Tillage-induced short-term soil organic matter turnover and respiration

S. R. Fiedler[1], P. Leinweber[1], G. Jurasinski[1], K.-U. Eckhardt[1], S. Glatzel[1,*]

[1]{University of Rostock, Faculty of Agricultural and Environmental Sciences, Rostock, Germany}

[*]{now at: University of Vienna, Department of Geography and Regional Research, Vienna, Austria}

Correspondence to: S. R. Fiedler (sebastian.fiedler@uni-rostock.de)

Abstract

Tillage induces decomposition and mineralisation of soil organic matter (SOM) by the disruption of macroaggregates and may increase soil CO_2 efflux by respiration, but these processes are not well understood at the molecular level. We sampled three treatments (mineral fertiliser = MF, biogas digestate = BD, unfertilised control = CL) of a stagnic luvisol a few hours before and directly after tillage, and four days later from a harvested maize field in Northern Germany and investigated these samples by pyrolysis-field ionization mass spectrometry (Py-FIMS) and hot-water extraction. Before tillage, the Py-FIMS mass spectra revealed distinct differences in relative ion intensities of undisturbed soil compared to BD most likely attributable to the cattle manure used for the biogas feedstock and to relative enrichments during anaerobic fermentation. After tillage, the CO_2 effluxes were increased in all treatments, but this increase was less pronounced in BD. We explain this by a restricted availability of readily biodegradable carbon compounds and, possibly an inhibitory effect of sterols from digestates. Despite high spatial variability, significant changes in SOM composition were observed following tillage. In particular, lignin decomposition and increased proportions of N-containing compounds were detected in BD. In MF, lipid proportions increased at the expense of ammonia, ammonium, carbohydrates and

peptides, indicating an enhanced microbial activity. SOM composition in CL was unaffected by tillage. In summary, combining all analyses data provided strong evidence for significant short-term SOM changes due to tillage in fertilised soils.

1 Introduction

The influence of tillage on soil organic matter (SOM) is generally well understood. Tillage stimulates decomposition of SOM resulting in increased CO_2 efflux (Alvarez et al., 2001; Dao, 1998; Liu et al., 2006), mostly by aeration and by the disruption of SOM that had been protected in macro-aggregates (Grandy and Robertson, 2007; Six et al., 1999). In the long-term, tillage promotes a shift of chemical structure and age towards more recent SOM (Grandy and Neff, 2008) due to both, the mineralisation of older SOM and the decomposition of recent plant residues (Balesdent et al., 1990). In addition, tilled soils contain lower amounts of readily biodegradable (hereinafter referred to as "labile") organic matter (Balota et al., 2003) and have an increased potential for mineralisation and nitrification (Doran, 1980) which implies a lower potential to immobilise mineral N (Follett, R. F. and Schimel, D. S., 1989; Schulten and Hempfling, 1992). However, the immediate, short-term effects of tillage events on SOM are almost unknown.

Research on short term effects of tillage on SOM has focussed largely on CO_2 efflux: several studies recorded the dynamics of CO_2 efflux immediately after tillage (cf., Table 5 in Fiedler et al., 2015) and some basic models have been developed that describe correlations between CO_2 efflux and the turnover of soil organic carbon (SOC) after tillage by first order kinetics (La Scala et al., 2008). Admittedly, SOC accounts for the majority of SOM, but these correlations do not causally explain which SOC constituents are mineralised. Furthermore, SOM-CO_2-efflux-relationships are influenced by the type of soil amendment (Fiedler et al., 2015).

Biogas digestate is a relatively new type of soil amendment, and its long-term stability in soil is still under debate as recently reviewed by Möller (2015). Consequently, it is not clear how long-term application of biogas digestates would alter the composition of SOM, and tillage effects on short-term SOM turnover in biogas digestate-amended soils

are almost unstudied. Even short-term changes of SOM may have strong effects on nutrient availability and plant productivity. A better understanding of the immediate impacts of tillage on SOM and its turnover may help to avoid adverse effects for plant growth (Doran, 2002; Franzluebbers et al., 1994; Mijangos et al., 2006).

In general, detecting changes in the molecular-chemical composition of SOM in time periods as short as days, requires extremely sensitive methods. Pyrolysis-field ionization mass spectrometry (Py-FIMS) is a very sensitive method and has been applied successfully to investigate differences in the chemical composition of SOM under different fertiliser treatments like mineral NPK-fertiliser or farmyard manure (Jandl et al., 2004; Leinweber et al., 2008b; Schmidt et al., 2000). Even very small alterations in the composition and stability of dissolved organic matter – a very reactive part of SOM – during storage in the fridge (Schulten et al., 2008) or diurnal cycles of CO_2-assimilation and respiration (Kuzyakov et al., 2003; Melnitchouck et al., 2005; Leinweber et al., 2008a) have been detected and resolved by multivariate statistics of mass-spectrometric fingerprints. Furthermore, Py-FIMS of bulk SOM revealed alterations in laboratory incubation experiments and linked these to respiration and enzyme activities (Leinweber et al., 2008b). However, it is unclear if the method is sensitive enough to detect tillage-induced SOM alterations under various fertilisation regimes and analyse its influence on CO_2 efflux at the field scale where spatial heterogeneity may interfere with the temporal dynamics much more than in the above cited laboratory studies.

Hot-water extraction is a relatively simple method to release labile SOM and to estimate how much of soil C and N can be easily utilised my microorganisms (Leinweber et al., 1995). These labile pools have been suggested to be an important indicator of short-term changes in SOM quality due to soil management (Haynes, 2005). Furthermore, a significant proportion of hot water-extracted organic matter originates from microbial biomass. Thus, this approach is a potential indicator for changes in microbial biomass or activity (Sparling et al., 1998), which may reflect sources of CO_2 efflux following tillage.

Here, we investigate (1) short-term effects of tillage on SOM composition and (2)

89 potential relationships between decomposable SOM fractions and measured CO_2 efflux
90 under the impact of different soil amendments by combining Py-FIMS with CO_2 efflux
91 measurements.

92

93 ## 2 Materials and methods

94 ### 2.1 Study site

95 The study site is located in northeast Germany in the ground moraine of the
96 Weichselian glacial period at 53° 48' 35" N and 12° 4' 20" E (elevation 10 m) within a
97 gently rolling relief. The soil is a stagnic luvisol (IUSS Working Group WRB, 2006)
98 with sandy loam texture (sand = 63 %, silt = 26 %, clay = 11 %) overlying bedrock of
99 till. The top soil (0-30 cm) has an organic carbon content of 1.16% (standard deviation
100 (SD) = 0.1, *n* = 3, measured with CN-analyser "vario MAX", Elementar, Hanau,
101 Germany), pH of 7.4 (SD = 0.9, *n* = 3, measured in H_2O with pH meter "CX-401",
102 Elmetron, Zabrze, Poland) and bulk density of 1.51 g cm^{-3} (SD = 0.08, *n* = 3, measured
103 on 250 cm^3 soil cores). The climate is characterized by maritime influence with annual
104 averages of 8.8° C temperature and 557 mm total precipitation for the 30-year-period
105 from 1985 until 2014 (LFA 2015). The experiment was conducted on a field which has
106 been cultivated with maize (*Zea Mays* L.), cultivar "Atletico", as feedstock for a biogas
107 plant. The previous crops were winter wheat (*Triticum aestivum* L.) followed by maize.

108 We compared three fertiliser treatments: CL – without fertiliser (control), MF – with
109 mineral fertiliser, and BD – with biogas digestate. The size of the three experimental
110 plots was 6 by 30 m each. In both fertilised treatments, equal overall amounts of plant-
111 available N were applied (160 kg ha^{-1}) on 26 April 2012. The mineral fertiliser calcium
112 ammonium nitrate was top-dressed whereas the biogas digestate was injected into the
113 soil down to 10 cm depth with a track width of 25 cm. Following the research facility
114 for agriculture and fisheries (LFA) of the federal state of Mecklenburg-Western
115 Pomerania, Germany (personal communication, 2014), a mineral fertiliser equivalent of
116 70% of total N in the biogas digestates (229 kg N ha^{-1}) was assumed. The biogas
117 digestate originated from the anaerobic fermentation of 91% cattle slurry, 7% rye groats

and 2% maize silage; it had pH 8.1, and 3.8% C, 0.5% total N and 0.3% NH_4-N in original matter.

Sixteen days after harvest of the maize (8 October 2012), the field site was first tilled with a disc harrow "Väderstad Carrier 300" down to 10 cm depth (24 October, about 9.15 a.m.) and then with a reversible mouldboard plough "Överum CX 490" down to 30 cm depth on the subsequent day (25 October, about 11.30 a.m.).

2.2 CO_2 concentration measurement and estimation of CO_2 efflux

For measuring CO_2 exchange we permanently installed three replicate collars in each treatment after fertilisation in spring which were removed for tillage and inserted back afterwards. The adjacent collars shared distances of 1m. The collars had a total height of 15 cm and were installed into the soil down to 12 cm depth. The CO_2 concentration measurements where performed with two LI-COR (Inc., Lincoln, NE, USA) LI-820 infrared gas analysers, each connected to a non-steady state closed chamber that was placed on the collars during measurements. The chambers had a square area of 0.62 m^2 and a height of 0.55 m, resulting in a chamber volume of 0.34 m^3 and were equipped with small fans (80 x 80 x 25 mm, 3000 rpm, 68 $m^3\ h^{-1}$) in order to mix and homogenize the air inside the chambers. Due to the successive measurement of the replicates in each treatment, we obtained pseudo-replications.

During chamber placement, we recorded CO_2 concentrations in the chamber headspace with 1.3 s intervals for 3 to 5 min, resulting in approximately 140 to 230 data points per measurement. Fluxes were estimated with function *fluxx* of package *flux* version 0.3-0 (Jurasinski et al., 2014) for the R statistical software version 2.15.2 (R Core Team, 2013). In short, the algorithm identifies the most linear part of the CO_2 concentration development during chamber placement time and fits a linear regression model (Eq. (1)):

$$f = \frac{MpV}{RTA}\frac{dc}{dt}10^6, \qquad (1)$$

with f the CO_2 flux (g m^{-2} h^{-1}), M the molar mass of CO_2 (g mol^{-1}), p the air pressure (Pa), V the chamber volume (m^3), R the gas constant (J mol^{-1} K^{-1}), T the temperature

146 inside the chamber (K), *A* the area covered by the chamber (m^2), and *dc/dt* the CO_2
147 concentration change over time (ppm h^{-1}). The minimum proportion of data points to be
148 kept for regression analyses was 70 % of a concentration measurement to discard data
149 noise at the beginning and the end resulting from chamber deployment and removal (for
150 details see help file for function *fluxx* of package *flux*). Thus, each CO_2 flux was
151 estimated at least from 98 concentration measurements. Only linear fluxes with a
152 concentration change of at least 10 ppm, a normalised root mean square error (NRMSE)
153 ≤ 0.15 and a coefficient of determination (R^2) of at least 0.85 were included in further
154 analyses. We assumed linearity of concentration change and did not test for non-
155 linearity since 95.1% of the obtained linear regressions had $R^2 \geq 0.95$.

156 To obtain reference data from before tillage operations, the undisturbed site was
157 measured hourly between 7 a.m. and 1 p.m. on 19 October 2012 (i.e. between harvest
158 and tillage). The intervals between measurements before, during and after tillage
159 operations were varied to effectively capture the development of CO_2. The
160 measurements immediately after the tillage operations were conducted within one
161 minute by inserting the collars and putting on the airtight chambers. The timeline (24
162 till 29 October) of tillage events, soil samplings and the respective CO_2 measurements,
163 together with soil temperature, is shown in Fig. 1. After this period, CO_2 measurements
164 were performed hourly before noon on 1, 5 and 9 November.

165 ## 2.3 Soil sampling and analyses

166 Three replicates of bulk soil samples were taken at 5 – 15 cm depth with soil sample
167 rings (V = 250 cm^3) in a triangular arrangement between the three collars for gas
168 sampling (see 2.2) in each treatment at three dates: 1) right before the first tillage
169 operation, 2) in the afternoon after the second tillage operation and 3) four days after the
170 second tillage operation. The resulting 27 soil samples were fixed immediately with
171 liquid nitrogen and splitted thereafter into subsamples for freeze-drying and for oven-
172 drying at 60° C.

173 For Pyrolysis-field ionization mass spectrometry (Py-FIMS), about 5 milligrams of the
174 freeze-dried, ground and homogenized samples were thermally degraded in the ion

source (emitter: 4.7 kV, counter electrode -5.5 kV) of a double-focusing Finnigan MAT 95 mass spectrometer (Finnigan, Bremen, Gemany). The samples were heated in a vacuum of 10^{-4} Pa from 50 °C to 700 °C, in temperature steps of 10 °C over a time period of 15 minutes. Between magnetic scans the emitter was flash heated to avoid residues of pyrolysis products. The Py-FIMS mass spectra of each sample were gained by the integration of 65 single scans in a mass range of 15 – 900 *m/z*. Ion intensities were referred to 1 mg of the sample. Volatile matter was calculated as mass loss in percentage of sample weight. The three replicates of each sample were then averaged to one final survey spectrum. Moreover, thermograms were compiled for the total ion intensities. The assignment of marker signals to chemical compounds from the survey spectra were interpreted according to Leinweber et al. (2013) to obtain the relative abundance of ten SOM compound classes: 1) carbohydrates, 2) phenols and lignin monomers, 3) lignin dimers, 4) lipids, alkanes, alkenes, bound fatty acids and alkyl monoesters, 5) alkylaromatics, 6) mainly heterocyclic N-containing compounds, 7) sterols, 8) peptides, 9) suberin, and 10) free fatty acids.

Subsamples of oven-dried and sieved soil (2 mm) were used for determination of total and hot water-extracted C and N. For determination of total C and N, 1 g of ground soil was analysed with a vario Max CN Element Analyzer (elementar Analysensysteme GmbH, Hanau, Germany) based on high temperature combustion at up to 1200 °C with subsequent gas analysis. For hot-water extraction, 20 g soil was boiled in 40 ml deionized water for 60 minutes (Leinweber et al., 1995). After filtration with pleated filter (240 mm, 80 g m^{-2}) by Munktell (Falun, Sweden), extracts were analysed with a DIMATOC 2000 (DIMATEC Analysentechnik GmbH, Essen, Germany) for determination of hot-water extractable organic C (HWC) and total nitrogen bound (HWN). These measurements of organic C and total nitrogen bound are based on the principle of thermal-catalytic oxidation with subsequent NDIR detection and the principle of chemiluminescence, respectively. For each sample, two replicates were analysed and results were averaged for further calculations.

2.4 Statistical analyses

All statistical analyses were run using R 2.15.2 (R Core Team, 2013). The cumulated

205 CO_2 effluxes were estimated by a bootstrap method with the function *auc.mc* of the R
206 package *flux* version 0.3-0 (Jurasinski et al., 2014). In detail, the CO_2 fluxes were
207 cumulated in 250 iterations, while for each run 25 fluxes were omitted randomly for the
208 period after tillage. For the reference period before tillage, in each iteration run 4 fluxes
209 were omitted randomly. The numbers of randomly omitted fluxes per run correspond
210 roughly to one fifth of the recorded fluxes per treatment in the respective periods. The
211 resulting data were used to calculate means and standard deviations. Tukey's HSD test
212 was applied to test for differences in means of CO_2 fluxes as well as of HWC and HWN
213 between sampling periods and treatments against a significance level of $\alpha < 0.05$. Py-
214 FIMS signals of the compound classes were tested for differences in means by Tukey's
215 HSD test against a significance level of $\alpha < 0.1$ since the number of replicates was
216 limited and the variances rather high.

217 A principal component analysis (PCA) was applied to the mass signals with significant
218 differences between the samples according to univariate Wilk's λ ($p < 0.001$) with
219 function *rda* of R package vegan version 2.3-0 (Oksanen et al., 2015).

220 Partial least squares regression (PLSR) was used for discrimination (Barker and Rayens,
221 2003) to maximally explore linkages between shifts in the *m/z* data by tillage and shifts
222 in CO_2 efflux. PLSR models were built by using the R package "autopls" version 1.3
223 (Schmidtlein et al., 2015) with stepwise backward selection combined with a 10-fold
224 cross-validation to substantially reduce the number of variables, *i.e.*, to extract the
225 variables with the highest explanatory power. The PLSR procedure was repeated 10.000
226 times to yield coherent results since the obtained PLSR models differed widely both in
227 the number and in the choice of variables, thus in their predictive performance. Based
228 on the performance index suggested by Bauwe et al. (2015), the 500 "best" models were
229 extracted and, finally, the mass signals which were utilised more than 50 times in the
230 latter models were chosen.

231

3 Results

3.1 Soil organic carbon, nitrogen, hot-water extractable carbon and hot-water extractable nitrogen

One of the replicates in MF exhibited exceptionally low HWC and HWN values. According to Dixon's Q-test, these values were outlier (one-third and half, respectively, as high as for the other replicates in MF) and thus excluded from further analysis. Before tillage, the soil of all treatments had similar C and HWC contents, while the N and HWN contents were slightly higher in MF, resulting in significantly narrower C/N and HWC/HWN ratios in MF (8.55 and 5.93, respectively) compared to BD (9.03 and 8.54, respectively) (Table 1). The C, N and HWC contents of all treatments were changed only slightly by tillage, but the HWN content of soil in BD increased from 0.05 mg g^{-1} (5.6 % of N) up to 0.07 mg g^{-1} (7.4 % of N), resulting in a significant ($p < 0.05$) narrowing of the HWC/HWN ratio from 8.5 down to 6.0 (Table 1).

3.2 Soil CO_2 efflux

Five days before the tillage operations (19 October 2012), the mean efflux rates (all in g CO_2-C m^{-2} h^{-1}) were 0.133 (CL), 0.192 (MF) and 0.173 (BD), with the efflux being significantly lower from CL than from the amended plots MF and BD ($p < 0.05$) (Fig. 2). In the morning before the first tillage operation with a disc harrow (24 October), the effluxes had similar magnitudes and proportions like five days before (CL = 0.147, MF = BD = 0.199, all in g CO_2-C m^{-2} h^{-1}). After harrowing, CO_2-effluxes increased to 0.849 (CL), 0.833 (MF) and 0.479 (BD). Over the next 5.5 hours, these values declined to 0.602 (CL), 0.460 (MF) and 0.276 (BD) resulting in overall mean effluxes of 0.554 (CL), 0.481 (MF) and 0.344 (BD), with the latter being now significantly lower ($p < 0.05$) than CL or MF during the measured period after harrowing. Directly before the second tillage operation with a reversible mouldboard plough in the morning of the following day (25 October), the mean effluxes were 0.299 (CL), 0.249 (MF) and 0.290 (BD) (all in g CO_2-C m^{-2} h^{-1}). Immediately after ploughing, they increased sharply up to 2.443 (CL), 2.654 (MF) and 3.347 (BD) and declined to 0.371 (CL), 0.718 (MF) and 0.223 (BD) after 4 hours, leading to overall mean effluxes of the measured period after

ploughing of CL = 1.012, MF = 1.392, and BD = 1.020. Although the mean CO_2 fluxes within each treatment differed significantly ($p < 0.05$) from the other measured days only after ploughing (25 October), BD on average showed significantly ($p < 0.05$) lower fluxes than CL or MF after tillage on 24 and 29 October (Fig. 3) as well as on 1 November (CL = 0.262, MF = 0.242, BD = 0.113, all in g CO_2-C m^{-2} h^{-1}) and 5 November (CL = 0.331, MF = 0.316, BD = 0.074, all in g CO_2-C m^{-2} h^{-1}).

3.3 Pyrolysis-Field Ionization Mass Spectroscopy

The thermograms of total ion intensity (TII) and the Py-FIMS mass spectra of the soil samples of CL and MF taken before tillage were similar whereas the ones of BD were different from those two (Fig. 4): The TII-thermograms of CL and MF had a peak at 480 °C, but BD displayed a pronounced bimodal shape with a first volatilisation maximum at about 390 °C which was less marked in CL and MF. Furthermore, the mass spectrum of BD differed distinctly from the mass spectra of MF and CL, especially in the abundance of marker signals for carbohydrates and peptides (e.g., *m/z* 58, 60, 84, 69, 110, 126 and 162) were lower. Apart from this the spectra are dominated by signals for lignin mono- and dimers (e.g., *m/z* 150, 208, 222, 244) as well as for homologous series of alkenes and alkadienes from *n*-C_{18} up (e.g., m/z 252, 264/266, 278/280, 294, 308, 322, 336, 364, 392, 406) (Fig. 4).

After discriminant function analysis with Wilk's λ, the resulting significant relative mass signals ($p < 0.001$, $n = 67$) were further explored by PCA. The first two principal components explained 78.3% and 8.3% of total variance. All treatments are well separated from each other (Fig. 5), with CL mainly in the 3rd quadrant, MF mainly in the 1st and BD spanning from the 2nd to the 4th quadrant. According to this analysis, samples from MF and BD taken before the tillage events (pre) showed the largest differences in composition. The PCA separated the samples taken at different dates (pre, post and post + 4) in the treatments MF and BD, but not in CL.

Basic data of the Py-FI mass spectra and the proportions of compound classes are compiled in Table 2. Approximately 46.9% of the TII in the mass spectra could be explained by m/z signals assigned to the compound classes. Additionally, non-specific

low-mass signals and isotope peaks contributed 2.6% and 14.2%, respectively. Before tillage, the volatised matter (VM) was highest in BD although the differences in means were not significant ($p > 0.1$). However, four days after tillage VM increased to 7.1% in BD and then significantly ($p < 0.05$) exceeded that in MF and CL. Such an increase over time was only observed for BD, but it was not significant ($p < 0.01$). In the other treatments, a temporal increase in VM occurred directly after the first tillage with disc harrow.

The relative (Table 2) and absolute (data not shown) ion intensities of the compound classes varied across treatments before tillage and changed differently after tillage. In the undisturbed soil, BD had the lowest proportions of carbohydrates, heterocyclic N-containing compounds and peptides and the highest proportions of lignin dimers, lipids, sterols, suberin and free fatty acids. CL was characterized by higher proportions of phenols and lignin monomers whereas MF ranged between BD and CL regarding the proportions of these compound classes. In BD, the relative proportions of the samples taken after tillage displayed significant ($p < 0.1$) increases of carbohydrates, phenols and lignin monomers, alkylaromatics, heterocyclic N-containing compounds and peptides while lignin dimers, lipids, sterols and free fatty acids decreased. In MF, the proportion of lipids increased while carbohydrates and peptides decreased. No changes were detected in the unfertilised treatment CL. The discrimination of relative mass signals with PLSR to explain cumulated CO_2 efflux revealed mainly functional groups from ketones and amides, peptides, carbohydrates as well as lignin building blocks and fatty acids (Table 3).

Linear correlations were calculated to investigate relationships between HWC, HWN and soil respiration as suitable indicators of SOM dynamics (Kuzyakov, 2006; Leinweber et al., 1995) and the absolute signal counts of the compound classes (Fig. 6). The latter was derived from Table 2 by Eq. (2).

$$CII_{abs} = \frac{TII \times CII_{rel}}{100}, \qquad (2)$$

with CII_{abs} the absolute ion intensity of the respective compound class, TII the total ion intensity and CII_{rel} the proportion of the ion intensity of the respective compound class.

In MF only the ion intensities for carbohydrates were positively correlated with HWC whereas in BD more compound classes correlated with the tested indicators of SOM dynamics. Here, HWC was positively correlated with the ion intensities of lignin dimers, lipids, alkylaromatics, sterols and suberin, but no such correlation was found for carbohydrates in disagreement to MF. However, HWN showed a positive correlation with carbohydrates in BD. HWN was also positively correlated to phenols, lignin monomers and heterocyclic N-containing compounds but negatively correlated to free fatty acids. CO_2 efflux increased with decreasing amounts of sterols and suberin in BD.

4 Discussion

4.1 Bulk soil and hot-water extracted carbon and nitrogen

The C- and HWC-contents of the treatments showed no significant differences before tillage (Tab. 1). However, the observed higher N- and HWN-contents in MF (Tab. 1) did not confirm the outcomes of other experiments with similar fertilisers. No significant differences in soil C and N were found between MF and BD in the field (Odlare et al., 2014). On the contrary, in a pot experiment with maize the soil N content was higher in the digestate than in the mineral fertiliser treatment (Bachmann et al., 2011). That study as well as the present investigation lasted for weeks and months, respectively. Therefore, the C- and N-contents obtained may not be representative for long-term effects of biogas digestate vs. mineral fertiliser.

The increase in HWN in BD after tillage indicates an increase of easily mineralisable organic N which probably originates from soil biomass and lysates (Ghani et al., 2003; Leinweber et al., 1995; Raich and Potter, 1995) and implies an accelerated microbial turnover of soil organic N. This seems reasonable since the microbial community is able to adjust its structure and activity relatively fast to utilise formerly protected organic matter after exposure due to disruption of aggregates by tillage (Jackson et al., 2003; La Scala et al., 2008; Mueller et al., 2014). Accordingly, Fiedler et al. (2015) observed a short-lived increase of HWC after the first of two days of several tillage operations which was not found in the present study. Most likely, we just did not detect it, because

348 we took no soil samples after the first day. Overall, a single amendment with biogas
349 digestates very likely is insufficient to initiate changes in bulk soil C- and N-levels.
350 However, the increased HWN-levels in BD can be ascribed to a tillage promoted
351 microbial turnover of soil organic N, confirming that the hot water extracts are a
352 particularly sensitive approach to detect early SOM changes (Haynes, 2005).

353 ### 4.2 Soil CO_2 efflux

354 The immediate and sharp increase of CO_2 efflux from soils just after tillage is a well-
355 documented response and seems to be mainly driven by the release of trapped CO_2 from
356 broken up aggregates by tillage (Calderon and Jackson, 2002; Ellert and Janzen, 1999;
357 Reicosky et al., 1997). It is commonly suggested that a few hours afterwards, waning of
358 this physical outgassing is accompanied by an increased soil respiration due to a better
359 substrate supply for microorganisms from disrupted aggregates as well as increased soil
360 aeration (Schulten et al., 1997; Grandy and Robertson, 2007). The amounts of the
361 observed fluxes are well in accordance with the findings of previous studies (e. g.,
362 Rochette and Angers, 1999) and can be explained both by the magnitude of the
363 disturbance, i.e. soil comminution, and the fertilisation history of the soil (Schulten et
364 al., 1997).

365 The smaller relative efflux from BD compared to MF and CL after tillage is remarkable
366 since before tillage the CO_2 fluxes in BD were of the same magnitude as those in MF
367 and exceeded those in CL (Fig. 2). This becomes particularly evident when one
368 considers the relation of cumulated CO_2 fluxes between the treatments before (19
369 October) and after tillage (24 – 29 October) (Fig. 3). Before tillage, the ratio of
370 cumulated CO_2 fluxes in CL : MF : BD was 1 : 1.27 : 1.21 and changed to
371 1 : 1.21 : 0.71 after tillage. The relatively lower CO_2 efflux from BD after tillage may
372 have different reasons. On the one hand, the organic matter originating from the
373 digestates is likely less available to soil microorganisms, i. e. more “recalcitrant”, since
374 the most labile C has been consumed already in the biogas reactor (Thomsen et al.,
375 2013; Möller, 2015; Wentzel et al., 2015). On the other hand, even a single application
376 of organic amendment can increase aggregate stability (Grandy et al., 2002). Therefore,
377 the resilience against disruption by tillage might be promoted, leading to a better

physical protection of labile soil C not contained within digestates. As a consequence, the effect of increased CO_2 efflux after tillage as observed in CL and MF, may have been substantially reduced by a relative shortage of labile substrate in BD that affects the above suggested increased soil respiration due to substrate supply after tillage. Furthermore, the narrower HWC/HWN ratio in BD after tillage suggests an improved N supply for soil microbes which might have enhanced their C use efficiency. Such an enhanced C use efficiency may be accompanied by decreased C losses to heterotrophic respiration as long as C availability is not limited (Schnitzer, 2001; Sinsabaugh et al., 2013). However, N addition decreased the respiration when C was limited in laboratory incubation experiments (Eberwein et al., 2015). Furthermore, Oades (1984) observed decreasing CO_2 fluxes from soil under N saturating conditions and dextrose amendments of 1.5 and 3 mg g^{-1} soil in comparison to non-saturating conditions, but increased CO_2 fluxes after dextrose amendments $\geq$ 7.5 mg g^{-1} soil. This supports the assumption of not limited, but rather low levels of available C in the soil of BD. Also the proportion of carbohydrates in BD derived from Py-FIMS, as discussed below, consolidates this assumption. But in fact, the HWC/HWN ratio of BD after tillage was not lower than that of MF, so, in conclusion, the above described mechanisms do not well explain why the CO_2 efflux was lower after tillage in BD when compared to MF.

4.3 Pyrolysis-Field Ionization Mass Spectroscopy and synthesis

Generally, the Py-FIMS basic data and mass spectra (Fig. 4) and the proportions of compound classes (Tab. 2) confirm published data from this method for Luvisols in terms of relatively high shares of lignin monomers, phenols and alkylaromatics (Leinweber et al., 2009). Lignin monomers and phenols might be complementarily attributed to residues of the just harvested maize. Indeed, Gregorich et al. (1996) found that these are important components of maize leaves and roots as well as the light fraction of the soil under this crop. However, the Py-FIMS data indicate differences in SOM composition between the fertilization treatments and a pronounced as well as distinct impact of tillage in the treatments MF and BD (Fig. 5).

In the spectra of samples from BD, the additional peak at 390° C in the TII-thermogram (Fig. 4) can be attributed mainly to phenols and lignin monomers which likely

originated from primary organic matter residues since this relatively low volatilization temperature indicates labile and fairly undecomposed organic matter (Leifeld and Lützow, 2014; Ludwig et al., 2015; Sleutel et al., 2011). It is reasonable to refer this organic matter to residues from the application of BD. The VM, which is an indicator of the SOM content (Sorge et al., 1993; Wilcken et al., 1997) but also of its stability (Ludwig et al., 2015; Leinweber and Schulten, 1995), was larger in BD before tillage than in MF and CL. This suggests a tendency to elevated SOM due to application of rather stable organic matter with biogas digestate. Its increase after tillage might be explained by a general destabilization, perhaps by an enhanced SOM turnover due to an improved microbial accessibility to relatively recalcitrant residues of BD after tillage (Dao, 1998; Dungait, et al., 2012). The temporal increase in VM directly after the first tillage with disc harrow in MF and CL may indicate a similar increased accessibility of SOM. But here, the newly available SOM has been depleted quickly by microbial respiration since the microbial community is able to respond rapidly to disturbances of arable soils (Jackson et al., 2003). In MF, this assumption is supported by the decreasing shares of carbohydrates and by significantly decreasing relative signals of *m/z* 17 and 18 (data not shown), which are assigned to ammonia and ammonium, pointing to a microbial immobilisation (Mengel, 1996). Accordingly, these two *m/z* were also selected by the PLSR as explanatory signals for CO_2 efflux (Tab. 3).

The compound classes of BD revealed the largest proportions of lignin dimers, lipids, sterols, suberin and free fatty acids at the expense of carbohydrates, heterocyclic N-containing compounds and peptides before tillage (Tab. 2). Such a SOM composition most likely reflects the cattle manure and plant residues of the biogas feedstock and their relative depletions (amides and polysaccharides) or enrichments (lignins and long-chain aliphatic compounds) during anaerobic fermentation (Leinweber et al., 1992; Möller, 2015; van Bochove et al., 1996). The pronounced tillage effect in this treatment, obvious from the increased relative signal intensities of carbohydrates, phenols and lignin monomers, alkylaromatics, heterocyclic N-containing compounds and peptides at the expense of lignin dimers, lipids, sterols and free fatty acids following tillage (Tab. 2), suggests the decomposition of lignin and the new formation of carbohydrates and peptides. This is in line with reports of lignin decomposition faster than that of the total

SOM (Leinweber et al., 2008b; Rasse et al., 2006; Thevenot et al., 2010). Kalbitz et al. (2003) suggested that lignin-derived moieties and lipids are utilised by microorganisms at low initial availability of carbohydrates, accompanied by an accumulation of the resulting microbial metabolites like carbohydrates and peptides. This suggestion is supported on the one hand by the effect of specific lignins on soil CO_2 efflux (Tab. 3) since CO_2 is an indicator for microbial decomposition activity (Kuzyakov, 2006). On the other hand, a relative increase of the signals for *m/z* 125, 167, 185 and 203 was observed in the BD treatment (data not shown) which are assigned to the bacterial cell wall products N-acetylmuramic acid and N-acetylmuramyl-L-alanyl-D-isoglutamine (Bahr and Schulten, 1983). Furthermore, the build-up of heterocyclic N-containing compounds might also imply a relative shortage of available carbohydrates since a reduced C availability during the microbial transformation of N is suggested to promote formation of heterocyclic N instead of N immobilisation (Follett and Schimel, 1989; Gillespie et al., 2014; Schulten and Hempfling, 1992). The increased proportion of lipids at the expense of carbohydrates and peptides in MF likely results from increased heterotrophic respiration of labile substrates driven by enhanced microbial activity after tillage (La Scala et al., 2008; Reicosky and Archer, 2007; Zakharova et al., 2014). The minor changes in SOM compounds in CL might be a consequence of the wider HWC/HWN ratio compared with MF since a lack of available N is known to decrease the efficiency of microbial activity (Schnitzer, 2001; Sinsabaugh et al., 2013).

The positive linear correlation of HWC with lignin dimers, lipids, alkylaromatics, sterols and suberin in BD (Fig. 6) indicates a reasonable linkage between the dynamic organic C fraction (as indicated by HWC) and the quantity of applied biogas digestate (as indicated by lignin dimers, lipids, alkylaromatics, sterols and suberin). At the same time, the microorganisms in BD may have been short in available labile C since there was no significant ($p > 0.05$) correlation between HWC and carbohydrates. In contrast, a significant and positive correlation was observed between HWC and carbohydrates in MF (Fig. 6). This linkage was previously described by Leinweber et al. (1995) and attributed to microbial biomass (Ghani et al., 2003) and labile soil C (Sparling et al., 1998).

469 Interestingly, HWN correlated positively with carbohydrates in BD. Since the major
470 part of carbohydrates in soils originate from microorganisms and their residues (Gunina
471 and Kuzyakov, 2015), this may suggest a metabolic coupling between carbohydrates
472 and HWN because many N-cycling processes are mediated microbially (Isobe and
473 Ohte, 2014). This idea is supported by the negative correlation between HWN and free
474 fatty acids, which also hints to a coupling of the dynamic N pool with microbial activity
475 in BD. Actually, free fatty acids are known as a major carbon source during nitrogen
476 immobilisation by microbial anabolism (Kirchmann and Lundvall, 1993).

477 In BD, the cumulated CO_2 efflux and the amounts of sterols were negatively correlated
478 (Fig. 6). This supports the suggestion of Heumann et al. (2011, 2013) that sterols may
479 have an inhibitory effect on microorganisms of the N cycle. Furthermore, Negassa et al.
480 (2011) reported a significant inhibition of the urease activity with increasing sterol
481 proportions in agro-industrial byproducts. Since microbial activity can affect
482 heterotrophic soil respiration (Ryan and Law, 2005), it is likely that increased amounts
483 of sterols as they are typically found in biogas digestates (Leinweber, 2015, unpublished
484 Py-FIMS data) delay the decomposition and, thus, may slow down soil respiration.
485 However, since the amounts of sterols decreased significantly after tillage in DB (Table
486 3), the actual sterol contribution to reduced CO_2-efflux in BD relative to the other
487 treatments cannot be ascertained by the present data set. In light of the contradicting
488 observation of increased labile N after tillage in BD, inhibitory effects of sterols as
489 reported in the above publications may be more pronounced in undisturbed soils.

490 Our data and analyses suggest a short-term induction of an enhanced microbial N-
491 turnover by tillage under fertilisation with biogas digestates. This is supported by the
492 results of each of the used methods and their cross-validation, i.e., (i) HWN as an
493 indicator for labile N increased, (ii) CO_2 efflux as an possible indicator for carbon use
494 efficiency in terms of improved microbial N-availability decreased, (iii) Py-FIMS data
495 pointing at an increase of N-containing compounds along with the decomposition of
496 lignins, and finally, (iv) significant correlations among data sets from these methods
497 (Fig. 6).

In MF, the depletion of HWC was linked to decreasing amounts of carbohydrates, certainly due to increased microbial respiration, though no significant correlation with CO_2 efflux was found. No modifications were detected in CL were the absence of amendment may have led to a shortage of N as indicated by the relatively high HWC/HWN-ratio which likely inhibited an enhanced microbial activity.

5 Conclusions

Combining Py-FIMS as a sensitive technique to detect differences and alterations of specific compound classes of SOM with classical methods like hot-water extraction and measurements of soil CO_2 efflux allowed us to gain a better understanding of short-term SOM turnover after tillage operations. After tillage, SOM composition changed in the temporal scale of days and the changes varied significantly under different types of amendment. Particularly obvious were the turnover of lignin-derived substances and the depletion of carbohydrates due to soil respiration. Thus, in BD, the SOM turnover was relatively fast, questioning the suggested recalcitrance of biogas digestates as stable leftovers of the anaerobic fermentation. Since we found indications for inhibitory effects of sterols on the CO_2 efflux, which were previously reported in three independent studies on parameters of the N-cycle, their long-term impact on SOM stocks should be examined more closely. Therefore, future investigations should address the short- and long-term turnover of SOM following various soil amendments, especially with the relatively new biogas digestates.

Acknowledgements

We thank the technicians Steffen Kaufmane and Sascha Tittmar for their assistance in field work and the research facility for agriculture and fisheries of the federal state of Mecklenburg-Western Pomerania (LFA) in Gülzow for their co-operation, especially Jana Peters and Andreas Gurgel. The joint research project underlying this report was funded by the German Federal Ministry of Food and Agriculture (funding identifier 22007910). Py-FIMS analyses in the Mass Spectrometry Laboratory of Soil Science

were funded by the “Exzellenzförderprogramm” of the Ministry of Education, Science and Culture, federal state of Mecklenburg-Vorpommern (Project UR 07 079) as well as by the German Federal Ministry of Food and Agriculture (funding identifier 22400112).

References

Alvarez, R., Alvarez, C. R., and Lorenzo, G.: Carbon dioxide fluxes following tillage from a mollisol in the Argentine Rolling Pampa, European Journal of Soil Biology, 37, 161–166, doi:10.1016/S1164-5563(01)01085-8, 2001.

Bachmann, S., Wentzel, S., and Eichler-Löbermann, B.: Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L, Journal of Plant Nutrition and Soil Science, 174, 908–915, doi:10.1002/jpln.201000383, 2011.

Bahr, U. and Schulten, H.-R.: Pyrolysis field ionization mass spectrometry of cell wall components and bacterial cell walls, Journal of Analytical and Applied Pyrolysis, 5, 27–37, doi:10.1016/0165-2370(83)80011-4, 1983.

Balesdent, J., Mariotti, A., and Boisgontier, D.: Effect of tillage on soil organic carbon mineralization estimated from 13C abundance in maize fields, Journal of Soil Science, 41, 587–596, doi:10.1111/j.1365-2389.1990.tb00228.x, 1990.

Balota, E. L., Colozzi-Filho, A., Andrade, D. S., and Dick, R. P.: Microbial biomass in soils under different tillage and crop rotation systems, Biology and Fertility of Soils, 38, 15–20, doi:10.1007/s00374-003-0590-9, 2003.

Barker, M. and Rayens, W.: Partial least squares for discrimination, J. Chemometrics, 17, 166–173, doi:10.1002/cem.785, 2003.

Bauwe, A., Jurasinski, G., Scharnweber, T., Schröder, C., and Lennartz, B.: Impact of climate change on tree-ring growth of Scots pine, common beech and pedunculate oak in northeastern Germany, iForest, 9, 1–11, doi:10.3832/ifor1421-008, 2015.

Calderon, F. J. and Jackson, L. E.: Rototillage, disking, and subsequent irrigation: Effects on soil nitrogen dynamics, microbial biomass, and carbon dioxide efflux, Journal of Environmental Quality, 31, 752–758, 2002.

557 Dao, T. H.: Tillage and crop residue effects on carbon dioxide evolution and carbon
558 storage in a Paleustoll, Soil Science Society of America Journal, 62, 250–256,
559 1998.

560 Doran, J. W.: Soil Microbial and Biochemical Changes Associated with Reduced
561 Tillage: Soil Science Society of America Journal, 44, 765–771,
562 doi:10.2136/sssaj1980.03615995004400040022x, 1980.

563 Doran, J. W.: Soil health and global sustainability: translating science into practice: Soil
564 Health as an Indicator of Sustainable Management, Agriculture, Ecosystems &
565 Environment, 88, 119–127, doi:10.1016/S0167-8809(01)00246-8, 2002.

566 Dungait, J. A. J., Hopkins, D. W., Gregory, A. S., and Whitmore, A. P.: Soil organic
567 matter turnover is governed by accessibility not recalcitrance, Global Change
568 Biology, 18, 1781–1796, doi:10.1111/j.1365-2486.2012.02665.x, 2012.

569 Eberwein, J. R., Oikawa, P. Y., Allsman, L. A., and Jenerette, G. D.: Carbon
570 availability regulates soil respiration response to nitrogen and temperature, Soil
571 Biology and Biochemistry, 88, 158–164, doi:10.1016/j.soilbio.2015.05.014,
572 2015.

573 Ellert, B. H. and Janzen, H. H.: Short-term influence of tillage on CO2 fluxes from a
574 semi-arid soil on the Canadian Prairies, Soil and Tillage Research, 50, 21–32,
575 doi:10.1016/S0167-1987(98)00188-3, 1999.

576 Fiedler, S. R., Buczko, U., Jurasinski, G., and Glatzel, S.: Soil respiration after tillage
577 under different fertiliser treatments – implications for modelling and balancing,
578 Soil and Tillage Research, 150, 30–42, doi:10.1016/j.still.2014.12.015, 2015.

579 Follett, R. F. and Schimel, D. S.: Effect of Tillage Practices on Microbial Biomass
580 Dynamics, 1091 pp., doi:10.2136/sssaj1989.03615995005300040018x, 1989.

581 Franzluebbers, A. J., Hons, F. M., and Zuberer, D. A.: Seasonal changes in soil
582 microbial biomass and mineralizable c and n in wheat management systems, Soil
583 Biology and Biochemistry, 26, 1469–1475, doi:10.1016/0038-0717(94)90086-8,
584 1994.

585 Ghani, A., Dexter, M., and Perrott, K. W.: Hot-water extractable carbon in soils: a
586 sensitive measurement for determining impacts of fertilisation, grazing and
587 cultivation, Soil Biology and Biochemistry, 35, 1231–1243, doi:10.1016/S0038-
588 0717(03)00186-X, 2003.

589 Gillespie, A. W., Diochon, A., Ma, B. L., Morrison, M. J., Kellman, L., Walley, F. L.,
590 Regier, T. Z., Chevrier, D., Dynes, J. J., and Gregorich, E. G.: Nitrogen input
591 quality changes the biochemical composition of soil organic matter stabilized in
592 the fine fraction: a long-term study, Biogeochemistry, 117, 337–350,
593 doi:10.1007/s10533-013-9871-z, 2014.

594 Grandy, A. S., Porter, G. A., and Erich, M. S.: Organic Amendment and Rotation Crop
595 Effects on the Recovery of Soil Organic Matter and Aggregation in Potato
596 Cropping Systems, Soil Science Society of America Journal, 66,
597 doi:10.2136/sssaj2002.1311, 2002.

598 Grandy, A. S. and Robertson, G.: Land-Use Intensity Effects on Soil Organic Carbon
599 Accumulation Rates and Mechanisms, Ecosystems, 10, 59–74,
600 doi:10.1007/s10021-006-9010-y, 2007.

601 Grandy, A. S. and Neff, J. C.: Molecular C dynamics downstream: The biochemical
602 decomposition sequence and its impact on soil organic matter structure and
603 function, Science of The Total Environment, 404, 297–307,
604 doi:10.1016/j.scitotenv.2007.11.013, 2008.

605 Gregorich, E. G., Monreal, C. M., Schnitzer, M., and Schulten, H. R.: Transformation
606 of plant residues into soil organic matter: Chemical characterization of plant
607 tissue, isolated soil fractions, and whole soils, Soil Science, 161, 680–693,
608 doi:10.1097/00010694-199610000-00005, 1996.

609 Gunina, A. and Kuzyakov, Y.: Sugars in soil and sweets for microorganisms: Review of
610 origin, content, composition and fate, Soil Biology and Biochemistry, 90, 87–
611 100, doi:10.1016/j.soilbio.2015.07.021, 2015.

612 Haynes, R. J.: Labile Organic Matter Fractions as Central Components of the Quality of
613 Agricultural Soils: An Overview, in: Advances in Agronomy, Academic Press,
614 221–268, 2005.

615 Heumann, S., Schlichting A., Boettcher, J., and Leinweber, P.: Sterols in soil organic
616 matter in relation to nitrogen mineralization in sandy arable soils, Journal of
617 Plant Nutrition and Soil Science, 174, 576–586, doi:10.1002/jpln.200900273,
618 2011.

619 Heumann, S., Rimmer, D. L., Schlichting, A., Abbott, G. D., Leinweber, P., and
620 Böttcher, J.: Effects of potentially inhibiting substances on C and net N
621 mineralization of a sandy soil—a case study, J. Plant Nutr. Soil Sci., 176, 35–39,
622 doi:10.1002/jpln.201200353, 2013.

623 Isobe, K. and Ohte, N.: Ecological Perspectives on Microbes Involved in N-Cycling,
624 Microbes and Environments, 29, 4–16, doi:10.1264/jsme2.ME13159, 2014.

625 IUSS Working Group WRB: World Reference Base for Soil Resources 2006. World
626 Soil Resources Report No. 103. FAO, Rome, 2006

627 Jackson, L. E., Calderon, F. J., Steenwerth, K. L., Scow, K. M., and Rolston, D. E.:
628 Responses of soil microbial processes and community structure to tillage events
629 and implications for soil quality, Geoderma, 114, 305–317, doi:10.1016/S0016-
630 7061(03)00046-6, 2003.

631 Jandl, G., Leinweber, P., Schulten, H.-R., and Eusterhues, K.: The concentrations of
632 fatty acids in organo-mineral particle-size fractions of a Chernozem, European
633 Journal of Soil Science, 55, 459–470, doi:10.1111/j.1365-2389.2004.00623.x,
634 2004.

635 Jurasinski, G., Koebsch, F., Guenther, A., Beetz, S.: flux: Flux rate calculation from
636 dynamic closed chamber measurements. R package version 0. 3-0.
637 http://CRAN.R-project.org/package=flux, 2014

638 Kalbitz, K., Schwesig, D., Schmerwitz, J., Kaiser, K., Haumaier, L., Glaser, B.,
639 Ellerbrock, R., and Leinweber, P.: Changes in properties of soil-derived

dissolved organic matter induced by biodegradation, Soil Biology and Biochemistry, 35, 1129–1142, doi:10.1016/S0038-0717(03)00165-2, 2003.

Kirchmann, H. and Lundvall, A.: Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry: Biology and Fertility of Soils, Biol Fertil Soils, 15, 161–164, doi:10.1007/BF00361605, 1993.

Kuzyakov, Y.: Sources of CO_2 efflux from soil and review of partitioning methods, Soil Biology and Biochemistry, 38, 425–448, doi:10.1016/j.soilbio.2005.08.020, 2006.

Kuzyakov, Y., Leinweber, P., Sapronov, D., and Eckhardt, K.-U.: Qualitative assessment of rhizodeposits in non-sterile soil by analytical pyrolysis, Journal of Plant Nutrition and Soil Science, 166, 719–723, doi:10.1002/jpln.200320363, 2003.

La Scala, N., Lopes, A., Spokas, K., Bolonhezi, D., Archer, D. W., and Reicosky, D. C.: Short-term temporal changes of soil carbon losses after tillage described by a first-order decay model, Soil and Tillage Research, 99, 108–118, doi:10.1016/j.still.2008.01.006, 2008.

Leifeld, J. and Lützow, M. von: Chemical and microbial activation energies of soil organic matter decomposition, Biology and Fertility of Soils, 50, 147–153, doi:10.1007/s00374-013-0822-6, 2014.

Leinweber, P., Schulten, H.-R., and Horte, C.: Differential thermal analysis, thermogravimetry and pyrolysis-field ionisation mass spectrometry of soil organic matter in particle-size fractions and bulk soil samples, Thermochimica Acta, 194, 175–187, doi:10.1016/0040-6031(92)80016-P, 1992.

Leinweber, P. and Schulten, H. R.: Composition, stability and turnover of soil organic matter: investigations by off-line pyrolysis and direct pyrolysis-mass spectrometry, Journal of Analytical and Applied Pyrolysis, 32, 91–110, doi:10.1016/0165-2370(94)00832-L, 1995.

667 Leinweber, P., Schulten, H. R., and Korschens, M.: Hot-water extracted organic-matter-
668 chemical-composition and temporal variations in a long-term field experiment,
669 Biology and Fertility of Soils, 20, 17–23, doi:10.1007/BF00307836, 1995.

670 Leinweber, P., Eckhardt, K.-U., Fischer, H., and Kuzyakov, Y.: A new rapid micro-
671 method for the molecular-chemical characterization of rhizodeposits by field-
672 ionization mass spectrometry, Rapid Communications in Mass Spectrometry,
673 22, 1230–1234, doi:10.1002/rcm.3463, 2008a.

674 Leinweber, P., Jandl, G., Baum, C., Eckhardt, K.-U., and Kandeler, E.: Stability and
675 composition of soil organic matter control respiration and soil enzyme activities,
676 Soil Biology & Biochemistry, 40, 1496–1505,
677 doi:10.1016/j.soilbio.2008.01.003, 2008b.

678 Leinweber, P., Kruse, J., Baum, C., Arcand, M., Knight, J. D., Farrell, R., Eckhardt, K.-
679 U., Kiersch, K., and Jandl, G.: Chapter Two - Advances in Understanding
680 Organic Nitrogen Chemistry in Soils Using State-of-the-art Analytical
681 Techniques, in: Advances in Agronomy, Sparks, D. L. (Ed.), Advances in
682 Agronomy, Academic Press, 83–151, 2013.

683 Liu, X., Herbert, S. J., Hashemi, A. M., Zhang, X., and Ding, G.: Effects of agricultural
684 management on soil organic matter and carbon transformation - a review, Plant,
685 Soil and Environment, 52, 531–543, 2006.

686 Ludwig, M., Achtenhagen, J., Miltner, A., Eckhardt, K.-U., Leinweber, P., Emmerling,
687 C., and Thiele-Bruhn, S.: Microbial contribution to SOM quantity and quality in
688 density fractions of temperate arable soils, Soil Biology and Biochemistry, 81,
689 311–322, doi:10.1016/j.soilbio.2014.12.002, 2015.

690 Melnitchouck, A., Leinweber, P., Eckhardt, K. U., and Beese, R.: Qualitative
691 differences between day- and night-time rhizodeposition in maize (Zea mays L.)
692 as investigated by pyrolysis-field ionization mass spectrometry, Soil Biology &
693 Biochemistry, 37, 155–162, doi:10.1016/j.soilbio.2004.06.017, 2005.

694 Mengel, K.: Turnover of organic nitrogen in soils and its availability to crops, Plant and
695 Soil, 181, 83–93, doi:10.1007/BF00011295, 1996.

Mijangos, I., Pérez, R., Albizu, I., and Garbisu, C.: Effects of fertilization and tillage on soil biological parameters: Papers from the 1st International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld-2005), Enzyme and Microbial Technology, 40, 100–106, doi:10.1016/j.enzmictec.2005.10.043, 2006.

Möller, K.: Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review: Agronomy for Sustainable Development, Agron. Sustain. Dev., 35, 1021–1041, doi:10.1007/s13593-015-0284-3, 2015.

Mueller, C. W., Gutsch, M., Kothieringer, K., Leifeld, J., Rethemeyer, J., Brueggemann, N., and Kögel-Knabner, I.: Bioavailability and isotopic composition of CO2 released from incubated soil organic matter fractions, Soil Biology and Biochemistry, 69, 168–178, doi:10.1016/j.soilbio.2013.11.006, 2014.

Negassa, W., Baum, C., and Leinweber, P.: Soil amendment with agro-industrial byproducts: molecular-chemical compositions and effects on soil biochemical activities and phosphorus fractions, Z. Pflanzenernähr. Bodenk., 174, 113–120, doi:10.1002/jpln.201000034, 2011.

Oades, J. M.: Soil organic matter and structural stability: mechanisms and implications for management, Plant Soil, 76, 319–337, doi:10.1007/BF02205590, 1984.

Odlare, M., Pell, M., Arthurson, J. V., Abubaker, J., and Nehrenheim, E.: Combined mineral N and organic waste fertilization - effects on crop growth and soil properties, JOURNAL OF AGRICULTURAL SCIENCE, 152, 134–145, doi:10.1017/S0021859612001050, 2014.

Ohkubo, S., Iwata, Y., and Hirota, T.: Influence of snow-cover and soil-frost variations on continuously monitored 5CO26 flux from agricultural land, Agricultural and Forest Meteorology, 165, 25–34, doi:10.1016/j.agrformet.2012.06.012, 2012.

Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H.: vegan:

725 Community Ecology Package. R package version 2.3-0. http://CRAN.R-
726 project.org/package=vegan, 2015

727 R Core Team: R: A language and environment for statistical computing. R Foundation
728 for Statistical Computing, Vienna, Austria, http://www.R-project.org, 2013

729 Raich, J. W. and Potter, C. S.: Global patterns of carbon dioxide emissions from soils,
730 Global Biogeochemical Cycles, 9, 23–36, doi:10.1029/94GB02723, 1995.

731 Rasse, D. P., Dignac, M.-F., Bahri, H., Rumpel, C., Mariotti, A., and Chenu, C.: Lignin
732 turnover in an agricultural field: from plant residues to soil-protected fractions,
733 European Journal of Soil Science, 57, 530–538, doi:10.1111/j.1365-
734 2389.2006.00806.x, 2006.

735 Reicosky, D. and Archer, D.: Moldboard plow tillage depth and short-term carbon
736 dioxide release, Soil and Tillage Research, 94, 109–121,
737 doi:10.1016/j.still.2006.07.004, 2007.

738 Reicosky, D. C., Dugas, W. A., and Torbert, H. A.: Tillage-induced soil carbon dioxide
739 loss from different cropping systems, Soil and Tillage Research, 41, 105–118,
740 doi:10.1016/S0167-1987(96)01080-X, 1997.

741 Rochette, P. and Angers, D. A.: Soil surface carbon dioxide fluxes induced by spring,
742 summer, and fall moldboard plowing in a sandy loam, Soil Science Society of
743 America Journal, 63, 621–628, 1999.

744 Ryan, M. G. and Law, B. E.: Interpreting, measuring, and modeling soil respiration,
745 Biogeochemistry, 73, 3–27, doi:10.1007/s10533-004-5167-7, 2005.

746 Schmidt, L., Warnstorff, K., Dörfel, H., Leinweber, P., Lange, H., and Merbach, W.:
747 The influence of fertilization and rotation on soil organic matter and plant yields
748 in the long-term Eternal Rye trial in Halle (Saale), Germany, Journal of Plant
749 Nutrition and Soil Science, 163, 639–648, doi:10.1002/1522-
750 2624(200012)163:6<639:AID-JPLN639>3.0.CO;2-L, 2000.

751 Schmidtlein, S., Feilhauer, H., and Bruelheide, H.: Mapping plant strategy types using
752 remote sensing, Journal of Vegetation Science, 23, 365–405,
753 doi:10.1111/j.1654-1103.2011.01370.x, 2012.

754 Schnitzer, M.: The in situ analysis of organic matter in soils, Canadian Journal of Soil
755 Science, 81, 249–254, doi:10.4141/S00-064, 2001.

756 Schulten, H.-R. and Hempfling, R.: Influence of agricultural soil management on humus
757 composition and dynamics: Classical and modern analytical techniques, Plant
758 and Soil, 142, 259–271, doi:10.1007/BF00010971, 1992.

759 Schulten, H.-R., Sorge-Lewin, C., and Schnitzer, M.: Structure of "unknown" soil
760 nitrogen investigated by analytical pyrolysis, Biology and Fertility of Soils, 24,
761 249–254, doi:10.1007/s003740050239, 1997.

762 Schulten, H. R., Leinweber, P., and Jandl, G.: Analytical pyrolysis of humic substances
763 and dissolved organic matter in water, in: Refractory organic substances in the
764 environment, Frimmel, F. H., Abbt-Braun, G., Heumann, K. G., Hock, B.,
765 Lüdemann, H.-D., and Spiteller, M. (Eds.), John Wiley & Sons, 163–187, 2008.

766 Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., and Richter, A.: Carbon use
767 efficiency of microbial communities: stoichiometry, methodology and
768 modelling, Ecol Lett, 16, 930–939, doi:10.1111/ele.12113, 2013.

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

772 Sleutel, S., Leinweber, P., van Ranst, E., Kader, M. A., and Jegajeevagan, K.: Organic
773 Matter in Clay Density Fractions from Sandy Cropland Soils with Differing
774 Land-Use History, 75, 521–532:
775 https://dl.sciencesocieties.org/publications/sssaj/abstracts/75/2/521, last access:
776 16 September 2014, 2011.

777 Sorge, C., Müller, R., Leinweber, P., and Schulten, H.-R.: Pyrolysis-mass spectrometry
778 of whole soils, soil particle-size fractions, litter materials and humic substances:
779 statistical evaluation of sample weight, residue, volatilized matter and total ion

intensity, Fresenius' Journal of Analytical Chemistry, 346, 697–703, doi:10.1007/BF00321275, 1993.

Sparling, G., Vojvodic-Vukovic, M., and Schipper, L. A.: Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C, Soil Biology and Biochemistry, 30, 1469–1472, doi:10.1016/S0038-0717(98)00040-6, 1998.

Thevenot, M., Dignac, M.-F., and Rumpel, C.: Fate of lignins in soils: A review, Soil Biology and Biochemistry, 42, 1200–1211, doi:10.1016/j.soilbio.2010.03.017, 2010.

Thomsen, I. K., Olesen, J. E., Møller, H. B., Sørensen, P., and Christensen, B. T.: Carbon dynamics and retention in soil after anaerobic digestion of dairy cattle feed and faeces, Soil Biology and Biochemistry, 58, 82–87, doi:10.1016/j.soilbio.2012.11.006, 2013.

van Bochove, E., Couillard, D., Schnitzer, M., and Schulten, H.-R.: Pyrolysis-Field Ionization Mass Spectrometry of the Four Phases of Cow Manure Composting, Soil Sci. Soc. Am. J., 60, 1781–1786, doi:10.2136/sssaj1996.03615995006000060024x, 1996.

Wentzel, S., Schmidt, R., Piepho, H.-P., Semmler-Busch, U., and Joergensen, R. G.: Response of soil fertility indices to long-term application of biogas and raw slurry under organic farming, Applied Soil Ecology, 96, 99–107, doi:10.1016/j.apsoil.2015.06.015, 2015.

Wilcken, H., Sorge, C., and Schulten, H. R.: Molecular composition and chemometric differentiation and classification of soil organic matter in Podzol B-horizons, Geoderma, 76, 193–219, doi:10.1016/S0016-7061(96)00107-3, 1997.

Zakharova, A., Midwood, A. J., Hunt, J. E., Graham, S. L., Artz, R. R. E., Turnbull, M. H., Whitehead, D., and Millard, P.: Loss of labile carbon following soil disturbance determined by measurement of respired ∂13CO2, Soil Biology and Biochemistry, 68, 125–132, doi:10.1016/j.soilbio.2013.10.001, 2014.

808 Table 1. Means and standard deviations of soil organic carbon (C), nitrogen (N), C/N ratio, hot-water extractable carbon (HWC) and
809 nitrogen (HWN) and HWC/HWN ratio before and after tillage. Different letters in each column indicate significant differences (Tukey's
810 HSD test, $p < 0.05$) in means. Additionally, significant changes within a treatment (BD, biogas digestate; MF, mineral fertiliser; CL,
811 control) are highlighted in bold.

Treatment	Date	C (mg g^{-1})	N (mg g^{-1})	C/N	HWC (mg g^{-1})	HWN (mg g^{-1})	HWC/HWN
BD	Pre	8.4 ± 0.0	0.9 ± 0.0 ^{b}	9.0 ± 0.1 ^{a}	0.44 ± 0.02	0.05 ± 0.00 ^{bc}	**8.5 ± 0.1 ^{a}**
	Post	8.5 ± 0.1	1.0 ± 0.0 ^{ab}	8.8 ± 0.3 ^{ab}	0.44 ± 0.03	0.07 ± 0.01 ^{ab}	**6.1 ± 0.4 ^{b}**
	Post + 4	8.4 ± 0.0	1.0 ± 0.0 ^{ab}	8.7 ± 0 ^{ab}	0.40 ± 0.02	0.07 ± 0.01 ^{abc}	**6.0 ± 0.4 ^{b}**
MF	Pre	8.7 ± 0.3	1.0 ± 0.0 ^{a}	8.5 ± 0.2 ^{b}	0.44 ± 0.05	0.08 ± 0.00 ^{ab}	5.9 ± 0.8 ^{b}
	Post	8.5 ± 0.3	1.0 ± 0.0 ^{ab}	8.5 ± 0.1 ^{b}	0.42 ± 0.04	0.09 ± 0.02 ^{a}	4.9 ± 0.7 ^{b}
	Post + 4	8.6 ± 0.3	1.0 ± 0.0 ^{a}	8.5 ± 0.2 ^{b}	0.39 ± 0.00	0.07 ± 0.01 ^{abc}	5.5 ± 0.5 ^{b}
CL	Pre	8.5 ± 0.2	1.0 ± 0.0 ^{ab}	8.8 ± 0.2 ^{ab}	0.50 ± 0.10	0.06 ± 0.02 ^{abc}	8.9 ± 1.3 ^{a}
	Post	8.6 ± 0.2	1.0 ± 0.0 ^{ab}	8.8 ± 0 ^{ab}	0.48 ± 0.04	0.06 ± 0.01 ^{bc}	8.8 ± 0.8 ^{a}
	Post + 4	8.5 ± 0.0	1.0 ± 0.0 ^{ab}	8.7 ± 0.1 ^{ab}	0.40 ± 0.03	0.04 ± 0.00 ^{c}	9.6 ± 0.3 ^{a}

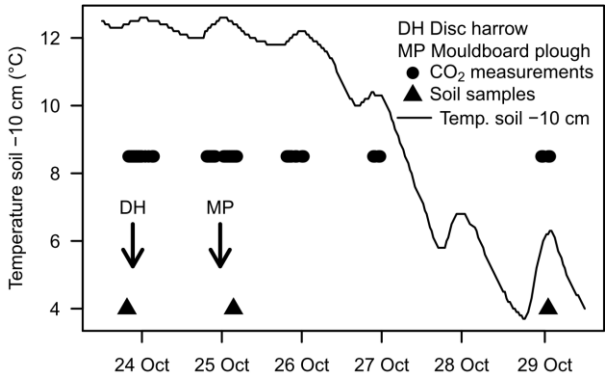
Table 2. Total ion intensity (TII), percentage of matter volatilised in pyrolysis (VM), and relative contribution of soil organic matter compound classes to the TII as detected by Py-FIMS in the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) close before (Pre) and after tillage (Post) and also four days after tillage (Post + 4) with standard deviations. Different letters in a column of each treatment indicate significant differences (Tukey's HSD test, $p < 0.1$) in means of the different dates towards tillage. Additionally, treatments with significant changes are highlighted in bold.

Treatment	Date	TII (10^6 counts mg^{-1})	VM (%)	Relative proportions of compound classes (% TII)*										
				CHYDR	PHLM	LDIM	LIPID	ALKYL	NCOMP	STEROL	PEPTI	SUBER	FATTY	Sum
	Pre	44.3 ± 11.5	5.2 ± 1.3	**3.7 ± 1.8** [a]	**9.8 ± 3.8** [a]	3.4 ± 1.4	**5.3 ± 1.0** [a]	11.9 ± 1.2	**1.8 ± 0.8** [a]	**1.6 ± 0.7** [a]	**4.3 ± 1.2** [a]	0.1 ± 0.1	**0.5 ± 0.2** [a]	**42.3 ± 5.4** [a]
BD	Post	40.3 ± 19.3	4.7 ± 1.3	**5.6 ± 0.3** [ab]	**13.3 ± 0.8** [ab]	2.5 ± 0.4	**4.1 ± 0.1** [b]	12.5 ± 0.7	**2.8 ± 0.2** [b]	**0.7 ± 0.2** [b]	**5.5 ± 0.3** [ab]	0 ± 0.1	**0.2 ± 0.1** [b]	**47.3 ± 0.9** [ab]
	Post + 4	35.1 ± 3.0	7.1 ± 1.2	**6.2 ± 0.3** [b]	**14.4 ± 0.3** [b]	1.9 ± 0.2	**3.9 ± 0.1** [b]	13.2 ± 0.1	**3.2 ± 0.2** [b]	**0.6 ± 0** [b]	**5.9 ± 0.2** [b]	0 ± 0	**0.2 ± 0** [b]	**49.4 ± 0.7** [b]
	Pre	34.2 ± 3.4	3.9 ± 1.1	5.6 ± 0.9	11.4 ± 0.7	2.9 ± 0.4	**4.6 ± 0.4** [a]	12.2 ± 0.9	2.7 ± 0.2	1 ± 0.4	5.4 ± 0.7	0 ± 0	0.3 ± 0.3	46.0 ± 0.3
MF	Post	39.1 ± 5.2	4.6 ± 1.0	4.6 ± 0.2	10.5 ± 0.6	3.5 ± 0.2	**5.1 ± 0.1** [ab]	12.4 ± 0.3	2.3 ± 0.1	1.2 ± 0.2	4.8 ± 0.2	0 ± 0	0.1 ± 0.1	44.5 ± 0.8
	Post + 4	46.5 ± 15.8	4.2 ± 0.5	4.3 ± 1.0	10.3 ± 1.6	3.3 ± 0.5	**5.4 ± 0.4** [b]	12.6 ± 0.5	2.2 ± 0.5	1.2 ± 0.3	4.5 ± 0.4	0 ± 0.1	0.3 ± 0.1	44.2 ± 2.8
	Pre	41.5 ± 15.5	**3.6 ± 0.6** [a]	5.5 ± 0.3	14.3 ± 0.4	2.2 ± 0.8	4.3 ± 0.1	13.6 ± 0.4	3.1 ± 0.2	0.6 ± 0	5.4 ± 0.2	0 ± 0	0.2 ± 0.2	49.2 ± 0.9
CL	Post	41.2 ± 7.8	**4.7 ± 0.4** [b]	5.6 ± 0.3	14.4 ± 0.2	1.8 ± 0.1	4.5 ± 0.2	13.9 ± 0.1	3.1 ± 0.1	0.6 ± 0.1	5.4 ± 0.3	0 ± 0	0.3 ± 0.1	49.6 ± 0.6
	Post + 4	47.9 ± 14.8	**3.2 ± 0.5** [a]	5.6 ± 0.5	14.4 ± 0.6	2.5 ± 0.8	4.3 ± 0	13.7 ± 0.5	3.1 ± 0.2	0.6 ± 0.1	5.3 ± 0.2	0 ± 0	0.1 ± 0.1	49.5 ± 1.3

817 *CHYDR, carbohydrates with pentose and hexose subunits; PHLM, phenols and lignin monomers; LDIM, lignin dimers; LIPID, lipids,
818 alkanes, alkenes, bound fatty acids, and alkyl monoesters; ALKY, alkylaromatics; NCOMP, mainly heterocyclic N-containing compounds;
819 STEROL, sterols; PEPTI, peptides; SUBER, suberin; FATTY, free fatty acids.

820 Table 3. Results of iterative partial least square regression for cumulated CO_2 efflux as
821 dependent variable and *m/z* data of all treatments and sampling times as explaining
822 variables.

m/z	Molecule/compound class
17/18	Ammonium
31	$[M+H]^+$ of formaldehyde
34	H_2S
43	C_2H_3O from ketones/amides and C_3H_7 propyl
46	Formic acid
55	C_3H_3O from ketones/amides
57	C_3H_5O from ketones/amdides and C_4H_9 butyl
73	Propanamide
83	C_5H_9N from peptides
85	$C_4H_5O_2$ from carbohydrates
91	Fragment from peptides
98/99	Carbohydrates
206, 222, 230/231, 246, 254, 258	Lignins
296, 299, 337, 418, 424	Fatty acids ($C_{19:1}$, $C_{19:0}$, $C_{22:2}$, $C_{28:3}$, $C_{28:0}$)

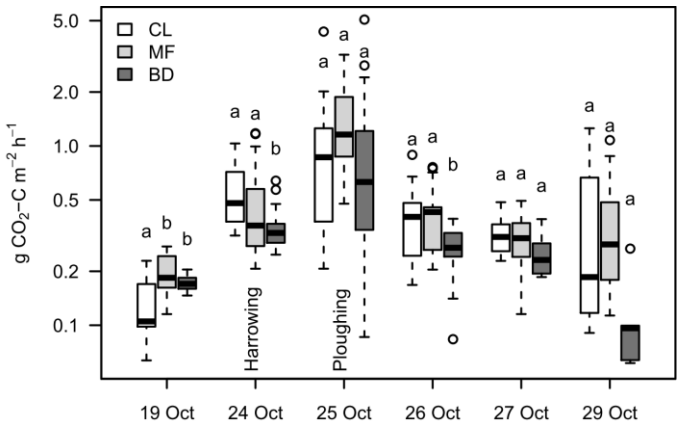


823

824

825 Figure 1. Timeline of the soil sampling and the CO_2 measurements in relation to the tillage
826 events. Additionally, soil temperature in 10 cm depth is plotted, recorded every 30 minutes
827 with an automated meteorological station (DALOS 535, F&C, Gülzow, Germany).

828



829

830

831 Figure 2. Soil CO_2 efflux and time of tillage operations (harrowing up to 10 cm depth and
832 ploughing up to 30 cm depth). Note that for the days of tillage (24 and 25 October) only the
833 fluxes after tillage are included in order to get a better attribution of the tillage effect.
834 Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$) in mean fluxes
835 of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) for each
836 measurement day.

837

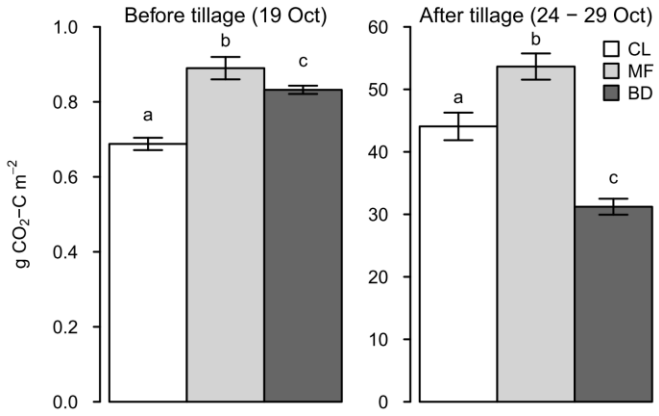


Figure 3. Cumulated soil CO_2 effluxes on a day before (19 October, between 7 a.m. and 1 p.m.) and the period (24 October, 7 a.m. – 29 October, 1 p.m.) tillage. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$) in means of the cumulated fluxes of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before and after, respectively. Error bars represent the standard deviation of interpolation by bootstrapping after 250 iteration runs.

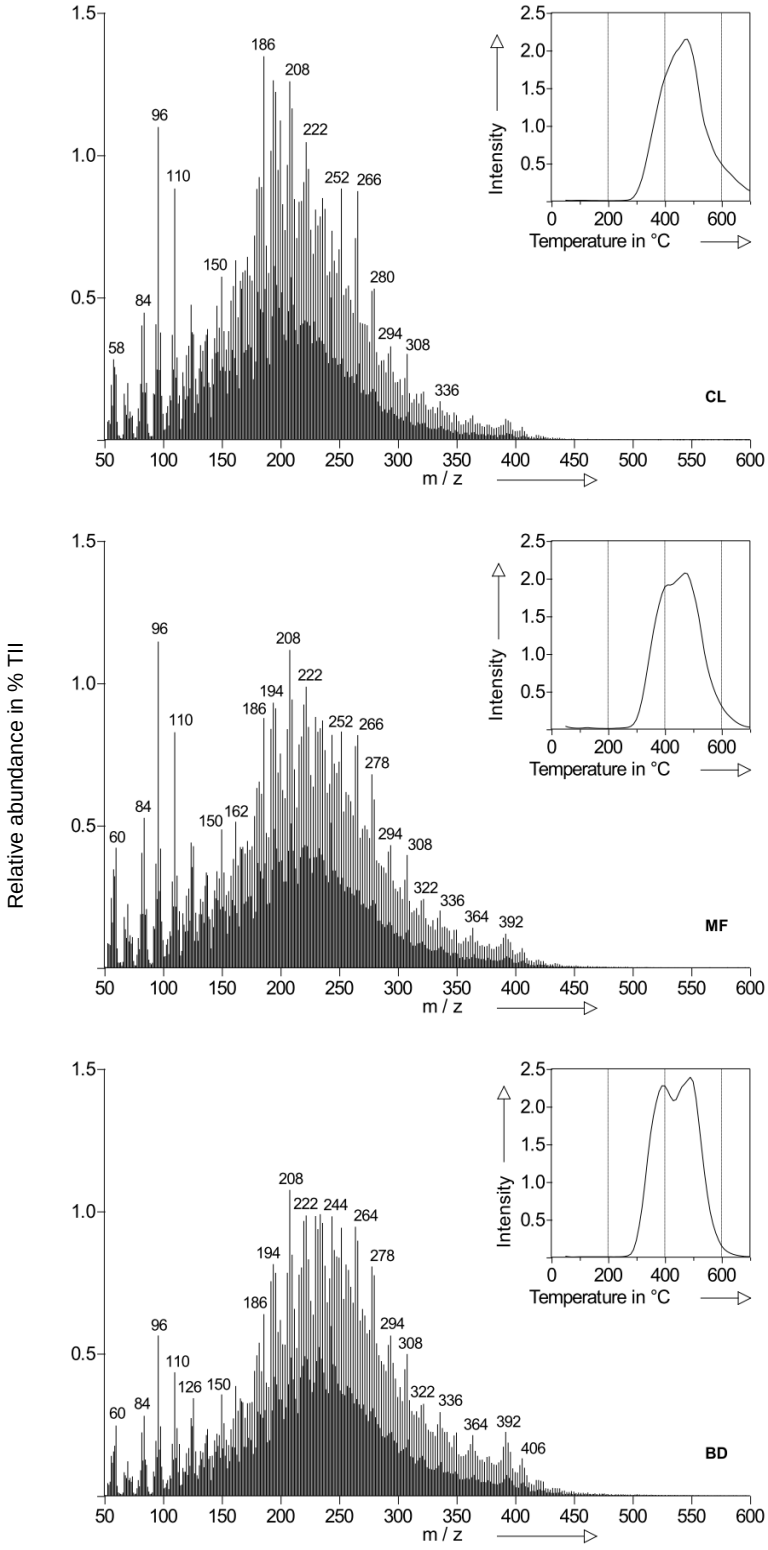


Figure 4. Thermograms of total ion intensity (TII, inserts upper right) and summed pyrolysis-field ionization mass spectra of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before tillage.

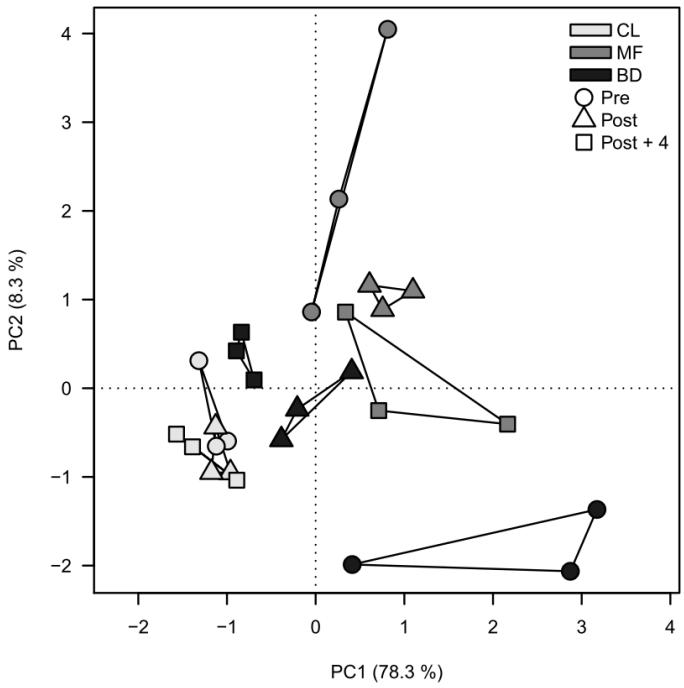


Figure 5. Principal component analysis of mass signals with significant differences according to Wilks' λ. Treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) and sampling times (pre-tillage, post-tillage and post-tillage + 4 days) are depicted by different colours and symbols, respectively. Since the areas integrated by the corresponding three sampling points do not overlap for the fertilised treatments, significant distinctions and changes of relative SOM composition can be assumed before and after tillage, respectively.

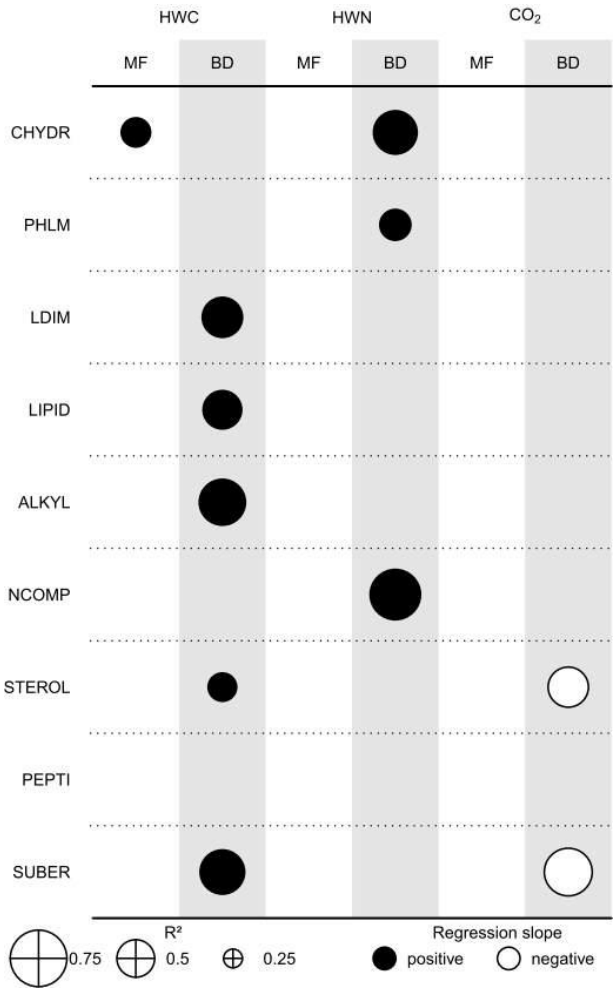


Figure 6. Significant ($p < 0.05$) linear correlations between absolute signal counts of the compound classes and hot-water extractable carbon (HWC), hot-water extractable nitrogen (HWN) and soil respiration (CO_2), respectively, with the corresponding coefficients of determination (R^2) and direction of regression slopes, derived from the three soil sampling dates. No significant correlations were observed for CL and thus omitted in the figure.