Department of Environmental Chemistry

-Faculty of Organic Agricultural Sciences-

University of Kassel

Effects of grassland renovation on carbon concentrations and aggregate distribution in temperate grassland soils

Dissertation

submitted to the Faculty of Organic Agricultural Sciences of the

University of Kassel

to fulfill the requirements for the degree "Doktor der Agrarwissenschaften"

(Dr. agr.)

by Dipl. Agr. Biol.

Deborah Linsler

Witzenhausen, April 2013

This work has been accepted by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doktor der Agrarwissenschaften (Dr. agr.).

1. Supervisor: Prof. Dr. Bernard Ludwig (University of Kassel)

2. Supervisor: PD Dr. Martin Potthoff (University of Göttingen)

Defence day: 10.10. 2013

Preface

This work is submitted to the Faculty of Organic Agricultural Sciences at the University of Kassel to fulfill the requirements for the degree “Doktorin der Agrarwissenschaften” (Dr. agr.). This thesis was prepared within the DFG Research Training Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture”.

The cumulative dissertation consists of three papers as first author, which are published, submitted, or in preparation to submission in international refereed journals. The manuscripts are included in chapter 4, 5, and 6.

Chapter 1 gives an English summary. A general introduction to all parts of the present work is given in chapter 2 whereas chapter 3 includes the objectives of the present work. A general conclusion covering the three papers is given in chapter 7. Chapter 8 contains a summary in German language.

The following papers conduced to this thesis:

Chapter 4:

Linsler, D., Geisseler, D., Loges, R., Taube, F., Ludwig, B. (2013): Temporal dynamics of soil organic matter composition and aggregate distribution in permanent grassland after a single tillage event in a temperate climate. Soil and Tillage Research 126, 90-99.

Chapter 5:

Linsler, D., Geisseler, D., Loges, R., Taube, F., Ludwig, B. (2013): Effects of tillage and organic fertilization on carbon, nitrogen and phosphorus pools in temperate grassland soils (submitted).

Chapter 6:

Linsler, D., Geisseler, D., Loges, R., Taube, F., Ludwig, B. (2013): Temporal variations of carbon concentration, aggregate distribution and microbial biomass in temperate grassland soils (in preparation for submission).

Table of contents

List of tables

List of figures

List of abbreviations

A-G	arable land – grassland
C_{mic}	microbial biomass carbon
C_{org}	organic carbon
C_t	total carbon
fLF	free light fraction
G-G	grassland – tillage – grassland
G-W-G	grassland – tillage – winter wheat – tillage – grassland
G-W-G_0	G – W – G without slurry application
G-W-G_+	G – W – G with slurry application (240 kg N ha^{-1} $year^{-1}$)
LF	light fraction
MF	mineral fraction
N_t	total nitrogen
oLF	occluded light fraction
PG	permanent grassland
PG_0	PG without slurry application
PG_+	PG with slurry application (240 kg N ha^{-1} $year^{-1}$)
SOM	soil organic matter
SPT	sodiumpolytungstate

1. Summary

The effects of continuous tillage systems on the amount and distribution of soil organic matter (SOM) and the dynamics of aggregates have been analyzed extensively for arable soils. However, less is known about the effects of single or sporadic tillage events on the amount and composition of SOM and aggregate dynamics of agriculturally used grassland soils. Such sporadic tillage events are aimed to maintain high yields in intensively used grasslands but may also result in the disruption of aggregates and considerable losses of organic carbon (C_{org}).

Therefore, the objectives of the present thesis were (I) to study the longer-term (two and five years) effects of sporadic tillage or temporal conversion of grassland into arable land on C_{org} stocks, water-stable aggregate and SOM distribution, (II) to investigate the combined effects of a temporal conversion from grassland into arable land and organic fertilization on carbon, nitrogen and phosphorus dynamics, and (III) to study the temporal dynamics of C_{org} concentrations, aggregate distribution and microbial biomass after the conversion from arable land into grassland, after a single tillage operation in grassland and in a permanent grassland.

Soil samples were taken from field trials at the experimental farm Lindhof (54°27 `N, 9°57 `E) in northern Germany. The study site is cha racterized by a mean annual temperature of 8.9 °C and a mean annual precipitation of 768 mm. S mall-scaled differences in geological conditions and pedogenetic processes resulted in a heterogeneous distribution of soil types (i.e. Cambisol, Eutric Luvisol, Stagnosol, and Anthrosol).

The aggregates were fractionated with a wet sieving procedure to gain five fractions: >2000 µm (large macroaggregates), 2000 - 1000 µm (medium macroaggregates), 1000 – 250 µm (small macroaggregates), 250 - 53 µm (microaggregates), and <53 µm (silt and clay fraction). For determination of SOM composition a density fractionation scheme was used and two different SOM fractions (free SOM light fraction and occluded SOM light fraction) were distinguished. The basal respiration rates, indicating the microbial activity, were determined in an

incubation experiment (14 days at 20 °C). The micro bial biomass carbon (C_{mic}) was determined with a chloroform - fumigation – extraction – method. To estimate variations in fungal biomass, the ergosterol (a fungal cell membrane component) concentration was determined.

For Objective I we used a grassland side, where in the years 2005 and 2008 two field trials were initiated. The treatments consist of a single tillage operation with grassland renovation (G-G: grassland – tillage – grassland) and a temporal conversion of grassland into arable land (G-W-G: grassland – tillage - winter wheat – tillage – grassland). Permanent grassland (PG; since 1994) served as control. Soil samples were taken in April 2010 in three depths (0 – 10 cm, 10 – 25 cm, 25 – 40 cm). Our results showed that tillage of grassland lead to a reduction of C_{org} stocks (calculated on an equivalent mass of soil), large macroaggregates (>2000 µm) and SOM in the top 10 cm soil depth. Two years after the single tillage operation, the C_{org} stocks were 34% lower in the surface soil (0 – 10 cm) in G-G in comparison with PG. Five years after the tillage operation, no more significant differences were found between PG and G-G; however, the C_{org} stocks were still 15% lower in G-G. Regarding the soil profile (0 – 40 cm) no significant differences between G-G and PG existed. The same pattern showed the distribution of large water-stable macroaggregates with 60% and 27% lower large macroaggregate concentrations in the surface soil two and five years after a tillage operation, respectively. Two years after tillage, the SOM concentration (free SOM light fraction and occluded SOM light fraction) is still decreased by 31% in G-G in comparison with PG, whereas five years after tillage no more significant differences existed. A second tillage event and the insertion of one season winter wheat in G-W-G did not lead to any further effects on C_{org} stocks, water-stable aggregates and SOM concentrations in comparison with G-G.

To investigate the effects of organic fertilization and sporadic tillage (Objective II), we used G-W-G tilled in 2005 and PG as presented above. Additionally to the plots without organic fertilization, which we used to investigate Objective I (0_1), we sampled plots that received an

organic fertilization with cattle slurry in a rate of 240 kg N ha^{-1} $year^{-1}$ since 2006 (+). Soil samples were also taken in April 2010 in three soil depths (0 – 10 cm, 10 – 25 cm, 25 – 40 cm). Regarding the combined effect of tillage and organic fertilization, we found a trend ($p < 0.1$) of lower C_{org} stocks and significantly lower N_t stocks in the surface soil and in the soil profile due to tillage (G-W-G_+ and G-W-G_0 vs. PG_+ and PG_0), whereas these findings were more pronounced in the slurry-amended plots than in the plots without organic fertilization. However, the C_{org} and N_t stocks mean of 73 t C_{org} and 7.4 t N_t in 5413 t soil) were not influenced due to four years of slurry application at common rates (PG_+ and G-W-G_+ vs. PG_0 and G-W-G_0). In the labile pools the organic fertilization resulted in the surface soil in significantly higher (32%) basal respiration rates and 2.5-fold lower free soil organic matter, which points to increasing microbial activity and decomposition rates. The net N mineralization was not influenced due to tillage or fertilization.

For studying the temporal dynamics of C_{org} concentration, aggregates and microbial biomass (Objective III) a further field trial was initiated in September 2010. Soil samples were taken in three soil depths (0 – 10 cm, 10 – 25 cm, 25 – 40 cm) six times within one year (from October 2010 to October 2011) after a tillage operation in grassland (G-G) or the conversion from arable land into grassland (A-G) using permanent grassland (PG) as control. Temporal variations in C_{org} stocks and concentrations were not detected for G-G, A-G, and PG. However, in G-G and PG the aggregate distribution varied markedly during sampling times, with the strongest variation in the large macroaggregates (>2000 μm) for the PG surface soil. While the C_{mic} concentration were only temporally influenced in A-G (increasing concentrations during the year of 44%), the ergosterol concentration were strongly influenced by sampling times in all three treatments and soil depths.

Overall, our results suggest that a single tillage operation in grassland soils decreased markedly the C_{org} concentrations, larger aggregates and SOM. However, this does not result in long-lasting effects on the above mentioned parameter. The additional organic fertilization

showed, that tillage-related effects were more pronounced in the slurry amended plots than in the plots without organic fertilization. However, while the C_{org} concentration is not subject to fluctuations within a year, there are large variations of the aggregate distribution even in a permanent grassland soil. Therefore conclusions of results from a single sampling time should be handled with care.

[1] To clarification of the difference, the plots without slurry amendments were marked with a 0 in experiment II

2. General introduction

Grasslands are traditionally known as vehicle for soil improvement, livestock feed, and also most recently for bioheat (Samson et al., 2005). However, grassland has further effects such as weed and pest management (Abawi and Widmer, 2000), nitrogen fixation (Russelle and Birr, 2004), and greenhouse gas mitigation. Grasslands also offer other important functions for humans by protecting water and air quality, maintaining the biodiversity of the flora and the micro-, meso-, and macrofauna, and reducing physical risks such as soil erosion (Vertès et al., 2007). In the last years increasing attention has focused on the use of agricultural soils to slightly decrease the CO_2 level in the atmosphere through the sequestration of carbon in soils. Grasslands, as agricultural used area, have in general high soil organic matter (SOM) contents and the conversion from arable land into grassland is known as good practice to increase the SOM and therefor organic carbon (C_{org}) stocks in the soil (Guo and Gifford, 2002). In grassland soils, the main inputs of organic material are from roots (dead roots and root exudates), which contributes to the carbon storage because of their location in the soil with good physical protection from degradation (Six et al., 2002). However, a common practice in intensively used grasslands or pastures is the sporadic renovation of the grassland to maintain high yields. The destruction of the grass sward is carried out either chemically (by using a broad-spectrum herbicide such as glyphosate) or physically (by tillage for instance with a moldboard plow). However, tillage of the soil is known to greatly influence the carbon dynamics by the destruction of the soil structure (e. g. of aggregates). Therefore the previously physically protected SOM may be mineralized, which lead to decreasing C_{org} stocks in the soil.

2.1 The C cycle in grasslands

The carbon cycle in agricultural soils is characterized by the carbon assimilation via photosynthesis, allocation of assimilates to above- and belowground plant biomass, biomass

removal by grazing of animals or harvesting, senescence, and the death of leaves and roots that are returned to the soil (Vertès et al., 2007). Organic carbon can be accumulated in the soil mainly coming from plant biomass, dead organisms, animal waste, or rhizodepositions (Arrouays et al., 2002). The SOM plays an important role in the maintenance and development of soil fertility, mainly in the cycling, retention, and supply of plant nutrients (Stevenson, 1994).

To maintain the organic material in the soil, it has to be protected from mineralization by microorganisms. The SOM is stabilized in the soil by different mechanisms, for instance the organo-mineral associations or the spatial inaccessibility (von Lützow et al., 2006). The spatial inaccessibility can be caused by the occlusion of organic in soil aggregates spatially separating potentially energy sources from microorganisms and their enzymes, which enhances the persistence of the occluded organic matter compared to the non-occluded organic matter.

In arable soils, the formation of macroaggregates (>250 µm; Tisdall and Oades, 1982) is considered as major process for spatial inaccessibility and protection of organic material from mineralization (Helfrich et al., 2008). A “lifecycle” of an aggregate was proposed by Six et al. (2000). Organic material, which is found free and unprotected in the soil (free light fraction), is encrusted with products of microorganisms and clay particles to form microaggregates within macroaggregates to form and stabilize them (Six et al., 1999). The in aggregates occluded organic material (occluded light fraction), which is located in the aggregates, occurs in larger amounts in soils, which are less cultivated (Six et al., 2000), for instance in arable no-till soils or in grassland soils.

However, the formation and disruption or break down of aggregates is a continuum and may vary with time. Temporal variations in C_{org} concentrations (Leinweber et al., 1994; Jacobs et al., 2010) or soil aggregates (Alvaro–Fuentes et al., 2007; Daraghmeh et al., 2009; da Veiga et al., 2009; Jacobs et al., 2010; Bamberg et al., 2011) were reported in arable soils. Whereas in some studies environmental factors (such as climatic conditions) were mentioned as reasons for

variations in aggregate dynamics (Cosentino et al., 2006; Dimoyiannis, 2009) other studies suggest that the cultivation of the soil (such as tillage, crop growth or harvest) caused temporal variations (Jacobs et al., 2010).

2.2 Effect of tillage and fertilization on soil organic matter and soil aggregates

The effect of regular tillage on SOM and aggregates was studied intensively in arable soil. Aggregates can be disrupted due to tillage releasing organic material, which is no longer protected and mineralizable by microorganisms (Bronick and Lal, 2005; Zotarelli et al., 2007). However, not only the presence of tillage has an effect on SOM and aggregates, but also the different types of tillage. For instance, the aggregate concentration was lower in the top 5 cm of regularly tilled soils with a plow than in tilled soils with a harrow (Jacobs et al., 2009; Andruschkewitsch et al., 2013). Also the frequency of tillage affects SOM and aggregates. It is known, that not only regular tillage has an effect on the above mentioned parameters, but also sporadic tillage or a single tillage event. In arable soils, a single tillage event leads to a reduction of C_{org} concentrations (VandenBygaart and Kay, 2004) and of larger aggregates (Stavi et al., 2011). However, the information about the effect of sporadic tillage of no-till arable land or of grassland on SOM and soil aggregate dynamics is scarce.

A contrary effect to tillage on SOM and aggregates has the fertilization especially with organic fertilizers (e.g., manure, slurry). In their review, Conant et al. (2001) reported that C was sequestered by fertilizing grassland with 0.3 t C ha^{-1} $year^{-1}$. Furthermore, manure plays a basic role in the formation and stabilization of soil aggregates, often leading to an enrichment of SOM and larger aggregates (Celik et al., 2004; Mikha and Rice, 2004; Mellek et al., 2010). Therefore, the application of organic fertilizers may be a good tool to reduce the negative impacts of a tillage event with the aim of grassland renovation on larger aggregates and C_{org} concentrations in the longer-term.

2.3 Impacts of grassland tillage

The sporadic tillage of grassland is a common praxis, especially in organic farming where synthetic herbicides are banned. Grassland tillage is carried out when the yield of the grassland decreased for instance due to increasing weed pressure, botanical degradation, soil compaction or damaged grass swards (for instance due to drought or frost) (Taube et al., 2002).

When grassland is tilled, the vegetation is destructed, which results in increased mineralization rates. This is useful for the following crops by providing nutrients, however, when the mineralized nutrients exceeds the demand of the re-established grassland this often leads to losses of the major nutrients, for instance of nitrogen as NO_3^- leaching and N_2O emissions (Davies et al., 2001; Djurhuus and Olsen, 1997; Webster et al., 1999; Vertès et al., 2007). Such nitrogen losses results in an environmental risk and an economical loss to the farmers. Furthermore, there is not only a loss of nutrients, but also C_{org} in the soil. By measuring the CO_2 fluxes, Eriksen and Jensen (2001) found that the cultivation of grassland leads to total emissions of 2.6 t C ha^{-1} in the first three months after the cultivation in comparison to 1.4 t C ha^{-1} emitted in the uncultivated grassland.

The large increase in the mineralization rates as mentioned above can be ascribed to the incorporation of fresh litter derived from the plowed-in grass sward (Sheperd et al., 2001). Furthermore, the tillage leads to increased aeration and disruption of the soil aggregates (Six et al., 2002) by which a large amount of organic material becomes available to microbial degradation. Beside direct tillage effects on the SOM and aggregate dynamics (e.g., translocation of the soil, increased aeration, disruption of aggregates, addition of organic material) it may also cause indirect effects (e.g., decreasing root and plant biomass in the re-established grassland, shift of grassland species composition resulting in different composition of the litter input). The direct effects are likely to influence the SOM and aggregate dynamic in the short-term directly after tillage whereas more time is probably needed to observe potential

effects resulting from the indirect effects. However, less is known about the temporary respond of the SOM and aggregates to the described direct and indirect effects of tillage.

2.4 References

Abawi, G.S., Widmer, T.L. (2000): Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology 15, 37-47.

Alvaro-Fuentes, J., Arrue, J. L., Gracia, R., Lopez, M. V. (2007): Soil management effects on aggregate dynamics in semiarid Aragon (NE Spain). Science of the Total Environment 378, 179–182.

Andruschkewitsch, R., Geisseler, D., Koch, H-J., Ludwig, B. (2013): Effects of tillage on contents of organic carbon, nitrogen, water-stable aggregates and light fraction for four different long-term trials. Geoderma 192, 368–377.

Arrouays, D., Balesdent, J., Germon, J. C., Jayet, P. A., Soussana, J. F., Stengel, P. (2002). Increasing carbon stocks in French agricultural soils?. INRA et MED, Paris.

Bamberg, A. L., Cornelis, W. M., Timm, L. C., Gabriels, D., Pauletto, E. A., Pinto, L. F. S. (2011): Temporal changes of soil physical and hydraulic properties in strawberry fields. Soil Use and Management 27, 385–394.

Bronick, C. J., Lal, R. (2005): Manuring and rotation effects on soil organic carbon concentration for different aggregate size fractions on two soils in northeastern Ohio, USA. Soil and Tillage Research 81, 239–252.

Celik, I., Ortas, I., Kilic, S. (2004): Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. Soil and Tillage Research 78, 59–67.

Conant, R.T., Paustian, K., Elliott E.T. (2001): Grassland management and conversion into grassland: Effects on soil carbon. Ecological Applications 11, 343–355.

Daraghmeh, O. A., Jensen, J. R., Petersen, C. T. (2009): Soil structure stability under conventional and reduced tillage in a sandy loam. Geoderma 150, 64–71.

da Veiga, M., Reinert, D. J., Reichert, J. M. (2009): Aggregate stability as affected by short and tongterm tillage systems and nutrient sources of a Hapludox in southern Brazil. Revista Brasileira de Ciencia do Solo 33, 767–777.

Davies, M.G., Smith, K.A., Vinten, A.J. (2001): The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. Biology and Fertility of Soils 33, 423–434.

Djurhuus, J., Olsen, P. (1997): Nitrate leaching after cut grass/clover leys as affected by time of ploughing. Soil Use and Management 13, 61-67.

Eriksen, J., Jensen, L. S. (2001): Soil respiration, nitrogen mineralization and uptake in barley following cultivation of grazed grasslands. Biology and Fertility of Soils 33, 139-145.

Guo, L. B., Gifford, R. M. (2002): Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360.

Helfrich, M., Ludwig, B., Potthoff, M., Flessa, H. (2008): Effect of litter quality and soil fungi on macroaggregate dynamics and associated partitioning of litter carbon and nitrogen. Soil Biology and Biochemistry 40, 1823-1835.

Jacobs, A., Rauber, R., Ludwig, B. (2009): Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. Soil and Tillage Research 102, 158–164.

Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B. (2010): Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46, 671–680.

Leinweber, P., Schulten, H. R., Korschens, M. (1994): Seasonal variations of soil organic matter in a long-term africultural *experiment*. Plant and Soil 160, 225–235.

Mellek, J.E., Dieckow, J., da Silva, V.L., Favaretto, N., Pauletti, V., Vezzani, F.M., Moretti Souza, J.L. de (2010): Dairy liquid manure and no-tillage: Physical and hydraulic properties and carbon stocks in a Cambisol of Southern Brazil. Soil and Tillage Research 110, 69–76.

Mikha, M.M., Rice, C.W. (2004): Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. Soil Science Society of America Journal 68, 809–816.

Russelle, M. P., Birr, A.S. (2004): Large-scale assessment of symbiotic dinitrogen fixation by crops: soybean and alfalfa in the Mississippi River Basin. Agronomy Journal 96, 1754-1760.

Samson, R., Mani, S., Boddey, R.M., Sokhansanj, S., Quesada, D., Urquiaga, S., Reis, V., Lem, C.H. (2005): The potential of C 4 perennial grasses for developing a global BIOHEAT Industry. Critical Reviews in Plant Science 24, 461 – 495.

Sexstone, A.J. Revsbech, N. P., Parkin, T.B., Tiedje, J.M. (1985): Direct measurement of oxygen profiles and denitrification rates in soil aggregates. Soil Science Society of America Journal 49, 645-651.

Shepherd, M., Hatch, D., Jarvis, S., Bhogal, A. (2001): Nitrate leaching from reseeded pasture. Soil and Use Management 17, 97-105.

Six, J., Elliott, E. T., Paustian, K. (1999): Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358.

Six, J., Elliott, E. T., Paustian, K. (2000): Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099–2103.

Six, J., Feller, C., Denef, K., Ogle, S. M., Moraes Sa, J. C., Albrecht, A. (2002): Soil organic matter, biota and aggregation in temperate and tropical soils – Effects of no-tillage. Agronomie 22, 755–775.

Sollins, P., Homann, P., Caldwell, B. A. (1996): Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Stavi, I., Lal, R., Owens, L. B. (2011): On-farm effects of no-till versus occasional tillage on soil quality and crop yields in eastern Ohio. Agronomy for Sustainable Development 31, 475–482.

Stevenson, F.J. (1994): Humus chemistry: Genesis, Composition, Reaction. John Wiley & Sons, New York.

Taube, F., Wachendorf, M., Trott, H. (2002): Future challenges in grassland cultivation in Germany. In: Grassland resowing and grass-arable crop rotations. Velthof G, Taube F, Conijn J (eds) 67-78.

Tisdall, J.M., Oades, J.M. (1982): Organic matter and water-stable aggregates in soils. Journal of Soil Science 33, 141-163.

VandenBygaart, A. J., Kay, B. D. (2004): Persistence of soil organic carbon after plowing a long-term no-till field in southern Ontario, Canada. Soil Science Society of America Journal 68, 1394–1402.

Vertès, F., Hatch, D., Velthof, G., Taube, F., Laurent, F., Loiseau, P., Recous, S. (2007): Short-term and cumulative effects of grassland cultivation on nitrogen and carbon cycling in ley-arable rotations. Grassland Science in Europe 9, 227-246.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. (2006): Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. European Journal of Soil Science 57, 426-445.

Webster, C. P., Poulton, P. R., Goulding, K. W. T. (1999): Nitrogen leaching from winter cereals grown as part of a 5-year ley-arable rotation. European Journal of Agronomy 10, 99-109.

Zotarelli, L., Alves, B. J. R., Urquiaga, S., Boddey, R. M., Six, J. (2007): Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols. Soil and Tillage Research 95, 196–206.

3. Objectives of the present study

As described above the dynamics of aggregates and SOM in arable soils are well studied. However, there are lacks of knowledge about the C_{org}, aggregates, and SOM dynamics in grassland soils with different cultivation. Furthermore, like regular tillage also sporadic tillage affected the carbon dynamics. Thus this thesis focuses on the effects of sporadic tillage of grassland in the longer-term and in the short-term. There was also investigated the combined effect of organic fertilization and tillage in grassland soils and the short-term effect of the conversion of arable land in grassland.

Specific objectives include:

i. Study of the longer-term effects of a single tillage operation in grassland soils on C_{org} stocks, water-stable aggregate distribution, and SOM composition (chapters 4 and 5).

ii. Study of the longer-term effects of temporal conversion of grassland into arable land on the above mentioned parameters (chapters 4 and 5).

iii. Quantification of the combined effects of temporal conversion grassland into arable land and organic fertilization on C, N and P dynamics (chapter 5).

iv. Comparison of soils with different cultivation histories (arable land and grassland) on the temporal dynamics of C_{org} concentrations, water-stable aggregates and microbial biomass in the first year after (re-)establishing grassland (chapter 6).

v. Determination of temporal and seasonal dynamics on C_{org} concentrations, water-stable aggregates and microbial biomass in permanent grassland soils (chapter 6).

4. Temporal dynamics of soil organic matter composition and aggregate distribution in permanent grassland after a single tillage event in a temperate climate

Deborah Linsler[a], Daniel Geisseler[a], Ralf Loges[b], Friedhelm Taube[b], Bernard Ludwig[a]

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

[b] *Institute of Crop Science and Plant Breeding, Grass and Forage Science/Organic Agriculture, Christian-Albrechts-University of Kiel, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany*

4.1 Abstract

The dynamics of soil organic matter (SOM) composition in grassland after a single tillage event is not completely understood. Our objective was to study the long-term effects of sporadic tillage on SOM stocks, aggregate distribution and density fractions in grassland soils. We hypothesized that tillage decreased organic carbon (C_{org}) stocks, concentration of water-stable macroaggregates, and SOM light fractions. In April 2010 soil samples were taken at three depths two and five years after either one or two tillage operation(s). The trial site has loamy sandy soils (Cambisols, Eutric Luvisols, Stagnosols, Anthrosols) and is situated north of Kiel in Germany, with a mean annual temperature of 8.9 °C a nd a precipitation of 768 mm. Treatments consisted of permanent grassland (control), tillage of grassland followed by a re-establishment of grassland (G-G) and tillage of grassland followed by a re-establishment of grassland with one season of winter wheat in between (G-W-G). Two years after tillage, the C_{org} stocks, corrected for differences in soil bulk density, were significantly reduced ($p \leq 0.05$) in the top 10 cm of the soil profile in both tilled treatments (25.4, 16.8 and 18.6 Mg C_{org} ha^{-1} in 1250 Mg soil in the control, G-G and G-W-G, respectively). However, in the entire soil profile (0 – 40 cm) the C_{org}

stocks in the G-G treatment were only 5% lower and in the G-W-G treatment 4% higher than in the control and no longer significant. The concentration of water-stable macroaggregates (>250 µm) in the top 10 cm was decreased by 10 g and 9 g in 100 g^{-1} soil in the G-G and G-W-G treatments, respectively, compared with the control. The water-stable aggregate-size classes >2000 µm and 250 - 1000 µm were most affected by the tillage treatments. The concentration of the SOM light fraction decreased by 31% and 41% in the surface soil of the G-G and G-W-G treatments, respectively. Five years after tillage, the effects of tillage treatments on C_{org} stocks, water-stable aggregates, and SOM composition were still detectable; however the differences were much smaller and no longer significant. Our results suggest that sporadic tillage of grassland may not result in marked effects on C_{org} stocks, aggregate stability or SOM composition.

4.2 Introduction

The effects of permanent conversion of grasslands to croplands on carbon (C) stocks have been studied intensively (Guo and Gifford, 2002; Soussana et al., 2004). In their review, Guo and Gifford (2002) reported an average loss of C stocks of about 59% after the conversion from pasture to arable land. For the meta analysis they used data from 74 publications, which included soils of different types and textures from all over the world. Soussana et al. (2004) emphasized the asymmetric kinetics of C accumulation. They estimated that over a period of 20 years, the C accumulation resulting from the conversion of arable to grassland is on average only half of that lost when grassland is converted to arable land. While much research has focused on C dynamics after permanent conversion, less is known about grass arable/ley-arable rotations (Vertès et al., 2007) and grasslands which are tilled only sporadically. Especially in organic farming systems where synthetic herbicides are banned, infrequent tillage may be a necessary practice to control weeds or improve species composition. In general, tillage of

grassland results in increased mineralization of soil organic matter (SOM), resulting in elevated losses of soil nutrients such as nitrogen (N) (Davies et al., 2001; Djurhuus and Olsen, 1997; Soussana et al., 2004; Webster et al., 1999; Vertès et al., 2007). Furthermore, the tillage of grassland leads to the death of most of the living plant biomass, which then serves as organic input to the soil.

Soil organic matter may be stabilized and protected from degradation by occlusion in aggregates or by interactions with mineral surfaces (von Luetzow et al., 2006). When aggregates are disrupted by tillage, organic matter is exposed (Bronick and Lal, 2005; Zotarelli et al., 2007). Microaggregates (aggregates <250 µm) are more stable than macroaggregates (aggregates >250 µm) and tillage generally destroys macroaggregates more easily than microaggregates, making the SOM contained in macroaggregates more vulnerable to mineralization (Cambardella and Elliott, 1993; Six et al., 1999). To assess the interactions between SOM and soil mineral surfaces, schemes of density fractionation are often used (Golchin et al., 1994; John et al., 2005; Cerli et al., 2012). These procedures separate SOM into the light fraction (LF) and mineral-associated SOM. The LF consists of partially decomposed plant residues, which are not closely associated with soil minerals. Two different types of LF can be distinguished: the free LF (fLF) and the LF which is occluded in aggregates (oLF) (Cerli et al., 2012).

Permanent grassland generally contains more SOM than arable land (Guo and Gifford, 2002; Soussana et al., 2004). The conversion of pasture or grassland to arable land does not only reduce SOM stocks as reported above, but may also affect the distribution and stability of aggregates in soil (Ross, 1993; Singh and Singh, 1996; Shepherd et al., 2001). For instance, Shepherd et al. (2001) found that the mean weight diameter of water-stable aggregates had declined by 64 – 71% four years after the conversion of pasture to arable land. Quincke et al. (2007) determined the water-stable macroaggregate concentration in the top 5 cm of no-till fields

after a one-time tillage event. Even though this study is not directly comparable with sporadic tillage in grassland soils, it is interesting to note that no differences in concentrations of water-stable aggregates between the tilled fields and the no-till control were found two years after a single tillage event.

We hypothesized that tillage operations in grassland soils lead to a decrease in organic C stocks and concentrations of water-stable macroaggregates of soil and SOM in light fractions (Hypothesis 1). However, these effects are reversible, and after a certain time the organic C stocks, the concentrations of water-stable macroaggregates and the concentrations of SOM in light fractions can be restored (Hypothesis 2).

Overall, only limited information is available about what effect sporadic tillage operations have on the composition of SOM and water-stable aggregates in grassland soils with one season of cropping. The objective was to study the long-term effects of sporadic tillage in grassland soils on C stocks, water-stable aggregate distribution and density fractions.

4.3 Material and methods

4.3.1 Study site

The sampling area is located near the Baltic Sea in Germany, north of Kiel (experimental farm Lindhof, 54°27 `N, 9°57 `E). The mean annual t emperature in the area is 8.9 °C and precipitation is 768 mm. For further information on temperature and precipitation conditions see figure 4.1 and figure 4.2. The soil at the site is heterogeneous and the soil types include Cambisols, Eutric Luvisols, Stagnosols, and Anthrosols (Schmeer et al., 2009).

In 1994, an experimental field at the site (which was arable land prior to the trial) was converted to permanent grassland. Between 1994 and 2005, the grassland was generally cut 1-2 times per year for silage production and grazed by cattle 3-4 times each year. In 2005, a field trial (trial B) was initiated to determine the short- and long-term effects of a single tillage

operation. The aim was to study the effects of grassland renovation on N fluxes and C storage. In one treatment a mixture of permanent grass species and white clover (*Trifolium repens* L.) was sown immediately after tillage. In another treatment, winter wheat (*Triticum aestivum* L.) was grown for one season before the same grass-clover mixture was sown as in treatment one. The latter treatment was included so as to investigate the effects of one season of winter wheat on nitrate leaching and to ascertain whether the crop can use mineralized N efficiently. For the investigation of C dynamics, this is an interesting treatment because of the addition of a high mass of organic material in the form of straw. The three treatments, each with three replicates arranged in a randomized plot design (plot size 3 x 14-20 m), were:

G-G: **g**rassland – tillage - **g**rassland

The plots (grassland since 1994) were tilled in September 2005 and a grass-clover mixture was sown. The mixture contained 67% perennial ryegrass (*Lolium perenne* L.), 17% timothy grass (*Phleum pratense* L.), 10% smooth meadow-grass (*Poa pratensis* L.) and 6% white clover (*Trifolium repens* L.).

G-W-G: **g**rassland – tillage – **w**inter wheat – tillage – **g**rassland

The plots (grassland since 1994) were tilled in October 2005 and winter wheat (*Triticum aestivum* L. variety Bussard) was sown. After the wheat harvest, the plots were tilled again in September 2006 to incorporate the straw (approximately 7 Mg ha^{-1}), which was left on the field after grain harvest. Afterwards, a grass-clover mixture as described above was sown.

Permanent grassland (since 1994) served as control.

In the plots where the trial was established in 2005/06, the soil (0 - 40 cm) had a pH of 5.6 (standard error ±0.2). The sand, silt and clay concentrations reached 530, 290 and 180 g kg^{-1}, respectively.

The trial was repeated in 2008/09 in an adjacent area (trial A) (approximately 50 m

away), also with three replicates each. In this area, the pH was 5.6 (±0.1) and the sand, silt and clay concentrations were 660, 210 and 130 g kg^{-1}, respectively.

In both trials, the plots were tilled with a moldboard plow to a depth of 25 cm. The grassland here is generally cut four times each year for forage production (Schmeer et al., 2009). In all three treatments, the plots were fertilized in 2007 and 2009 with 100 kg K ha^{-1}, 24 kg Mg ha^{-1}, 68 kg S ha^{-1} (Potassium sulfate with magnesium) and 45 kg P ha^{-1} (rock phosphate). These fertilizers are accredited by the German organic growers association “Bioland”. The site has been managed according to their guidelines since 1993 (Bioland, 2012).

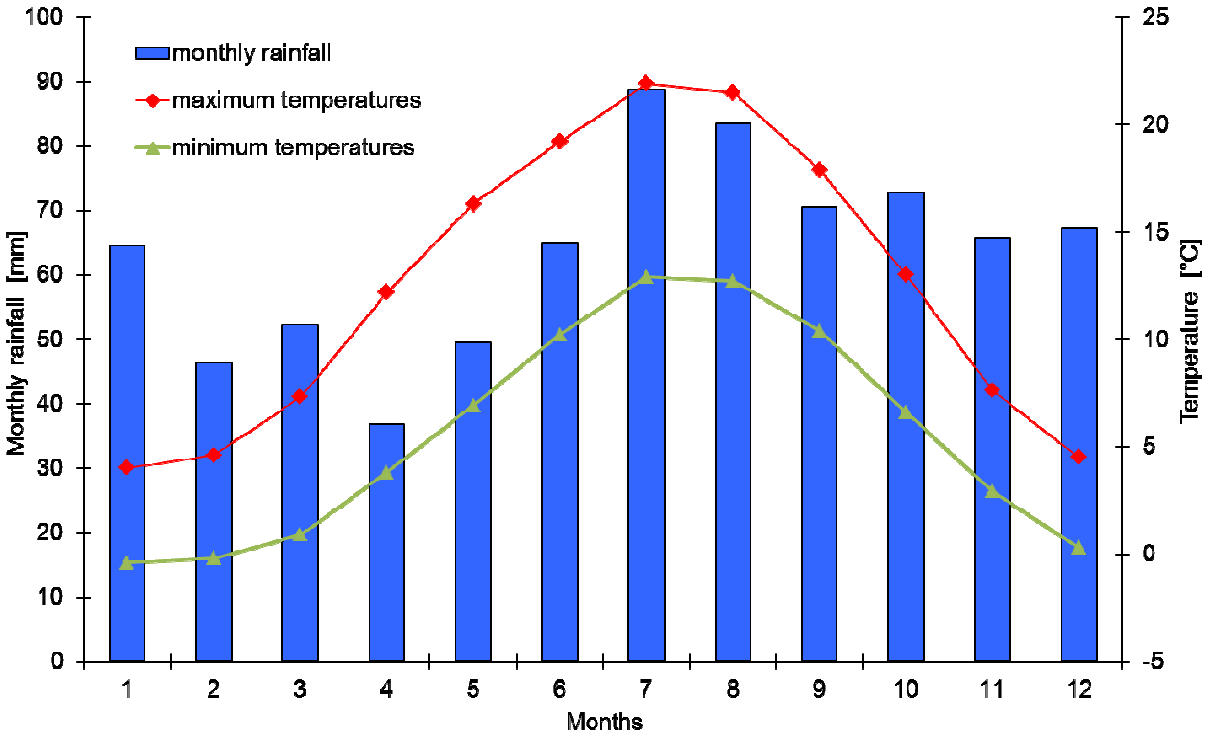


Figure 4.1: Monthly minimum and maximum temperatures and precipitation in the long-term (1987-2011) at the study site (Lindhof, North of Kiel, Germany).

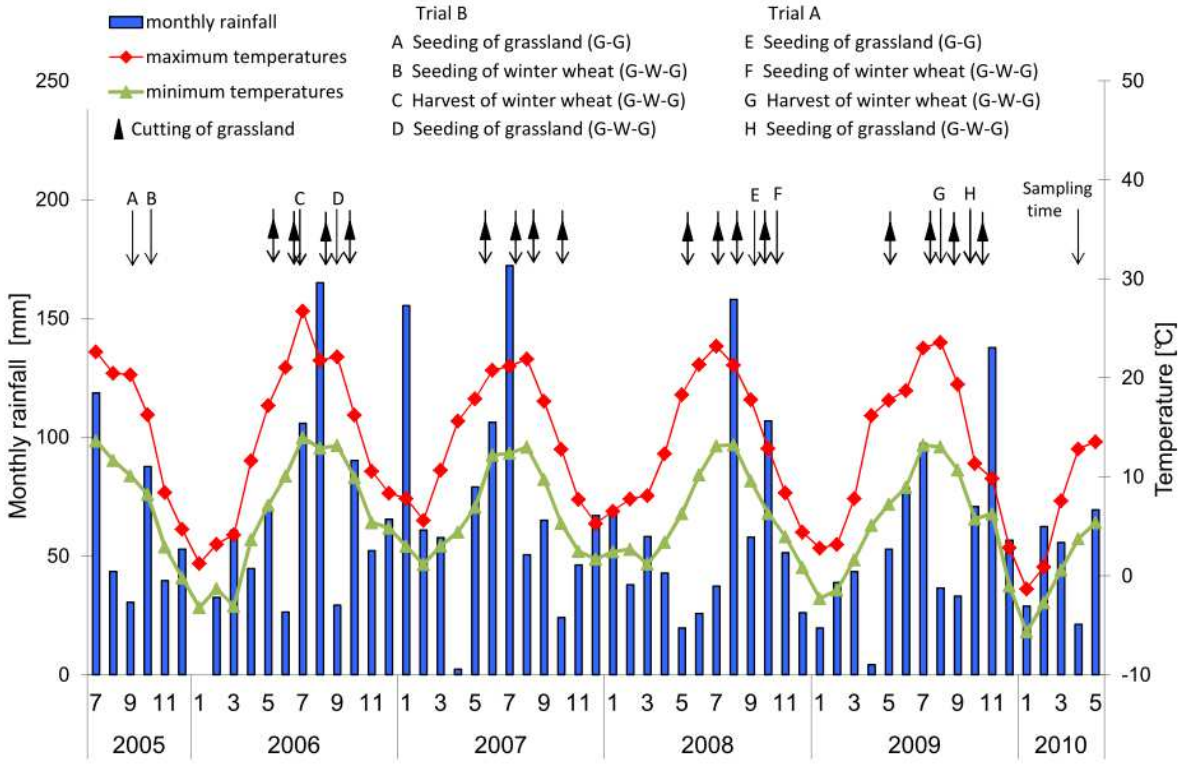


Figure 4.2: Monthly minimum temperatures, maximum temperatures, and precipitation from the beginning of the experiment (Sept. 2005) until sampling time (April 2010) at the study site (Lindhof, North of Kiel, Germany), including time of sampling, the dates of sowing and the dates of harvest in the different treatments (permanent grassland; G-G: grassland – tillage – grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland).

4.3.2. Sampling procedure

Soil samples were taken in April 2010. From each plot, four cores were combined to a composite sample. The samples were taken in the three field replicates more than 50 cm away from the border to the next plot at three depths (0 - 10 cm, 10 - 25 cm and 25 - 40 cm). The samples were stored at 4 ℃ before processing.

4.3.3. Aggregate fractionation

Water-stable aggregates of soil were fractionated by a wet-sieving procedure described by Cambardella and Elliott (1993) and slightly modified by Jacobs et al. (2009). Briefly, the field-

moist soil samples were gently sieved (<10 mm) and dried for 48 hours at 40℃. For the fractionation, approximately 100 g of soil were placed on a 2000 µm sieve and submerged into distilled water for 10 min to allow slaking. Afterwards, the sieve was lifted out of the water. The sieve was resubmerged when all the water left in the sieve had run out. This step was repeated 50 times. The time of the process depended on the mass of soil / water-stable aggregates on the sieve. Water-stable aggregates remaining on the sieve (large water-stable macroaggregates: 10 mm – 2000 µm) were collected, vacuum filtered (<0.45 µm), dried at 40 ℃ for 48 h, and weighed. Water-stable aggregates which passed the 2000 µm sieve were transferred to a sieve with the next smaller mesh size and the fractionation procedure was repeated as described above. The mesh sizes used were: 2000 µm for large water-stable macroaggregates, 1000 µm for medium water-stable macroaggregates, 250 µm for small water-stable macroaggregates and 53 µm for water-stable microaggregates. Material passing through the 53 µm sieve (silt and clay together with very small water-stable microaggregates) was precipitated with a 0.5 M $AlCl_3$ solution (5 mL for 2 L of suspension). To recover the <53 µm class after precipitation, the water was siphoned off and the deposit filtered, dried at 40 ℃ for 48 h, and weighed.

The concentrations of water-stable aggregates and C_{org} concentrations in water-stable aggregate classes were corrected for the sand concentration.

In the present study the entire procedure of aggregate fractionation was carried out by a single person, since this method has a marked operator variability (Jacobs et al., 2010).

Additionally, the concentrations of roots in the water-stable aggregate-size class >2000 µm were determined. The water-stable aggregates >2000 µm were crushed by hand and the class was wet-sieved again with a 2000 µm sieve. Stones and roots remaining on the sieve were separated by decantation. The fractions were filtered, dried at 40 ℃ for 48 h, and weighed.

4.3.4 Density fractionation

A scheme of density fractionation was used to determine the composition of SOM (Golchin et al., 1994, modified after Jacobs et al. (2009). However, due to high sand and root concentrations in our samples, the following adaptations of the procedure described by Jacobs et al. (2009) were necessary. Prior to the density fractionation, the field-moist soil samples were sieved through a 5 mm sieve, rather than the 2 mm sieve used by Jacobs et al. (2009), to protect the larger aggregates. The mass of soil (8 g instead of 10 g), the volume of sodium polytungstate (SPT) (30 mL instead of 40 mL) and the centrifugation speed (2000 x g instead of 4000 x g) were reduced due to the higher concentration of roots in grassland than in arable land. Additionally, the density of SPT was raised (1.8 g cm^{-3} instead of 1.6 g cm^{-3}) because of the higher sand concentration in comparison with Jacobs et al. (2009). To gain the oLF, the solution was centrifuged and decanted three times instead of once as reported by Jacobs et al. (2009). This was done because larger root pieces became stuck in the pellet during the first centrifugation, preventing them from floating to the surface of the solution.

Briefly, 8 g of field-moist soil were placed into a 70 mL centrifugation tube and 30 mL of a SPT solution was added as described above. When the water content of the soil was taken into account, the density of the SPT solution was 1.8 g cm^{-3}. The tube was then inverted 5 times and the solution was allowed to stand for 30 min before centrifugation at 2000 x g for 30 min (Multifuge 3 S-R, Heraeus, Hanau, Germany). The supernatant with floating particles was then vacuum filtered (<0.45 µm) and washed with 2 L of distilled water. To obtain the oLF, the soil pellet was dispersed with 30 mL of SPT solution (1.8 g cm^{-3}). To destroy the aggregates of soil, ten glass balls with a diameter of 5 mm were added and the tubes were then shaken for 18 hours at 175 rotations per minute (SM-30, Edmund Bühler, Hechingen, Germany). After disaggregation, the solution was centrifuged and decanted three times and the supernatant was filtered and washed as described above. The remaining mineral fraction (MF) in the tube was

separated into a sand fraction ($MF_{>53\ \mu m}$) and a silt plus clay fraction ($MF_{<53\ \mu m}$) by wet-sieving. The $MF_{>53\ \mu m}$ was filtered immediately, while the $MF_{<53\ \mu m}$ was put in a beaker with 1.5 L of distilled water and precipitated with 3.75 mL of a 0.5 M $AlCl_3$ solution. After precipitation the water was siphoned off and the $MF_{<53\ \mu m}$ was vacuum filtered. All fractions were dried at 40℃ for 48 h , and weighed.

4.3.5 Analytics and soil characterization

The bulk soil, the water-stable aggregate classes and the MFs of the density fractionation were ball-milled (MM 200, Retsch, Haan, Germany) and stored in plastic vials at room temperature.

Total C (C_t) and N (N_t) concentrations in the bulk soil as well as in the water-stable aggregate classes and density fractions were determined by dry combustion on a CN elemental analyzer (Elementar Vario El, Heraeus, Hanau, Germany). Since no carbonates were detectable, C_t corresponds to organic C (C_{org}).

Soil pH was determined in a 0.01 M $CaCl_2$ solution (2.5 mL of solution g^{-1} of soil). Soil texture was determined with the pipette method according to DIN ISO 11277 (2002) and bulk density according to DIN ISO 11272 (1998).

4.3.6 Calculations and statistical analyses

To account the differences in bulk density, the C_{org} mass per unit area was calculated for an equivalent mass of soil. The data are given in Mg C_{org} ha^{-1} in 1250 Mg soil for the surface soil (0 – 10 cm) and in 5758 Mg soil for the soil profile (0 – 40 cm).

The stone concentrations in our samples averaged 20 (in the area tilled 2005/06) and 60 g kg^{-1} soil (in the area tilled 2008/09). For the calculation of C_{org} stocks, we subtracted the concentration of stones (>2 mm) from the total mass of the volumetric samples (Ellert et al.,

2001), since C_{org} concentrations were determined in the fine earth (<2 mm).

The concentrations of C_{org} in water-stable aggregates were expressed on a sand-free basis as suggested by Six et al. (2000b) and were calculated as described by John et al. (2005): Concentration of sand-free C_{org} (g kg^{-1}) = C_{org} concentration (g kg^{-1}) / (1-sand proportion (g g^{-1})).

Statistical analyses were performed with the SAS program (SAS Institute, 1990). The two years of tillage were analyzed separately. The data sets were analyzed as a split-plot design with tillage treatment as the main factor and depth as sub factor. Normality of the residuals was tested with the Shapiro-Wilk test and homogeneity of variances with Levene´s test. When necessary, the data were transformed. The Tukey test was used for mean comparisons. Effects were considered significant for $p \leq 0.05$.

4.4 Results

4.4.1 Effect of tillage on C_{org} concentrations and C_{org} stocks

Two years after tillage (trial A), the C_{org} concentration in the surface soil (0 – 10 cm) was significantly lower ($p \leq 0.05$) in the tilled treatments than in the control (Table 4.1). The N_t concentration closely followed the C_{org} concentration and the C to N ratio averaged 10.3.

Five years after tillage (trial B), the C_{org} and N_t concentrations as well as the C to N ratio decreased in all treatments with depth. However, no significant differences between the treatments were detected (Table 4.1).

In trial A, the significant reduction of C_{org} concentrations in the top 10 cm soil depth due to tillage resulted in significantly lower C_{org} stocks. However, differences were limited to the topsoil; in the sampled soil profile (0 – 40 cm) no significant differences between the treatments were found.

Five years after tillage no differences in C_{org} stocks between treatments were found.

Table 4.1: Soil properties (bulk soil <2 mm) among different treatments (permanent grassland; G-G: grassland – tillage – grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland) and soil depths in a long-term trial, north of Germany, in loamy sandy grassland soils sampled two and five years after the tillage operation(s); mean values and standard errors (n=3)

	Trial A: Tillage 2 years before sampling			Trial B: Tillage 5 years before sampling		
Soil properties	0-10 cm	10-25 cm	25-40 cm	0-10 cm	10-25 cm	25-40 cm
N_t (g kg^{-1})						
Permanent grassland	1.97 (0.13) Aa	1.11 (0.15) b	0.95 (0.07) b	2.10 (0.29) a	1.37 (0.09) b	1.16 (0.05) b
G – G	1.21 (0.07) Ba	1.42 (0.02) a	0.79 (0.10) b	1.61 (0.09) a	1.47 (0.09) ab	1.09 (0.09) b
G – W – G	1.42 (0.06) B	1.39 (0.10)	1.14 (0.14)	1.67 (0.09) a	1.33 (0.05) b	0.94 (0.06) c
C_{org} (g kg^{-1})						
Permanent grassland	21.58 (1.48) Aa	11.39 (1.48) b	9.61 (0.86) b	21.91 (3.04) a	13.36 (0.92) b	11.29 (0.50) b
G – G	12.43 (0.70) Ba	14.98 (0.53) a	7.73 (1.13) b	17.15 (1.31) a	14.49 (1.02) ab	10.62 (0.96) b
G – W – G	14.41 (0.83) B	14.41 (0.94)	11.57 (1.60)	17.16 (1.07) a	13.02 (0.54) b	9.04 (0.61) c
C to N ratio						
Permanent grassland	10.95 (0.06) Aa	10.30 (0.10) b	10.04 (0.13) b	10.41 (0.08) a	9.75 (0.07) b	9.74 (0.06) b
G – G	10.25 (0.05) ABa	10.53 (0.21) a	9.80 (0.16) b	10.65 (0.18) a	9.84 (0.11) b	9.69 (0.09) b
G – W – G	10.14 (0.28) B	10.35 (0.04)	10.14 (0.18)	10.30 (0.14)	9.79 (0.12)	9.61 (0.24)
Bulk density (g cm^{-3})						
Permanent grassland	1.25 (0.10) b	1.51 (0.08) a	1.50 (0.11) a	1.16 (0.11)	1.43 (0.08)	1.43 (0.03)
G – G	1.45 (0.13)	1.50 (0.06)	1.62 (0.10)	1.24 (0.01) b	1.38 (0.04) b	1.55 (0.06) a
G – W – G	1.44 (0.09)	1.41 (0.07)	1.46 (0.04)	1.26 (0.13)	1.41 (0.06)	1.56 (0.04)

Table 4.2: C_{org} stocks calculated on an equivalent mass of soil among different treatments (permanent grassland; G-G: grassland – tillage – grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland) and soil depths in a long-term trial, north of Germany, in loamy sandy grassland soils sampled two and five years after the tillage operation(s); mean values and standard errors (n=3)

Trial A: Tillage 2 years before sampling

	Depth	C_{org} stock (Mg C ha^{-1} in 1250 Mg soil)	Depth	C_{org} stock (Mg C ha^{-1} in 5758 Mg soil)
Permanent grassland	0 - 10.0 cm	25.4 (1.6) A	0 - 40.0 cm	69.3 (3.6)
G - G	0 - 8.7 cm	16.8 (0.7) B	0 - 37.7 cm	66.1 (3.1)
G - W - G	0 - 8.7 cm	18.6 (1.3) B	0 - 40.2 cm	72.3 (6.7)

Trial B: Tillage 5 years before sampling

	Depth	C_{org} stock (Mg C ha^{-1} in 1250 Mg soil)	Depth	C_{org} stock (Mg C ha^{-1} in 5758 Mg soil)
Permanent grassland	0 - 10.8 cm	24.9 (2.6)	0 - 42.3 cm	77.2 (5.6)
G - G	0 - 10.0 cm	21.1 (1.6)	0 - 40.9 cm	73.2 (4.7)
G - W - G	0 - 9.9 cm	21.3 (2.0)	0 - 40.4 cm	68.9 (5.0)

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

4.4.2 Distribution of water-stable aggregates two and five years after tillage

The two water-stable aggregate-size classes most affected by tillage were the large water-stable macroaggregates (>2000 µm) and the small water-stable macroaggregates (250 – 1000 µm). In the surface soil layer of trial A, significantly lower concentrations of large water-stable macroaggregates but higher concentrations of small water-stable macroaggregates were found in the tilled treatments than in the permanent grassland (Figure 4.3).

In trial B (5 years after tillage), the concentrations of the different water-stable aggregate-size classes were no longer different among the three treatments (Figure 4.3).

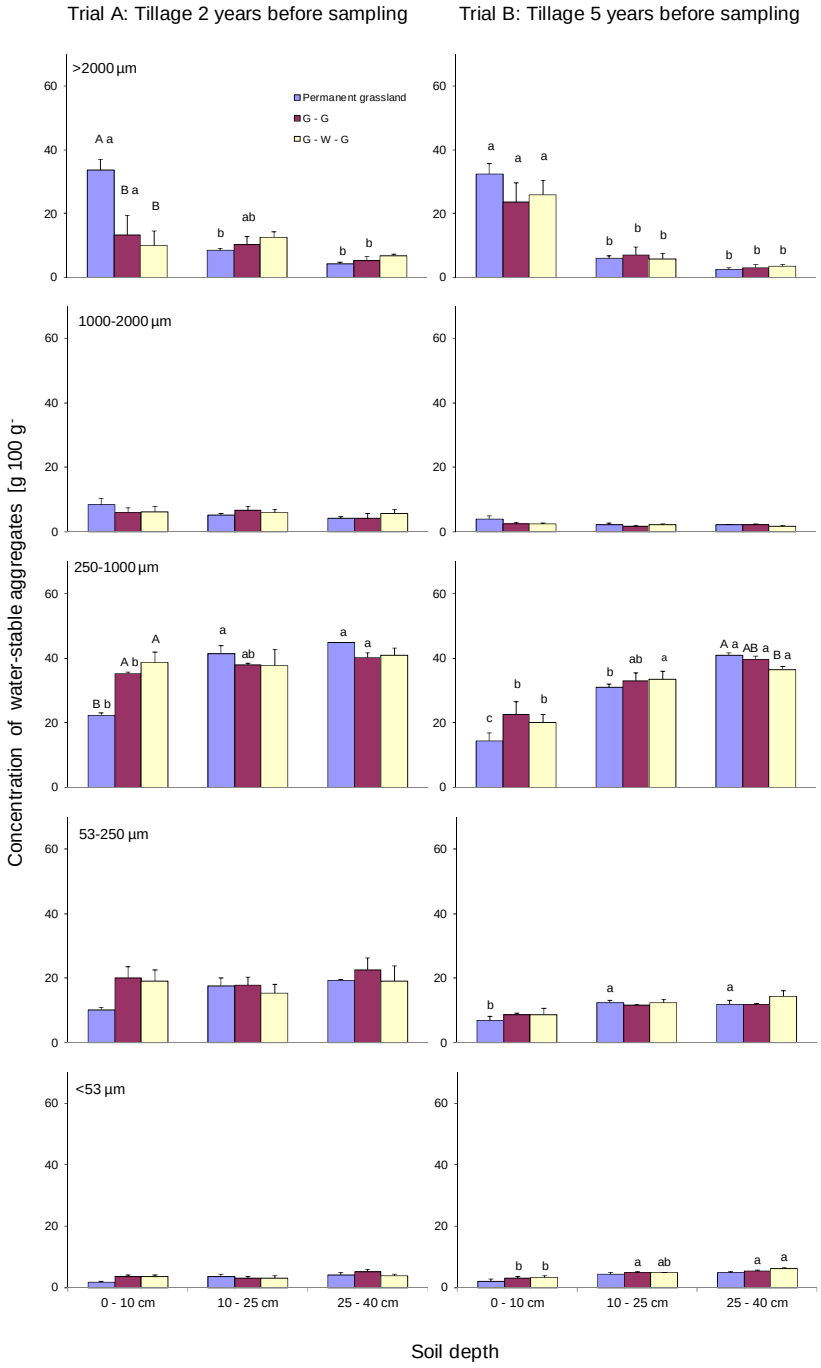


Figure 4.3: Concentrations (g 100 g^{-1} soil) of water-stable aggregates among different treatments (permanent grassland; G-G: grassland – tillage – grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland) and soil depths in a long-term trial, North Germany, in loamy sandy grassland soils sampled two and five years after the tillage operation(s); mean values and standard errors (n=3).

Lowercase letters indicate significant differences between sampling depths. Uppercase letters indicate significant differences among treatments; exclusively those values which are significantly different ($p<0.05$) are followed by different letters.

The C_{org} concentrations in water-stable aggregate classes varied considerably. In the water-stable macroaggregates, the C_{org} concentrations ranged from 21 to 208 g kg^{-1} sand-free class and in water-stable microaggregates from 17 to 40 g kg^{-1} sand-free class (Table 4.3). The C_{org} concentrations in water-stable aggregate classes decreased in the order 1000 - 2000 µm (75 ± 7 g kg^{-1} sand-free class)^{A} > >2000 µm (47 ± 3 g kg^{-1})^{B} > 250 - 1000 µm (36 ± 2 g kg^{-1})^{BC} > 53 - 250 µm (28 ± 1 g kg^{-1})^{CD} > <53 µm (20 ± 1 g kg^{-1})^{D} (mean values and standard errors of all treatments, soil depths, and replicates; letters indicate significant differences between C_{org} in water-stable aggregate classes; n = 54). This comparison reflects the general trend of decreasing C_{org} concentration with decreasing water-stable aggregate-size class. This was true with the exception of the water-stable aggregate-size class 1000 - 2000 µm, which had high sand concentrations (830 g kg^{-1} in trial A and 660 g kg^{-1} in trial B). The differences in C_{org} concentrations in water-stable aggregates between the tilled plots and the control were more pronounced two years after tillage than five years after tillage (Table 4.3). As in the bulk soil, the N_t concentrations closely followed the C_{org} concentrations in water-stable aggregate-size classes.

Table 4.3: Mean values of C_{org} concentrations (g kg^{-1} fraction) calculated on a sand-free basis among water-stable aggregate size classes, different treatments (permanent grassland; G-G: grassland – tillage – grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland) and soil depths in a long-term trial, north of Germany, in loamy sandy grassland soils sampled two and five years after the tillage operation(s); mean values and standard errors (n=3).

	Trial A: Tillage 2 years before sampling			Trial B: Tillage 5 years before sampling		
Aggregate fractions and treatments	0-10 cm	10-25 cm	25-40 cm	0-10 cm	10-25 cm	25-40 cm
>2000 µm						
Permanent grassland	97.2 (7.2) Aa	39.2 (2.4) Bb	31.4 (5.2) b	60.5 (7.9) a	31.4 (2.5) b	26.4 (2.9) b
G – G	67.6 (1.0) Ba	59.6 (3.7) Aa	45.0 (2.2) b	45.2 (3.0) a	35.4 (4.5) ab	22.5 (2.7) b
G – W – G	85.1 (7.0) ABa	49.1 (2.1) ABb	43.2 (2.5) b	50.3 (4.4) a	30.7 (1.2) ab	21.4 (6.6) b
1000-2000 µm						
Permanent grassland	208.2 (11.0) a	60.3 (8.6) b	31.8 (6.3) c	60.1 (2.1)	45.8 (4.3)	50.0 (7.2)
G – G	122.2 (1.0) a	130.7 (39.1) a	51.1 (4.2) b	59.1 (4.6) a	51.9 (2.9) ab	41.6 (4.1) b
G – W – G	125.7 (30.3)	96.8 (9.4)	63.3 (15.4)	57.6 (4.0)	49.3 (1.9)	46.6 (6.6)
250-1000 µm						
Permanent grassland	52.9 (5.5) Aa	42.1 (1.1) ab	27.1 (2.1) b	49.3 (5.7) a	29.5 (2.3) b	29.9 (3.4) b
G – G	35.3 (2.9) Ba	55.1 (11.7) ab	23.1 (3.3) b	40.2 (0.2) a	34.4 (1.2) b	28.9 (1.0) c
G – W – G	41.5 (1.9) AB	39.1 (1.2)	31.4 (4.9)	37.4 (2.0) a	29.4 (1.4) b	23.9 (1.4) b
53-250 µm						
Permanent grassland	39.6 (0.7)	32.4 (1.5) b	22.8 (1.4) c	30.5 (2.8) a	29.2 (1.3) ab	21.6 (1.1) b
G – G	30.7 (1.4)	33.6 (1.9) a	16.8 (2.2) b	29.6 (1.5) a	27.4 (1.5) ab	22.6 (1.7) b
G – W – G	36.8 (3.2)	36.3 (2.1)	24.9 (3.5)	28.6 (1.2) a	26.0 (1.7) a	17.8 (0.1) b
<53 µm						
Permanent grassland	26.3 (3.0)	23.1 (1.6)	20.0 (2.5)	21.7 (2.0)	20.0 (1.5)	18.0 (2.0)
G – G	19.1 (1.2)	19.9 (1.3)	14.2 (3.7)	20.2 (0.8)	18.7 (0.6)	17.0 (1.4)
G – W – G	24.5 (1.6)	24.5 (1.9)	22.3 (4.4)	19.0 (0.6) a	18.0 (0.8) a	13.0 (0.5) b

Lowercase letters indicate significant differences between sampling depths. Uppercase letters indicate significant differences among treatments; exclusively those values which are significantly different ($p < 0.05$) are followed by different letters.

4.4.3 Distribution of SOM two and five years after tillage

Two years after tillage the fLF concentration was decreased significantly by 63% in the surface soil of the G-G treatment compared with the control (Table 4.4). The fLF concentrations in the G-W-G treatment closely followed those in the G-G treatment. In contrast, the tillage treatments had no effect on the oLF concentrations.

Five years after tillage, there were no significant differences in fLF and oLF concentrations between the tilled treatments and the control. However, both fLF and oLF decreased with soil depth (Table 4.4).

The C_{org} concentrations in the two LFs were roughly one and two orders of magnitude higher than in the $MF_{<53\ \mu m}$ and the $MF_{>53\ \mu m}$, respectively.

The N_t concentrations in density fractions generally followed the trend of C_{org} concentrations with the exception that the highest values were in the oLF and not in the fLF (data not shown).

4.4.4 Correlation analysis of different C fractions

The two largest water-stable aggregate classes were positively correlated with the LFs, the root concentrations in the water-stable aggregates >2000 µm and the C_{org} concentrations in the bulk soil. In contrast, the two largest water-stable aggregate classes were negatively correlated with the water-stable aggregate classes 250 - 1000 µm and 53 - 250 µm (Table 4.5). The latter two classes were also negatively correlated with the LFs, the roots in the largest water-stable aggregates and the C_{org} concentration in the bulk soil. All these correlations were highly significant ($p < 0.01$).

Table 4.4: SOM concentrations and types (g 100 g^{-1} soil) and Corg concentrations (g kg^{-1} fraction) in density fractions among different treatments (permanent grassland; G-G: grassland – tillage – grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland) and soil depths in a long-term trial, north of Germany, in loamy sandy grassland soils sampled two and five years after the tillage operation(s); mean values and standard errors (n=3).

	Trial A: Tillage 2 years before sampling				Trial B: Tillage 5 years before sampling			
	Concentration of density fraction (g 100 g^{-1} soil)		Concentration of C_{org} in density fraction (g kg^{-1})		Concentration of density fraction (g 100 g^{-1} soil)		Concentration of C_{org} in density fraction (g kg^{-1})	
SOM types and treatments	0-10 cm	10-25 cm	0-10 cm	10-25 cm	0-10 cm	10-25 cm	0-10 cm	10-25 cm
Free light fraction								
Permanent grassland	0.5 (0.1) Aa	0.1 (0.0) b	249.4 (13.6)	243.7 (7.3)	0.3 (0.1) a	0.1 (0.0) b	244.4 (17.2)	200.0 (18.1)
G – G	0.2 (0.1) B	0.2 (0.1)	278.5 (9.4)	280.4 (14.9)	0.4 (0.1) a	0.1 (0.0) b	279.7 (26.4)	239.1 (16.6)
G – W – G	0.1 (0.1) B	0.2 (0.1)	272.5 (5.5)	314.8 (3.0)	0.2 (0.0) a	0.1 (0.0) b	286.6 (15.4)	246.3 (19.8)
Occluded light fraction								
Permanent grassland	1.7 (0.2)	0.9 (0.2)	228.8 (20.5)	215.2 (19.4)	2.3 (0.3) a	1.3 (0.1) b	207.8 (11.2)	203.7 (11.8)
G – G	1.4 (0.3)	1.5 (0.3)	216.7 (20.7)	217.8 (22.0)	1.6 (0.3)	1.2 (0.3)	240.8 (8.7)	193.7 (15.7)
G – W – G	1.2 (0.2)	1.2 (0.1)	180.6 (11.5)	191.7 (15.5)	1.6 (0.1) a	1.1 (0.1) b	224.6 (19.9)	223.1 (8.5)
Mineral fraction >53 µm								
Permanent grassland	64.8 (4.0)	67.4 (4.3)	3.5 (0.4) Aa	1.1 (0.2)b	54.5 (2.7)	55.7 (2.8)	3.1 (0.2) a	1.6 (0.2) b
G – G	63.2 (6.1)	62.7 (6.1)	1.5 (0.3) B	1.9 (0.1)	51.6 (1.6)	52.1 (1.9)	2.6 (0.2) a	1.9 (0.3) ab
G – W – G	66.8 (4.4)	66.2 (3.6)	1.6 (0.4) B	1.6 (0.5)	51.6 (2.1)	51.1 (1.3)	2.6 (0.4)	2.0 (0.5)
Mineral fraction <53 µm								
Permanent grassland	32.3 (4.4)	31.4 (4.8)	40.4 (6.8)	28.1 (5.9)	42.2 (3.6)	43.5 (3.1)	34.0 (5.3)	24.1 (2.6)
G – G	35.5 (6.0)	35.7 (6.1)	24.9 (3.4)	30.3 (2.5)	45.4 (2.5)	46.4 (2.1)	24.2 (1.3)	23.1 (1.6)
G – W – G	32.0 (4.2)	32.6 (3.4)	32.6 (3.1)	31.9 (3.5)	46.6 (2.2)	46.9 (1.8)	24.9 (0.7) a	21.4 (0.9) a

Lowercase letters indicate significant differences between sampling depths. Uppercase letters indicate significant differences among treatments; exclusively those values which are significantly different ($p < 0.05$) are followed by different letters.

Table 4.5: Correlation coefficients between concentrations of aggregates, roots in >2000 µm aggregates, concentrations of density fractions (fLF: free light fraction, oLF: occluded light fraction, MF: mineral fraction) and C_{org} concentrations in the bulk soil. Soil for analysis was sampled in a long-term trial, north of Germany, in loamy sandy grassland soils two and five years after plowing operation(s). Data are only shown for significant ($p \leq 0.05$, marked as *) and highly significant ($p \leq 0.01$, marked as **) correlations (n=54).

	Concentration of aggregate fractions					Roots	Concentration of density fractions				Texture			C_{org} concentration
Aggregate fractions	2000 - 1000 µm	1000 - 250 µm	250 - 53 µm	<53 µm	>250 µm		fLF	oLF	MF $_{>53\ \mu m}$	MF $_{<53\ \mu m}$	clay	silt	sand	
>2000 µm	0.49 **	-0.96 **	-0.77 **		0.77 **	0.85 **	0.77 **	0.72 **						0.83 **
2000 - 1000 µm		-0.43 **	-0.63 **	-0.62 **	0.69 **	0.46 **	0.54 **	0.42 **	0.56 **	-0.62 **	-0.60 **	-0.52 **	0.59 **	0.52 **
1000 - 250 µm			0.58 **		-0.57 **	-0.83 **	-0.76 **	-0.71 **						-0.79 **
250 - 53 µm				0.32 *	-0.96 **	-0.63 **	-0.59 **	-0.53 **		0.32 *	0.36 **	0.27 *	-0.33 *	-0.68 **
<53 µm					-0.36 **				-0.98 **	1.00 **	0.90 **	0.92 **	-0.98 **	
>250 µm						0.64 **	0.62 **	0.53 **	0.30 *	-0.36 **	-0.37 **	-0.32 *	0.37 **	0.69 **

4.5 Discussion

4.5.1 Effect of tillage on C_{org} concentrations and C_{org} stocks

Two years after tillage, the C_{org} stocks were significantly lower in the top 10 cm of the soil profile in the G-G treatment compared with the soil under permanent grassland (Table 4.2). This reduction supports Hypothesis 1, that a tillage event leads to a reduction of C_{org} stocks. However, over the whole sampled soil profile (0 – 40 cm) the C_{org} stocks of these two treatments did not differ. The lower stocks in the surface soil layer were mainly the result of significantly lower C_{org} concentrations (Table 4.1). This was presumably caused by the death of living biomass and subsequent decomposition after the tillage operation and by lower root biomass in recently established grasslands than in older ones (Bolinder et al., 2002). However, in the 10 - 25 cm layer the C_{org} concentrations were equal in the tilled soil compared with the soil under permanent grassland. This indicates that most of the organic material incorporated by the plow had been mineralized by the time of sampling. Five years after tillage, the differences in C_{org} concentrations between the tilled treatments and the permanent grassland were no longer significant, which supports Hypothesis 2. This result also supports the above-mentioned suggestions about the effects of root concentration and incorporated organic material on C_{org}.

The effect of one-time tillage on C_{org} concentrations was investigated by several studies in arable land, which are not directly comparable with our research in permanent grassland soils. In a study conducted at two arable sites with silty clay loam soil (Typic Argiudolls and Mollic Hapludalfs; Soil Survey Staff, 2006), Quincke et al. (2007) found a significant decrease in the C_{org} concentration in the top 5 cm of the soil profile 1 and 6 months after tilling no-till fields. Wortmann et al. (2010) reported in their study at the same sites that the effects of tillage were still significant in the top 5 cm of the profile after 5 years at one of the two sites. In another study with different soil types and textures, VandenBygaart and Kay (2004) found significantly decreased C_{org} concentrations in the top 10 cm of the profile 18 months after no-till fields were tilled. However, the lower concentrations

in this study did not translate into decreased C_{org} stocks due to differences in the bulk density. Therefore, based on these results, one-time tillage is likely to result in a decrease in C_{org} in the topsoil in both arable land and grassland. However, without any further tillage, the original C_{org} concentration seems to be restored within a few years both in arable land (VandenBygaart and Kay, 2004) and grassland (this study).

In the G-W-G treatment, the C_{org} concentrations were in the same magnitude as those in the G-G treatment and in general showed a similar behavior. The incorporation of approximately 7 Mg straw ha^{-1} had no effect on C_{org} concentrations. The main reason is most likely to be that the incorporated straw had already been decomposed to a large extent.

4.5.2 Distribution of water-stable aggregates two and five years after tillage

The lower C_{org} concentration in the tilled treatments in trial A (Table 4.1; Table 4.2) may be due to the destruction of aggregates, which has been shown in many studies in arable fields (Tisdall and Oades, 1982; Six et al., 2000a; Kushwaha et al., 2001; Bronick and Lal, 2005; Jacobs et al., 2009). In the G-G treatment significantly lower concentrations of large water-stable macroaggregates were found in the surface soils two years after tillage compared with the control. Simultaneously, significantly more small water-stable macroaggregates (1000 - 250 µm) were found (Figure 4.3).

We hypothesized (Hypothesis 1) that tillage immediately reduces the concentration of water-stable macroaggregates in the soil. Two years after tillage we found a shift from large to small water-stable macroaggregates, but we found no increasing concentrations in water-stable microaggregates or non-aggregated soil. Five years after tillage no differences in water-stable aggregate concentrations were found, which supports Hypothesis 2. In contrast to our results, at two sites Quincke et al. (2007) found the water-stable aggregate distribution for an arable silty clay loam soil (Typic Argiudolls and Mollic Hapludalfs) in the top 5 cm of the soil profile was not different

two years after a one-time tillage event of a no-till field compared with the no-till control. This may be an indication that the effects of one-time or sporadic tillage on water-stable aggregate concentration differ between arable land and grassland, presumably due to the regular cultivation in arable land.

Tillage incorporates the aboveground biomass by turning the soil of the plow layer. This may result in an increased concentration of large water-stable macroaggregates in the lower part of the plow layer. In our study, however, such an increase in the 10 – 25 cm soil layer was not observed.

Our study indicates that tillage mainly decreased the concentration of large water-stable macroaggregates (Figure 4.3). Tillage may disrupt large water-stable macroaggregates by physical impact or reduce their concentration by increasing the decomposition rate of the root system of the tilled-in plants. Large macroaggregates consist of smaller aggregates held together by a network of roots and hyphae (Tisdall and Oades, 1982; Six et al., 2000a). The root biomass increases with time after seeding a grass-clover mixture (Bolinder et al., 2002). Therefore, the root biomass was presumably smaller in the tilled plots two years after tillage than after five years and also smaller than in the permanent grassland. The decomposition of roots after tillage may have also affected macroaggregate stability in grassland soils. We also found a strong positive correlation between the water-stable aggregate class >2000 µm and its root concentration. For instance, in the surface soil layer in trial A we found in 100 g soil 46 g, 19 g and 14 g water-stable aggregates >2000 µm in the permanent grassland, G-G and G-W-G treatment, respectively, and in this water-stable aggregate class 0.55 g, 0.22 g and 0.15 g of roots, respectively. Therefore, roots seem to be very important for the formation of large water-stable macroaggregates. This is in line with the hierarchical model by Tisdall and Oades (1982).

The decreased concentration of large water-stable macroaggregates in trial A due to tillage (Figure 4.3) is in agreement with several other studies. In these studies, smaller aggregates were

found to be more stable than bigger ones and, therefore, less susceptible to disruption by tillage (Christensen, 1986; Cambardella and Elliott, 1994; Six et al., 1999). If tillage had an effect on smaller water-stable aggregates in our study, it must have been of short duration.

The additional incorporation of straw did not lead to greater concentrations of water-stable aggregates of soil in the G-W-G treatment compared with the G-G treatment either two years after tillage or five years after tillage (Figure 4.3). This is in contrast to studies on arable land with different soil types and textures, which reported increased aggregate concentrations due to the incorporation of straw (Christensen, 1986; Duiker and Lal, 1999; Sodhi et al., 2009). Aggregates and organic material interact, as proposed by Six et al. (2000a). According to their model, fLF is encrusted with microbial products and clay particles to form microaggregates, which in turn combine to form macroaggregates (Six et al., 1999). The differences between our results and the above mentioned results in arable land may be attributed to the higher SOM concentrations in grassland than in arable land. Presumably, the fLF to build aggregates is less limited in grassland than in arable land and because of this, the incorporation of organic material had no effect.

In our study, small water-stable macroaggregates (250 – 1000 µm) behaved more like water-stable microaggregates than larger water-stable macroaggregates in both trials in all treatments and soil depths (Table 4.5). First, tillage resulted in a shift from water-stable macroaggregates >1000 µm to water-stable aggregates <1000 µm. Second, while the concentration of LF and the concentration of C_{org} in the bulk soil were positively correlated with water-stable aggregates >1000 µm, the correlation with water-stable aggregates <1000 µm was negative. The same was true for the roots found in large water-stable macroaggregates. This suggests that roots are a dominant factor holding together water-stable aggregates >1000 µm in these grassland soils, while other factors, such as texture, microbial biomass or fungal hyphae, seem to be more important for water-stable aggregates <1000 µm.

However, the wet-sieving procedure may result in a redistribution of water-stable

aggregates. The fast rewetting of aggregates can lead to a confinement of air in aggregates. The resulting buildup of air pressure causes aggregates to slake (Kemper et al., 1985), thus disrupting less stable aggregates and increases the concentration of smaller aggregates.

In our study, concentrations of C_{org} within water-stable aggregates generally increased (with the exception of one class) with increasing water-stable aggregate-size class in all treatments in both trials (Table 4.3). This is in agreement with other studies which focused on aggregates in grassland soils (Cambardella and Elliott, 1993; John et al., 2005). This is also in agreement with the hierarchical model by Tisdall and Oades (1982), which suggests that smaller macroaggregates are bound together by binding agents which contain C_{org}. Thus, C_{org} is expected to increase with increasing aggregate-size class. Other studies focused on the effect of tillage on C_{org} concentrations within aggregate-size classes. Six et al. (2000b) did not find any differences in C_{org} concentrations within aggregate-size classes among no-till and conventional-till soils at four sites with different soil types and textures. However, Oorts et al. (2007) found higher C_{org} concentrations within aggregates in no-till fields compared with conventional-till fields on a Haplic Luvisol in northern France. Thus, reliable generalizations on the effect of tillage on C_{org} concentrations in aggregates are not yet possible.

4.5.3 Distribution of SOM two and five years after tillage

The results of the density fractionation in trial A showed that tillage reduced the fLF concentration in the top 10 cm of the soil profile with no effect on the oLF (Table 4.4). We hypothesized that there is a reduction in LF in soil due to tillage. However, two years after tillage there was only a reduction in fLF visible. Golchin et al. (1997) proposed that occluded organic material with a density of 1.8 g cm^{-3} derives from microaggregates. However, the oLF also derives from macroaggregates, where it acts as a binding component in microaggregates.

Two years after tillage, the concentration of water-stable macroaggregates in the surface

soil layer was lower than in the permanent grassland. In our study the correlations indicate a positive relationship between the LFs and the large water-stable macroaggregates and the medium water-stable macroaggregates and a negative correlation between the LFs and the small water-stable macroaggregates and the water-stable microaggregates.

The additional incorporation of straw in the G-W-G treatment had no effect on the concentration of LF in soil (Table 4.4). This was surprising, as the mass of straw incorporated (approximately 7 Mg ha^{-1}) exceeded the concentration of LF found in the top 40 cm of the two tilled treatments two years after tillage (5.1 and 6.9 Mg ha^{-1} under G-W-G and G-G treatment, respectively). In contrast, the LF under the permanent grassland reached 10.8 Mg ha^{-1}. Therefore, our results suggest that the effect of straw on the LF was no longer detectable less than a year after its incorporation. In wheat straw buried in litter bags Christensen (1985) found a weight loss of 30% and 50% after one month and six months, respectively. In addition, the C to N ratios in fLF and in oLF in our study were 20.7 and 13.9, respectively, with no significant differences between depths and treatments. This is much closer to the 21.3 measured for roots under grassland by van Eekeren et al. (2009), than the C to N ratio of straw which is generally not below 100 (Nieder and Richter, 1989; Gaind et al., 2009). Therefore, it seems likely that the straw was decomposed rapidly and that roots were the main source of LF in these grassland soils.

4.6 Conclusions

In line with our first hypothesis a single tillage operation in grassland has an effect on C_{org} stocks, the concentrations of water-stable aggregates and SOM. However, this effect was restricted to the top 10 cm of the soil profiles. Two years after tillage there were still significant differences between the tilled treatments and the control. Five years after tillage the effects were no longer significant. Hence, the effect of tillage lasts only a few years, supporting our second hypothesis. Based on our results, sporadic tillage of grassland, for example for weed control, should not result

in long-lasting effects on C stocks and aggregate stability.

4.7 Acknowledgements

We would like to thank Anja Sawallisch for technical assistance and anonymous reviewers for their valuable comments. This project is financed by the Deutsche Forschungsgemeinschaft (DFG-Research Training Group 1397 ‘‘Regulation of soil organic matter and nutrient turnover in organic agriculture’’).

4.8 References

Bioland (2012): http://www.bioland.de/bioland/richtlinien.html. Accessed 07.05.2012

Bolinder, M. A., Angers, D. A., Belanger, G., Michaud, R., Laverdiere, M. R. (2002): Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. Canadian Journal of Plant Science 82, 731–737.

Bronick, C. J., Lal, R. (2005): Manuring and rotation effects on soil organic carbon concentration for different aggregate size fractions on two soils in northeastern Ohio, USA. Soil and Tillage Research 81, 239–252.

Cambardella, C. A., Elliott, E. T. (1993): Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071–1076.

Cambardella, C. A., Elliott, E. T. (1994): Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal 58, 123–130.

Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G., Kaiser, K. (2012): Separation of light and heavy organic matter fractions in soil - Testing for proper density cut-off and dispersion level. Geoderma 170, 403-416.

Christensen, B. T. (1985): Wheat and barley straw decomposition under field conditions- Effect of soil type and plant cover on weight loss, nitrogen and potassium content. Soil Biology and Biochemistry 17, 691–697.

Christensen, B. T. (1986): Straw incorporation and soil organic matter in macroaggregates and particle-size separates. Journal of Soil Science 37, 125–135.

Davies, M. G., Smith K. A., Vinten A. J. A. (2001): The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. Biology and Fertility of Soils 33, 423-434.

DIN ISO 11272 (1998): Bodenbeschaffenheit - Bestimmung der Trockenrohdichte, ISO 11272:1998. Beuth Verlag, Berlin, Germany.

DIN ISO 11277 (2002): Bodenbeschaffenheit – Bestimmung der Partikelgrößenverteilung in Mineralböden – Verfahren mittels Siebung und Sedimentation, ISO 11277: 1998/Cor.1:2002. Beuth Verlag, Berlin, Germany.

Djurhuus, J., Olsen, P. (1997): Nitrate leaching after cut grass/clover leys as affected by time of ploughing. Soil Use and Management 13, 61-67.

Duiker, S. W., Lal, R. (1999): Crop residue and tillage effects on carbon sequestration in a Luvisol in central Ohio. Soil and Tillage Research 52, 73–81.

Eekeren, N. van, van Liere, D., Vries, F. de, Rutgers, M., Goede, R. de, Brussaard, L. (2009): A mixture of grass and clover combines the positive effects of both plant species on selected soil biota. Applied Soil Ecology 42, 254–263.

Ellert, B. H., Janzen H. H., McConkey B. G. (2001): Measuring and comparing soil carbon storage, in: Lal R., Kimble J. M., Follet R. F., Stewart B. A. (Eds.), Assessment Methods for Soil Carbon. CRC Press, Boca Raton, FL, pp. 131–146.

Gaind, S., Nain, L., Patel, V. B. (2009): Quality evaluation of co-composted wheat straw, poultry droppings and oil seed cakes. Biodegradation 20, 307–317.

Golchin, A., Baldock, J. A., Oades, J. M. (1997): A model linking organic matter decomposition, chemistry and aggregate dynamics, in: Lal, R., Kimble, J. M., Follett, R. F., Stewart, B. A. (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton, FL, pp. 245–266.

Golchin, A., Oades, J. M., Skjemstad, J. O., Clarke, P. (1994): Study of free and occluded particulate organic matter in soils by solid state 13C CP-MAS NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research 32, 285–309.

Guo, L. B., Gifford, R. M. (2002): Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360.

Jacobs, A., Rauber, R., Ludwig, B. (2009): Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. Soil and Tillage Research 102, 158–164.

Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B. (2010): Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46, 671-680.

John, B., Yamashita, T., Ludwig, B., Flessa, H. (2005): Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128, 63–79.

Kemper, W.D., Rosenau, R., Nelson, S. (1985): Gas displacement and aggregate stability of soils. Soil Science Society of America Journal 49, 25–28.

Kushwaha, C. P., Tripathi, S. K., Singh, K. P. (2001): Soil organic matter and water-stable aggregates under different tillage and residue conditions in a tropical dryland agroecosystem. Applied Soil Ecology 16, 229–241.

Luetzow, M. von, Koegel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. (2006): Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. European Journal of Soil Science 57, 426–445.

Nieder, R., Richter, J. (1989): The significance of wheat straw decomposition with regard to the C-cycle and N-cycle of a cultivated Loess soil. Zeitschrift für Pflanzenernährung und Bodenkunde 152, 415–420.

Oorts, K., Bossuyt, H., Labreuche, J., Merckx, R., Nicolardot, B. (2007): Carbon and nitrogen stocks in relation to organic matter fractions, aggregation and pore size distribution in no-tillage and conventional tillage in northern France. European Journal of Soil Science 58, 248–259.

Quincke, J. A., Wortmann, C. S., Mamo, M., Franti, T., Drijber, R. A., Garcia, J. P. (2007): One-time tillage of no-till systems: Soil physical properties, phosphorus runoff, and crop yield. Agronomy Journal 99, 1104–1110.

Ross, S. M. (1993): Organic matter in tropical soils: current conditions, concerns and prospects for conservation. Progress in Physical Geography 17, 265–305.

SAS Institute (1990): SAS/STAT User´s Guide, Version 6. SAS Institute Inc., Cary, NC, USA.

Schmeer, M., Loges, R., Taube, F. (2009): Der Einfluss von Grünlanderneuerung mit einer eingeschobenen Ackerzwischennutzung auf die Stickstoffflüsse im System Boden-Pflanze. Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau 10, 88-91.

Shepherd, T. G., Saggar, S., Newman, R. H., Ross, C. W., Dando, J. L. (2001): Tillage-induced changes to soil structure and organic carbon fractions in New Zealand soils. Australian Journal of Soil Research 39, 465–489.

Singh, S., Singh, J. S. (1996): Water-stable aggregates and associated organic matter in forest, savanna, and cropland soils of a seasonally dry tropical region, India. Biology and Fertility of Soils 22, 76–82.

Six, J., Elliott, E. T., Paustian, K. (1999): Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358.

Six, J., Elliott, E. T., Paustian, K. (2000a): Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099–2103.

Six, J., Paustian, K., Elliott, E. T., Combrink, C. (2000b): Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681–689.

Sodhi, G. P. S., Beri, V., Benbi, D. K. (2009): Soil aggregation and distribution of carbon and nitrogen in different fractions under long-term application of compost in rice-wheat system. Soil and Tillage Research 103, 412–418.

Soil Survey Staff (2006): Keys to Soil Taxonomy, 10th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Soussana, J. F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T. Arrouays, D. (2004): Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management 20, 219-230.

Tisdall, J. M., Oades, J. M. (1982): Organic matter and water stable aggregates in soils. Journal of Soil Science 33, 141–163.

VandenBygaart, A. J., Kay, B. D. (2004): Persistence of soil organic carbon after plowing a long-term no-till field in southern Ontario, Canada. Soil Science Society of America Journal 68, 1394–1402.

Vertès, F., Hatch, D., Velthof, G., Taube, F., Laurent, F., Loiseau, P., Recous, S. (2007): Short-term and cumulative effects of grassland cultivation on nitrogen and carbon cycling in ley-arable rotations. Grassland Science in Europe 9, 227-246.

Webster, C. P., Poulton, P. R., Goulding, K. W. T. (1999): Nitrogen leaching from winter cereals grown as part of a 5-year ley-arable rotation. European Journal of Agronomy 10, 99-109.

Wortmann, C. S., Drijber, R. A., Franti, T. G. (2010): One-time tillage of no-till crop land five years post-tillage. Agronomy Journal 102, 1302–1307.

Zotarelli, L., Alves, B. J. R., Urquiaga, S., Boddey, R. M., Six, J. (2007): Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols. Soil and Tillage Research 95, 196–206.

5. Effects of tillage and organic fertilization on carbon, nitrogen and phosphorus pools in temperate grassland soils

Deborah Linsler[1], Daniel Geisseler[1], Ralf Loges[2], Friedhelm Taube[2], Bernard Ludwig[1]

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

[2] *Institute of Crop Science and Plant Breeding, Grass and Forage Science/Organic Agriculture, Christian-Albrechts-University of Kiel, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany*

5.1 Abstract

Fertilization and tillage of grassland soils may affect the dynamics of soil carbon, nitrogen and phosphorus, but the temporal dynamics are not completely understood. The objective was to study the effects of tillage and organic fertilization on the organic C (C_{org}) stocks, concentrations of density fractions and water-stable aggregates, basal respiration rates, net N mineralization rates and Olsen-P concentrations in temperate grassland soils. Soil samples were taken north of Kiel, Germany, at three depths five years after the start of the trial. Treatments included permanent grassland (PG) and tillage of a grassland followed by one season of winter wheat and grassland (G-W-G). The plots were split and received either 240 kg N ha^{-1} $year^{-1}$ in the form of cattle slurry (PG_+, G-W-G_+) or no organic fertilization (PG_0, G-W-G_0). In the surface soil (0 – 10 cm) and in the entire soil profile (0 – 40 cm) tillage and one season of winter wheat (G-W-G_+ and G-W-G_0 vs. PG_+ and PG_0) resulted in a trend ($p < 0.1$) of decreasing C_{org} stocks and significantly ($p < 0.05$) lower N_t stocks. However, no differences were found due to organic fertilization (PG_+ and G-W-G_+ vs. PG_0 and G-W-G_0) indicating that four years of slurry application at common rates did not markedly affect total stocks (mean of 73 t C_{org} and 7.4 t N_t ha^{-1} in 5413 t $soil^{-1}$). In the surface soil, however, labile

pools were affected markedly: basal respiration rates were significantly higher (32%) due to organic fertilization in the surface soil and significantly lower (37%) due to tillage and one season of winter wheat. In contrast, the free light fraction decreased significantly 2.2 – 2.5-fold in the top 25 cm soil depths due to organic fertilization, which suggests that microbial activity and therefore decomposition of labile organic material increased. The aggregate distribution was not influenced by fertilization. However, a decrease of 22% in the concentrations of large macroaggregates in the surface soil layer was observed four years after the last tillage event. Net N mineralization rates were not influenced by organic fertilization or by tillage. Organic fertilization led to significantly higher Olsen-P concentrations in the surface soil layer, while tillage had a significant negative effect on Olsen-P concentrations in the deepest soil layer. Overall, this study showed that a period of four years of organic fertilization sufficed to stimulate microbial activity as indicated by increased basal respiration rates and Olsen-P concentrations and decreased light fraction concentration in the surface soil. Tillage-related effects on aggregate distribution and basal respiration were restricted to the surface soil and were more pronounced in the slurry-amended plots than in the plots without slurry.

5.2 Introduction

The effects of organic fertilization on total nitrogen (N_t), organic C (C_{org}) and phosphorus (P) stocks as well as labile stocks in arable soils have been investigated in a number studies and quantitative information has been reported for several long-term experiments (for reviews see, e.g., Edmeades, 2003; Diacono and Montemurro, 2010; Ludwig et al., 2011). Similarly, several studies assessed fertilization effects on total stocks in grassland soils. For instance, Conant et al. (2001) reported in their review that on average 0.3 t C ha^{-1} $year^{-1}$ was sequestered by fertilizing grassland with organic or mineral fertilizers. Moreover, the application of organic fertilizers to grassland resulted in increased biomass production (Lambert et al., 1996), and increased stocks of N and P have also been reported (Hao et al., 2003; Reddy et al., 1999; Singh et al., 2001; Sharpley, 2003).

Less information, however, is available for the effects of an application of manure or slurry on labile stocks in grassland soils. Repeated amendments of organic material increase the labile C pool (Cambardella and Elliott, 1992; Aoyama et al., 1999; Griffin and Porter, 2004) and also increase the microbial biomass and its activity (Gunapala and Scow, 1994; Houot and Chaussod, 1995), which in turn affects mineralization rates, but quantitative data are scarce (Mallory and Griffin, 2007).

Soil aggregate dynamics are affected by the availability of C_{org}, soil texture and landuse. For instance, Guo et al. (2010) investigated bulk density, soil aggregate distribution and stability, and soil organic carbon and total nitrogen associated with soil aggregates in soil after cropland conversion to grassland in the semiarid Loess Plateau and reported that physical properties significantly improved after conversion to grassland. Moreover, it is well known for arable soils that manure plays a basic role in the formation and stabilization of aggregates. This often leads to an enrichment of larger aggregates (Celik et al., 2004; Mikha and Rice, 2004; Mellek et al., 2010). However, less information is available about the effects of organic fertilization on aggregate distribution in grassland soils.

Another practice in grassland, sporadic tillage, may also affect C_{org} stocks and aggregates. Sporadic tillage of grasslands may be done to improve species composition or to control weeds. Tillage kills a large part of the plant biomass, which then becomes a source of soil organic matter (SOM). However, tillage also disrupts aggregates (Bronick and Lal, 2005; Jacobs et al., 2009), and exposes organic matter, making it more susceptible for microbial degradation and mineralization (Bronick and Lal, 2005; Zotarelli et al., 2007). Therefore, tillage generally results in lower C_{org} concentrations (VandenBygaart and Kay, 2004) and concentrations of macroaggregates (Quincke et al., 2007). Furthermore, a tillage event also often leads to losses of the major nutrients, such as N (Davies et al., 2001; Soussana et al., 2004; Vertès et al., 2007).

Recently we had studied the effects of sporadic tillage in grassland soils on C_{org} stocks, aggregates and SOM (Linsler et al., 2013). In the present study, we extended our analysis to the

combined effects of organic fertilization and tillage on the above mentioned parameters. Moreover, we included additional properties such as microbial activity, net N mineralization and Olsen-P concentrations. The objective in the present study was to quantify the effects of tillage and organic fertilization on C, N, and P dynamics for a five-year trial on a loamy sandy grassland soil.

5.3 Material and Methods

5.3.1 Study site

The sampling area is situated north of Kiel (experimental farm Lindhof, 54°27 `N, 9°57 `E), Germany, near the Baltic Sea. The mean annual temperature and precipitation in the area is 8.9 °C and 768 mm, respectively. The soil at the site is heterogeneous. The soil types include Cambisols, Eutric Luvisols, Stagnosols, and Anthrosols (Schmeer et al., 2009). The soil (0 – 40 cm) at the site has a sand, silt and clay concentration of 530, 290 and 180 g kg^{-1}, respectively. The pH (mean and standard error) was 5.6 ± 0.1 and 5.7 ± 0.1 in the plots with and without slurry application, respectively (see treatment description below).

In 1994, arable land at the experimental trial was converted to permanent grassland. Between 1994 and 2005 the grassland was generally cut 1 – 2 times for silage production and grazed 3 – 4 times with cattle each year. In 2005, a field trial was initiated to determine the short- and long-term effects of tillage and fertilization on N fluxes and C storage in grassland soils. The treatments were:

PG: **P**ermanent **g**rassland (since 1994)

G-W-G: **g**rassland – tillage – **w**inter wheat – tillage – **g**rassland

The plots (grassland since 1994) were tilled with a mouldboard plow to a depth of 25 cm in October 2005 and winter wheat (*Triticum aestivum* L. variety Bussard) was sown. After the wheat harvest, the plots were tilled again in September 2006 to incorporate the straw (7.7 and 7.4 t ha^{-1} in the treatment with and without slurry

application, respectively), which was left on the field after grain harvest. Afterwards, a grass clover mixture was sown. The mixture contained 63% perennial ryegrass (*Lolium perenne* L.), 16% timothy-grass (*Phleum pratense* L.), 9% smooth meadow-grass (*Poa pratensis* L.), and 12% white clover (*Trifolium repens* L.).

In early spring 2006, the plots were split in two subplots. One subplot received no organic fertilization (PG_0, G-W-G_0), while the other was fertilized with cattle slurry (PG_+, G-W-G_+) at an annual rate of 240 t N ha^{-1}. Since the composition of slurry varied among the applications, the amount of slurry added had to be adjusted to match 240 kg N ha^{-1} $year^{-1}$ and it ranged from 73 to 117 t fresh matter ha^{-1} $year^{-1}$. The amounts of C and P added with the slurry applications ranged from 1.7 to 2.5 t C ha^{-1} $year^{-1}$ and from 30 to 45 kg P ha^{-1} $year^{-1}$. Slurry was applied in the season with winter wheat three times with 80 kg N ha^{-1} each. The grasslands received slurry four times each year at N rates of 80, 60, 60, and 40 kg N ha^{-1} applied to each cut.

Tillage (in the treatments G-W-G_+ and G-W-G_0) and organic fertilization (in the treatments PG_+ and G-W-G_+) may affect C and N dynamics directly (translocation of soil, aeration, destruction of macroaggregates, addition of organic matter), but also indirectly (plant species composition with differing biomass yields and thus root inputs and different decomposability). For instance, S. Chen (pers. comm.) reported that in adjacent plots of the same field trial, clover accounted for 29% of the aboveground biomass in a fertilized 17 years old permanent grassland (corresponding to PG_+) and for 13% in a fertilized 5 years old grassland (similar to G-W-G_+, tillage in 2005, however, no winter wheat was included). In the unfertilized plots with 17 years old grassland (corresponding to PG_0) and 5 years old grassland (similar to G-W-G_0) clover accounted for 23 and 31% of the biomass, respectively. A reason for that difference may be that grasses are favored when a higher content of N is available. This effect was even more pronounced in the G-W-G_+ treatment than in the PG_+ treatment, where the grasses in the resown grass-clover mixture developed better than the clover.

Each year, the grassland is generally cut four times for forage production (Schmeer et al., 2009). In 2007 and 2009, the field trial was limed with 450 kg CaO ha^{-1} $year^{-1}$ and 30 kg MgO ha^{-1}

year^{-1}. In the same years, potassium sulfate with magnesium and rock phosphate were applied to all treatments. These applications amounted to 100 kg K ha^{-1}, 24 kg Mg ha^{-1}, 68 kg S ha^{-1}, and 45 kg P ha^{-1} in each of those years. The site has been managed organically since 1993 according to the guidelines of the organic association “Bioland” (Bioland, 2012).

5.3.2 Sampling procedure

Soil was sampled in April 2010. The treatments, each with three replicates, are arranged in a randomized plot design (plot size 6 x 18 m). In each plot, four cores were taken and combined to a composite sample. The samples were taken at least 50 cm away from the border to the next plot at three depths (0 – 10 cm, 10 – 25 cm and 25 – 40 cm). The samples were stored at 4 °C before processing.

5.3.3 Short-term incubation experiment

Labile C and N were determined using a short-term incubation experiment. Unfortunately, no standardized procedure for aerobic incubation experiments exists (Khanna et al., 2001) and incubation parameters may vary widely. For instance, Chang and Trofymow (1996) used an incubation period of 3 hours, whereas Gamboa and Galicia (2011) incubated samples for 90 days, and Kotzerke et al. (2011) employed an incubation temperature of 20 °C, whereas Yang et al. used 30 °C. In the present experiment, we used an incubation period of 14 days and an incubation temperature of 20 °C.

The incubation experiment was carried out with samples from all treatments and soil depths. Soil samples were passed through a 2 mm sieve prior to the incubation. Twenty grams of field-moist soil were placed in a 120 mL plastic beaker. Distilled water was added to bring the soil moisture to 60% of its water holding capacity (Linn and Doran, 1984). The plastic beakers were then placed into 1.5 L glass jars and the soil was incubated in a climate cabinet (Kühlbrutschrank ICP 800, Memmert, Schwabach, Germany). Before and after the incubation, inorganic N (NO_3 – N

and NH_4 – N) were extracted from the soil with 0.5 M K_2SO_4 (4 mL of solution g^{-1} of soil) (Kuderna et al., 1993) and determined on a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria). Net N mineralization was calculated by subtracting the initial inorganic N from the inorganic N present at the end of the incubation. The basal respiration rates were measured according to Isermeyer (1952) by trapping the CO_2 in 10 mL of 1 M NaOH. The traps were replaced after 7 days. The CO_2 evolved was determined by titrating the solution to pH 8.3 with 1 M HCl after the addition of 5 mL of 0.5 M $BaCl_2$.

5.3.4 Olsen-Phosphorus

Olsen-P concentrations were measured on 1 g field-moist soil. The soil was shaken with 20 mL extractant solution for 30 min with 200 rotations per minute (SM-30, Edmund Bühler, Hechingen, Germany) and the resulting solution was filtered (Whatman 595 1/2). The extractant solution was 0.5 M $NaHCO_3$, adjusted to pH 8.5 with 10 M NaOH (Olsen et al., 1954). The P concentration in the extracts was determined with the method described by Murphy and Riley (1962) and Watanabe and Olsen (1965).

5.3.5 Aggregate fractionation

Soil aggregates were fractionated with a wet-sieving procedure described by Cambardella and Elliott (1993) and slightly modified by Linsler et al. (2013). Approximately 100 g of air-dried soil were put on a 2000 µm sieve and submerged into distilled water for 10 min. Afterwards the sieve was lifted out of the water and submerged again. This step was repeated 50 times. Soil aggregates retained on the sieve were collected, dried, and weighed. Aggregates, which passed the 2000 µm 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. The silt and clay together with very small microaggregates passing through the 53 µm sieve were

precipitated with a 0.5 M $AlCl_3$ solution (5 mL for 2 L of suspension). To recover the <53 µm class after precipitation, the water was siphoned off and the deposit was filtered, dried, and weighed.

Since a marked operator variability was found for this method (Jacobs et al., 2010), aggregates were fractionated by a single person in the present study.

5.3.6 Density fractionation

A scheme of density fractionation (Golchin et al. 1994), modified by Linsler et al. (2013), was used. Briefly, 8 g of field-moist soil (gently sieved with 5 mm mesh size) were placed in a centrifugation tube and 30 mL of a sodium polytungstate solution (SPT; density of 1.8 g cm^{-3}) was added. The tube was then inverted 5 times and centrifuged (Multifuge 3 S-R, Heraeus, Hanau, Germany). Afterwards, the supernatant with floating particles was vacuum filtered (<0.45 µm) and washed with distilled water. To obtain the occluded light fraction (oLF), 30 mL of SPT solution and ten glass balls were added to the pellet and the tubes were shaken (SM-30, Edmund Bühler, Hechingen, Germany) to destroy the aggregates. Afterwards, the supernatant was filtered and washed as described above. The remaining mineral fraction (MF) in the tube was separated by wet sieving in a sand ($MF_{>53\ µm}$) and silt plus clay fraction ($MF_{<53\ µm}$). The $MF_{>53\ µm}$ was filtered immediately. The $MF_{<53\ µm}$ was transferred to a beaker with 1.5 L of distilled water and precipitated with 3.75 mL of a 0.5 M $AlCl_3$ solution. After precipitation, the water was carefully siphoned off and the $MF_{<53\ µm}$ was vacuum filtered. All fractions were dried and weighed.

5.3.7 Analytics and soil characterization

The bulk soil, the aggregate classes and the MFs of the density fractionation were ball-milled (MM 200, Retsch, Haan, Germany). Total C (C_t) and N (N_t) concentrations in all samples were determined by dry combustion on a CN elemental analyzer (Elementar Vario El, Heraeus, Hanau, Germany). C_t corresponds to C_{org}, because no carbonates were detectable.

Soil pH was determined in a 0.01 M $CaCl_2$ solution (2.5 mL of solution g^{-1} of soil). Soil texture was

determined according to DIN ISO 11277 (2002) and bulk density according to DIN ISO 11272 (1998).

5.3.8 Calculations and statistical analyses

The C_{org} mass per unit area was calculated for an equivalent mass of soil to account for differences in bulk density. The data are given in t C_{org} ha^{-1} in 1149 t and 5413 t soil in the surface soil (0 – 10 cm) and in the entire soil profile (0 – 40 cm), respectively. For the calculation of C_{org} stocks, we subtracted the mass of stones (>2 mm) from the total mass of the volumetric samples (Ellert et al., 2001).

Organic C concentrations in aggregate classes were expressed on a sand-free basis. They were calculated as described by John et al. (2005):

Concentration of sand-free C_{org} (g kg^{-1})

= C_{org} concentration (g kg^{-1}) / (1 - sand proportion (g g^{-1}))

Concentrations of aggregate classes were not corrected for the sand contents, since sand particles were found to be parts of macro aggregates (e.g., Yang and Wander, 1998; John et al., 2005 and own observations).

Statistical analyses were carried out with the SAS program (SAS Institute, 1990). The data sets were analyzed with one-way ANOVAs. For the C_{org} and N_t stocks, basal respiration rates, net N mineralization rate and Olsen-P concentrations we first analyzed the four treatments separately. For all collected data, we determined the effect of tillage by comparing the tilled (G-W-G_+, G-W-G_0) and untilled (PG_+, PG_0) treatments. For the determination of the effect of organic fertilization we compared the treatments with regular slurry applications (PG_+, G-W-G_+) and without slurry applications (PG_0, G-W-G_0). The different soil depths were analyzed separately. Normality of the residuals was tested with the Shapiro-Wilk test and homogeneity of variances with Levene´s test. If necessary the data were transformed. The Tukey test was used for mean comparisons. Effects were considered significant for $p \leq 0.05$. Trends were also reported ($0.05 < p \leq 0.1$).

5.4 Results and discussion

5.4.1 Effects of organic fertilization and tillage on C_{org} and N_t stocks

There were no significant differences among the treatments in C_{org} and N_t stocks (calculated on an equivalent mass of soil) in the surface soil layer (0- ~10 cm) and the soil profile (0- ~40 cm), when the four treatments were analyzed separately (Table 5.1). This indicates that the fertilization period was too short to see differences and that the last tillage event four years before sampling did not lead to long lasting marked negative effect on C_{org} and N_t stocks.

A comparison of plots with slurry application and plots without slurry application indicated that organic fertilization (total application of 960 kg N ha^{-1} and approximately 8 t C ha^{-1} in 4 years) had no effect on C_{org} and N_t stocks in the surface soil and in the soil profile (Table 5.1). However, in the 10 – 25 cm soil layer, there was a trend ($p = 0.09$) of higher C_{org} stocks due to fertilization (data not shown). Conant et al. (2001) summarized in their review that C was sequestered on average at a rate of 0.3 t C ha^{-1} $year^{-1}$ in grassland soils fertilized with mineral fertilizer or organic fertilizer. In the sampled plots, variability of C_{org} and N_t stocks were too high to significantly detect expected increases of 1.5 t C ha^{-1}.

Tillage (carried out in October 2005 and September 2006) led to a trend of lower C_{org} stocks and significantly lower N_t stocks in the surface soil layer and in the entire soil profile (0 – 40 cm, Table 5.1). The decrease of C_{org} and N_t stocks after tillage may be caused by the death of the plant biomass and subsequent decomposition and by lower root biomass in younger grasslands than in older ones (Bolinder et al., 2002). The difference between tilled and no-tilled plots was greater in the plots with organic fertilization. Presumably the re-established plants in the plots with slurry application received enough nutrients with the slurry in the surface soil layer, so that less roots grew into deeper soil layers, reducing the C_{org} and N_t input into the 25 – 40 cm soil layer.

Table 5.1: C_{org} stocks [t ha^{-1}] calculated on an equivalent mass of soil among different treatments (PG: permanent grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland; +: with slurry application); 0: without slurry application) and soil depths; mean values and standard errors (n = 3).

	Depth	Stocks (t ha^{-1} in 1149 t soil)		Depth	Stocks (t ha^{-1} in 5413 t soil)	
		C_{org}	N_t		C_{org}	N_t
PG_+	0 - 10.0 cm	25.9 (1.2)	2.5 (0.1)	0 - 40.0 cm	80.0 (6.1)	8.2 (0.6)
GWG_+	0 - 8.8 cm	22.6 (0.7)	2.2 (0.1)	0 - 37.3 cm	67.9 (4.3)	6.9 (0.5)
[a] PG_0	0 - 10.0 cm	25.2 (3.5)	2.4 (0.3)	0 - 39.7 cm	77.6 (6.3)	7.8 (0.6)
[a] GWG_0	0 - 9.1 cm	19.7 (1.2)	1.9 (0.1)	0 - 37.9 cm	66.5 (3.5)	6.7 (0.3)
[b] Slurry		ns	ns		ns	ns
[b] Tillage		0.05	0.04		0.06	0.03

[a] Data for PG_0 and G-W-G_0 are taken from Linsler et al. (2013) and are recalculated for 1149 and 5413 t soil.
[b] Probability values of a two way ANOVA of tillage and slurry application; ns: not significant.

5.4.2 Effects of organic fertilization and tillage on basal respiration and light fraction C

Labile C responded markedly to the organic fertilization (PG_+, G-W-G_+: the last slurry application was carried out approximately 7 months prior to sampling) (Figure 5.1). In the surface soil layer, basal respiration rates were significantly higher in the PG_+ treatment than in the other three treatments and also in the slurry-fertilized treatments compared to the treatments without slurry (Figure 5.1). The higher rates in the plots with slurry application compared with the unfertilized treatments was due to the presence of readily available C and N in the slurry, which stimulates the soil microbial biomass (Mallory and Griffin, 2007). Similarly, Hopkins and Shiel (1996) found in a grassland soil (Typic Ochraqualf) an increase of about 15% in basal respiration rates in soils fertilized for about 100 years with 20 t ha^{-1} $year^{-1}$ farmyard manure in comparison with the unfertilized control.

Organic fertilization significantly lowered fLF concentrations, both in the surface soil and in 10 – 25 cm soil depth (Table 5.2). In contrast, organic fertilization had no effect on the oLF and MF fractions. The application of C in slurry can promote and increase the size and activity of microbial

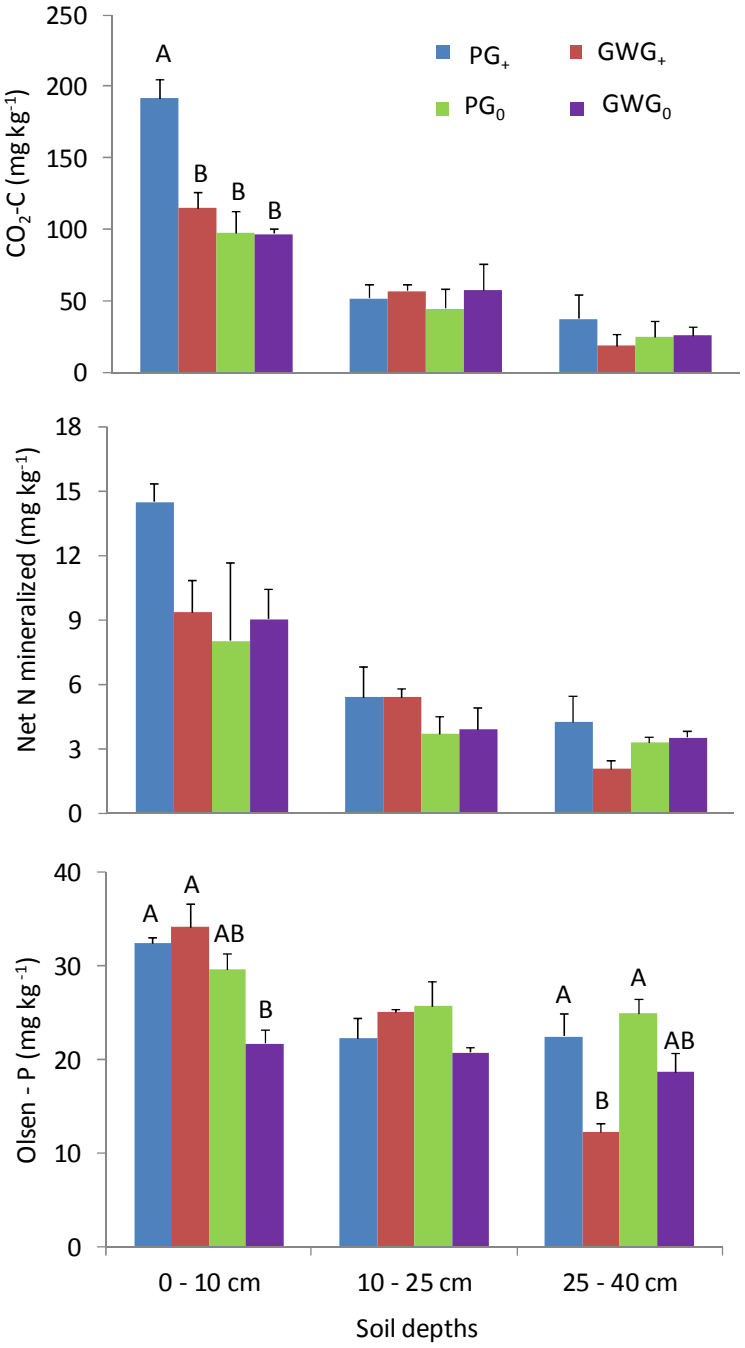
biomass (Hatch et al., 2000). Presumably the higher activity of microbial biomass led to increased mineralization of fLF.

Application of slurry led to significant higher C_{org} concentrations in the fLF in the soil layer 10 – 25 cm (Table 5.2). However, due to significantly lower concentrations of fLF in the fertilized plots the C_{org} stored in fLf in the top 25 cm soil depths were much higher in the plots without slurry (990 kg ha^{-1}) in comparison with the fertilized plots (540 kg ha^{-1}), whereas the C_{org} stored in LF (sum of fLF and oLF) was similar in the fertilized (8.9 t ha^{-1}) and unfertilized (8.6 t ha^{-1}) plots. In long-term fertilization studies in arable land the C_{org} concentrations stored in the LF increased with increasing fertilizer rate in varying soil types and textures (Gong et al., 2009; Heitkamp et al., 2011). This indicates that the fertilization period in our study was too short to see differences in C_{org} stored in LF or that grassland do not react or react slower than arable land in this context.

Table 5.2: Concentrations of density fractions (g kg^{-1}) and C_{org} concentrations in density fractions (g kg^{-1}) in plots with slurry (PG_+, G-W-G_+), without slurry (PG_0, G-W-G_0), tilled plots (G-W-G_+, G-W-G_0) and permanent grassland plots (PG_+, PG_0) (PG: permanent grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland; +: with slurry application; 0: without slurry application) in different soil depths; mean values and standard errors (n = 3).

	Concentrations of density fractions (g kg^{-1})		C_{org} concentrations in density fractions (g kg^{-1})	
	0-10 cm	10-25 cm	0-10 cm	10-25 cm
free light fraction				
Plots with slurry	1.1 (0.2)	0.3 (0.1)	265 (14)	223 (5)
Plots without slurry	2.8 (0.3)	0.7 (0.2)	300 (3)	299 (15)
Tilled plots	1.6 (0.2)	0.6 (0.2)	298 (4)	272 (16)
Permanent grassland	2.7 (0.6)	0.5 (0.1)	264 (17)	250 (11)
[a] Slurry	0.01	0.04	ns	<0.01
[a] Tillage	ns	ns	ns	ns
occluded light fraction				
Plots with slurry	19 (1)	9 (1)	216 (13)	213 (6)
Plots without slurry	19 (2)	12 (1)	245 (9)	223 (3)
Tilled plots	17 (2)	10 (1)	227 (22)	227 (13)
Permanent grassland	21 (2)	11 (1)	224 (15)	209 (17)
[a] Slurry	ns	0.09	ns	ns
[a] Tillage	0.06	ns	ns	ns
Mineral fraction >53				
Plots with slurry	498 (22)	527 (11)	2.8 (0.2)	1.8 (0.1)
Plots without slurry	531 (13)	534 (9)	2.7 (0.1)	1.9 (0.1)
Tilled plots	508 (22)	508 (21)	2.4 (0.3)	2.0 (0.3)
Permanent grassland	541 (25)	553 (21)	3.1 (0.2)	1.6 (0.1)
[a] Slurry	ns	ns	ns	ns
[a] Tillage	ns	0.07	0.02	ns
Mineral fraction <53				
Plots with slurry	488 (22)	464 (11)	29 (2)	23 (1)
Plots without slurry	444 (18)	452 (7)	26 (2)	24 (2)
Tilled plots	481 (20)	479 (24)	23 (0)	22 (2)
Permanent grassland	429 (29)	438 (23)	34 (3)	25 (2)
[a] Slurry	ns	ns	ns	ns
[a] Tillage	ns	ns	0.01	ns

Data for PG_0 and G-W-G_0 are taken from Linsler et al. (2013).
[a] Probability values of a two way ANOVA of tillage and slurry application; ns: not significant.



	0-10 cm	10-25 cm	25-40 cm
Slurry	<0.01	ns	ns
Tillage	0.01	ns	ns

	0-10 cm	10-25 cm	25-40 cm
Slurry	ns	ns	ns
Tillage	ns	ns	ns

	0-10 cm	10-25 cm	25-40 cm
Slurry	<0.01	ns	0.04
Tillage	ns	ns	<0.01

Figure 5.1: Basal respiration rates (mg CO_2-C kg^{-1}), net N mineralization rates (mg N kg^{-1}) and Olsen-P concentrations (mg P kg^{-1}) among different treatments (PG: permanent grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland; +: with slurry application; 0: without slurry application) and soil depths The tables shows the probability values of a two way ANOVA of tillage and slurry application in different soil depths; ns: not significant; mean values and standard errors (n = 3).

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

There was a significant decrease in basal respiration rates in the surface soil layer of the tilled treatments compared to the permanent grasslands (Figure 5.1). This effect was mainly due to the big difference between the PG_+ and G-W-G_+ treatment (191 and 114 mg CO_2 kg^{-1} soil, respectively) and the main reason was presumably due to the different plant species' composition in the treatments (described in section 2.1). Clover and perennial ryegrass differ in their C to N ratios (18 – 22 and 52 – 53, respectively) (Kumar and Goh, 2002). Plant residues with lower C to N ratios generally decompose faster (Moorhead et al., 1998), which may have resulted in higher decomposition rates in the PG_+ treatment compared to the G-W-G_+ treatment in the incubation experiment.

Four years after the last tillage event, only small effects of tillage on density fractions were still visible (Table 5.2). This indicates that the incorporated straw had been completely decomposed and the re-sown grassland had restored the pre-tillage SOM contents and LF concentrations.

5.4.3 Effects of organic fertilization and tillage on the distribution of aggregates

The application of slurry had no significant effects on the concentration of aggregates (Table 5.3). This was in contrast with several studies in arable land, which found a positive effect of slurry or manure applications on the concentration and size of aggregates (Celik et al., 2004; Mikha and Rice, 2004; Mellek et al., 2010). The fertilization periods in these studies (2 – 9 years) were in the same magnitude than in our study (4 years). This suggests that grassland soils react slower to the amendments of organic fertilizers in comparison to arable soils.

There were significant higher C_{org} concentrations in the medium macroaggregates in the top 10 cm soil depth in the plots with slurry application (Table 5.3). In studies on arable land, manure application significantly increased the C_{org} concentration in macroaggregates and in microaggregates (Mikha and Rice, 2004; Gulde et al., 2008). Our results suggest that slurry applications to grassland soils have similar effects.

Table 5.3: Concentrations of aggregate classes (g (100 g)$^{-1}$) and C_{org} concentrations in aggregate classes (g kg^{-1}) calculated on a sand-free basis in plots with slurry (PG_+, G-W-G_+), without slurry (PG_0, G-W-G_0), tilled plots (G-W-G_+, G-W-G_0) and permanent grassland plots (PG_+, PG_0) (PG: permanent grassland; G-W-G: grassland – tillage – winter wheat – tillage – grassland; +: with slurry application; 0: without slurry application) in different soil depths; mean values and standard errors (n = 3).

	Concentrations of aggregate classes (g (100 g)$^{-1}$)			C_{org} concentrations in aggregate classes (g kg^{-1})		
	0-10 cm	10-25 cm	25-40 cm	0-10 cm	10-25 cm	25-40 cm
>2000 µm						
Plots with slurry	60 (2)	17 (4)	7.4 (1.1)	59 (5)	32 (2)	21 (3)
Plots without slurry	50 (4)	13 (2)	6.5 (1.1)	55 (5)	31 (1)	24 (4)
Tilled plots	48 (6)	17 (4)	7.0 (1.6)	50 (4)	30 (2)	19 (5)
Permanent grassland	61 (2)	13 (1)	6.9 (1.7)	65 (3)	32 (3)	26 (3)
[a] Slurry	ns	ns	ns	ns	ns	ns
[a] Tillage	0.03	ns	ns	0.03	ns	ns
1000-2000 µm						
Plots with slurry	3.9 (0.5)	2.9 (0.4)	3.1 (0.3)	71 (5)	44 (6)	25 (4)
Plots without slurry	5.7 (1.1)	3.3 (0.7)	2.7 (0.1)	59 (2)	48 (3)	48 (7)
Tilled plots	3.9 (0.4)	2.8 (0.1)	2.5 (0.2)	64 (4)	46 (2)	32 (4)
Permanent grassland	5.7 (1.5)	3.4 (0.4)	3.2 (0.2)	66 (2)	46 (5)	41 (6)
[a] Slurry	ns	ns	ns	0.01	ns	<0.01
[a] Tillage	ns	ns	ns	ns	ns	ns
250-1000 µm						
Plots with slurry	21 (1)	51 (3)	54 (3)	48 (2)	30 (2)	25 (2)
Plots without slurry	28 (2)	56 (3)	62 (1)	43 (2)	29 (2)	27 (2)
Tilled plots	29 (3)	53 (5)	54 (4)	42 (0)	30 (0)	22 (2)
Permanent grassland	20 (2)	54 (1)	62 (1)	49 (1)	30 (1)	30 (2)
[a] Slurry	ns	ns	ns	ns	ns	ns
[a] Tillage	0.03	ns	ns	0.09	ns	<0.01
53-250 µm						
Plots with slurry	12 (1)	24 (1.6)	29 (3)	30 (1)	27 (0)	19 (2)
Plots without slurry	13 (1)	20 (1.2)	24 (2)	30 (1)	28 (1)	20 (1)
Tilled plots	15 (3)	22 (1.6)	29 (3)	29 (1)	27 (1)	16 (2)
Permanent grassland	10 (1)	22 (1.8)	23 (2)	30 (1)	28 (1)	23 (1)
[a] Slurry	ns	ns	ns	ns	ns	ns
[a] Tillage	0.04	ns	ns	ns	ns	<0.01
<53 µm						
Plots with slurry	2.4 (0.3)	5.0 (0.4)	6.7 (1.3)	21 (1)	18 (1)	14 (2)
Plots without slurry	2.7 (0.2)	4.6 (0.3)	5.5 (0.3)	20 (1)	19 (0)	15 (1)
Tilled plots	3.2 (0.5)	5.0 (0.2)	7.2 (1.2)	19 (1)	18 (1)	11 (2)
Permanent grassland	1.9 (0.4)	4.6 (0.6)	5.0 (0.4)	22 (1)	20 (2)	18 (2)
[a] Slurry	ns	ns	ns	ns	ns	ns
[a] Tillage	0.03	ns	ns	0.03	ns	<0.01

Data for PG_0 and G-W-G_0 are taken from Linsler et al. (2013).
[a] Probability values of a two way ANOVA of tillage and slurry application; ns: not significant.

In the surface soil layer, tillage resulted in a significant decrease in the concentration of large macroaggregates and a significant increase in the concentrations of small macroaggregates, microaggregates and the <53 µm class (Table 5.3). The difference between the tilled plots and the permanent grasslands were bigger in the plots with slurry application than in the plots without slurry application. For arable soils different results of occasional tillage on aggregate concentrations were reported. Whereas in one study in arable land no differences in aggregate distribution were reported two years after a one-time tillage event of no-till fields (Quincke et al., 2007), in another study the mean weight diameter of aggregates was decreased significantly three years after a one-time tillage event in no-till fields (Stavi et al., 2011).

Tillage had a negative effect on C_{org} concentrations in the large macroaggregates, the small macroaggregates and the <53 µm class in the surface soil layer and on the three smallest size classes in the deepest soil layer (Table 5.3). For arable soils, there were higher values in no-till soils reported (Oorts et al., 2007). Other studies, however, found that C_{org} concentrations in aggregate classes were not influenced by tillage (Six et al., 2000b). Thus, generalizations on the effect of tillage on C_{org} concentrations in aggregates are not yet possible.

5.4.4 Effects of organic fertilization and tillage on Olsen-P concentrations

The application of slurry led to significantly higher Olsen-P concentrations in the surface soil (Figure 5.1). An increase of Olsen-P concentration due to fertilization was reported for arable soils after 3 – 6 years of manure application (Reddy et al., 1999; Shepherd and Withers, 1999; Singh et al., 2001). Our results suggest that a similar fertilization period (4 years) sufficed to affect also grassland soils in the surface soil layer. In this layer was an increase due to slurry amendments on average of 33% observed. However, the slurry application had no effect on the 10 – 25 cm soil layer and even led to significantly lower Olsen-P concentrations in the deepest soil layer (25 – 40 cm) (Figure 1). The lower concentrations in the deepest soil layer were mainly due to 52 - 103% lower Olsen-P concentrations in the G-W-G_+ treatment in comparison to the other three treatments.

In this plots the re-established plants presumably received enough nutrients with the slurry in the upper soil layer. Hence, presumably less roots grew in deeper soil layers and served as input of organic material and Olsen-P.

Tillage and one season of winter wheat led to significantly lower Olsen-P concentrations in the deepest soil layer (25 - 40 cm, Figure 5.1). Heyland et al. (1996) reported that P contents in wheat straw, wheat grains and grass-clover are approximately 1.7, 3.5 and 3.1 kg P t yield^{-1}. A comparison of the P exports in the year in which winter wheat was cultivated (wheat grains (25 kg P ha^{-1}), harvested grass-clover (25 kg P ha^{-1}) and re-distribution (wheat straw in the plow layer, 12 kg P ha^{-1}) suggests that uptake of labile P by winter wheat from the surface and subsoil and subsequent removal (grains) and re-distribution of P due to the mixing of straw in the plowing horizon may have partly contributed to a depletion of Olsen-P concentrations in the 25 - 40 cm layer.

5.4.5 Effects of organic fertilization and tillage on net N mineralization rates

The net N mineralization rates showed a similar behavior as the basal respiration rates. However, neither tillage nor organic fertilization had a significant effect on net N mineralization rates (Figure 5.1). This is in contrast to other studies, where higher net N mineralization rates were reported due to mineral fertilization or organic manure addition (Gill et al., 1995; Hatch et al., 2000; Gong et al., 2011). However, the fertilization periods in these studies cited were much longer (12 – 18 years), suggesting that four years of slurry application in our study did not affect N dynamics sufficiently to obtain significant differences.

5.5 Conclusions

Four years of cattle slurry application resulted in significantly higher microbial activity, Olsen-P concentrations and significantly lower LF concentrations. The growing of one season of winter wheat and the last tillage four years before sampling had opposite effects on basal respiration rates

and Olsen-P concentration and also resulted in significant lower N_t stocks and concentrations of large macroaggregates. As tillage and organic fertilization also influenced the species composition in these grassland soils, generalizations on the direct effect of tillage and organic fertilization in grassland soils are not yet possible.

5.6 Acknowledgements

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

5.7 References

Aoyama, M., D.A. Angers, N’Dayegamiye, A. (1999): Particulate and mineral-associated organic matter in water-stable aggregates as affected by mineral fertilizer and manure applications. Canadian Journal of Soil Science 79, 295–302.

Bolinder M.A., Angers D.A., Belanger G., Michaud R., Laverdiere M.R. (2002): Root biomass and shoot to root ratios of perennial forage crops in eastern Canada Canadian Journal of Soil Science 82, 731–737.

Bronick, C.J, Lal, R. (2005): Manuring and rotation effects on soil organic carbon concentration for different aggregate size fractions on two soils in northeastern Ohio, USA. Soil and Tillage Research 81, 239–252.

Cambardella, C.A., Elliott, E.T. (1993): Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071–1076.

Cambardella, C.A., Elliott, E.T. (1994): Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal 58: 123–130.

Celik, I., Ortas, I., Kilic, S. (2004): Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. Soil and Tillage Research 78, 59–67.

Chang, S.X., Trofymow, J.A. (1996): Microbial respiration and biomass (substrate-induced respiration) in soils of old-growth and regenerating forests on northern Vancouver Island, British Columbia. Biology and Fertility of Soils 23, 145-152.

Christensen, B.T. (1986): Straw incorporation and soil organic matter in macroaggregates and particle-size separates. Journal of Soil Science 37, 125–135.

Conant, R.T., Paustian, K., Elliott E.T. (2001): Grassland management and conversion into grassland: Effects on soil carbon. Ecological Applications 11, 343–355.

Davies, M.G., Smith, K.A., Vinten, A.J. (2001): The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. Biology and Fertility of Soils 33, 423–434.

Diacono, M., Montemurro, F. (2010): Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30: 401-322.

DIN ISO 11272 (1998): Bodenbeschaffenheit - Bestimmung der Trockenrohdichte, ISO 11272: Beuth Verlag Berlin, Germany.

DIN ISO 11277 (2002): Bodenbeschaffenheit – Bestimmung der Partikelgrößenverteilung in Mineralböden – Verfahren mittels Siebung und Sedimentation, ISO 11277: 1998/Cor.1:2002. Beuth Verlag Berlin, Germany.

Edmeades, D.C. (2003): The long-term effects of manures and fertilisers on soil productivity andquality: a review. Nutrient Cycling in Agroecosystems 66, 165–180.

Ellert, B. H., Janzen H. H., McConkey B. G., (2001): Measuring and comparing soil carbon storage, in: Lal R., Kimble J. M., Follet R. F., Stewart B. A. (Eds.), Assessment Methods for Soil Carbon. CRC Press, Boca Raton, FL, pp. 131–146.

Gamboa, A.M., Galicia, L. (2011): Differential influence of land use/cover change on topsoil carbon and microbial activity in low-latitude temperate forests. Agriculture, Ecosystems and Environment 142, 280–290.

Gill, K., Jarvis, S.C., Hatch, D.J. (1995): Mineralization of nitrogen in long-term pasture soils – Effects of management. Plant and Soil 172, 153–162.

Golchin, A., Oades, J. M., Skjemstad, J. O., Clarke, P. (1994): Study of free and occluded particulate organic matter in soils by solid state 13C CP-MAS NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research 32, 285–309.

Gong, W., Yan, X., Wang, J., Hu, T., Gong, Y. (2009): Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat-maize cropping system in northern China. Geoderma 149, 318–324.

Gong, W., Yan, X., Wang, J., Hu, T., Gong, Y. (2011): Long-term applications of chemical and organic fertilizers on plant-available nitrogen pools and nitrogen management index. Biology and Fertility of Soils 47, 767–775.

Griffin, T.S., Porter, G.A. (2004): Altering soil carbon and nitrogen stocks in intensively tilled two-year rotations. Biology and Fertility of Soils 39, 366–374.

Gulde, S., Chung, H., Amelung, W., Chang, C., Six, J. (2008): Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Science Society of America Journal 72, 605–612.

Gunapala, N., Scow, K. (1998): Dynamics of soil microbial biomass and activity in conventional and organic farming systems. Soil Biology and Biochemistry 30, 805–816.

Guo, Z.B., Yan, G.J., Zhang, R.H., Li, F.M., Zeng, Z.X., Liu, H. (2010): Improvement of soil physical properties and aggregate-associated C, N, and P after cropland was converted to grassland in semiarid loess plateau. Soil Science 175, 99-104.

Hao, X.Y., Chang, C., Travis, G.R., Zhang, F.R. (2003): Soil carbon and nitrogen response to 25 annual cattle manure applications. Journal of Plant Nutrition and Soil Science 166, 239–245.

Hatch, D.J., Lovell, R.D., Antil, R.S., Jarvis, S.C., Owen, P.M. (2000): Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. Biology and Fertility of Soils 30, 288–293.

Heitkamp, F., Raupp, J., Ludwig, B. (2011): Effects of fertilizer type and rate on labile soil fractions of a sandy Cambisol-long-term and short-term dynamics. Journal of Plant Nutrition and Soil Science 174, 121–127.

Heyland, K-U., Aldag, R., Braun, H., Brugger, G., Paul, V.H., Ripke, F.O., Schmid, R., Schulze, R., Spielhaus, G. (1996): Allgemeiner Pflanzenbau. Eugen Ulmer Verlag, Stuttgart, Germany.

Hopkins, D.W., Shiel, R.S. (1996): Size and activity of soil microbial communities in long-term experimental grassland plots treated with manure and inorganic fertilizers. Biology and Fertility of Soils 22: 66–70.

Houot, S., Chaussod, R. (1995): Impact of agricultural practices on the size and activity of the microbial biomass in a long-term field experiment. Biology and Fertility of Soils 19, 309–316.

Isermeyer, H. (1952) Eine einfache Methode zur Bestimmung der Bodenatmung und der Karbonate im Boden. Zeitschrift für Pflanzenernährung und Bodenkunde 56, 26–38.

Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B. (2010): Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46, 671–680.

Jacobs, A., Rauber, R., Ludwig, B. (2009): Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. Soil and Tillage Research 102, 158–164.

John, B., Yamashita, T., Ludwig, B., Flessa, H. (2005): Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128, 63–79.

Kotzerke, A., Fulle, M., Sharma, S., Kleineidam, K., Welzl, G., Lamshoeft, M., Schloter, M., Wilke, B-M. (2011): Alterations in total microbial activity and nitrification rates in soil due to amoxicillin-spiked pig manure. Journal of Plant Nutrition and Soil Science 174, 56–64.

Khanna, P.K., Ludwig, B., Bauhus, J., O'Hara, C. (2001): Assessment in the oak lysimeter. and significance of labile organic C pools in forest soils. p. 167–182. In: R. Lal, J.M. Kimble, R.F. Follett, and B.A. Stewart (ed.) Assessment methods for soil carbon. CRC Press, Boca Raton, FL.

Kuderna, M., Potsch, E., Blum, W.E. (1993): Effect of extractants on the results of Nmin determination. Bodenkultur 44, 7–14.

Kumar, K., Goh, K.M. (2002): Management practices of antecedent leguminous and non-leguminous crop residues in relation to winter wheat yields, nitrogen uptake, soil nitrogen mineralization and simple nitrogen balance. European Journal of Agronomy 16, 295–308.

Lambert, M. G., D. J. Barker, A. D. Mackay, Springett, J. (1996): Biophysical indicators of sustainability of North Island hill pasture systems. Proceedings of New Zealand Grassland Association 57, 31–36.

Ledgard, S.F., Jarvis, S.C., Hatch, D.J. (1998): Short-term nitrogen fluxes in grassland soils under different long-term nitrogen management regimes. Soil Biology and Biochemistry 30, 1233–1241.

Linn, D.M., Doran, J.W. (1984): Effect of water filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48, 1267–1272.

Linsler, D., Geisseler, D., Loges, R., Taube, F., Ludwig, B. (2013): Temporal dynamics of soil organic matter composition and aggregate distribution in permanent grassland after a single tillage event in a temperate climate. Soil and Tillage Research 126: 90–99.

Ludwig, B., Geisseler, D., Michel, K., Joergensen, R.G., Schulz, E., Merbach, I., Raupp, J., Rauber, R., Hu, K., Niu, L., Liu, X. (2011): Effects of fertilization and soil management on crop yields and carbon stabilization in soils. A review. Agronomy for Sustainable Development 31, 361-372.

Mallory, E.B., Griffin, T.S. (2007): Impacts of soil amendment history on nitrogen availability from manure and fertilizer. Soil Science Society of America Journal 71: 964–973.

McDowell, R.W., Condron, L.M. (2004): Estimating phosphorus loss from New Zealand grassland soils. New Zeal. Journal of Agricultural Research 47, 137–145.

Mellek, J.E., Dieckow, J., da Silva, V.L., Favaretto, N., Pauletti, V., Vezzani, F.M., Moretti Souza, J.L. de (2010): Dairy liquid manure and no-tillage: Physical and hydraulic properties and carbon stocks in a Cambisol of Southern Brazil. Soil and Tillage Research 110: 69–76.

Mikha, M.M., Rice, C.W. (2004): Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. Soil Science Society of America Journal 68: 809–816.

Moorhead, D.L., Westerfield, M.M., Zak, J.C. (1998): Plants retard litter decay in a nutrient-limited soil: a case of exploitative competition? Oecologica 113, 530–536.

Murphy, J., Riley, J. P. (1962): A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.

Olsen, S., Cole, C., Watanabe, F., Dean, L. (1954): Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, D.C.

Oorts, K., Bossuyt, H., Labreuche, J., Merckx, R., Nicolardot, B. (2007): Carbon and nitrogen stocks in relation to organic matter fractions, aggregation and pore size distribution in no-tillage and conventional tillage in northern France. European Journal of Soil Science 58, 248–259.

Persson, J., Kirchmann, H. (1994): Carbon and nitrogen in arable soils as affected by supply of N fertilizers and organic manures. Agriculture, Ecosystems and Environment 51, 249–255.

Quincke, J.A., Wortmann, C.S., Mamo, M., Franti, T., Drijber, R.A., Garcia, J.P. (2007): One-time tillage of no-till systems: Soil physical properties, phosphorus runoff, and crop yield. Agronomy Journal 99, 1104–1110.

Reddy, D.D., Rao, A.S., Takkar, P.N. (1999): Effects of repeated manure and fertilizer phosphorus additions on soil phosphorus dynamics under a soybean-wheat rotation. Biology and Fertility of Soils 28: 150–155.

SAS Institute (1990): SAS/STAT User´s Guide, Version 6. SAS Institute Inc., Cary, NC, USA.

Schmeer, M,, Loges, R., Taube, F. (2009): Der Einfluss von Grünlanderneuerung mit einer eingeschobenen Ackerzwischennutzung auf die Stickstoffflüsse im System Boden-Pflanze. Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau, 88–91.

Sharpley, A.N. (2003): Soil mixing to decrease surface stratification of phosphorus in manured soils. Journal of Environmental Quality 32, 1375–1384.

Shepherd, M.A., Withers, P.J. (1999): Applications of poultry litter and triple superphosphate fertilizer to a sandy soil: effects on soil phosphorus status and profile distribution. Nutrient Cycling in Agroecosystems 54, 233–242.

Singh, M., Tripathi, A.K., Reddy, K.S., Singh, K.N. (2001): Soil phosphorus dynamics in a Vertisol as affected by cattle manure and nitrogen fertilization in soybean-wheat system. Journal of Plant Nutrition and Soil Science 164, 691–696.

Six, J., Elliott, E.T., Paustian, K. (1999): Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63: 1350–1358.

Six, J., Elliott, E.T., Paustian, K. (2000b): Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. Soil Science Society of America Journal 64, 1042–1049.

Soussana, J.F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., Arrouays, D. (2004): Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management 20, 219–230.

Tisdall, J.M., Oades, J.M. (1982): Organic matter and water stable aggregates in soils. Journal of Soil Science 33, 141–163.

VandenBygaart, A.J., Kay, B.D. (2004): Persistence of soil organic carbon after plowing a long-term no-till field in southern ontario, Canada. Soil Science Society of America Journal 68, 1394–1402.

Vertès, F., Hatch, D., Velthof, G., Taube, F., Laurent, F., Loiseau, P., Recous, S. (2007): Short-term and cumulative effects of grassland cultivation on nitrogen and carbon cycling in ley-arable rotations. Grassland Science in Europe 9, 227–246.

Watanabe F. S., Olsen, S. (1965) Test of an ascorbic acid method for determining P in water and NaHCO3 extract from soil. Soil Science Society of America Proceedings 29, 677–678.

Yang, X.M., Wander, M.M. (1998): Temporal changes in dry aggregate size and stability: tillage and crop effects on a silty loam Mollisol in Illinois. Soil and Tillage Research 49, 173–183.

Yang, C., Wang, M., Chen, H., Li, J. (2011): Responses of butachlor degradation and microbial properties in a riparian soil to the cultivation of three different plants. Journal of Environmental Science 23, 1437–1444.

Zotarelli, L., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Six, J. (2007): Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols. Soil and Tillage Research 95, 196–206.

6. Temporal variations of carbon concentration, aggregate distribution and microbial biomass in temperate grassland soils

Deborah Linsler[1], Ralf Loges[2], Friedhelm Taube[2], Daniel Geisseler[1], Rainer Georg Joergensen[3], Bernard Ludwig[1]

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

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

[3] Department of Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

6.1 Abstract

In grassland soils, the temporal dynamics in stocks of soil organic carbon (C_{org}) and different fractions is not completely understood. We investigated the temporal variations of C stocks, soil aggregates, microbial biomass C and ergosterol concentrations in a temperate loamy sandy grassland soil. Soil samples were taken in a trial in northern Germany at six sampling times between October 2010 and October 2011 in different soil depths between 0 and 40 cm from a permanent grassland, an arable plot converted to grassland and grassland with a one-time tillage operation. The C_{org} concentrations and stocks in the permanent grassland were not influenced by sampling times, whereas the ergosterol concentrations were markedly affected. There was also a strong variation in the concentrations of large macroaggregates (>2000 µm) in the surface soil (0-10 cm) with the highest concentration in October 2011 (67 g 100 g^{-1} soil) and a 3.2-fold lower concentration in May. The marked decrease in May was probably mainly due to a markedly

decreased gravimetric moisture content (11%) which may have decreased the stability of this fraction. A one-time tillage operation in grassland with subsequent grassland renovation led to significantly lower concentration and stocks of C_{org} from April 2011 to October 2011 in the surface soil layer in comparison with the permanent grassland. Similar patterns showed the concentrations of large macroaggregates, microbial biomass C and ergosterol. The conversion of arable land into grassland and therefore the growing of perennial plants and the absence of regular soil cultivation did not affect concentrations of macroaggregates in the first year, but there was an increase in microbial biomass C (1.4-fold) and ergosterol concentration (3.3-fold) in the surface soil layer. This study indicates that grassland soils may exhibit a large temporal variability in the dynamics of water-stable aggregates, because the stability of large macroaggregates > 2000 µm (which are generally not present in arable soils) can be greatly affected by environmental conditions such as water content and temperature. The ergosterol concentrations showed large temporal variations in all treatments, whereas temporal variations in microbial biomass C concentrations were much less pronounced, indicating a higher sensitivity of ergosterol to soil cultivation or changing environmental conditions.

6.2 Introduction

Over the last years increasing attention has focused on the management of agricultural soils in order to slightly decrease the CO_2 level in the atmosphere through the sequestration of carbon in soils. It is well established that the conversion of arable land into pasture or grassland generally increases the soil organic carbon (C_{org}) stocks (Guo and Gifford, 2002; Mensah et al., 2003; Soussana et al., 2004; Su, 2007). However, the mechanisms underlying the increased carbon sequestration are not completely understood. Furthermore, little is known about the temporal dynamics of C_{org} pools in soils after the conversion of arable land into grassland.

Sporadic tillage is a common practice in managed grassland soils to control weeds, reduce soil compaction or shift the species composition in reseeded grasslands. However, these benefits of

sporadic tillage may be accompanied by negative impacts. Tillage of grassland often results in losses of important nutrients, for instance nitrogen (Davies et al., 2001; Soussana et al., 2004; Vertès et al., 2007). Furthermore, a tillage event may lead to a destruction of soil aggregates which protect occluded organic material from mineralization (Stavi et al., 2011; Linsler et al., 2013). When aggregates are disrupted the organic material is exposed (Bronick and Lal, 2005; Zotarelli et al., 2007) and can be mineralized. This may lead to decreasing C_{org} concentrations in the soil. Indeed, one single tillage event in arable soils (VandenBygaart and Kay, 2004) or grassland soils (Linsler et al., 2013) may result in a loss of C_{org}, yet information about the temporal dynamics after a tillage event in grassland with grassland renovation is scarce.

Many studies found seasonal variations in C_{org} concentration (Leinweber et al., 1994; Jacobs et al., 2010), aggregates (Alvaro–Fuentes et al., 2007; Daraghmeh et al., 2009; da Veiga et al., 2009; Jacobs et al., 2010; Bamberg et al., 2011) or microbial biomass (Salinas-Garcia, 1997; Kuhnert et al., 2012; Zhang et al., 2012) in arable land. Whereas some studies suggest that temporal changes in aggregate dynamics correspond to variations in environmental factors such as temperature or soil water content (Cosentino et al., 2006; Dimoyiannis, 2009), other studies stated that the cultivation of the soil is the reason for variations (Jacobs et al., 2010). Grassland soils are less affected by external influences than arable soils, since no regular tillage takes place and there is variation in the crops grown. Therefore, the main cause of variations in grassland soils is most likely to be environmental factors. However, less is known about the occurrence and the magnitude of temporal dynamics of C_{org} concentrations, aggregates, and microbial biomass in permanent grassland soils.

We hypothesized that (i) there is an increase in larger water-stable aggregates (>250 μm) and microbial biomass in the first year after the conversion from arable land into grassland; (ii) there is an initial decrease in these properties after a grassland renovation (carried out by a tillage event) because of the destruction of the soil structure and afterwards an increase in the year after the re-establishment of grassland; and (iii) that the C_{org} concentration, aggregate distribution, and

microbial biomass concentration in a permanent grassland have only slight variations within one year because of the permanent soil cover throughout the year as well as the lack of regular tillage and cultivation of various crops. The objective of this study was to quantify the temporal dynamics of water-stable aggregates and the microbial biomass in recently established grasslands and in permanent grassland.

6. 3 Material and Methods

6.3.1 Study site

The field trial was initiated on the experimental farm Lindhof (54°27 `N, 9°57 `E) in northern Germany, close to Kiel. The mean annual temperature in the area is 8.9 °C and the mean annual precipitation is 768 mm. For further information on the temperature and precipitation at the sampling site in the year of sampling see Figures 6.1a and 6.1b. The soil there is heterogeneous and the soil types consist of Cambisols, Eutric Luvisols, Stagnosols, and Anthrosols (Schmeer et al., 2009). The soil (0 – 40 cm; mean ± standard deviation) in this area has a sand, silt, and clay content of 67 (7), 23 (4), and 10 (4) %, respectively. The pH value is 5.7 (0.6).

The experimental farm Lindhof has been managed organically since 1993 in accordance with the guidelines of the German organic growers association “Bioland”. An experimental field at the site (which was arable land prior to the trial) was split in 1994 and one part converted to permanent grassland whereas the other part was left as arable land. Between the years 1994 and 2005 the grassland was generally cut 1-2 times per annum for silage production and grazed 3-4 times with cattle each year. After the year 2005 the grassland is generally cut four times each year for forage production (Schmeer et al., 2009). The crops cultivated in the arable land varied. The plants cultivated in recent years were potatoes (*Solanum tuberosum* L.) in 2010, oats (*Avena sativa* L.) in 2009, grass-clover in 2008, and winter wheat (*Triticum aestivum* L.) in 2007.

In autumn 2010 a field trial was started to determine the effects of grassland renovation and conversion of arable land into grassland. The aim was to study these effects on nitrogen fluxes and

carbon storage. The three treatments (each with three replicates) were:

G-G: **g**rassland – tillage - **g**rassland

Plots, which were grassland since 1994, were plowed with a moldboard plow to a depth of 25 cm in September 2010 and afterwards sown with a grass-clover mixture. The standard grassland seed mixture contained 63% perennial ryegrass (*Lolium perenne* L.), 16% Timothy grass (*Phleum pratense* L.), 9% smooth meadow-grass (*Poa pratensis* L.), and 12% white clover (*Trifolium repens* L.).

A-G: **a**rable land - **g**rassland

Plots, which were previously arable land, were plowed with a moldboard plow to a depth of 25 cm in September 2010 and afterwards sown with the grass-clover mixture as described above.

Permanent grassland (since 1994) served as control.

Fertilization was carried out in 2011 and amounted to 100 kg K ha^{-1}, 24 kg Mg ha^{-1}, 68 kg S ha^{-1}, and 45 kg P ha^{-1} in each treatment.

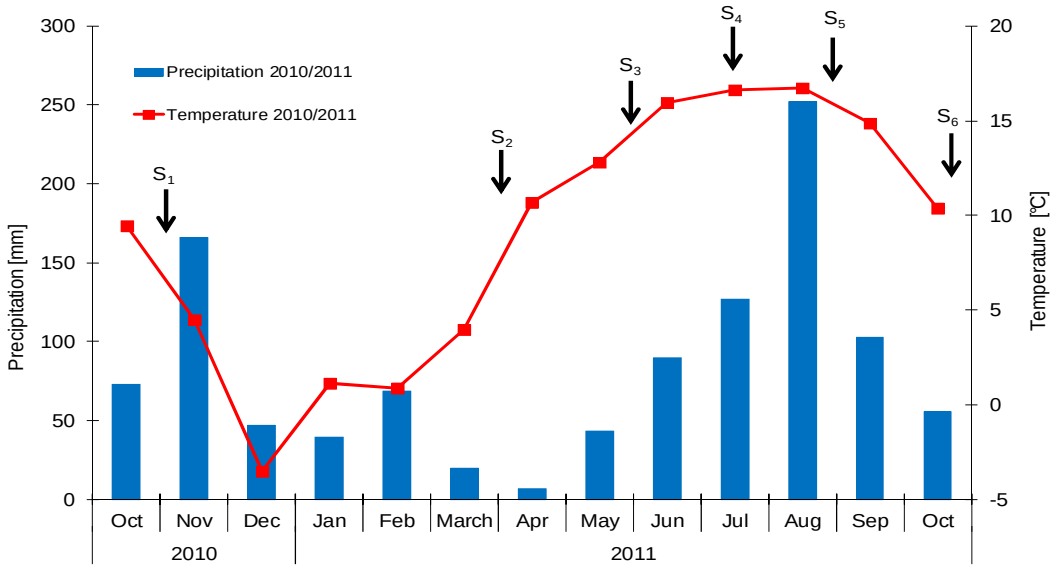


Figure 6.1a: Monthly precipitation and temperature during the sampling period at the study site with sampling times (S_1 –S_6) marked with an arrow (experimental farm “Lindhof”, northern Germany).

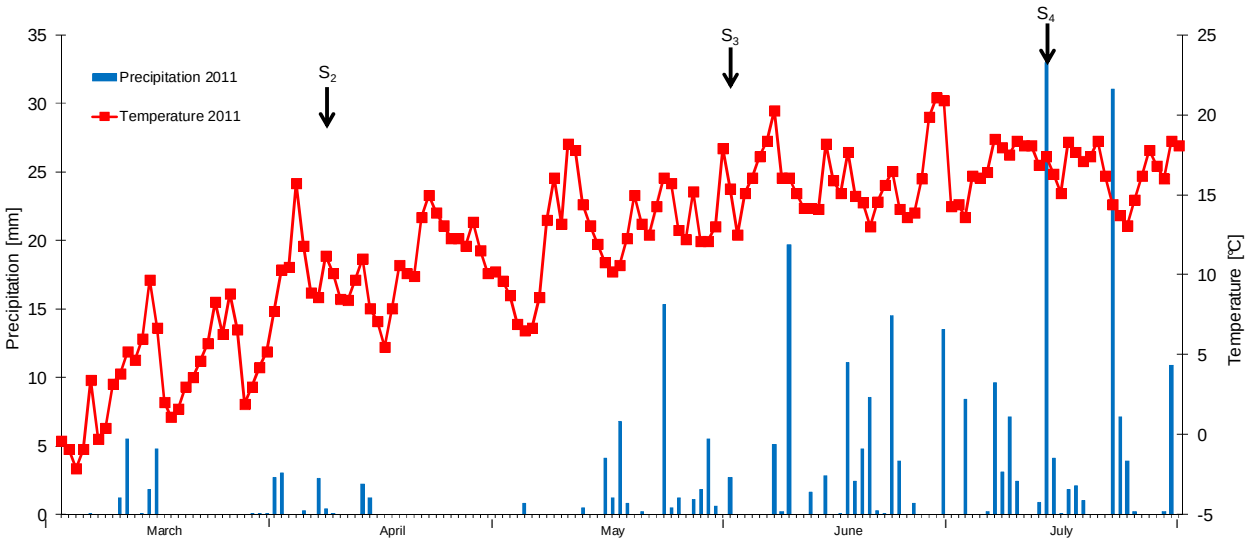


Figure 6.1b: Precipitation and temperature from March to July 2011 at the field trial in a higher resolution with sampling times (S_2 – S_4; marked with an arrow).

6.3.2 Sampling procedure

Soil samples were taken six times within one year from October 2010 to October 2011. The sampling dates were: 25.10.2010 (after seeding of grassland), 07.04.2011 (after winter), 31.05.2011 (after the 1st grassland cut in 2011), 13.07.2011 (after the 2nd grassland cut), 23.08.2011 (after the 3rd grassland cut), and 26.10.2011 (after the 4th grassland cut). Two cores were taken in each plot and combined to a composite sample. Samples for bulk density were taken on the 07.04.2011 and the 26.10.2011 for calculation of stocks. Samples were taken from the three field replicates at three depths (0 - 10 cm, 10 - 25 cm, and 25 - 40 cm). The samples were stored at 4 ℃ before processing.

6.3.3 Aggregate fractionation

For aggregate fractionation, we used a wet-sieving method developed by Cambardella and Elliott (1993) and slightly modified by Linsler et al. (2013). Briefly, approximately 100 g of air-dried soil (<10 mm) were placed on a 2000 µm sieve and submerged into distilled water for 10 min to

allow slaking. Afterwards, the sieve was moved up and down into the water with 50 repetitions. Water-stable aggregates retained on the sieve were collected, dried, and weighed. Aggregates, which passed the 2000 µm sieve, were put onto the next smaller mesh size and the fractionation procedure was continued as described above. Altogether, 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. Finally, the material passing through the 53 µm sieve (silt and clay together with very small microaggregates) was precipitated with 0.5 M $AlCl_3$ (5 mL on 2 L of supernatant). To recover the <53 µm fraction after precipitation, the water was siphoned off and the deposit was filtered, dried, and weighed.

An operator variability was found for that method (Jacobs et al., 2010). Therefore, the whole wet-sieving procedure for all samples was carried out by the first author.

We have calculated the C_{org} concentrations in aggregates on a sand-free basis (John et al., 2005):

Sand-free C_{org} (g kg^{-1}) = C_{org} concentration (g kg^{-1}) / (1 - sand proportion (g g^{-1})).

6.3.4 Microbial biomass C and ergosterol

The chloroform-fumigation-extraction method was used for estimating microbial biomass (Vance et al., 1987). Two portions of 10 g field-moist soil were taken from each soil sample. One portion was fumigated for 24 h at a temperature of 25 °C with ethanol-free $CHCl_3$. The fumigated and non-fumigated soil samples were extracted with 40 ml of 0.5 M K_2SO_4 by shaking for 30 min with 200 rev min^{-1}. Organic C in the extracts was determined (multi N/C 2100S, Analytik Jena, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990).

Ergosterol, a fungal cell-membrane component, was extracted from 2 g of field-moist soil with 100 ml distilled ethanol (Djajakirana et al., 1996). The solution was shaken at 250 rev min^{-1} for 30 min, filtered (Whatmann GF/A), evaporated at 40 °C and 55 mbar, and taken up in 10 ml

methanol. Ergosterol was then determined by reversed-phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm.

6.3.5 Analytics and soil characterisation

The bulk soil and the aggregate-size classes were ball-milled after drying (MM 200, Retsch, Haan, Germany) and stored in plastic vials at room temperature.

Concentrations of total carbon (C_t) and total nitrogen (N_t) were determined in the bulk soil and in the aggregate-size classes by dry combustion on a CN elemental analyzer (Elementar Vario El, Heraeus, Hanau, Germany). No carbonates were present in the soil, therefore total C corresponds to C_{org}. The pH values were measured in 0.01 M $CaCl_2$ (2.5 ml of solution g^{-1} of soil). The texture of the soil was measured with the pipet method according to DIN ISO 11277 (2002) and the bulk density according to DIN ISO 11272 (1998).

6.3.6 Statistical analyses

Statistical analyses were carried out with the SAS program (SAS Institute, 1990). For determination of differences among the sampling times we used a repeated measures-analysis of variance. If the test of orthogonal components was significant we used a multivariate test (Wilk's Lambda) to determine statistical significance. If the test of orthogonal components were not significant, the Huynh-Feldt conditions are complied and we used this univariate test. The treatments were compared with a one-way ANOVA and the mean comparisons were performed with the Tukey test. Effects were considered significant for $p \leq 0.05$.

6.4 Results

6.4.1 C_{org} stocks

C_{org} stocks in the permanent grassland were significantly higher than those in A-G and G-G in

both sampling times in which bulk density was determined (Table 6.1). In the surface soil layer of the permanent grassland in April there were 30 t C_{org} ha^{-1} in 1251 kg soil, whereas in the recently established grasslands the C_{org} stocks were 55 – 58% lower. Roughly the same was found in October 2011, with 46 – 54% lower C_{org} stocks in the recently established grasslands in comparison with the permanent grassland. However, the significantly lower C_{org} stocks in A-G and G-G were limited to the surface soil layer. Regarding the soil profile (0 – 40 cm) no significant differences among treatments existed either in April or in October 2011.

Table 6.1: C_{org} stocks (t ha^{-1}) calculated on an equivalent mass of soil among different treatments (G-G: grassland – tillage – grassland; A-G: arable land – grassland) and soil depths at two sampling times; mean values and standard errors (n=3).

	Depths (cm)	C_{org} stock (t C ha^{-1} in 1251 kg soil)	Depths (cm)	C_{org} stock (t C ha^{-1} in 5521 kg soil)
Sampling time April 2011				
Permanent grassland	0 - 10 cm	30.4 (3.6)^{A}	0 - 40 cm	82.3 (15.2)
G - G	0 - 8.5 cm	12.8 (2.3)^{B}	0 - 39.2 cm	65.6 (13.8)
A - G	0 - 8.4 cm	13.6 (1.2)^{B}	0 - 35.0 cm	50.3 (9.3)
Sampling time October 2011				
Permanent grassland	0 - 10.6 cm	26.3 (0.5)^{A}	0 - 38.6 cm	74.0 (4.1)
G - G	0 - 8.7 cm	14.1 (1.7)^{B}	0 - 37.8 cm	59.8 (9.9)
A - G	0 - 8.4 cm	12.2 (1.8)^{B}	0 - 37.8 cm	51.4 (7.9)

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

6.4.2 Aggregate distribution during the year of sampling

In the surface soil layer, the different sampling times affected significantly the aggregate-size distribution in the permanent grassland and in the G-G, but not in the A-G treatment (Figure 6.2). The strongest variation was found in the permanent grassland in the large macroaggregates (>2000 µm), with the lowest concentration (mean ± standard error) in May (21 ± 5%) and more than three times higher concentrations in October 2011 (67 ± 1%). The aggregates without any temporal

variation were the medium macroaggregates (2000 – 1000 µm). In the deeper soil layer (10 – 40 cm), there was less variation in aggregate distribution. Only the small macroaggregates (1000 – 250 µm) were affected by sampling time in the permanent grassland and the microaggregates in the G-G and A-G treatments.

In all aggregate-size classes at least at one sampling time difference was found among treatments in the surface soil layer (Figure 6.2). The highest concentrations of large macroaggregates and medium macroaggregates were observed in the permanent grassland and subsequently the lowest concentrations of small macroaggregates and microaggregates. This effect was significant for all sampling times except in May for large macroaggregates. No significant differences existed between A-G and G-G, except for the large macroaggregates in July and the small macroaggregates in October 2011. In the deeper soil layer the differences among treatments were much less pronounced. Only the small macroaggregates had significantly higher concentrations in A-G in comparison with the permanent grassland and G-G.

The C_{org} concentrations in aggregate-size classes (calculated on a sand-free basis) ranged from 24 g kg^{-1} to 76 g kg^{-1} fraction for macroaggregates, 25 g kg^{-1} to 40 g kg^{-1} fraction for microaggregates and 18 g kg^{-1} to 22 g kg^{-1} fraction for the silt and clay fraction (Table 6.2). The C_{org} concentrations in aggregates were only affected by sampling times in the permanent grassland in the medium macroaggregates and in the microaggregates. In general, there was a decrease of C_{org} concentration in aggregates in the order permanent grassland > G-G > A-G in the macroaggregates and microaggregates (on average 56, 51 and 37 g kg^{-1}, respectively) and no difference in the silt and clay fraction (20 g kg^{-1}).

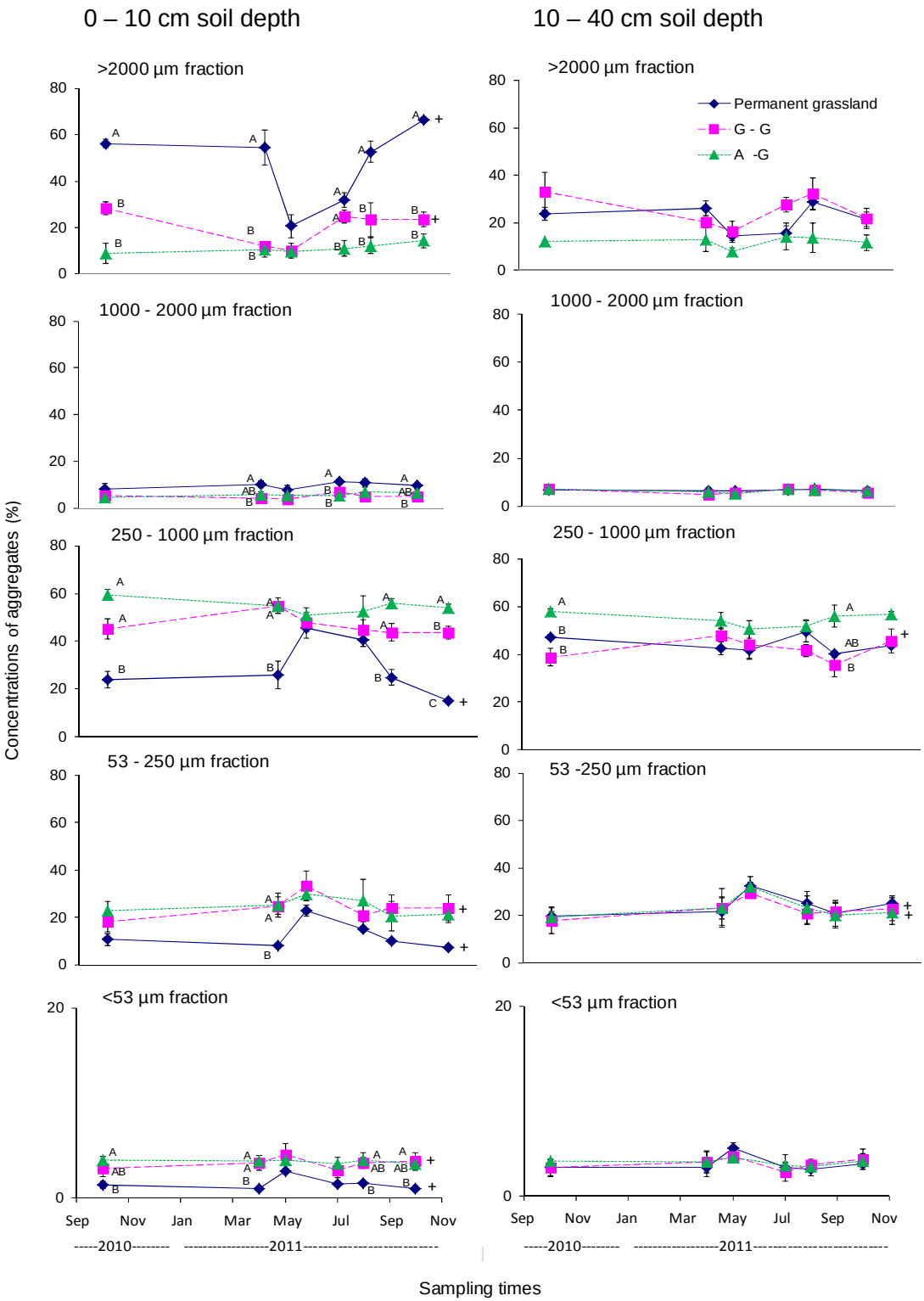


Figure 6.2: Water-stable aggregates (%) in 0 – 10 cm and 10 – 40 cm soil depths among different treatments (Permanent grassland; G-G: grassland – tillage – grassland; A-G: arable land – grassland) and sampling times; letters indicate significant ($p \leq 0.05$) differences among treatments, + indicates a significant ($p \leq 0.05$) sampling time effect; mean values and standard errors (n=3).

Table 6.2: C_{org} concentrations (g kg^{-1} fraction) calculated on a sand-free basis in water-stable aggregate classes in the soil profile (0 – 40 cm) among different treatments (G-G: grassland – tillage – grassland; A-G: arable land – grassland) and sampling times; mean values and standard errors (n=3).

	October 2010	April 2011	May 2011	July 2011	August 2011	October 2011	Effect of sampling time
>2000 µm							
Permanent grassland	49 (7)	67 (8)	76 (9)	61 (7)	63 (8)	68 (9)	0.02
G-G	54 (16)	53 (12)	57 (18)	65 (13)	56 (14)	57 (16)	ns
A-G	51 (12)	43 (8)	41 (4)	44 (3)	43 (5)	50 (5)	ns
2000 - 1000 µm							
Permanent grassland	42 (9)	55 (10)	69 (9)	58 (5)^{AB}	60 (9)	55 (7)	0.02
G-G	49 (15)	48 (14)	55 (19)	67 (10)^{A}	55 (15)	49 (17)	ns
A-G	27 (6)	29 (9)	24 (5)	31 (7)^{B}	30 (6)	33 (3)	ns
1000 - 250 µm							
Permanent grassland	39 (5)	52 (10)	54 (6)	53 (6)	50 (8)	44 (6)	ns
G-G	41 (14)	37 (13)	39 (11)	51 (14)	38 (11)	39 (15)	ns
A-G	40 (3)	37 (7)	35 (6)	40 (4)	38 (3)	37 (4)	ns
250 - 53 µm							
Permanent grassland	27 (6)	30 (7)	40 (8)	36 (7)	30 (4)	25 (3)	0.02
G-G	28 (9)	25 (8)	37 (15)	27 (14)	27 (10)	26 (10)	ns
A-G	28 (5)	26 (7)	33 (6)	27 (5)	26 (3)	26 (5)	ns
< 53 µm							
Permanent grassland	19 (4)	19 (3)	24 (3)	20 (1)	21 (3)	20 (2)	ns
G-G	21 (6)	18 (5)	20 (6)	20 (5)	21 (6)	20 (7)	ns
A-G	22 (2)	19 (4)	19 (2)	20 (3)	22 (2)	21 (3)	ns

Letters indicate significant differences among treatments; exclusively those values which are significantly different ($p \leq 0.05$) are followed by different letters.

6.4.3 Concentrations of microbial biomass C and ergosterol during the year of sampling

In the surface soil layer, the microbial biomass C concentrations were only affected by sampling times in A-G (Figure 6.3) where they increased from 132 mg kg^{-1} in October 2010 to 190 mg kg^{-1} in October 2011. In the deeper soil layer, microbial biomass C concentrations were affected by sampling time only in the permanent grassland with highest concentration in May (363 mg kg^{-1}) and lowest in October 2011 (162 mg kg^{-1}). In the surface soil layer, in all sampling times the permanent grassland had significant higher microbial biomass C concentrations than the recently established grasslands. No significant differences existed between A-G and G-G.

The ergosterol concentrations were strongly affected by sampling times in all treatments and in both soil layers (Figure 6.3). In the G-G and A-G treatments, the ergosterol concentrations increased from 0.3 to 0.7 mg kg^{-1} and 0.2 to 0.7 mg kg^{-1}, respectively, in the surface soil layer. In the surface soil layer of the permanent grassland, the lowest ergosterol concentrations were found in October 2010 (0.5 mg kg^{-1}) and the highest in August (1.5 mg kg^{-1}). Ergosterol concentrations in the surface soil layer of the permanent grassland were significantly higher in comparison with A-G and G-G with no difference between A-G and G-G. However, significant differences among treatments occurred in the deeper soil layer only in October 2010.

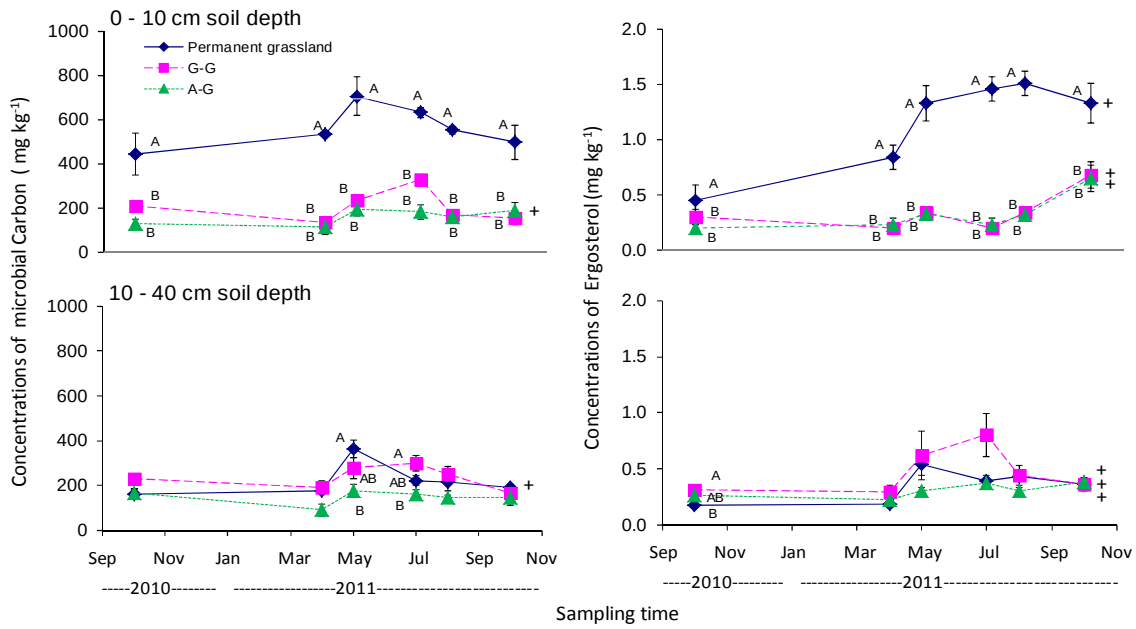


Figure 6.3: Concentrations of microbial carbon (mg kg^{-1}) and concentrations of ergosterol (mg kg^{-1}) in the surface soil (0 – 10 cm) and in 10 – 40 cm soil depths among different treatments (Permanent grassland; G-G: grassland - tillage - grassland; A-G: arable land - grassland) and sampling times; letters indicate significant ($p \leq 0.05$) differences among treatments, + indicates a significant ($p \leq 0.05$) sampling time effect; mean values and standard errors (n=3).

6.5 Discussion

6.5.1 C_{org} stocks

The concentrations (not shown) and stocks of C_{org} in the three soil depths of A-G were not affected by sampling times (Table 6.1). Since the accumulation of C_{org} in grasslands can be explained by the high root production by grasses (Cerri et al., 1991) the well-known C_{org} sequestration after the conversion from arable land into grassland (Mensah et al., 2003; Su, 2007) may need more time than one year.

C_{org} stocks in the surface soil of the permanent grassland were significantly higher than those of the G-G and A-G treatments, probably mainly due to the effects of tillage and the initially reduced C input from the newly established grasses.

6.5.2 Temporal dynamics of aggregates in permanent grassland

We hypothesized that there is only small variation in aggregate-size distribution in the permanent grassland because of the lack of cultivation. However, in the permanent grassland there was a large difference among different sampling times in concentrations of aggregates (Figure 6.2). For instance, in the surface soil layer the concentrations of large macroaggregates (mean ± standard error) ranged from 21 ± 5% in May to 67 ± 1% in October 2011. The difference was presumably caused by rainfall and water contents in the soil. Rainfall in March (20 mm) and April (7 mm) was only small, which caused a dry soil (gravimetric moisture content had a mean of 11% in May in 0 – 40 cm; Table 6.3). We observed a strong correlation between every aggregate size-class and the gravimetric moisture content of the bulk soil in the soil profile (0 – 40 cm). The correlation (Pearson correlation; $p < 0.001$ for every aggregate size-class) was positive for the large macroaggregates ($r = 0.69$) and medium macroaggregates ($r = 0.35$) and negative for the small macroaggregates ($r = -0.53$), microaggregates ($r = -0.53$), and the <53 µm size class ($r = -0.41$; Figure 6.4). Presumably water retention is low in the grassland soil with a high sand content (mean of 67%), which causes a desiccating of the binding agents of aggregates (such as root exudates, excretions of microbial biomass) in May and thus a reduction in their functionality.

The strong decrease in concentration was mainly restricted to the large macroaggregates, which were the dominant aggregate-size class until April in the surface soil layer of the permanent grassland. It is well known, that larger aggregates are less stable than smaller ones and, therefore, more susceptible to disruption (Christensen, 1986; Cambardella and Elliott, 1994; Six et al., 1999). In A-G and G-G there were higher concentrations in more stable smaller aggregates and therefore the drying and rewetting of the soil had minor impacts (discussed below).

Table 6.3: Gravimetric moisture content (%) in the different treatments (G-G: grassland – tillage – grassland; A-G: arable land – grassland) and soil depths at the sampling times.

	October 2010	April 2011	May 2011	July 2011	August 2011	October 2011	Effect of sampling time
0 - 10 cm							
Permanent grassland	20.7 (2.0)	25.6 (2.5)^{A}	15.9 (2.1)	22.4 (1.3)^{A}	24.2 (2.5)^{A}	27.9 (1.2)^{A}	<0.01
G-G	19.1 (0.1)	15.9 (0.1)^{B}	13.2 (0.1)	16.9 (0.7)^{B}	15.5 (1.4)^{B}	17.5 (0.6)^{B}	<0.01
A-G	17.1 (1.0)	14.4 (1.1)^{B}	10.4 (0.7)	13.7 (0.8)^{B}	14.3 (1.4)^{B}	15.6 (1.1)^{B}	<0.01
10 - 25 cm							
Permanent grassland	17.5 (0.8)	15.9 (1.4)	10.8 (1.7)	15.1 (0.8)	17.6 (2.6)^{AB}	18.2 (1.1)	0.02
G-G	20.6 (1.4)	16.1 (0.6)	11.5 (0.8)	19.8 (1.9)	18.9 (1.4)^{B}	14.9 (4.0)	0.05
A-G	16.8 (0.8)	15.0 (1.5)	9.5 (0.3)	13.4 (0.9)	14.9 (0.9)^{A}	15.4 (1.1)	<0.01
25 - 40 cm							
Permanent grassland	16.0 (1.1)	14.3 (1.8)	9.4 (1.6)	13.9 (1.2)	14.7 (1.8)	16.3 (1.1)	0.02
G-G	18.3 (0.7)	17.2 (0.5)	11.9 (1.6)	15.2 (0.6)	19.0 (2.4)	17.9 (1.3)	0.05
A-G	16.8 (0.6)	14.5 (1.5)	8.8 (0.4)	13.6 (0.9)	13.2 (1.5)	15.3 (1.4)	<0.01

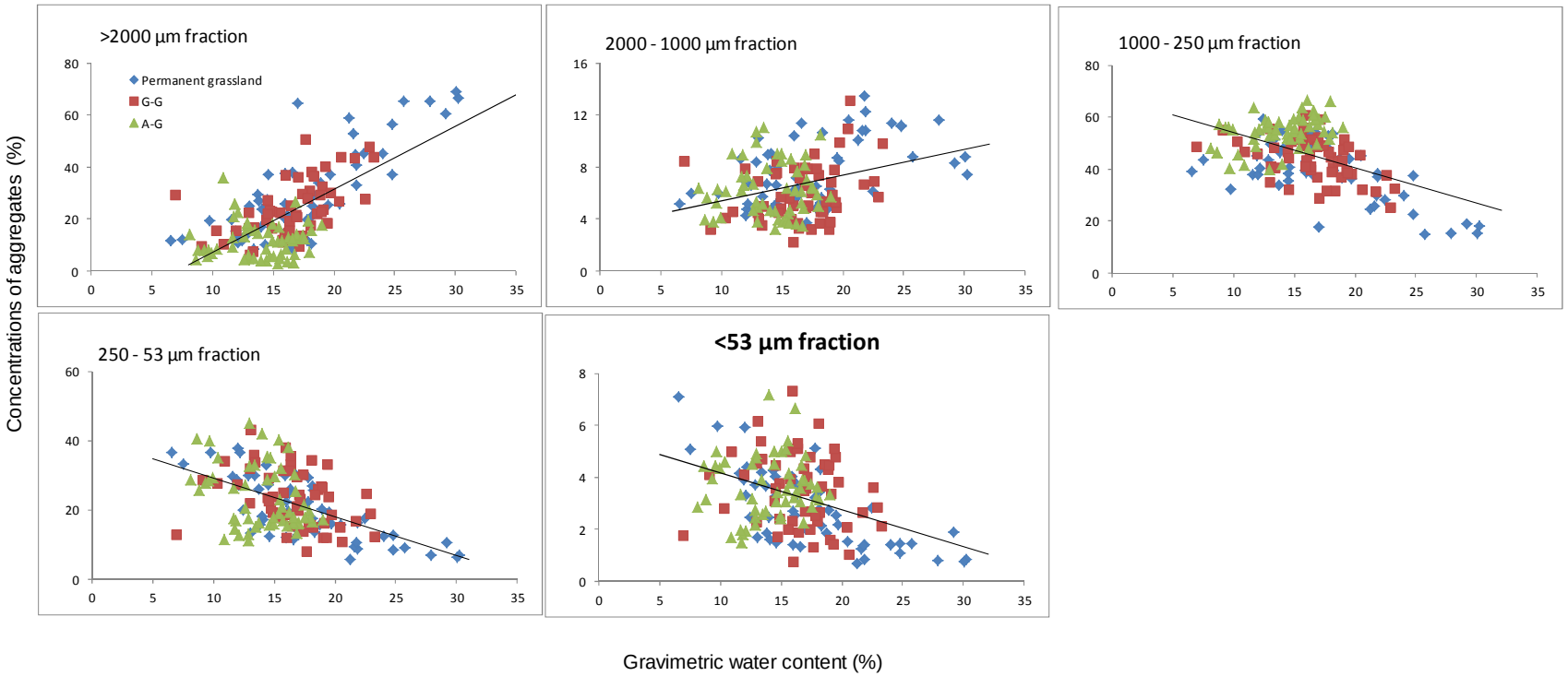


Figure 6.4: Correlations of the concentration of water-stable aggregates and the gravimetric moisture content of the bulk soil. The different symbols mark the different treatments (Permanent grassland; G-G: grassland - tillage - grassland; A-G: arable land - grassland).

6.5.3 Temporal dynamics of aggregates in A-G and G-G

It is well known, that arable soils contain fewer large aggregates than do grassland soils (John et al., 2005; Haghighi et al., 2010). Therefore, we hypothesized an increase in larger aggregates in A-G in the first year after the conversion from arable land into grassland. However, such an increase was not observed in the first year after the conversion (Figure 6.2). Presumably the hypothesized increase in macroaggregate concentration needs a longer time span.

We hypothesized that there is first a decrease in macroaggregates in G-G after the grassland renovation and the tillage event and afterwards an increase during the year of sampling. In the first sampling time after the tillage event (October 2010) there was a significantly lower concentration of large macroaggregates and a significantly higher concentration of small macroaggregates in the surface soil layer in G-G in comparison with the permanent grassland (Figure 6.2). The opposite case was found in the deeper soil layer, but less strongly. Overall, this resulted in no differences in the sampled soil profiles. This suggests that a tillage event in grassland soils caused a shift of aggregates from the surface soil in deeper soil layers presumably by turning the soil upside down due to the plow and the incorporation of organic material in deeper soil layer. The physical destruction of aggregates due to the tillage event seems to be non-existent or low.

However, there was a decrease in the concentrations of large macroaggregates of about 44% in the soil profile between October 2010 and April in G-G. This might be due to indirect effects of tillage killing most of the living plant biomass and therefore a reduction in roots and microbial and fungal biomass, parameters known to build aggregates (Tisdall and Oades, 1982; Six et al., 2000).

There were significantly lower concentrations of large and medium macroaggregates in the surface soil layer in G-G in comparison with permanent grassland in four of the six sampling times within the first year after the tillage event. The reduction of larger aggregates after occasional tillage was reported for arable soils (Stavi et al., 2011) and for grassland soils (Linsler et al., 2013). However, whereas in the surface soil two years after a tillage event no differences in aggregate distribution were found for arable soils (Quincke et al., 2007) there was still a significant reduction in

grassland soils (Linsler et al., 2013). This indicates that grassland soils need more time than arable soils for the previous aggregate concentrations to be restored.

The C_{org} concentrations in aggregates indicate that the major part of C_{org} was sequestered in the macroaggregates (>250 µm) in the three treatments (for the permanent grassland 78%, for G-G 76%, and for A-G 69%; Table 6.2). In all treatments most of the C_{org} was sequestered in the small macroaggregates (for the permanent grassland, G-G, and A-G 36%, 37%, and 50%, respectively) followed by the large macroaggregates in the permanent grassland and G-G (33% and 32%, respectively) and the microaggregates in A-G (23%). This indicates that the macroaggregates, especially the small macroaggregates, are very important for the storage of C_{org} in all three treatments. However, whereas for the permanent grassland and G-G also large macroaggregates have a high importance, for the previous arable land the microaggregates are more important than the large macroaggregates. When comparing the different sampling times, in all three treatments the lowest C_{org} storage in large macroaggregates and the highest C_{org} storage in microaggregates were found in May. This indicates that for these grassland soils after a dry period and therefore lower water contents in the soil the large macroaggregates become less important and the microaggregates more important for C_{org} storage, whereas no difference seems to exist for the other aggregate-size classes.

6.5.4 Concentrations of microbial carbon and ergosterol during the year of sampling

The microbial biomass C concentrations were affected by sampling times in the top 10 cm soil depth in A-G, with no effect in the deeper soil layer (10 – 40 cm; Figure 6.3). In the surface soil layer, there was an increase of about 43% in microbial biomass C concentration in the first year after the conversion from arable land to grassland. An increase of 138% in the microbial biomass was found after five years of continuous pasture on a Wakanui silt loam soil in New Zealand (Haynes et al., 1999). The higher concentration of microbial biomass C after the conversion from arable land to grassland or pasture was presumably a result of the buildup of organic material due

to increased root biomass production and the exudation of organic compounds from roots.

The microbial biomass C concentration was not affected by sampling times in the top 10 cm and in the 10 – 40 cm soil layer after the grassland renovation in G-G (Figure 6.3). Hence, no increase in microbial biomass C concentrations was observed during the year of sampling. This was in contrast to findings in arable soils; there was reported a significant reduction of bacteria and fungi 6 - 7 months after a one-time tillage event in no-till soil in different soil types (Wortmann et al., 2008). However, the comparison of microbial biomass after a tillage event in arable soils and in grassland soils are not directly comparable, because the amount of easily decomposable organic material in arable soils may limit the growth of microorganisms.

The ergosterol concentration increased in the surface soil and upper soil 2.3 and 1.2 fold, respectively. This indicates that the fungal component of the microbial biomass increased more quickly after a tillage event and grassland re-establishment. The faster increase in fungal biomass than in other microbial groups was also found after a one-time tillage event in no-till arable soil (Wortmann et al., 2008). Presumably fungal biomass (in comparison with the whole microbial biomass) reacts more strongly or faster to the tilled-in organic material.

In the surface soil layer, the microbial biomass C and ergosterol concentrations in all sampling times were significantly lower in G-G than in the permanent grassland. This indicates that there was a rapid and strong decrease in microbial and fungal biomass in the surface soil layer in the first month after tillage (before the first sampling time). Within one year it was not possible to restore the previous concentration of microbial and fungal biomass in G-G.

In the permanent grassland, the microbial biomass C concentration was significantly affected by sampling times in the deeper soil layer (Figure 6.3). The microbial biomass C concentration in the surface soil layer tended to be also affected ($p = 0.06$) by sampling times. In arable land, temporal differences in microbial biomass were reported in soils with different soil types and textures (Franzleubbers et al., 1994; Salinas-Garcia, 1997; Kuhnert et al., 2012; Zhang et al., 2012). The microbial biomass C concentration in the permanent grassland was highest in

May in both soil layers. At this sampling time, the concentration of large macroaggregates was lowest in both soil layers. Presumably at this date a higher amount of organic material previously occluded in aggregates was no longer protected due to the breakdown of the large macroaggregates and it became mineralized and stimulated the microbial biomass.

As in the recently established grasslands, the ergosterol concentration in the permanent grassland was also affected by sampling times in both soil depths (Figure 6.3). The ergosterol concentration was lowest in October 2010 and increased until August and afterwards there was a slightly decrease. A decrease in autumn was also found in other studies in pasture soils (Turgay and Nonaka, 2002) and could be related to climatic conditions which may affect the availability of decomposable substrates.

6.6 Conclusion

In grassland soils variations within one year were found for aggregate distribution, microbial biomass and fungal biomass. The C_{org} concentrations were not affected by sampling times. In contrast to our hypothesis there were high variations found in the permanent grassland in the ergosterol concentration and in the aggregate distribution, which suggests that environmental factors have a marked influence here. In the first year after the conversion of arable land to grassland an increase in microbial biomass C and saprotrophic fungi existed in the surface soil layer, whereas after grassland renovation only an increase in fungal biomass existed.

6.7 Acknowledgements

We thank Anja Sawallisch and Gabriele Dormann for technical advice and her help. This project was funded by the German Research Foundation (DFG Research Training Group 1397 ‘‘Regulation of soil organic matter and nutrient turnover in organic agriculture’’).

6.8 References

Alvaro-Fuentes, J., Arrue, J. L., Gracia, R., Lopez, M. V. (2007): Soil management effects on aggregate dynamics in semiarid Aragon (NE Spain). Science of the Total Environment 378, 179–182.

Bamberg, A. L., Cornelis, W. M., Timm, L. C., Gabriels, D., Pauletto, E. A., Pinto, L. F. S. (2011): Temporal changes of soil physical and hydraulic properties in strawberry fields. Soil Use and Management 27, 385–394.

Bolinder, M. A., Angers, D. A., Belanger, G., Michaud, R., Laverdiere M. R. (2002): Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. Canadian Journal of Plant Sience 82, 731–737.

Bronick, C. J., Lal, R. (2005): Manuring and rotation effects on soil organic carbon concentration for different aggregate size fractions on two soils in northeastern Ohio, USA. Soil and Tillage Research 81, 239–252.

Cambardella, C. A., Elliott, E. T. (1993): Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071–1076.

Cambardella, C. A., Elliott, E. T. (1994): Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal 58, 123–130.

Cerri, C. C., Volkoff, B., Andreux, F. (1991): Nature and behavior of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus. Forest Ecology and Management 38, 247–257.

Christensen, B. T. (1986): Straw incorporation and soil organic matter in macro-aggregates and particle-size separates. Journal of Soil Science 37, 125–135.

Cosentino, D., Chenu, C., Le Bissonnais, Y. (2006): Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38, 2053–2062.

da Veiga, M., Reinert, D. J., Reichert, J. M. (2009): Aggregate stability as affected by short and tongterm tillage systems and nutrient sources of a Hapludox in southern Brazil. Revista Brasileira de Ciencia do Solo 33, 767–777.

Daraghmeh, O. A., Jensen, J. R., Petersen, C. T. (2009): Soil structure stability under conventional and reduced tillage in a sandy loam. Geoderma 150, 64–71.

Davies, M. G., Smith, K. A., Vinten, A. J. (2001): The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. Biology and Fertility of Soils 33, 423–434.

Dimoyiannis, D. (2009): Seasonal soil aggregate stability variation in relation to rainfall and temperature under Mediterranean conditions. Earth Surface Processes and Landforms 34, 860–866.

DIN ISO 11272 (1998): Bodenbeschaffenheit - Bestimmung der Trockenrohdichte, ISO 11272: Beuth Verlag Berlin, Germany.

DIN ISO 11277 (2002): Bodenbeschaffenheit – Bestimmung der Partikelgrößenverteilung in Mineralböden – Verfahren mittels Siebung und Sedimentation, ISO 11277: 1998/Cor.1:2002. Beuth Verlag Berlin, Germany.

Djajakirana, G., Joergensen, R. G., Meyer, B. (1996): Ergosterol and microbial biomass relationship in soil. Biology and Fertility of Soils 22, 299–304.

Franzleubbers, A. J., Hons, F. M., Zuberer, D. A. (1994): Seasonal changes in soil microbial biomass and mineralizable C and N in wheat management systems. Soil Biology and Biogeochemistry 26, 1469–1475.

Guo, L. B., Gifford, R. M. (2002): Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360.

Haghighi, F., Gorji, M., Shorafa, M. (2010): A study of the effects of land use changes on soil physical properties and organic matter. Land Degradation and Development 21, 496–502.

Haynes, R. J. (1999): Size and activity of the soil microbial biomass under grass and arable management. Biology and Fertility of Soils 30, 210–216.

Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B. (2010): Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. Biology and Fertility of Soils 46, 671–680.

John, B., Yamashita, T., Ludwig, B., Flessa, H. (2005): Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128, 63–79.

Kemper, W. D., Rosenau, R., Nelson, S. (1985): Gas displacement and aggregate stability of soils. Soil Science Society of America Journal 49, 25–28.

Kuhnert, R., Oberkofler, I., Peintner, U. (2012): Fungal growth and biomass development is boosted by plants in snow-covered soil. Microbial Ecology 64, 79–90.

Leinweber, P., Schulten, H. R., Korschens, M. (1994): Seasonal variations of soil organic matter in a long-term africultural experiment. Plant and Soil 160, 225–235.

Linsler, D., Geisseler D., Loges, R., Taube, F., Ludwig, B. (2013): Temporal dynamics of soil organic matter composition and aggregate distribution in permanent grassland after a single tillage event in a temperate climate. Soil and Tillage Research 126, 90–99.

Mensah, F., Schoenau, J. J., Malhi, S. S. (2003): Soil carbon changes in cultivated and excavated land converted to grasses in east-central Saskatchewan. Biogeochemistry 63, 85–92.

Okano, S., Sato, K., Inoue, K. (1991): Negative relationship between microbial biomass and root amount intopsoil of a renovated grassland. Soil Science and Plant Nutrition 37, 47–53.

Quincke, J. A., Wortmann, C. S., Mamo, M., Franti, T., Drijber, R. A., Garcia, J. P. (2007): One-time tillage of no-till systems: Soil physical properties, phosphorus runoff, and crop yield. Agronomy Journal 99, 1104–1110.

SalinasGarcia, J.R., Hons, F. M., Matocha, J. E., Zuberer, D. A. (1997): Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. Biology and Fertility of Soils 25, 182–188.

SAS Institute (1990): SAS/STAT User´s Guide, Version 6. SAS Institute Inc., Cary, NC, USA.

Schmeer, M,, Loges, R., Taube, F. (2009): Der Einfluss von Grünlanderneuerung mit einer eingeschobenen Ackerzwischennutzung auf die Stickstoffflüsse im System Boden-Pflanze. Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau, 88–91.

Six, J., Elliott, E. T., Paustian, K. (1999): Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358.

Six, J., Elliott, E. T., Paustian, K. (2000): Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099–2103.

Soussana, J. F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., Arrouays, D. (2004): Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management 20, 219–230.

Stavi, I., Lal, R., Owens, L. B. (2011): On-farm effects of no-till versus occasional tillage on soil quality and crop yields in eastern Ohio. Agronomy for Sustainable Development 31, 475–482.

Su, Y. Z. (2007): Soil carbon and nitrogen sequestration following the conversion of cropland to alfalfa forage land in northwest China. Soil and Tillage Research 92, 181–189.

Tisdall, J. M., Oades, J. M. (1982): Organic matter and water stable aggregates in soils. Journal of Soil Science 33, 141–163.

Turgay, O. C., Nonaka, M. (2002): Effects of land-use and management practices on soil ergosterol content in andosols. Soil Science and Plant Nutrition 48, 693–699.

Vance, E. D., Brookes, P. C., Jenkinson, D. S. (1987): An extraction method for measuring soil microbial biomass-C. Soil Biology and Biochemistry 19, 703–707.

VandenBygaart, A. J., Kay, B. D. (2004): Persistence of soil organic carbon after plowing a long-term no-till field in southern Ontario, Canada. Soil Science Society of America Journal 68, 1394–1402.

Vertès, F., Hatch D., Velthof, G., Taube, F., Laurent, F., Loiseau, P., Recous, S. (2007): Short-term and cumulative effects of grassland cultivation on nitrogen and carbon cycling in ley-arable rotations. Grassland Science in Europe, 227–246.

West, A. W., Grant, W. D., Sparling, G. P. (1987): Use of ergosterol diaminopimelic acid and gluosamine contents of soils to monitor changes in microbial populations. Soil Biology and Biochemistry 19, 607–612.

Wortmann, C. S., Quincke, J. A., Drijber, R. A., Mamo, M., Franti, T. (2008): Soil Microbial Community Change and Recovery after One-Time Tillage of Continuous No-Till. Agronomy Journal 100, 1681–1686.

Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R., Brookes, P. C. (1990): Measurement of soil microbial biomass by fumigation extraction – an automated procedure. Soil Biology and Biochemistry 22, 1167–1169.

Young, I. M., Feeney, D. S., O'Donnell, A. G., Goulding, K. W. T. (2012): Fungi in century old managed soils could hold key to the development of soil water repellency. Soil Biology and Biochemistry 45, 125–127.

Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., Filley, T. R. (2012): Soil microbial community dynamics over a maize (Zea mays L.) growing season under conventional- and no-tillage practices in a rainfed agroecosystem. Soil and Tillage Research 124, 153–160.

Zhang, T., Wang, Y., Wang, X., Wang, Q., Han, J. (2009): Organic carbon and nitrogen stocks in reed meadow soils converted to alfalfa fields. Soil and Tillage Research 105, 143–148.

Zotarelli, L., Alves, B. J. R., Urquiaga, S., Boddey, R. M., Six, J. (2007): Impact of tillage and crop rotation on light fraction and intra-aggregate soil organic matter in two Oxisols. Soil and Tillage Research 95, 196–206.

7. General conclusion

There were significant reductions in C_{org} stocks, large water-stable aggregates and SOM concentrations in the top 10 cm soil depths two years after a one-time tilled grassland soil in comparison with non-tilled permanent grassland. Regarding the sampled profile (0 – 40 cm) these negative impacts are no longer evident. Five years after the tillage event differences in the surface soil layer in C_{org}, aggregates and SOM are still detectable between the tilled and the permanent grassland; however, these effects were much smaller and no longer significant. Based on the results of this thesis, sporadic tillage of grassland has negative impacts on C_{org} stocks, soil organic matter and water-stable aggregates but the tillage should not result in long lasting marked effects.

There were no differences found in C_{org} stocks, water-stable aggregates and SOM between one-time tilled grassland and a grassland which is tilled twice with the growing of one-season winter wheat in between. Therefore, the negative impacts of the insertion of cropping in a grassland on C_{org} stocks, aggregate distribution and SOM composition were small after five years small and no longer significant. This suggests that a temporal conversion from grassland into arable land for one season has no further effects in comparison with a one-time tillage event on C_{org} stocks, water-stable aggregates and SOM dynamics.

The organic fertilization over a timespan of four years resulted in higher basal respiration rates, Olsen-P concentrations and in significantly lower SOM (determined with a density fractionation scheme). The growing of one season winter wheat and two tillage events had opposite effects on the basal respiration rates and Olsen-P concentrations. Regarding the combined effect of tillage and fertilization this two practices have only contrary influences in the microbial activity and Olsen-P concentrations. However, since fertilization and tillage greatly influence the plant species composition, general implications from the direct effects of tillage or organic fertilization on nutrient and carbon dynamics in grassland soils cannot be deduced in the moment.

In the first year after the conversion from arable land into grassland, there was an increase in large and medium macroaggregates, C_{mic} and ergosterol concentrations in the surface soil layer.

The C_{org} concentrations did not change. Our results suggest that there is a continuous increase in these parameters from the beginning of the established grassland. In contrast, after a one-time tillage event there was only an increase in ergosterol concentration in the surface soil layer. In general the lowest values in large macroaggregates, C_{mic} and ergosterol concentrations were found in April or May, which indicates that there is no or small direct physical destruction due to the plow. Therefore, observed patterns are more likely a result of indirect tillage effects such as turning of the soil upside down or lower root biomass in the younger grassland.

The data also revealed a high temporal variation in the permanent grassland in aggregate distribution (especially in the large macroaggregates) and in ergosterol concentration. This confirms that not only the regular cultivation of soils (e.g., arable soils with regularly tillage, crop growth and harvest) affect variations; also environmental factors such as increased rainfall or drought may have a large influence especially on soil aggregates.

8. Zusammenfassung

Um einen gleichbleibend hohen Ertrag in Grünland zu erzielen, ist es in der Praxis eine gängige Methode, das Grünland regelmäßig zu erneuern. Vor allem im ökologischen Landbau, in dem der Einsatz von Herbiziden verboten ist, ist die physikalische Grünlanderneuerung mittels eines Pflugereignisses eine gängige Praxis. Dauergrünland wird ganz oder teilweise erneuert, wenn der Ertrag sinkt, beispielsweise durch erhöhten Unkrautdruck, Rückgang hochwertiger Futtergräser, Bodenverdichtung oder einer beschädigten Grasnarbe (Trockenschäden, Frostschäden).

Wenn in Grünland ein Pflugereignis stattfindet, wird die bestehende Vegetation zerstört, eine erhöhte Mineralisierungsrate ist die Folge. Erhöhte Mineralisierungsraten gehen einher mit einer erhöhten Nährstoffverfügbarkeit. Allerdings kann dies auch zu einem Verlust an Nährstoffen führen, wenn die mineralisierten Nährstoffe die Nachfrage von dem neu angesäten Grünland übersteigen. Dies ist vor allem bei Stickstoff (N) zu beobachten, bei dem ein Verlust durch Nitratauswaschung (NO_3) oder Lachgasemissionen (N_2O) nach einem Pflugereignis in Grünland beobachtet werden kann (Davies et al., 2001; Djurhuus and Olsen, 1997; Webster et al., 1999; Vertès et al., 2007). Solche Stickstoffverluste bedeuten ein erhöhtes Umweltrisiko und gleichzeitig auch finanzielle Verluste für den Landwirt. Neben Nährstoffen kommt es auch zum Verlust an Kohlenstoff (C) im Boden, was durch erhöhte Kohlenstoffdioxidemissionen (CO_2) belegt werden kann (Eriksen und Jensen, 2001).

Erhöhte Mineralisierungsraten können der Einarbeitung des Grünlandbestandes zugeschrieben werden (Sheperd et al., 2001). Darüber hinaus bewirkt das Pflügen eine erhöhte Durchlüftung des Bodens und eine Zerstörung der Bodenaggregate (Six et al., 2002), wodurch zusätzlich eine große Menge an zuvor geschütztem organischen Material der mikrobiellen Biomasse zur Mineralisierung zur Verfügung steht. Somit hat das Pflügen in Grünland direkte Auswirkungen auf die Dynamik von Bodenaggregaten und organischem Material (Umverteilung des Bodens, erhöhte Durchlüftung, Zugabe an organischem Material), aber auch indirekte Effekte

(Abnahme der Pflanzen- und Wurzelmasse im neu angesäten Grünland und dadurch geringere Nachlieferung an organischem Material, möglicherweise auch mit einer anderen Abbaubarkeit). Die direkten Auswirkungen des Pflügens auf die Bodenaggregate und das organische Material finden wahrscheinlich direkt oder kurz nach dem Pflugereignis statt, während die indirekten Effekte vermutlich eine längere Zeitspanne benötigen. Von daher ist es notwendig, sowohl die Kurzzeit- als auch die Langzeiteffekte eines Pflugereignisses in Grünland zu untersuchen, was in dieser Arbeit durchgeführt wurde.

Die Ziele dieser Arbeit umfassen:

i. Untersuchungen der Langzeiteffekte eines Pflugereignisses in Grünland auf Vorräte von organischem C (C_{org}), wasserstabilen Bodenaggregaten und der organischen Bodensubstanz (OBS).
ii. Untersuchungen der Langzeiteffekte einer temporären Umwandlung von Grünland in Ackerland auf die oben genannten Parameter.
iii. Quantifizierung der kombinierten Effekte einer temporären Umwandlung von Grünland in Ackerland und einer organischen Düngung auf die C-, N- und Phosphor- (P) Dynamiken.
iv. Vergleich von Böden mit verschiedenen Bewirtschaftungsvorgeschichten (Ackerland und Grünland) auf die zeitlichen Veränderungen der C_{org}-Konzentrationen, Bodenaggregate und der mikrobiellen Biomasse innerhalb des ersten Jahres einer Grünlandetablierung.
v. Bestimmung von zeitlichen Schwankungen der C_{org}-Konzentrationen, Bodenaggregate und der mikrobiellen Biomasse in einem Dauergrünland.

Zur Bearbeitung dieser Ziele wurden Grünlandflächen des Versuchsgutes `Lindhof` der Universität Kiel in Norddeutschland beprobt. Die Jahresdurchschnittstemperatur dort beträgt 8,9 ℃ und der Jahresniederschlag 768 mm. Der lehmige sandige Boden ist relativ heterogen, die vorhandenen Bodentypen sind Braunerde, Parabraunerde, Pseudogley und Kolluvisol.

Die vorliegende Arbeit ist in drei Teile gegliedert:

Teil 1: Zeitliche Veränderungen in der Zusammensetzung der organischen Bodensubstanz und in der Aggregatverteilung nach einem Pflugereignis in Grünlandböden der gemäßigten Breiten.

Die zeitlichen Änderungen der organischen Bodensubstanz im Dauergrünland nach einem einmaligen Pflugereignis wurden noch nicht ausreichend untersucht. In diesem Teil der vorliegenden Arbeit wurden die Langzeiteffekte von sporadischem Pflügen auf die Vorräte an C_{org}, auf die Aggregatverteilung und auf die organischen Bodensubstanz (ermittelt mittels Dichtefraktionierung) untersucht (Ziele i und ii). Wir vermuteten durch das Pflugereignis eine Reduzierung der C_{org}-Vorräte, der Aggregatkonzentrationen und der OBS. Im April 2010 wurden für die Durchführung der Laboranalysen Bodenproben in drei Tiefenstufen zwei bzw. fünf Jahre nach einem Pflugereignis oder der temporären Umwandlung von Grünland in Ackerland entnommen. Die Behandlungen bestehen aus Dauergrünland (Kontrolle), Pflügen des Grünlandes gefolgt von einer Grünland-Neuansaat (G-G) und Pflügen des Grünlandes mit anschließender Ansaat von Winterweizen und der darauffolgenden Grünland-Neuansaat (G-W-G). Zwei Jahre nach dem Pflugereignis waren die C_{org}-Vorräte (korrigiert auf Unterschiede in der Lagerungsdichte) in den obersten 10 cm des Bodens in beiden gepflügten Behandlungen noch signifikant ($p \leq 0.05$) reduziert (die Vorräte betrugen 25,4 t C_{org} ha^{-1} im Dauergrünland, 16,8 t C_{org} ha^{-1} in G-G und 18,6 t C_{org} ha^{-1} in G-W-G in 1250 t Boden). Im gesamten beprobten Profil (0 – 40 cm) waren die C_{org} Vorräte in G-G allerdings nur 5% geringer und in G-W-G 4% höher als im Dauergrünland und nicht signifikant unterschiedlich. Die Konzentrationen an wasserstabilen Makroaggregaten (>250 µm) in der obersten Bodenschicht war in G-G um 10 g und in G-W-G um 9 g je 100 g Boden im Vergleich zum Dauergrünland reduziert. Die Aggregatgrößenklassen, die durch das Pflugereignis am meisten beeinflusst waren, waren die Aggregate >2000 µm und 250 - 1000 µm. Die OBS war in der obersten Bodenschicht in G-G um 31% und in G-W-G um 41% reduziert. Fünf Jahre nach dem Pflugereignis waren Unterschiede zwischen dem Dauergrünland und den gepflügten Behandlungen

in den C_{org}-Vorräten, Bodenaggregaten und der OBS noch sichtbar, allerdings waren die Unterschiede sehr viel geringer und nicht mehr signifikant.

Basierend auf diesen Ergebnissen kann davon ausgegangen werden, dass ein Pflugereignis in Dauergrünland C_{org}-Vorräte, wasserstabile Makroaggregate und die OBS negativ beeinflusst. Allerdings dauert dieser Effekt nur wenige Jahre an und resultiert nicht in langanhaltenden deutlichen Auswirkungen auf die genannten Parameter. Die zeitlich begrenzte Umwandlung von Grünland in Ackerland und ein zweites Pflugereignis haben keine zusätzlichen negativen Auswirkungen auf die untersuchten Parameter im Vergleich zu einem einmaligen Pflugereignis.

Teil 2: Auswirkungen von Bodenbearbeitung und organischer Düngung auf Kohlenstoff-, Stickstoff- und Phosphor-Pools in Grünlandböden der gemäßigten Breiten.

Düngung wie Bodenbearbeitung von Grünlandböden sind in der Lage C-, N- und P-Pools zu beeinflussen, jedoch konnten die vorhandenen Dynamiken und Langzeiteffekte noch nicht vollständig verstanden werden. Das Ziel dieses Teils der Arbeit ist die Untersuchung der Auswirkungen von sporadischem Pflügen und organischer Düngung in einem Dauergrünland der gemäßigten Breiten auf die Vorräte von C_{org}, Konzentrationen an OBS (bestimmt mittels Dichtefraktionierung) und wasserstabilen Aggregaten, Basalatmungsraten, Netto-N-Mineralisationsraten und Konzentrationen an Olsen-P (Ziel iii). Bodenproben wurden in drei Bodentiefen fünf Jahre nach dem Beginn des Versuches genommen. Die Behandlungen beinhalten Dauergrünland (DG) und eine temporäre Umwandlung von Grünland in Ackerland (Grünland-Pflügen-Winterweizen-Pflügen-Grünland; G-W-G). Die Parzellen wurden geteilt und erhielten entweder 240 kg N ha^{-1} in der Form von Rindergülle (DG_+, G-W-G_+) oder keine organische Düngung (DG_0, G-W-G_0). In der obersten Bodenschicht (0 – 10 cm), genauso wie im beprobten Profil (0 – 40 cm), resultiert das Pflügen und die eine Saison Winterweizen (DG_+ und G-W-G_+ vs. DG_0 und G-W-G_0) in einem Trend ($p < 0.1$) zu verminderten Vorräten an C_{org} und signifikant ($p <$

0.05) geringeren Vorräten an Stickstoff (N_t). Im Gegensatz hierzu verursachte die organische Düngung (DG_+ und G-W-G_+ vs. DG_0 und G-W-G_0) keine Änderungen an C_{org}- oder N_t-Vorräten, was darauf hinweist, dass Gülle-Applikationen in geläufigen Ausbringungsraten keinen deutlichen Einfluss auf Gesamtvorräte haben (Mittelwert von 73 t C_{org} und 7.4 t N_t ha^{-1} in 5413 t Boden). In der obersten Bodenschicht sind die labilen Pools allerdings deutlich beeinflusst: die Basalatmungsraten waren signifikant höher (32%) durch die Düngung und signifikant geringer (37%) durch das Pflügen und die Saison Winterweizen. Im Gegensatz hierzu war die freie leichte Fraktion (OBS, die frei im Boden vorliegt) in den obersten 25 cm des Bodens signifikant und 2,2- bis 2,5-fach durch die Düngung reduziert, was auf eine erhöhte mikrobielle Aktivität und dadurch einen erhöhten Abbau an labilem organischen Material deutet. Die Aggregatverteilung wurde durch die Düngung nicht beeinflusst. Vier Jahre nach dem letzten Pflugereignis fand man jedoch eine um 22% reduzierte Konzentration an großen Makroaggregaten (>2000 μm) in der obersten Bodenschicht. Die Netto-N-Mineralisation wurde weder durch die organische Düngung noch durch die Pflugereignisse beeinflusst. Die organische Düngung führte zu signifikant erhöhten Olsen-P-Konzentrationen in der obersten Bodenschicht und die Saison Winterweizen mit den Pflugereignissen führte zu signifikant geringeren Olsen-P-Konzentrationen in der tiefsten Bodenschicht (25 – 40 cm).

Im Großen und Ganzen zeigt dieser Teil der Studie, dass vier Jahre organische Düngung ausreichen, um in der obersten Bodenschicht die mikrobielle Aktivität anzuregen, was aus erhöhten Basalrespirationsraten geschlossen werden kann, sowie die Olsen-P-Konzentration zu steigern und die OBS zu verringern. Die Pflugereignisse vier Jahre vor der Probenahme und die temporäre Umwandlung von Grünland in Ackerland haben entgegengesetzte Auswirkungen auf die Basalrespirationsraten und Olsen-P-Konzentrationen und führt zusätzlich zu signifikant geringeren N_t-Vorräten und Konzentrationen von großen Makroaggregaten. Diese Effekte sind in den Parzellen mit Gülledüngung stärker ausgeprägt als in den Parzellen ohne Gülledüngung. Die Düngung und die Neuansaat des Grünlandes beeinflussen allerdings auch die Artenzusammensetzung der Vegetation, weswegen Verallgemeinerungen der direkten Effekte von

Pflugereignissen oder Düngung in Grünland nicht möglich sind.

Teil 3: Zeitliche Schwankungen der Kohlenstoffkonzentrationen, Aggregatverteilung und mikrobiellen Biomasse in Grünlandböden der gemäßigten Breiten

In Grünlandböden wurden das Vorkommen und die Größenordnung von Schwankungen in C_{org}-Vorräten und verschiedenen weiteren Fraktionen noch nicht ausreichend untersucht. In diesem Teil der vorliegenden Arbeit sollten die Vorräte von C_{org} und Konzentrationen von Bodenaggregaten, mikrobiellem C und Ergosterol in regelmäßigen Abständen für die Dauer eines Jahres in Grünlandböden untersucht werden (Ziel iv und v). Die Bodenproben wurden an sechs Terminen zwischen Oktober 2010 und Oktober 2011 in einer Bodentiefe von 0 – 40 cm entnommen. Die Behandlungen beinhalten Dauergrünland, Ackerland, welches im September 2010 in Grünland umgewandelt wurde (A-G), und das Pflügen von Grünland gefolgt von einer Grünland-Neuansaat im September 2010 (G-G). Im Dauergrünland waren die C_{org}-Konzentrationen und Vorräte bei den Probenahmezeitpunkten nicht verschieden, während die Aggregatverteilung und die Ergosterol-Konzentrationen signifikant beeinflusst waren. In den Aggregatfraktionen wurde die höchste Variabilität in der obersten Bodenschicht in den großen Makroaggregaten (>2000 µm) im Dauergrünland gefunden. Hier wurde die höchste Konzentration mit 67% im Oktober 2011 gemessen. Eine 3,2-mal geringere Messung im Mai 2011 konnte durch ein Austrocknen des Bodens erklärt werden. Die Umwandlung von Ackerland in Grünland und dadurch die Abwesenheit von regelmäßiger Bodenbearbeitung führte zu einer stetigen Zunahme an mikrobiellem C (C_{mik}; 1,4-fach) und Ergosterol (3,3-fach) in der obersten Bodenschicht im ersten Jahr. Die Konzentrationen und Vorräte an C_{org} wurden im ersten Jahr nach der Umwandlung noch nicht beeinflusst. Ein einmaliges Pflugereignis in Grünland führte in der obersten Bodenschicht zu signifikant geringeren C-Konzentrationen und Vorräten von April 2011 bis Oktober 2011 im Vergleich zum Dauergrünland. Wenn man allerdings das beprobte Profil auf Unterschiede in C_{org}-Konzentrationen und -Vorräten vergleicht, findet man innerhalb des ersten Jahres nach dem

Pflugereignis keinen signifikanten Unterschied. Ähnliche Muster zeigen die großen Makroaggregate, sowie die mikrobiellen C- und Ergosterol-Konzentrationen. Dies deutet darauf hin, dass Pflugereignisse in Grünland lediglich einen Einfluss auf die oberste Bodenschicht besitzen.

Man kann aus diesem Teil der Studie schlussfolgern, dass die Aggregatverteilung und die Konzentrationen an C_{mik} und Ergosterol Schwankungen während des Jahres unterliegen, während die C_{org}-Konzentrationen stabil bleiben. Die Schwankungen wurden in kürzlich angesätem Grünland sowie im Dauergrünland gefunden, was darauf hindeutet, dass nicht nur die Bodenbearbeitung Schwankungen verursacht, sondern auch Umweltfaktoren, wie beispielsweise Trockenheit oder starker Regenfall.

Danksagung

Zunächst möchte ich mich bei meinem Betreuer Prof. Dr. Ludwig für die stetige Unterstützung und Motivation bedanken, sowie für die Bereitstellung des interessanten Forschungsthemas, welches mir die Möglichkeit gab, diese Arbeit anzufertigen.

Für die Nutzung der Versuchsflächen möchte ich mich bei der Universität Kiel, Abteilung Grünland und Futterbau / Ökologischer Landbau, bedanken. Hierbei gilt mein besonderer Dank dem Team der Versuchsstation Lindhof sowie Herrn Prof. Taube und Herrn Dr. Loges.

Mein besonderer Dank gilt meinen Kollegen am Fachgebiet Umweltchemie, die mich stets unterstützten und mit wertvollen Diskussionen zum Gelingen dieser Arbeit beitrugen. Ich werde mich lange, mit einem Lächeln, an die regen Diskussionen über manch „elementare“ Themen unserer gemeinsamen Kaffeepausen erinnern!
Hierbei gilt mein Dank Dr. Daniel Geisseler für das Heranführen an das wissenschaftliche Arbeiten und die anschließende Betreuung während der Erstellung von Teilen dieser Arbeit. Ich möchte mich außerdem bei meinen Mit-Doktoranden Anja Sänger, Rouven Andruschkewitsch, Christiane Piegholdt und Shafique Maqsood für das Teilen von Freud und Leid des Doktoranden-Daseins bedanken. Für die unermüdliche technische Unterstützung sowohl im Labor als auch auf dem Feld möchte ich Anja Sawallisch meinen Dank aussprechen. Ein großes Dankeschön auch an Dr. Stefanie Heinze, Dr. Christel Ross und Dr. Michael Kaiser.

Außerdem bedanke ich mich für die fachgebietsübergreifende Hilfe von Gabi Dormann, Sabine Ahlers und Elsa Zwicker. Einen zusätzlichen Dank auch an alle „Grakos“ und Assoziierten, mit denen ich bei Kolloquien, Workshops, der Vorbereitung der Tagung und der Auslandsexkursion viele Stunden verbringen durfte.

Sebastian Opitz, Sarah Bender und Oska Bender möchte ich nicht nur für den warmen Ofen im Winter danken, sondern auch für ein immer offenes Ohr, welches mir beim Bewältigen von vielen Herausforderungen der Arbeit und des Lebens sehr geholfen hat.

Ich danke Johanna Pinggera für jedes Frühstück, jeden Tee und dafür, dass sie mich bei all den verrückten Dingen des Lebens begleitet hat.

An dieser Stelle möchte ich mich auch ganz herzlich bei meiner Familie bedanken, die mich stets unterstützt und mich durch viele Höhen und Tiefen meines Lebens begleitet hat.

Für das Ermöglichen dieser Arbeit durch die Finanzierung des Graduiertenkollegs 1397 möchte ich mich bei der Deutschen Forschungsgemeinschaft bedanken.

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt und andere als die in der Dissertation angegebenen Hilfsmittel nicht benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden.

Witzenhausen, April 2013

_____

(Deborah Linsler)