbers and Stuedemann, 2008; Follett et al., 2009). However, more studies are yet required for site conditions like the ones in temperate wet climates, where both high organic carbon inputs and SOC decomposition are combined, and a reduction in crop residue inputs might follow after conversion or renovation.

50 No less important is to consider the effects of LUC and tillage methods on deeper soil layers. LUC can affect SOC in the subsoil indirectly through the change in vegetation when for example perennial forages are displaced by annual crops (VandenBygaart et al., 2011; Ellert et al., 2008; Jobbagy and Jackson, 2000). Both vertical root growth from taproots and high rhizodeposition production in perennial forages can mobilize C below the tillage layer and throughout the soil profile, in the form of dissolved organic carbon (DOC) (Johnson et al., 2006; Lal and Follett, 2009; Nüsse et al., 2018). Increasing SOC

55 stocks in the subsoil have been observed in the arable to grassland conversion (Gentile et al., 2005; Guo and Gifford, 2002), while the opposite was reported for grassland conversion or cessation (Don and Schulze, 2008; MacDonald et al., 2011). Furthermore, tillage methods can affect the vertical distribution of crop residues, thereby affecting SOC in the subsoil. While CT incorporates crop residues into the soil directly, thereby increasing their contact to microorganisms and to the soil matrix, NT accumulates these on the soil surface, limiting its decomposition and movement to the subsoil (Paul et al., 1996; Reinsch

60 et al., 2021; Luo et al., 2010; Angers and Eriksen-Hamel, 2008). A major criticism of many studies is that they have been restricted to measure SOC changes in the topsoil where ploughing takes place, neglecting potential changes occurring in the subsoil during conversion and renovation (Gál et al., 2007; Ogle et al., 2019a). Therefore, the effects of LUC- and tillage-

induced effects on SOC stocks over the whole soil profile need to be studied more intensively (VandenBygaart et al., 2011; Luo et al., 2010; Lal and Follett, 2009).

65 In this study, the effects of LUC and tillage methods on SOC stocks after the conversion to silage maize cropping or renovation of a 10-year-old permanent grassland are assessed for the 0-90 cm soil layer over 6 years. We hypothesize that 1) no-till can reduce SOC losses after LUC from grassland to arable land and maintain SOC stocks when used for grassland renovation, 2) the conversion or renovation of a permanent grassland negatively affects subsoil SOC, and 3) soil C inputs from plant residues are the main controlling factor for SOC change rates in no-till systems.

70 2 Material and Methods

2.1 Study site

The long-term experiment was located at the research farm "Hohenschulen" of the Christian-Albrechts-University of Kiel in northwest Germany (54°31'N; 9°19'E; 30 m a.s.l.). The climate is maritime with a mean annual temperature of 8.9 °C and a mean annual precipitation of 847 mm (1978-2010). The dominant soil type is stagnic Luvisols (FAO classification) with a
75 sandy loam texture (Table 1), and organic C contents between 1 and 1.5 %. The site has a slope of 9-11 °. Due to this, very high variability in subsoil SOC stocks between the upper and lower half of the site was observed, most likely as a result of historical erosion and sedimentation processes. To exclude these site-specific effects, this study only reports results from the upper half of the experimental site.

Before 2015, a 10-year-old seeded grassland experiment (established in 2004) organized in a split-plot design was located on
80 the field, comparing different cutting frequencies (3 vs 5 cuts yr^{-1}) as main factor and N fertilization levels (0 vs 360 kg N ha^{-1} yr^{-1}) as subplot factors in 6 x 6 m plots. The established grassland consisted of a mixture of different grass and legume species, including *Lolium perenne, Festuca pratensis*, *Poa pratensis*, *Phleum pratense*, *Dactylis glomerata*, *Trifolium repens*, and *Medicago sativa*. Between 2005 and 2014, the botanical composition of the swards differed between the treatments, dominated by *Medicago sativa* and *Trifolium repens* from the legume species, and by *Dactilys glomerata* and *Lolium perenne* from the
85 grass species in the 3- and 5-cut treatments, respectively (Nüsse et al., 2018; Schmeer et al., 2014). Despite these differences, by the end of 2014 the SOC stocks barely differed between the pre-management levels in the different soil layers (provided in Supplementary: Table S1, S2 and S3).

In spring 2015, three new cropping systems were introduced randomly into the previous grassland sward. Part of the grassland was converted to a continuous silage maize system (CM) using CT and NT, part of the grassland was renovated (GR) using
90 NT, and part remained unchanged as a control (GC). In addition to the new cropping systems, two N application levels were introduced using calcium ammonium nitrate (CAN). The N levels were non-fertilized (N0) and fertilized with CAN (N1), according to a demand of 180 kg N ha^{-1} yr^{-1} for the silage maize systems and 380 kg N ha^{-1} yr^{-1} for the cut-used grassland systems. The number of replicates used in each treatment in this study is provided in Appendix A: Table A1. A diagram of the

pre-management factors existing between 2004 and 2014, and the treatments introduced in the current study from 2015 is

95   provided in Supplementary: Figure S1.

At the experimental setup, all plots except for the GC plots were sprayed with glyphosate-containing herbicides (Round-Up Ultra and Round-Up Powerflex). Plots with the CT treatment were subsequently rotovated (10 cm depth), mouldboard ploughed (25 - 30 cm depth), and treated with rotary harrow for seedbed preparation. The plots with the NT treatment received no soil cultivation. The seeding was performed with direct drilling seeders in both CM systems (Horsch, Germany) and the

100   GR treatment (Vredo, Netherlands). The maize (*Zea Mays*) variety Ronaldinio (KWS) was sown each year in May at a rate of 10.5 seeds m^{-2}, with a row distance of 0.75 m and at a sowing depth of 5 cm. In the GR treatment, a 5:1 seed mixture of *Lolium perenne* to *Trifolium repens* was sown at a rate of 40 kg ha^{-1} once in spring 2015. Nutrients besides 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).

105   **2.2 Measurements**

2.2.1 Soil sampling and carbon analysis

SOC concentration was measured annually from 2014 (the year prior conversion) to 2020 at three different soil layers, namely 0-30, 30-60, and 60-90 cm, at the end of each growing season. To do so, a sample from each plot was collected using a hydraulic-driven soil auger (inner Ø 2 cm), oven-dried at 30 °C, and sieved to pass a 2 mm mesh size for further analyses. The

110   C and N contents were determined using a CN elemental analyzer (Vario Max CN, Elementar) (ISO).

To calculate SOC stocks, soil bulk density measurements were conducted in 2020 in all the treatments at 15 cm soil depth just before tillage in CT-CM. No significant differences between soil bulk densities were detected between the treatments. Therefore, the mean soil bulk density of 1.61 g cm^{-3} was adopted in all treatments for the 0-30 cm soil layer, regardless of the tillage treatment. To calculate SOC stocks for the 30-60 and 60-90 cm soil layers, a soil bulk density of 1.65 and 1.70 g cm^{-3}

115   was assumed, respectively, based on the boulder clay features of the subsoil layers (Struck et al., 2019).

2.2.2 Plant biomass sampling and soil 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, before sowing.

120   The grassland systems were harvested with a cutting frequency of 4 cuts yr^{-1} (May, Jul, Aug, and Oct), 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 annual yields (Y_P) measured for the period 2015 – 2020 are provided in Supplementary: Table S4.

To obtain yield-based allocation coefficients, total above- (AGB) and belowground biomass (BGB) measurements conducted
125 during and after the growing seasons (March – October and November – March , respectively) by Struck (2018) in the years
2015 and 2016 were used. During the growing season, AGB samples were taken at each grassland cutting and maize harvest
on 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 (Steingrobe et al., 2001), starting from
mid-March and following the grassland cutting intervals (every 6 weeks). Roots were washed from soil using a hydro-
130 pneumatic elutriation system (Smucker et al., 1982) and collected using a screen with a mesh size of 0.63 mm. After drying
the samples, the ash-corrected dry matter (ACDM) 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
135 (Nov, Feb, and Mar). A detailed description of the measurements is available in Struck (2018).

The annual soil C inputs (C_i) were estimated system wise, using the measured Y_P and yield-based allocation coefficients to
calculate the above (AG) and belowground (BG) C_i produced during the growing season, and adding fixed inputs to account
for the residues produced AG and BG (AW–AG_i and AW–BG_i, respectively) in the grassland systems after the growing season.
For the AG residues produced during the growing season, stubbles were calculated using the estimated SI in the CM systems,
140 whereas in the grassland systems harvest residues and litter deposition were calculated using a SI equal to 15 % on a DM basis,
as proposed by Bolinder et al. (2007). 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 produced after the growing season in the grassland
systems, measured BGB of the first two experimental years was used. Since AGB was not measured in this period, we assumed
these inputs using measured AGB in winter from a similar grassland experiment located at a nearby experimental farm (Loges
145 et al., 2018). To account for 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_i calculations, as suggested by Pausch and Kuzyakov (2018). Ash-corrected
dry matter (ACDM) values were used for the C_i calculations, as these are preferred to total dry matter (Bolinder et al., 2007).
Carbon content of 48 % was calculated on the ash-corrected AG and BG plant material. The steps and equations used to
calculate annual soil C inputs in each cropping system, using the annual yields (Y_P) and using the yield-based allocation
150 coefficients and fixed inputs are provided in Supplementary: Tables S5 and S6.

2.4 Statistical analysis

The interaction of the factors cropping system, N rate and soil layer with time (year) were tested for their effects on SOC
stocks, using analysis of covariance (ANCOVA) within a linear mixed-effects model. To do so, the lme package (Bates et al.
2015) was used in R statistical software (R Core Team, 2020). Three separate ANCOVAs were performed: one using all the
155 treatments to assess the influence of the cropping systems and N rates on ΔSOC (Mg C ha^{-1} yr^{-1}) in the different soil layers
after grassland conversion (i.e., the LUC), another using only the CM systems to assess the influence of tillage method and N

rates on ΔSOC after conversion, and a third one using all the treatments to assess the influence of CS and N rates on ΔSOC across the soil profile (0-90cm). When the study factors significantly interacted with time, separate regressions were estimated and tested whether the mean ΔSOC statistically differed from zero. All the tested effects were considered significant with a p-value < 0.05.

In addition, the changes in ΔSOC by a unit change in annual soil C_i were calculated by linear regression in the 0-30 cm soil layer, using the observations of the unploughed systems (GC, NT-GR, and NT-CM) (Ferreira et al., 2012; Johnson et al., 2006). The minimum amount of annual C_i required to maintain the SOC stocks at its initial levels (ΔSOC = 0) was calculated using the regression estimates, by solving for x when y = 0.

3. Results

3.1 Soil layer-specific effects of grassland conversion and renovation on soil organic carbon stocks

After grassland conversion and renovation, the SOC stocks were significantly affected by the interaction between soil layer, cropping system and year (p < 0.001, Table 2), thus the development of SOC over the years differed between the cropping systems and soil layers. However, there was no significant effect or interaction induced by the different N rates. This was the same for the analysis with the CM systems only, where the ANCOVA revealed significant effects over time induced by the tillage method (p < 0.01) and the soil layer (p < 0.001), but not by the N rates (Table 3). Since the N rates showed no significant influence nor any interaction over the years, cropping systems were pooled over the N rates and separated by soil depth.

Table 2

Table 3

At the 0-30 cm layer, significant SOC losses over time were observed in CM for both tillage methods (p < 0.0001, Figs 1 and 2). By using NT during conversion, SOC losses were reduced from -2.7 to -1.5 Mg C ha^{-1} yr^{-1}, equal to 44 % lower annual SOC losses. By the end of the study period, SOC stocks in CT-CM and NT-CM had decreased by 23 and 12 % (-3.8 and -2.0 % yr^{-1}), respectively, in relation to the initial SOC levels before conversion. In contrast, SOC stocks remained unchanged in the topsoil of NT-GR and GC.

In the subsoil, significant increases in SOC was observed only in GC at the 30-60 cm layer (1.1 Mg C ha^{-1} yr^{-1}, p < 0.01, Figs 1 and 2). In the other systems, no significant changes in SOC stocks were observed after conversion or renovation by using any of the tillage methods. At the 60-90 cm soil layer, ΔSOC was not significant in all four cropping systems.

Figure 1

Figure 2

3.2 Effects of grassland conversion and renovation on soil organic carbon stocks in the whole profile

When SOC stocks were summed up across the whole soil profile (0-90 cm), they were significantly affected by the cropping systems over time (p < 0.001, Table 4). The ΔSOC was significantly negative in both CT-CM (p < 0.0001) and NT-CM (p <

0.01) with losses of -3.4 and -1.9 Mg C ha^{-1} yr^{-1}, respectively. In the grassland systems, highly positive but non-significant ΔSOC was observed for GC (1.3 Mg C ha^{-1} yr^{-1}), whereas SOC remained unchanged in NT-GR (-0.1 Mg C ha^{-1} yr^{-1}) (Figs 3
190 and 4).

Table 4

Figure 3

Figure 4

3.3 Effects of soil C inputs on topsoil SOC stocks

195 The mean annual soil C_i for the period 2015–2020 are shown in Table 5. After grassland conversion to CM, the total C_i decreased by 72 % (from 6.4 to 1.8 Mg C ha^{-1} yr^{-1}) when averaged over CM systems and N rates, whereas after grassland renovation, total C_i decreased by 13 % (from 6.8 to 6.0 Mg C ha^{-1} yr^{-1}) compared to the GC. The reduced C_i after conversion was mainly due to lower BG C_i with an 82 % reduction (from 5.6 to 1 Mg C ha^{-1} yr^{-1}) compared to 33 % lower C_i from AGB (from 1.2 to 0.8 Mg C ha^{-1} yr^{-1}). After grassland renovation, BG C_i decreased by 13 % (from 5.6 to 4.9 Mg C ha^{-1} yr^{-1}), whereas
200 AG C_i decreased by 8 % (from 1.2 to 1.1 Mg C ha^{-1} yr^{-1}). Only marginal differences between the C_i of the two tillage methods in the CM systems were observed. In all four systems, N fertilization increased AG C_i, while it reduced BG C_i except for CT-CM where it remained unchanged.

Table 5

The linear regression of ΔSOC against total annual soil C_i (Fig. 5) for the no-till systems (GC, NT-GR, and NT-CM) indicated
205 that SOC loss rates were reduced by 0.32 Mg C ha^{-1} yr^{-1} (C.I.: 0.17 – 0.46, p < 0.001, R^2 = 0.47) with every additional Mg of C_i from plant residues. Approximately 6.5 Mg C_i ha^{-1} yr^{-1} from plant residues were required across N rates to maintain the SOC stocks at the initial level (~75 Mg SOC ha^{-1}).

Figure 5

4 Discussion

210 ### 4.1 No-till (NT) effects on topsoil SOC stocks after grassland conversion and renovation

The use of NT has frequently been designated as a promising measure to maintain or increase SOC stocks in arable lands (West and Post, 2002; West and Marland, 2002). However, its suitability to conserve SOC after grassland renovation and conversion to arable land, particularly over several years, has barely been studied. In this study, it was hypothesized that NT can reduce SOC losses after LUC from grassland to arable land and maintain SOC stocks when used for grassland renovation.
215 In agreement with the initial hypothesis, annual SOC losses were reduced by almost half when NT was used instead of CT, despite the observed substantial losses (Figs 1 and 2).

These results both agree and disagree with those of previous research with a similar experimental setup, but with different site conditions. Our study showed a similar reduction in annual SOC losses (~1.2 Mg C ha^{-1} yr^{-1}) by NT compared to CT as those

found in a 3-year study by Franzluebbers and Stuedemann (2008). The same study, however, reported no losses in SOC stocks

220   over time by using NT after grassland conversion. These results were obtained at a site with similar soil texture as in the present study, but with much higher precipitation (1250 vs. 847 mm yr^{-1}) and much lower initial SOC stocks (~35 vs. ~74 Mg ha^{-1}). In another study, no losses in SOC stocks were found 6 years after a grassland was converted to continuous maize using NT, at a site with high initial SOC stocks (~91 Mg ha^{-1}) but with different soil texture (silt loam vs. sandy loam) combined with an extended drought period (Follett et al., 2009). However, a previously applied soil carbon model calibrated with soil carbon

225   and yield data from the presented experimental site confirm the decay of SOC regardless of soil tillage method used (Reinsch et al., 2021).

Possible explanations for these differences might be associated with factors related to site conditions. Site conditions can play an important role in regulating both the rate of SOC formation and decomposition. For instance, high precipitation and a positive soil water balance can stimulate both high SOC formation and decomposition rates (Guo and Gifford, 2002). These

230   rates can be proportional to the initial C content, the soil C balance, and the amount of silt and clay protecting the SOC (Paul et al., 1996). At our study site, a moist temperate climate is combined with comparably high SOC stocks in sandy loam soil. Under these conditions, high decomposition rates likely caused the loss of SOC after grassland conversion also in the NT-CM treatment.

Contrary to the results observed for grassland conversion, using NT during grassland renovation (NT-GR) conserved SOC

235   stocks and showed no differences in the 0-30 cm layer compared to the GC (Figs 1 and 2). Results for NT use for the conservation of SOC stocks during grassland renovation are scarce. Existing studies report non-conclusive results regarding the advantages of NT over CT during grassland renovation. For instance, Linsler et al. (2013) reported no differences in SOC stocks between a grassland that was renovated using CT and the adjacent undisturbed grassland system, sampled five years after renovation. Similarly, Gaweł and Grzelak (2020) observed no changes in SOC stocks over time in neither NT nor CT

240   during grassland renovation in the 0-30 cm layer sampled 3 years after renovation. In contrast, a 25 % reduction in SOC stocks was observed 2.5 years after a single use of CT for grassland renovation, compared to the adjacent undisturbed grassland in a study located in a temperate wet region (Necpálová et al., 2014). Since our study did not include a grassland renovation treatment using CT, conclusions about how much SOC losses were avoided by replacing CT with NT for renovation cannot be drawn directly. However, by comparing GR with GC it can be deduced that the SOC balance was not affected by the

245   grassland renovation measure using NT. However, in intensive grassland systems renovation occurs usually in intervals after 5 and 10 years, respectively. Reinsch et al., 2018 found that regular renovation can reduced the C sequestration rate of grasslands substantially compared to permanent grassland, thereof, improved grassland management instead of frequent grassland renovation should be sought.

Additionally, our observations are based on sequential annual soil sampling to determine annual SOC change rates, compared

250   to single soil samplings several years after ploughing and renovation events in other studies. This difference in methodology supports our results, by eliminating potential unseen effects caused by the sampling interval.

4.2 LUC and tillage effects on SOC in the subsoil

Due to a lack of data, it is often recommended to observe the SOC dynamics in deeper soil layers to study the effects of LUC and tillage methods on the whole soil profile. It was hypothesized that the conversion or renovation of a permanent grassland

255   negatively affects subsoil SOC. In this study, the cropping system effect on SOC was different for the different soil layers, while this was not the case for the effect of the tillage method in the subsoil (Table 2 and Table 3). Subsoil SOC stocks were significantly increasing in the 30-60 cm layer of the GC, whereas in the other systems it remained unchanged or was only slightly and insignificantly affected (Table 2 and Table 3, Figure 1 and Figure 2).

Reasons for the observed differences between the GC and the other systems might be attributed to changes in vegetation and

260   its effects on the amount and distribution of BG C_i, mainly roots and water-soluble C compounds, as indicated in other studies (Guo and Gifford, 2002; Post and Kwon, 2000). Due to its high solubility in water, DOC can be the most important C source and flux in subsoils (Kaiser and Kalbitz, 2012; Don and Schulze, 2008). It has been found highly associated not only with the production of AG and BG crop residues, rhizodeposits, and with the easily degradable C fraction, but also to be highly sensitive to soil disturbance and soil properties (Haynes, 2000; Guo and Gifford, 2002). Higher amounts of DOC have been observed

265   in soils under both permanent grasslands and the grassland phase of a ley-arable rotation compared to arable soils (Gentile et al., 2005; Haynes, 2000). It has been found that chemical fallowing with or without ploughing reduced DOC levels in the subsoil compared to an undisturbed grassland (MacDonald et al., 2011). Don and Schulze (2008) observed a reduction in the export of DOC after ploughing a grassland, and that this export was higher in a sandy than in a clayey soil, indicating the adsorption of DOC by the clay fraction.

270   As frequently observed, the combination of low temperatures and waterlogging, as typical conditions in many subsoils, impedes the decomposition of SOC fractions (Necpálová et al., 2014; Freibauer et al., 2004). Although it was not measured, it is likely that high amounts of DOC are being produced and leached from the rooting zone of the GC, facilitated by the sandy texture of the topsoil and accumulated in the clay-enriched and frequently waterlogged subsoil, typical for stagnic Luvisols, where SOC is slowly decomposed (Jobbagy and Jackson, 2000).

275   A reduction in soil C sequestration was also observed in NT-GR, where it is likely that the existing root formation and decay dynamics were affected temporarily or permanently by eliminating the old grassland containing a well-developed root system formed by several grass and legume species, an effect that was also described by Kayser et al. (2018). Increasing SOC stocks in the subsoil, as observed under GC, could be expected in NT-GR if the initial conditions promoting SOC in the subsoil are restored after some time, as implied in other studies (Ogle et al., 2019a). These results suggest that SOC stocks in the subsoil

280   can be affected by changes in vegetation and management as previously mentioned (VandenBygaart et al., 2011; Murugan et al., 2014; Nüsse et al., 2018).

When the whole soil profile was considered, the overall impact of grassland conversion and renovation on SOC was negative compared to the GC. This occurred as a result of the ceased C sequestration in the subsoil after both renovation and conversion, as well as from the strong decline in topsoil SOC after conversion (Table 4, Figs 3 and 4).In other studies, opposite results

285   have been observed down to 1 m depth, when arable land and forests soils have been converted to grasslands. Grassland soils have been shown to increase the amount and proportion of SOC in deeper layers,compared to other land use types like arable lands (Post and Kwon, 2000; Guo and Gifford, 2002; Jobbagy and Jackson, 2000). These findings address the sensitivity of subsoil SOC to LUC, and thus emphasize the need of sampling deeper than the common 30 cm layer, as suggested in several studies (VandenBygaart et al., 2011; Christopher et al., 2009; Gál et al., 2007; Wanniarachchi et al., 1999).

290   **4.3 Soil C input effects on annual SOC changes (ΔSOC)**

It was hypothesized that soil C inputs from plant residues are the main controlling factor for SOC change rates in no-till systems. Confirming this hypothesis, we found a strong significant relationship between ΔSOC and C_i when data was pooled over the treatments NT-GR, NT-CM, and GC (Figure 5). This confirms the expected importance of C_i to maintain SOC stocks at initial levels when management or land-use changes occur. According to earlier studies (Wilhelm et al., 2004; Follett, 2001;

295   Lal and Kimble, 1997), the equilibrium level of SOC can be linearly related to the amount of crop residues, given that all management practices with potential effects on SOC (e.g., soil erosion and SOC degradation rates by tillage) remain constant. Thus, conserving SOC stocks mainly relies on adequate levels of C_i to balance system-specific SOC decomposition rates. Consequently, reduced amounts of mean annual crop residues returned to the soil would lead to losses of SOC from cropping systems (Poyda et al., 2019). In NT-CM, the mean annual soil C_i was reduced by 74 %, whereas in NT-GR this reduction was

300   only 6 % compared to GC (Table 5). Approximately, 6.5 Mg ha^{-1} yr^{-1} of soil C_i from plant residues was required to maintain the SOC stocks at the initial level of the permanent grassland before the experiment (~75 Mg SOC ha^{-1}; i.e., ΔSOC = 0) (Johnson et al., 2006 and Johnson et al., 2014).

Our results indicate that NT is a suitable option to maintain SOC stocks when a grassland renovation measure seems unavoidable. The most likely explanation is that a lower soil C_i due to reduced productivity in the renovation year is

305   compensated by reduced microbial decomposition rates as a result of lower amounts of labile C entering the soil, which remains undisturbed. In the case of grassland conversion, however, these effects were not sufficient to compensate for the strongly reduced soil C_i under NT-CM. These results indicate that site conditions are of overriding importance for the efficiency of NT for SOC conservation after grassland conversion, as postulated by Guo and Gifford (2002) and Conant et al. (2001).

5 Conclusions

310   In this study, the impacts of LUC from grassland to arable land as well as that of tillage methods on SOC stocks after grassland conversion and renovation were evaluated for the whole soil profile. Opposite to what some studies suggested, NT could not conserve SOC stocks after LUC, but it strongly reduced their losses compared to CT and demonstrated that SOC stocks can be conserved when NT was used for grassland renovation under the conditions of the study site. The much lower soil C_i compared to the grassland systems was the most important reason for the observed SOC losses under NT-CM. Interestingly,

315   the permanent grassland was still accumulating SOC, which could not have been observed if only the topsoil was sampled,

and this process was interrupted even by a grassland renovation using NT. This strengthens the need to consider the SOC changes occurring in the whole profile, as ongoing C sequestration might be occurring in deeper soil layers, even when soil C saturation has been already achieved in the topsoil.

Appendix A

320   Table A1

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325 J.DLR.; Writing—review and editing, A.P., T.R., and F.T; Visualization, J.DLR.; Supervision, A.P., T.R., and F.T.; Funding acquisition, T.R. and F.T.

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475

480   **Table 1.** Soil textures and types of different layers at the study site (German system).

Layer (cm)	Texture (%)			Soil type
	Sand	**Silt**	**Clay**	
0-25	54	30	16	Highly loamy sand
25-45	50	31	19	Medium sandy loam
45-70	49	33	18	Medium sandy loam
70-	55	31	14	Highly loamy sand

Table 2. Analysis of covariance (ANCOVA) to determine the influence of cropping system, N rate, soil layer, time, and their interactions on soil organic carbon (SOC) stocks after a 10-year utilization period as permanent grassland.

Fixed-effects	Degrees of freedom†	F-value	P-value
(Intercept)	**1, 995**	**1321.14**	**<.0001**
Soil layer	**2, 995**	**556.96**	**<.0001**
N rate	1, 36	0.21	0.65
Cropping System	3, 9	0.61	0.63
Time	**1, 424**	**53.81**	**<.0001**
Soil layer × N rate	2, 955	0.54	0.58
Soil layer × Cropping system	6, 955	1.95	0.07
N rate × Cropping system	3, 36	0.18	0.91
Soil layer × Time	**2, 955**	**19.12**	**<.0001**
N rate × Time	1, 424	0.51	0.48
Cropping System × Time	**3, 424**	**9.11**	**<.0001**
Soil layer × N rate × Cropping system	6, 955	1.20	0.30
Soil layer × N rate × Time	2, 955	0.28	0.76
Soil layer × Cropping system × Time	**6, 955**	**4.90**	**<.0001**
N rate × Cropping system × Time	3, 424	0.20	0.90
Soil layer × N rate × Cropping system × Time	6, 955	1.08	0.37

†: Degrees of freedom (Numerator, Denominator)

Model R^2 marginal = 0.88; conditional = 0.92

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Table 3. Analysis of covariance (ANCOVA) to determine the influence of tillage method, N rate, soil layer, time, and their interactions on soil organic carbon (SOC) stocks in the continuous silage maize systems (CM) after conversion of a 10-year-old permanent grassland.

Fixed-effects	Degrees of freedom†	F-value	P-value
(Intercept)	**1, 641**	**1310.64**	**<.0001**
Soil layer	**2, 641**	**556.75**	**<.0001**
N rate	1, 22	0.19	0.66
Tillage	1, 3	1.28	0.34
Time	**1, 284**	**53.21**	**<.0001**
Soil layer × N rate	2, 641	0.55	0.58
Soil layer × Tillage	2, 641	1.67	0.19
N rate × Tillage	1, 22	0.24	0.63
Soil layer × Time	**2, 641**	**18.99**	**<.0001**
N rate × Time	1, 284	0.50	0.48
Tillage × Year	**1, 284**	**6.84**	**<0.01**
Soil layer × N rate × Tillage	2, 641	1.01	0.37
Soil layer × N rate × Time	2, 641	0.28	0.75
Soil layer × Tillage × Time	2, 641	1.86	0.16
N rate × Tillage × Time	1, 284	0.10	0.75
Soil layer × N rate × Tillage × Time	2, 641	1.30	0.27

†: Degrees of freedom (Numerator, Denominator)

Model R^2 marginal = 0.86; conditional = 0.91

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Table 4. Analysis of covariance (ANCOVA) to determine the influence of cropping system, N rate, time, and their interactions on soil organic carbon (SOC) stocks across the whole soil profile (0-90 cm) after a 10-year utilization period as permanent grassland.

Fixed-effects	Degrees of freedom†	F-value	P-value
(Intercept)	1, 424	691.39	<.0001
N rate	1, 36	1.71	0.20
Cropping System	3, 9	0.77	0.54
Time	**1, 424**	**27.47**	**<.0001**
N rate × Cropping system	3, 36	0.99	0.41
N rate × Time	1, 424	0.58	0.45
Cropping system × Time	**3, 424**	**5.85**	**<.001**
N rate × Cropping system × Time	3, 424	1.49	0.22

†: Degrees of freedom (Numerator, Denominator)

Model R^2 marginal = 0.13; conditional = 0.99

505   **Table 5.** Mean and standard deviation (S.D.) of annual soil carbon inputs (C_i) from above- (AG) and belowground (BG) plant residues calculated for the different cropping systems and N rates at the study site. Estimates are representative of 16 replicates for GC, 8 replicates for NT-GR, and 24 replicates each for NT-CM and CT-CM.

Cropping System	N rate	AG C_i	BG C_i	Total C_i
		Mean (SD) *(Mg C ha^{-1} yr^{-1})*		
GC	N0	1.0 (0.3)	7.3 (2.8)	8.2 (3.1)
GC	N1	1.4 (0.3)	3.8 (0.9)	5.3 (1.1)
NT-GR	N0	0.9 (0.3)	6.1 (2.7)	7.0 (3.0)
NT-GR	N1	1.3 (0.3)	3.7 (0.9)	5.0 (1.2)
NT-CM	N0	0.6 (0.2)	1.2 (0.3)	1.8 (0.5)
NT-CM	N1	0.8 (0.2)	1.0 (0.3)	1.9 (0.5)
CT-CM	N0	0.7 (0.2)	0.9 (0.2)	1.6 (0.5)
CT-CM	N1	0.9 (0.1)	0.9 (0.1)	1.8 (0.3)

GC: grassland control (undisturbed permanent grassland); NT-GR: grassland renovation with no-tillage;

NT-CM: continuous silage maize with no-tillage; CT-CM: continuous silage maize with conventional tillage;

N0: non-fertilized; N1: fertilized with CAN based on a crop demand of 380 kg N ha^{-1} yr^{-1} for the grassland

systems of 180 kg N ha^{-1} yr^{-1} for the silage maize systems.
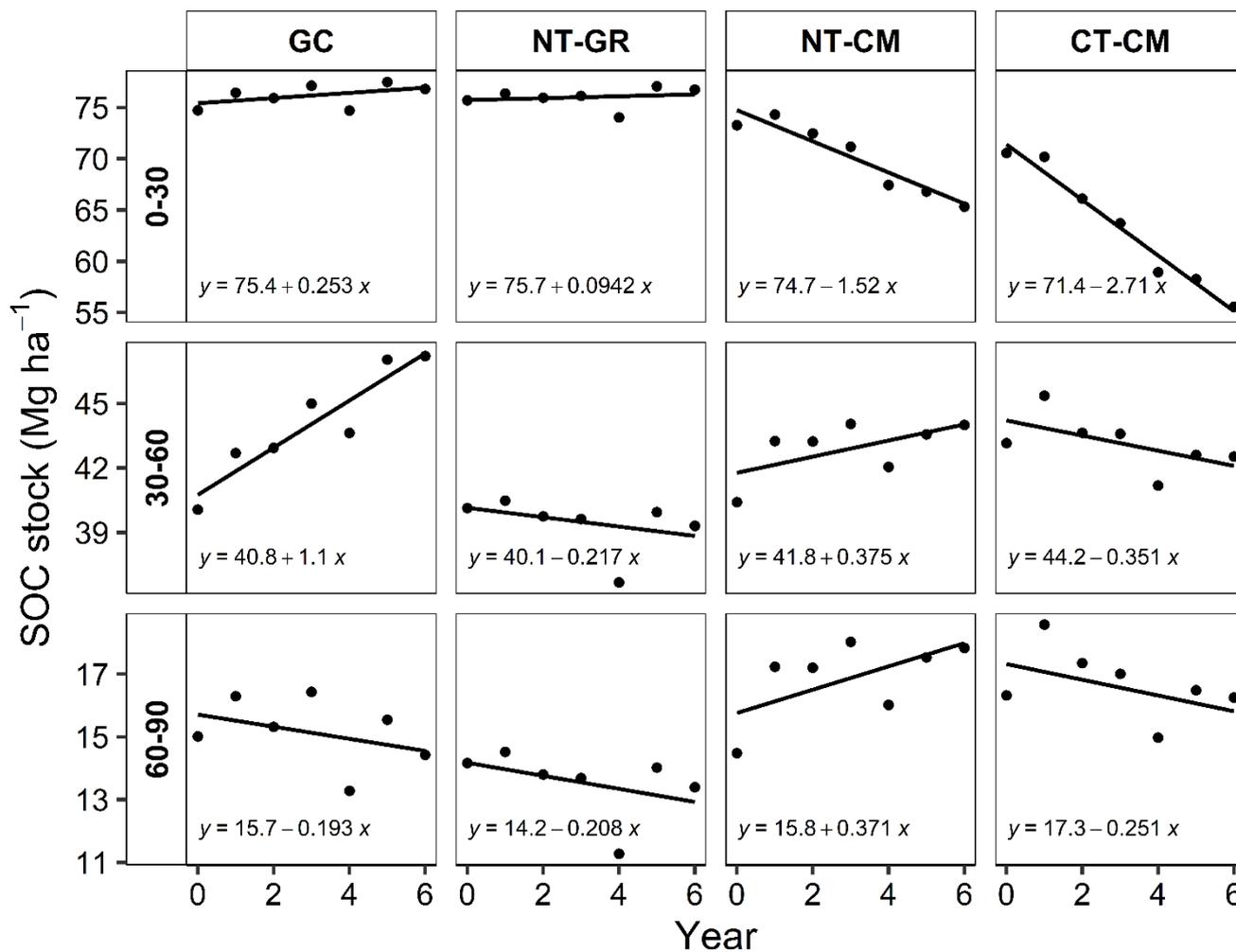
Figure 1. Mean temporal development of soil organic carbon (SOC) stocks in the different cropping systems and layers for the period 2014 – 2020, pooled over the two N rates. GC stands for undisturbed grassland control, NT-GR for no-till grassland renovation, NT-CM for no-till continuous silage maize, and CT-CM for conventionally tilled continuous silage maize. Regression estimates are representative of 16 replicates for GC, 8 replicates for NT-GR, and 24 replicates each for NT-CM and CT-CM.

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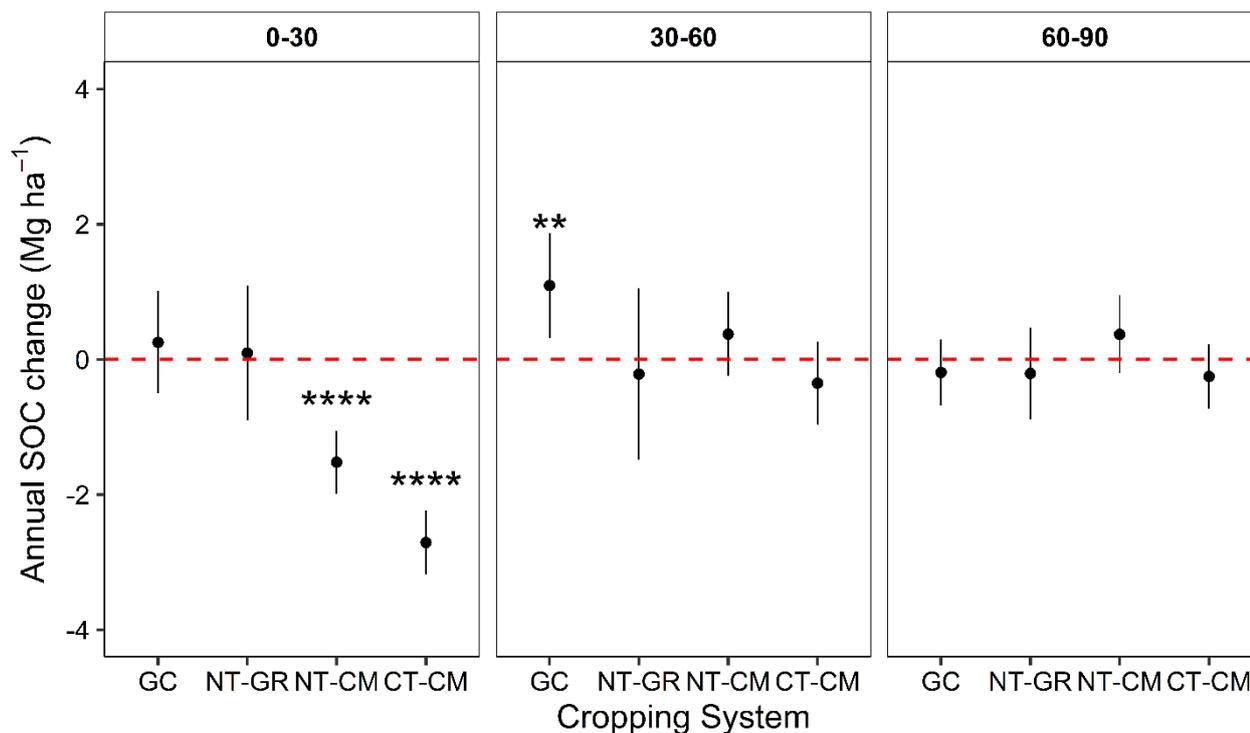


Figure 2. Mean annual SOC change rates (± 95 % confidence intervals) for the different cropping systems during the period 2014 – 2020, pooled over the two N rates. GC stands for grassland control, NT-GR for no-till grassland renovation, NT-CM for no-till continuous silage maize, and CT-CM for conventionally tilled continuous silage maize. Estimates are representative of 16 replicates for GC, 8 replicates for NT-GR, and 24 replicates each for NT-CM and CT-CM. Cropping systems with *,**,***, or **** indicate ΔSOC significantly differed from zero at p < 0.05, p < 0.01, p < 0.001 or at p < 0.0001, respectively.

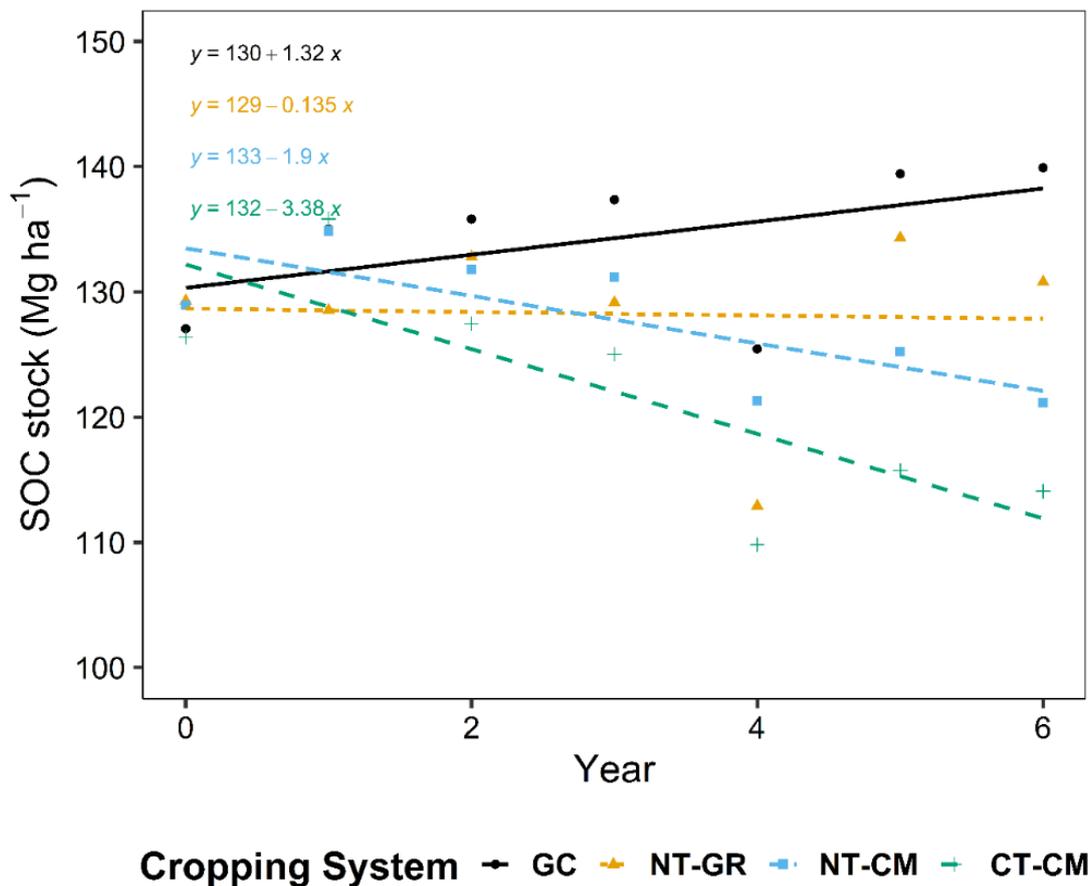



Figure 3. Mean temporal development of soil organic carbon (SOC) stocks for the different cropping systems across the 0-90 cm profile for the period 2014 – 2020, pooled over the two N rates. GC stands for grassland control, NT-GR for no-till grassland renovation, NT-CM for no-till continuous silage maize, and CT-CM for conventionally tilled continuous silage maize. Regression estimates are representative of 16 replicates for GC, 8 replicates for NT-GR, and 24 replicates each for NT-CM and CT-CM.

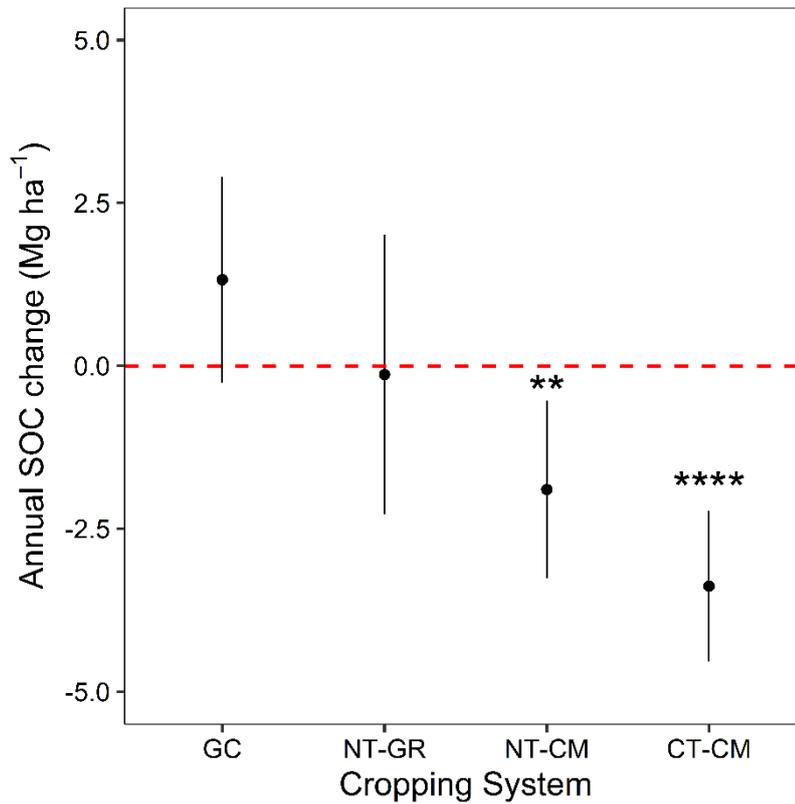



Figure 4. Mean annual SOC change rates (± 95 % confidence intervals) in the 0-90 cm soil profile, pooled over the two N rates in the different cropping systems during the period 2014 – 2020. GC stands for grassland control, NT-GR for no-till grassland renovation, NT-CM for no-till continuous silage maize, and CT-CM for conventionally tilled continuous silage maize. Means are representative of 16 replicates for GC, 8 replicates for NT-GR, and 24 replicates each for NT-CM and CT-CM. Cropping systems with *,**,***, or **** indicate ΔSOC significantly differed from zero at $p < 0.05$, $p < 0.01$, $p < 0.001$ or at $p < 0.0001$, respectively.

540

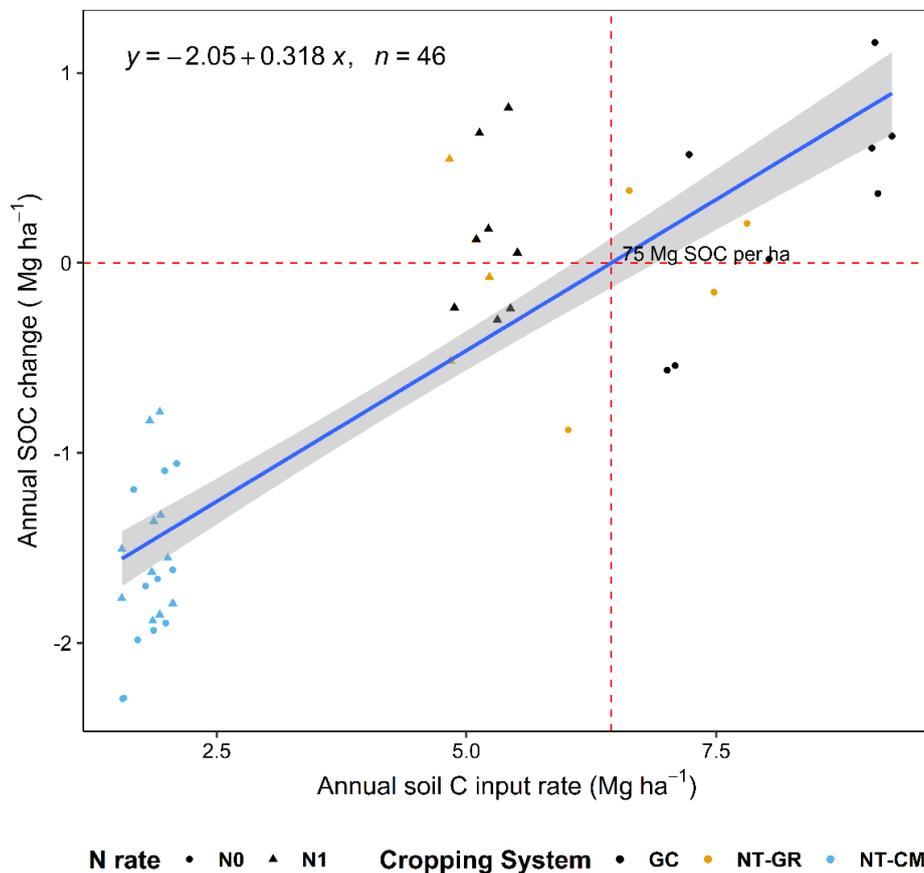
N rate • N0 ▲ N1 **Cropping System** • GC • NT-GR • NT-CM

545

Figure 5. Linear regression of annual soil organic carbon (SOC) change rates against annual soil C input rates from both above- and belowground biomass in the unploughed systems. Colors and shapes of the symbols indicate the replicates for the different cropping systems and N rates, respectively. GC stands for grassland control, NT-GR for no-till grassland renovation, and NT-CM for no-till continuous silage maize. The N0 and N1 refer to the non-fertilized and fertilized systems using CAN, respectively, based on a crop demand of 380 kg N ha^{-1} 550 yr^{-1} for the grassland systems, and 180 kg N ha^{-1} yr^{-1} for the silage maize systems.

555

560

Table A1. Number of replicates observed in the different cropping systems and N rates, using the upper half of the experimental site since 2015, after their introduction into the 10-year-old permanent grassland (2004-2014). In the permanent grassland, the cutting frequencies comprised of 3 and 5 cuts yr^{-1}, whereas the N application rates comprised of 0 and 360 kg N ha^{-1} yr^{-1}. From 2015, the cropping systems comprised of an undisturbed permanent grassland as control (GC), a no-till grassland renovation (NT-GR), a no-till continuous silage maize (NT-CM) and a conventionally tilled continuous silage maize (CT-CM), whereas the N rates comprised of non-fertilized (N0) and fertilized systems (N1) with CAN, based on a crop demand of 380 kg N ha^{-1} yr^{-1} for the grassland systems and 180 kg N ha^{-1} yr^{-1} for the silage maize systems.

| Cropping Systems since 2015 | N rate | Permanent Grassland (2004-2014) | | | | Replicates by treatment |
| | | Cutting frequency x N rate levels | | | | |
		3×0	5×0	3×360	5×360	
GC	N0	2	2	2	2	8
	N1	2	2	2	2	8
NT-GR	N0	-	-	2	2	4
	N1	-	-	2	2	4
NT-CM	N0	2	2	4	4	12
	N1	2	2	4	4	12
CT-CM	N0	2	2	4	4	12
	N1	2	2	4	4	12
Total number of replicates		12	12	24	24	72