PHD THESIS

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Litter quality, temperature, and soil water content as drivers of decomposition and respiration in a long-term tillage trial

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Dissertation

Submitted to the Faculty of Organic Agriculture Sciences of the University of Kassel to fulfill the requirements for the degree Doktor der Agrarwissenschaften (Dr. agr.)

by

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Dedication

To my sister Isabelle and my parents.

Preface

This project was supported by the Research Training Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture” (Graduiertenkolleg 1397/3) of the German Research Fundation (DFG). The cumulative thesis is submitted to the University of Kassel, Faculty of Organic Agricultural Sciences (FB 11), Department of Soil Biology and Plant Nutrition, to fulfil the requirements for the degree “Doktor der Agrarwissenschaften” (Dr. agr.) – “kumulative Dissertation am Fachbereich Ökologische Agrarwissenschaften der Universität Kassel”. The chapters 2, 3, and 4 are based on three manuscripts as first author, which are published in refereed international scientific journals. Chapter 1 comprises a general introduction to the research topics and the objectives of the thesis. Chapter 5 contains the general conclusions. Supplementary materials are given in chapter 6.

The following publications are included in the here presented cumulative thesis:

Chapter 2

Faust, S., Koch, H.-J., Joergensen, R.G., 2019. Respiration response to different tillage intensities in transplanted soil columns. Geoderma 352, 289-297.

Chapter 3

Faust, S., Koch, H.-J., Dyckmans, Joergensen, R.G., 2019. Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial. Applied Soil Ecology 141, 38-44.

Chapter 4

Faust, S., Kaiser, K., Wiedner, K., Glaser, B., Joergensen, R.G., 2018. Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant residues. Plant and Soil 433, 7-18.

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List of abbreviations

α	Significance level
a	Annum
AcBr	Acetyl bromide method for lignin determination
adj. R^2	Adjusted R^2
ADL	Acid detergent lignin
Alt.	Altitude
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
Ap	A horizon, which is directly affected by tillage
asl	Above see level
ASON	Acetosyingone (3,5-dimethoxy-4-hydroxyacetophenone)
AVON	Acetovanillone (4-hydroxy-3-methoxyacetophenone)
BAD	Benzoic acid
BBCH-Code	Identification key of phenological growth stages of a plant; BBCH: Biologische Bundesanstalt, Bundessortenamt, Chemische Indurstie
BSTFA	Bis-(trimethlysilyl)-triflouroacetamide
C	Carbon (element)
$C4C_{sample}$, $C3C_{sample}$	Content of maize derived C and C3 plant-derived C, respectively, in the analysed sample
C4-MBC, C3-MBC	Maize-derived MBC and C3 plant-derived MBC, respectively
C4-MRC	Content of maize-derived microbial residue C
C4-POM, C3-POMC	Content of maize derived and C3 plant derived, respectively, particulate organic matter C
C4-SOC; C3-SOC	Content of maize and C3 plant derived SOC, respectively
C_{fum}	Content of K_2SO_4-extracted C of fumigated soil
C_{nonfum}	Content of K_2SO_4-extracted C of not fumigated soil
Ct_{sample}	Content of total C in the analysed sample, i.e. the sum of C3 and C4
C3 plant; C4 plant	Plant with C3 and C4 pathway, respectively, for C fixation in photosynth.
C	Cinnamic units, i.e. p-coumaric, ferulic acid
°C	Degree Celsius
CAD	p-coumaric acid
CFE	Chloroform fumigation extraction
$CHCl_3$	Chloroform
CIRAS	Combined infrared gas analysis system
cm	Centimetre
CO_2	Carbon dioxide
CUE	Microbial carbon use efficiency
CUE_{TP}	CUE of the total microbial products
CuO	Cupric oxide oxidation method for determining lignin concentration
CV	Coefficient of variation
cv.	Cultivar
d	Day
$\delta^{13}C$	$^{13}C/^{12}C$ ratio expressed relative to the PDB standard
$\delta^{13}C_{control}$	Isotopic value of the respective fractions without maize leaf litter

$\delta^{13}C_{fum}$	Isotopic value of the K_2SO_4-extracted C of the fumigated soil samples
$\delta^{13}C_{maize}$	Isotopic value of the maize leaf litter
$\delta^{13}C_{MB}$	Isotopic value of the microbial biomass
$\delta^{13}C_{nonfum}$	Isotopic value of the K_2SO_4-extracted C of the not fumigated soil samples
$\delta^{13}C_{sample}$	Isotopic value of the respective fractions with maize leaf litter amendment
DFG	Deutsche Forschungsgemeinschaft; German Research Fundation
diam.	Diameter
DiOHBAD	3,5-dihydroxybenzoic acid
DM	Dry matter
DW	Dry weight
E	East
E_C	(Organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils)
e.g.	Exempli gratia
EPS	Extracellular polymeric substances
Establ.	Establishment
et al.	et alii (and others)
FAD	Ferulic acid
FAO	Food and Agriculture Organization of the United Nations
$Fe(NH_4)_2(SO_4)_2 \times 6\ H_2O$	Ammonium iron (II) sulfate hexahydrate
Fig.	Figure
FR	Friemar (Thuringia)
g	Gramm
g	force of centrifuge
GCMS	Gas chromatograph with mass-sensitive detector
GR	Grombach (Baden-Wuerttemberg)
h	Hour
ha	Hectare
HAD	Heptadecanoic acid
HCl	Hydrochloric acid
H_2O	Water
HPLC	High performance liquid chromatography
H_2SO_4	Sulfuric acid
i.e.	Id est
IfZ	Institut für Zuckerrübenforschung (Engl.: Institute of Sugar Beet Research, Göttingen, Germany)
IRGA	Infrared gas analyser
IRMS	Isotope-ratio mass spectrometry
k	Decay constant; mass loss rate constant
k_{EC}	Extractable part of microbial biomass C after fumigation
kg	Kilogram
K_2SO_4	Potassium sulphate
L.	Linné
ln	Logarithmus naturalis (ln-transformed data)
LÜ	Lüttewitz (Saxony)
m	Metre
M	Molarity
MBC, MBN	Microbial biomass carbon and nitrogen, respectively

mg	Milligram
µg	Microgram
min	Minute
ml	Millilitre
µl	Microlitre
mm	Millimetre
µm	Micrometre
mmol	Millimole
MRC	Microbial residue C, i.e. the C derived from non-biomass microbial materials (metabolites + necromass)
MRT	Mean residence time
N	North
N	Nitrogen (element)
N_2	Molecular nitrogen
NaOH	Sodium hydroxide
NaCl	Sodium chloride
n	Number of samples
nm	Nanometre
NS	Not significant
OH goup	Hydroxy group
OHBAD	3-hydroxybenzoic acid
OHBAL	Hydroxybenzaldehyde
OHAPON	4-hydroxyacetophenone
P	Probability value for significance
PAD	Phthalic acid
pH	Potentia hydrogenii; pondus hydrogenii
POM	Particulate organic matter
POMC; POMN	Carbon and nitrogen, respectively, in the particulate organic matter
$POM^{13}C$ ($\delta^{13}C$ (‰))	Isotopic value of the particulate organic matter
ppm	Parts per million
Precip.	Precipitation
PVC	Polyvinyl chloride
qCO_2	Metabolic quotient
r	Correlation coefficient
r^2	Coefficient
rev min^{-1}	Revolutions per minute
s	Second
S	Syringic units, i.e. syringaldehyde, syringic acid, acetosyringone
S0	Beginning of the litterbag and soil bag experiments (sampling 0)
S3	Third sampling date of litterbags and soil bags
SAAD	Salicylic acid (2-hydroxybenzoic acid) (SAAD)
SAD	Syringic acid
SAL	Syringaldehyde
SOC	Soil organic carbon
$SO^{13}C$ ($\delta^{13}C$ (‰))	Isotopic value of SOC
$SO^{13}C_{t0}$	Maize-derived soil organic C content before burial (time 0)
$SO^{13}C_{ti}$	Maize-derived soil organic C content at the sampling date ti
SOM	Soil organic matter
sp.	Species
sqrt	Square root transformed data

t	Time between burial and sampling date
t	Tonne
Temp.	Temperature
T_S	Soil temperature
UV	Ultraviolet irradiation
V	Vanillic units, i.e. vanillin, vanillic acid, acetovanillone
VAD	Vanillic acid (4-hydroxy-3-methoxybenzoic acid)
VAL	Vanillin (4-hydroxy-3-methoxybenzaldehyde)
VIF	Variance inflation factor
V-PDB	Vienna Pee Dee Belemnite; Standard for $\delta^{13}C$ measurement
VSC	Sum of the vanillic, syringic and cinnamic units
v/v	Volume percent
VWC	Volumetric water content
wt.%	Weight percent
w/v	Weight by volume
w/w	Mass fraction
ZS	Zschortau (Saxony)

Summary

For sugar beet cultivation in most parts of central Europe especially water erosion is a serious problem, as for in the beginning of the growing season in late spring inherent to the cultivation system large soil areas of bare soil are left poorly protected up to the time when the plants are closing in the rows (beginning of crop covering; phenological growth stages of BBCH 31 - 39) (Koch et al., 2009). Non-inversion tillage systems are an effective measure for reducing erosion risks by diminishing soil compaction and increasing the water infiltration rate and cumulative seepage. However, a serious drawback is the considerable reduction of these systems in terms of sugar beet yield production, especially in the no-tillage treatment (Koch et al., 2009; Murugan et al., 2014). To investigate the effects of tillage intensity on all relevant aspects for sugar beet cultivation the German sugar industry in collaboration with the Institute for Sugar Beet Research (Göttingen/Germany) established a series of on-farm long-term tillage trials in the early 1990s at initially ten loessial study sites typical for sugar beet cultivation in southern and eastern Germany (Jacobs et al., 2015; Koch et al., 2009). The three tillage intensities were (1) annual mouldboard ploughing (25-30 cm depth), (2) grubber (10-15 cm), i.e. a rigid tine field cultivator, and (3) no-tillage, in a winter wheat - winter wheat - sugar beet crop rotation. In order to improve sugar beet establishment the seedbed of the no-tillage treatment was prepared to a depth of 3-5 cm before sugar beet sowing.

At the four sites where both, soil organic C (SOC) and microbial biomass C (MBC), were examined, no or only moderate increases in SOC stocks were assessed in the 0-30 cm soil layer of the grubber as well as in the no-tillage treatments, as compared to the treatment including ploughing of the land (Andruschkewitsch et al., 2013; Jacobs et al., 2015; Murugan et al., 2014), where, especially in terms of increases in MBC stocks (Murugan et al., 2014), the observed changes were quite strong. Under the assumption

that the C input into the soil is similar amongst the different tillage treatments, the higher MBC to SOC ratio in grubber and no tillage treatments indicates a lower microbial turnover (Murugan et al., 2014).

Non-inversion tillage systems may reduce soil temperature (T_S) as higher SOC contents are accumulating close to the surface (Heinze et al., 2010; Kaiser et al., 2014), which at the same time enables the soil to store more water. This leads to increased soil moisture contents (Abdullah, 2014; Frasier et al., 2016; Jacobs et al., 2011b; Silva-Olaya et al., 2013) in the uppermost centimetres of top soil and thus also boost to a certain extend the heat storage capacity (Frasier et al., 2016; Lakshmi et al., 2003). On the other hand lower T_S reduces microbial maintenance energy requirements and furthermore increases the microbial substrate use efficiency (Manzoni et al., 2012).

Three distinct studies were conducted. The common objectives for study 1 and 2 were to investigate the dependencies between tillage intensity, volumetric water content (VWC), T_S, as well as soil respiration and beyond that to draw conclusions about the microbial turnover from the experimentally generated data sets gained within the three tillage treatments. Additionally, decomposition of maize (*Zea maize* L.) leave litter and microbial carbon use efficiency (CUE) were assessed in the context of a real farming surrounding at one site (Friemar/Thuringia) of the experimental trial in study 2 as to get a more detailed insight in the microbially mediated decomposition work with the goal of getting a more complete picture together. Study 3 deals with a methodological topic which is relevant for plant litter decomposition studies itself, i.e. the comparison of different methods for determining lignin concentration.

In study 1, i.e. "*Respiration response to different tillage intensities in transplanted soil columns*", undisturbed soil core samples were collected in PVC columns (30 cm diameter, 20 cm height) from four sites of the on-farm tillage experiments in southern and

eastern Germany (Koch et al., 2009) and transferred to the experimental field of the University of Kassel at Neu-Eichenberg (North Hessen) with the objective to exclude site-specific climatic variation having artless a vast influence on both soil moisture together with T_S. Carbon dioxide (CO_2) flux, T_S and VWC were measured over one year in either an unplanted period as well as in a period with growing maize (*Zea mays* L.).

At 5 cm depth, VWC was lowest with plough tillage throughout the year. At 15 cm depth, VWC was highest with grubber tillage during the utterly observed period with growing maize. Throughout the unplanted period, mean T_S was generally highest with grubber tillage. Whereas during the period with growing maize, the mean value in T_S at 5 cm of depth increased in the order no-tillage < plough < grubber and at 15 cm of depth in the order plough < grubber < no-tillage, respectively. Mean CO_2 flux measured and upscaled was 1.12 t CO_2-C ha^{-1} in the unplanted and 2.85 t CO_2-C ha^{-1} in the period with growing maize. A multiple linear relationship model showed that T_S and VWC together were able to explain 70.4% of the variance in CO_2 evolution rates in the unplanted and 37.2% in the period with growing maize. T_S effects generally dominated and resulted similar regression coefficients for both periods. VWC had smaller effects on CO_2 evolution, which were positive for the unplanted period and negative for the period with growing maize. Significant tillage × T_S interactions in the unplanted period and tillage × VWC interactions in the period with growing maize were observed. Interactions were caused by strong positive T_S effects with grubber tillage in the unplanted period as well as by strong negative VWC effects with plough tillage in the period with growing maize. The ratio of CO_2 flux to MBC was lowest under no-tillage, indicating a slower microbial turnover and a more efficient substrate use of the soil microbial community. However, this was not observed in the grubber treatment, contrasting the hypothesis. The ratio of CO_2 flux to MBC measured in the field is described by the metabolic quotient qCO_2, an ecophysiological parameter which is ordinarily assessed under standardized conditions in

the laboratory and gives information on the catabolic requirements of a microbial community (Anderson and Domsch, 1989). Even though a small part of the CO_2 which was measured during the period with growing maize derived from autotrophic root respiration of the maize plants (~13%), the CO_2 to MBC ratio proofed to be useful to reflect a reduced MBC turnover in the no-tillage system, which probably led to the highest MBC content. Similar to other studies, the CO_2 to MBC ratio was negatively related to the MBC to SOC ratio.

In study 2, i.e. "*Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial*", VWC, T_S, and soil respiration were measured in a field experiment under practical farming conditions and with the above mentioned tillage treatments plough, grubber, and no-tillage. In contrast to study 1, soil from only one site was investigated, i.e. the field trial in Friemar/Thuringia. Furthermore, the decomposition of maize leaf litter was monitored for eight months in litterbags as well as in so called soil bags, i.e. mesh bags which were filled with an intimate mixture of autochthonous soil and non-decomposed, chopped maize leaves, which were recovered after a specific exposure time in the field as particulate organic matter (POM). The main objective was to determine which methodological approach is more suitable to reflect tillage effects on litter decomposition and microbial turnover.

Maize is a C4 plant and therefore bears higher $\delta^{13}C$ values than the autochthonous SOC mainly derived from C3 plants (Balesdent and Mariotti, 1996; Ryan and Aravena, 1994). Accordingly the conversion of maize substrate into MBC and SOC can be measured and the microbial C use efficiency (CUE) for complex substrates can be calculated (Joergensen and Wichern, 2018). Soil respiration, i.e. CO_2 flux, was monitored for a prolonged period of time of 22.5 months in the field to level the year specific and crop specific effects.

Under no-tillage it was found that CO_2 flux was lowest, even though this soil contained the highest amounts of soil organic C and microbial biomass C at a soil depth of 0-5 cm. One reason that could explain the finding is the slow warming of the soil in spring due to the highest VWC, leading to the lowest mean T_S. Continuously measured CO_2 evolution rates indicated a slower microbial turnover in the no-tillage (lowest CO_2 evolution) in comparison with mouldboard ploughing. The highest CO_2 evolution rate was found in the grubber treatment. Overall maize leaf litter was more rapidly decomposed in the soil bags, with a mean mass loss rate constant of $k = 0.0108\ d^{-1}$, than in the litterbags ($k = 0.0063\ d^{-1}$). Within the soil bags, the mass loss rate constant for maize leaf litter was significantly higher in the no-tillage treatment ($k = 0.0119\ d^{-1}$) compared to the plough treatment ($k = 0.0092\ d^{-1}$), contrasting the lowest CO_2 evolution rates in the no-tillage treatment. Low CO_2 evolution rates in general do indicate a slow microbial turnover. Hence no relationship could be found between the CO_2 evolution and the decomposition of added maize leaf litter, calculated as recovered maize leaf litter C in percent of the initially added maize leaf litter C. CO_2 evolution was highest in the grubber treatment. In contrast, the mean microbial C use efficiency of the maize leaf litter of 0.27 in the soil bags was not affected by tillage intensity. The reason for the most rapid maize leaf litter decomposition in the no-tillage treatment was probably the highest MBC content at 0-5 cm of depth. However, litter mass loss was only measured in the uppermost soil layer (1-6 cm) which was probably not sufficient to mirror the average turnover of the whole soil profile. Jacobs et al. (2011b) revealed a significant effect of soil depth on litter C loss.

Although both methods, i.e. the soil bag and the litterbag method, are appropriate for monitoring decomposition processes, the soil bag method has pertinent the big advantage that decomposing microorganisms have spatially unimpeded access to the litter right from the beginning on, which does probably better simulate the natural field conditions.

Summary

The quantification of lignin is vital in ecophysiological research (Brinkmann et al., 2002). Decomposition of plant material, compost, or mulch may be monitored or predicted by measuring certain key parameters in plant, or more general, organic material. Lignin is one major parameter amongst those key parameters (Rowland, Roberts, 1999). Beyond that one of the methods for lignin determination, i.e. the cupric oxide oxidation (CuO) method, gives additional information on the origin and composition of the lignin.

Acid detergent lignin (ADL), acetyl bromide (AcBr), and CuO methods for lignin determination have been developed largely independently in the different research disciplines and are nowadays often parallel in use, without sufficient knowledge on their possibilities and restrictions. Consequently, the central objective of study 3, i.e. "*Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant residues*", was to compare ADL, AcBr, and CuO methods for lignin determination and quality in organic tissue relevant for agricultural practice and in soil biological research.

Twenty-seven plant species and organic materials from different groups belonging to the legumes, crucifers, herbs and grasses along with also some trees species were analysed. Median lignin concentrations of the 27 organic and plant materials were 4.5% ADL and 6.0% AcBr lignin, significantly exceeding the median of 2.1% CuO lignin. ADL concentrations varied from 0.8 to 27.0%, those of AcBr and CuO lignin ranged from 1.8 to 12.2% and from 0.6 to 9.7%, respectively. AcBr lignin showed a significant negative, non-linear relationship with total N. In addition, the relationship of ADL and CuO data was negatively affected by total N. The primary advantage of the ADL method lies in its simplicity and well reproducibility. However, it is not recommended for litter from immature plants, where ADL results in underestimation of lignin. It is also not appropriate for litter that contains high concentrations of protein, cutins, waxes, and tannins, where the ADL method may lead to an overestimation of lignin. The AcBr procedure is less

interfered from non-lignin products compared to ADL. The CuO method is not interfered with by any other organic component in the plant material and gives additional information on the composition of the lignin. However, the release of phenolic units is probably incomplete, pointing to the relevance of the ongoing need to keep up the search for method improvements with regard to the digestion procedure.

Zusammenfassung

Für den Anbau von Zuckerrüben stellt in vielen mitteleuropäischen Anbaugebieten insbesondere die durch Wasser bedingte Bodenerosion eines der größten systemimmanenten Probleme dar, da weite Teile des Bodens bis zum Bestandesschluss (BBCH 31-39), also wenn über 90% der Pflanzen benachbarter Reihen sich berühren, unbedeckt bleiben (Koch et al., 2009). Nicht-wendende Bodenbearbeitung ist eine wirksame Maßnahme die Erosionsrisiken beim Rübenanbau zu mindern, indem gleichzeitig die Bodenverdichtung verringert und das kumulative Wasserinfiltrationsvermögen erhöht wird. Ein erheblicher Nachteil der pfluglosen Bodenbearbeitung ist, insbesondere bei Direktsaat, der Minderertrag bei Zuckerrüben (Koch et al., 2009; Murugan et al., 2014). Die deutsche Zuckerrübenindustrie (Südzucker AG, Ochsenfurt, Deutschland) hat daher in Zusammenarbeit mit dem Institut für Zuckerrübenforschung (IfZ, Göttingen, Deutschland) einen Langzeitversuch zur Bodenbearbeitung auf ursprünglich zehn Lössstandorten in typischen Zuckerrübenanbaugebieten im südlichen- sowie im östlichen Deutschland angelegt (Jacobs et al., 2015; Koch et al., 2009).

Die drei untersuchten Bodenbearbeitungsintensitäten waren jährliches Pflügen (25-30 cm Bearbeitungstiefe), Grubbern (10-15 cm) und Direktsaat (3-5 cm tiefe Bearbeitung zur Saatbettvorbereitung vor der Zuckerrübeneinsaat) in einer Winterweizen – Winterweizen – Zuckerrübenfruchtfolge.

An den Standorten, an welchen neben dem organischen Kohlenstoff auch die mikrobielle Biomasse gemessen wurde, wurden entweder keine oder nur moderate Zunahmen der Vorräte von organischem Kohlenstoff in einer Bodentiefe von 0-30 cm in der Direktsaat- und Grubbervariante im Vergleich zur Pflugvariante gemessen (Andruschkewitsch et al., 2013; Jacobs et al., 2015; Murugan et al., 2014), hingegen aber

eine starke Erhöhung der Vorräte des in der mikrobiellen Biomasse gebundenen Kohlenstoffs (Murugan et al., 2014). Unter der Annahme, dass die Einträge an organischem Kohlenstoff in Form von Ernterückständen, Wurzeln und Rhizodepositionen aufgrund der vergleichbaren Ertragshöhe in der Pflug- und der Grubbervariante in etwa gleich sein müssten, und in der Direktsaatvariante aufgrund des niedrigsten Ertrages am geringsten ausfallen dürften, deutet ein höherer Quotient von mirkrobiellem Biomasse-C zu organischem Kohlenstoff auf einen geringeren mikrobiellen Umsatz hin (Murugan et al., 2014). Nichtwendende Bodenbearbeitung führt zu einer niedrigeren Bodentemperatur, da ein höherer Gehalt an organischem Kohlenstoff (und somit Humus) in der oberen Bodenschicht (Heinze et al., 2010; Kaiser et al., 2014) die Bodenfeuchtigkeit erhöht (Abdullah, 2014; Frasier et al., 2016; Jacobs et al., 2011b; Silva-Olaya et al., 2013) und somit grundsätzlich auch die Wärmespeicherkapazität (Frasier et al., 2016; Lakshmi et al., 2003). Niedrigere Bodentemperaturen verringern weiterhin den Erhaltungsenergiebedarf der mikrobiellen Biomasse und erhöhen deren Substratnutzungseffizienz (Manzoni et al., 2012).

Es wurden insgesamt drei verschiedene Studien durchgeführt. In den Studien 1 und 2 wurden die Abhängigkeiten zwischen Bodenbearbeitungsintensität, dem volumetrischen Wassergehalt, der Bodentemperatur und der Bodenatmung untersucht. Daraus wurden Rückschlüsse auf den mikrobiellen Umsatz in den drei Bodenbearbeitungsvarianten gezogen. Studie 3 befasst sich mit einer methodischen Frage, die im Zusammenhang mit Untersuchungen zum Abbau von Pflanzen- bzw. Ernteresten relevant ist, nämlich der Messung von Lignin mittels drei unterschiedlichen Methoden.

Studie 2 beschränkt sich auf nur einen Versuchsstandort, nämlich auf das in Friemar/Thüringen angelegte Versuchsfeld, aber dafür wurde diese Studie unter den gegebenen landwirtschaftlichen Management- und natürlichen Standortbedingungen

durchgeführt. Zusätzlich zu den oben genannten Messgrößen wurden der Abbau von Maisblattstreu und die mikrobielle C-Substratnutzungseffizienz bestimmt.

Für Studie 1, "*Respiration response to different tillage intensities in transplanted soil columns*", wurden ungestörte Bodenproben in PVC-Säulen (30 cm Durchmesser, 20 cm Höhe) von vier im östlichen und südlichen Deutschland gelegenen Feldern des Langzeitbodenbearbeitungsversuches (Koch et al., 2009) gezogen und auf die Versuchsfläche der Universität Kassel in Neu-Eichenberg (Nordhessen, nahe Witzenhausen) verbracht, sodass dieselben Standortbedingungen (z.B. Witterung, Klima, Untergrundboden) auf die Böden einwirkten.

Das aus dem Boden entweichende CO_2, die Bodentemperatur und der volumetrische Wassergehalt wurden wöchentlich ein Jahr lang während einer Periode ohne jegliches Pflanzenwachstum und während einer Periode mit Wachstum von Mais (*Zea mays* L.) gemessen.

In 5 cm Bodentiefe war der volumetrische Wassergehalt in der Pflugvariante im Schnitt über das ganze Jahr am niedrigsten. In 15 cm Bodentiefe war der Wassergehalt in der Grubbervariante während der mit Mais bewachsenen Periode am höchsten. Während der Periode ohne Mais war die durchschnittliche Bodentemperatur in der Grubbervariante am höchsten. In der Periode mit Mais stiegen die mittleren Bodentemperaturen in 5 cm Bodentiefe in der Reihenfolge Direktsaat < Pflug < Grubber an; in 15 cm Tiefe stiegen sie in der Reihung Pflug < Grubber < Direktsaat an. Die mittleren kumulativen CO_2-Emissionen betrugen 1,12 t CO_2-C ha^{-1} in der Periode ohne Mais und 2,85 t CO_2-C ha^{-1} in der Periode mit Mais. Die Regressionen zeigten, dass Bodentemperatur und volumetrischer Wassergehalt 70,4% der Varianz der CO_2-Raten in der Periode ohne Mais und 37,2% in der Periode mit Mais erklärten. Die Bodentemperatur hatte generell einen stärkeren Effekt auf die CO_2-Raten als der volumetrische Wassergehalt und zeigte in beiden Perioden ähnliche Regressionskoeffizienten. Die geringeren Effekte des

volumetrischen Wassergehalts hatten in der Periode ohne Mais einen positiven und in der Periode mit Mais einen negativen Einfluss auf die Bodenatmung (CO_2-Freisetzung). Signifikante Wechselwirkungen zwischen der Bodenbearbeitungsintensität und der Bodentemperatur gab es in der Periode ohne Mais. Weiterhin wurden ebenfalls signifikante Bodenbearbeitungs- × Wassergehalts-Wechselwirkungen in der Periode mit Mais gefunden. Diese Wechselwirkungen wurden durch die stark positive Wirkung der Bodentemperatur in der Grubbervariante während der Periode ohne Mais und die stark negative Wirkung des Wassergehalts in der Pflugvariante während der Periode mit Mais erzeugt. Der Quotient von kumulativer CO_2–Freisetzung zu mikrobiellem Biomasse-C war am niedrigsten bei Direktsaat, was ein Hinweis darauf sein könnte, dass dort im Vergleich zu den anderen beiden Varianten der mikrobielle Umsatz geringer und die Substratnutzungseffizienz der mikrobiellen Gemeinschaft höher ist. Allerdings war der Quotient von kumulativer CO_2–Freisetzung zu mikrobiellem Biomasse-C wider Erwarten in der Grubbervariante nicht geringer als in der Pflugvariante. Der Quotient von kumulativer CO_2–Freisetzung zu mikrobiellem Biomasse-C im Feld ist vergleichbar mit dem metabolischen Quotienten, qCO_2, einer ökophysiologischen Messgröße, welche normalerweise unter standardisierten Bedingungen im Labor gemessen wird und Aussagen zum Katabolismus der Mikroorganismengemeinschaft erlaubt (Anderson and Domsch, 1989). Selbst wenn ein geringer Teil des während der Periode mit Mais gemessenen CO_2 aus der autotrophen Wurzelrespiration (~13%) stammt, erwies sich der Quotient von CO_2 zu mikrobiellem Biomasse-C als nützlich, um einen verringerten mikrobiellen Umsatz in der Direktsaatvariante festzustellen, welcher wiederum zu der höchsten mikrobiellen Biomasse in dieser Variante führte. Wie auch in anderen Studien war der Quotient von CO_2 zu mikrobiellem Biomasse-C negativ mit dem Quotienten von mikrobiellem Biomasse-C zu organischem Kohlenstoff korreliert.

In Studie 2, "*Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial*", wurden volumetrischer Wassergehalt, Bodentemperatur und Bodenatmung (CO_2-C) in einem Langzeit-Bodenbearbeitungsveruch auf einem der Versuchsstandorte unter Praxisbedingungen im Feld gemessen. Die Bodenbearbeitungsvarianten und die Fruchtfolge waren dieselben, wie die in Studie 1 genannten (Pflug, Grubber, Direktsaat). Im Unterschied zu Studie 1 wurden die Untersuchungen nur mit einem Versuchsboden an einem Standort durchgeführt, nämlich auf dem Versuchsfeld in Friemar/Thüringen. Außerdem wurde der Streuabbau von Maisblättern (*Zea maize* L.) über einen Zeitraum von 8 Monaten mit zwei unterschiedlichen Methoden vergleichend untersucht: nämlich mittels (1) Streubeuteln mit einer Maschenweite von 1 mm, die mit reinem Maisstreu gefüllt wurden, und (2) mit Netzbeuteln mit einer Maschenweite von 100 µm, die mit einem Gemisch aus autochthonem Boden und Maisstreu gefüllt wurden und im Folgenden als Boden-Streu-Beutel-Methode bezeichnet wird. Diese Maisstreu wurde als sogenannte partikuläre organische Substanz nach Ende der Expositionszeit im Feld wieder zurückgewonnen, in ein entsprechend ausgerüstetes Labor der Universität Kassel überführt und dort quantifiziert. Bei der Boden-Streu-Beutel-Methode kam die dem Boden innewohnende mikrobielle Zersetzergemeinschaft direkt und von Anbeginn an in Kontakt mit dem Maissubstrat, welches grundsätzlich stärker den natürlichen Gegebenheiten entspricht. Das vorrangige Ziel dieser Studie war es zu ergründen, welche der beiden Methoden besser den Einfluss der Bodenbearbeitung auf den Streuabbau und den mikrobiellen Umsatz widerspiegelt.

Mais hat als C4-Pflanze ein anderes C-Isotopenverhältnis und höhere δ^{13}C-Werte als der autochthone organische Kohlenstoff des Feldes, der zu allergrößten Teilen von C3 Pflanzen stammt (Balesdent and Mariotti, 1996; Ryan and Aravena, 1994). Aufgrund dessen konnte die Inkorporation von Maissubstrat in die mikrobielle Biomasse, den

direkten und indirekten Eintrag von maisbürtigem Kohlenstoff in den bereits vorhandenen organischen Kohlenstoffpool des Bodens sowie die Effizienz von Mikroorganismen in ihrer Nutzung von Mais als komplexem Substrat, quantifiziert werden (Joergensen and Wichern, 2018). Die Bodenatmung, also die CO_2-C-Freisetzung aus dem Boden, wurde über knapp zwei Jahre (22,5 Monate) gemessen, um Jahresschwankungen und mögliche Effekte durch die zwei aufeinanderfolgenden Kulturen Winterweizen und Zuckerrübe auszugleichen.

In der Direktsaatvariante war die Bodenatmung (CO_2-Emission) am niedrigsten, obwohl dort der organische Kohlenstoff und die mikrobielle Biomasse in 0-5 cm Bodentiefe am höchsten waren. Ein Grund dafür war wahrscheinlich die langsamere Erwärmung des Bodens aufgrund des höchsten volumetrischen Wassergehaltes, was die geringste Durchschnittsbodentemperatur zur Folge hatte. Die niedrigen CO_2-Raten in Direktsaat deuten auf einen langsameren mikrobiellen Umsatz im Vergleich zur Pflugvariante hin, was aber in der Grubbervariante nicht festgestellt werden konnte. Dort waren die kumulativen CO_2-Emissionen sogar am höchsten. Maisblattstreu wurde in den Boden-Streu-Beuteln mit einer mittleren Massenverlustkonstante von $k = 0{,}0108\ Tag^{-1}$ durchweg schneller abgebaut als in den Streubeuteln ($k = 0{,}0063\ Tag^{-1}$). In den Boden-Streu-Beuteln war die C-Massenverlustkonstante k in der Direktsaat- signifikant höher im Vergleich zur Pflugvariante ($k = 0{,}0119\ Tag^{-1}$ *versus* $k = 0{,}0092\ Tag^{-1}$), was im Widerspruch zu den niedrigsten CO_2-Raten bei Direktsaat steht, die auf einen niedrigen mikrobiellen Umsatz schließen lassen. Am höchsten waren die CO_2-Freisetzungsraten in der Grubbervariante. Es wurde somit kein Zusammenhang zwischen der Abbaugeschwindigkeit des Maisstreus und den CO_2-Raten oder den kumulativen CO_2-Emissionen gefunden. Die mittlere mikrobielle C-Nutzungseffizienz in den Boden-Streu-Beuteln betrug 0,27 und unterschied sich im Gegensatz zum Streuabbau in den Boden-Streu-Beuteln nicht signifikant zwischen den Bodenbearbeitungsvarianten. Der Grund für

den schnellen Streuabbau in der Direktsaatvariante ist vermutlich, dass die mikrobielle Biomasse in der oberen Bodenschicht (1-6 cm) am größten ist. Aber da der Streuabbau nur in dieser obersten Bodenschicht gemessen wurde, repräsentiert er sehr wahrscheinlich nicht eine über 0-30 cm Bodentiefe gemittelte Streuabbaurate. Gleiches gilt für den mikrobiellen Umsatz: So zeigten Jacobs et al. (2011b), dass die Bodentiefe durchaus einen signifikanten Effekt auf die Streuabbaurate hat.

Sowohl die Streubeutel- als auch die Boden-Streu-Beutel-Methode erwiesen sich als geeignet für das Monitoring von Streuabbauprozessen. Allerdings hat die Boden-Streu-Beutel-Methode, bei welcher die Streu von Anfang an mit der den Boden bewohnenden Zersetzergemeinschaft in direktem und engem Kontakt ist, den Vorteil, dass der Abbau sofort beginnen kann und somit die realen Feldbedingungen (zumindest wenn die Ernterückstände in den Boden eingearbeitet werden) besser nachzuahmen vermag.

Die Quantifizierung von Lignin ist ein wichtiger Baustein in der ökophysiologischen Forschung (Brinkmann et al., 2002). Der Abbau von Pflanzenstreu, Kompost oder Mulch kann durch Messung von bestimmten Schlüsselparametern des zu inkorporierenden Pflanzenmaterials prognostiziert werden. Lignin ist einer dieser Schlüsselparameter (Rowland, Roberts, 1999). Mit einer der in dieser Studie angewandten Methoden zur Messung von Lignin, namentlich mit der Kupferoxidationsmethode (CuO), kann man zusätzlich noch wertvolle Informationen über den Ursprung des Lignins und das Stadium der Zersetzung gewinnen.

Die in Studie 3, "*Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant residues*", durchgeführten drei Methoden, nämlich die (1) Säure-Detergenz-Lignin-Methode nach Van Soest (acid detergent lignin (ADL)), die (2) CuO-Methode und die (3) Acetylbromid-Methode (AcBr) haben sich weitgehend unabhängig voneinander in unterschiedlichen Wissenschaftsdisziplinen entwickelt und werden heutzutage parallel angewandt ohne

ausreichende Kenntnisse der Möglichkeiten und Unwägbarkeiten. Deshalb war es vorrangiges Ziel von Studie 3 die mittels der ADL-, AcBr- und CuO-Methoden erhobenen Ligninkonzentrationen von organischen Materialien, welche für die landwirtschaftliche Praxis und die bodenbiologische Forschung relevant sind, untereinander zu vergleichen.

Siebenundzwanzig pflanzenbasierte Materialien und Pflanzenarten unterschiedlicher Gruppen, darunter Leguminosen, Kreuzblütler, Kräuter, Gräser und Bäume, wurden auf ihre Lignin-, C- und N-Konzentrationen hin analysiert. Die Mediane der Ligninkonzentrationen der 27 analysierten Pflanzenmaterialien betrugen 4,5% für ADL und 6,0% für AcBr-Lignin, welche beide signifikant über dem Median von 2,1% gemessen als CuO-Lignin lagen. Die ADL-Konzentrationen bewegten sich in einem Bereich von 0,8 bis 27,0%, die von AcBr lagen zwischen 1,8 bis 12,2% und die von CuO-Lignin erstreckten sich von 0,6 bis 9,7%. AcBr-Lignin zeigte einen signifikant negativen, nicht-linearen Zusammenhang mit dem Gesamt-N (Gesamtstickstoff). Außerdem wurde der Quotient zwischen den ADL- und CuO-Messwerten negativ durch Gesamt-N beeinflusst.

Die ADL-Methode ist einfach und gut reproduzierbar. Allerdings ist sie nicht empfehlenswert zur Ligninmessung in unreifem Pflanzenmaterial, bei welchem die ADL-Methode zu einer Unterschätzung des Lignins führen kann. Ebenso wenig ist sie geeignet für Streu, welches viel Protein, Cutin, Wachse und Tannine enthält, da dort wiederum die ADL-Methode zu einer Überschätzung der Ligningehalte führt. Bei der AcBr-Methode hingegen wirken gelöste Produkte, die keine Derivate von Lignin sind, weit weniger störend. Die CuO-Methode schließlich wird durch keine organischen Komponenten des Pflanzenmaterials beeinträchtigt. Ein weiterer Vorteil ist, dass zusätzliche Informationen zum Ursprung des Lignins und zum Zersetzungsstatus abgeleitet werden können. Allerdings ist die Freisetzung der Phenoleinheiten sehr wahrscheinlich unvollständig,

sodass eine Optimierung des Aufschlusses von Lignin in detektierbare Lignineinheiten erstrebenswert wäre.

1. General introduction

1.1 Reduction of tillage intensity

In former times, tillage of agricultural soils has been predominantly executed by ploughing, i.e. by soil inversion (Holland, 2004). For maintaining soil fertility the agricultural management, which includes soil cultivation, needs to be site-adapted, to conserve soil structure, and should minimise erosion and compaction (Dieckmann et al., 2006). Depending on the biotic and abiotic site charatcteristics, continuous soil inversion may degrade soil structure leading to soil compaction and depletion of soil organic matter (SOM) (Holland, 2004). These soils are much more prone to water and wind erosion, which in some regions may result in desertification (Holland, 2004).

Measures to reduce tillage intensity

Soil ecosystem services are indispensable in arable land. A reduction of tillage intensity is one measure to preserve the quality of soils and their diverse functions. Measures for a reducted tillage intensity, also named conservation tillage, as well as no-tillage, also named direct drilling, are considered as agroecological practices for a sustainable agriculture (Wezel et al., 2014). The term conservation tillage conveys a wide variety of soil management measures which conserve soil moisture and diminish erosion by sustaining at least 30% of the soil surface covered by plant residues after drilling (Peigné et al., 2007). In general, conservation tillage involves soil management practices, which aim to minimise the disruption of soil structure, composition, and natural biodiversity, thereby reducing erosion, degradation, and water contamination (Holland, 2004). The working depth of conservation tillage is shallow and in most cases without soil inversion, like no-tillage (direct drilling) or shallow tillage (10-15 cm) with discs or rigid-tine cultivator (Peigné et al., 2007). In contrast, conventional tillage involves

inversion of the soil by mouldboard or disc plough or spading machine to a depth of 20-35 cm and less than 30% of crop residues are left on the soil surface after crop establishment (Peigné et al., 2007).

Development of reduced tillage and reasons for utilisation

Soil conservation techniques were developed in the USA to cope with soil loss and preserve soil moisture (Holland, 2004). First experiments with no-tillage were established in the US Corn Belt and the Great Plains in the late 50s and early 60s (Six et al., 2002). Because of the introduction of the herbicides atrazine and paraquat during these years, the adoption of no-tillage by the farmers progressed fast (Six et al., 2002). In the Great Plains, which is a semi-arid region, the main advantage was the high moisture preservation under no-tillage (Army et al., 1961; Black and Power, 1965; Smika and Wicks, 1968; Wiese and Army, 1958). In the Corn Belt, soil erosion control was a major incentive to implement no-tillage. In both regions, the economic advantage was a compelling reason for the adoption of no-tillage (Six et al., 2002).

In Central Europe, agriculture is under structural socio-economic change since some decades. A major reason is the high price pressure, which leaded to bigger agricultural work units. In order to carry out the work on time and cost efficiently, the reduction of tillage intensity gained more importance, as tillage management holds high saving potentials (Koch et al., 2009; Nail et al., 2007). The reduced costs for fuel, lubricants and machinery are perceived as a major benefit of no-tillage adaptation (Baker et al., 2007; Soane et al., 2012).

Physicochemical advantages and soil restoration

Conservation tillage is advantageous for the physicochemical soil properties as the organic mulch at the soil surface increases the organic matter content in the surface layer of the soil profile and promotes the aggregate stability (Franzluebbers, 2002b; Schjønning and Rasmussen, 1989) which reduces water erosion (Obalum et al., 2019).

Aggregation also provides habitat space for soil organisms as well as adequate oxygen supply to roots and soil organisms (Franzluebbers, 2002b). The soils under no-tillage and grubber tillage revealed higher yields of macro-aggregates, increased C contents of macro-aggregates, and more micro-aggregates within macro-aggregates in 0-5 cm soil depth compared to conventional tillage (Andruschkewitsch et al., 2014; Fuentes et al., 2012). Further, macro-aggregate turnover was higher under conventional tillage (Andruschkewitsch et al., 2014). Bruce et al. (1990) observed a positive relationship between soil organic carbon (SOC) contents and water stable aggregation. Aggregation increases the bearing capacity and trafficability and thus protects the soil against compaction (Dieckmann et al., 2006).

A higher aggregate stability raises the water infiltration rate and hence reduces run-off and soil erosion (Franzluebbers, 2002b)**.** Mitigation of run-off is also important to reduce negative off-site impacts on the environment, like the pollution of surface water with sediment, pesticides and nutrients (Franzluebbers, 2002b; Holland, 2004; Horn et al., 1995). Water infiltration was higher in soils from long-term no-tillage compared to conventional tillage treatment and thus also the subsequent water transmission and storage in soil (Franzluebbers, 2002b; Holland, 2004). Better drainage and increased water holding capacity reduces the extremes of water logging and drought, respectively (Holland, 2004). Furthermore, a high cumulative infiltration is fundamental to replenish aquifers, which poses a contribution to mitigate implications of climate change.

In contrast, a lack of plant residue cover and thus an exposure of the soil surface to high-intensity rainfall result in poor aggregation, crusting, reduced water permeability, and lower plant-water availability (Franzluebbers, 2002b). A soil management, which leads to SOC losses, increases the clay content in the Ap soil horizon (the part of the soil horizon which is directly affected by tillage) followed by a decline in rainwater infiltration and an insufficient water recharging of the plant root zone (Bruce et al., 1986, 1988).

It has been proved that long-term conservation tillage is a vital procedure to reclaim soil fertility, e.g. if applied at moderately and severely eroded fields where cotton (*Gossypium hirsutum* L.) was produced with intensive tillage for more than 150 years on sloping Southern Piedmont soils (USA) (Langdale et al., 1992). Restoration processes were initiated by increasing the SOC content in the upper 0-1.5 cm soil layer of the soil profile, which were accompanied by a rise of soil nitrogen (N), water-stable aggregation, infiltration, and a considerable reduction of the run-off (Langdale et al., 1992).

Soil erosion

In Europe, water erosion is one of the most serious threats to soil fertility (Clemens and Stahr, 1994; Prasuhn, 2012). The risk of water erosion is especially high for soils of high silt content and in areas with steep slopes and unfavourable distribution of precipitation (Wegener, 2001). Fertile silt loams are suitable for sugar beet cultivation (Götze et al., 2016; Trimpler et al., 2017) where large soil areas are left uncovered in late spring (Koch et al., 2009). Therefore, erosion is a special problem for sugar beet cultivation. Long term conservation tillage into crop residues is beneficial in ameliorating soils prone to erosion (Langdale et al., 1992). More stable physical condition of the soil including reduced risks for soil erosion is one of the mostly described benefits of no-tillage (Baker et al., 2007; Soane et al., 2012). The accumulation of crop residues at the surface reduces soil erosion by buffering the soil surface against strong rainfall impact

(Langdale et al., 1992). This was also observed at the long term tillage trial established by the Institute of Sugar Beet Research (IfZ, Göttingen, Germany) were soil erosion almost completely disappeared in the grubber and no-tillage treatments (Dieckmann, 2008).

CO_2 saving potentials

In the past it was controversially discussed if reduced tillage intensity and no-tillage are effective management methods for SOC sequestration. Generally, the C-stratification is modified in reduced and no tilled fields: The top soils of fields under no-tillage (Fuentes et al., 2009; Six et al., 2002) and reduced tillage (Jacobs et al., 2015; Murugan et al., 2014) with residue retention reveal higher SOC contents and lower SOM decomposition rates (Six et al., 2002) compared to the top soils with conventional tillage (i.e. ploughing). When considering the whole soil profile, results have been less consistent or did not show an accumulation under conservation tillage measures at all (Barker et al., 2007; Jacobs et al., 2015; Ludwig et al., 2011). There are other methods like legume-based cropping systems which have been proved to be suitable to increase C (and N) stocks (Diekow et al., 2005). However, the application of minimum tillage management technics, which lead to a C-accumulation in the surface layer of the soil profile, has several other advantages like a significant reduction of soil erosion and conservation of moisture and nutrients (Franzluebbers, 2002a; Spargo et al., 2008). Additionally, the application of reduced tillage measures is an option to reduce greenhouse gas emissions as these procedures are less energy (fuel) consuming compared to ploughing (Nail et al., 2007).

Ecological aspects

In addition, also the edaphon benefits from a reduced tillage intensity. The abundance of earthworms and nematodes is higher under conservation tillage relative to conventional tillage (Birkas et al., 2004; Briones and Schmidt, 2017; Chan, 2001; Ehlers, 1975; Kladivko, 2001; Marwitz et al., 2012). The richer soil biota under conservation tillage can improve nutrient recycling, which may also help combat crop pests and diseases by enhancing the suppressive potential of the soil. Abundant crop residues and weed seeds improve food supply for insects, birds and small mammals (Holland, 2004). Furthermore, a higher soil biodiversity can develop under conservation tillage (Holland, 2004). For example, arbuscular mycorrhizal fungi (AMF) spore density and species richness increased in the top-soils under reduced tillage as compared to the ploughed plots in clay soils (Säle et al., 2015).

Challenges of non-inversion tillage systems in sugar beet

Generally, non-inversion tillage systems have been optimized and are well established for producing winter cereals (Epperlein, 2001) but have been less developed for sugar beet cultivation and showed negative yield impacts (Ahl et al., 1998; Heuer et al., 2006; Hoffmann, 1997; Koch et al., 2009; Tomanová et al., 2006). In the IfZ-long term field trial the cereal yields of the grubber and no-tillage treatments were almost similar to those of the plough tillage treatment after the first three crop rotations, each encompassing one year of sugar beet (*Beta vulgaris* L.) followed by two years of winter wheat (*Triticum aestivum* L.) (Dieckmann, 2008). However, sugar beet yield decreased in grubber and especially in no-tillage compared to plough (Koch et al., 2009). The yield gap between plough and grubber tillage to no-tillage was about 10-15 % (Koch et al., 2009; Murugan et al., 2014). A common reason besides others is the low plant density

(Pringas and Märländer, 2004; Richard et al., 1995). In the IfZ-trial plant density was also lower under reduced and no-tillage, whereas penetration resistance and bulk density increased and air filled pore volume decreased in the topsoil down to 0.27 m of depth (Koch et al., 2009). This in turn diminished the sugar beet yields, especially in no-tillage (Koch et al., 2009). Generally, ploughing could lead to an excessive looseness throughout the ploughing depth and the topsoil is highly susceptible to re-compaction. Further, a pan below the ploughing depth could occur (Soane et al., 2012). But also no-tillage bears a risk of topsoil compaction (Soane et al., 2012), particularly during the first year of transition from conventional to conservation tillage (Peigné et al., 2007). Additionally, high amounts of harvest residues, which cover the soil surface, lead to an inadequate embedding of the seeds (Dieckmann, 2008; Pringas and Märländer, 2004), which are lacking water for germination and which are more prone to damage from mice and slugs (Koch et al., 2009).

Other possible disadvantages of conservation tillage, especially in organic farming, are greater pressure from grass weeds and less suitable measures than ploughing for poorly drained, unstable soils or high rainfall areas. A restriction in N availability may occure and the crop choice could be limited (Peigné at al., 2007; Pringas et al., 2002). The abandonment of ploughing often involves an increased incidence of plant diseases and pests such as snails, slugs and mice, higher mycotoxin content in the grain and the need for a higher application of crop protection products (Pringas et al., 2002). Thus, the pressure of weeds and soil-borne pathogens may threaten in some cases the quantity or the quality of the harvests (Säle et al., 2015).

1.2 Long-term field experiments

Long-term agroecosystem experiments have a duration of over 20 years and aim to investigate crop production, nutrient cycling, as well as specific environmental impacts. The oldest long-term agroecosystem experiment was initiated by J. B. Lawes and J. H. Gilbert in 1843 at Rothamsted Experimental Station in England (Rasmussen et al., 1998). Whereas the aim of the first installed long-term experiments was to investigate the impact of certain management techniques on crop yields, later other research questions like sustainability, environmental impacts, species adaptation to changes and further issues were addressed (Rasmussen et al., 1998). SOM, estimated by quantifying the SOC contents, is perhaps the most important determinant of soil quality (Rasmussen et al., 1998), as increases in SOC provide several ecosystem services such as retention of nutrients and water, prevention of erosion and increased soil biodiversity (Christensen, 2001; Janzen, 2004; Lal and Kimble, 1997). As SOC reacts only gradually to changes in agricultural management such as crop rotation, fertilizer input, manure application, or tillage, sometimes soil C changes may require at least 20 years to be detectable by present analytical methods, which underpins the importance of long-term agro-ecosystem experiments (Rasmussen et al., 1998).

Long-term tillage trial of Südzucker AG and the Institute of Sugar Beet Research (IfZ)

In order to test the cultivation of sugar beet under minimum or no-tillage, an on-farm long-term field experiment was established by the sugar industry (agricultural division of Südzucker AG Mannheim/Ochsenfurt) in collaboration with the Institute of Sugar Beet Research (IfZ Göttingen) in the early 1990s on 10 loessial sites of typical arable regions for sugar beet cultivation in southern and eastern Germany (Jacobs et al., 2015; Koch et al., 2009). For the experimental fields, on which the first study (*Respiration response to different tillage intensities in transplanted soil columns*; Chapter 2) (Fig. 1.1 and 1.2), and the second study (*Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial*, Chapter 3) were based, the effects of the three tillage intensities (1) annual mouldboard ploughing to a depth of 25-30 cm, (2) grubber tillage (rigid-tine cultivator) to a depth of 10-15 cm, and (3) no-tillage (direct drilling) on several agronomic and (soil) biological as well as soil physical and chemical parameters had already been investigated (Fig. 1.3). As in the no-tillage treatment sugar

Fig. 1.1. Insertion of 48 columns from four sites and three treatments at the experimental station of Uni Kassel in Neu-Eichenberg.

Fig. 1.2. Weekly soil respiration, temperature and volumetric water content measurements of the soils in the columns grown with maize.

Fig. 1.3. Experimental site in Friemar/Thuringia with winter wheat on 25 October 2013; (a) plough tillage treatment, (b) grubber tillage treatment, (c) no-tillage treatment.

beet establishment repeatedly failed on large field spots, a seedbed cultivation to a depth of 3-5 cm was introduced before sowing (Koch et al., 2009).

Depending on the sampling and calculation procedure, no or moderate increases in SOC stocks were found in the non-inversion tillage treatments compared with mouldboard ploughing (Andruschkewitsch et al., 2013; Jacobs et al., 2009, 2015; Murugan et al., 2014), but strong increases in microbial biomass C (MBC) stocks (Murugan et al., 2014). Under the assumption that the C inputs in grubber and no-tillage did not exeed those of the plough treatment, an increased MBC to SOC ratio indicate a reduced microbial turnover in grubber and no-tillage. This is probably due to a higher SOC content in the upper soil layer which leads to an increased volumetric water content (VWC) that in turn reduces the warming of the soil. In order to investigate those dependencies, VWC, soil

Fig. 1.4. Biweekly measurements of soil respiration, temperature and volumetric water contents in the Friemar site; here in the culture of sugar beet.

Fig. 1.5. Sugar beet under plough tillage treatment on the left side and no-tillage treatment on the right side in the Friemar site.

Fig. 1.6. Alternating burial of soil bags and litterbags at the Friemar site on 16 October 2013.

temperature and soil respiration, as a parameter for microbial activity, were measured in study 1 (Chapter 2) and study 2 (Chapter 3).

In study 1, VWC, soil temperature and soil respiration measurements were taken weekly from November 2013 for one year. The data collected during a period without plant growing and a period of growing maize were analyzed separately. In study 2, VWC, soil temperature and soil respiration were measured every second week from 17 October 2013 until 2 September 2015, i.e. during 22.5 months, to level annual effects (weather conditions) and effects of the crops which were winter wheat in the first year (2013/2014) and sugar beet in the second year (2015) (Fig. 1.3, 1.4, 1.5).

1.3 Litterbag method and particulate organic matter (POM)

Litterbag method

For measuring the C-mass loss of plant residues in the field, the litterbag method developed by Bocock and Gilbert (1957) is a suitable approach (Joergensen et al., 2009). This method was used for litter decomposition in the organic layers of forest ecosystems (Berg, 2000; Joergensen et al., 2009; Potthoff and Loftfield, 1998) and for decomposition experiments with green manure and harvest residues in arable fields (Christensen, 1985; Knacker et al., 2003; Soon and Arshad, 2002). Litterbag experiments allow to study the effects of tillage management on decomposition (Burgess et al., 2002; House and Parmalee, 1985; Jacobs et al., 2011a, 2011b). Jacobs et al., (2011b) used litterbags to investigate degradation kinetics under different tillage treatments and in two depths. Furthermore, litterbag-plant material was investigated for amino sugars, neutral sugars, and lignin components (vanillyl, syringyl, and cinnamyl units), based on which rations were calculated, e.g. the acid/aldehyde ratios of vanillyl and syringyl units, and the ratios of syringyl/vanillyl and cinnamyl/vanillyl units, which give information on microbial

degradation (Jacobs et al., 2011a). The litterbag method was also used to study the effects of fertilization, different crops (Rottmann et al., 2011), and salinity (Muhammad et al., 2006) on decomposition of straw from several plant species. Generally, an important advantage of the litterbag method is the simple recovery of litter transferred to the field and the possibility of excluding specific groups from the decomposition process by choosing the mesh size (House and Parmalee, 1985; Knacker et al., 2003).

In a study to compare methods for the assessment of the effects of plant protection products on organic matter breakdown in arable fields, the litterbag method fulfilled all relevant criteria. It showed also distinct advantages when compared to the mini-container, cotton-strip and bait-lamina assays, and the application and tracking of stable isotopes (Knacker et al., 2003). However, the disadvantage of the litterbag approach is that the plant residues are not from the beginning on in a close contact to the autochthonous microbial decomposers of the soil, which may lower decompositions rates (Potthoff et al., 2005). Furthermore, the litter is sticking together in the bag, has higher water contents compared to the surrounding soil and is less aerated (Dunger and Fiedler, 1997). Hence, the C-loss rate of the litter determined by the litterbag method is slightly lower than that of the natural occurring litter when it is incorporated in soil by tillage measures.

In study 2, litterbags were applied to measure the effects of tillage intensity on the decomposition of maize leaf litter, more precisely, on the C mass loss rate constants. Further, the results of the litterbag method were compared with the so called litter-soil-bag method, where a mixture of litter and authochtonous soil was mixed and filled in nylon mesh bags with a mesh size of 100 µm (Fig. 1.6).

Particulate organic matter (POM)

Given the disadvantages of the litterbag method (see above) a second type of mesh bag was prepared to investigate the decomposition when soil and litter are in direct contact from the beginning on. Therefore, a defined amount of litter, i.e. 2% maize leaf litter related to soil DM, i.e. 3.08–3.35 g maize leaf litter DM, was mixed thoroughly with a defined amount of autochthonous soil (200 g fresh soil) and put into the 100 µm-mesh bag (6 cm×28.5 cm). Soil bags and litterbags were exposed simultaneously in the field and also recovered at the same time (after 3, 6, and 8 months). After the field exposure over these defined time spans, the soil bags were collected from the field and the litter within the soil bags was recovered as particulate organic matter (POM) according to Magid and Kjærgaard (2001). Jannoura et al. (2014) used the POM method to investigate the decomposition of different organic fertilizers even without bags. Aleklett and Wallander (2012) used mesh bags of nylon mesh (50 µm pore size) squares at a size of 10 cm × 10 cm, which were folded and filled each with 2.5 g organic amendments and 17.5 g sand to determine the conditions under which organic amendments stimulate AMF growth. Struecker et al. (2016) used small soil bags to analyse mechanisms controlling SOC in subsoil by measuring among others organic C associated with different soil density fractions. Soil samples (20 g) were mixed with 1.5 wt.% of maize roots. Sanaullah et al. (2011) also used this type of soil-filled bags where soil was mixed with ^{13}C and ^{15}N labelled wheat root material. These elements were tracked in different depths by physical SOM fractionation.

In study 2, the soil bag-POM method was used to investigate if the mean mass loss rate constants k and the mean microbial carbon use efficiencies (CUE) of maize leaf litter differed between the tested three tillage intensities. As a C4 plant maize (*Zea mays* L.) exhibits a higher $\delta^{13}C$ value than the autochthonous SOC, which is mainly derived from C3 plants (Balesdent and Mariotti, 1996; Ryan and Aravena, 1994). Therefore it

was possible to assess the incorporation of the maize substrate into MBC and SOC and to calculate the CUE for this complex substrate (Joergensen and Wichern, 2018).

1.4 Lignin

Decomposition of compost, mulch or plant material, like it was examined in study 2 for maize in litterbags and soil bags, may be monitored or predicted by measuring certain key parameters like the lignin content (Rowland and Roberts, 1999). The decomposition depends on the physicochemical environment (e.g. soil properties, climate), the initial litter quality, e.g. the concentration and composition of structural components like hemicellulose, cellulose, and lignin, and the decomposer community of microorganisms and soil animals (Knacker et al., 2003). The quantitative determination of lignin is vital in ecophysiological research (Brinkmann et al. 2002). Lignin is made up of aromatic compounds and contains various types of bondings leading to a stronger resistance to degradation at initial stages of microbial decomposition than other plant constituents (Derenne and Largeau, 2001; Glasser and Kelley, 1987; Sollins et al., 1996). The lignin units which are released during the cubric oxide oxidation (CuO) - one method for determining lignin - give information on the lignin origin and the microbial degradation state of plant materials (Jacobs et al., 2011a; Kalbitz et al., 2006). Further, these lignin units can be used as markers for land-use effects on vegetation and soil organic matter (e.g. Thevenot et al., 2010).

In many plant residues, high protein concentrations are related to low lignin concentrations, leading to low C/N ratios. This results in the often stated negative relationship between substrate C/N ratio and decomposition rate (e.g., Jacobs et al., 2011b). However, for N-free starch, pectin, hemicellulose, and cellulose the C/N ratio is not an appropriate parameter for predicting their decomposition rate, as they are all

rapidly decomposed nearly exclusively by soil fungi (Schneider et al., 2012). Therefore, the lignin/N ratio has been proposed as a more accurate indicator for decomposition rates than the C/N ratio (Lobe et al., 2002; Melillo et al., 1982), which reveals the importance of exact lignin quantification.

Increasing interdisciplinary research requires common links between livestock nutrition and soil biology (e.g. Jost et al., 2013), forest and invertebrate ecology (e.g. Harrop-Archibald, et al., 2016), soil biology and biogeochemistry (e.g. Dao et al., 2018), grassland ecology and biogas production (e.g. Hensgen et al., 2014) and a common understanding in the reliability of different methods for determining lignin concentration in plants. However, ADL, AcBr, and CuO as methods for lignin determination have been developed largely independently in the different research disciplines and are nowadays often parallel in use, without sufficient knowledge on their possibilities and restrictions. To the best of our knowledge, no comparison of these three methods for lignin determination in organic tissue relevant for agricultural practice and soil biological research has been conducted.

1.5 Objectives

Objectives of the first study "Respiration response to different tillage intensities in transplanted soil columns"

The study "*Respiration response to different tillage intensities in transplanted soil columns*" addressed the following questions: (1) Do higher SOC contents in the top layers of grubber and no-tillage systems increase the VWC in comparison with mouldboard ploughing? (2) Does an increased VWC reduce T_S, especially in the unplanted period? (3) Does lower T_S reduce the CO_2 efflux, which would indicate a lower microbial

turnover? (4) Are the relationships between SOC, VWC, T_S and CO_2 efflux affected by site-specific soil properties?

The objective was to measure the CO_2 flux from transplanted soil columns over one year in an unplanted period and in the period with growing maize, and to relate it to the MBC stocks.

Objectives of the second study "Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial"

As a comparison of the litterbag and the soil bag methods under field conditions does not exist to the best of our knowledge, the central objective of the second study "*Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial*" was to monitor the decomposition of maize leaf litter by these methods at one site of the long-term tillage trial, i.e. at Friemar/Thuringia. The objectives were to investigate if (1) the rates of litter decomposition differs between the soil bag and the litterbag methods, and (2) if a slower microbial turnover in the grubber and no-tillage treatments in comparison with mouldboard ploughing is reflected by slower decomposition rates of the maize leaf litter.

Objectives of the third study "Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant residues"

Because of the importance of lignin in ecophysiology, reproducible and exact methods for lignin determination are important. Therefore, in study 3, "*Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant residues*", the central objective was to compare three common methods of

lignin determination, i.e., ADL, CuO, and AcBr, in organic tissue relevant for agricultural practice and soil biological research.

2. Respiration response to different tillage intensities in transplanted soil columns

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Abstract

Undisturbed soils columns were transplanted from three tillage treatments at four sites in Central Germany to one site to investigate the relations between the CO_2 efflux, soil temperature (T_S) and volumetric water content (VWC) over one year in an unplanted period and maize (*Zea mays* L.) planted period. No-tillage and grubber, i.e. rigid-tine cultivator, (10-15 cm) systems contain higher stocks of microbial biomass C (MBC) in comparison with mouldboard ploughing (25-30 cm). This must be due to a reduction in microbial turnover, because higher VWC reduces T_S. At 5 cm depth, VWC was lowest with plough tillage throughout the year. At 15 cm depth, VWC was highest with grubber tillage during the planted period. During the unplanted period, mean T_S was generally highest with grubber tillage. During the planted period, mean T_S increased in the order no-tillage < plough < grubber at 5 cm depth and in the order plough < grubber < no-tillage at 15 cm depth. Mean CO_2 efflux was 1.12 t C ha^{-1} in the unplanted and 2.85 t C ha^{-1} in the planted period. Multiple linear relationships showed that T_S and VWC explained 70.4% of the variance in CO_2 evolution rates in the unplanted and 37.2% in the planted period. T_S effects generally dominated and showed similar regression coefficients in both

periods. VWC had smaller effects, which were positive in the unplanted period and negative in the planted period. Significant tillage × T_S interactions were observed in the unplanted period and tillage × VWC interactions in the planted period. Interactions were caused by strong positive T_S effects with grubber tillage in the unplanted period and by strong negative VWC effects with plough tillage in the planted period. From a soil ecological viewpoint, grubber and no-tillage can be recommended as it improves microbial life conditions.

Key words: Soil temperature; Soil moisture; CO_2 efflux; Microbial respiration; Maize growth

2.1 Introduction

For sugar beet cultivation, water erosion is a special problem, as large soil areas are left uncovered in late spring, during a period when heavy rainfall events regularly occur (Koch et al., 2009). For reducing erosion risks by increasing the water infiltration capacity, non-inversion tillage systems are an important option as they promote macro-aggregate formation (Andruschkewitsch et al., 2014; Fuentes et al., 2012) and earthworm abundance (Briones and Schmidt, 2017; Ehlers, 1975; Marwitz et al., 2012). However, non-inversion tillage systems have been less well developed for sugar beet cultivation due to several reports of negative yield impact (Koch et al., 2009). For this reason, the German sugar industry established an on-farm long-term field experiment in the early 1990s at different sites typical for sugar beet cultivation (Jacobs et al., 2015; Koch et al., 2009). This experiment exhibited no or only moderate increases in soil organic C (SOC) stocks (Andruschkewitsch et al., 2013; Jacobs et al., 2015; Murugan et al., 2014), but strong increases in microbial biomass C (MBC) stocks (Murugan et al., 2014). This is in

line with several other experiments in Ireland (van Groenigen et al., 2010), south Sweden (Hydbom et al., 2017) and Argentina (Frasier et al., 2016).

The on-farm long-term field experiment (Koch et al., 2009) compared mouldboard ploughing (25-30 cm) with grubber (10-15 cm), i.e. a rigid-tine cultivator, and no-tillage treatments (Jacobs et al., 2015; Murugan et al., 2014). These three tillage treatments had no significant effects on grain yield and only minor effects on sugar beet yield (Murugan et al., 2014; Jacobs et al., 2015), which were sometimes moderately declined in the no-tillage treatment. This suggests similar C input rates into the soil by the different tillage treatments, assuming a constant root to shoot ratio (Ludwig et al., 2007) and considering that the contribution of sugar beets to the C input is relatively low (Lavahun et al., 1996; Ludwig et al., 2007). In this case, the specific increase in MBC stocks must be due to a reduction in microbial turnover (Ussiri and Lal, 2009).

In spring, a retarded N mineralization has been repeatedly observed in non-inversion tillage in comparison with mouldboard ploughing (Rial-Lovera et al., 2016; Ruisi et al., 2016). This indicates a reduction in microbial turnover, although over-proportionately more mineralizable N is accumulated close to the soil surface (Sharifi et al. 2008). Substrate use efficiency and maintenance energy requirements are the two main components of the soil microbial turnover (Jenkinson et al., 1987; Joergensen and Wichern, 2018). Both factors are strongly affected by soil environmental conditions, e.g. by clay content, soil pH, soil temperature (T_S) and soil moisture (Allison and Goulden, 2017; Manzoni et al., 2012, 2016). Lower T_S reduces microbial maintenance energy requirements and increases their substrate use efficiency (Manzoni et al., 2012). Non-inversion tillage systems may reduce T_S as higher SOC contents close to the surface (Heinze et al., 2010; Kaiser et al., 2014) increase soil moisture (Abdullah, 2014; Frasier et al., 2016; Jacobs et al., 2011b; Silva-Olaya et al., 2013) and, thus, heat storage capacity (Frasier et al., 2016; Lakshmi et al., 2003).

In the field, microbial maintenance energy requirements can be estimated by measuring the CO_2 efflux from soil (Joergensen and Wichern, 2018). CO_2 evolution has been repeatedly measured in many studies of tillage effects, usually in combination with soil moisture and T_S assessments (Euster et al., 2010; Morell et al., 2010, 2011; Silva-Olaya et al., 2013). However, direct evidence is still missing that non-inversion tillage increases soil moisture and reduces T_S (Moyano et al., 2013), although indications exists that CO_2 efflux is lower in no-tillage treatment in comparison with mouldboard ploughing (Faust et al., 2019). However, the reverse has been repeatedly measured (Dong et al., 2017; Oorts et al., 2007).

The different sites of the on-farm long-term tillage experiments considerably differed in long-term annual mean air temperature and precipitation (Koch et al., 2009), which may mask the interrelated effects of SOC, volumetric water content (VWC), and T_s on the microbial turnover in the surface soil. To reduce site-specific climatic variation in the relationship between soil moisture and T_S, undisturbed soil columns were taken from four sites of the on-farm tillage experiment (Koch et al., 2009) and transferred to an experimental field close to Witzenhausen. The proximity to this research environment facilitated the use of hand-held devices for measuring CO_2 flux, soil moisture and T_S.

Soil transplantation has been used for investigating site-specific soil and climatic effects on microbial processes in forest soils (Berg et al., 1997; Raubuch et al., 1998), but not extensively (Bockheim and Gennadiyev, 2009), especially not in arable systems. However, the current study solely investigates site-specific soil effects and not climatic effects due to sparing reciprocal transplantation. In contrast to these previous forest soil transplantation experiments, maize (*Zea mays* L.) plants were grown on the current columns to admit plant transpiration effects on soil moisture and, thus, most likely T_S. The depth of the transplanted columns was restricted to 20 cm as possible SOC effects on soil moisture and temperature of the tillage treatments mainly occur in the top layer. This

is most likely also true for root growth. However, this depth restriction hampers further evaluating the C sequestration potential of the different tillage treatments.

The current study is based on the following hypotheses: (1) A higher SOC content in the top layer of grubber and no-tillage systems increases the VWC in comparison with mouldboard ploughing. (2) An increased VWC reduces T_S, especially in the unplanted period. (3) A lower T_S reduces the CO_2 efflux, indicating lower microbial turnover. (4) Site-specific soil properties do not affect relationships between CO_2 efflux, VWC, and T_S. The objective was to measure the CO_2 efflux from transplanted soil columns over one year in an unplanted period and maize planted period, which was finally related to the MBC stocks.

2.2 Material and methods

2.2.1 Tillage systems

Soil columns were taken from three tillage systems, which had been investigated as on-farm long-term field experiments at four sites (Friemar, Grombach, Lüttewitz and Zschortau) since the early 1990s by the Institute of Sugar Beet Research, Göttingen, Germany, in cooperation with Südzucker AG, Mannheim/Ochsenfurt (Jacobs et al., 2015; Koch et al., 2009; Murugan et al., 2014). At the four sites, mean annual temperature and precipitation ranged from 7.8 to 9.3°C and 512 to 776 mm, respectively. Site characteristics, soil pH and texture are given in Table 2.1.

At each site, three similar sized tillage treatment plots were formed on one large field with spatially homogeneous soil properties. The different tillage systems were: annual mouldboard ploughing to a depth of 25-30 cm, grubber tillage to a depth of 10-15 cm and no-tillage with direct drilling. Before sugar beet sowing, 3-5 cm deep seedbed cultivation was introduced in the no-tillage treatment to improve sugar beet crop establishment.

Table 2.1. Climatic and soil characteristics (3-27 cm) of the four experimental sites (Murugan et al., 2014).

Site	Establ.	Alt. (m)	Temp. (°C)	Precip. (mm)	Soil pH (H_2O)	Clay	Silt	Sand	Soil type (FAO, 2014)
						(g kg^{-1})			
FR	1992	310	7.8	517	8.1	290	680	30	Haplic Phaeozem
GR	1990	95	9.3	776	7.2	230	760	10	Haplic Luvisol
LÜ	1992	290	8.6	572	7.4	160	810	30	Haplic Luvisol
ZS	1997	110	8.8	512	7.6	140	530	320	Gleyic Luvisol

FR: Friemar, GR: Grombach, LÜ: Lüttewitz, ZS: Zschortau; Establ.: Establishment Alt.: Altitude; Temp.: Temperature; Precip.: Precipitation; pH: Soil pH.

Depending on the site, tillage plot size ranged from 2.5 to 8 ha per treatment. The crop rotation had consisted of sugar beet (*Beta vulgaris* L.), winter wheat (*Triticum aestivum* L.), and winter wheat at all sites for the past 15 years (Koch et al., 2009). White mustard (*Sinapis alba* L.) was sown after harvest of the second wheat as green manure. Crop residues were left on the field and sugar beet was sown in March–April using a single-seed drill adapted to crop residues lying on the soil surface. The crop management was carried out following the regional standards of agricultural practice, including the use of non-selective herbicides. Based on the infestation level between treatments, sugar beet selective herbicides, molluscicides and rodenticides were used (Koch et al., 2009). Application of N fertilizer varied between the sites but was identical for all treatments at one site. Information on mean annual crop yield and the mean annual N fertilization rate is provided by Murugan et al. (2014).

2.2.2 Soil transplantation and experimental layout

Undisturbed soil core samples were collected in PVC columns (30 cm diameter, 20 cm height) from the above mentioned four sites in August 2013 after winter wheat harvest and before soil tillage. At each site, four replicate columns per treatment were taken,

resulting in a total of 48 columns. The four sampling points formed a line in the middle of each treatment strip, with a distance of between 44 and 75 m from the field edge and a distance between 88 and 150 m between each sampling, depending on the length of each field. All columns were installed randomly in one row at the experimental site of the University of Kassel in Neu Eichenberg (51° 23′ N, 9° 55′ E, 220-250 m asl) to expose them to the same climate and weather conditions. The long-term average air temperature and precipitation were 8.7 °C and 625 mm, respectively.

A thin layer (~1 cm) of soil from the respective experimental site was spread beneath the soil columns to guarantee homogeneous porosity with a better water conductivity. All weeds growing on the soil columns were manually removed before measuring CO_2 evolution. To imitate real conditions in the field with regard to temperature and water conditions due to shading and water uptake, one maize plant per column was grown in May 2014. Maize was also sown in three rows around the row of soil columns (50 cm × 20 cm spacing). The maize plants were not fertilized. After one year in November 2014, the aboveground maize biomass of the plants grown in the columns was collected and dried at 60°C, and soil samples were taken at 0-10 and 10-20 cm depth. The samples were taken from the soil columns with a steel corer of 53 mm of diameter (Eijkelkamp Soil & Water, Giesbeek, The Netherlands) at a defined distance from the maize stem/plant, i.e. 8 cm and right at the position where the CIRAS gas chamber was placed for the CO_2-C measurements. Fresh soil samples were sieved (< 2 mm) and homogenized.

2.2.3 Soil respiration

Measurements were taken weekly from November 2013 for one year. Temperature and volumetric water content were measured at two depths in all 48 columns with portable devices: at 5 and 15 cm depth for temperature (calibrated digital electronic thermometer,

No. 620-0914, VWR, Darmstadt, Germany) and 2-8 and 12-18 cm for VWC (HH2 moisture meter-readout unit equipped with a ThetaProbe soil moisture sensor, type ML2x Delta-T Devices, England). Additionally, sensors were installed in nine columns with soils from Friemar, each treatment in three replicates, in which data for temperature (Th2-f, Fenwall Thermistor, UMS, Munich, Germany) and VWC (Theta-ML2x) were logged hourly at the two depths with a data logger (DL2e Data Logger, Delta-T Devices, Cambridge UK). The ML2x sensors were calibrated for each field and each treatment according to the manufacturer's manual to improve reliability. Correction factors for the T_S and VWC data collected by handheld devices were established separately for each treatment and each depth, using all measurements taken over the experimental period, i.e. regardless the season. The correction factors are based on the mean differences over the 46 different sampling days between logger data and those collected by the handheld devices. The error was systematic as both T_S and VWC data were biased in that way that data collected by handheld devices were higher than the simultaneously taken data by the loggers.

Soil respiration was also measured weekly concurrently to soil temperature and VWC measurements, using a single transportable infrared gas analyser CIRAS-1 (PP Systems, Hitchin, UK, Blanke, 1996). The measurements of the 48 columns were carried out at random, usually between 11:00 am and 2:00 pm, with times falling between 8:15 am and 6:25 pm to exclude systematic effects of daytime. The dynamic system consisted of a chamber (100 mm diameter, 150 mm height) coupled to a portable infrared gas analyser (IRGA) in a closed circuit. For CO_2 measurement, the steel ring at the bottom of the cylindrical chamber was pushed gently into the soil to avoid leaks.

For calculating the total amount of CO_2 evolved during the experimental period, the CO_2 evolution rate data expressed as mg CO_2-C m^{-2} h^{-1} were taken as representative for the whole day and for the whole period until the next measuring point (Jannoura et al.,

2014). The CO_2 evolution rate was corrected for the difference in air temperature between time of measuring at the sampling day and the daily mean air temperature (Terhoeven-Urselmans et al., 2009). The rate-modifying factor of the RothC model ($y = 47.9 / (1 + e^{(106/(T + 18.3))})$) was used for this temperature correction (Coleman and Jenkinson, 2005; Jenkinson et al., 1987). The main advantage of the current approach is that the actual rates were corrected to the daily mean temperature, as the measurement of 48 soil columns required roughly three hours or more. On sunny days, a significant shift in temperature occurred and probably in CO_2 evolution rate, which would be carried to the next respiration rate measurement if calculated by the moving average of simple linear interpolation (Dong et al., 2017).

2.2.4 Soil analysis

In soil samples, total C and total N were determined after combustion, using a Vario Max CN analyser (Elementar, Hanau, Germany). Inorganic C content was determined using the Scheibler method. After the addition of 10% HCl to the soil the evolving CO_2 was measured gas-volumetrically, using a Scheibler apparatus (Blume et al., 2011). SOC content was calculated as total C minus carbonate C. MBC was estimated by fumigation-extraction (Vance et al., 1987). Organic C concentrations in the 0.5 M K_2SO_4 extracts were measured using an automatic Multi N/C 2100S analyser (Analytik Jena, Germany). MBC was calculated as E_C/k_{EC}, where E_C = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Joergensen, 1996; Wu et al., 1990).

2.2.5 Statistical analysis

The results presented in the tables are the arithmetic means of the treatments and sites and expressed on an oven-dry basis (24 h at 105°C). Normality of distribution of the residuals of the dependent variables were tested by Shapiro-Wilk test accompanied by a graphical assessment of qq-plots. Data were ln-transformed for variables that violated the normality of distribution. The effects of the independent factors "treatment", i.e. the three tillage intensities plough, grubber, and no-tillage, and "site", i.e. the four long-term tillage field trials from which the samples were taken, were examined by a two-way analysis of variance (ANOVA) without blocking. Significant differences between the groups were detected by the Tukey-HSD post hoc test. P-values <0.05 were considered as significant. Multiple regression models were calculated between the CO_2 evolution rate as a dependent variable and T_S and VWC as independent variables. All regression models were tested for normality (Shapiro-Wilk), constancy of variance, absence of correlation between the residuals (Durban-Watson statistics) and absence of multi-collinearity, calculating the variance inflation factor (VIF), which did not exceeded 4.0. All statistical analyses were performed by SPSS 24 (IBM-SPSS Inc., USA).

2.3 Results

At the end of the experiment, plough tillage exhibited 17% lower SOC and 26% lower MBC stocks at 0-20 cm depth than grubber and no-tillage (Table 2.2), leading to a 12% lower MBC/SOC ratio. Differences between tillage treatments were mainly caused by the lower SOC (Fig. 2.1) and MBC (not shown) stocks at 0-10 cm in plough tillage. Highest SOC and MBC stocks were measured in the Friemar soil (Table 2.2), whereas the highest MBC/SOC ratio was observed in the Grombach soil. No significant

Table 2.2. Stocks of SOC and MBC, the MBC/SOC ratio at the end of the experiment and maize biomass at harvest.

	SOC (t ha^{-1} 20 cm^{-1})	MBC (kg ha^{-1} 20 cm^{-1})	MBC/SOC (%)	Maize biomass (g $plant^{-1}$)
Plough	34.7 B	655 B	1.9 B	15 B
Grubber	42.7 A	839 A	2.0 AB	23 A
No-tillage	41.2 A	938 A	2.3 A	21 A
Friemar	50.1 a	951 a	1.9 b	20 a
Grombach	34.0 b	847 ab	2.5 a	20 a
Lüttewitz	34.8 b	738 b	2.1 ab	20 a
Zschortau	39.2 b	707 b	1.8 b	18 a
Probability values				
Treatment	<0.01	<0.01	0.02	<0.01
Site	<0.01	<0.01	<0.01	NS
Treatment × site	NS	NS	NS	NS
CV (± %)	14	19	18	26

CV = mean coefficient of variation between replicate measurements (n = 4), different letters between treatments or sites indicate a significant difference ($P < 0.05$).

interactions occurred between tillage and site. Maize biomass was even 32% lower with plough tillage than with grubber and no-tillage, without significant differences between soils.

In the Friemar soil, continuous logger data of VWC varied between 11.2 and 43.1% at 5 cm depth (Fig. 2.2a) and between 29.2 and 39.7% at 15 cm depth (Fig. 2.2b). In the Friemar soil, discrete VWC hand-held device data were 19% higher at 5 cm depth and 6% at 15 cm depth than continuous logger data (Fig. 2.3). For this reason, VWC hand-held device data of the other three soils were corrected accordingly, assuming identical differences between the two methods for each soil. During the unplanted period, mean VWC varied around 26.8% at 5 cm depth and around 33.3% at 15 cm depth (Table 2.3). During the planted period, mean VWC varied around 28.7% at 5 cm depth and around

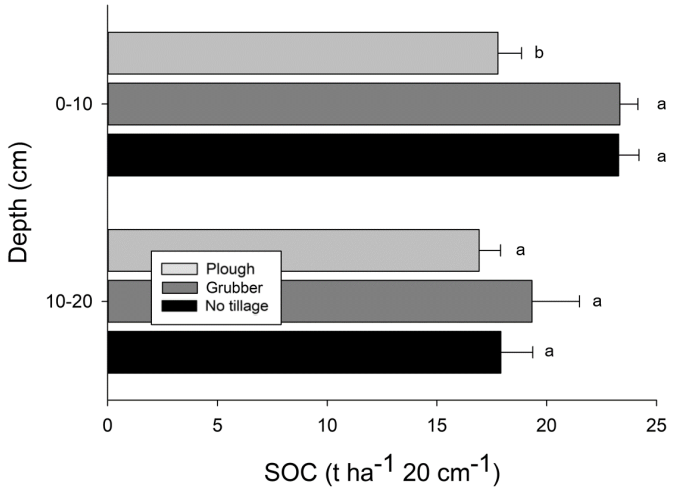

Fig. 2.1. SOC stocks at 0-10 and 10-20 cm depth in the three tillage treatments; different small letters indicate a depth-specific significant difference (Tukey test, P < 0.05).

33.1% at 15 cm depth. Mean VWC was lowest with plough tillage throughout the year at 5 cm depth; it was highest with grubber tillage during the planted period at 15 cm depth. Mean VWC was always significantly lowest in the Zschortau soil, leading to significant tillage × site interactions. Highest VWC was measured in Grombach soil, but the differences to the Friemar and Lüttewitz soils were not always significant.

In the Friemar soil, continuous logger data of T_S varied between 0.0 and 26.5 °C at 5 cm (Fig. 2.4) and between 0.5 and 24.0 °C at 15 cm depth. Mean air T ranged between -5.0 and 27.0 °C at 2 m height. In the Friemar soil, discrete T_S hand-held device data were 0.2 °C lower at 5 cm and 0.07 °C higher at 15 cm depth than continuous logger data (Fig. 2.5). For this reason, T_S hand-held device data of the other three soils were corrected accordingly, again assuming identical differences between the two methods for each soil. In the Friemar soil, the daily T_S fluctuations were strongest with grubber tillage at 5 cm (Fig. 2.6). During the unplanted period, mean T_S varied around 9.1 °C at 5 cm depth and around 7.0 °C at 15 cm depth and were always highest with grubber tillage (Table 2.4),

but the differences to the other tillage treatments were not always significant. During the planted period, mean T_S varied around 18.5 °C at 5 cm depth and around 15.8 °C at 15 cm depth. The difference in T_S increased in the order no-tillage < plough < grubber at 5 cm depth and in the order plough < grubber < no-tillage at 15 cm depth. Mean T_S was always highest in the Zschortau soil, leading to significant effects of "site". However, the differences to the soils of the other three sites were not always significant.

Mean cumulative CO_2 evolution varied around 1.12 t C ha^{-1} in the unplanted period and 2.85 t C ha^{-1} in the planted period and was highest with grubber tillage (Table 2.5). However, the differences were only significant in the planted period in comparison with plough tillage. Mean cumulative CO_2 evolution was highest from the Friemar soil throughout the year, although the difference was not significant to the Grombach soil in the unplanted period and to the Lüttewitz soil in the planted period. The CO_2-C/MBC ratio was approximately 30% lower with no-tillage in comparison with the other tillage treatments. Multiple linear relationships showed significant effects of T_S and VWC on the CO_2 evolution rate (Table 2.6), explaining between 64.5 and 74.8% of the variance in the unplanted period (Fig. 2.7a) and between 31.7 and 41.7% in the planted period (Fig. 2.7b). T_S effects generally dominated and showed similar regression coefficients in both periods (Table 2.6). VWC had smaller effects, which were positive in the unplanted period and negative in the planted period. Tillage had generally no significant main effect, but significant tillage × T_S interactions were observed in the unplanted period and tillage × VWC interactions in the planted period. Interactions were caused by strong positive T_S effects with grubber tillage in the unplanted period and by strong negative VWC effects with plough tillage in the planted period.

Table 2.3. Mean volumetric water contents without (11/16/2013-05/29/2014) and with (05/30/2014-11/11/2014) maize.

	Mean volumetric water content (%)			
	5 cm 11/16-05/29	5 cm 05/30-11/11	15 cm 11/16-05/29	15 cm 05/30-11/11
Plough	23.8 C	26.1 B	32.8 A	32.5 B
Grubber	27.1 B	29.9 A	34.2 A	34.2 A
No-tillage	29.4 A	30.0 A	33.0 A	32.5 B
Friemar	27.8 ab	29.2 a	34.4 ab	34.4 ab
Grombach	29.7 a	29.9 a	36.1 a	35.9 a
Lüttewitz	26.5 b	29.9 a	33.1 b	33.0 b
Zschortau	22.9 c	25.6 b	29.7 c	29.1 c
Probability values				
Treatment	<0.01	<0.01	NS	0.01
Site	<0.01	<0.01	<0.01	<0.01
Treatment × site	<0.01	<0.01	0.02	<0.01
CV (± %)	11	6.6	7.6	7.4

Hand-held device data, corrected by the logger data from Friemar, CV = mean coefficient of variation between replicate measurements (n = 4); different capital letters between treatments or different small letters between sites indicate a significant difference (Tukey test, $P < 0.05$).

Table 2.4. Mean temperature without (11/16/2013-05/29/2014) and with (05/30/2014-11/11/2014) maize.

	Temperature (°C)			
	5 cm 11/16-05/29	5 cm 05/30-11/11	15 cm 11/16-05/29	15 cm 30/05-11/11
Plough	9.07 AB	18.48 B	6.89 B	15.60 C
Grubber	9.26 A	18.85 A	7.01 A	15.77 B
No-tillage	8.81 B	18.17 C	7.00 AB	15.88 A
Friemar	8.93 b	18.54 ab	6.85 b	15.70 bc
Grombach	8.81 b	18.30 b	6.91 b	15.63 c
Lüttewitz	9.13 ab	18.53 ab	6.98 b	15.78 b
Zschortau	9.31 a	18.63 a	7.12 a	15.90 a
Probability values				
Treatment	<0.01	<0.01	<0.05	<0.01
Site	<0.01	<0.05	<0.01	<0.01
Treatment × site	NS	NS	NS	NS
CV (± %)	8.6	5.4	5.7	2.7

Hand-held device data, corrected by the logger data from Friemar, CV = mean coefficient of variation between replicate measurements (n = 4); different capital letters between treatments or different small letters between sites indicate a significant difference (Tukey test, $P < 0.05$).

Table 2.5. Cumulative CO_2 evolution without (11/11/2013-05/29/05/2014, i.e. 195 days) and with (05/30/2014-11/11/2014, i.e. 165 days) maize and the ratios of cumulative CO_2 evolution to SOC and MBC at the end of the experiment.

	CO_2-C (t C ha^{-1})		CO_2-C/MBC
	11/16-05/29	05/30-11/11	(mg CO_2-C g^{-1} biomass C d^{-1})
Plough	1.04 A	2.64 B	20.4 A
Grubber	1.27 A	3.20 A	19.5 A
No-tillage	1.05 A	2.72 AB	14.2 B
Friemar	1.33 a	3.59 a	19.1 a
Grombach	1.22 ab	2.50 b	16.4 a
Lüttewitz	0.97 b	2.82 ab	18.7 a
Zschortau	0.96 b	2.49 b	17.9 a
Probability values			
Treatment	NS	0.02	<0.01
Site	<0.01	<0.01	NS
Treatment × site	0.02	NS	NS
CV (± %)	20	17	20

CV = mean coefficient of variation between replicate measurements (n = 4); different letters between treatments or sites indicate a significant difference ($P < 0.05$).

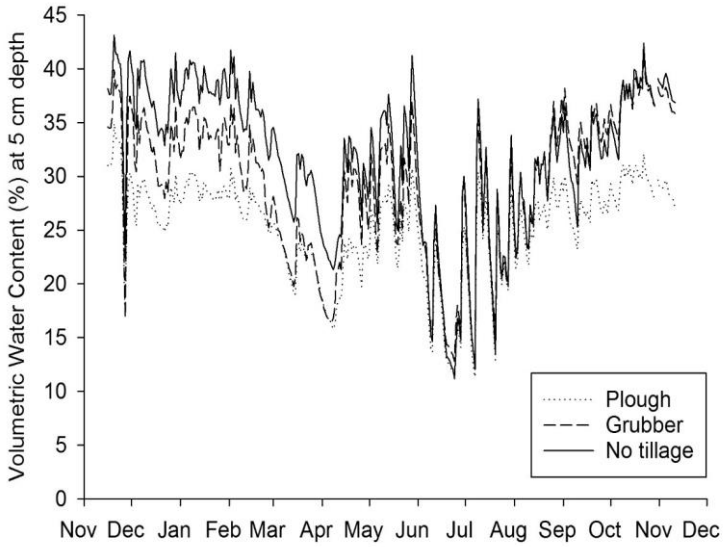

Fig. 2.2a.

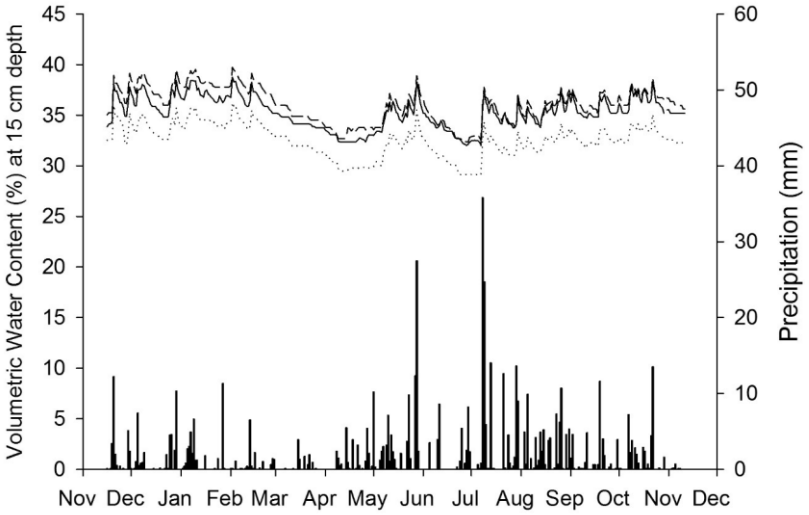

Fig. 2.2b.

Fig. 2.2. Volumetric water contents (logger data) of the Friemar soil over the (11/16/2013-11/11/2014) at (a) 5 cm depth and (b) 15 cm depth in the three tillage treatments (including precipitation); mean coefficients of variation between replicates within one treatment and depth (n=3) were at 5 cm depth: Plough: 8.6%, Grubber: 11.6%, No Till 7.3%; 15 cm depth: Plough: 2.2%, Grubber: 6.8%, No Till: 4.2%.

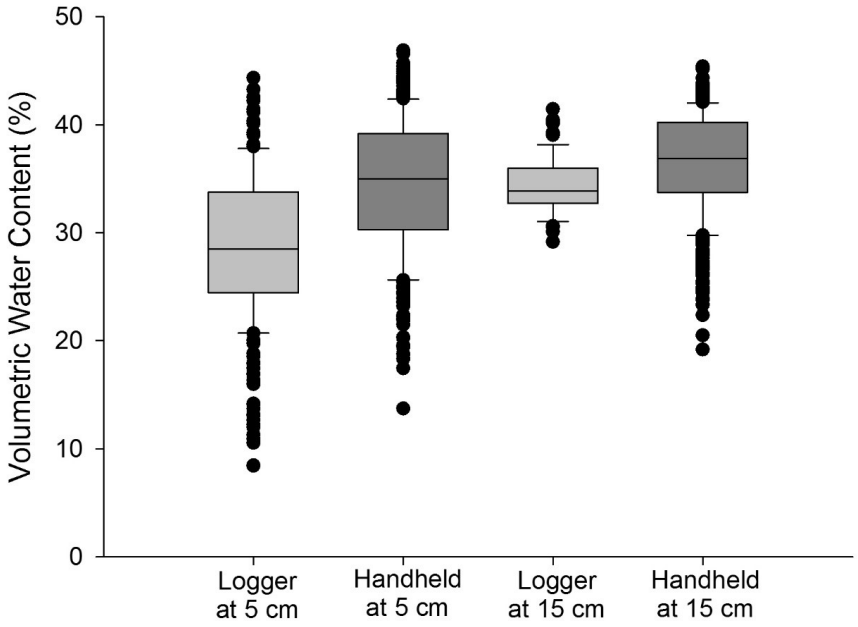

Fig. 2.3. Median volumetric water contents of the Friemar soil (boxplot over all tillage treatments), comparing logger and hand-held device data (n = 846).

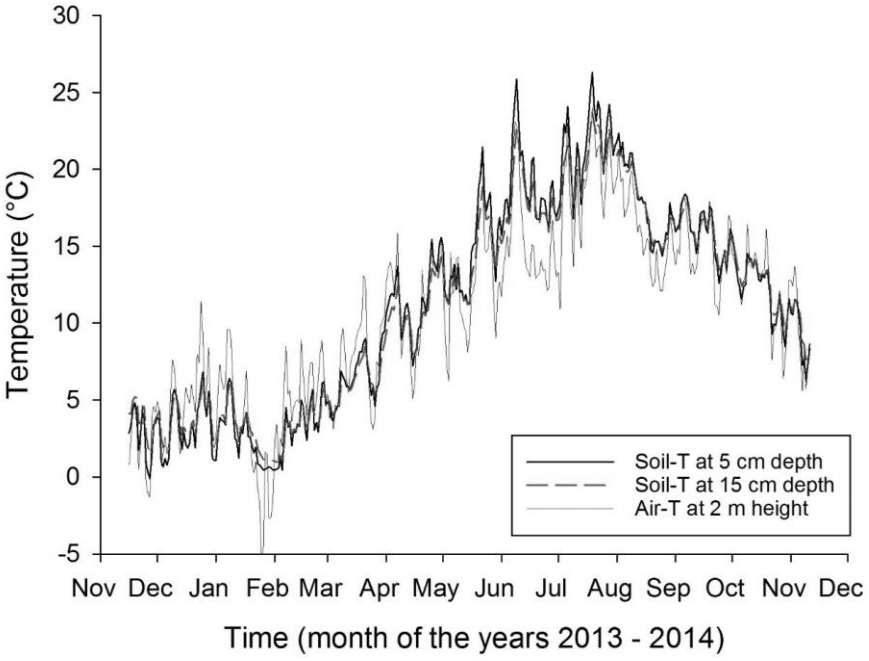

Fig. 2.4. Temperature (°C, logger data) at Friemar over the trial period (11/16/2013-11/11/2014) at (a) 5 cm depth and (b) 15 cm depth and air temperature in 2 m height. Mean of the three tillage treatments; mean coefficients of variation between treatments within one depth (n = 3): 5 cm depth: 2.3%; 15 cm depth: 1.0%.

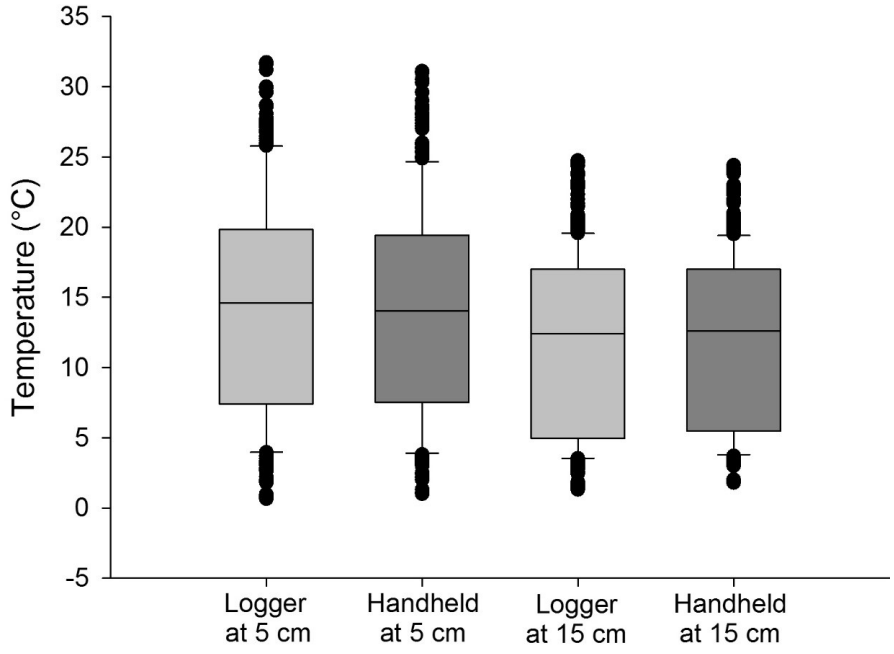

Fig. 2.5. Median temperature (°C) of the Friemar soil (boxplot over all tillage treatments), comparing logger and hand-held device data (n = 846).

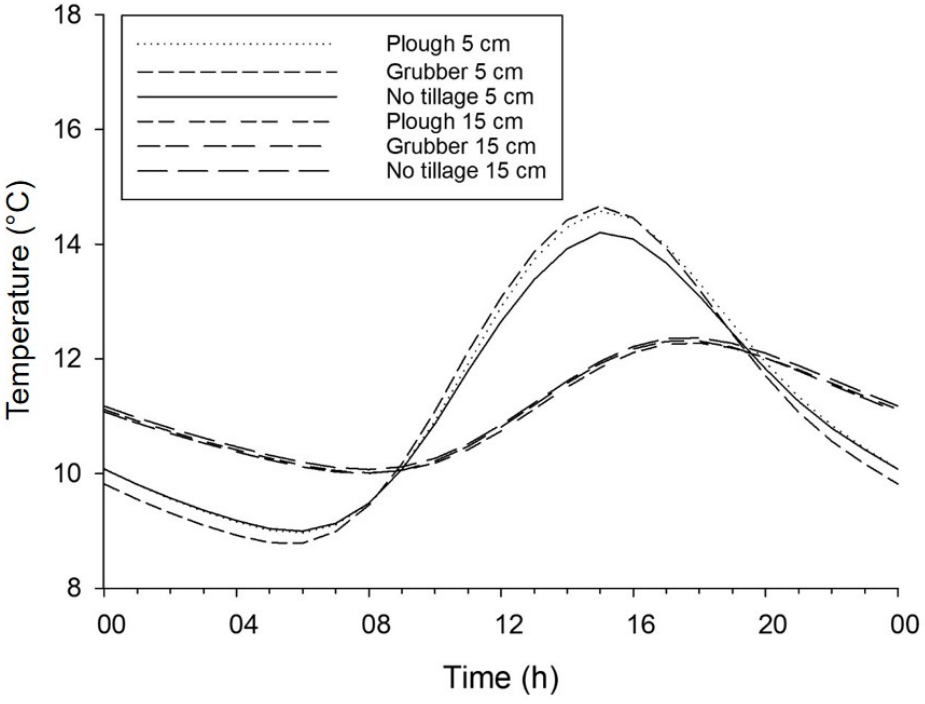

Fig. 2.6. Mean changes in temperature (°C) over the day in the three tillage treatments.

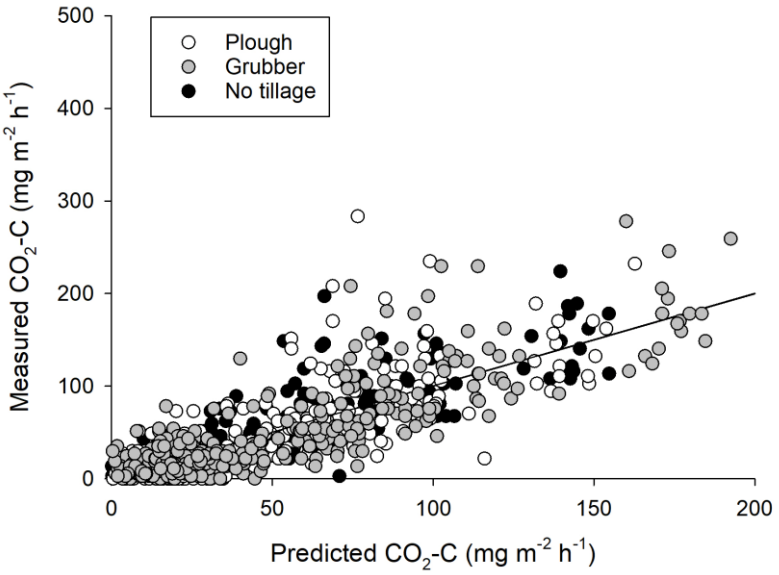

Fig. 2.7a.

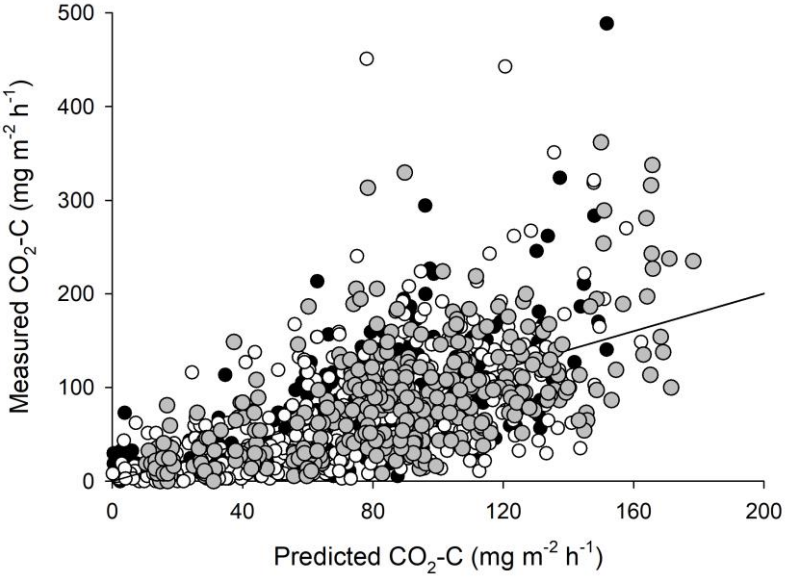

Fig. 2.7b.

Fig. 2.7. Multiple linear relationships between measured and predicted (see Table 2.6) values of CO_2 evolution, volumetric water content (mean 5 and 15 cm), and temperature (mean 5 and 15 cm) for the three tillage treatments (a) without maize (11/16/2013-05/29/2014) and (b) with growing maize plants (05/30/2014-11/11/2014).

Table 2.6. Multiple linear relationships between CO_2 evolution and daily mean temperature and daily mean volumetric water content for the periods without (11/16/2013-05/29/2014) and with (05/30/20014-11/112014) maize; probability values for the interaction models.

Dependent variable	Constant	Independent variables		Adjusted	Probability values	
CO_2-C (mg m^{-2} h^{-1})		T_S (°C)	VWC (%)	r^2 (%)		
16/11/2013 – 05/29/2014 without maize						
Plough	-43.34	7.77	0.682	64.5	Treatment	NS
Grubber	-72.14	9.36	1.339	74.8	T_S	<0.01
No-tillage	-49.11	7.85	0.792	71.9	VWC	<0.01
					Treatment × T_S	<0.01
					Treatment × VWC	NS
05/30/2013 – 11/11/2014 with maize						
Plough	13.71	7.42	-2.311	31.7	Treatment	NS
Grubber	-50.38	8.07	-0.165	38.4	T_S	<0.01
No-tillage	-41.06	7.67	-0.644	41.7	VWC	0.01
					Treatment × T_S	NS
					Treatment × VWC	0.03

2.4 Discussion

2.4.1 Tillage and plant effects on CO_2 efflux

No general tillage effects on CO_2 efflux were observed in the current experiment, using soil columns from a long-term field experiment under practical farming conditions (Koch et al., 2009). However, season-specific significant interactions occurred between tillage and T_S in the unplanted period as well as between tillage and VWC in the planted period, according to the multiple linear regression analysis. T_S and VWC explained on average 70% of the variation in CO_2 efflux in the unplanted and 37% in the planted period. Weissert et al. (2016) explained 71% (urban parkland) and 54% (urban parkland) by

VWC and T_S. Dong et al. (2017) attributed 71% to these two factors, investigating a field cropped with winter wheat.

A mean CO_2 efflux of 4.0 t C ha^{-1} a^{-1} is in the range obtained by others from arable sites, considering the differences in soil and climatic conditions as well as land use management. Franzluebbers et al. (1995) measured 4.1 to 7.0 t CO_2-C ha^{-1} a^{-1}, Piao et al. (2000) 1.6 to 4.3 t CO_2-C ha^{-1} a^{-1}, Oorts et al. (2007) 3.5 to 4.5 t CO_2-C ha^{-1} a^{-1}, Dong et al. (2017) 5.6 to 7.2 t CO_2-C ha^{-1} a^{-1}, and Faust et al. (2019) 5.0 to 8.1 t CO_2-C ha^{-1} a^{-1} at Friemar, which is one of the experimental sites, from which the current soil columns were obtained. It cannot be excluded that this range of CO_2 effluxes is in part affected by the correction model for closing the long gaps in time between the short measuring periods of CO_2. A large variety of correction approaches has been presented by Hoffmann et al. (2015), but none of them is perfect, as certain model parameters were always determined with insufficient precision. In several studies, e.g. Franzluebbers et al. (1995), Piao et al. (2000), Jannoura et al. (2014), and Faust et al. (2019) the CO_2 evolution rate, measured at a certain day, was taken as representative for the whole period until the next measuring point without further correction. This was done to keep the statistical evaluation close to the measured data, especially as the difference to a current correction approach is often small. The most similar data to the current study were obtained by daily measurements at four points per day with a permanently installed chamber, which can be closed during the CO_2 measurements (Oorts et al., 2007).

In the planted period, the CO_2 efflux consisted of heterotrophic and autotrophic respiration. However, the presence of maize plants did not apparently affect the relationship between CO_2 efflux, T_S and VWC but added considerable variation to this relationship, due to the different response of plants and soil microorganisms to T_S and VWC. Only the intercept shifted from -54.8 in the unplanted period to -25.9 in the planted period, averaged over all three tillage treatments, due to autotrophic root respiration.

Approximately 3.9 g root dry matter or 1.8 g root C was presumably present per column at harvest, assuming a shoot to root ratio of 5, derived from pots of similar size to the current soil columns (Muhammad et al., 2007). It can be assumed that the total C input by root respiration + roots + rhizodeposition is approximately 50% of the aboveground dry matter C (Franzluebbers et al., 1995; Ludwig et al. 2007). This would amount to 4.4 g C column^{-1} (mean = 0.5 × 8.8 g C plant^{-1} or column^{-1}, see Table 2.2). In this case, the total amount of CO_2-C evolved by root respiration and mineralization of rhizodeposition would be 2.6 g CO_2-C column^{-1} or 0.37 t CO_2-C ha^{-1}, which is equivalent to 13% of the CO_2 efflux in the planted period.

2.4.2 Temperature and moisture effects on CO_2 efflux

The linear regression coefficients between T_S and CO_2 efflux were similar in the unplanted but also in the planted period. The linearity, which is in agreement with Piao et al. (2000) and Weissert et al. (2016), does not necessarily contradict the exponential relationships often observed between soil temperature and CO_2 evolution rate (Dong et al., 2017; Ooerts et al., 2007; Terhoeven-Urselmans et al., 2009). The exponential relationship might be masked by the high variation of the many measuring points, especially in the planted period (Fig. 2.7b). However, it is unlikely that the differences observed between the hand-held device and logger data significantly affected the relationship between T_S and CO_2 efflux. In fact, the correction of hand-held devices with logger data at a certain subset, i.e., the columns from Friemar in the current study, are a useful option, when only a limited number of loggers can be installed at experimental areas.

In contrast to the relationship between T_S and CO_2 efflux, that between soil water content and CO_2 efflux is more complex, especially under field conditions. Linear

relationships have been observed in laboratory incubations, which are strictly limited to heterotrophic respiration in the absence of autotrophic plants (Orchard and Cook, 1983; Cook and Orchard, 2008). This implies the possibility that VWC rectified any exponential relationship between T_S and CO_2 efflux values in a multiple regression analysis, as suggested by Lloyd and Taylor (1994). Another reason might be that the current T_S data were measured at deeper depths, i.e. not only at 5 cm but also at 15 cm depth, which may have dampened the T_S response (Gaumont-Guay et al., 2006). Also the measurements at discreet points in time, i.e. weekly, as in the current study, might be a problem, as some change in VWC can be missed out. At low VWC, both autotrophic root and microbial respiration declines, due to limitation in available water. As VWC starts to increase, soil respiration will increase. However, once a soil becomes water saturated, autotrophic root and microbial respiration might be O_2 limited due to restricted gas diffusion. Therefore, the relationship between VWC and CO_2 flux is most likely curvilinear, the exact equation greatly depending on gas transport conditions (Luo and Zhou, 2006). Structure related soil physical properties such as bulk density, texture, aggregation, porosity, and surface states are one important factor group for CO_2 flux (Dong et al., 2017; Franzluebbers et al., 1995). Weather conditions such as drought or rainfall events are another important factor group (Dong et al., 2017; Euster et al., 2010; Morell et al., 2010, 2011).

VWC effects on CO_2 efflux were generally smaller than T_S effects, in accordance with others (Davidson et al., 1998; Dong et al., 2017; Weissert et al., 2016), and showed strong season-specific differences. The higher the VWC, the more CO_2 was evolved in the unplanted period, as observed by Davidson et al. (1998) in a mixed hardwood forest. This relationship suggests that the water buffers against cold temperatures in winter, as the VWC never dropped to values that would limit microbial respiratory activity (Gaumont-Guay et al., 2006; Moyano et al., 2013). The lower the VWC, the more CO_2 was evolved in the planted period, which could not be solely explained by autotrophic

root respiration. This relationship suggests that a stronger water uptake of maize plants, especially under plough tillage, increases in rhizodeposition and thus microbial respiratory activity but most likely also CO_2 diffusion from the deeper soil layers to the soil surface.

The strong variation of CO_2 efflux data in the planted period is in line with the data of others (Dong et al., 2017; Müller et al., 2011; Terhoeven-Urselmans et al., 2009), who did not observe any relationships between CO_2 efflux and T_S around a mean of 16 °C. Reasons might be rapid shifts in plant development and autotrophic root respiration, substrate availability to soil microorganisms or gas diffusion after rainfall events. Soil temperature and soil moisture data were unable to explain the day to day variation of the CO_2 efflux over short periods (Jensen et al., 1996). Also, application of the mechanistic CO_2 transport model SOILCO2 (Šimůnec and Suarez, 1993) to these data failed to predict day-to-day variation (Jensen et al., 1996), although this model includes not only CO_2 production, dispersion, and convection but also water and heat flow. An important reason for this failure is rainfall, which changes the effective diffusion coefficient and affects the CO_2 transport from the site of production to the soil surface in an unpredictable way (Šimůnec and Suarez, 1993). Small water filled pores close to the soil surface are able to trap CO_2 produced at lower soil depth (Jensen et al., 1996). This points to another central problem of measuring soil CO_2 efflux: Soil depth and, thus, the amount of soil microorganisms contributing to the CO_2 efflux from the surface, may vary with the conditions for gas diffusion (Rottmann et al., 2010).

Only in the Zschortau soil was it possible to measure the negative relationship between low VWC and high T_S, as hypothesized in the introduction. Grubber and no-tillage treatments have higher VWC than plough tillage but only no-tillage has lower T_S than plough tillage. Heat transfer in soil depends on a complex interplay of VWC, texture, bulk density, and soil structure, which causes significant T_S × tillage interactions on the

CO_2 flux in the unplanted period, due to the grubber treatment of the current study. In contrast, plant water uptake and rhizodeposition led to significant VWC × tillage interactions on the CO_2 flux in the planted period, caused by the plough treatment.

2.4.3 Relationships between MBC and CO_2 efflux

No-tillage led to the significantly lowest ratio of CO_2 efflux to MBC, indicating a slower microbial turnover, as hypothesized in the introduction at least for this treatment. The results of the grubber-tilled soil point in the same direction, although they are not significantly different from the ploughed soil. One reason might be that the volumes of the soil columns were too small to prevent excessive heat transfer to or from the surrounding soil. This methodological problem of the current soil column experiment for measuring the relationships between T_S, VWC and microbial turnover points to the necessity to find alternative approaches. Suitable approaches could be the integration of microbial turnover factors into inverse modelling of the relationship between SOC content, T_S and VWC (Bauer et al., 2012) on large scales (Euster et al., 2010; Falloon et al., 2011; Moyano et al., 2013). The SOC contents had significant effects as covariate on the relationships between the total CO_2 efflux and tillage but could not be integrated into the current linear multiple regression models, using parameters with a high temporal resolution.

The lowest CO_2 efflux to MBC ratio indicates a more efficient substrate use of the soil microbial community in the no-tillage soil, i.e. the microorganisms were able to transfer more substrate into their biomass due to a reduced MBC turnover. The CO_2 efflux/MBC ratio measured in the field from undisturbed soil columns over one year with varying VWC, neglecting the contribution of autotrophic respiration in the planted period, draws a similar eco-physiological relationship to the metabolic quotient qCO_2. This is the

ratio of basal respiration to MBC (Anderson and Domsch 1990, 2010), measured under defined laboratory conditions, using sieved and pre-incubated soil adjusted to about 50% water holding capacity. Then, the qCO_2 gives information on the catabolic requirements of a microbial community (Anderson and Domsch, 1990, 2010) and is usually negatively related to the MBC/SOC ratio (Anderson and Domsch, 1989; Goenster et al., 2017; Heinze et al., 2010; Murugan et al., 2014). In the current experiment, this negative relationship was also observed between the CO_2 efflux/MBC and the MBC/SOC ratio.

2.5 Conclusions

A reduction in tillage intensity to systems without soil inversion generally increased SOC and VWC at 0-10 cm, whereas T_S was only decreased with no-tillage but not with grubber tillage. However, the lowest CO_2 efflux to MBC ratio indicates a reduced MBC turnover in the no-tillage soil, leading to the highest MBC content in this treatment, which is in accordance with our hypotheses. The absence of this relationship in the grubber treatment might be caused by the restriction of our study to 20 cm depth or by a limitation of the soil transplantation approach, due to insufficient heat buffering to the surrounding soil. From a soil ecological viewpoint, grubber and no-tillage can be recommended as it improves microbial life conditions.

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2. Respiration response to different tillage intensities in transplanted soil columns

Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture” of the German Research Foundation (DFG).

3. Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial

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Abstract

In a long-term tillage field trial under practical farming conditions in Central Germany, the decomposition of maize (*Zea maize* L.) leaf litter was monitored for 8 months in litterbags and soil bags, where non-decomposed litter was mixed into soil and recovered as particulate organic matter (POM). The objective was to determine which methodological approach is more suitable to reflect tillage effects on litter decomposition and microbial turnover in no-tillage and grubber (15 cm), i.e. a rigid tine cultivator, treatments in comparison with mouldboard ploughing (25 cm). Under no-tillage, CO_2-C efflux monitored for 22.5 months was lowest, although this soil contained the highest soil organic C and microbial biomass C contents at 0-5 cm depth. One reason is the slow warming in spring due to the highest volumetric water content, leading to the lowest mean soil temperature. Maize leaf litter was more rapidly decomposed in the soil bags, with a mean mass loss rate constant k = 0.0108 d^{-1}, than in the litterbags, with k = 0.0063 d^{-1}. This difference mainly occurred during the initial 3-month period after burying. In the

soil bags, the mass loss rate constant for maize leaf litter was significantly higher in the no-tillage than in the plough treatment. In contrast, the mean microbial C use efficiency of the maize leaf litter of 0.27 in the soil bags was not affected by the tillage treatments. Although both methods are in principle suitable for monitoring decomposition processes, the soil bag method has advantages, as initial accessibility to decomposing microorganisms is facilitated.

Keywords: Mouldboard ploughing; Grubber; No-tillage; Decomposition; Loss rate; Microbial turnover; Carbon use efficiency

3.1 Introduction

Water erosion is one of the most serious threats to fertile silt loams in hilly Central European landscapes, which is a special problem for sugar beet cultivation, as large soil areas are left uncovered in late spring (Koch et al., 2009). To optimize grubber tillage systems for sugar beet cultivation, an on-farm long-term field experiment was established at typical arable sites used for sugar beet cultivation in central Germany (Jacobs et al., 2015; Koch et al., 2009). In this experiment, no or moderate (Andruschkewitsch et al., 2013; Jacobs et al., 2009, 2015; Murugan et al., 2014) increases in soil organic C (SOC) stocks were found in the non-inversion tillage treatments compared with mouldboard ploughing, but strong increases were observed in microbial biomass C (MBC) stocks (Murugan et al., 2014). Their results agree with several other experiments (Frasier et al., 2016; Hydbom et al., 2017; van Groenigen et al., 2010). This different response of SOC and MBC stocks suggests a reduction in microbial turnover if non-inversion tillage does not increase the C input into soil (Ussiri and Lal, 2009).

The increased SOC contents of the top layer (Ahl et al., 1998; Heinze et al., 2010; Kaiser et al., 2014; Luo et al., 2010) result in higher soil moisture (Abdullah, 2014; Frasier et al., 2016; Frey et al., 1999; Jacobs et al., 2011b; Moraru and Rusu, 2012) and heat storage capacity, lowering the soil temperature (Frasier et al., 2016; Jacobs et al., 2011b; Lakshmi et al., 2003). These relationships most likely reduce the turnover of the microbial biomass (Moyano et al., 2013). Carbon use efficiency (CUE), i.e. the percentage of substrate converted to microbial biomass and metabolites, is an important component of microbial turnover in soil (Joergensen and Wichern, 2018). CUE has gained considerable research interest (Geyer et al., 2016; Manzoni et al., 2012; Spohn et al., 2016a, 2016b), e.g. as a parameter for models simulating C and N fluxes in agroecosystems (Brilli et al., 2017). Microbial C use can be measured as CO_2 production but also as mass loss of substrate added to soil.

For measuring the mass loss of plant residues under field conditions, the litterbag method developed by Bocock and Gilbert (1957) is a useful approach (Joergensen et al., 2009). Litterbags have been widely used for studying litter decomposition in forest ecosystems (Berg, 2000; Joergensen et al., 2009; Potthoff and Loftfield, 1998), but also for investigating tillage effects on straw decay in arable soils (Burgess et al., 2002; House and Parmalee, 1985; Jacobs et al., 2011a, 2011b). An important advantage of this method is the simple recovery of litter transferred to the field and the possibility of excluding specific soil invertebrates from decomposition by using different mesh sizes (House and Parmalee, 1985; Knacker et al., 2003). However, litterbags reduce the intimate contact between plant residues and autochthonous microbial decomposers, which may lower decomposition rates (Potthoff et al., 2005).

An alternative approach to litterbags is the recovery of non-decomposed plant residues as particulate organic matter (POM) according to Magid and Kjærgaard (2001), which enables natural contact between organic substrates and the autochthonous soil

microbial community. This approach has been successfully used in incubation experiments (Rottmann et al., 2011) and pot experiments (Muhammad et al., 2006), but rarely in field experiments (Jannoura et al., 2013, 2014). There, the precision of the POM method can be improved by adding the plant residues, e.g. maize litter, to soil bags with a defined amount of soil, similar to the mycorrhiza in-growth mesh bags (Aleklett and Wallander, 2012). Maize (*Zea mays* L.) has the advantage of being a C4 plant, exhibiting higher $\delta^{13}C$ values than the autochthonous SOC mainly derived from C3 plants (Balesdent and Mariotti, 1996; Ryan and Aravena, 1994). This enables to measure the incorporation of the substrate into MBC and SOC as well as to calculate CUE value for complex substrates (Joergensen and Wichern, 2018).

As a comparison of these two approaches is not available under field conditions, the central objective of the current experiment was to fill this gap by monitoring the decomposition of maize leaf litter in litterbags and soil bags at Friemar. This site is part of the long-term field experiment of the German sugar beet industry (Jacobs et al., 2015; Koch et al., 2009; Murugan et al., 2014) and exhibited the strongest difference between the tillage treatments mouldboard ploughing, grubber and no-tillage, especially at 0-5 cm depth. The underlying hypotheses are: (1) CO_2 evolution rates indicate a slower microbial turnover in the grubber and no-tillage treatments in comparison with mouldboard ploughing. (2) This slower microbial turnover is also reflected by lower decomposition rates of maize leaf litter and higher CUE values. (3) Litter is decomposed more rapidly throughout the year in soil bags than in litterbags. (4) Soil bags are more suitable for detecting tillage effects than litterbags.

3. Response of maize leaf decomposition in litterbags and soil bags to tillage intensity

3.2 Material and Methods

3.2.1 Experimental site, design and soil sampling

The experimental site Friemar is part of a long-term tillage experiment designed and operated by the Institute for Sugar Beet Research (Koch et al., 2009). All sites were placed on commercial fields at a variety of locations, typical for sugar beet production in Central Germany and cultivated by the agricultural division of Südzucker AG Mannheim/Ochsenfurt. At each site, one large field with spatially homogeneous soil properties was divided into equally sized tillage plots (Koch et al. 2009). Due to mechanization, it was not possible to repeat the tillage plots at a certain site.

Three tillage systems were investigated at four selected sites, i.e. Friemar, Grombach, Lüttewitz, and Zschortau, for specific studies carried out by the Research Training Group 1397 of the German Research Foundation (Murugan et al., 2014). The different sites were often used as independent replicates without further statistical evaluation of the field replicates at one site (Koch et al., 2009 Andruschkewitsch et al. 2013, 2014). All sites showed similar tillage effects after 10-13 experimental years (Murugan et al., 2014; Jacobs et al., 2015), although the differences between the treatments has been developed to different extent, leading to significant tillage × site interactions (Murugan et al., 2014).

Table 3.1. Soil and autochthonous particulate organic matter (POM), characteristics of the experimental site in Friemar, Thuringia.

	Soil pH[a] (H_2O)	SOC[b] (t ha^{-1})	Soil C/N[b]	MBC[b] (t ha^{-1})	MB-C/N[b]	$SO^{13}C$[a]	$POM^{13}C$[a]
						$\delta^{13}C$ (‰)	
Plough	7.9	61.5	8.6	1.2	7.6	-26.4	-27.1
Grubber	7.3	71.4	9.0	1.2	6.4	-26.7	-27.9
No-tillage	7.2	70.8	9.2	1.4	7.8	-27.0	-27.7

[a] At 0-5 cm depth; $POM^{13}C$: Mean of the two fractions 0.4-2 mm and > 2 mm; [b] At 0-40 cm depth, recalculated data according to Murugan et al. (2014).

The previous studies were based on one sampling per year, largely independent from season. In contrast, the current study is based on repeated samplings and CO_2 measurements over the year, which was only possible at one site that has the closest distance to Witzenhausen.

The experimental field in Friemar was established in 1992/1993 in an arable loess region of central Germany (50° 57′ North, 10° 47′ East, 310 m asl) on a Haplic Phaeozem. Long-term mean annual temperature is 7.8°C and annual precipitation 517 mm (Andruschkewitsch et al., 2013). Mean daily air temperature (2 m) varied between -7.9 and 26.8°C from 17 October 2013 until 2 September 2015, with a mean of 9.3°C (Fig. 3.1a). Mean annual temperatures in 2013, 2014 and 2015 were 8.0, 9.6 and 9.4°C, respectively, and mean annual precipitation 604, 539 and 405 mm, respectively. The soil consists of 31% clay, 65% silt, and 5% sand (Andruschkewitsch et al., 2013). Further soil properties are given in Table 3.1.

The field with spatially homogenous soil properties was divided into three plots (660 m × 140-144 m), each being assigned to one of the three tillage treatments: (i) annual mouldboard ploughing to a depth of 25-30 cm, (ii) grubber tillage to a depth of 10-15 cm, and (iii) no-tillage, with direct drilling. Before sugar beet sowing, the seedbed of the no-tillage treatment was prepared to a depth of 3-5 cm to improve sugar beet crop establishment (Koch et al., 2009). The crop rotation consisted of sugar beet (*Beta vulgaris* L.), winter wheat (*Triticum aestivum* L.), and winter wheat (Andruschkewitsch et al., 2013). Crop residues were left on the field and sugar beet was sown in March–April using a single-seed drill adapted to crop residues lying on the soil surface. The crop management was carried out following the regional standards of agricultural practice, including the use of non-selective herbicides in grubber and no-tillage treatments. Sugar beet selective herbicides, molluscicides and rodenticides were used, the applications depending on the infestation level. Application of N fertilizer did not vary between the

treatments (Koch et al., 2009). Information on the mean annual N fertilization rate is provided by Murugan et al. (2014). Average wheat yields from 1997 to 2015 were 8.1 t ha^{-1} in the plough, 8.3 t ha^{-1} in the grubber, and 7.8 t ha^{-1} in the no-tillage treatment. Corresponding mean sugar beet yields were 67.7, 66.3, and 60.6 t ha^{-1}, respectively.

Since it was the explicit goal of the trial to use machinery sizes relevant to agricultural practice, it was not possible to establish field replications at the individual sites (Jacobs et al., 2015). At each site, one large field with spatially homogeneous soil properties was divided into three equally sized tillage plots (Koch et al. 2009). For our study, each of these three plots was divided into seven subplots of approximately 142 m × 86 m. Then, each subplot was again divided into nine squares (approximately 47 m × 29 m). Out of these nine squares, one was chosen randomly in which soil samples were taken, soil bags and litterbags were buried, and soil temperature, volumetric water content and soil respiration was measured. On 9 October 2013, after sowing of winter wheat, soils were sampled at 0-5 cm depth. Fresh soil samples were sieved to pass a 2 mm sieve and homogenized. The initial soil samples were analysed for their chemical and microbial properties, similarly to the soils in the soil bags as described below.

3.2.2 Measurement of temperature, water content, and soil respiration

Soil moisture, temperature and respiration measurements were carried out at approximately two-week intervals from 17 October 2013 until 2 September 2015 with portable devices using an infrared gas analyser CIRAS-1 (PP-Systems, Hitchin, UK, Blanke, 1996). The system consisted of a dynamic chamber (100 mm diameter, 150 mm height) coupled to a portable infrared gas analyser (IRGA) in a closed circuit. The measurements were taken in random order, i.e., data collection started at a different treatment and at a different end of the field on each measuring day. The measurements

were carried out between 10:00 am and 6:20 pm. For the CO_2 measurements, the steel ring at the bottom of the cylindrical chamber was pushed gently about 2 cm into soil. In the chamber, CO_2 enrichment was measured for 120 s or until an increase of about 50 ppm CO_2 was achieved. For calculating the total amount of CO_2 evolved during the experimental period, the CO_2 evolution rate data expressed as mg CO_2-C m^{-2} h^{-1} were taken as representative for the whole day and for the whole period until the next measurement (Jannoura et al., 2014). Soil temperature at 5 cm depth (T_S) and volumetric water content (VWC; Theta-ML2x) at 0-6 cm depth were measured concurrently.

3.2.3 Litterbag and soil bag experiments

Maize leaves for litterbags and soil bags were harvested on 27 September 2013, dried (60°C), chopped < 5 mm, and sieved > 2 mm. Polyamide litterbag (5.5 cm × 20.5 cm; 1 mm mesh) were filled with 5 g dry matter (DM) maize leaf litter. Three treatments, 3 samplings, and 7 replicates resulted in a total of 63 litterbags. They were closed with a plastic clip and buried vertically at a depth of one cm (top end of the litterbag) to 6.5 cm (bottom end of the litterbag) beneath the soil surface on 16 October 2013. The litterbags were removed after 92 d (16 January 2014), 178 d (12 April 2014), and 244 d (17 June 2014), respectively. Litterbag maize leaf litter was dried (60°C) and ball milled for C and N analysis.

For the soil bag experiment, polyamide bags (6 cm × 28.5 cm, 100 µm mesh size) were filled with a mixture of 200 g of fresh soil from the respective plots (between 154.2-167.6 g soil DM) and 2% maize leaf litter related to soil DM, i.e. 3.08-3.35 g maize leaf litter DM. The initial C and N contents, and $\delta^{13}C$ of the dried maize leaf litter were 46% C, 2.1% N and -13.2 ‰ $\delta^{13}C$, respectively. The ergosterol content of maize leaf litter was 0.9 µg g^{-1} DM. On 16 October 2013, 63 soil bags, i.e. 3 treatments, 3 sampling dates, 7

replicates, were buried 1 cm below the soil surface and sampled after 92, 178, and 244 days at the same dates as the litterbags. Each soil bag was buried in the same square from which the soil sample for this soil bag had been taken. Half of the soil bag content, i.e. around 100 g fresh soil, was used for POM analysis, the other half for chemical and microbial analysis. All visible particles of maize straw residues were removed by sieving (< 2 mm) and with tweezers in advance (Lukas et al., 2013).

In the soil bags, maize leaf litter was recovered as particulate organic matter (POM) according to Magid and Kjærgaard (2001) as described by Muhammad et al. (2006). An aliquot of 100 g moist soil was taken from the soil bag and dispersed in 400 ml 5% NaCl, stirred by hand and allowed to stand for at least 45 min. Then, the samples were poured gradually onto two sieves of 2 mm and 0.4 mm mesh size and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve during the washing procedure until the water passing the sieve became clear. The material retained on the 0.4 mm sieve was transferred into a beaker. Tap water was added and organic material was separated from the mineral material by repeated flotation-decantation, until organic particles were no longer visible in the mineral fraction. The mineral fraction was discarded. The fractions POM 0.4-2 mm and POM > 2 mm were washed with distilled water and transferred into crucibles, dried at 60°C, weighed and milled for C, N, and $\delta^{13}C$ analysis. Approximately 1.5% of maize leaf litter is soluble (unpublished results from Potthoff et al., 2006) and might be lost by the POM recovery method.

3.2.4 Chemical analysis

A subsample of litter material and soil was dried (80°C) and ball milled to fine powder for chemical analysis. Total C and $\delta^{13}C$ isotope composition was measured by isotope-ratio mass spectrometry (IRMS) (Delta plus, Finnigan, Bremen, Germany) via

open split interface (Conflo III, Finnigan, Bremen, Germany). Carbonate C (≈ 1.0 mg g^{-1} soil) was not removed from soil samples before $\delta^{13}C$ analysis. Soil organic C (SOC) content was determined as total C minus carbonate C, which was gas-volumetrically determined after the addition of 10% HCl to the soil using a Scheibler apparatus (Blume et al., 2011). Soil pH was measured using a soil to water ratio of 1 to 2.5 (w/v).

3.2.5 Microbial biomass

Ergosterol was extracted from 2 g fresh soil or 0.5 straw with 100 ml ethanol (96%) at 250 rev min^{-1} for 30 min (Djajakirana et al., 1996), followed by reversed-phase HPLC with 100% methanol as mobile phase and detection at a wavelength of 282 nm.

MBC was estimated by fumigation-extraction (Vance et al., 1987). A sub-sample of 20 g moist soil was separated into two portions. One portion was fumigated at 25°C with ethanol-free $CHCl_3$, which was removed after 24 h. Fumigated and non-fumigated samples were extracted for 30 min with 40 ml of 0.05 M K_2SO_4 (Bruulsema and Duxbury, 1996; Lukas et al., 2013) at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C concentrations in the 0.05 M K_2SO_4 extracts were measured using an automatic analyser (Multi N/C 2100S, Analytik Jena, Germany). MBC was E_C/k_{EC}, where E_C = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990; Joergensen, 1996).

For determining the $\delta^{13}C$-isotope composition of MBC, 20 ml aliquots of 0.05 M K_2SO_4 extracts of fumigated and non-fumigated samples were freeze dried for 3 days and afterwards dried at 60°C in an oven. The freeze-dried K_2SO_4 extracts were analysed as described above (Lukas et al., 2013).

3.2.6 Calculations and statistical analysis

Isotope values are expressed in delta notation relative to V-PDB for ^{13}C. The $\delta^{13}C$ value of the microbial biomass ($\delta^{13}C_{MB}$) was calculated according to Potthoff et al. (2003):

$$\delta^{13}C_{MB} = (\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{nonfum} \times C_{nonfum}) / (C_{fum} - C_{nonfum})$$

where $\delta^{13}C_{fum}$ and $\delta^{13}C_{nonfum}$ are the $\delta^{13}C$ values of the fumigated and the non-fumigated extract, respectively. C_{fum} and C_{nonfum} are the C content of the fumigated and the non-fumigated extract, respectively. The amount of maize derived C (C_4–C_{sample}) was calculated for each single replicate of all treatments by the following equation (Balesdent and Mariotti, 1996):

$$C4C_{sample} = Ct_{sample} \times (\delta^{13}C_{sample} - \delta^{13}C_{control}) / (\delta^{13}C_{maize} - \delta^{13}C_{control})$$

$$C3C_{sample} = Ct_{sample} - C4C_{sample}$$

where Ct_{sample} is the total C in the analysed sample; $\delta^{13}C_{sample}$ is the isotopic value ($\delta^{13}C$) in SOC, the two POM-C fractions, and MBC in the soil bags with maize leaf litter amendment; $\delta^{13}C_{control}$ is the isotopic value of the respective fractions in the respective treatments that did not receive maize leaf litter; and $\delta^{13}C_{maize}$ is the isotopic value of the maize leaf litter.

Assuming a first order decay kinetic (Balesdent and Mariotti, 1996), the degradation can be described by the following equation:

$$SO^{13}C_{ti} = SO^{13}C_{t0} \times e^{-kt} \text{ or } -k = ln\ (SO^{13}C_{ti}/SO^{13}C_{t0})/t$$

where $SO^{13}C_{ti}$ equals the maize-derived C content at the sampling date ti, $SO^{13}C_{t0}$ the maize-derived C content before burial, k the decay constant, and t the time between burial and sampling date. Then, mean residence time (MRT) of the maize litter is $1/k$.

The results presented in the tables are the arithmetic means of each treatment or treatment per sampling date and are expressed on an oven-dry basis (80°C for soils; 60°C for plant material). Normality of distribution of the residuals of the dependent variables was tested. Data were ln- or square-root-transformed for the variables that violated the normality of distribution. The effects of the independent factors were examined by a general linear model for repeated measures ANOVA, with sampling date as within-subject-factor and tillage treatment as between-subject-factor. The Tukey test was used as post-hoc test. All statistical analyses were performed using SPSS 24 (IBM-SPSS, Chicago, US).

3.3 Results

At 0-6 cm depth, VWC was on average 12% higher in the grubber and 46% higher in the no-tillage treatment than in the plough treatment over the whole experimental period (Table 3.2, Fig. 3.1b). In contrast, mean T_S at 5 cm was on average 7% higher in the plough and grubber treatments than in the no-tillage treatment (Table 3.2, Fig. 3.1c). CO_2-C flux rates (Fig. 3.2) and cumulative CO_2-C evolution of the grubber treatment significantly exceeded the respective values of the plough and no-tillage treatments by approximately 20 and 60%, respectively (Table 3.2).

In the field-exposed soil bags, the mean C3-SOC content was 24.7 mg g^{-1} soil in the grubber and no-tillage treatments over all three sampling dates and, thus, 55% higher than in the plough treatment (Table 3.3). Averaging all data, 1.7 mg g^{-1} soil or 19% of the initially added maize leaf litter C was determined as C4-SOC, without significant effects of tillage treatments or soil bag exposure time. Mean ergosterol and C3-MBC contents always declined in the order no-tillage > grubber > plough treatment, although the differences between the treatments were not always significant at each sampling day. In

the no-tillage treatment, mean ergosterol and C3-MBC contents were 34% higher than in the grubber and roughly 100% higher than in the plough treatment. The tillage treatments did not affect the maize-derived C4-MBC contents. However, the C4-MBC/C3-MBC ratio declined from 2.1 in the plough, to 1.4 in the grubber and to 1.1 in the no-tillage treatment at sampling date 1. Averaged over all treatments, C4-MBC contents decreased from 528 µg g^{-1} soil at sampling date 1 to 387 µg g^{-1} soil at sampling date 2, and finally to 305 µg g^{-1}soil at sampling date 3, which was equivalent to 5.8, 4.2, and 3.3%, respectively, of the initially added maize leaf litter C.

At sampling date 1, 20% of the initially added maize leaf litter C (Fig. 3.3) or 1.9 mg g^{-1} soil was recovered in the soil bags as C4-POM, without significant tillage treatment effects (Table 3.3). This percentage declined to 11% in the plough and to approximately 7% in grubber and no-tillage treatments at sampling date 3 (Fig. 3.3). In the litterbags, on average 44% of the maize leaf litter C were recovered at sampling date 1. This percentage declined to 23% at sampling date 3. The recovery was always lowest in the no-tillage treatment, but the differences to the plough and grubber treatments were not always significant. Mean mass loss rate constants k = 0.0063 d^{-1} and k = 0.0108 d^{-1} were calculated for litterbags and soil bags, respectively. These rate constants result in mean residence times of 171 and 96 days. In contrast to the maize leaf litter C in the litterbags, the mean residence time of C4-POM in the soil bags was significantly shorter in the no-tillage (86 days) than in the plough treatment (111days).

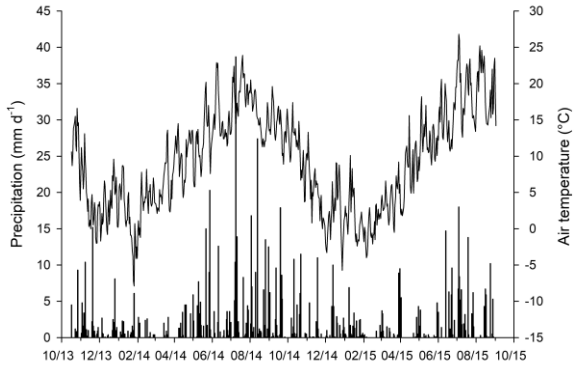

Fig. 3.1a

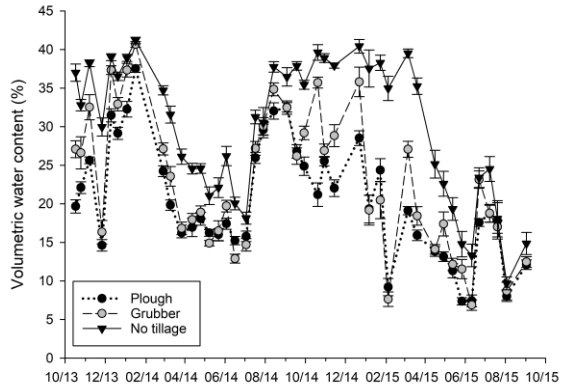

Fig. 3.1b

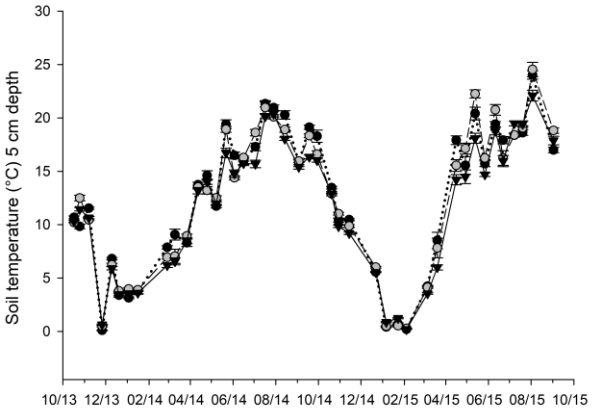

Fig. 3.1c

Fig. 3.1. (a) Daily mean temperatures at 2 m height and daily precipitation; (b) mean VWC at 0-6 cm depth in the three tillage treatments; (c) mean soil temperatures at 5 cm depth in three tillage treatments; bars indicate one standard error.

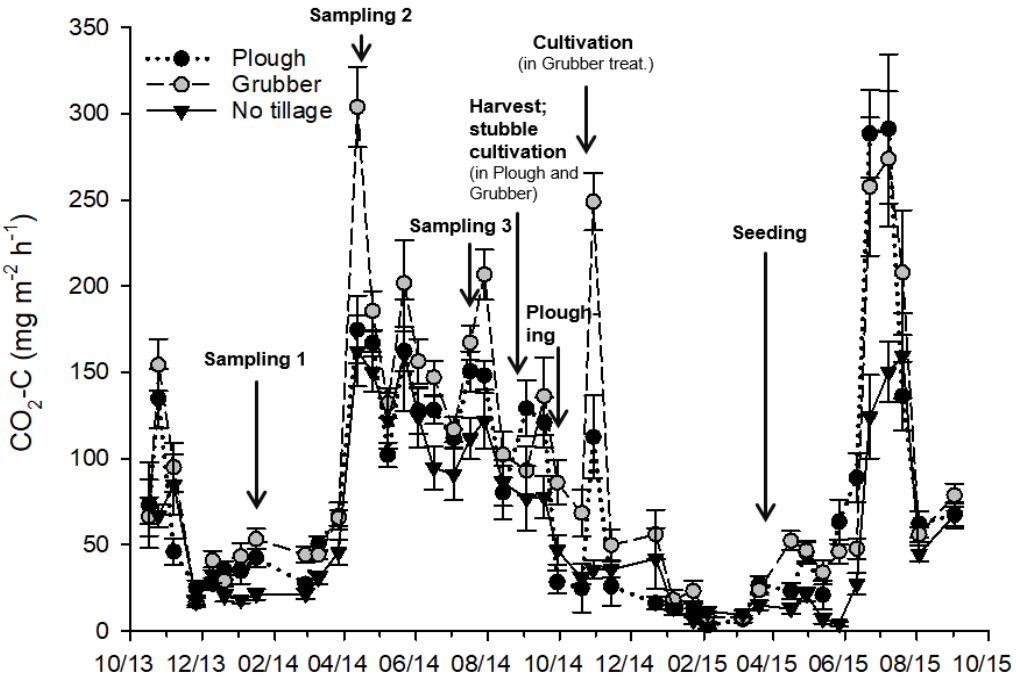

Fig. 3.2. Mean CO_2-C flux rates from the three tillage treatments; bars indicate one standard error.

Table 3.2. Mean volumetric water contents (VWC) at 0-6 cm depth, soil temperature at 5 cm depth, and cumulative CO_2-C flux in three tillage treatments.

	VWC (%)	Temperature (°C)	CO_2-C (t C ha^{-1} 365 $days^{-1}$)
Plough	19.9 c	12.8 a	6.5 b
Grubber	22.2 b	12.8 a	8.1 a
No-tillage	29.1 a	12.0 b	5.0 c
CV (± %)	16	7.6	9.7

CV = mean coefficient of variation between replicate measurements (n = 7); different letters within a column indicate a significant difference between the treatments (Tukey test, $P < 0.05$).

Table 3.3. Mean contents of C3-SOC, C4-SOC, ergosterol, C3-MBC, C4-MBC, and C4-POM in three tillage treatments for each sampling date; probability values of an ANOVA for repeated measures.

Sampling	Treatment	C3-SOC	C4-SOC	Ergosterol	C3-MBC	C4-MBC	C4-POM
(days)		(mg g^{-1} soil)		(µg g^{-1} soil)			
1 (92)	Plough	17.0 b	2.2 a	1.9 b	239 b	492 a	2.1 a
	Grubber	33.0 a	1.0 b	2.1 b	397 a	546 a	1.7 a
	No-tillage	26.0 a	2.0 a	2.9 a	514 a	545 a	1.8 a
2 (178)	Plough	15.4 b	1.4 a	1.3 c	190 c	378 a	1.8 a
	Grubber	21.2 a	1.7 a	2.1 b	353 b	389 a	1.2 b
	No-tillage	23.5 a	1.7 a	3.0 a	476 a	395 a	1.4 b
3 (244)	Plough	15.3 c	1.5 a	1.3 c	203 b	303 a	1.0 a
	Grubber	20.5 b	1.9 a	2.0 b	386 a	280 a	0.7 ab
	No-tillage	24.1 a	1.8 a	2.8 a	486 a	332 a	0.6 b
Probability values							
Treatment		<0.01	NS	<0.01	<0.01	NS	<0.01
Time		<0.01	NS	NS	<0.01	<0.01	<0.01
Treatment × time		<0.01	<0.01	NS	NS	NS	NS
CV (± %)		12	29	18	21	14	26

NS = not significant; CV = mean coefficient of variation between replicate measurements (n = 7); different letters within a column indicate a sampling date-specific significant difference between the treatments (Tukey test, $P < 0.05$).

3. Response of maize leaf decomposition in litterbags and soil bags to tillage intensity

Table 3.4. Mass loss rate constants of maize leaf litter C in litterbags and soil bags from the beginning on 16 October 2013 (S0) to the last sampling date on 17 June 2014 (S3), i.e. after 244 days.

	Mass loss rate constants of C (k d^{-1})	
	Litterbags	Soil bags
Plough	0.0054 a	0.0092 b
Grubber	0.0064 a	0.0112 ab
No-tillage	0.0071 a	0.0119 a
CV (± %)	29	16

CV = mean coefficient of variation between replicate measurements (n = 7); different letters within a column indicate a significant difference between the treatments (Tukey test, $P < 0.05$).

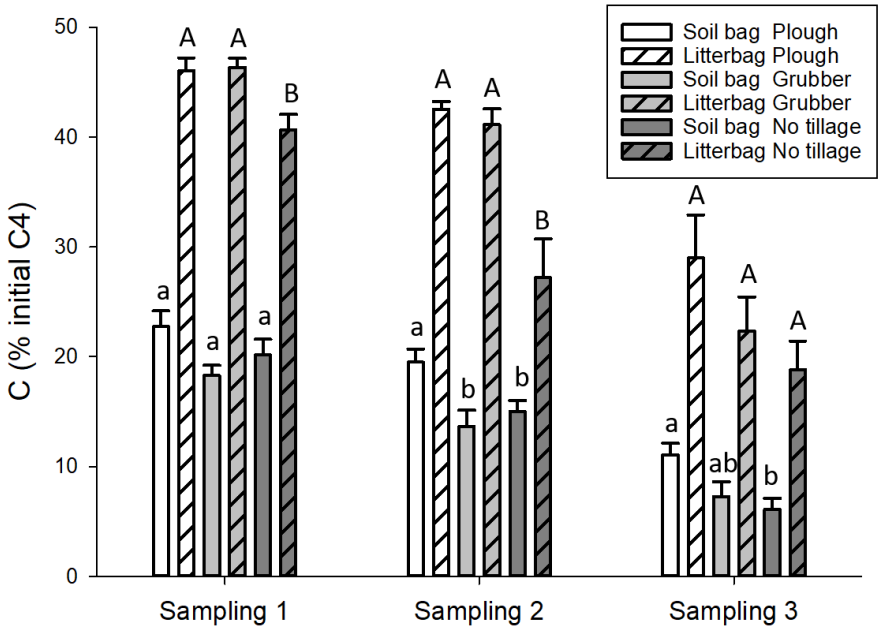

Fig. 3.3. Maize leaf litter C recovered as C4-POM from the soil bags and maize leaf litter C recovered from the litterbags in percent of the initially added maize leaf litter C at the three sampling dates; different small letters on top of the bars indicate a sampling date-specific significant difference between the treatments (Tukey test, P < 0.05) for the soil bags; different capital letters on top of the bars indicate a sampling date-specific significant difference between the treatments (Tukey test, P < 0.05) for the litterbags.

3.4 Discussion

3.4.1 CO_2 efflux

Lowest CO_2-C efflux was measured in the no-tillage treatment, although this soil contained the highest mean SOC and MBC contents at 0–5 cm depth. One reason is the slow warming in spring due to the highest VWC, leading to the lowest mean soil temperature. Another reason might be that the lower yields of the no-tillage treatment in comparison with the other treatments led to lower C input rates by root and harvest residues (Murugan et al., 2014). The highest CO_2 efflux was measured in the grubber treatment, where usually the highest wheat yields were obtained at Friemar (Murugan et al., 2014). Root and harvest residues of wheat exhibit a much higher C input into soil than those of sugar beets (Ludwig et al., 2007). In addition, the root to shoot ratio of wheat might be increased in the grubber treatment, contrasting the view of fixed root to shoot ratios, proposed by SOM turnover models (Brilli et al., 2017; Ludwig et al., 2007).

The confounding of tillage and site effects cannot be excluded, due to the absence of true treatment replicates at Friemar. However, the long distances between the sampling points and the relatively homogenous soil conditions most likely keep this type of error low. This view is supported by similar tillage effects after 10-13 experimental years at each of the four experimental sites Friemar, Grombach, Lüttewitz, and Zschortau (Murugan et al., 2014; Jacobs et al., 2015).

3.4.2 Microbial CUE

The formation of C4-MBC was not clearly related to the size of the autochthonous C3-MBC, i.e., a larger MBC did not incorporate a larger percentage of substrate C, as repeatedly observed after glucose addition (Bremer and Kuikman, 1994; Witter and Kanal, 1998; Chander and Joergensen, 2001). The decline in C4-MBC was not affected

by the tillage treatment, suggesting that the same was true for microbial turnover. The mean CUE of the present study was 0.27, calculated as proposed by Joergensen and Wichern (2018): CUE_{TP} = (MRC + MBC) / (substrate C - POMC). In the current experiment, this calculation is equivalent to C4-MRC = C4-SOC – C4-MBC (as C4-POMC was removed before C4-SOC determination), where MRC is microbial residue C. This fraction is the sum of non-biomass microbial products such as exo-enzymes, extracellular polymeric substances (EPS), secondary metabolites, and necromass (Joergensen and Wichern, 2018). Maize-derived C4-MBC was also calculated using a k_{EC} value of 0.45 (Joergensen, 1996). In addition, it was assumed that C4-POM represents non-decomposed substrate, neglecting the presence of approximately 1 to 2% microbial biomass (Potthoff et al., 2006; Rottmann et al., 2011).

The CUE calculated for the current field experiment was markedly lower than values reported by others of 0.39 (Muhammad et al., 2006) and 0.60 (Rottmann et al., 2010). These CUE values were obtained under controlled laboratory or greenhouse conditions, respectively, using maize straw litter as a substrate. The most likely reason is the long time-span between application of the maize leaf litter to the soil bags and the first sampling date. The optimum sampling point would be a period of maximum substrate incorporation into MBC, which requires more samplings in the initial period after placement in the field. The mean CUE was 0.45 for complex organic polymers such as cellulose, straw and MBC, ranging from 0.3 to 0.6 (Joergensen and Wichern, 2018).

3.4.3 Maize leaf litter decomposition

91% of added maize leaf litter C was decomposed in the soil bags and recovered as POM, but only 77% in the litterbags during the 244-d experimental period, which is equivalent to mean mass loss rate constants k = 0.0108 d^{-1} in the soil bags and k = 0.0063

d^{-1} in the litterbags. In a field experiment using POM recovery, a mass loss rate constant $k = 0.0055\ d^{-1}$ can be calculated for maize leaf straw with a C/N ratio of 32 from the data of Mueller et al. (1998). In litterbag field experiments, mass loss rate constants were $k = 0.0039\ d^{-1}$ for maize leaf straw with a C/N ratio of 42 (Burgess et al., 2002) and $k = 0.0074\ d^{-1}$ for maize leaf litter with a C/N ratio of 21 (Jacobs et al., 2011b). The current mass loss rate constants of the litterbags were within the range of those previously published. However, to the best of our knowledge, no direct comparisons between the soil bag and litterbag approaches are currently available in one experiment.

Lower mass loss rate constants in litterbags agree with the general view stated in the literature that litterbag decay is slower than that observed in soil (Dunger and Fiedler, 1997). The differences between these two approaches mainly developed during the first 92-d autumn interval up to 16 January 2014. Especially November 2013 was characterized by relatively high soil temperatures (Fig. 3.1c) and high CO_2-C evolution rates (Fig. 3.2). Rapid decomposition of organic substrates with a low C/N ratio at autumn and winter temperatures has been observed by others (Burgess et al., 2002; Lukas et al., 2013; Magid et al., 1997, 2004). In the initial decomposition phase, the lack of an immediate direct contact to the soil and thus to the autochthonous microbial decomposer community (Henriksen and Breland, 2002) is probably more important than in later phases, where ratios of mass loss rate constants in soil bags and litterbags remained stable at a similar level. Decomposition of organic residues depends not only on the C/N ratio but also on their microbial colonisation (Flessa et al., 2002; Potthoff et al., 2008; Scheller and Joergensen, 2008; Jacobs et al., 2011a). The current maize leaf litter had an initial ergosterol content of 0.9 µg g^{-1} DM, which is considerably lower than the 12 to 24 µg g^{-1} DM measured on wheat straw after harvest (Scheller and Joergensen, 2008). The current data supports the view that substrate decomposition depends only partially on the initial microbial colonization of substrate (Chander et al., 2002a; Jacobs et al., 2011a).

Another reason for lower mass loss rate constants in litterbags may be the agglutination of wet leaf litter particles, reducing aeration during the sensible period of initial decomposition in autumn (Dunger and Fiedler, 1997). It is unlikely that differences in water content play a decisive role in this general wet period, although higher water contents have often been observed in litterbags (Dunger and Fiedler, 1997; Knacker et al., 2003). Litterbag enclosures can lead to non-uniform changes in decay rate constants in different types of environments (Berhe, 2013). For these reasons, the results differ from natural decomposition of harvest residues. Maize leaf litter was most rapidly decomposed in the no-tillage treatment with highest MBC contents at 0-5 cm depth and the slowest in the plough treatment with lowest MBC contents. This means that there are no apparent relationships between decomposition of added maize leaf litter and CO_2 evolution, which was highest in the grubber treatment. Decomposition patterns were similar for the litterbags and soil bags, if the pattern of significances is not considered. However, it should be considered that our investigations were limited to the 0-5 cm soil layer.

3.5 Conclusions

CO_2 evolution rates indicated a slower microbial turnover in the no-tillage but not in the grubber treatment in comparison with mouldboard ploughing. However, a slower microbial turnover was not reflected by lower decomposition rates of maize leaf litter and also not by higher CUE values. Maize leaf litter was decomposed more rapidly in the soil bags than in the litterbags. This difference mainly occurred during the initial 3-month period after burying, i.e. both methods showed the same decomposition trend until the end of the experiment. This suggests that both methods are in principle suitable for monitoring decomposition processes. However, the mass loss rate constant for maize leaf litter, recovered as POM, was significantly higher in the no-tillage than in the plough

treatment. The soil bag method has apparently advantages, as the initial accessibility to the decomposing microorganisms is facilitated, which also reduced the variation between the replicates and led to significant differences between the tillage treatments.

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4. Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant residues

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Abstract

Aims Acid detergent lignin (ADL), acetyl bromide (AcBr), and cupric oxide oxidation (CuO) were compared as methods for determining lignin concentration and quality in plant residues.

Methods These three methods were used to analyze 27 plant residues from different groups of species, i.e., legumes, crucifers, herbs, grasses, and trees.

Results Median lignin concentrations of the 27 plant materials were 4.5% ADL and 6.0% AcBr lignin, significantly exceeding the median of 2.1% CuO lignin. ADL concentrations varied from 0.8 to 27.0%; those of AcBr and CuO lignin ranged from 1.8 to 12.2% and

from 0.6 to 9.7%, respectively. AcBr lignin showed a significant negative, non-linear relationship with total N. In addition, the relationship of ADL and CuO data was negatively affected by total N.

Conclusion The ADL method is simple and well reproducible, and large datasets are available for comparison. The AcBr procedure is fast, with less interference from non-lignin products than ADL. The CuO method is not interfered with by any other organic component in the plant material and gives additional information on the composition of the lignin. However, the release of phenolic units may be incomplete.

Key words: Acetyl bromide; Acid detergent lignin; Cupric oxide oxidation; Lignin determination

4.1 Introduction

In vascular plants, lignin is embedded in inter-cellular space between cellulose, hemicellulose, and pectin to give compressive strength (Hatfield and Fukushima 2005). Further, lignin facilitates water transport and impedes the degradation of cell wall polysaccharides by pathogens, insects, and other herbivores (Hatfield and Fukushima 2005). It is a hydrophobic complex polymer formed through enzyme-mediated radical coupling of mono-lignols, mainly coumaryl, coniferyl, and sinapyl alcohols, but also of a large variety of other phenyl-propanoid units (Sarkanen and Ludwig 1971; Sederoff et al. 1999). Lignin is indigestible for livestock and most soil animals and hardly decomposable for most soil microorganisms. It is mainly decomposed by fungi, especially white rot Basidiomycota (Martinez et al. 2005). Although some actinobacterial species seem to have the ability to degrade lignin, their general contribution to lignin decomposition is likely insignificant (Větrovský et al. 2014).

Despite its inherent recalcitrance, lignin is not accumulated in soil organic matter under aerobic, humid temperate environmental conditions at intermediate pH (Hamer and Marschner 2002; Kögel-Knabner et al. 2008; Marschner et al. 2008; Schmidt et al. 2011). This is different in acidic soils (e.g., Derenne and Largeau 2001), in mountain soils with low mean annual temperatures and low mean annual rainfall (e.g., Glaser et al. 2000), and under wet, submerged, and anoxic soil conditions (Bierke et al. 2008; Derenne and Largeau 2001; Olk et al. 2006), where lignin-rich material accumulates.

In many plant residues, high protein concentrations are related to low lignin concentrations, leading to increasingly low C/N ratios. This results in the often-stated negative relationship between substrate C/N ratio and decomposition rate (e.g., Jacobs et al. 2011b). However, this is basically only true for non-decomposed plant residues, not for highly microbially processed materials such as sugar cane filter cake (Rasul et al. 2006), cattle faeces (Jost et al. 2013) or soil organic matter (Chander et al. 2002b). As N-free starch, pectin, hemicellulose, and cellulose are all rapidly decomposed nearly exclusively by soil fungi (Schneider et al. 2012), the lignin/N ratio has been proposed by some authors as a more relevant indicator for decomposition rates than the C/N ratio (Lobe et al. 2002; Melillo et al. 1982). The increasing use of the lignin/N ratio in the past decades (Mehring et al. 2015; Taylor et al. 1989) has intensified demand for accurate lignin determination.

Several methods are available for determining lignin in organic matter, which all have specific advantages and disadvantages. In livestock nutrition, beside Klason and permanganate lignin, acid detergent lignin (ADL) is the most widespread approach (Hatfield and Fukushima 2005; Oestmann et al. 1995; Van Soest 1963). In forest and animal ecology, the acetyl bromide (AcBr) method has been repeatedly used (Brinkmann et al. 2002; Fukushima and Hatfield 2004). In soil biogeochemistry, the sum of lignin-derived cupric oxide (CuO) oxidation products can be used as an index for the lignin

concentration in soil (Hedges and Ertel 1982; Thevenot et al. 2010). These CuO oxidation products give additional information on lignin origin and degradation state (Jacobs et al. 2011a; Kalbitz et al. 2006). In particular, decreases in syringyl/vanillyl and cinnamyl/vanillyl ratios have been repeatedly used as indices for microbial lignin alteration, due to the higher stability of vanillyl units (Goñi et al. 1993; Hedges et al. 1988). Consequently, CuO oxidation products can be used as markers for land-use effects on vegetation and soil organic matter (e.g. Thevenot et al. 2010).

Increasing interdisciplinary research requires common links between livestock nutrition and soil biology (e.g. Jost et al. 2013), forest and invertebrate ecology (e.g. Harrop-Archibald et al. 2016), soil biology and biogeochemistry (e.g. Dao et al. 2018), grassland ecology and biogas production (e.g. Hensgen et al. 2014) and a common understanding in the reliability of different methods for determining lignin concentration in herbaceous plant material. ADL, AcBr, and CuO have been developed largely independently in the different research disciplines and are nowadays often parallel in use, without sufficient knowledge on their possibilities and restrictions. Consequently**,** our central objective was to compare ADL, AcBr, and CuO for lignin determination in organic tissue relevant for agricultural practice and in soil biological research. For this purpose, plant materials, which are important as fodder, green manure, harvest residue, and organic fertilizer, were selected from different groups, i.e., legumes, crucifers, herbs, grasses, and trees, and analyzed for their contrasting lignin concentrations.

4.2 Material and methods

Plant material

Plant material, which contributes to soil organic matter stocks and C and nutrient cycling, such as catch crops, green manure, and crop residues, was collected in September 2013. Most of the catch crops were grown at the Hessian State Manor of Frankenhausen, northern Hessia, Germany (51° 24′ N; 9° 25′ E), the experimental farm of Kassel University. Poplar roots and leaf litter of poplar and willow were collected from short-rotation coppices. Other plant species used in this study are often utilized in soil biological research for decomposition and mineralization experiments, e.g., maize and wheat straw for litterbag and incubation studies (Jacobs et al. 2011b; Rottmann et al. 2010). Generally, woody and herbaceous plant materials were selected to cover a large range of lignin concentrations.

A selection of 27 different plant material was sampled: amaranth (*Amaranthus* sp. L.), bell bean (*Vicia faba* L.), buckwheat (*Fagopyrum tatricum* Gaert), common vetch (*Vicia sativa* L.), field pea (*Pisum sativum* subsp. *sativum* var. *arvense* L. Poir.), fodder radish (*Raphanus sativus* var. *longipinnatus* L. cv. Structurator), maize leaf and stem (*Zea mays* L.), malva (*Malva* sp. L.), mustard (*Sinapis alba* L. cv. Asta), oat straw (*Avena sativa* L.), pea straw (*Pisum sativum* L.), hay (meadows; mainly grass), lucerne (*Medicago sativa* L.), perennial ryegrass (*Lolium perenne* L. cv. Cancan), phacelia (*Phacelia* sp. JUSS.), poplar leaf litter of two clones (*Populus* sp. L. clone AF2 and *Populus* sp. L. clone Max), poplar root (*Populus* sp. L), Sudan grass (*Sorghum x drummondi* (STEUD.) MILLSP. & CHASE), sugarcane (*Saccharum officinale* L.) filter cake, sunflower (*Helianthus annuus* L.), vetchling (*Lathyrus sativus* L.), willow leaf litter (*Salix* sp. L), winter oilseed rape (*Brassica napus* L.), wheat straw (*Triticum aestivum* L.), and white lupin (*Lupinus albus* L.).

Some plant material was sampled as standing green biomass (bell bean, buckwheat, common vetch, field pea, fodder radish, malva, mustard, perennial ryegrass, phacelia, Sudan grass, sunflower, vetchling, winter oilseed rape, white lupin), the other after harvest of mature plants (amaranth, hay, lucerne, maize stem and leaf straw, oat straw, pea straw, poplar root (70 % of roots > 5 mm diam.; 30% of roots < 5 mm diam.), after litter fall (poplar leaf litter of two clones, willow leaf litter) or processed residues (sugarcane filter cake). After drying at 60°C, the plant materials were ground and homogenized with a high-speed rotor-cutting mill for ADL, followed by a ball mill for AcBr and CuO. Total C and total N concentrations were determined after combustion, using a Vario Max CN analyzer (Elementar, Hanau, Germany).

Acid detergent lignin

For gravimetric lignin determination (Van Soest 1963), 1 g of ground dry plant material was weighed into sintered glass crucibles with porous membrane and boiled for 1 h in 100 ml acid-detergent solution, i.e., 20 g N-acetyl-N,N,N,-trimethyl ammonium bromide/ l in 0.5 M H_2SO_4, plus one drop of antifoam agent (Malophen NP11) in a refluxing apparatus (FT Fibertec 1020, FOSS, Hilleroed, Denmark). The solution was vacuum filtered, and the residues were washed thoroughly with hot distilled water. Glasses were transferred to an FT 1021 Fibertec cold extraction unit and 25 ml of cold 72% H_2SO_4 were added and stirred repeatedly. After 3 h, the acid solution was filtered under vacuum. Then, the residue was washed with hot distilled water, and then with acetone. The porous glass crucible was dried at 105 °C, cooled, and weighed.

Acetyl bromide method

Before determining the concentrations of lignin, lipids were extracted from 1 g oven-dried (60°C) samples in a Soxhlet apparatus for 3 h with 80% ethanol and for 1 h with 100% chloroform (Iiyama and Wallis 1990; Rottmann et al. 2011). The possible interference by remaining lipids was expected to be negligible, as the lipid concentration in the analyzed plant materials was generally low. The samples were air-dried in a fume hood and then oven-dried at 60°C. Then, lignin was determined according to Iiyama and Wallis (1988). A lipid-free 1-mg sample was mixed with 300 µl 25% acetyl bromide (v/v in glacial acetic acid) and 10 µl 70% perchloric acid. Subsequently, the samples were shaken and incubated for 30 min at 70°C. After 10, 20, and 30 min, the samples were shaken again. Then, the samples were rapidly cooled on ice, mixed with 300 µl 2 M NaOH, and centrifuged for 5 min at 3,000 × *g*. A 250-µl aliquot of the supernatant was mixed with 1.25 ml glacial acetic acid. The absorbance of the solution was determined at 280 nm (FLUOstar, BMG, Offenburg, Germany) in UV-transparent 96-well microwell plates (Th. Geyer). Calibration curves were generated with increasing concentrations of coniferyl alcohol (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mmol l^{-1}) (Sigma Aldrich), dissolved in UV-adequate ethanol, and processed by the same procedure as the plant samples.

CuO oxidation

Lignin-derived phenols were determined after alkaline CuO oxidation (Hedges and Ertel 1982), as described by Bierke et al. (2008). Depending on the C concentration, samples of 5–20 mg ground plant material were mixed with 500 mg of CuO, 100 mg of $Fe(NH_4)_2(SO_4)_2 \times 6\ H_2O$, 50 mg of glucose and 15 ml of 2 M NaOH. As an internal standard, 1 ml of 100 µg ml^{-1} ethyl vanillin in 2 M NaOH was added and the mixture was

oxidized for 3 h at 170°C in a pressure digester (Groteklaes, Jülich, Germany) under constant stirring. The mixture was then centrifuged for 15 min at 5000 × *g* and the pH of the supernatant was adjusted to 1.8–2.2, using 6 M HCl. In order to precipitate the humic acids, the samples were left in the dark for 1 h and then centrifuged again for 25 min at 5000 × *g*. The phenols released were solid-phase extracted by C18 columns (Bakerbond SPE, J.T. Baker, Phillipsburg, USA), and then re-eluted from the dried columns under N_2 with ethyl acetate. Ethyl acetate was removed by rotary evaporation (40°C) and the residue was re-dissolved in 1 ml of phenylacetic acid solution (internal standard for derivatization; concentration 25 µg ml^{-1} of methanol), and then dried under N_2.

For derivatization, 100 µl of pyridine and 200 µl of BSTFA (bis-(trimethlysilyl)-triflouroacetamide) were added and the solution was left in the dark for 2 h at room temperature (Bierke et al. 2008; Jacobs et al. 2011a; Kögel-Knabner 1995). Trimethylsilyl derivatives were separated by gas chromatography (GC-2010, Shimadzu, Tokyo, Japan) using a high-resolution GC column (30 m length, 0.25 mm diameter, 0.25 µm film thickness; Agilent, Santa Clara, USA) and detected by a mass-sensitive detector (GCMS-QP 2010, Shimadzu, Tokyo, Japan). The injection volume was 1 µl. The temperature setting was heating to 100°C (3 min), temperature increase to 250°C at a heating rate of 10°C min^{-1}, keeping the temperature at 250°C for 10 min, heating to 300°C at a heating rate of 30°C min^{-1} and keeping the temperature constant at 300°C for 5 min. Identification and quantification was carried out with an external standard mixture, containing known amounts of phenols. Lignin components yielded by CuO oxidation only account for the aryl ether-bonded lignin phenols (Bierke et al. 2008). CuO oxidation products are vanillin, vanillic acid and acetovanillone (vanillic units, V), syringaldehyde, syringic acid and acetosyringone (syringic units, S), p-coumaric and ferulic acid (cinnamic units, C) (Bierke et al. 2008; Jacobs et al. 2011a). The sum of these three units (V+S+C=VSC)

reflects the total lignin gained by the CuO method and is referred to as “CuO lignin” in this study.

Statistical analysis

The results presented in tables 4.1, 4.2 and in the supplementary tables 6.4 to 6.6 are arithmetic means (C, N and C/N: n = 2; ADL: n = 2; AcBr: n = 5; CuO: n = 2-3) and expressed on an oven-dry basis (60°C). Normality was tested by the Shapiro–Wilk test and equal variance by the Levene test. Data were ln- or square-root transformed for the variables that violated normal distribution. A one-way ANOVA followed by the Holm-Sidak post hoc test was used to test the significance of differences between the lignin concentrations yielded by the three methods for each plant material (Table 4.1). General difference between the three methods was analysed using paired t-tests on the method-specific means for each plant material (Fig. 4.1; total N 0-6%). For comparing the mean lignin concentrations of the two N-groups 0-2% and 2-6% total N for each method (Fig. 4.1), a one way ANOVA was performed, followed by the Holm-Sidak post hoc test. The differences of the means of the CuO product groups vanillyl, syringyl, and cinnamyl of the five plant groups and their ratios, i.e., syringyl/vanillyl and cinnamyl/vanillyl, were analyzed by a one way ANOVA, followed by the Holm-Sidak post hoc test (Fig. 4.4). Regression and multiple linear regression models were calculated between total N and the three methods for lignin determination. All regression models were tested for normality, constancy of variance, the absence of correlation between the residuals (Durbin–Watson statistics) and the absence of multi-collinearity, calculating the variance inflation factor (VIF). P-values <0.05 were considered as significant. All statistical analyses were performed using SigmaPlot 13.0 (Systat Inc., San José, USA).

4.3 Results

The 27 plant litter samples had median values of 4.5% ADL and 6.0% AcBr lignin, significantly exceeding the median of 2.1% CuO lignin (Table 4.1). ADL concentrations varied from 0.8 to 27.0% (Fig. 4.1); those of AcBr and CuO lignin ranged from 1.8 to 12.2% and from 0.6 to 9.7%, respectively. The lignin concentrations obtained by the three methods did not significantly differ for three plant materials only, i.e., vetchling, buckwheat and wheat straw (Table 4.1). ADL and AcBr lignin did not significantly differ for a further seven plant materials, ADL and CuO for a further ten plant materials, and AcBr and CuO for a further three plant materials (Table 4.1). In contrast to the between plant species variation, the variation between replicates decreased in the order CuO > AcBr >> ADL, as indicated by the mean of the coefficients of variation of the replicates of each plant material (Table 4.1).

AcBr lignin showed a significant negative non-linear relationship with total N (Fig. 4.2). In addition, the positive relationship between ADL and CuO data was negatively affected by total N, as indicated by multiple regression analysis (Fig. 4.3). Based on this analysis, two groups of plant species were formed: one with low (0-2%) and one with high N concentration (2-6%) (Fig. 4.1). The low N group always contained more lignin than the high N group. This difference was only significant for ADL and CuO, but not for AcBr lignin.

Legume and crucifer species always contained lowest median concentrations of vanillyl and syringyl units, but the differences were not significant for herbs and grass species (Fig. 4.4a). Tree species exhibited the highest median concentrations of vanillyl and syringyl units, but the differences to herbs and grass species were not significant. Grass species showed the highest median concentration of cinnamyl units, which was significantly higher than those of all other plant species. The highest median

syringyl/vanillyl ratios were found in herbs and grass species (Fig. 4.4b), significantly exceeding the lowest median ratio of the legume species. Grass species revealed the highest median cinnamyl/vanillyl ratio, which was significantly higher than those of herbs and tree species.

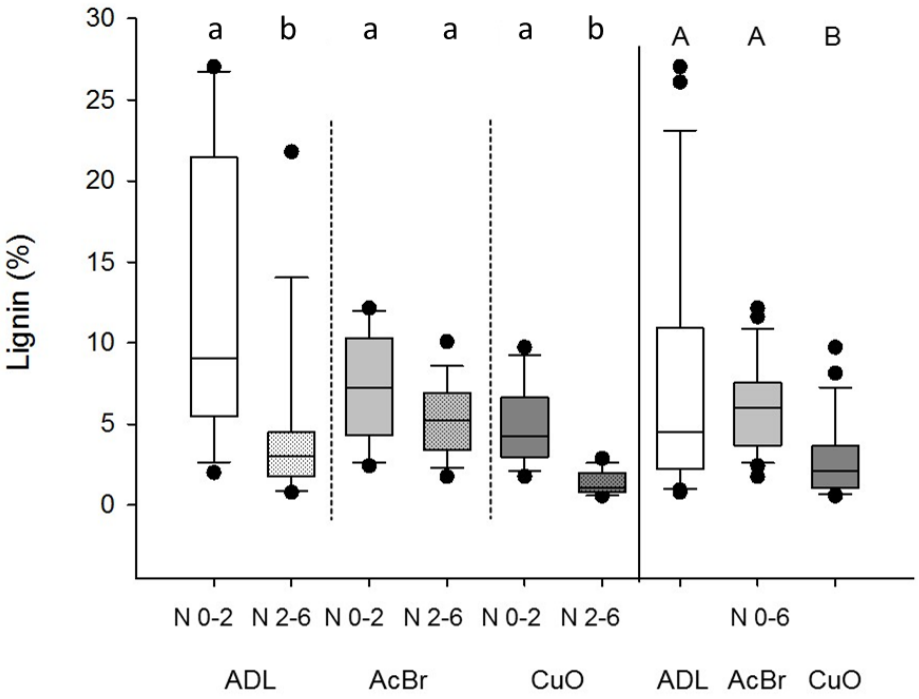

Fig. 4.1. Boxplots for lignin concentrations obtained by the acid detergent lignin (ADL), acetyl bromide (AcBr), and cupric oxide oxidation (CuO) methods for plant material containing 0 - 2 % N (n = 12), 2 - 6 % N (n = 15), and 0 - 6 % N (N = 27). Different capital letters indicate significant differences of the lignin concentrations obtained by the three methods; different small letters indicate significant differences between the lignin concentrations of two N-groups measured by the same method (P < 0.05).

Table 4.1. Chemical composition of 27 plant materials, obtained by acid detergent lignin (ADL), acetyl bromide (AcBr), and cupric oxide oxidation (CuO) methods sorted by plant groups. Different letters within a row indicate significant different means (ANOVA followed by Holm-Sidak post hoc test; $p<0.05$).

Plant group	Sample	C	N	C/N	ADL	AcBr	CuO
		(% DW)			(% DW)		
Legume	Common vetch	45	6.0	7	3.4 b	6.9 a	0.8 c
Legume	Vetchling	45	5.5	8	3.0 a	1.8 a	1.4 a
Legume	Field pea leaves	46	5.4	9	2.2 b	3.8 a	1.1 b
Legume	Bell bean	44	5.3	8	3.0 b	5.3 a	0.6 c
Legume	White lupin	46	4.3	11	2.4 b	6.8 a	0.9 b
Legume	Pea straw	44	4.0	11	8.9 a	10.1 a	1.1 b
Legume	Lucerne	45	4.0	11	4.5 ab	5.2 a	2.0 b
Crucifer	Mustard	43	3.8	11	3.7 a	3.7 a	2.1 b
Crucifer	Fodder radish	41	3.7	11	0.8 b	3.2 a	0.9 b
Crucifer	Winter oilseed rape	42	3.7	11	0.9 b	3.4 a	0.7 b
Herb	Malva	42	4.7	9	1.0 b	3.7 a	0.6 b
Herb	Sunflower	42	3.0	14	6.2 a	2.7 b	1.0 c
Herb	Phacelia	43	1.3	34	6.5 a	4.6 ab	3.7 b
Herb	Amaranth	41	1.0	40	11.1 a	7.2 ab	5.3 b
Herb	Buckwheat	45	0.9	49	5.1 a	3.1 a	3.0 a
Grass	Perennial ryegrass	44	4.4	10	2.2 b	7.1 a	1.0 b
Grass	Sugarcane filter cake	43	3.1	14	21.8 a	7.6 b	2.9 c
Grass	Sudangrass	45	3.1	15	1.8 b	6.0 a	2.4 b
Grass	Maize stem	35	1.5	24	7.1 a	7.3 a	3.1 b
Grass	Grass hay	44	1.2	37	4.1 b	10.7 a	3.0 b
Grass	Wheat straw	48	0.5	94	7.2 a	8.1 a	8.1 a
Grass	Maize leaf straw	46	0.3	154	2.0 c	12.2 a	4.9 b
Grass	Oat straw	45	0.3	170	10.9 ab	11.6 a	7.0 b
Tree	Poplar leaf litter, AF2	47	1.6	29	26.1 a	4.2 b	2.9 c
Tree	Poplar leaf litter, MAX	47	1.0	45	18.7 a	7.2 b	1.8 c
Tree	Willow leaf litter	48	0.9	52	27.0 a	7.3 b	5.5 b
Tree	Poplar root	49	0.4	109	22.4 a	2.4 c	9.7 b
	CV (±%)	1.2	2.3	1.6	5.6	20	29

CV = mean coefficient of variation between replicates per method (C, N, C/N: $n = 2$; ADL: $n = 2$; AcBr: $n = 5$; CuO: $n = 2$-3).

Table 4.2. Concentrations of vanillyl, syringyl, and cinnamyl units as well as the ratios of syringyl to vanillyl and cinnamyl to vanillyl units of 27 plant materials, obtained by cupric oxide oxidation (CuO) method sorted by plant groups.

Plant group	Sample	Vanillyl units	Syringyl units	Cinnamyl units	Syringyl / vanillyl units	Cinnamyl/ vanillyl units
		(mg g^{-1} DW)				
Legume	Common vetch	2.9	1.8	3.7	0.6	1.3
Legume	Vetchling	5.4	2.4	6.0	0.5	1.2
Legume	Field pea leaves	5.3	2.2	4.0	0.4	0.8
Legume	Bell bean	2.6	1.5	2.0	0.5	0.8
Legume	White lupin	5.7	2.0	1.5	0.4	0.3
Legume	Pea straw	4.4	2.6	3.6	0.6	0.8
Legume	Lucerne	8.2	7.1	4.8	0.9	0.6
Crucifer	Mustard	9.0	10.1	2.2	1.1	0.2
Crucifer	Fodder radish	2.6	1.4	4.8	0.5	1.9
Crucifer	Winter oilseed rape	2.1	1.7	3.3	0.8	1.7
Herb	Malva	1.9	1.2	2.5	0.6	1.4
Herb	Sunflower	5.1	4.3	1.0	0.8	0.2
Herb	Phacelia	15.5	18.5	2.5	1.2	0.2
Herb	Amaranth	13.3	37.7	2.2	2.9	0.2
Herb	Buckwheat	8.7	17.0	3.8	2.0	0.4
Grass	Perennial ryegrass	3.1	2.2	5.1	0.7	1.7
Grass	Sugarcane filter cake	6.0	10.9	12.0	1.9	2.2
Grass	Sudangrass	7.0	4.5	12.6	0.6	1.8
Grass	Maize stem	7.5	11.9	11.9	1.6	1.6
Grass	Hay	9.4	9.8	10.9	1.1	1.2
Grass	Wheat straw	24.0	39.3	18.2	1.6	0.8
Grass	Maize leaf straw	6.5	10.3	32.1	1.6	4.9
Grass	Oat straw	21.0	28.9	20.5	1.4	1.0
Tree	Poplar leaf litt. AF2	14.6	10.6	3.3	0.7	0.2
Tree	Poplar leaf litt. MAX	8.8	6.5	2.5	0.8	0.3
Tree	Willow leaf litter	27.8	24.7	2.3	0.9	0.1
Tree	Poplar root	55.5	39.1	2.6	0.9	0.1
	CV (±%)	30	33	29	13	21

CV = mean coefficient of variation between replicates (CuO: n = 2-3).

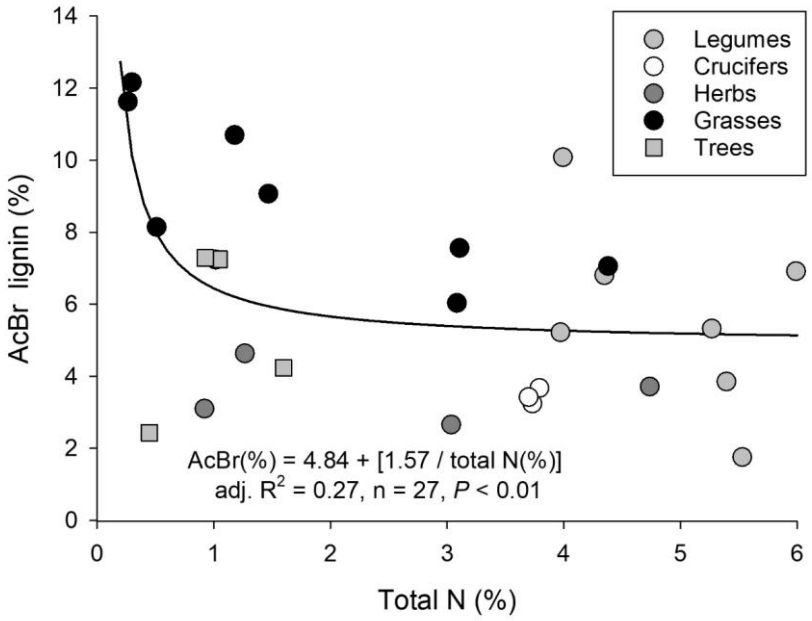

Fig. 4.2. Relationship between AcBr-lignin and total N in the 27 organic materials.

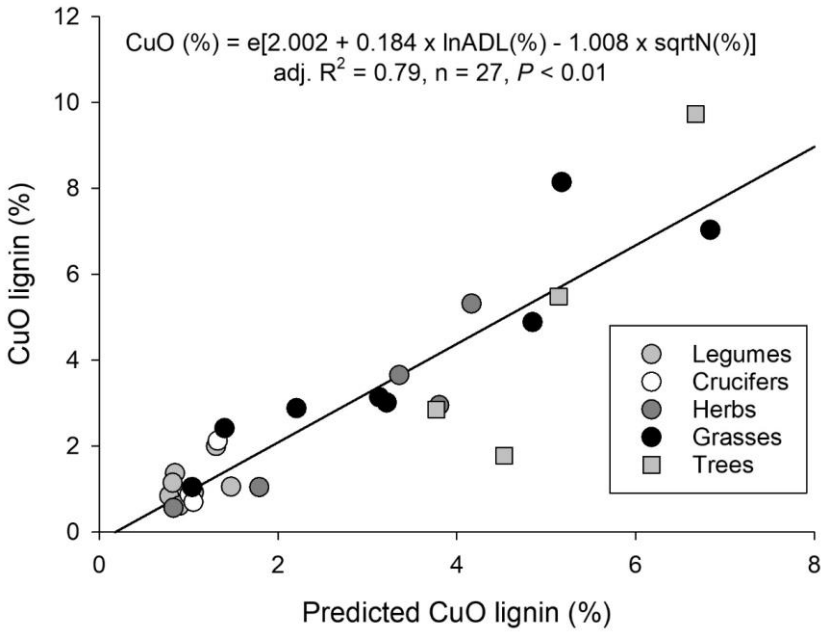

Fig. 4.3. Multiple linear regression model for CuO lignin predicted by ADL and total N in the 27 organic materials.

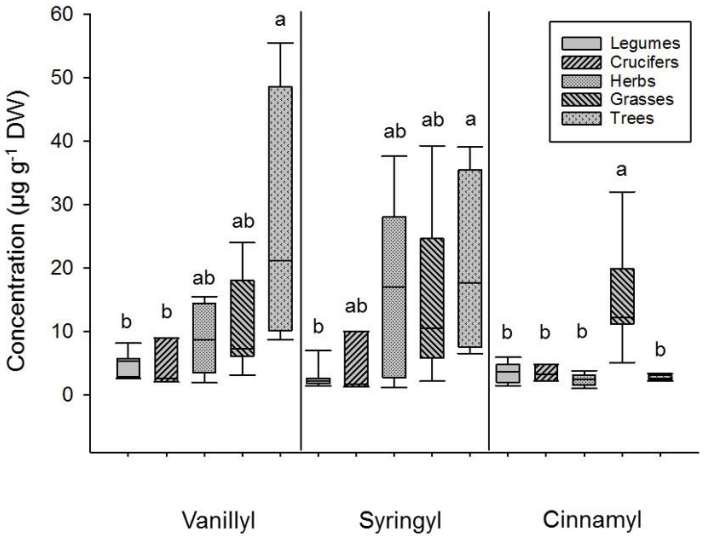

Fig. 4.4a.

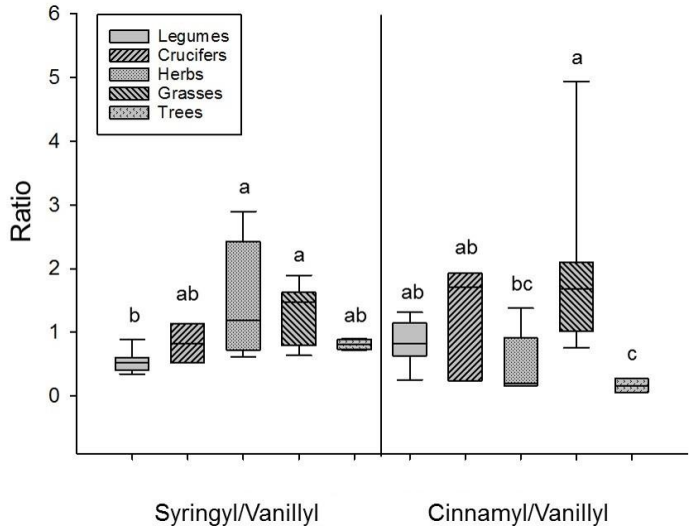

Fig. 4.4b.

Fig. 4.4. Boxplot for (a) vanillyl, syringyl, and cinnamyl units as well as (b) syringyl/vanillyl units and cinnamyl/vanillyl units in the plant groups legumes (n = 7), crucifers (n = 3), herbs (n = 5), grasses (n = 8), and trees (n = 4) obtained by the cupric oxide oxidation (CuO) method. Different letters indicate significant differences of the concentrations of the given units between the plant groups (P < 0.05).

4.4 Discussion

Each of the three methods for determining lignin in plant material has specific advantages and drawbacks. The ADL method is simple and reproducible, i.e., the variation is low between replicate samples, and large datasets are available for comparison (Fukushima and Hatfield 2004; Hatfield and Fukushima 2005; Van Soest 1963). ADL data are also available as NIRS estimates for many organic substrates after appropriate calibration (Althaus et al. 2013; Marten et al. 1983). The AcBr procedure is fast as well as relatively simple and inexpensive, with less interference from non-lignin products than ADL (Dence 1992; Hatfield and Fukushima 2005). The CuO method is more compound-specific and gives additional information on the composition of the lignin. However, each procedure has drawbacks, i.e., the lignin concentration is systematically affected by plant groups and N concentration in a method-specific way.

ADL

Most ADL concentrations were within the ranges reported in the literature. ADL concentrations were 4.2 to 9.7% in legumes obtained by Fukushima and Hatfield (2004) and Iiyama and Wallis (1990), 2.8% in young grass found by Fukushima and Hatfield (2004), 8.3 to 11.2% in mature cereal straw obtained by Fukushima and Hatfield (2004) and Iiyama and Wallis (1990), and 10.1 to 24.1% in tree leaves measured by Melillo et al. (1982). In green leaves of forage legume, but especially in grass species, H_2SO_4 can dissolve large amounts of the lignin, leading to an underestimation of ADL concentrations (Hatfield et al. 1994; Hatfield and Fukushima 2005; Kondo et al. 1987; Lowry et al. 1994). This might explain the lower ADL than AcBr lignin concentrations in many legume, crucifer, and grass species.

In contrast to these three groups of plant species, the ADL concentrations were considerably higher than the AcBr lignin concentrations in the group of tree species. Mehring et al. (2015) obtained 8 to 14% ADL in different leaf litter types, which is lower than in the recent study. In contrast, Brinkmann et al. (2002) measured 12.5 to 20% ADL in leaves of different tree species, which is comparable with the ADL of the tree leaves of our study. However, Brinkmann et al. (2002) detected considerable amounts of bound protein in the ADL fraction and estimated their contribution to ADL in the range of 14 to 18% in beech leaves. These results indicate that the ADL method tends to overestimate lignin to different degrees in leaves and wood. This view is supported by the negative effect of the total N concentration on the relationship between ADL and CuO. Acid dissolution of lignin, on the one hand, and lignoproteins in ADL (Brinkmann et al. 2002), on the other, are reasons for the absence of a linear relationship between ADL and AcBr lignin, as already observed by Brinkmann et al. (2002). It is important to note that not only lignoproteins but also the presence of cutins, surface waxes, and condensed tannins may result in an overestimation of ADL (Johansson et al. 1986; Preston et al. 1997; Zech et al. 1987). The highest range between minimum and maximum values is a specific feature of the ADL method in comparison with the other methods. The ADL method is not recommended for litter from immature plants, where ADL results in underestimation of lignin. The ADL method is also not recommended for litter that contains high concentrations of protein and cutins, waxes, and tannins, where ADL leads to an overestimation of lignin. According to the current results, ADL is certainly useful for all kinds of straw.

AcBr lignin

Literature AcBr concentrations were 8.0% for herbs (Iiyama and Wallis 1990) and 7.1 to 13.5% for legumes (Fukushima and Hatfield 2004; Iiyama and Wallis 1990), which differs only slightly from the present AcBr lignin concentrations. Rottmann et al. (2011) measured higher AcBr lignin concentrations in alfalfa (18.0%). Also, the AcBr-lignin concentrations ranged from 12.3 to 16.5% for grasses (Fukushima and Hatfield 2004; Iiyama and Wallis 1990), which is more than twice that of our study. In the present study, AcBr lignin in mature cereal straw was lower than that reported by Fukushima and Hatfield (2004), i.e. 12.4 to 21.3%, and Rottmann et al. (2011), i.e., 29.0% in wheat straw. Rottmann et al. (2011) revealed 19.0% AcBr lignin concentration in canola, which is 5 times more than that in the present study. The main reason is probably that all crucifer plants analyzed were young, having been harvested 50 days after sowing.

The AcBr procedure is based on the formation of acetyl derivatives in non-substituted OH groups and a bromide replacement of the α-C-OH groups to solubilize lignin completely under acidic conditions (Moreira-Vilar et al. 2014). The required AcBr digestion time may also be a source of error, as it perhaps varies between different plant species. Also, esterified non-ligneous phenolic components may interfere at 280-nm (Brinkmann et al. 2002), although the preceding fat extraction in the AcBr method should remove most of this material (Zimmer 2002).

One difficulty with the AcBr procedure is the need for a well-defined lignin standard that reflects the composition of lignin units (Dence 1992). It has been reported that the absorption maximum of lignin phenols shifts towards 270 nm in samples with a high sinapyl concentration (Hatfield and Fukushima 2005). However, there is no evidence to suggest a serious underestimation of AcBr lignin in the current plants species, containing high concentrations of syringyl units, i.e., CuO oxidation products of sinapyl alcohol. It

has been considered that xylans are partially degraded to furfurals, which absorb in the 280-nm region under acidic conditions and in the presence of acetyl bromide and that this is exacerbated by the addition of perchloric acid (Hatfield et al. 1999; Hatfield and Fukushima 2005). However, a serious interference of xylans did not always explain the difference between the tested methods (Moreira-Vilar et al. 2014) and is also not obvious in the present set of samples, including grass materials known to contain high concentrations of xylans (Faik 2010).

Problems can be caused by proteins remaining insoluble in the acid solution, causing light scattering and excessive irregular absorption (Hatfield and Fukushima 2005), which might be another reason for the variation between replicate samples. However, the negative regression between AcBr lignin and total N concentration points to the inverse relationship between cell-wall components and protein, probably indicating increasingly unstable and inhomogeneous distributed lignin at low C/N ratios. This results in the often-stated negative relationship between substrate C/N ratio and decomposition rate (e.g., Scheller and Joergensen 2008). The AcBr method did not exhibit any specific weaknesses in most cases for analyzing the current plant material except the relatively high coefficient of variation. This might be the main reason for the absence of any correlation to the ADL and CuO procedures.

CuO lignin

Hedges and Mann (1979) reported 4.5% CuO lignin as a mean of six species of non-woody angiosperm tissues, and Klotzbücher et al. (2011) reported 3.2 to 4.6% CuO lignin in litter and roots of young herbal species, which is within the range of the present study. Higher CuO lignin concentrations in grasses (8.5%) and mature cereal straw (9.7%) were reported by Lobe et al. (2002). Higher CuO lignin concentrations were also measured by

Dignac et al. (2005), i.e. 9.0 and 17.9% and in wheat leaves and stems, respectively, and by Jacobs et al. (2011a) and Bierke et al. (2008), i.e. 16.8 to 17.8% in mature cereal straw.

Cupric oxide (CuO) oxidation cleaves lignin and releases a set of lignin-derived phenols that can be analyzed (Hedges and Ertel 1982). However, the optimum CuO digestion time varies between different plant species (Thevenot et al. 2010). Consequently, the release of phenolic units may be incomplete (Dignac et al. 2009), especially from the inner part of thick cell wall units (Otto and Simpson 2006). The release of phenolic units also might be affected by processing of the organic substrates, e.g., by drying and milling (Klotzbücher et al. 2011). Despite the highest coefficient of variation, the CuO method is an interesting alternative to the ADL and AcBr methods after further optimization of digestion time and processing. This is especially true for root and mature plant litter, i.e. the organic material that dominates C input into soil. There, phenolic product ratios of CuO can then be used as indicators of lignin sources for SOC sequestration (Crow et al. 2009; Otto and Simpson 2006).

The ratio of cinnamyl/vanillyl units can be used to trace organic matter from non-woody, especially gramineous vascular plants (Hedges and Ertel 1982). A decrease in this ratio also suggests microbial lignin alteration, as cinnamyl are more labile than vanillyl units (Kiem and Kögel-Knabner 2003). The ratio of syringyl/vanillyl units has been used even more frequently as an index of lignin oxidation (Goñi et al. 1993; Hedges et al. 1988) and microbial lignin alteration (Kiem and Kögel-Knabner 2003; Thevenot et al. 2010). In an arable soil, the ratio of cinnamyl/vanillyl units was 0.4 (Jacobs et al. 2011a), exceeding the ratio of 9 of the 27 plant species analyzed, especially of the tree litter. Such relatively low values in fresh organic substrates obscure any alteration during fungal decomposition. This points to the importance of including the original composition of VSC units of organic substrates added to soil when using CuO oxidation products for tracking lignin decomposition (Jacobs et al. 2011a).

4.5 Conclusions

The ADL method is simple and reproducible, i.e., the variation between sample replicates is small, and large datasets are available for comparison. The ADL method is not recommended for litter from immature plants, where ADL results in underestimation of lignin. The ADL method is also not recommended for litter that contains high concentrations of protein and cutins, waxes, and tannins, where ADL leads to overestimation of lignin. According to the current results, ADL is certainly useful for all kinds of straw. The AcBr procedure is fast, with less interference from non-lignin products than ADL and did not exhibit any specific weaknesses in most cases for analyzing the current plant material. Despite the highest coefficient of variation, the CuO method is an interesting alternative to the ADL and AcBr methods after further optimization of digestion time and processing. This is especially true for root and mature plant litter, i.e. the organic material that dominates C input into soil. There, phenolic product ratios of CuO can then be used as indicators of lignin sources for SOC sequestration. The variation in the initial ratios of syringyl/vanillyl and cinnamyl/vanillyl between different plant species needs to be taken into account when using these ratios as indices for fungal degradation of lignin in soil.

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5. General conclusions

Study 1 and 2 of the presented research provide insights on the dependencies between tillage intensity, SOC contents, VWC, soil temperature, maize leaf litter mineralization and soil respiration. Also, a method comparison was executed by applying two different types of mesh bags to investigate litter decomposition under plough, grubber and no-tillage treatments.

Soil organic carbon content, volumetric water content, soil temperature and CO_2 flux under different tillage intensities

Soil organic carbon (SOC) stocks were higher under grubber and no-tillage treatments in the 0-10 and 0-20 cm soil layers of study 1, and in the 0-5 cm soil layer of study 2. In study 1, the VWC in 5 cm soil depth in grubber and no-tillage treatments were also significantly higher compared to plough tillage on average over one year, matching the higher SOC contents. Similarly, in study 2, the highest SOC contents in no-tillage and grubber tillage lead also to the highest VWC.

In study 1, no-tillage had the highest VWC and at the same time lowest mean temperature in 5 cm of depth, however, a similar inverse relationship between VWC and soil temperature was not detected in the grubber and plough treatments. Regarding the site, a negative relationship between VWC and soil temperature was only discovered in the soil samples of one experimental site (Zschortau), indicating a site specific effect, which was probably caused by the higher sand content. The soil of the experimental site in Zschortau contains 320 g kg^{-1} sand and thus significantly more than the soils of the other three sites, which contain 10-30 g kg^{-1} sand (Murugan et al., 2014). Similarly, in study 2, the highest VWC occurred with lowest mean soil temperatures in 5 cm of soil

depth in no-tillage, whereas plough and grubber treatment revealed the same mean temperature.

In study 1, despite the lowest soil temperature in no-tillage (5 cm of depth) during the unplanted period, CO_2 flux was not significantly lower compared to grubber and plough. In the period with growing maize plants, CO_2 flux was highest in grubber treatment, which is similar to study 2. In this period, lowest CO_2 flux was measured in plough, although soil temperature was not lowest in this treatment in the 5 cm layer, revealing that soil temperature alone cannot predict the respiration response. This was confirmed by the regressions, which revealed that soil temperature had the biggest effect on CO_2 flux, but CO_2 flux was also significantly affected by VWC, treatment × soil temperature and treatment × VWC interactions.

In study 1, against the expectations a slower microbial turnover was only indicated in the no-tillage, but not in the grubber treatment, by an approximately 30% lower CO_2-C/MBC ratio in comparison to the plough and grubber treatments.

A limitation of the methodological approach of study 1 was the soil depth investigated, i.e. 0-20 cm, which did not comprise the whole soil layer affected by plough, i.e. down to 30 cm. If possible, soil sampling should even include the soil layer below the depth of the horizon which is influenced by plough tillage, as also recommended by Jacobs et al. (2015) for the estimation of SOC stocks. Further, maybe the PVC rings encompassed a too small soil volume to buffer against the temperatures of the surrounding soil. Also, the method of soil core collection after harvest might have increased the bulk density, but if so, it would have been a systematic error affecting all 48 soil columns. It cannot be excluded that a minor share of the measured CO_2 flux originated from the subsoil beneath the soil columns, however, most probably this was a relatively small amount and in addition also a systematic error.

In study 2, an indicator which was pointing to a lower microbial turnover in no-tillage treatment was the lowest CO_2 flux, most probably because of the slow soil warming also of deeper soil layers due to the highest volumetric water content. However, grubber treatment revealed counter-intuitively the highest CO_2 fluxes, also exceeding those of the plough treatment which is similar to study 1.

Mass loss rate constant of maize-C

The mass loss rate constant of maize-C in the soil bags was unexpectedly highest in no-tillage despite the highest mean volumetric water content, lowest soil temperature as well as lowest CO_2 flux in this treatment. Probably the dominant factor controlling C mass loss was the microbial biomass C content in the 0-5 cm soil layer, which was highest in the no-tillage treatment. Additionally, the measured temperature difference was presumably too small to have a more enhancing effect on litter decomposition in plough and grubber treatments, where the soil temperature means were slightly higher compared to no-tillage.

Microbial carbon use efficiency and microbial turnover

Against the expectations, the microbial carbon use efficiency was not higher and the microbial turnover was not lower in grubber and no-tillage treatments compared to the plough treatment. Both parameters were not significantly affected by the factor tillage intensity (results for microbial turnover not shown).

Litterbag versus soil bag method

Litter was decomposed more rapidly throughout the eight months in soil bags than in litterbags. Although both methods are suitable for monitoring litter decomposition, the

soil bag method has the advantage that the litter is from beginning on in intimate contact to the autochthonous microbial decomposer community, which is a more realistic simulation of the field conditions, at least for tilled soils where crop residues are incorporated into the soil. Further, the difference between plough and no-tillage treatment regarding C loss was significant and the variation was lower in the soil bag compared to the litterbag method.

Regarding the soil bag experiment, it is recommended to choose shorter and higher numbers of exposure intervals in the first phase of decomposition in order to measure the peak of C4-C incorporation into the microbial biomass. This would enable to assess the microbial carbon use efficiency more exactly.

Maybe study 2 would be more conclusive if soil and litterbags also would have been buried in deeper soil layers to assess the recovered maize litter C, the microbial carbon use efficiency as well as the microbial turnover. Jacobs et al. (2011b) detected significant depth effects on the amount of recovered maize residue C in a litterbag experiment under different tillage intensities.

Lignin method comparison

In study 3 a method comparison was executed by assessing the lignin contents analysed by three different methods.

The ADL method is simple and reproducible, i.e., the variation between sample replicates is small, and large datasets are available for comparison. The ADL method is not recommended for litter from immature plants, where ADL results in underestimation of lignin. It is also not appropriate for litter that contains high concentrations of protein and cutins, waxes, and tannins, which lead to overestimation of lignin. However, ADL seems to be a useful method for different kinds of straw.

Comparing the AcBr with the ADL procedure, non-lignin products interfere less with the AcBr than with the ADL procedure. The CuO method is not interfered with by any other organic component in the plant material and gives additional information on the composition of the lignin. However, the release of phenolic units is incomplete. Despite the highest coefficient of variation, the CuO procedure is a promissing alternative to the ADL and AcBr procedures after further optimization of digestion time and processing. This is especially true for root and mature plant litter, i.e. the organic material that dominates C input into soil. There, phenolic product ratios of the CuO method could then be used as indicators of lignin sources for SOC sequestration.

6. Supplementary material

6.1 Study 2 - Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial

Supplementary Table 6.1. $\delta^{13}C$ values of SOC, MBC, and POMC in the tillage treatments at three sampling times. Probability values of an ANOVA for repeated measures (P < 0.05).

	SOC	MBC	POMC	
			0.4-2 mm	> 2 mm
	δ13C (‰)			
Sampling 1				
Plough	-24.8	-17.0	-16.9	-14.3
Grubber	-26.3	-18.1	-20.5	-16.1
No-tillage	-25.9	-18.9	-20.6	-15.8
Sampling 2				
Plough	-25.3	-17.1	-16.9	-14.4
Grubber	-25.7	-18.7	-20.5	-16.0
No-tillage	-26.0	-19.8	-20.5	-15.5
Sampling 3				
Plough	-25.2	-17.8	-17.7	-16.0
Grubber	-25.5	-20.0	-22.4	-19.2
No-tillage	-26.0	-20.3	-22.2	-19.1
Probability values				
Treatment	<0.01	<0.01	<0.01	<0.01
Time	NS	<0.01	<0.01	<0.01
Treatment × time	<0.01	<0.01	NS	NS
CV (± %)	1.3	3.7	4.7	6.5

NS = not significant; CV = mean coefficient of variation between replicate measurements (n = 7).

Supplementary Table 6.2. Contents of total N in soil, C3 and N in POM (C3-POMC and POMN, respectively) as well as the POM-C/N ratio in the tillage treatments at three sampling times. Probability values of an ANOVA for repeated measures (P < 0.05).

	Total N	C3-POMC		POMN		POM-C/N	
	(mg g^{-1} soil)	0.4-2 mm	> 2 mm	0.4-2 mm	> 2 mm	0.4-2 mm	> 2 mm
		($\mu g\ g^{-1}$ soil)					
Sampling 1							
Plough	1.8	0.26	0.13	56	63	17	25
Grubber	2.1	0.73	0.24	79	37	19	32
No-tillage	2.5	0.78	0.23	80	44	19	32
Sampling 2							
Plough	1.7	0.22	0.12	57	61	15	21
Grubber	2.1	0.66	0.12	89	29	16	24
No-tillage	2.5	0.70	0.13	92	38	15	22
Sampling 3							
Plough	1.7	0.25	0.12	54	25	15	23
Grubber	2.2	0.59	0.17	67	23	15	21
No-tillage	2.6	0.63	0.12	67	12	16	25
Probability values							
Treatment	<0.01	<0.01	NS	<0.01	<0.05	NS	NS
Time	NS	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Treat × time	NS	NS	NS	NS	<0.05	NS	<0.01
CV (± %)	5.6	22	48	21	47	7.3	15

NS = not significant; CV = mean coefficient of variation between replicate measurements (n = 7).

Supplementary Table 6.3. Contents of MBC and MBN as well as the MB-C/N ratio in the tillage treatments at three sampling times. Probability values of an ANOVA for repeated measures (P < 0.05).

	MBC	MBN	MB-C/N
	($\mu g\ g^{-1}$ soil)		
Sampling 1			
Plough	731	147	5.0
Grubber	943	178	5.6
No-tillage	1059	207	5.1
Sampling 2			
Plough	568	117	4.9
Grubber	742	156	4.8
No-tillage	871	178	4.9
Sampling 3			
Plough	506	86	5.9
Grubber	666	125	5.4
No-tillage	819	150	5.6
Probability values			
Treatment	<0.01	<0.01	NS
Time	<0.01	<0.01	<0.01
Treatment × time	NS	NS	NS
CV (± %)	12	16	9.4

NS = not significant; CV = mean coefficient of variation between replicate measurements (n = 7).

6.2 Study three - Comparison of different methods for determining lignin concentration and quality in herbaceous and woody plant recidues

Supplementary Table 6.4. Cupric oxide oxidation (CuO) products, i.e. benzoic acid (BAD), 4-hydroxybenzaldehyde (OHBAL), 4-hydroxyacetophenone (OHAPON), salicylic acid (2-hydroxybenzoic acid) (SAAD), vanillin (4-hydroxy-3-methoxybenzaldehyde) (VAL), 3-hydroxybenzoic acid (OHBAD), of 27 plant materials sorted by plant groups.

Plant group	Sample	BAD	OHBAL	OHAPON	SAAD	VAL	OHBAD
		(mg g^{-1} DW)					
Legume	Common vetch	1.97	0.46	0.25	0.44	1.81	0.31
Legume	Vetchling	1.59	0.63	0.34	0.42	3.78	0.27
Legume	Field pea leaves	1.64	0.46	0.31	0.52	3.59	0.30
Legume	Bell bean	1.85	1.53	0.67	0.52	1.22	0.33
Legume	White lupin	1.80	0.51	0.37	0.50	2.09	0.31
Legume	Pea straw	1.12	0.19	0.19	0.31	3.13	0.25
Legume	Lucerne	1.83	0.44	1.84	0.69	5.03	0.26
Crucifer	Mustard	1.87	1.17	0.48	0.55	6.54	0.29
Crucifer	Fodder radish	1.45	0.37	0.28	0.45	1.43	0.29
Crucifer	Winter oilseed rape	1.23	0.63	0.39	0.38	1.17	0.24
Herb	Malva	1.42	0.21	0.32	0.38	1.10	0.29
Herb	Sunflower	1.12	0.22	0.20	0.40	3.72	0.27
Herb	Phacelia	0.91	0.18	0.20	0.34	11.76	0.24
Herb	Amaranth	0.98	0.36	0.31	0.57	10.11	0.32
Herb	Buckwheat	0.67	0.26	0.19	0.32	6.66	0.22
Grass	Perennial ryegrass	2.34	0.53	0.35	0.57	1.56	0.40
Grass	Sugarcane filter cake	1.72	1.42	0.80	0.88	3.52	0.51
Grass	Sudangrass	1.13	1.96	0.75	0.39	5.17	0.27
Grass	Maize stem	0.70	0.22	0.17	0.20	5.86	0.21
Grass	Hay	0.65	0.10	0	0.18	7.34	0.24
Grass	Wheat straw	0.68	0.55	0.27	0.35	18.82	0.20
Grass	Maize leaf straw	0.43	0.13	0.03	0.10	5.19	0.18
Grass	Oat straw	0.38	0.25	0	0.05	17.02	0.17
Tree	Poplar leaf litter, AF2	2.04	0.44	0.53	1.52	10.15	0.40
Tree	Poplar leaf litter, MAX	2.11	0.21	0.26	1.46	6.10	0.52
Tree	Willow leaf litter	1.27	0.37	0.34	0.79	20.29	0.41
Tree	Poplar root	1.92	0.56	0.46	1.21	38.73	0.44
	CV (±%)	25	114	100	77	27	22

CV = mean coefficient of variation between replicates (n = 2-3).

Supplementary Table 6.5. Cupric oxide oxidation (CuO) products, i.e. acetovanillone (4-hydroxy-3-methoxyacetophenone) (AVON), 4-hydroxybenzoic acid (OHBAD), phthalic acid (PAD), syringaldehyde (SAL), vanillic acid (4-hydroxy-3-methoxybenzoic acid) (VAD), acetosyingone (3,5-dimethoxy-4-hydroxyacetophenone) (ASON), of 27 plant materials sorted by plant groups.

Plant group	Sample	AVON	OHBAD	PAD	SAL	VAD	ASON
		(mg g^{-1} DW)					
Legume	Common vetch	0.72	0.36	0.60	0.83	0.40	0.43
Legume	Vetchling	1.02	0.52	0.50	1.40	0.55	0.58
Legume	Field pea leaves	1.11	0.51	0.60	1.02	0.59	0.63
Legume	Bell bean	0.93	0.72	0.62	0.51	0.49	0.48
Legume	White lupin	0.92	0.74	0.67	0.99	2.72	0.57
Legume	Pea straw	0.83	0.42	0.48	1.53	0.40	0.68
Legume	Lucerne	2.41	0.53	0.58	3.02	0.72	3.25
Crucifer	Mustard	1.54	1.09	0.66	6.69	0.88	2.06
Crucifer	Fodder radish	0.69	0.65	0.53	0.53	0.44	0.43
Crucifer	Winter oilseed rape	0.50	0.54	0.45	0.78	0.38	0.53
Herb	Malva	0.53	0.25	0.53	0.47	0.29	0.31
Herb	Sunflower	0.94	0.35	0.45	2.91	0.45	0.86
Herb	Phacelia	2.67	0.25	0.47	12.58	1.11	3.72
Herb	Amaranth	2.05	0.48	0.64	26.01	1.13	7.58
Herb	Buckwheat	1.44	0.34	0.39	12.24	0.59	3.11
Grass	Perennial ryegrass	0.80	0.75	0.70	0.95	0.75	0.68
Grass	Sugarcane filter cake	1.65	0.51	1.04	6.62	0.83	2.57
Grass	Sudangrass	1.24	0.97	0.48	1.30	0.62	2.13
Grass	Maize stem	1.17	0.03	0.29	5.63	0.49	4.06
Grass	Hay	1.51	0.05	0.30	4.78	0.53	3.38
Grass	Wheat straw	3.52	0.25	0.42	21.05	1.68	10.77
Grass	Maize leaf straw	1.00	0.02	0.24	6.30	0.32	2.63
Grass	Oat straw	2.79	0.02	0.25	15.96	1.14	8.03
Tree	Poplar leaf litter, AF2	2.90	0.48	0.75	6.96	1.54	2.22
Tree	Poplar leaf litter, MAX	1.75	0.29	0.76	4.03	0.92	1.49
Tree	Willow leaf litter	5.42	0.54	0.60	17.43	2.14	4.41
Tree	Poplar root	12.46	3.94	0.87	26.53	4.35	7.70
	CV (±%)	33	71	35	29	54	40

CV = mean coefficient of variation between replicates (n = 2-3).

Supplementary Table 6.6. Cupric oxide oxidation (CuO) products, i.e. 3,5-dihydroxybenzoic acid (DiOHBAD), syringic acid (SAD), p-coumaric acid (CAD), ferulic acid (FAD), and heptadecanoic acid (HAD), of 27 plant materials sorted by plant groups.

Plant group	Sample	DiOHBAD	SAD	CAD	FAD	HAD
		(mg g^{-1} DW)				
Legume	Common vetch	0.83	0.55	2.66	1.03	0.68
Legume	Vetchling	0.63	0.44	4.15	1.80	0.56
Legume	Field pea leaves	0.77	0.54	2.63	1.35	0.69
Legume	Bell bean	0.71	0.47	1.03	0.96	0.70
Legume	White lupin	0.69	0.48	0.74	0.75	0.68
Legume	Pea straw	0.60	0.43	2.23	1.32	0.47
Legume	Lucerne	0.67	0.78	1.69	3.10	0.62
Crucifer	Mustard	0.82	1.31	0.64	1.56	0.70
Crucifer	Fodder radish	0.67	0.41	1.73	3.07	0.60
Crucifer	Winter oilseed rape	0.57	0.39	0.92	2.32	0.52
Herb	Malva	0.77	0.44	0.88	1.64	0.60
Herb	Sunflower	0.60	0.54	0.40	0.62	0.54
Herb	Phacelia	0.88	2.16	0.90	1.64	0.54
Herb	Amaranth	1.02	4.11	0.86	1.31	0.71
Herb	Buckwheat	0.56	1.65	2.15	1.66	0.77
Grass	Perennial ryegrass	0.92	0.60	1.15	3.97	0.80
Grass	Sugarcane filter cake	1.74	1.68	9.60	2.35	1.20
Grass	Sudangrass	0.57	1.07	3.69	8.95	0.53
Grass	Maize stem	0.61	2.26	7.69	4.23	0.38
Grass	Hay	0.72	1.67	3.94	6.97	0.35
Grass	Wheat straw	0.89	7.47	7.17	11.00	0.49
Grass	Maize leaf straw	0.67	1.35	16.17	15.90	0.32
Grass	Oat straw	0.53	4.91	8.76	11.75	0.28
Tree	Poplar leaf litter, AF2	1.07	1.44	2.23	1.11	0.78
Tree	Poplar leaf litter, MAX	1.42	1.03	1.50	0.99	0.95
Tree	Willow leaf litt.	1.26	2.89	0.71	1.56	0.69
Tree	Poplar root	1.41	4.88	1.20	1.44	1.01
	CV (±%)	39	42	28	30	34

CV = mean coefficient of variation between replicates (n = 2-3).

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