Effects of grassland management intensification on dynamics of soil organic carbon and nitrogen in temperate grassland soils

Dissertation

Submitted to the Faculty of Organic Agriculture Science (Fachbereich ökologische Agrarwissenschaften) of the University of Kassel to fulfill the requirements for the degree Doktor der Naturwissenschaften (Dr. rer. nat.)

by

Anja Marie Nüsse

born in

Bad Driburg

Göttingen, 12.09.2017

Date of thesis defense: 20.04.2018

First Supervisor: Prof. Dr. Bernard Ludwig

Second Supervisor: Prof. Dr. Rainer Georg Jörgensen
Preface

This thesis was prepared within the Research Training Group “Regulation of soil organic matter and nutrient turnover in organic agriculture” (Graduiertenkolleg 1397/3) and funded by the Deutsche Forschungsgemeinschaft (DFG). The Thesis is submitted to the Faculty of Organic Agriculture Sciences to fulfil the requirements for the degree “Doktor der Naturwissenschaften” (Dr. rer. nat). The dissertation is based on three papers, two as first author, one as co-author, which are published in or submitted to international refereed journals. The manuscripts are included in chapters 2, 3, and 4. Chapter 1 comprises a general introduction to the research topic as well as the objectives this thesis was based on and Chapter 5 contains the overall conclusion.

The following papers are included in this thesis:

Chapter 2:


Chapter 3:


Chapter 4:

Table of contents

Table of contents ........................................................................................................... I
List of Figures ............................................................................................................... III
List of Tables ................................................................................................................. IV
List of Abbreviations ..................................................................................................... V
Summary ....................................................................................................................... 7
Zusammenfassung ......................................................................................................... 13

1 General Introduction .......................................................................................... 22
1.1 Types of Grassland .......................................................................................... 22
1.2 Function, Actual Development, and Management Practices of Grasslands .. 22
1.3 Grassland Management Affects Carbon and Nitrogen Cycles and Sequestration
   mechanisms ............................................................................................................. 23
   1.3.1 Effects of Grazing on Carbon and Nitrogen Cycles and Sequestration Mechanisms .... 24
   1.3.2 Effects of mowing and nitrogen supply on carbon and nitrogen cycles and storage
       mechanisms ........................................................................................................ 25
   1.3.3 Effects of Grassland Renewal and Different Practices of Grassland Renewal on
       Carbon and Nitrogen Cycles and Sequestration Mechanisms ............................. 27
1.4 Objectives of the Present Study ........................................................................ 28

2 Effect of grazing intensity and soil characteristics on soil organic carbon
   and nitrogen stocks in a temperate long-term grassland ............................... 30
   ABSTRACT ............................................................................................................. 31
   2.1 Introduction ..................................................................................................... 32
   2.2 Materials and Methods .................................................................................. 34
       2.2.1 Study area ................................................................................................. 34
       2.2.2 Plot description and sampling design ....................................................... 34
       2.2.3 Analytics and sampling design ................................................................. 35
   2.3 Results and Discussion .................................................................................. 37
       2.3.1 Weighted stocks of SOC and N ................................................................. 37
       2.3.2 Weighted stocks of C_{mic}, N_{min} and basal respiration rates ................. 38
       2.3.3 Data variability – influences of mineral soil characteristics and pH .......... 39
   2.4 Conclusion ..................................................................................................... 40
   2.5 Acknowledgments ......................................................................................... 41

3 Effect of grassland harvesting frequency and N fertilization on stocks and
   dynamics of soil organic matter in the temperate climate ............................. 42
   ABSTRACT ............................................................................................................. 43
## Table of contents

3.1 Introduction ......................................................................................................................... 44  
3.2 Materials and Methods ........................................................................................................ 45  
\hspace{12pt} 3.2.1 Study area ................................................................. 45  
\hspace{12pt} 3.2.2 Plot description and sampling design ..................................... 45  
\hspace{12pt} 3.2.3 Analytics and soil characterization ........................................ 46  
\hspace{12pt} 3.2.4 Statistical analysis .......................................................... 47  
3.3 Results and Discussion ........................................................................................................ 48  
\hspace{12pt} 3.3.1 SOC and N, stocks and C/N-ratios ...................................... 48  
\hspace{12pt} 3.3.2 C_{mic} and ergosterol contents ............................................ 49  
\hspace{12pt} 3.3.3 Water-stable aggregate size classes ..................................... 51  
3.4 Conclusion .......................................................................................................................... 52  
3.5 Acknowledgments ............................................................................................................... 52  
4 Effect of chemical and physical grassland renovation on the temporal dynamics of organic carbon stocks and water-stable aggregate distribution in a temperate grassland soil ................................................................. 53  
4.1 Introduction ........................................................................................................................ 55  
4.2 Materials and Methods ....................................................................................................... 56  
\hspace{12pt} 4.2.1 Study Area ................................................................. 56  
\hspace{12pt} 4.2.2 Plot description and sampling design ..................................... 57  
\hspace{12pt} 4.2.3 Analytics and soil characterization ........................................ 58  
\hspace{12pt} 4.2.4 Aggregate fractionation .................................................... 58  
\hspace{12pt} 4.2.5 Statistical analyses .......................................................... 58  
4.3 Results and discussions ...................................................................................................... 60  
\hspace{12pt} 4.3.1 SOC stocks ................................................................. 60  
\hspace{12pt} 4.3.2 Aggregate distribution .................................................... 62  
\hspace{12pt} 4.3.3 SOC storage in water-stable aggregates ................................ 67  
4.4 Conclusion .......................................................................................................................... 69  
4.5 Acknowledgements ........................................................................................................... 69  
5 General Conclusions ............................................................................................................. 70  
6 Acknowledgement ................................................................................................................ 73  
References ............................................................................................................................... 75
List of Figures

Figure 1.1: Extensively grazed grassland in Relliehausen, Germany. Right side of the fence: Grazing intensity adjusted to a target compressed pasture height (CPH) of about 18 cm. Left side of the fence: Grazing intensity adjusted to a target CPH of about 6 cm. 25

Figure 1.2: Plant species composition affected by varying grassland management. The photos on the right side show a three-cut (3C) and a five-cut (5C) grassland with an addition of 360 kg of mineral N ha⁻¹ per year. The pictures on the left side show a 5C and a 3C grassland with no addition of mineral fertilizers. 26

Figure 2.1: Results of multiple linear regression analyses in soil depth 0-25 cm for the contents of soil organic C (SOC), total N (Nᵣ), microbial biomass C (Cₑ₀), and the basal respiration rate. For each regression analysis only one significant explanatory variable (p ≤ 0.05, top: Feox, bottom: SOC) remained in the stepwise variable selection procedure. 40

Figure 3.1: a) and b): Content of water-stable aggregate size classes; c) and d): contents of Cₑ₀ (soil microbial biomass); e) and f): ergosterol contents under different harvesting frequencies with or without N fertilization in 0-10 cm and 10-30 cm, respectively. Means and standard deviations (n=3). Asterisks show significant effects. 50

Figure 3.2: Scatter plot of Cₑ₀ (soil microbial biomass C) content against content of soil organic carbon (SOC) (a), ergosterol content against SOC content (b), ergosterol content against content of water-stable aggregates (> 2000 µm) (c), and Cₑ₀ content against content of water-stable aggregates (> 2000 µm) (d) for the surface soil. Spearman’s rho coefficients are also shown. 51

Figure 4.1: Water-stable aggregate concentrations (g/kg soil) in 0–10 cm and 10–40 cm soil depths among different treatments and sampling times; letters indicate significant (p ≤ 0.05) differences among treatments. Shown are the mean values and standard deviations (n=3). 63

Figure 4.2: Scatter plots of the concentrations of aggregate fractions and the gravimetric moisture content in two soil depths. The lines are the modelled lines of the linear regression analyses. 65
List of Tables

**Table 2.1:** Weighted soil organic C (SOC) stock, total N (N_t) stock, microbial biomass C (C_{mic}) stock and mineral N (N_{min}) stock and basal respiration among three grazing pressures (GP) and three soil depths; mean values and standard deviations (standard deviations of field replicates are given in parentheses; n=3). ................................................................. 38

**Table 3.1:** Soil organic C (SOC) and total N (N_t) stocks calculated on an equivalent mass of soil and C/N ratios of different cutting frequencies, either with or without N fertilization, in 3 soil depths. Means and standard deviations (n=3). Results of analyses of variances (ANOVAs) are also shown: significant differences (p ≤ 0.05) between the factor levels 3C and 5C for the factor cut, between the levels N fertilization and no N fertilization for the factor fertilization and significant interactions are labeled as ≤ 0.05. ................................................................................................. 49

**Table 4.1:** Management details and sampling times of the different treatments at the field trial in Wehnen (Buchen et al. 2017). ................................................................................................... 57

**Table 4.2:** Bulk density (g/cm³) and soil organic carbon (SOC) stocks (t/ha) calculated on an equivalent mass of soil in different treatments and sampling times (T_0: before grassland renovation; T_{6_days}: six days after grassland renovation; T_{2_mo}: two months after grassland renovation; T_{7_mo}: seven months after grassland renovation and T_{12_mo}: twelve months after grassland renovation) in the surface soil layer. Shown are the mean values and standard deviations (n=3). ............................................................................... 61

**Table 4.3:** Results of linear regression analyses for various aggregate fractions (g/kg soil) in two soil depths. ................................................................................................................................ 64

**Table 4.4:** Organic carbon concentrations stored in aggregate fractions (g/kg bulk soil) in the different treatments and sampling times (T_{6_days}: six days after grassland renovation; T_{2_mo}: two months after grassland renovation; T_{7_mo}: seven months after grassland renovation and T_{12_mo}: twelve months after grassland renovation) in the surface soil layer (0-10 cm). Shown are the mean values and standard deviations (n=3). .......................... 68
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl₃</td>
<td>Aluminium chloride</td>
</tr>
<tr>
<td>Al₂ox</td>
<td>Oxalate soluble aluminium</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>Barium chloride</td>
</tr>
<tr>
<td>Bzw.</td>
<td>beziehungsweise</td>
</tr>
<tr>
<td>DIN</td>
<td>Deutsches Institut für Normierung</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Chem</td>
<td>chemical sward killing with glyphosate, followed by direct seeding of grassland in 1 cm depth; treatment</td>
</tr>
<tr>
<td>Cmic</td>
<td>Soil microbial biomass carbon</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CPH</td>
<td>Target compressed pasture height</td>
</tr>
<tr>
<td>DFG</td>
<td>Deutsche Forschungsgemeinschaft</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>Fe₂ox</td>
<td>Oxalate soluble iron</td>
</tr>
<tr>
<td>Fig</td>
<td>Figure</td>
</tr>
<tr>
<td>flF</td>
<td>Free light fraction</td>
</tr>
<tr>
<td>GP</td>
<td>Grazing pressure</td>
</tr>
<tr>
<td>GMC</td>
<td>Gravimetric moisture content</td>
</tr>
<tr>
<td>GRK</td>
<td>Graduiertenkolleg</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloride acid</td>
</tr>
<tr>
<td>H₂O</td>
<td>Water</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>ICP</td>
<td>Inductively coupled plasma</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>Potassium sulphate</td>
</tr>
<tr>
<td>K₂O</td>
<td>Potassium oxide</td>
</tr>
<tr>
<td>kEC, kEN</td>
<td>Extractable portion of total C, and N from microbial biomass</td>
</tr>
<tr>
<td>MgO</td>
<td>Magnesium oxide</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>n</td>
<td>Number of replicates</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NH₄</td>
<td>Ammonium</td>
</tr>
<tr>
<td>Nₘin</td>
<td>Mineral Nitrogen</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Nitrate</td>
</tr>
<tr>
<td>Nₜ</td>
<td>Total Nitrogen</td>
</tr>
<tr>
<td>N₂O</td>
<td>Nitrous oxide</td>
</tr>
<tr>
<td>olF</td>
<td>Occluded light fraction</td>
</tr>
<tr>
<td>FORBIOBEN</td>
<td>For Biodiversity Benefit</td>
</tr>
<tr>
<td>R₂</td>
<td>Spearman’s rank correlation coefficient</td>
</tr>
<tr>
<td>p</td>
<td>Probability value</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical analysis system</td>
</tr>
<tr>
<td>SOC</td>
<td>Soil organic carbon</td>
</tr>
</tbody>
</table>
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOM</td>
<td>Soil organic matter</td>
</tr>
<tr>
<td>Phys</td>
<td>chemical sward killing with glyphosate followed by the usage of a rotary cultivator, a moldboard plow (25 cm deep) and land packer, afterwards seeding of grassland</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>Phosphorus pentoxide</td>
</tr>
<tr>
<td>T₀</td>
<td>Sampling time before grassland renovation</td>
</tr>
<tr>
<td>T₆_days</td>
<td>Sampling time six days after grassland renovation</td>
</tr>
<tr>
<td>T₂_mo</td>
<td>Sampling time two months after grassland renovation</td>
</tr>
<tr>
<td>T₇_mo</td>
<td>Sampling time seven months after grassland renovation</td>
</tr>
<tr>
<td>T₁₂_mo</td>
<td>Sampling time 12 months after grassland renovation</td>
</tr>
<tr>
<td>UNESCO</td>
<td>United Nations Educational Scientific and Cultural Organization</td>
</tr>
<tr>
<td>3C</td>
<td>Three grassland cuts</td>
</tr>
<tr>
<td>5C</td>
<td>Five grassland cuts</td>
</tr>
</tbody>
</table>
Summary

In Germany, about one third of the agricultural area is managed as permanent grassland. The predominant use of permanent grassland in Germany is mostly the production of fodder for meat or milk production, achieved by grazing or mowing. In addition, an increased demand for biomass to produce renewable energy has influenced grassland management in the last decade. Until 2013, a continuous decrease in grassland due to grassland conversion was recorded (from 2003 to 2013, by a total of about 5%). The reduction in permanent grassland was accompanied by an increase in intensification of grassland in many places. Therefore, the intensification of grassland management is typically conducted via higher grazing pressure or increased average use by mowing, combined with the application of fertilizers or an increase in fertilizer delivery.

However, especially for farming in naturally small-scale, mountainous landscapes with less productive soils, extensive grazing is recommended for coincidently achieving meat and milk production as well as biodiversity goals.

Also of interest is the renewal of grassland that is entirely or partially carried out, especially in intensively-used grassland, to improve efficiency. Grassland renewal can be performed with or without subsequent tillage.

It is generally known that management intensification of grassland, grassland renewal, or conversion to arable land influences carbon and nitrogen stocks and dynamics in soil.

From these current circumstances, the following objectives have been deduced for my doctoral thesis:

(I) to evaluate the impact of different extensive grazing pressures on carbon and nitrogen in soil, on the coupling of carbon and nitrogen in soil, on soil microbial carbon, on the basal respiration of soil, and on mineral nitrogen in soil.

(II) to evaluate the impact of varying, frequent mowing in combination with and without mineral N fertilization on carbon and nitrogen in soil, on the coupling of carbon and nitrogen in soil, on soil microbial carbon, on ergosterol as a marker of fungal biomass, on soil aggregate size class distribution and its carbon contents, and on the composition of soil organic matter.
(III) to investigate the temporal dynamics during grassland renewal from the chemical destruction of the existing vegetation and reseeding either by direct seeding or by prior tillage, and to investigate their effects on organic carbon and nitrogen in soil, on soil microbial carbon, on soil aggregate size class distribution, and on their carbon contents.

For the processing of (I), soil samples were taken during a long-term grazing experiment northwest of Göttingen (FOR BIOdiversity BENefit trial in Solling, Relliehausen), from three different extensively-grazed treatments in three soil depths (0-10 cm, 10-25 cm, and 25-40 cm) in April 2013. The different grazing pressures were determined by measuring the compressed pasture height during the vegetation period, using a rising-plate meter and subsequently adjusting the stocking rate.

The soil samples were analyzed for soil organic carbon (SOC) and nitrogen (N) contents, the coupling of carbon and nitrogen, soil microbial carbon (C$_{mic}$), basal respiration, and mineral nitrogen (N$_{min}$). Furthermore, pH, clay content, oxalate soluble aluminum, and iron contents were determined. Before data analysis, weighted means were calculated for the stocks of SOC, N, N$_{min}$, and C$_{mic}$ concentrations and basal respiration rates with the proportion of each compressed pasture height class as a weighting factor. A two-factorial analysis of variance (ANOVA) with the factor grazing intensity and the factor block was used in case of normality of residuals and homoscedasticity; otherwise, a Welch ANOVA was used. The weighted stocks of SOC, N, C$_{mic}$, and basal respiration were not significantly affected (p ≤ 0.05) by grazing intensity.

The data were highly heterogeneous, which was probably caused by the heterogeneous soil mineralogy as well as by uncertainties in the analytical determination of the respective contents, the determination of bulk densities, and the stone contents. Thus, a large part of the variation of the organic carbon and total nitrogen contents can be explained regarding the content of oxalate soluble iron (multiple linear regression: R$^2 = 0.64$). However, the contents of microbial carbon (R$^2 = 0.96$) and the basal respiration rate (R$^2 = 0.9$) are, in turn, explained to a significant extent by the organic carbon contents. Furthermore the possible effects of grazing intensity on the SOC and N, stocks are presumably explained by the mineralogical variability. However, the low variability of the C/N ratios of different grazing intensities is attributed to a coupling of C and N and, in turn, suggests sufficient SOC and N, supply in the extensive FORBIOBEN grazing trial.
For the processing of (II), soil samples were taken in three soil depths up to 60 cm in depth in a grassland trial near Kiel managed by the Christian Albrechts University of Kiel. The grassland was established uniformly in 2004 after its preceding management as arable land. The trial was established in a randomized block design with three replicates and included these treatments: three cuts (3C) and five cuts (5C) per year; with and without N fertilization (N fertilization: 360 kg N ha\(^{-1}\) year\(^{-1}\) as calcium ammonium nitrate). The soil samples were analyzed for SOC and N\(_{t}\) stocks, C\(_{\text{mic}}\) and ergosterol as a marker for fungal biomass, soil aggregate size class distribution, their carbon contents, and the composition of soil organic matter (SOM). Three factorial ANOVAs with the factors cut (levels: 3 cuts and 5 cuts), fertilization (levels: N fertilization and no N fertilization), interaction between cut and fertilization, and factor block (three replicates organized in blocks) were conducted.

The SOC stocks from the 5C regime compared to the 3C regimes in soil depths of 0 ~ 10 cm were significantly higher. This was presumably caused by higher harvesting frequencies, which promote the growth of tiller and leaf, are high in photosynthesis, and can stimulate biomass production. Additionally, the SOC stocks were significantly higher in the treatment without N fertilization in a soil depth of 0 ~ 10 cm. N fertilization may result in a decrease of SOC stocks by increasing microbial activity and altering the C substrate utilization pattern through changes in plant biomass composition. Furthermore, higher biomass yields under the 3C compared to the 5C regime as well as different plant species compositions in the treatments may have also contributed to SOM dynamics. Plant species composition was mainly influenced by the differing cut and fertilization regime. The main plant species under the 5C regime without N fertilization at the time of soil sampling were *Lolium perenne* and *Trifolium repens* (a highly productive grass and a legume, respectively). A dense root growth in *Lolium perenne* in a soil depth of 0 ~ 10 cm and the positive effects of legumes on SOC sequestration presumably caused the increase of the SOC stock under the 5C regime without N fertilization. The N\(_{t}\) stocks were significantly higher under the 5C regime in a depth of 0 ~ 10 cm in comparison to the 3C regime, which is presumably related to the occurrence of *Lolium perenne* under the 5C regime.

Soil microbial C contents were significantly higher under the 5C regime than under the 3C regime, whereas N fertilization in significantly lower C\(_{\text{mic}}\) contents resulted in topsoil. This is presumably related to stimulated root exudation due to a higher harvesting frequency and complex changes in microbial competition and community structure due to the addition of N. Ergosterol contents in the surface soil were significantly higher under the 5C regime in comparison with the 3C regime, presumably caused by different root
growth, exudation, and substrate quality. $C_{\text{mic}}$ and the ergosterol contents were closely correlated to SOC stocks ($C_{\text{mic}}$: Spearman $R_s = 0.81$; ergosterol: Spearman $R_s = 0.87$) in topsoil. This indicates that, in the treatments that most likely resulted in higher root productions due to stimulation and plant species composition, microbial and fungal biomasses were stimulated.

For the aggregate distribution in the topsoil layer, a shift from small (250-1000 $\mu$m) to large (> 2000 $\mu$m) macroaggregates from the 3C to the 5C regime was detected, presumably caused by a higher concentration of roots and root exudates related to *Lolium perenne*, with its high amount of fine roots under the 5C treatment in a soil depth of 0-10 cm. Five cuts per year affects SOC and $N_t$ stocks positively, as well as contents of large macro-aggregates, $C_{\text{mic}}$, and ergosterol, which indicate positive effects on the soil fertility of 5C in comparison with 3C.

The N fertilization resulted in slightly negative effects on SOC stocks and $C_{\text{mic}}$ contents. However, as the plant species composition was strongly influenced by cut and fertilization, it is not possible to assign the results found to direct effects (e.g., stimulation of biomass production and root exudation) or to indirect effects due to a different plant species composition.

For the processing of (III) a grassland trial was established on a continuous cut grassland located in Oldenburg in 2013. The trial was managed and supervised by the Chamber of Agriculture of Lower Saxony and the Thünen Institute. In June 2013, a field trial was established and the treatments were arranged in a randomized complete block design with three replicates and consist of

- Treatment (i), chem: chemical sward killing with glyphosate, followed by direct seeding of grassland in 1-cm depth;
- Treatment (ii), phys: chemical sward killing with glyphosate followed by the use of a rotary cultivator, a moldboard plow (25-cm deep), and a land packer, afterwards seeding of grassland;
- Treatment (iii), continuous cut grassland as control.

Soil samples were taken five times during August 2013 to October 2014, each replicated four times in three soil depths. SOC stocks, aggregate size classes, and organic carbon stored in aggregate size classes were analyzed using two-way analysis of variance.
with the factors grassland renovation (control, chem, and phys) and block (blocks 1 to 3). For further data analysis, Spearman’s rank correlation and regression analysis was conducted.

Soil carbon stocks showed no significant differences between the grassland renewal with or without plowing and control. This suggests that a grassland renovation, after neither a purely chemical destruction of sward nor a grassland renovation using a plow, had a direct impact on the loss of carbon stocks in the grassland soils. This is perhaps related to the rather low pH in the soil (on average, 4.8), resulting in hampered microbial biomass, which, in turn, hampered the degradation of organic material.

However, grassland renewal after plowing leads to an increase in microaggregate concentrations six days after plowing, compared to the grassland control in the surface soil and in the soil profile. This indicates that one-time plowing had a direct impact on soil aggregates and destroyed macroaggregates, which fragmented into microaggregates.

However, significant seasonal variations of macro- and microaggregate concentrations over time were visible. Higher macroaggregate concentrations were found during periods with increased rainfall following higher gravimetric water content in the soil, compared to periods with less rainfall and less gravimetric water content. Correlation analysis and multiple linear regression analysis have shown that the gravimetric soil water content had a putatively major influence on the distribution of aggregate size classes. Thus, lower gravimetric soil water content led to a higher concentration of microaggregates and a lower concentration of macroaggregates, while higher gravimetric soil water content led to lower microaggregate and higher macroaggregate concentrations.

The physical renovation two and seven months after plowing also led to a significantly higher microaggregate concentration in the surface soil compared to the grassland renewal without plowing, while the concentrations in the permanent grassland were in between. In the chemical renovation, the larger roots of the dead plants might still be largely undecomposed, protecting macroaggregates from degeneration. In the physical renovation, plowing affects macroaggregates negatively by dismantling them into microaggregates via mechanical forces. Furthermore, dead roots were rearranged and breached, which caused a loss of stability in the soil matrix. However, a year after plowing and grassland renovation, no significant difference existed compared to the other treatments. In the beginning, no effect of grassland renovation on SOC in aggregates was visible. However, later microaggregates become more important than macroaggregates in the sequestration of SOC compared to the unplowed control. Because of the late response in SOC in
aggregates after the plowing event, it seems that the indirect effects (for instance, the
degradation of the root systems of the dead plants) on soil aggregates had a wider influence
than the direct physical impact of the plow. The plowing event in the physical renovation
cause direct physical destruction of the aggregates and, indirectly, destruction of the root
systems of the dead plants. However, impacts on soil macroaggregates were nullified one
year after grassland renovation.

Chemical renovation resulted in higher macroaggregate concentrations compared with
the physical renovation, especially two-to-seven months after the renovation, and in similar
concentrations compared with the permanent grassland in any sampling time within one
year. Presumably, dead plant roots could act as binding agents and stabilize aggregates for
some time. The high temporal variation in the aggregate distribution within one year in the
renovated as well as in the permanent grassland indicates that the soil moisture had a wide
influence and that dry conditions in the soil led to a breakdown of larger aggregates.

From the findings obtained above, it can be concluded that the impact of the
intensified use of grassland on carbon and nitrogen dynamics is highly dependent on
location. First, in the implementation and the degree of intensification, major differences
have been observed. Thus, a direct comparison are not directly achievable. However, it can
be stated that intensified grassland management, except with a high application rate of
mineral N fertilizer and a large removal of biomass (with a mean of 1459 g m$^{-2}$), does not
or only has a low tendency to have negative impacts on the SOC and N, stocks and
sequestration mechanisms. Unlike the loss of biodiversity through intensified use, the SOC
stocks and sequestration mechanisms, which are inevitably linked to plant species diversity
and the variety of soil organisms, tend to react more slowly to management changes, while
showing relatively rapid regeneration. Thus, targeted and appropriate management seems
to be important when favoring SOC stocks and sequestration mechanisms.
Zusammenfassung


Allerdings wird besonders auf schlechter zu bewirtschaftenden, häufig gebirgigen Standorten, mit vergleichsweise geringer Bodenqualität, eine extensivere Beweidung zur Deckung der Nachfrage nach extensiv erzeugten Fleischprodukten, aber auch zur Förderung von Biodiversität und Naturschutz empfohlen.

Ebenfalls von Interesse für eine Ertragssteigerung ist die Erneuerung von Grünland, die besonders in intensiver genutztem Grünland regelmäßig, ganz oder teilflächig, erfolgen kann. Hier ist es einerseits möglich Grünlandsaatmischungen ohne vorherige Bodenbearbeitung per Direktsaat einzubringen oder andererseits eine vorhergehende Bodenbearbeitung durchzuführen und Grünland neu einzusäen.

Allgemein bekannt ist, dass eine Nutzungsintensivierung im Grünland, eine Grünlanderneuerung oder gar eine Umwandlung zu Ackerland, die Kohlenstoff- und Stickstoffspeicherung im Boden beeinflusst.

Aus diesen aktuellen Gegebenheiten leiten sich für meine Dissertation folgende Ziele ab:


(II) Die Untersuchung des Einflusses von Schnittnutzungshäufigkeiten in Kombination mit mineralischer Düngung auf Kohlenstoff- und Stickstoffvorräte im
Zusammenfassung

Boden, die Kopplung von Kohlenstoff und Stickstoff im Boden, den mikrobiellen Bodenkohlenstoff, Ergosterol als Marker für die pilzliche Biomasse, die Bodenaggregatgrößenklassenverteilung und deren Kohlenstoffgehalte und die Zusammensetzung der organischen Bodensubstanz.


Zur Bearbeitung von (I) wurde ein Langzeit-Beweidungsexperiment nordwestlich von Göttingen beprobt. Die FORBIOBEN (FOR BIOdiversity BENefit) Versuchsfläche im Solling bei Relliehausen gliedert sich in neun, jeweils ca. 1 ha große, gezäunte und durch Rinder beweidete Wiesen. Drei unterschiedlich extensive Beweidungsintensitäten in drei Wiederholungen wurden in drei Tiefenstufen (0-10 cm, 10-25 cm, 25-40 cm) beprobt. Die Beweidungsintensität wurde ermittelt und regelmäßig auf jeder der neun Flächen mittels diagonaler Messung der Grasnarbenhöhen überprüft. Abhängig davon, ob die Zielvorgabe (mäßige Beweidungsintensität: Grasnarbenhöhe ~ 6 cm, geringe Beweidungsintensität: Grasnarbenhöhe ~ 12 cm und sehr geringe Beweidungsintensität: Grasnarbenhöhe ~ 18 cm) der entsprechenden Beweidungsintensität erreicht wurde bzw. nicht erreicht wurde, wurde die Anzahl der Rinder auf der jeweiligen Parzelle angepasst.

durchgeführt, sofern die Normalverteilung der Residuen und Homoskedastizität vorlag, andernfalls wurde eine Welch-ANOVA durchgeführt.

Es wurden keine signifikanten Effekte (\( p \leq 0.05 \)) der Beweidungsintensität auf die gewichteten Vorräte von Kohlenstoff- und Stickstoff, des mikrobiellen Kohlenstoffs und der Basalatmung festgestellt. Abgesehen von den C/N-Verhältnissen war die Datenlage stark heterogen, was vermutlich auf die heterogene Bodenmineralogie und auf mögliche Unsicherheiten in der Analytik und der Bestimmung der Lagerungsdichte und des Skelettanteils zurückzuführen ist. So konnte ein großer Teil der Variation der organischen Kohlenstoff- und Stickstoffgehalte über die Gehalte an oxalatlöslichem Eisen erklärt werden (multiple lineare Regression: \( R^2 = 0.64 \)). Die Gehalte an mikrobiellem Kohlenstoff (\( R^2 = 0.96 \)) und die Basalatmungsrate (\( R^2 = 0.9 \)) wurden hingegen wiederum zu einem großen Teil durch die organischen Kohlenstoffgehalte erklärt.


Zur Bearbeitung von (II) wurde Grünland in der Nähe von Kiel auf einer Versuchsfläche der Christian-Albrechts-Universität zu Kiel in drei Bodentiefen bis 60 cm beprobt. Das Grünland wurde 2004 nach vorheriger Ackernutzung einheitlich angelegt. Die vier Behandlungen 3-Schnittnutzung und 5-Schnittnutzung, jeweils ungedüngt, bzw. gedüngt (mit 360 kg N ha\(^{-1}\) a\(^{-1}\) in Form von Kalkammonsalpeter), wurden unterteilt durch ein randomisiertes Blockdesign mit drei Feldwiederholungen. Die Bodenproben wurden auf Kohlenstoff- und Stickstoffvorräte, die Kopplung von Kohlenstoff und Stickstoff, die mikrobielle und pilzliche Biomasse, die Bodenaggregategrößenklassenverteilung, die Kohlenstoffgehalte der unterschiedlichen Aggregategrößenklassen und die Zusammensetzung der organischen Bodensubstanz untersucht. Zur Datenanalyse wurden dreifaktorische Varianzanalysen mit den Faktoren Schnitt (3-Schnitt und 5-Schnitt),
Zusammenfassung

Düngung (Stickstoffdüngung und keine Stickstoffdüngung), sowie die Interaktion von Schnitt und Düngung und Block (Block 1-3) durchgeführt.

Signifikant höhere organische Kohlenstoffvorräte wurden unter der 5-Schnittnutzung im Vergleich zur 3-Schnittnutzung in der Bodentiefe 0 ~ 10 cm festgestellt. Eine vermehrte Schnittnutzung kann zu einer Stimulation des Wachstums von photosynthetisch aktiven Pflanzenteilen führen und daraus resultierend zu einem erhöhten Kohlenstoffeintrag beitragen. Außerdem wurden ebenfalls in der Bodentiefe 0 ~ 10 cm höhere organische Kohlenstoffvorräte unter den N-ungedüngten im Vergleich zu den N-gedüngten Flächen festgestellt. Unter den N-gedüngten Flächen wurde möglicherweise die mikrobielle Aktivität durch die Stickstoffgabe angeregt, was zu einer stärkeren Mineralisierung von organischem Kohlenstoff führte. Weiterhin vermuten wir, dass die Veränderung der Pflanzenartenzusammensetzung in Folge der unterschiedlichen Bewirtschaftung diese Ergebnisse verstärkte. Die Flächen, die nicht N gedüngt wurden, aber durch eine 5-Schnittnutzung bewirtschaftet wurden, zeigten nach 8 Jahren vor allem *Lolium perenne* als Hauptbestandsbildner und am zweithäufigsten die Leguminose *Trifolium repens*. *Lolium perenne* ist ein Hochleistungsgras und zeigt ein dichtes Wurzelfilz in 0 ~ 10 cm Bodentiefe. Der mikrobielle Kohlenstoff war signifikant höher unter der 5-Schnittnutzung im Vergleich zur 3-Schnittnutzung, wohingegen N-Düngung zu geringeren mikrobiellen Kohlenstoffgehalten im Oberboden führte. Dieser ist vermutlich auf eine Stimulation der Wurzelexsudation, ausgelöst durch eine höhere Schnittnutzungsintensität einerseits und komplexe Veränderungen in der mikrobiellen Gemeinschaft auf Grund der Stickstoffgabe andererseits, zurückzuführen. Die Ergosterolgehalte waren signifikant erhöht unter der 5-Schnittnutzung, vermutlich auch begründet durch ein erhöhtes Wurzelwachstum, erhöhte Wurzelexsudation und eine veränderte Pflanzenartenzusammensetzung. Der mikrobielle Kohlenstoff reagierte entsprechend der organischen Kohlenstoffvorräte. Ähnliche Ergebnisse zeigten auch die Ergosterolgehalte, die zur Quantifizierung der pilzlichen Biomasse herangezogen wurden. Sowohl der mikrobielle Kohlenstoff als auch die Ergosterolgehalte waren eng mit den organischen Bodenkohlenstoffvorräten korreliert (mikrobieller Bodenkohlestoff: Spearman $R_s = 0.81$; Ergosterolgehalt: Spearman $R_s = 0.87$). Dies deutet daraufhin, dass auch die Gehalte an mikrobieller und pilzlicher Biomasse, neben den direkten Einflüssen der Behandlungen, ebenfalls auf die Pflanzenartenzusammensetzung zurückzuführen sind. In der Bodentiefe 0-10 cm wurde eine Verschiebung von kleinen zu großen wasserstabilen Makroaggregaten von der 3-Schnitt- zur 5-Schnittnutzung festgestellt. Dies ist vermutlich bedingt durch den höheren Anteil an *Lolium perenne* unter der 5-Schnittnutzung, das wiederum zu einem höheren Anteil
an Feinwurzeln und Pilzhyphen unter der 5-Schnittnutzung in 0-10 cm Bodentiefe führt und die Aggregierung kleiner Makroaggregate in großen Makroaggregaten fördert.

Die Ergebnisse der Dichtefraktionierung weisen darauf hin, dass die leichte Fraktion nicht durch die unterschiedlichen Behandlungen beeinflusst wurde.

Zusammenfassend zeigt diese Studie, dass eine 5-Schnittnutzung im Vergleich zu einer 3-Schnittnutzung zu einem erhöhten Eintrag von organischem Kohlenstoff führen kann und sich somit prinzipiell positiv auf Bodenfruchtbarkeit und die Kohlenstoffspeicherung auswirken kann, während eine konventionelle N-Düngung leichte negative Effekte zeigte. Grundsätzlich kann allerdings nicht differenziert werden, ob die unterschiedliche Bewirtschaftung direkte Auswirkungen auf den Boden hatte oder ob dies indirekte Effekte, ausgelöst durch die stark veränderte Pflanzenartenzusammensetzung in Folge der varierten Bewirtschaftung, waren.


1. Keine Vorbehandlung des Bodens, Grünlanderneuerung nach chemischer Abtötung der bestehenden Vegetation und Direktsaat;

2. nach chemischer Abtötung der bestehenden Vegetation folgte die Vorbehandlung des Bodens durch Pflug (25 cm tief) und Packer, anschließend wurde das Grünland neu angesät;

3. Dauergrünland als Kontrolle.

Die Bodenproben wurden auf organische Kohlenstoff- und Stickstoffvorräte, Aggregatgrößenklassenverteilung und deren Kohlenstoffgehalte untersucht. Zur Datenanalyse wurden zwei-faktorielle ANOVAs mit den Faktoren Grünlanderneuerung (Kontrolle, Grünlanderneuerung nach chemischer Abtötung der bestehenden Vegetation und Grünlanderneuerung nach Pflugeinsatz) und Block (Block 1-3) angewandt. Für weitergehende Datenanalysen wurden Spearman’s rank Korrelationen und Regressionsanalysen angewandt.


Die Grünlanderneuerung mit Umbruch führt auch zwei und sieben Monate nach dem Umbruch zu geringeren Mikroaggregatkonzentrationen im Oberboden im Vergleich zu der rein chemischen Grünlanderneuerung, während die Mikroaggregatkonzentrationen der Kontrolle zwischen der Grünlanderneuerung mit Umbruch und der rein chemischen lagen. Dies ist darauf zurückzuführen, dass durch den Pflugeinsatz im Vergleich zur chemischen Grünlanderneuerung das durch die chemische Abtötung teils abgestorbene Wurzelfilz umgelagert und durchbrochen wird, was zu einem Stabilitätsverlust der Bodenmatrix führt.


1 General Introduction

1.1 Types of Grassland

Grassland is defined by UNESCO as land vegetated with herbaceous plants, covering less than 10 % tree and shrub cover (White 1983). According to FAO, grassland cover 40.5 % of the Earth’s landmass and is among the largest habitat type in the world (Reynolds et al. 2005). The term “grassland” can be differentiated as native, semi-natural, and cultivated grassland. This differentiation refers to the origin and management of grassland. Native grassland occurs naturally without direct human interventions, in locations where forests and scrublands cannot be established, as they are inhibited by climatic and soil conditions (Silva et al. 2008). Native grassland is comparatively rare in Central Europe; dry grassland and alpine grassland are, for example, native grassland types. Semi-natural grassland is established by humans and is created and maintained by agricultural activities, although their plant communities are predominantly natural (Silva et al. 2008). However, the influence of humans on semi-natural grassland is lower compared to that on cultivated grassland, where the first priority is the production goal. In cultivated grassland, only a low number of highly productive plant species is sown and fertilization is a current practice (Öckinger & Smith 2007).

1.2 Function, Actual Development, and Management Practices of Grasslands

The differentiation of the term “grassland” depicts the different functions grasslands provide (Conant et al. 2010). On the one hand, grassland habitats encompass a wide range of benefits for the conservation of the environment and nature. Grassland ecosystems can support an increased quality of ground- and surface-water, they can contribute beneficially to flood control, they can reach biodiversity goals and provide recreational functions for human beings, and they, after all, support climate mitigation, as they store approximately 34 % of the global stock of carbon in terrestrial ecosystems (Becker et al. 2014; Silva et al. 2008). On the other hand, grasslands provide agricultural products in the form of fodder for livestock. Furthermore, biofuel products have been progressively produced on grasslands for approximately 20 years. Around 25 % of world milk and beef production
occur on grasslands managed solely for those purposes (Conant et al. 2001). In Germany, more than one quarter of the agricultural area was used as grasslands in 2016 (Anonymous 2017). However, from 2003 to 2012, the loss of grassland to cultivated land was about 5% (Behm et al. 2012), whereas, in 2013 to 2016, the amount of grassland area was consistent (Anonymous 2017). The decrease in the area utilized as grassland is often accompanied by the intensification of grassland management (Anonymous 2017; Becker et al. 2014). The grassland in Germany is mainly used as meadows or pastures for the production of hay and silage, as well as for dairy production and cattle husbandry. Intensification of grassland management is characterized by increased harvesting frequencies and increased grazing pressure, as well as by mineral and organic fertilization and increased fertilization rates (Becker et al. 2014). However, particularly in less valuable locations with less productive soils, extensive herding of cattle is recommended in order to achieve biodiversity and production goals (Isselstein et al. 2007). Furthermore, for consistently high yields, partial or complete grassland renovation is regularly conducted, especially in intensively managed grassland (Velthof et al. 2010). Grassland renovation can be conducted by reseeding or by chemical mortification of the old sward and direct seeding. Additionally, it is also possible to combine the above-mentioned procedure by plowing after mortification of the old sward and subsequent to the seeding of the new sward. A dilating aspect is the conversion of grassland predominantly for the cultivation of energy plants (e.g., Zea mays).

1.3 Grassland Management Affects Carbon and Nitrogen Cycles and Sequestration mechanisms

Management intensification in grassland influences the carbon and nitrogen stocks and dynamics (Soussana et al. 2006; Sousana & Lemaire 2014; Rumpel et al. 2015; Conant et al. 2001, 2011; Jones & Donelly 2004). Soil organic carbon (SOC) sequestration in grassland soils is of interest because soils serve a large reservoir of organic carbon in terrestrial ecosystems (Post et al. 1982). Thus, carbon stored in grassland soils is climate relevant (Ciais et al. 2010). Furthermore, SOC in combination with N in soil is important for a productive and functioning ecosystem and influences soil functions and, thereby, soil fertility (Soussana et al. 2014; Conant et al. 2001; Haferkamp & MacNeil 2004).

The carbon cycle in grasslands starts with the net C flow from the atmosphere to the vegetation and soil. Afterwards, the mean residence time of C within the ecosystem is an important aspect for investigating the C cycle in grasslands (Soussana & Lemaire 2014).
The residence time of above-ground C compared to below-ground C is short. The above-ground C residence time depends on the grazing or harvesting regime as well as on the associated release of C into the atmosphere. The residence time of above-ground C is between only a few days and weeks to months (Soussana & Lemaire 2014). The main pathways of C into soil are root and shoot tissues and root exudations (Rasse et al. 2005). In contrast to above-ground sequestration, the residence time of C in soil is much longer; C can be stored in soil from about one year or less to more than 1000 years and is a more complex process (Soussana & Lemaire 2014). Reasons for the differences in residence time of SOC in soil lie in pedoclimatic aspects, but there are further different sequestration mechanisms that could be influenced by grassland management (Soussana et al. 2006; Soussana & Lemaire 2014; Rumpel et al. 2015; Conant et al. 2001, 2011; Jones & Donelly 2004). One important mechanism is the alteration of organic matter into chemical forms that are more recalcitrant to microbial attack or are more likely to adsorb on the soil solids. The mechanisms that cause the biochemical alteration are decomposition, condensation, and polymerization (Jastrow et al. 2007). The sorption of labile organic C or partly humidified soil organic matter (SOM) to soil minerals is another important stabilization process (Baldock & Skjemstad 2000). Greater preservation against mineralization in comparison to chemical protection is gained through the physical protection of SOM in soil aggregates of different size classes and different stabilities (Elliot et al. 1991; Six et al. 2002).

1.3.1 Effects of Grazing on Carbon and Nitrogen Cycles and Sequestration Mechanisms

Grazing influences SOC in the defoliation of plant tissue, trampling, defecation, and urination of grazing animals (Figure 1.1). For an evaluation of the impact of grazing on grassland ecosystems as positive or negative, knowing the pedoclimatic conditions as well as the intensity of grazing is crucial. Depending on the pedoclimatic conditions combined with the level of grazing pressure, the effect of grazing can increase, decrease, or maintain the size of SOC stock in grassland soil unaltered (Milchunas & Lauenroth 1993; Derner et al. 2006; Piñeiro et al. 2010). C and N cycles are strongly coupled in grassland soils as compared to permanent cropping because of a more stable C/N ratio of organic matter inputs to soil (Rumpel et al. 2015). However, grazing can also uncouple C and N cycles (Soussana & Lemaire 2014). Less-intensively grazed pastures are typically dominated by slow-growing plant species producing litter of lower quality and a higher C/N ratio, whereas the more intensively grazed pastures are dominated by faster growing plants with a
higher litter quality and a lower C/N ratio (Klumpp et al. 2007, 2009; Klumpp & Soussana 2009). With an increasing C/N ratio in plants, mineralization by microorganisms decreases, which may result in a longer residence time for organic matter in the soil (Klumpp et al. 2007, 2009; Klumpp & Soussana 2009).

Figure 1.1: Extensively grazed grassland in Relliehausen, Germany. Right side of the fence: Grazing intensity adjusted to a target compressed pasture height (CPH) of about 18 cm. Left side of the fence: Grazing intensity adjusted to a target CPH of about 6 cm.

1.3.2 Effects of mowing and nitrogen supply on carbon and nitrogen cycles and storage mechanisms

Mowing of grasslands affects SOC dynamics in the regular harvest of plant tissue. The same as with grazing, an effect of mowing on SOC dynamics depending on pedoclimatic conditions and harvesting frequency can be expected. Higher harvesting frequencies cause a higher export of C via removal of above-ground biomass and hence lower SOC stocks (Parsons et al. 2013; Jacob 1987). However, defoliation can also have a stimulating effect
on photosynthesis and carbon sequestration in plant material, and thus on SOC stocks (Frame & Laidlaw 2011). Furthermore, due to higher harvesting frequencies, it is possible for a shift in plant species composition to take place and influence the accumulation of carbon stocks in soils (Figure 1.2). As root exudation increased due to frequent mowing, microbial biomass C ($C_{mic}$) is likewise stimulated (Mawdsley & Bardgett 1997; Guitian & Bardgett 2000). Compared to grazing, mowing can have a higher detrimental effect and negatively influences macroaggregation and aggregate stability (Franzluebbers et al. 2000). Few studies exist that examine the influence of mowing on the quality of SOM. Herold et al. (2014) found a reduced turnover of mineral associated SOM and a depletion of young light fraction C due to increased harvesting frequency.

![Figure 1.2: Plant species composition affected by varying grassland management. The photos on the right side show a three-cut (3C) and a five-cut (5C) grassland with an addition of 360 kg of mineral N ha$^{-1}$ per year. The pictures on the left side show a 5C and a 3C grassland with no addition of mineral fertilizers.](image)
Different studies treating mineral N fertilization as a tool for grassland intensification have arrived at controversial results. The review by Conant et al. (2001) found mostly increased soil carbon concentrations caused by fertilization related to stimulated biomass production. On the contrary, a decrease in carbon concentration after continuous fertilization was explained in a study by Li et al. (2014) as being due to the altering of plant species composition and thereby of the biomass composition. Furthermore, N fertilization was related to stimulating effects on C\textsubscript{mic}. Again, increased C\textsubscript{mic} concentrations caused by the stimulating effects of fertilization, as well as decreased C\textsubscript{mic} concentrations because of less root exudation, was observed (Cole et al. 2005; van der Wal et al. 2009). However, studies dealing with water-stable aggregate size class distribution influenced by fertilization in grassland soils are scarce. Nevertheless, Mosquera-Losada et al. (2015) found increasing macroaggregate concentrations in a silvopastoral system due to N fertilization. Accordingly, the effect of N fertilization on SOC stocks and dynamics is not sufficiently understood and depends on pedoclimatic conditions (Rumpel et al. 2015).

1.3.3 Effects of Grassland Renewal and Different Practices of Grassland Renewal on Carbon and Nitrogen Cycles and Sequestration Mechanisms

Grassland renovation is a tool to obtain high yields and a premium fodder quality in intensively used grasslands. Renovation can be conducted by chemical mortification of the existing vegetation and re-sowing with an adjusted plant species mixture (chem) or by chemical mortification of the existing vegetation and then a subsequent plowing before re-sowing the adjusted seed mixture (phys), as already mentioned above. However, besides the positive effects grassland renovation has on production goals, it is already known that grassland renovation can cause losses of N\textsubscript{2}O emissions and NO\textsubscript{3} leaching, and can thereby cause environmental harm. Knowledge of the chem treatment on SOC and aggregate dynamics is scarce, perhaps because it is not as common as the phys treatment for grassland renovation. Ammann et al. (2013) found a decrease in carbon stocks of about 130 g C h\textsuperscript{-1} y\textsuperscript{-1} on average over three years after a physical grassland renovation. Before grassland renovation, the grassland studied acted as a carbon sink and accumulated around 100 g C ha\textsuperscript{-1} y\textsuperscript{-1} over a period of six years. The authors explained this loss as mainly due to respiration losses in the fallow phase between plowing and reseeding. The results match those of different studies working on water-stable soil aggregate classes. These studies have found decreasing macroaggregate concentrations after plowing or tillage in general, which was caused by the physical destruction of macroaggregates and followed by a release of SOC that was occluded before and thereby protected against mineralization.
1 General Introduction

(Linsler et al. 2013; Stavi et al. 2011; Zotarelli et al. 2007; Bronick & Lal 2005). Additionally, plowing of grasslands often results in losses of nitrogen (Davies et al. 2001; Soussana et al. 2006) and can also further cause a reduction in C\textsubscript{mic} (Wortmann et al. 2008). Besides the influence of a tillage event, Linsler et al. (2015) found a temporal variation in water-stable aggregates, C\textsubscript{mic}, and ergosterol as a proxy for fungi in temperate grassland soil. Here, climatic conditions seem to have played an important role, because increased gravimetric moisture content also led to an increased macroaggregate concentration.

1.4 Objectives of the Present Study

It can be concluded from the findings above that influences of management intensification on grassland SOC dynamics are not fully understood. Even though – or especially because – grassland soils are basically high in SOC stocks and favorable systems in terms of SOM storage, grassland management and intensification may decouple C and N cycles. Decoupled C and N cycles are harmful because losses of reactive N are facilitated, and, furthermore, most of the ecosystem services that grassland provides are linked to C and N cycles via processes such as photosynthesis, respiration, and SOM decomposition. Grassland intensification influences the biogeochemical cycles of C and N, mostly by affecting plant activity and thereby rhizosphere processes and nutrient flows. For optimized grassland management processes dependent to pedoclimatic conditions and management, it is important for SOC and N dynamics to be better known and understood to ensure ecosystem services and productivity of grassland systems. Currently we lack knowledge of the effect of varying extensive grazing intensity, of combined management intensification (such as varying fertilization and cutting regimes), and of different grassland renovation practices regarding SOC dynamics. Investigations into disentangle below-ground complexity are needed.

Motivated by the aforementioned lack of knowledge, the research objectives of my thesis are to:

(I) determine the influence of varying extensive grazing on SOC and N\textsubscript{t} stocks, on the coupling of C and N cycles, on C\textsubscript{mic} concentrations, on basal respiration, and on mineral N in soil.

(II) determine the influence of cutting frequency combined with mineral N fertilization and no N fertilization on SOC and N\textsubscript{t} stocks in soil, the coupling of C
and N cycles, C_{mic} concentrations, ergosterol content as a proxy for fungi, soil aggregate size class distribution and their C content, and the composition of SOM.

(III) investigate the temporal dynamic of physical and chemical grassland renovation practices regarding SOC stocks in soil, soil aggregate size class distribution, and their C content.
2 Effect of grazing intensity and soil characteristics on soil organic carbon and nitrogen stocks in a temperate long-term grassland

Anja Nüsse¹, Deborah Linsler¹, Michael Kaiser¹, Dorothee Ebeling², Bettina Tonn², Johannes Isselstein² and Bernard Ludwig*¹

¹Department of Environmental Chemistry University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

²Institute of Grassland Science, Georg-August-University, Göttingen, von-Sieboldt-Str. 8, 37075 Göttingen, Germany

*Corresponding author: + 49 5542 981631; e-mail: bludwig@uni-kassel.de
ABSTRACT

The effects of different grazing pressures on soil properties are not sufficiently understood. The objectives were to analyze the effects of three different extensive grazing pressures on stocks of soil organic C and total N, soil microbial biomass C, basal respiration and mineral N in three different soil depths of a long-term pasture in Central Germany (FORBIOBEN field trial). No significant \( p \leq 0.05 \) effects of grazing pressure on weighted stocks of soil organic C, total N, soil microbial biomass C, mineral N and basal respiration rate were observed, suggesting that the C and N cycles are coupled in the three grazing treatments. Oxalate soluble Fe contents explained a marked part of the variation of soil organic C (multiple linear regression: \( R^2 = 0.64 \)) and total N contents \( (R^2 = 0.64) \) in the soils, whereas almost all of the variability of soil microbial biomass C contents and basal respiration was explained by soil organic C contents. Overall, variabilities of soil organic C and N contents were largely explained by oxalate soluble Fe contents, whereas grazing intensity did not affect the C and N dynamics.

Keywords: pasture; grazing pressure; microbial biomass; mineral N; basal respiration rate
2 Effect of grazing intensity and soil characteristics on soil organic carbon and nitrogen stocks in a temperate long-term grassland

2.1 Introduction

Nearly one third of the German agricultural area is used as grassland (Socher et al. 2013). Intensity of grassland management is varying but often driven by the demand for forage production (Conant et al. 2001). A recommended management practice for low-productivity grasslands in Germany is an extensive herding of cattle for meat production to serve biodiversity and production goals (Isselstein et al. 2007). Type and intensity of grassland management exert strong influence on soil organic carbon (SOC) and total nitrogen (N) stocks of pasture soils (Conant et al. 2001). SOC stored in grasslands is climate relevant (Ciais et al. 2010) and is in conjunction with N, sequestration important for the productivity of pasture ecosystems (Conant et al. 2001; Haferkamp & MacNeil 2004).

In general, temperate grassland soils are typically rich in SOC because of rhizodeposition (Jones & Donelly 2004) and because of the activity of soil fauna that promote aggregation of soil organic matter and stabilize it for extended periods (Six et al. 2002). C and N cycles are more strongly coupled in grassland soils as compared to permanent cropping because of a more stable C/N ratio of organic matter inputs to soil (Rumpel et al. 2015). Soussana and Lemaire (2014) suggested grazing pressure (GP) in intensively used pastures as the main factor for C return to soil, since the reduced leaf area and the excretal return may affect the C and N cycle markedly. Up to 60 % of above-ground dry-matter production is ingested by domestic herbivores on intensively used pastures (Lemaire & Chapman 1996). Especially moderate grazing on naturally nutrient poor grasslands could favor nutrient cycling and increase primary production (Klumpp et al. 2007). The leaf area determines the capacity to capture atmospheric CO₂ and may be mainly affected by the GP due to defoliation intensity and frequency and treading by animals (Hodgson 1996). Grazing has an influence on the plant species composition and the development stages of plants (Hodgson 1996; Frame & Laidlaw 2011). Klumpp et al. (2007; 2009) and Klumpp and Soussana (2009) described a shift from less intensively grazed pastures dominated by slow growing plant species producing litter of less quality (high C/N ratio of ~ 40 and high lignin content) to more intensively grazed pastures dominated by faster growing plants with a higher litter quality. With increasing C/N ratio of plants the mineralization by microorganisms may decrease, which may result in a longer residence time of organic matter in soil. Moreover, higher digestible plants and plant parts (lower C/N ratio of ~ 20), dominating on intensively grazed pastures, may induce a decoupling of C and N cycles (Soussana & Lemaire 2014).
Quantity and quality of the impact of varying GP on SOC and N\textsubscript{t} pools may depend on environmental conditions. Previous results show diverse effects of grazing on SOC, whereas many of these differences appear to be the result of variations in climate, soil properties, landscape position, plant community, soil sampling and grazing management practices (Reeder 2002; Haferkamp & MacNeil 2004; Derner et al. 2006; Piñeiro et al. 2010). Consequently, it is not possible to extrapolate from global data sets or from data of arid or semi-arid environments to pasture conditions in the temperate zone. So far only few studies investigated the effect of varying GP in Germany or Central Europe on SOC stocks using long term field experiments.

Besides an effect of varying GP on SOC and N\textsubscript{t} pools, these pools are also affected by mineral characteristics and pH (Eusterhues et al. 2005, Wiseman & Püttmann 2006). For sites with small variabilities in mineral characteristics and pH, effects of varying GP on SOC and N\textsubscript{t} pools may be detectable, whereas for sites with high variabilities, effects of varying GP may be masked.

Based on the results summarized above, we hypothesized a lower carbon sequestration under higher GP and a decoupling of C and N cycles with increasing GP. Moreover, we hypothesized changed species decomposition and substrate quality at increasing GP, which may markedly affect stocks of soil microbial biomass C (C\textsubscript{mic}) and basal respiration rates. Besides the management effect GP, we hypothesized that SOC and N\textsubscript{t} stocks may be described to a marked extent by regression analyses using pH and contents of clay and oxalate soluble Fe (Fe\textsubscript{ox}) and Al (Al\textsubscript{ox}) as predictors. The objectives were to analyze the effects of three different GPs on weighted stocks of SOC and N\textsubscript{t} in three different soil depths for the extensively grazed FORBIOBEN (Isselstein et al. 2007) field trial in Relliehausen, Germany. Further, the correlation of SOC and N\textsubscript{t} concentrations were used to examine coupling of C and N cycles under varying GP. Additional objectives were to analyze the effect of GP on C\textsubscript{mic}, basal respiration rate and plant available N. Moreover, soil characteristics such as pH, texture and Fe\textsubscript{ox} and Al\textsubscript{ox} were analyzed that are known as alternative controlling factors of the soil C and N dynamics (Eusterhues et al. 2005, Wiseman & Püttmann 2006).
2.2 Materials and Methods

2.2.1 Study area

The study site is located near Relliehausen at the Scharfenberg in one of the central German uplands, the Solling (51° 46’ N, 9° 42’ E), 250 m above sea level. The mean annual temperature is 8.2 °C and the long-time average annual precipitation is 879 mm year⁻¹. The experimental field site is a mesotrophic, moderately species-rich hill grassland on a Vertisol with an average number of plant species of 10.9 m⁻² (Şahin Demirbağ et al. 2009). The pH is between 4.82 and 7.51, the soil texture ranges between a silt loam and a clay soil. The vegetation type is a Lolio-Cynosuretum (Scimone et al. 2007) which is dominated by grasses like *Agrostis* spec., *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa* spec., *Taraxacum officinale*, *Trifolium repens* (Şahin Demirbağ et al. 2008).

2.2.2 Plot description and sampling design

A grazing experiment with cattle (Simmentaler suckler cows) and no input of fertilizers, pesticides or cutting was established in spring 2005. Grazing management was conducted similar to traditionally extensive grazing. The target compressed pasture height (CPH) classes, which were the result of different grazing pressures, were selected after Isselstein et al. (2007) and Wrage et al. (2012). During winter, the cattle was kept in a barn and during the vegetation period on pasture (without feeding supplements). Start and end of the grazing period were dependent on weather conditions. The study site was subdivided into nine paddocks, each one hectare large, with following grazing pressures each replicated three times (Wrage et al. 2012):

- medium GP, target CPH of 6 cm, designed to utilize herbage growth for optimum livestock production (ca. 3.4 standard livestock units (one standard livestock unit being 500 kg) per ha)
- lenient GP, target CPH of 12 cm, designed to increase biodiversity by not fully utilizing herbage growth (ca. 1.8 standard livestock units per ha)
- very lenient GP, CPH of 18 cm, designed to increase biodiversity by not utilizing herbage growth (ca. 1.3 standard livestock units per ha)

The different grazing pressures were determined by measuring the CPH in the vegetation period at intervals of two weeks using a rising-plate meter (Corell et al. 2003) and subsequently increasing or decreasing stocking rate, if measured CPH deviated from
target CPH. The paddocks did not show a homogeneous CPH because of selective grazing of cattle. Therefore, three CPH classes were defined: short (CPH ≤ 5 cm), medium (5 cm < CPH < 12 cm) and tall (CPH ≥ 12 cm). Sample locations within the site were selected from two random points per paddock. Starting from these points, the closest 50 x 50 cm plots of short (CPH ≤ 5 cm), medium (5 cm < CPH < 12 cm) and tall (CPH ≥ 12 cm) CPHs were chosen for soil sampling. In each of these 50 x 50 cm plots, we took two combined samples (in total about 1 kg soil) with an open sided auger (Edelmann, Eijkelkamp, Giesbeek, the Netherlands; diameter of 6 cm) for basic characterization of soil properties and three combined samples for bulk density with sampling rings (Cylinder Set Model A, Eijkelkamp, Giesbeek, the Netherlands; volume of 100 cm³). In total, we took soil samples at 18 plots (3 paddocks as field replicates x 2 random points per paddock x 3 plots for each CPH per random point) per GP representing the heterogeneity of sward and geology of the study location. Soil samples were taken in three depths (0 - 10 cm, 10 - 25 cm and 25 - 40 cm) in April 2013, resulting in 54 soil samples for basic characterization and bulk density each. The samples for soil characterization were sieved (with 2 mm mesh-size) and stored at 4 °C before analyzing and the samples for bulk density were dried at 105 °C for at least 24 hours before weighing. In our study, a focus was put on weighted soil parameters with the proportion of each CPH class as a weighting factor.

2.2.3 Analytics and sampling design

Basic characterization

Soil bulk density was determined according to DIN ISO 11272 (1998). The pH was analyzed by extraction with CaCl₂ (25 mL 0.01 M CaCl₂, 10 g soil) (ISO 10390 2005). Soil texture was determined by applying the pipet method (DIN ISO 11277 2002). Gravimetric soil moisture content was determined by weighing field-moist soil, drying at 105 °C for 24 h and weighing soil again after drying. The determination of Feox and Alox followed DIN 19684-6 (1997). Briefly, a 5 g sample was shaken for 2 hours with 50 mL extraction solution (0.1 M ammonium oxalate and 0.1 M oxalic acid) and then filtrated through a fiberglass filter. Afterwards, the filtrates were measured with an atomic absorption spectrometer (SO6AA, GBC, Braeside, Australia) for Fe and Al concentrations.

Determination of total carbon, organic carbon, total nitrogen and carbon stocks
The concentrations of total C (Cₜ) and N in the bulk soil were determined by dry combustion on a CN elemental analyzer (Elemental Vario El, Heraeus, Hanau, Germany). The SOC content was calculated as the difference between the contents of Cₜ and inorganic C. The inorganic C concentration was measured with the Scheibler method following DIN 19682-13 (2009). The SOC stocks of the different soil layers were calculated on an equivalent mass basis as suggested by Ellert and Bettany (1995) to take differences in bulk density of the respective soil layers into account.

**Basal respiration and soil microbial biomass**

Basal respiration rates were measured using a slightly modified method of Heinze et al. (2010). Thirty grams of soil (adjusted to 50% of their waterholding capacity) were weighed into plastic beakers. That beaker and another one, containing 5 mL 0.5 M NaOH, were placed into 1500 mL Pyrex glass jars containing 20 mL dest. H₂O. The prepared Pyrex glass jars were incubated in a climate cabinet (ICP 800, Memmert, Schwabach, Germany) for 7 days at 22°C in the dark. The evolved CO₂ was determined by titration of the excess NaOH to pH 8.3 with 0.5 M HCl after addition of BaCl₂ solution. The incubated soil was used for measuring Cₘₐᵋ with the chloroform-fumigation-extraction method. Two subsamples, each 10 g moist soil, were processed per sample. One was fumigated with chloroform and one was not fumigated. The non-fumigated subsample was extracted with 40 mL of 0.5 M K₂SO₄ (30 min by oscillating shaking at 200 rev min⁻¹). To fumigate the other subsamples, they were incubated for 24 hours at 25°C with ethanol-free CHCl₃ and then extracted like the non-fumigated samples. In the extracts, organic C was determined by dry combustion on a CN elemental analyzer (Multi N/C 2100S, Analytik Jena, Germany). Afterwards, Cₘₐᵋ was calculated as EC / kEC, where EC = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and kEC = 0.45 (Wu et al. 1990).

**Mineral N**

Mineral N (Nₘᵋᵋ: sum of NO₃⁻ – N and NH₄⁺ – N) was measured using a slightly modified method of Kuderna et al. (1993). The Nₘᵋᵋ was extracted from 10 g of moist soil with 40 mL of 0.5 M K₂SO₄ (30 min by oscillating shaking at 200 rev min⁻¹). Afterwards, the extracts were analyzed for NO₃⁻ – N and NH₄⁺ – N on a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria).

**Calculations and statistical analyses**
All statistical analyses were conducted with the statistic software R (Version 3.2.2; R Development Core Team 2015). The two pseudo-replicates per paddock and CPH class were averaged for all analyses. Further, weighted means per paddock and soil depth were calculated for the stocks of SOC, N_t, N_min and C_mic concentrations and basal respiration rate with the proportion of each CPH class as a weighting factor (for weighted comparisons see e.g., Bland & Kerry 1998). The weighted means were analyzed with two-factorial ANOVAs with the factor grazing intensity (levels: medium, lenient and very lenient GP) and the factor block in case of normality of residuals and homoscedasticity. For stocks of N_min in soil depth 0-10 cm and for stocks of C_mic in soil depth 25-40 cm heteroscedasticity existed; therefore, a Welch ANOVA was used. Effects were considered significant for \( p \leq 0.05 \).

Multiple linear regression analyses with stepwise variable selection were carried out for SOC, N_t and C_mic concentrations and basal respiration rate in soil depth 0 – 25 cm with the soil parameters pH and clay, Fe_ox, Al_ox and SOC concentrations. Only factors with significant contributions were considered and the models with the lowest Akaike information criterion were chosen. Residuals were visually inspected for normality and homoscedasticity.

Spearman’s rank correlation was carried out for not normally distributed SOC and N_t concentrations of all treatments and soil depths.

### 2.3 Results and Discussion

#### 2.3.1 Weighted stocks of SOC and N_t

For the weighted stocks of SOC and N_t per ha no significant differences between different GPs were observed (Table 2.1). Further, the unweighted SOC and N_t contents were also not affected by GP or CPH (data not shown). Accordingly, a lower carbon sequestration under higher GP as described by Soussana and Lemaire (2014) was not detected for the study site in Relliehausen. We assume that the extensive cattle herding is adjusted to the location in all three GPs, which has also been reported for some other trials (Haferkamp and Macneil 2004; Piñeiro et al. 2010). One has to keep in mind that the determination of SOC and N_t stocks is affected by uncertainties in the analytical determination of the respective contents, the determination of bulk densities and the stone contents. In our study, we used the equivalent soil mass approach to compare different treatments. For a critical discussion on factors affecting the determination, which is
especially important for repeated sampling in time (which is not a focus of this study) readers may refer to Schrumpf et al. (2011).

The SOC and N\textsubscript{t} concentrations were positively correlated (r=0.95, p<0.05; data not shown). Accordingly, the strong correlation of SOC and N\textsubscript{t} concentrations suggest that C and N cycles are coupled over all treatments and depths. A decoupling of C and N cycles with an increased GP as described by Soussana and Lemaire (2014) was not observed for the site in Relliehausen.

**Table 2.1:** Weighted soil organic C (SOC) stock, total N (N\textsubscript{t}) stock, microbial biomass C (C\textsubscript{mic}) stock and mineral N (N\textsubscript{min}) stock and basal respiration among three grazing pressures (GP) and three soil depths; mean values and standard deviations (standard deviations of field replicates are given in parentheses; n=3).

<table>
<thead>
<tr>
<th></th>
<th>Medium GP</th>
<th>Lenient GP</th>
<th>Very lenient GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC stock [t ha\textsuperscript{-1}]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>32.8 (7.4)</td>
<td>39.2 (9.7)</td>
<td>31.6 (12.6)</td>
</tr>
<tr>
<td>10-25 cm</td>
<td>35.5 (6.5)</td>
<td>40.6 (2.2)</td>
<td>30.9 (4.2)</td>
</tr>
<tr>
<td>25-40 cm</td>
<td>30.1 (10.0)</td>
<td>31.1 (5.0)</td>
<td>24.3 (12.4)</td>
</tr>
<tr>
<td>N\textsubscript{t} stock [t ha\textsuperscript{-1}]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>3.24 (0.6)</td>
<td>3.76 (0.9)</td>
<td>3.11 (1.1)</td>
</tr>
<tr>
<td>10-25 cm</td>
<td>3.42 (0.7)</td>
<td>4.00 (0.5)</td>
<td>3.21 (0.3)</td>
</tr>
<tr>
<td>25-40 cm</td>
<td>2.80 (0.7)</td>
<td>2.20 (0.6)</td>
<td>1.68 (0.3)</td>
</tr>
<tr>
<td>C\textsubscript{mic} stock [kg ha\textsuperscript{-1}]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>934 (246)</td>
<td>1010 (250)</td>
<td>834 (210)</td>
</tr>
<tr>
<td>10-25 cm</td>
<td>826 (164)</td>
<td>969 (208)</td>
<td>812 (130)</td>
</tr>
<tr>
<td>25-40 cm</td>
<td>554 (272)</td>
<td>462 (95)</td>
<td>329 (14)</td>
</tr>
<tr>
<td>Basal respiration [kg CO\textsubscript{2}-C ha\textsuperscript{-1} day\textsuperscript{-1}]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>24.8 (9.7)</td>
<td>25.5 (3.8)</td>
<td>19.0 (6.2)</td>
</tr>
<tr>
<td>10-25 cm</td>
<td>25.2 (9.0)</td>
<td>26.4 (2.7)</td>
<td>22.2 (5.0)</td>
</tr>
<tr>
<td>25-40 cm</td>
<td>22.6 (7.6)</td>
<td>28.5 (3.6)</td>
<td>19.9 (10.6)</td>
</tr>
<tr>
<td>N\textsubscript{min} stock [kg ha\textsuperscript{-1}]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>48.9 (4.0)</td>
<td>39.9 (6.9)</td>
<td>46.1 (28.7)</td>
</tr>
<tr>
<td>10-25 cm</td>
<td>9.1 (0.6)</td>
<td>10.5 (2.1)</td>
<td>10.9 (2.1)</td>
</tr>
<tr>
<td>25-40 cm</td>
<td>11.9 (5.0)</td>
<td>11.9 (1.4)</td>
<td>12.5 (5.6)</td>
</tr>
</tbody>
</table>

2.3.2 Weighted stocks of C\textsubscript{mic}, N\textsubscript{min} and basal respiration rates

The weighted stocks of C\textsubscript{mic}, N\textsubscript{min} and basal respiration rates per ha were not affected by GP (Table 2.1) or CPH, respectively. Differences of plants between the GPs, e.g. varying plant species compositions, varying plant development stages and above ground
biomass may influence litter and root quality (Frame & Laidlaw 2011). Further, it is well known that litter and root quality of plants (e.g., using C/N ratio and lignin content as simple indicators) may influence the mineralization by microorganisms (Klumpp et al. 2007; Klumpp et al. 2009; Klumpp & Soussana 2009) and thus the stocks of C_{mic} and also the N mineralization dynamics. However, no effect of the GP on these properties were observed in the trial, presumably because of only small effects of the GPs on the species compositions and substrate quality or because of large spatial heterogeneity of SOC stocks (standard deviations of three field replicates are given in Table 2.1) which made a detection of treatment effects difficult.

2.3.3 Data variability – influences of mineral soil characteristics and pH

The SOC and N_t contents (data not shown) and also the stocks of SOC, N_t, C_{mic} and basal respiration rate are highly variable for the same GP. We hypothesized that much of this variability is due to the variability of mineral characteristics and pH. The results of the multiple linear regression analysis confirmed this expectation for Fe_{ox}, which is known to stabilize SOC by sorptive mechanisms and has been suggested as a predictor for SOC concentrations (Wiseman & Püttmann 2006). A marked part of the variability of SOC and N_t contents in 0-25 cm was explained with Fe_{ox} as predictor for SOC (R^2=0.64) and N_t (R^2=0.64) (Figure 2.1). The underlying mechanisms for the stabilization of SOC by different Fe fractions including Fe_{ox} are discussed by Eusterhues et al. (2005).

Almost all of the variability of C_{mic} contents (R^2=0.96) and basal respiration rate (R^2=0.9) in 0 – 25 cm was explained by SOC contents, which emphasizes the importance of SOC as nutrient source for the microorganisms.
2 Effect of grazing intensity and soil characteristics on soil organic carbon and nitrogen stocks in a temperate long-term grassland

2.4 Conclusion

The strong correlation between SOC and N, concentrations in the FORBIOBEN field study over all treatments and soil depths suggests that the C and N cycles are coupled among all GPs. The different CPH classes of each GP did not have any effect on SOC and N stocks and no significant effects of the GP on weighted stocks of SOC, N, Cmic, Nmin and basal respiration rate were observed, presumably due to a marked spatial variability of soil mineral characteristics (Figure 2.1 shows the range of Feox in 0-25 cm). Particularly,
Fe$_{ox}$ contents explained a marked fraction of the variation of SOC ($R^2 = 0.64$) and $N_i$ contents ($R^2 = 0.64$) in the soils. SOC in turn is an important nutrient source for microorganisms and positively related to $C_{mic}$ contents and basal respiration.

### 2.5 Acknowledgments

This work was supported by the German Research Foundation under grant DFG, GRK 1397.
3 Effect of grassland harvesting frequency and N fertilization on stocks and dynamics of soil organic matter in the temperate climate

Anja Nüsse¹, Deborah Linsler¹, Ralf Loges², Thorsten Reinsch², Friedhelm Taube², Bernard Ludwig¹*

¹Department of Environmental Chemistry University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

²Institute of Crop Science and Plant Breeding, Working group: Grass and Forage Science/ Organic Agriculture, Christian-Albrechts-University, Hermann-Rodewald Str. 9, 24118 Kiel

*Corresponding author: + 49 5542 981631; e-mail: bludwig@uni-kassel.de
ABSTRACT

Management of grassland may affect the dynamics of soil organic carbon (SOC). Objectives were to analyze the effect of different harvesting frequencies and nitrogen fertilization regimes on SOC and total N stocks in a field trial on a sandy loam to loamy sand soil of a grassland site near Kiel (Germany). Additionally, effects on microbial biomass C (C_{mic}) and ergosterol (as proxy for fungi) contents, water-stable aggregate size-classes and density fractions were studied. In the surface soil (0–10 cm), SOC and total N stocks, amounts of large water-stable macro-aggregates (>2000 µm) and contents of C_{mic} and ergosterol were significantly higher under a five cut regime. Moreover, C_{mic} (r_s = 0.61) and ergosterol contents (r_s = 0.67) were correlated with amounts of large water-stable macroaggregates suggesting that fungi and microbial biomass play an important role in binding of small macroaggregates into large macroaggregates. The free light fraction of SOM showed significantly higher C concentrations under three cut compared to five cut at 30–60 cm, presumably related to the C/N ratio and the decomposability of root litter. This study indicates the importance of cutting frequency on SOC and total N stocks, amounts of large macroaggregates and contents of C_{mic} and ergosterol.
3 Effect of grassland harvesting frequency and N fertilization on stocks and dynamics of soil organic matter in the temperate climate

3.1 Introduction

Intensity of grassland management is often driven by the demand for forage production (Conant et al. 2001) causing increased harvesting frequencies and N application amounts (Soussana et al. 2014; Rumpel et al. 2015). These factors may affect sward composition and further may affect stocks of soil organic carbon (SOC) and total nitrogen (N\textsubscript{t}) because of differences in root biomass, root growth and root distribution (Conant et al. 2001; Rumpel et al. 2015). Temperate grassland soils are typically rich in SOC because of a permanent plant coverage and thus high in root and shoot tissue deposition, active rhizodeposition (Jones & Donnelly 2004) and activity of soil fauna that promote aggregation of soil organic matter (SOM) and stabilize it for extended periods (Six et al. 2002). SOM is regarded as important for the functionality and productivity of grassland ecosystems (Conant et al. 2001; Rumpel et al. 2015).

For different grassland sites (e.g. differing in soil type, vegetation, climate, management) different effects of harvesting frequencies on SOC dynamics were observed. For instance, Parsons et al. (2013) described an increased export of harvestable C and nutrients due to an increased harvesting frequency and hence lower SOC and N\textsubscript{t} stocks. However, additional processes may also be relevant. To the contrary, higher harvesting frequencies could promote growth of tiller and leaf, which are high in photosynthesis and can stimulate above and below ground biomass production (Frame & Laidlaw 2011).

Nitrogen fertilization (200-400 kg N ha\textsuperscript{-1} year\textsuperscript{-1}) is generally expected to increase SOC stocks in grasslands (see e.g., review by Conant et al. 2001) by a higher aboveground gross primary production and thus result in increased root C-inputs for a formation of SOM. However, the decrease of SOC stock in an alpine meadow in China after 5 years of continuous fertilization was explained due to altering plant biomass composition, especially of the C/N ratios of different plant functional groups (Li et al. 2014). Frequent harvesting may result in increased contents of soil microbial biomass C (C\textsubscript{mic}) presumably due to stimulating root exudation (Guitian & Bardgett 2000). N fertilization increased C\textsubscript{mic} contents in some studies (e.g., van der Wal et al. 2009). A decrease, however, was also reported and explained by a decrease of C allocated by plants into roots and via root exudates into the rhizosphere (Bazot et al. 2006). Overall, effects of N addition on microorganisms in grasslands are not sufficiently understood.

Knowledge about the influence of N fertilization on water-stable aggregate size class distribution in grassland soils is also limited. The few studies available suggest that in
silvopastoral systems increasing contents of macroaggregates due to N fertilization may be found (Mosquera-Losada et al. 2015). Additionally, N fertilization is only a proxy for N availability for crop growth, as reduced N fertilization rates promote legume abundance in grassland (e.g. white clover), often resulting in dinitrogen fixation rates exceeding 200 g N/ha (Schmeer et al., 2014). Harvesting may influence macroaggregation and aggregate stability compared to grazing with cattle negatively and thus carbon sequestration (Franzluebbers et al. 2000). Additionally, frequent harvesting may cause a reduced turnover of mineral associated SOM (Herold et al. 2014).

The objectives were to analyze the effect of different harvesting frequencies and fertilization regimes on SOC and total N stocks in a field trial on a sandy loam to loamy sand soil of a grassland site near Kiel, Germany. Additionally, effects on C_{mic} and ergosterol contents, water-stable aggregate size classes and density fractions were studied.

### 3.2 Materials and Methods

#### 3.2.1 Study area

The study site of the Christian-Albrechts-University Kiel is located in north-west Germany in a morainic landscape about 10 km west of Kiel. The field was converted to grassland in 2004. The dominant soil type is a stagnic Luvisol and soil texture ranges from a sandy loam to a loamy sand. The mean annual temperature was about 8.3 °C and the mean annual precipitation was about 777 mm (Schmeer et al. 2014).

#### 3.2.2 Plot description and sampling design

The experiment was established in 2004 by seeding a diverse species seed mixture on all plots. The seeded species were *Lolium perenne*, *Festuca pratensis*, *Dactylis glomerata*, *Poa pratensis*, *Phleum pratense*, *Trifolium repens* and *Medicago sativa* (Schmeer et al. 2014). The field trial was fertilized with 140 kg P₂O₅ ha⁻¹, 370 kg K₂O ha⁻¹ and 100 kg MgO ha⁻¹ per year. The trial was established in a randomized block design with three replicates and included the treatments: three cuts (3C) and five cuts (5C) per year; without and with N fertilization (fertilization: 360 kg N ha⁻¹ year⁻¹ (Calcium ammonium nitrate separated in 120/100/80/60/0 kg N ha⁻¹ and 160/120/80 kg N ha⁻¹ at 5C and 3C regime, respectively). Biomass yields were measured annually for each cut and on each plot with a forage plot harvester (Haldrup, Logstor, Denmark). Additionally biomass sub-samples were taken on
0.5 m$^{-2}$ on each plot with a shear. Dry matter content of sub-samples were estimated after oven drying; yield percentage of each species were identified by weight after separation.

Soil samples were taken as composite samples of three subsamples in three soil depths (0–10 cm, 10–30 cm and 30–60 cm) with an auger for aggregate sampling (Edelmann, Eijkelkamp, Giesbeek, The Netherlands; diameter of 6 cm) in May 2014. Additional samples for determination of bulk density were taken with a known volume for the calculation of stocks. The samples were stored at 4°C before analyzing.

### 3.2.3 Analytics and soil characterization

For a basic soil characterization, bulk density was determined according to DIN ISO 11272 (1998). The pH was analyzed by extraction with CaCl$_2$ (25 mL 0.01 M CaCl$_2$, 10 g soil) (ISO 10390, 2005). The concentrations of total C ($C_t$) and N, in the bulk soil were determined by dry combustion with a CN elemental analyzer (Elemental Vario El, Heraeus, Hanau, Germany). Since no carbonates were detectable the $C_t$ content corresponds to SOC content. The SOC stocks of the different soil layers were calculated on an equivalent mass of soil as suggested by Ellert and Bettany (1995). $C_{mic}$ was determined with the chloroform-fumigation-extraction method after Wu et al. (1990). Ergosterol, a fungal cell-membrane component, was extracted using 2 g field-moist soil shaken with 100 mL distilled ethanol at 259 revolutions min$^{-1}$ for 30 min (Djajakirana et al. 1996). Afterwards, the solution was filtered (Whatmann GF/A), evaporated at 40 °C and 55 mbar, and taken up in 10 mL methanol. Determination of ergosterol was done using a reversed-phase HPLC with 100 methanol and detected at a wavelength of 282 nm (Heinze et al. 2010).

Aggregate fractionation was done using a wet-sieving method developed by Cambardella and Elliott (1994) and modified for grassland soils by Linsler et al. (2013). We placed 100 g air-dried soil ($< 10$ mm) on a 2000 µm sieve and let it slake for 10 min in distilled water. Then, the sieve was dipped out and in the water 50 times. The water-stable aggregates were separated from the water phase by vacuum filtration, dried and weighed. The fractionation procedure was continued with the soil which passes the 2000 µm, placed onto the next smaller sieve (1000 µm). The fractionation procedure was continued as described above for mesh sizes of 2000 µm for large macroaggregates; 1000 µm for medium macroaggregates; 250 µm for small macroaggregates and 53 µm for microaggregates. The material passing through the 53 µm sieve (silt and clay and very small microaggregates) was precipitated with 0.5 M AlCl$_3$ (5 mL on 2 L of supernatant) for
recovering due to siphoning the water off. As the variability of this method is affected by operator (Jacobs et al. 2010), the wet sieving was carried out for all samples by the first author.

Density fractionation was carried out as described by Cerli et al. (2012). Thirty mL of a sodium polytungstate solution (density 2.0 g cm\(^{-3}\)) were placed in a 50 mL centrifugation tube. The tube was moved slowly 5 times upside down. After 30 min a centrifugation with 4000 x g (Multifuge 3 S-R, Hareaus, Hanau, Germany) for 30 min was applied. After additional 30 min the supernatant containing the free light fraction was decanted, vacuum filtered (0.45 µm) and washed with 2 L of distilled water. Afterwards, 30 mL sodium polytungstate solution (density 1.8 g cm\(^{-3}\)) was added to the pellet. Then, the suspension was dispersed by ultrasonic (300 J) while cooling. After 30 min, the suspension was centrifuged and decanted again, as described above. The fraction was subsequently washed with 2 L of distilled water. The remaining pellet was transferred on a sieve with a mesh size of 53 µm and the silt and clay fraction was washed out. The fraction larger than 53 µm was transferred with a minimum volume of sodium polytungstate solution (density 1.8 g cm\(^{-3}\)) to a beaker. Afterwards, the remaining occluded light fraction could be decanted to the occluded light fraction out of the first step of separation. The occluded light fraction was then also washed with 2 L of distilled water. The mineral fractions smaller and larger than 53 µm were merged again, vacuum filtered and washed with 2 L of distilled water as described above. After drying of the fractions, the masses of the free light (fLF), occluded light (oLF) and mineral fractions were recorded.

### 3.2.4 Statistical analysis

All statistical analyzes were conducted with the statistic software R (Version 3.0.1, R Development Core Team, 2010). Three factorial ANOVAs with the factors cut (levels: 3 cuts and 5 cuts), fertilization (levels: N fertilization and no N fertilization), the interaction between cut and fertilization and the factor block (3 replicates organized in blocks) were conducted. Stepwise model reductions were carried out, eliminating first a non-significant interaction, then non-significant main effects (Crawley 2012). Effects were considered significant for p ≤ 0.05. Residuals of the final model were checked for homoscedasticity graphically and for normal distribution by the Shapiro-Wilk test as well as graphically. In 0-10 cm the aggregate contents 53-250 µm and < 53 µm and the ergosterol content were not normally distributed. Thus, the data were log-transformed to meet the requirements of the ANOVA.
For the surface soil, correlation analyses were conducted for the pairs SOC content and 
C_{mic} content, SOC content and ergosterol content, aggregate content > 2000 \, \mu m and 
erygosterol content, aggregate content 1000 – 2000 \, \mu m and C_{mic} content, aggregate content 
250 - 1000 \, \mu m and content of free light fraction. Spearman’s rho (R_s) correlation was 
calculated because the C_{mic} content, ergosterol content, content of free light fraction, 
content of aggregates > 2000 \, \mu m and 1000 – 2000 \, \mu m were not normally distributed.

3.3 Results and Discussion

3.3.1 SOC and N, stocks and C/N-ratios

The SOC stocks were significantly higher under the 5C regime in the soil depth 
0 ~ 10 cm (Table 3.1), presumably as higher harvesting frequencies promote growth of 
tiller and leaf, which are high in photosynthesis and can stimulate biomass production 
(Frame & Laidlaw 2011). Moreover, the SOC stocks were significantly higher in the 
treatment without N fertilization (Table 3.1). N fertilization may result in a decrease of 
SOC stocks by increasing microbial activity and altering C substrate utilization pattern 
through changes of plant biomass composition (Li et al. 2014).

Furthermore, different aboveground biomass yields (3C regime: 1459 ± 33 g/m² and 
1309 ± 38 g/m² for the treatments with and without N fertilization, respectively, and 5C 
regime: 1147 ± 31 g/m² and 851 ± 27 g/m², respectively; mean ± standard error of 10 
years) and a changing plant species composition in the treatments may have also 
contributed to SOM dynamics (Schmeer et al. 2014, Rumpel et al. 2015). All treatments in 
our study had the same initial plant species composition. Soon after the start of the field 
trial, a shift in plant species composition started and eight years after – the time of 
sampling, a marked shift in plant species composition had taken place. In the 3C regime 
the grasses reached 69 % and 34 % and the legumes 28 % and 64 % in the treatments with 
and without N fertilization, respectively, and in the 5C regime the grasses reached 98 % 
and 55 % and the legumes 1 % and 43 %, respectively (mean of ten years, the remaining 
percent are unsown species). More in detail, the main plant species under the 5C regime 
without N fertilization were Lolium perenne (a highly productive grass, 
Kutschera & Lichtenegger 2009) and Trifolium repens (a legume). A dense root growth of 
Lolium perenne in soil depth 0 ~ 10 cm and positive effects of legumes on SOC 
sequestration (Rodrigues et al. 2015) presumably caused the increase of the SOC stock 
under the 5C regime without N fertilization.
The N stocks were significantly higher under the 5C regime in 0 ~ 10 cm in comparison to the 3C regime (Table 3.1), which may mainly be related to the occurrence of *Lolium perenne* under the 5C regime.

The C/N-ratio was significantly higher under the 5C regime than under the 3C regime in 0 ~ 10 cm and 10 ~ 30 cm (Table 3.1), but differences between the C/N ratios of the two harvesting regimes were only small.

### Table 3.1: Soil organic C (SOC) and total N (N\textsubscript{t}) stocks calculated on an equivalent mass of soil and C/N ratios of different cutting frequencies, either with or without N fertilization, in 3 soil depths. Means and standard deviations (n=3). Results of analyses of variances (ANOVAs) are also shown: significant differences (p \leq 0.05) between the factor levels 3C and 5C for the factor cut, between the levels N fertilization and no N fertilization for the factor fertilization and no N fertilization for the factor fertilization and significant interactions are labeled as \leq 0.05.

<table>
<thead>
<tr>
<th>soil depth</th>
<th>3 cuts</th>
<th>5 cuts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-fertilization</td>
<td>no N-fertilization</td>
</tr>
<tr>
<td>SOC stock</td>
<td>0-10 cm</td>
<td>20.1 (1.5)</td>
</tr>
<tr>
<td>[t ha\textsuperscript{-1}]</td>
<td>10-30 cm</td>
<td>32.5 (7.9)</td>
</tr>
<tr>
<td>30-60 cm</td>
<td>30.6 (15.6)</td>
<td>39.3 (2.4)</td>
</tr>
<tr>
<td>N\textsubscript{t} stock</td>
<td>0-10 cm</td>
<td>1.9 (0.2)</td>
</tr>
<tr>
<td>[t ha\textsuperscript{-1}]</td>
<td>10-30 cm</td>
<td>3.3 (0.8)</td>
</tr>
<tr>
<td>30-60 cm</td>
<td>3.3 (1.5)</td>
<td>3.9 (0.2)</td>
</tr>
<tr>
<td>C/N-ratio</td>
<td>0-10 cm</td>
<td>10.5 (0.2)</td>
</tr>
<tr>
<td>10-30 cm</td>
<td>9.7 (0.1)</td>
<td>9.9 (0.1)</td>
</tr>
<tr>
<td>30-60 cm</td>
<td>9.2 (0.5)</td>
<td>10.2 (0.2)</td>
</tr>
</tbody>
</table>

ns: not significant.

### 3.3.2 $C_{mic}$ and ergosterol contents

Contents of $C_{mic}$ were significantly higher under the 5C regime than under the 3C regime in 0-10 cm (Figure 3.1), presumably due to stimulated root exudation (Guitian & Bardgett 2000). N fertilization resulted in significantly lower $C_{mic}$ contents in the surface soil (Figure 3.1). Potential reasons described in the literature are manifold and refer to possible changes in microbial competition and community structure, repression of enzyme activity, the built up of recalcitrant and toxic compounds, differences in root growth, exudation and substrate quality of varying plant species compositions (Lovell et al. 1995).
Ergosterol contents in the surface soil were significantly higher under the 5C regime in comparison with the 3C regime. Root growth, exudation and substrate quality may have caused higher fungal biomass under the 5C compared with the 3C regime (Bardgett & McAlister 1999). No effects of fertilization were observed for ergosterol content in the surface soil.

**Figure 3.1:** a) and b): Content of water-stable aggregate size classes; c) and d): contents of C_{mic} (soil microbial biomass); e) and f): ergosterol contents under different harvesting frequencies with or without N fertilization in 0-10 cm and 10-30 cm, respectively. Means and standard deviations (n=3). Asterisks show significant effects.

In 30-60 cm the C_{mic} contents (data not shown) were significantly higher under the 3C regime, presumably due to a higher root biomass and rhizodeposition at this depth range under the 3C regime related to the plant species composition: The main plant species under the 3C regime were *Medicago sativa* with a taproot and a dense root growth deeper in soil and *Dactylis glomerata* with a dense root growth in 30-60 cm (Kutschera & Lichtenegger 2009).

Contents of C_{mic} (Spearman R_s = 0.81) and ergosterol (R_s = 0.87) in soil depth 0-10 cm are strongly correlated with SOC contents (Figure 3.2), suggesting that in the treatments
which most likely resulted in higher root productions (see above), microbial and fungal biomasses were stimulated.

**Figure 3.2**: Scatter plot of $C_{\text{mic}}$ (soil microbial biomass C) content against content of soil organic carbon (SOC) (a), ergosterol content against SOC content (b), ergosterol content against content of water-stable aggregates (> 2000 $\mu$m) (c), and $C_{\text{mic}}$ content against content of water-stable aggregates (> 2000 $\mu$m) (d) for the surface soil. Spearman’s rho coefficients are also shown.

### 3.3.3 Water-stable aggregate size classes

In 0-10 cm the contents of large macroaggregates (> 2000 $\mu$m) and small macroaggregates (250-1000 $\mu$m) were significantly affected by the factor cut. Contents of large macroaggregates were higher under the 5C regime, whereas the small macroaggregates were lower under the 5C regime (**Figure 3.1**). This shift from small to
large macroaggregates might be explained by higher concentrations of roots and root exudates related to *Lolium perenne* with its high amount of fine roots (Kutschera & Lichtenegger 2009) under the 5C regime in soil depth 0-10 cm (Tisdall & Oades 1982).

The SOC contents stored in all water-stable aggregate size classes were not affected due to cut or fertilization (data not shown). In 10-30 cm and 30-60 cm, no significant treatment effects were found with only one exception: at 10-30 cm, a significant effect of cut and a significant interaction between two factors cut and fertilization was found for the content of large macroaggregates > 2000 µm.

Ergosterol and $C_{mic}$ contents in 0-10 cm were positively correlated with large water-stable macroaggregates (>2000 µm, $R_s = 0.67$ and $R_s = 0.64$, respectively, Figure 3.2) suggesting that fungi (Tisdall & Oades 1982) and $C_{mic}$ play a role in building of large macroaggregates.

At all depth ranges, density fractions were not significantly affected by harvesting or fertilization (not shown). However, the carbon concentration of the free light fraction showed significantly higher SOC concentrations under three-cut compared to five-cut at 30-60 cm (not shown), which is presumably related to the C/N ratio and the decomposability of root litter.

### 3.4 Conclusion

In comparison with three cuts per year, five cuts per year have positive effects on SOC and Nt stocks as well as on amounts of large macroaggregates and contents of $C_{mic}$ and ergosterol, which indicate positive effects on soil fertility. The N-fertilization resulted in slightly negative effects on $C_{mic}$ contents. Plant species composition was strongly influenced by cut and fertilization.

### 3.5 Acknowledgments

We would like to thank Anja Sawallisch for technical assistance. This project is financed by the Deutsche Forschungsgemeinschaft (“Research Training Group 1397: Regulation of soil organic matter and nutrient turnover in organic agriculture”).

52
Effect of chemical and physical grassland renovation on the temporal dynamics of organic carbon stocks and water-stable aggregate distribution in a temperate grassland soil

Deborah Linsler1*, Anja Nüsse1, Caroline Buchen2, Miriam Helfrich2, Hans-Peter Piepho3, Bernard Ludwig1

1Department of Environmental Chemistry University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

2Thünen Institute of Climate-Smart Agriculture, Federal Research Institute for Rural Areas, Forestry and Fisheries, Bundesallee 50, 38116 Braunschweig, Germany

3Biostatistics Unit, Institute of Crop Science, University of Hohenheim, Fruwirthstr. 23, 70599 Stuttgart, Germany

*Corresponding author: + 49 551 39 9341; e-mail: deborah.linsler@uni-goettingen.de
ABSTRACT

Grassland renovation can be done physically by plowing or chemically using herbicides, but information on influences of grassland renovation on soil structure is scarce. Our objective was to compare physically and chemically renovated grasslands and to quantify temporal variations of soil organic carbon (SOC) stocks, water-stable aggregates and SOC stored in aggregate fractions. Soil samples were taken on a sandy soil before (T₀), six days (T₆_days), two months (T₂_mo), seven months (T₇_mo) and twelve months (T₁₂_mo) after grassland renovation and in permanent grassland. Neither grassland renovation practice led to SOC losses in 0 ~ 10 cm after 12 months. The physical renovation led to an increase in microaggregates (53-250 µm) in the surface soil between T₀ and T₆_days and to highest microaggregate concentrations in T₂_mo and T₇_mo. Therefore, plowing seems to have direct negative effects on macroaggregates (10000-250 µm) followed by indirect longer-lasting negative effects, which were nullified after one year. Chemical renovation resulted in no different aggregate distribution than permanent grassland. Strong temporal variations in aggregate distribution were found especially for large macroaggregates with lowest concentrations in T₆_days and 7.6-fold higher concentrations in T₂_mo. A linear regression suggested that the soil gravimetric moisture content might have caused this observation.

Keywords: water-stable aggregates, seasonal dynamics, chemical grassland renovation, physical grassland renovation
4.1 Introduction

Grassland renovation is a common practice in case of decreasing yields of agriculturally used grasslands (Velthof et al., 2010). While especially in organic farming systems the existing grass swards are killed off physically (by tillage with a plow in general), in conventional farming systems this could also be done chemically (by using a broad-spectrum herbicide). However, knowledge about the effect of grassland renovation on soils is scarce.

During chemical grassland renovation the soil is only minimally disturbed, which has contrasting results on soil biota (Kremer & Means, 2009), whereas during physical renovation a large interference takes place, generally by plowing. Plowing is known to disrupt aggregates (Bronick and Lal, 2005; Jacobs et al., 2009), making organic material (OM) more susceptible for microbial degradation and mineralization (Bronick and Lal, 2005). Research has focused on aggregate dynamics in continuously tilled arable land, however, much less is known about sporadic tillage in arable or grassland soils. For arable soils, Quincke et al. (2007) found similar concentrations of water-stable macroaggregates (>250 µm) in the top 5 cm in a no-tilled silty clay loam soil two years after a one-time tillage event compared with the untilled control. In the same time span, a plowing event in a loamy sandy grassland soil resulted in significantly lower concentrations in medium (1-2 mm) and large (10-2 mm) macroaggregates in the top 10 cm compared with permanent grassland (Linsler et al., 2013). However, the influences, especially short-term, of a one-time tillage event on soil aggregate dynamics in grassland soils are largely uninvestigated.

Although the soil in chemically renovated grasslands is not physically disturbed, the death of the plants may also have a large impact on the soil structure. Roots can enmesh soil particles and form stable aggregates (Tisdall & Oades, 1982) and root exudates can furthermore act as aggregate binding agents (Oades, 1993). When these binding mechanisms are lost due to plant death, especially the amount of large macroaggregates may decrease, since that fraction was found to be built by roots (Bearden & Petersen, 2000). However, the magnitude and duration of these impacts of killing off the grass sward on aggregates are largely unknown.

The killed grass sward then turns into a source of soil organic matter. However, in contrast to the chemical renovation, during physical renovation the aboveground biomass is incorporated into the soil leading to a closer contact between residues and microorganisms and therefore may intensify decomposition processes (Jacobs et al., 2011).
On the other hand, the incorporated residues are in closer contact with the soil minerals and aggregate formation may increase. These mechanisms were mostly studied in arable soils and the impacts of the incorporation of OM in grassland soils are largely unknown.

However, the formation and disruption of aggregates is a continuum and may vary with time. Temporal variations in soil organic carbon (SOC) concentrations (Leinweber et al., 1994; Jacobs et al., 2010) or aggregate yields (Daraghmeh et al., 2009; Jacobs et al., 2010) were reported in arable soils. These variations in aggregate distribution were suggested to be caused by soil cultivation (Jacobs et al., 2010) or by environmental factors such as temperature, soil water content (Dimoyiannis, 2009) or freeze-thaw cycles (Li & Fan, 2014). A strong decrease in large macroaggregates due to low precipitation, causing a dry soil, was also reported for grassland soils (Linsler et al., 2015). However, the occurrence and the magnitude of temporal dynamics of aggregate concentrations in grassland soils are poorly understood.

The objective of this study was to quantify the effects of grassland renovation with different practices (physical and chemical) on SOC stocks, water-stable aggregate distribution and carbon storage in aggregate fractions as well as to quantify temporal dynamics for these parameters in renovated grasslands and in permanent grassland within the first year after renovation.

### 4.2 Materials and Methods

#### 4.2.1 Study Area

The study site is located in Germany northwest of Oldenburg (53°10' N, 8°2' E, 10 m a.s.l.). The mean annual temperature and the annual precipitation in the area is 9.9 °C and 760 mm, respectively (data from the nearby station on the experimental site of the Chamber of Agriculture, Lower Saxony). The soil type is a Plaggic Anthrosol and the soil (0-40 cm; mean ± standard deviation) has a sand, silt and clay content of 90.7±3.8, 3.3±3.8 and 7.8±5.3 %, respectively. The pH value is 4.8±0.3. The study site was managed by the Chamber of Agriculture, Lower Saxony and the Thünen-Institute of Climate-Smart Agriculture.
4.2.2 Plot description and sampling design

Before starting the experiment the site had been managed as continuous cut grassland for 15 years. In June 2013 a field trial was established and the treatments were arranged in a randomized complete block design with three replicates (plot size: 90 m²) consisting of (i) chemical renovation: a grassland renovation carried out by chemical sward killing with glyphosate and a subsequent reseeding by direct seeding in 1 cm depth, (ii) physical renovation: a grassland renovation by killing the sward with glyphosate followed by cutting and mixing with a rotary cultivator, plowing with a moldboard plow to a depth of 25 cm and a subsequent reseeding of grassland, (iii) continuous grassland serving as control. A timeline of the management practices within the year of in Table 4.1 (Buchen et al. 2017).

Table 4.1: Management details and sampling times of the different treatments at the field trial in Wehnen (Buchen et al. 2017).

<table>
<thead>
<tr>
<th>Date</th>
<th>Measures</th>
<th>Treatments</th>
<th>Agents</th>
<th>Application rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 27, 2013</td>
<td>1st soil sampling time (T₀)</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 29, 2013</td>
<td>chemical killing</td>
<td>chemical and physical renovation</td>
<td>Round Up Power Flex</td>
<td>3.75 L/ha</td>
</tr>
<tr>
<td>September 2, 2013</td>
<td>seeding</td>
<td>physical renovation</td>
<td>grassland seed mixture¹</td>
<td>40 kg/ha</td>
</tr>
<tr>
<td>September 2, 2013</td>
<td>rotovating and plowing (25 cm depth)</td>
<td>physical renovation</td>
<td>chemical reseeding</td>
<td></td>
</tr>
<tr>
<td>September 3, 2013</td>
<td>seeding</td>
<td>chemical renovation</td>
<td>grassland seed mixture¹</td>
<td>40 kg/ha</td>
</tr>
<tr>
<td>September 5, 2013</td>
<td>2nd soil sampling time (T₆_days)</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 31, 2013</td>
<td>cut</td>
<td>permanent grassland and physical renovation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 31, 2013</td>
<td>3rd soil sampling time (T₃_mo)</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 1, 2014</td>
<td>reseeding</td>
<td>chemical renovation</td>
<td>grassland seed mixture¹</td>
<td>20 kg/ha</td>
</tr>
<tr>
<td>March 27, 2014</td>
<td>N-fertilization</td>
<td>all</td>
<td>Ammonium nitrate²</td>
<td>100 kg/ha</td>
</tr>
<tr>
<td>April 10, 2014</td>
<td>4th soil sampling time (T₄_mo)</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 23, 2014</td>
<td>1st cut</td>
<td>all</td>
<td>Ammonium nitrate²</td>
<td>80 kg/ha</td>
</tr>
<tr>
<td>July 10, 2014</td>
<td>2nd cut</td>
<td>all</td>
<td>Hydrosulfan</td>
<td>60 kg/ha</td>
</tr>
<tr>
<td>July 11, 2014</td>
<td>N-fertilization</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 21, 2014</td>
<td>3rd cut</td>
<td>all</td>
<td>Hydrosulfan</td>
<td>40 kg/ha</td>
</tr>
<tr>
<td>August 22, 2014</td>
<td>N-fertilization</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 15, 2014</td>
<td>4th cut</td>
<td>all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 22, 2014</td>
<td>5th soil sampling time (T₅_mo)</td>
<td>all</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ containing 54% Lolium perenne, 20% Festuca pratensis, 17% Phleum pratense, 10% Poa pratensis
² containing 15.7% N-NH₃ and 9.5% N-NO₃

The soil was sampled five times during August 2013 and October 2014 in three soil depths (0-10 cm, 10-25 cm and 25-40 cm) with an auger for aggregate sampling (Edelmann, Eijkelkamp, Giesbeek, the Netherlands; diameter of 6 cm). The soil was sampled before grassland renovation (T₀) as well as six days after (T₆_days), two months after (T₂_mo), seven months after (T₇_mo) and twelve months after (T₁₂_mo) grassland renovation.
In each plot three cores were taken and combined to a composite sample. Additionally, samples for bulk density were taken.

### 4.2.3 Analytics and soil characterization

The bulk soil (<2 mm) and the aggregate-size fractions were dried, ball-milled and analyzed for total C and N concentrations by dry combustion (Elementar Vario EL, Heraeus, Hanau, Germany). As there were no carbonates present in the soil, total C corresponds to SOC. The pH was analyzed by extraction with CaCl₂ (25 mL 0.01 M CaCl₂, 10 g soil) and soil texture with the pipet method (DIN ISO 11277, 2002). The microbial biomass carbon concentrations were determined with chloroform fumigation extraction method after Wu et al. (1990).

The SOC stocks were calculated for an equivalent mass of soil to account for differences in bulk density as suggested by Ellert and Bettany (1995).

### 4.2.4 Aggregate fractionation

Soil aggregate-size fractionation was performed using a wet-sieving procedure described by Cambardella & Elliott (1993) and slightly modified by Linsler et al. (2013). Hundred gram of air-dried soil were placed on a sieve with 2000 µm mesh size and submerged into distilled water for 10 min to allow slaking. Afterwards the sieve was lifted out of the water and then resubmerged for 50 times. Soil aggregates retained on the sieve were collected, dried and weighed. Aggregates which passed the sieve were put onto the next smaller mesh size and the fractionation procedure was continued as described above. The mesh sizes used were: 2000 µm for large macroaggregates, 1000 µm for medium macroaggregates, 250 µm for small macroaggregates, and 53 µm for microaggregates.

### 4.2.5 Statistical analyses

The statistical analyses were carried out with the software programs R (version 3.4.0, R Core team, 2017) and SAS (SAS Institute Inc., 2014). Analyses of variance were calculated to study the effects of grassland renovation on (i) SOC stocks, (ii) aggregate-size classes and (iii) organic carbon stored in aggregate-size classes. We hypothesized that the grassland renovation treatments result (i) in decreased SOC stocks compared to the control due to the death of the plants and the plowing event; (ii) in decreased macroaggregate (larger stronger than smaller ones) yields and increased microaggregate yields due to the
degradation of the root systems and the physical disruption; and (iii) in a shift in aggregate stored carbon from macroaggregates to microaggregates.

To test these hypotheses, each of the response variables was subjected to two-way analyses of variance with the factors block (blocks 1 to 3) and grassland renovation (factor levels: control, chemical renovation and physical renovation). Groups were checked for homoscedasticity by Levene’s test (package car) and residuals of the final model were checked for homoscedasticity graphically and for normal distribution by the Shapiro-Wilk test as well as graphically. Mean comparisons were performed using the Tukey-test. In three cases, in the 0-10 cm soil layer (aggregate concentrations >2000 \(\mu\)m \((T_{7_{-\text{mo}}})\) and 1000-2000 \(\mu\)m \((T_{2_{-\text{mo}}})\); SOC stored in 250-1000 \(\mu\)m \((T_{6_{-\text{days}}})\) the data were square-root transformed to achieve an approximate normal distribution. The different depths and sampling times were analyzed separately in all cases.

In case of large differences between the standard deviations between factor levels of the factor grassland renovation, factor levels with similar variances were combined into one group and the remaining factor level was treated as a second group. Variance heterogeneity was considered using the mixed procedure of SAS (PROC MIXED) with the Satterthwaite approximation for the degrees of freedom. These calculations were carried out for the bulk densities at \(T_0\) and \(T_{12_{-\text{mo}}}\), for SOC stored in aggregate fractions >2000 \(\mu\)m \((T_{6_{-\text{days}}}, T_{7_{-\text{mo}}})\), 1000-2000 \(\mu\)m \((T_{0})\), 250-1000 \(\mu\)m \((T_{6_{-\text{days}}}, T_{2_{-\text{mo}}})\), and 53-250 \(\mu\)m \((T_{12_{-\text{mo}}})\), at 0-10 cm for the aggregate concentrations >2000 \(\mu\)m \((T_{7_{-\text{mo}}})\) and 250-1000 \(\mu\)m \((T_{6_{-\text{days}}}, T_{2_{-\text{mo}}}, T_{7_{-\text{mo}}})\) and at 10-40 cm for the aggregate concentrations >2000 \(\mu\)m \((T_{6_{-\text{days}}})\), and 250–1000 \(\mu\)m \((T_{2_{-\text{mo}}, T_{7_{-\text{mo}}}})\). In case of significant treatment effects, multiple comparisons were carried out with the Edwards-Berry-test.

Spearman’s rank correlation was calculated with the single values for gravimetric moisture content (GMC) (which was not normally distributed) and the bulk density of all treatments and in both soil depths to check our hypothesis that these variables are correlated.

Regression analyses with the aggregate concentrations as response variables and the GMC as explanatory variable was calculated for both soil depths. We hypothesized that the GMC has a positive relationship with large and medium macroaggregates, an intermediate relationship with small macroaggregates (because of that we have chosen a model with a squared term) and a negative relationship with microaggregates. Standardized residuals of the models were inspected for normality using the Shapiro-Wilk test and for
homoscedasticity graphically. A Box-Cox transformation was carried out for large and medium macroaggregates and microaggregates in both soil depths to reach normal distribution of the standardized residuals. For small macroaggregates in 10-40 cm soil depth a normal distribution of the standardized residuals was only reached after exclusion of one outlier-suspected value (the content of 426 g/kg at 13 % gravimetric moisture content). Effects were considered to be significant at p≤0.05.

4.3 Results and discussions

4.3.1 SOC stocks

We hypothesized that grassland renovation leads to a reduction in SOC stocks because of a lower subsequent contribution of organic material from the newly established plants and a potential destruction of larger aggregates. However, there was no significant loss of SOC in the renovated treatments within one year after the renovation in the surface soil (Table 4.2) as well as in the subsoil (10-40 cm) and the soil profile (0-40 cm) (data not shown). Several studies showed a reduction in SOC stocks after grassland or pasture renovation by plowing (Necpálová et al., 2014; Linsler et al., 2013) or a one-time tillage event in arable no-till soils (Quincke et al., 2007) up to two years after the plowing event. Therefore, the SOC seems to be somehow protected from being lost in the sampled grassland soil. One possible factor being responsible for this protection could be the rather low pH in the soil (on average 4.8) hampering the degradation of organic material. This is supported by the relatively high C/N ratio (13.7), indicating a high amount of largely undecomposed organic material in the soil. The reduction in SOC after plowing is assumed to be a consequence of a breakdown of aggregates and a release of previously aggregate-protected organic material (Six et al., 2000; Bronick and Lal, 2005). This no longer physically protected organic material might have decomposed slowly after the plowing event because of hampered microorganisms due to a low pH, resulting in a non-evident SOC loss.
Table 4.2: Bulk density (g/cm³) and soil organic carbon (SOC) stocks (t/ha) calculated on an equivalent mass of soil in different treatments and sampling times (T₀: before grassland renovation; T₆_days: six days after grassland renovation; T₂_mo: two months after grassland renovation; T₇_mo: seven months after grassland renovation and T₁₂_mo: twelve months after grassland renovation) in the surface soil layer. Shown are the mean values and standard deviations (n=3).

<table>
<thead>
<tr>
<th></th>
<th>Specific soil depths</th>
<th>Bulk density (g/cm³)</th>
<th>SOC stock (t/ha in 1473 t soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T₀</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent grassland 0-10.0 cm</td>
<td>1.5 (0.3)</td>
<td>35.6 (8.6)</td>
<td></td>
</tr>
<tr>
<td>Chemical renovation 0-11.0 cm</td>
<td>1.4 (0.3)</td>
<td>34.7 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Physical renovation 0-10.1 cm</td>
<td>1.5 (0.0)</td>
<td>36.7 (2.3)</td>
<td></td>
</tr>
<tr>
<td><strong>T₆_days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent grassland 0-9.6 cm</td>
<td>1.5 (0.1)</td>
<td>37.7 (4.6)</td>
<td></td>
</tr>
<tr>
<td>Chemical renovation 0-9.8 cm</td>
<td>1.5 (0.3)</td>
<td>37.5 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Physical renovation 0-10.2 cm</td>
<td>1.4 (0.1)</td>
<td>34.0 (2.9)</td>
<td></td>
</tr>
<tr>
<td><strong>T₂_mo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent grassland 0-10.8 cm</td>
<td>1.4 (0.1) A</td>
<td>34.3 (5.7)</td>
<td></td>
</tr>
<tr>
<td>Chemical renovation 0-11.3 cm</td>
<td>1.3 (0.1) AB</td>
<td>37.7 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Physical renovation 0-11.6 cm</td>
<td>1.3 (0.1) B</td>
<td>33.8 (3.5)</td>
<td></td>
</tr>
<tr>
<td><strong>T₇_mo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent grassland 0-13.6 cm</td>
<td>1.1 (0.1)</td>
<td>37.8 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Chemical renovation 0-11.4 cm</td>
<td>1.3 (0.1)</td>
<td>34.0 (5.4)</td>
<td></td>
</tr>
<tr>
<td>Physical renovation 0-12.0 cm</td>
<td>1.2 (0.1)</td>
<td>31.9 (1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>T₁₂_mo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent grassland 0-12.7 cm</td>
<td>1.2 (0.2)</td>
<td>37.7 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Chemical renovation 0-12.0 cm</td>
<td>1.2 (0.0)</td>
<td>37.0 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Physical renovation 0-11.3 cm</td>
<td>1.3 (0.0)</td>
<td>32.9 (6.5)</td>
<td></td>
</tr>
</tbody>
</table>

Letters show significant differences among treatments; exclusively those values which are significantly different (p<0.05) are followed by different letters.

We did not find any significant variations in SOC stocks due to management at the various sampling times, whereas we found strong variations in the bulk density, leading to differences in the calculated specific soil depths (same soil mass between the top 9.6 and 13.6 cm) (Table 4.2). The bulk density was strongly negatively correlated with the GMC of the bulk soil (Spearman correlation; for both soil depths p<0.001; r=-0.51 and -0.63 for
4 Effect of chemical and physical grassland renovation on the temporal dynamics of organic carbon stocks and water-stable aggregate distribution in a temperate grassland soil

0-10 and 10-40 cm soil depths) and we suggest that during a dry period aggregates were destroyed (presented below), leading to a compaction of the sandy soil and therefore an increase in bulk density.

4.3.2 Aggregate distribution

Impacts of grassland renovation on aggregates after 6 days in the surface soil.

Before grassland renovation (T₀), no significant differences in aggregate distribution in both soil layers were found among the three treatments (Figure 4.1). In T₆_days, there were significantly lower small macroaggregate (Figure 4.1) and total macroaggregate concentrations (not shown) in the surface soil and in the soil profile, respectively, in the physical renovation compared with the permanent grassland. This indicates that one-time plowing had a direct impact on soil aggregates and destroyed macroaggregates, which fragmented into microaggregates. Negative impacts of plowing on larger aggregates after occasional or one-time plowing were found for arable (Stavi et al., 2011) and grassland soils (Linsler et al., 2013). However, in these studies no distinction between direct and longer-lasting indirect effects of plowing was possible since they compared the unplowed with the plowed soil more than half a year after the last plowing event. Andruschkewitsch et al. (2014b) sampled soil before and after a plowing event in regularly plowed arable soil and found no difference in aggregate distribution. That result and our study suggest that the direct physical destruction of macroaggregates might be more evident in grassland than in arable soils.
Effect of chemical and physical grassland renovation on the temporal dynamics of organic carbon stocks and water-stable aggregate distribution in a temperate grassland soil

Figure 4.1: Water-stable aggregate concentrations (g/kg soil) in 0–10 cm and 10–40 cm soil depths among different treatments and sampling times; letters indicate significant (p \leq 0.05) differences among treatments. Shown are the mean values and standard deviations (n=3).

Impacts of grassland renovation on aggregates after 2 to 7 months in the surface soil.

The total macroaggregate concentrations in the soil profile were 2.2- to 2.7-fold higher in $T_{2_{-mo}}$ compared with $T_{6_{-days}}$, which was mainly caused by 6.7- to 10.2-fold higher large macroaggregate concentrations (Figure 4.1). Since this effect was also found in the
permanent grassland, environmental factors rather than the grassland renovation might have caused this huge impact on aggregates. The rainfall in the research area was rather low in July (22 mm) and August (36 mm), leading to a very low GMC in late August/early September (mean of 5.2 and 4.9 % in T_0 and T_6_days, respectively). In contrast, in T_2,mo the GMC reached 19.2 % (13.7 % in T_7_mo and T_12,mo). We calculated linear regressions and found that in all aggregate-size fractions the GMC plays the only or a major role in the explanation of variations in aggregate distribution (Table 4.3, Figure 4.2). Whereas the relationship between GMC and large as well as medium macroaggregates was positive, it was intermediate for small macroaggregates and negative for microaggregates. The loss of (macro)aggregate stability due to drying of the soil is known to be dependent on the soil texture with clayey soils becoming more stable and sandy soils tending to lose their stability upon drying (Kaiser et al., 2015). Linsler et al. (2015) also found a reduction in larger macroaggregate concentrations in a dried out permanent grassland soil with a lower sand content (67 %) than in our study (91 %). The larger aggregates are known to be less stable than microaggregates and therefore more susceptible to disruption (Cambardella and Elliott, 1994, Six et al., 1999), leading to a shift from macro- to microaggregates. The much lower macroaggregate concentrations in the sampling times with low GMC might be due to a reduction in the functionality of desiccated aggregate binding agents like root exudates or microbial excretions. When the soil gets rewetted, macroaggregate formation is induced, leading to a very strong increase of that aggregate fraction.

Table 4.3: Results of linear regression analyses for various aggregate fractions (g/kg soil) in two soil depths.

<table>
<thead>
<tr>
<th>Aggregate fraction</th>
<th>Intercept</th>
<th>GMC</th>
<th>(GMC)^a</th>
<th>R^2</th>
<th>( \lambda )</th>
<th>Intercept</th>
<th>GMC</th>
<th>(GMC)^a</th>
<th>R^2</th>
<th>( \lambda )</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2000 ( \mu m )</td>
<td>3.86</td>
<td>0.38</td>
<td>0.85</td>
<td>0.25</td>
<td>2.15</td>
<td>0.040</td>
<td>1.60</td>
<td>0.030</td>
<td>0.60</td>
<td>-0.30</td>
</tr>
<tr>
<td>2000-1000 ( \mu m )</td>
<td>3.08</td>
<td>0.40</td>
<td>0.29</td>
<td>0.04</td>
<td>1.60</td>
<td>0.030</td>
<td>1.60</td>
<td>0.030</td>
<td>0.60</td>
<td>-0.30</td>
</tr>
<tr>
<td>1000-250 ( \mu m )</td>
<td>87.31</td>
<td>30.85</td>
<td>-1.00</td>
<td>-0.56</td>
<td>50.58</td>
<td>34.370</td>
<td>-1.11</td>
<td>0.81</td>
<td>0.90</td>
<td>1.47</td>
</tr>
<tr>
<td>250-53 ( \mu m )</td>
<td>493.61</td>
<td>-10.67</td>
<td>0.74</td>
<td>0.92</td>
<td>13558.20</td>
<td>-522.50</td>
<td>-1.11</td>
<td>0.81</td>
<td>0.90</td>
<td>1.47</td>
</tr>
</tbody>
</table>

a: \( \lambda \) of Box-Cox-transformation, which was carried out for all aggregate fractions except for 1000-250 \( \mu m \).
4 Effect of chemical and physical grassland renovation on the temporal dynamics of organic carbon stocks and water-stable aggregate distribution in a temperate grassland soil

Figure 4.2: Scatter plots of the concentrations of aggregate fractions and the gravimetric moisture content in two soil depths. The lines are the modelled lines of the linear regression analyses.

In T2_1mo and T7_1mo the chemical renovation reached significantly higher total macroaggregate concentrations and significantly lower microaggregate concentrations in the surface soil compared with the physical renovation, while the concentrations in the permanent grassland were in between. In the chemical renovation, larger roots of the dead plants might still be largely undecomposed protecting macroaggregates from degeneration, as was also found after cover crop death by freezing (Linsler et al., 2016). In the physical
renovation, plowing of the soil led to a destruction of the root system of the previous grass sward and therefore the positive effects of dead roots might be lower. In both renovated grasslands, new macroaggregate formation might occur by the growing roots of the newly established grasslands (Tisdall and Oades, 1982). However, concentrations of root-built aggregates in the renovated grasslands might be lower than in the permanent grassland since the root biomass in recently established grasslands is lower than in older ones (Bolinder et al., 2002). A combination of macroaggregate protection by dead roots on the one hand and the build-up of aggregates from the growing plants on the other hand might lead to a slight increase in total macroaggregate concentrations in the chemical renovation compared with the permanent grassland in T2_mo. The overall lowest concentrations of macroaggregates in the physical renovation may indicate that after the direct and physical destruction of the aggregates by the plow, other and indirect mechanisms lead to a further breakdown of macroaggregates or interferences in the build-up of new macroaggregates.

In contrast to the chemical renovation, in the physical renovation the stubbles of the cut grassland were incorporated into the soil, serving as additional source of organic material. We hypothesized that this incorporation may lead to a faster build-up of aggregates, as a positive relation between the amount of added organic material and the build-up of aggregates was found before (Christensen, 2001). In arable soils, the aggregate disruption caused by crop residue incorporation by tillage is postulated to be compensated by a faster building of aggregates (Andruschkewitsch et al., 2014a). In our experiment, the amount of added organic material in form of dead roots and the generally high soil organic matter content in grassland soils might have been sufficient to reach a maximum of aggregate formation due to addition of organic material. This is supported by a similar increase in macroaggregates in the subsoil in the renovated grasslands.

**Impacts of grassland renovation on aggregates after 12 months in the surface soil.**

At T12_mo, differences in aggregate distribution among the treatments were no longer present. In the studied grassland soil, one year thus seems to be enough to compensate negative impacts following grassland renovation. At a different German site, Linsler et al. (2013) still found a negative impact of grassland renovation on large macroaggregates in the surface soil two years after the event, whereas Quincke et al. (2007) found for arable land the negative impacts of one-time tillage in no-till soil was nullified in that time span. It seems that the duration until the previous macroaggregate concentration is restored is not dependent on soil use but on other factors such as soil texture or environmental conditions.
Impacts of grassland renovation on aggregates in the subsoil.

In the subsoil (10-40 cm), only for the small macroaggregates in T six_days significantly higher concentrations were found in the physical compared with the chemical renovation and the variations among the treatments within one sampling time were in general lower than in the surface soil, indicating a very low or non-existing impact of grassland renovation. However, the sampling time and therefore the moisture content of the soil seems to have a strong influence especially on the large macroaggregates and microaggregates, suggesting that the influence of environmental factors in the subsoil is larger than that of cultivation practices.

4.3.3 SOC storage in water-stable aggregates

Mostly, there were no significant differences in SOC stored in aggregate fractions among treatments (Table 4.4), except for T seven_mo in the surface soil layer, when SOC concentrations stored in aggregates were lowest for the medium macroaggregates and highest for the microaggregates in the physical renovation. The plowing event at first had no impact on SOC storage in aggregates; however, after a certain time microaggregates become more important than macroaggregates in the storage of SOC compared to the unplowed control. Because of the late response in SOC storage in aggregates after the plowing event it seems that indirect effects (for instance the degradation of the root system of the dead plants) on soil aggregates have a wider influence than the direct physical impact of the plow.
4 Effect of chemical and physical grassland renovation on the temporal dynamics of organic carbon stocks and water-stable aggregate distribution in a temperate grassland soil

Table 4.4: Organic carbon concentrations stored in aggregate fractions (g/kg bulk soil) in the different treatments and sampling times (T<sub>6_days</sub>: six days after grassland renovation; T<sub>2_mo</sub>: two months after grassland renovation; T<sub>7_mo</sub>: seven months after grassland renovation and T<sub>12_mo</sub>: twelve months after grassland renovation) in the surface soil layer (0-10 cm). Shown are the mean values and standard deviations (n=3).

<table>
<thead>
<tr>
<th>Aggregate Size</th>
<th>Treatment</th>
<th>T&lt;sub&gt;0&lt;/sub&gt;</th>
<th>T&lt;sub&gt;6_days&lt;/sub&gt;</th>
<th>T&lt;sub&gt;2_mo&lt;/sub&gt;</th>
<th>T&lt;sub&gt;7_mo&lt;/sub&gt;</th>
<th>T&lt;sub&gt;12_mo&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2000 μm</td>
<td>Permanent grassland</td>
<td>1.6 (0.6)</td>
<td>1.2 (0.1)</td>
<td>5.3 (1.5)</td>
<td>4.4 (0.5)</td>
<td>4.0 (0.7)</td>
</tr>
<tr>
<td></td>
<td>Chemical renovation</td>
<td>1.2 (0.3)</td>
<td>1.4 (0.7)</td>
<td>11.2 (4.0)</td>
<td>7.5 (3.6)</td>
<td>6.7 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Physical renovation</td>
<td>1.7 (0.2)</td>
<td>1.9 (0.1)</td>
<td>4.6 (2.0)</td>
<td>2.6 (1.2)</td>
<td>4.2 (1.4)</td>
</tr>
<tr>
<td>1000 - 2000 μm</td>
<td>Permanent grassland</td>
<td>1.6 (0.5)</td>
<td>1.7 (0.5)</td>
<td>1.2 (0.7)</td>
<td>2.6 (0.5) A</td>
<td>1.4 (0.1)</td>
</tr>
<tr>
<td></td>
<td>Chemical renovation</td>
<td>1.6 (0.1)</td>
<td>1.6 (0.4)</td>
<td>1.9 (0.9)</td>
<td>2.1 (0.8) AB</td>
<td>1.6 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Physical renovation</td>
<td>1.8 (1.1)</td>
<td>1.2 (0.3)</td>
<td>1.3 (0.4)</td>
<td>0.9 (0.2) B</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>250 - 1000 μm</td>
<td>Permanent grassland</td>
<td>7.7 (3.7)</td>
<td>8.6 (3.4)</td>
<td>7.5 (1.9)</td>
<td>10.8 (2.7)</td>
<td>11.5 (2.3)</td>
</tr>
<tr>
<td></td>
<td>Chemical renovation</td>
<td>12.9 (5.9)</td>
<td>6.9 (2.8)</td>
<td>7.5 (4.6)</td>
<td>9.7 (1.2)</td>
<td>9.7 (0.6)</td>
</tr>
<tr>
<td></td>
<td>Physical renovation</td>
<td>7.8 (2.4)</td>
<td>5.5 (0.5)</td>
<td>6.2 (0.6)</td>
<td>6.8 (1.6)</td>
<td>8.5 (2.1)</td>
</tr>
<tr>
<td>53 - 250 μm</td>
<td>Permanent grassland</td>
<td>22.5 (8.5)</td>
<td>15.7 (3.9)</td>
<td>7.1 (0.4)</td>
<td>7.6 (1.1) AB</td>
<td>8.1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Chemical renovation</td>
<td>15.3 (2.2)</td>
<td>14.6 (2.7)</td>
<td>6.0 (0.8)</td>
<td>6.0 (1.8) B</td>
<td>7.0 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Physical renovation</td>
<td>15.4 (2.9)</td>
<td>19.3 (0.5)</td>
<td>8.8 (1.1)</td>
<td>9.6 (1.6) A</td>
<td>8.8 (0.1)</td>
</tr>
</tbody>
</table>

Letters show significant differences among treatments; exclusively those values which are significantly different (p<0.05) are followed by different letters.

In T<sub>0</sub> and in T<sub>6_days</sub> 28-38% of the SOC stored in aggregates were found in total macroaggregates in all treatments in the soil profile (0-40 cm). In contrast, in T<sub>2_mo</sub>, T<sub>7_mo</sub> and T<sub>12_mo</sub> the SOC stored in macroaggregates was in general higher and partly almost doubled, reaching 44-69% in the three treatments. These differences were mainly caused by large and small macroaggregates, which generally showed lower values in the first two and higher values in the last three sampling times. The variations in SOC storage can be attributed to the GMC in the bulk soil, which was 5.1% in the first two sampling times, whereas it increased to 15.5% in the last three. This indicates that large and small macroaggregates generally had a high importance in storing SOC in the studied grassland soil, however, when the soil was very dry, microaggregates and also the silt and clay
fraction become more important. Our findings thus indicate a redistribution of SOC in the aggregate-size fractions when the soil dried out and was subsequently rewetted. We have found effects of grassland renovation on SOC concentration stored in aggregate-size classes only in the surface soil, whereas in the subsoil (10-40 cm) or in the soil profile (0-40 cm) no significant differences could be detected (data not shown). However, since the subsoil content of OM is typically low, effects in the subsoil may be generally difficult to detect.

4.4 Conclusion

Grassland renovation in a temperate grassland soil did not lead to losses in SOC stocks in the bulk soil, independent whether it was carried out chemically or physically. The plowing event in the physical renovation had direct (i.e. physical destruction of aggregates) and indirect (i.e. destruction of the root system of the dead plants) negative impacts on soil macroaggregates, however, one year after grassland renovation they were nullified. Chemical renovation resulted in higher macroaggregate concentrations compared with the physical renovation, especially two to seven months after the renovation, and in similar concentrations compared with the permanent grassland in any sampling time within one year. Presumably, dead plant roots could act as binding agents and stabilize aggregates for some time.

There was high temporal variation in the aggregate distribution within one year in the renovated as well as in the permanent grassland. Linear regression analyses suggested that the soil moisture had a wide influence and that dry conditions in the soil led to a breakdown of larger aggregates.

4.5 Acknowledgements

We thank Anja Sawallisch for her technical advice and help. Many thanks are due to Hauke Heeren providing the area for study site as well as Robert Klippert und Christian Thomßen for implementation and management of the field trial. We thank the Chamber of Agriculture Lower Saxony for supplying weather information for the research site. This project was funded by the German Research Foundation (Research Training Group 1397).
5 General Conclusions

From the findings obtained concerning the three different grassland trials, it can be concluded that the impact of the intensified use of grassland on carbon and nitrogen dynamics is highly dependent on location. First, major differences were observed in the implementation and the degree of intensification. Thus, a direct comparison is not directly achievable. An impression of the diversity of grassland as well as grassland management limited to Germany is illustrated via the present studies. In a predominantly extensively-managed mountainous grassland location in Relliehausen, a high grazing pressure showed no significant influence on the SOC or N, stocks. However, it is possible that influences can be explained by high mineralogical variability. However, a coupling of SOC and N, can be assumed, as the variability of the C/N ratios is very low, and no significant differences between treatments can be found. Due to the coupling of SOC and N, it can be assumed that neither SOC nor N, stocks are influenced by the grazing intensities studied in soil. This is a strong indication for stable SOC and N, dynamics under all three extensive grazing intensities.

However, a higher cutting frequency in cut grassland has positive effects on the sequestration of carbon and nitrogen. Both SOC and N, stocks, as well as further stocks of microbial and fungal biomass, increased under the 5C regime compared to the 3C regime in the location investigated in Kiel. Moreover, the proportion of small macroaggregates decreased under the 5C regime. All these parameters indicate an improved soil function and ecology in the 5C regime. However, N fertilization with 360 kg N ha⁻¹ annually over eight years showed slightly adverse effects on the SOC and N, sequestration mechanisms and parameters for efficient soil ecology. Furthermore, the highest biomass yields were harvested under the 3C regime, and, therefore, the 3C regime is basically of more interest for the farmer because of a better cost-benefit ratio. The use of mineral N fertilizers leads to higher biomass yields and would be preferred by conventional management. The 3C regime in combination with N fertilization can thus be regarded as the most intensive form of farming of the field trial in Kiel, as, there, the greatest yields of biomass were provided. However, this treatment or farming system showed fewer positive effects on soil function and ecology.

Grassland renovation is less invasive when using solely chemical destruction of the sward, and it has a fewer negative impact on soil function and ecology in comparison to
treatment with subsequent plowing. Both types of grassland renewal have no influence on the SOC stocks. However, the solely chemically destructed sward is not affected in its aggregate structure because the root system that is killed is not influenced in its structure and thus remained a structuring frame, in contrast to the grassland renewal with plowing. After the decomposition of dead roots, the reseeded herb and grass roots act as a structuring element and serve the known positive effects on the aggregate formation. Significant factors for aggregate concentrations were weather and season, as large fluctuations were noticed among the variants of grassland renewal as well as in the grassland control. The fluctuations in the concentration of large macroaggregates over time are in close positive correlation with the soil water content. This means that the concentrations of macroaggregates are strongly dependent on the weather conditions at this plaggic anthrosol, with a sand content of around 90 %. Thus, macroaggregate concentration increase with damp, cold weather. However, these variations do not affect the SOC stocks. Basically, it can be concluded that the grassland renovation is less invasive only via chemical destruction of the sward and has less negative impact on soil function and ecology in comparison to the treatment with subsequent plowing.

Management intensification did not influence climate-relevant SOC sequestration in the extensive grazing trial in Relliehausen or the grassland renewal trial in Oldenburg, as the SOC stocks were not affected by varying treatments. For the cut grassland trial in Kiel, only slight losses in the amount of SOC stocks after eight years of application of mineral N fertilizer and simultaneous removal of large amounts of biomass (20.1 ± 1.5 t ha⁻¹ SOC of 3C with N fertilization in comparison to 26.3 ± 0.8 t ha⁻¹ SOC 5C without N fertilization; mean ± standard deviation) were determined. However, whether the shift in plant species composition or the management practice itself causes these effects cannot be determined. In addition, grassland renewal with plowing has reversible negative influence on SOC sequestration mechanisms due to the fact that grassland renewal that is normally not annually conducted is negligible for climate relevant SOC sequestration.

Future research needs include the investigation of the relation of soil parameters to the root biomass and the root habit of the different plant species, complemented by plant species compositions under different management varieties.

In principle, it can be stated that intensified grassland management, except for the case of a high application rate of mineral N fertilizer and a large removal of biomass (with a mean of 1459 g m⁻²), does not or only has a low tendency to have negative impacts on the SOC and Nᵣ stocks and sequestration mechanisms. Unlike the loss of biodiversity through
intensified use, the SOC stocks and sequestration mechanisms, which are inevitably linked to plant species diversity and the variety of soil organisms, tend to react more slowly to management changes while showing relatively rapid regeneration. Therefore, it is important to have targeted and appropriate management, which seems to be a basic principle for the three field trials conducted.
6 Acknowledgement

I would like to thank my supervisor Prof. Dr. Bernard Ludwig, who provided me the opportunity to work on this topic. He supervised me with an enormous expertise in the field of research and scientific writing. I am grateful for the experiences I made while working on this thesis. I also thank Prof. Dr. Rainer Georg Jörgensen for his readiness being second supervisor.

For the introduction into the topic, its methods and the continuous scientific advice I would like to thank Dr. Deborah Linsler.

I also thank Gabriele Dormann, Sabine Ahlers and Elsa Zwicker for the support and the technical guidance in the laboratory. I am thankful for the practical help in the laboratory and the field of former and current staff of the Department of Environmental Chemistry and Soil Biology of the University of Kassel (especially Maren Bohnert, Oliver Schäfer, Sven Thorwirth). I am especially thankful for Anja Sawallisch always being counterpart - in the field, in the laboratory and for discussing all other questions which came up during the work at this thesis. I also thank Marion Hoeck and Susanne Beck for the friendly atmosphere and the organizational support.

Further on, I thank the technical and scientific staff of the University of Göttingen, the Thünen Institute, the Chamber of Agriculture of Lower Saxony and the University of Kiel, who made it possible for me to take samples on their field trials and for a good and fruitful collaboration.

I also thank my former colleagues (Dr. Dennis Grunwald, Dr. habil. Michael Kaiser, Dr. Johanna Pingerra, Dr. Christel Roß, Svendja Vormstein) for the good working atmosphere, the support in any situation and their encouragement. These thanks are extended to the third cohort of the DFG-Research Training Group 1397. Thank you very much making the meetings, the trip to Sweden and Denmark and our Conference to such a joyful time.

This work, implemented in the Research Training Group 1397 was funded by the German Research Foundation (DFG). I am grateful for the opportunity to create this work, which was given to my be the DFG.
Finally, I would like to thank my family and my friends for their continuous support and encouragement during the time of this work. Special thanks go to my parents and my friend Moritz for their love and trust and endless encouragement.
References


References


References


Mawdsley, J.L., Bardgett, R.D., 1997. Continuous defoliation of perennial ryegrass (Lolium perenne) and white clover (Trifolium repens) and associated changes in the composition and activity of the microbial population of an upland grassland soil. Biology and Fertility of Soils 24, 52–58. doi:10.1007/BF01420220


R Development Core Team. 2014. R: A language and environment for statistical computing. Vienna.


Cuiusvis hominis est errare, nullius nisi insipientis in errore perseverare.

Cicero, Orationes Philippicae (12,2)