Dear Karsten,

thank you for the good news. We followed your suggestion and removed the respective parts. Additionally, lines 395 to 399 have been moved to lines 418 to 422; and the phrase “due to application of organic matter with biogas digestate” in line 387 has been changed to “through application of BD.”

Thank you for your time and expertise.

Best regards,
Sebastian

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

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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 differences in relative ion intensities of MF and CL 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. 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. Our analyses provide 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 (Dao, 1998), mostly by aeration and by the disruption of macro-aggregates, leading to release of protected SOM (Grandy and Robertson, 2007). 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 and Schimel, 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, these correlations do not causally explain which organic components 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

59 impacts of tillage on SOM and its turnover may help to avoid adverse effects for plant
60 growth (Franzluebbers et al., 1994).

61 In general, detecting changes in the molecular-chemical composition of SOM in time
62 periods as short as days, requires extremely sensitive methods. Py-FIMS is a very
63 sensitive method and has been applied successfully to investigate differences in the
64 chemical composition of SOM under different fertiliser treatments like mineral NPK-
65 fertiliser or farmyard manure (Jandl et al., 2004; Leinweber et al., 2008; Schmidt et al.,
66 2000). Even very small alterations in the composition and stability of dissolved organic
67 matter – a very reactive part of SOM – during storage in the fridge (Schulten et al.,
68 2008) or diurnal cycles of CO_2-assimilation and respiration (Kuzyakov et al., 2003;
69 Melnitchouck et al., 2005) have been detected and resolved by multivariate statistics of
70 mass-spectrometric fingerprints. Furthermore, Py-FIMS of bulk SOM revealed
71 alterations in laboratory incubation experiments and allowed to link these to respiration
72 and enzyme activities (Leinweber et al., 2008). However, it is unclear if the method is
73 sensitive enough to detect tillage-induced SOM alterations under various fertilisation
74 regimes and analyse its influence on CO_2 efflux at the field scale where spatial
75 heterogeneity may interfere with the temporal dynamics much more than in the above
76 cited laboratory studies.

77 Hot-water extraction is a relatively simple method to release labile SOM and to estimate
78 how much of soil C and N can be easily utilised by microorganisms (Leinweber et al.,
79 1995). These labile pools have been suggested to be an important indicator of short-
80 term changes in SOM quality due to soil management (Haynes, 2005). Furthermore, a
81 significant proportion of hot water-extracted organic matter originates from microbial
82 biomass. Thus, this approach is a potential indicator for changes in microbial biomass
83 or activity (Sparling et al., 1998), which may reflect sources of CO_2 efflux following
84 tillage.

85 Here, we investigate (1) short-term effects of tillage on SOM composition and (2)
86 potential relationships between decomposable SOM fractions and measured CO_2 efflux
87 under the impact of different soil amendments by combining Py-FIMS with CO_2 efflux
88 measurements.

89

2 Materials and methods

2.1 Study site

The study site is located in northeast Germany in the ground moraine of the Weichselian glacial period at 53° 48' 35" N and 12° 4' 20" E (elevation 10 m) within a gently rolling relief. The soil is a stagnic luvisol (IUSS Working Group WRB, 2006) with sandy loam texture (sand = 63 %, silt = 26 %, clay = 11 %) overlying bedrock of till. The top soil (0-30 cm) has an organic carbon content of 8.5 mg g^{-1} ± 0.2 (mean ± standard deviation, n = 9), pH of 7.4 ± 0.9 (n = 3) and bulk density of 1.51 g cm^{-3} ± 0.08 (n = 3), measured according to Fiedler et al. (2015). The climate is characterized by maritime influence with annual averages of 8.8° C temperature and 557 mm total precipitation for the 30-year-period from 1985 until 2014 (LFA, 2015). The experiment was conducted on a field which has been cultivated with maize (*Zea Mays* L.), cultivar 'Atletico', as feedstock for a biogas plant. Before our study period, during other trials, winter wheat (*Triticum aestivum* L.) followed by maize were grown on the field.

We compared three fertiliser treatments: CL – without fertiliser (control), MF – with mineral fertiliser, and BD – with biogas digestate. The size of the three experimental plots was 6 by 30 m each. In both fertilised treatments, equal overall amounts of plant-available N were applied (160 kg ha^{-1}) on 26 April 2012. The mineral fertiliser calcium ammonium nitrate was top-dressed whereas the biogas digestate was injected into the soil down to 10 cm depth with a track width of 25 cm. Following the research facility for agriculture and fisheries (LFA) of the federal state of Mecklenburg-Western Pomerania, Germany (2012, personal communication), a mineral fertiliser equivalent of 70% of total N in the biogas digestates (229 kg N ha^{-1}) was assumed. The BD for this single application originated from 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 undried material. During the cropping season 2012, maize was grown according to conventional agricultural practice.

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 bases in each treatment after fertilisation in spring which were removed for tillage and inserted back afterwards. The adjacent bases were placed 1 m apart. The bases had dimensions of 79 x 79 cm, 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 bases during measurements. The chambers had a square area of 0.6241 m² and a height of 0.55 m, resulting in a chamber volume of 0.34 m³ 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 replicate bases 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 inside the chamber (K), A the area covered by the chamber (m^2), and dc/dt the CO_2 concentration change over time ($ppm\ h^{-1}$). The minimum proportion of data points to be kept for regression analyses was 70 % of a concentration measurement. This allowed

discarding data noise at the beginning and the end resulting from chamber deployment and removal (for details see help file for function *fluxx* of package *flux*). Thus, each CO_2 flux was estimated at least from 98 concentration measurements. Only linear fluxes with a concentration change of at least 10 ppm, a normalised root mean square error (NRMSE) ≤ 0.15 and a coefficient of determination (R^2) of at least 0.85 were included in further analyses. We assumed linearity of concentration change and did not test for non-linearity since 95.1% of the obtained linear regressions had $R^2 \geq 0.95$.

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

2.3 Soil sampling and analyses

Three replicates of bulk soil samples were taken between 0 – 10 cm depth (depending on unevenness of soil surface due to tillage) directly with three soil sample rings (h = 6.1 cm, V = 250 cm^3) in a triangular arrangement around the three bases for gas sampling (see 2.2) in each treatment at three dates: 1) right before the first tillage operation, 2) in the afternoon after the second tillage operation and 3) four days after the second tillage operation. The resulting 27 soil samples were fixed immediately with liquid nitrogen and splitted thereafter into subsamples for freeze-drying and for oven-drying at 60° C.

For Py-FIMS, the freeze-dried samples were finally ground and homogenized by a planetary ball mill. Then, about 2 g were transferred into a Petri dish with a spatula and three crucibles were filled by drawing them across. These subsamples of about 5 mg 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. For plotting, 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 of soil were boiled in 40 ml deionized water for 60 minutes (Leinweber et al., 1995). After filtration with pleated filters (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) as well as of organic and inorganic bound N, often referred to as ‘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 CO_2 effluxes were estimated by a bootstrap method with the function *auc.mc* of the R package *flux* version 0.3-0 (Jurasinski et al., 2014). In detail, the CO_2 fluxes were cumulated in 250 iterations, while for each run 25 fluxes were omitted randomly for the period after tillage. For the reference period before tillage, in each iteration run 4 fluxes were omitted randomly. The numbers of randomly omitted fluxes per run correspond roughly to one fifth of the recorded fluxes per treatment in the respective periods. The resulting data were used to calculate means and standard deviations. Tukey's HSD test was applied to test for differences in means of CO_2 fluxes as well as of HWC and HWN between sampling periods and treatments against a significance level of $\alpha < 0.05$. Py-FIMS signals of the compound classes were tested for differences in means by Tukey's HSD test against a significance level of $\alpha < 0.1$ since the number of replicates was limited and the variances rather high.

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

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

233 # 3 Results

234 ## 3.1 Soil organic carbon, nitrogen, hot-water extractable carbon and hot-
235 water extractable nitrogen

236 One of the replicates in MF exhibited exceptionally low HWC and HWN values.
237 According to Dixon's Q-test, these values were outliers (one-third and half,
238 respectively, as high as for the other replicates in MF) and thus excluded from further
239 analysis. Before tillage, the soil of all treatments had similar C and HWC contents, but
240 differences appeared between MF and BD, where the N and HWN contents were
241 slightly higher in MF, resulting in narrower C/N and HWC/HWN ratios in MF (8.5 and
242 5.9, respectively) compared to BD (9.0 and 8.5, respectively) (Table 1). The C, N and
243 HWC contents of all treatments changed only slightly after tillage, but the HWN
244 content of soil in BD increased significantly ($p < 0.05$) from 0.05 mg g^{-1} (5.6 % of N)
245 up to 0.07 mg g^{-1} (7.4 % of N), resulting in a significant ($p < 0.05$) narrowing of the
246 HWC/HWN ratio from 8.5 down to 6.0 (Table 1).

247 ## 3.2 Soil CO_2 efflux

248 Five days before the tillage operations (19 October 2012), the mean efflux rates (all in g
249 CO_2-C m^{-2} h^{-1}) were 0.133 (CL), 0.192 (MF) and 0.173 (BD), with the efflux being
250 significantly lower from CL than from the amended plots MF and BD ($p < 0.05$) (Fig.
251 2). In the morning before the first tillage operation with a disc harrow (24 October), the
252 effluxes had similar magnitudes and proportions like five days before (CL = 0.147, MF
253 = BD = 0.199, all in g CO_2-C m^{-2} h^{-1}). After harrowing, CO_2-effluxes increased to 0.849
254 (CL), 0.833 (MF) and 0.479 (BD). Over the next 5.5 hours, these values declined to
255 0.602 (CL), 0.460 (MF) and 0.276 (BD) resulting in overall mean effluxes of 0.554
256 (CL), 0.481 (MF) and 0.344 (BD), with the latter being now significantly lower
257 ($p < 0.05$) than CL or MF during the measured period after harrowing. Directly before
258 the second tillage operation with a reversible mouldboard plough in the morning of the
259 following day (25 October), the mean effluxes were 0.299 (CL), 0.249 (MF) and 0.290
260 (BD) (all in g CO_2-C m^{-2} h^{-1}). Immediately after ploughing, they increased sharply up to
261 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 Spectrometry

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 differed markedly 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 the abundance of marker signals for carbohydrates and peptides (e.g., *m/z* 58, 60, 84, 69, 110, 126 and 162) was 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 accounted for 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 and increased from 5.2 to 7.1% during the days after tillage. Such an increase over time was only observed for BD, but it was not significant ($p > 0.1$). 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 check relationships between HWC, HWN and soil respiration as 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, the ion intensities for carbohydrates were positively correlated with HWC (R^2 =

0.44), whereas, in contrast, no such a correlation was found in BD. However, HWN showed a positive correlation with carbohydrates in BD ($R^2 = 0.61$). Further, CO_2 efflux increased with decreasing amounts of sterols in BD ($R^2 = 0.40$).

4 Discussion

4.1 Bulk soil and hot-water extracted carbon and nitrogen

The C-, HWC-, N- and HWN-contents of the treatments showed no differences before tillage (Table 1), thus confirming the outcomes of other field experiments with similar fertilisers (Makádi et al., 2016; Odlare et al., 2014). However, the C- and N-contents obtained may not be representative for long-term effects of biogas digestate vs. mineral fertiliser which may also depend on soil texture (Makádi et al., 2016).

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) 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). 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. Possibly, we did not detect it, because we took no soil samples after the first day. Overall, a single amendment with biogas digestates very likely is insufficient to initiate changes in bulk soil C- and N-levels. However, the increased HWN-levels in BD can be ascribed to a tillage promoted microbial turnover of soil organic N, confirming that the hot water extracts are a particularly sensitive approach to detect early SOM changes (Haynes, 2005).

4.2 Soil CO_2 efflux

The immediate and sharp increase of CO_2 efflux from soils just after tillage is a well-documented response and seems to be mainly driven by the release of trapped CO_2 from

348 broken up aggregates by tillage (Reicosky et al., 1997). It is commonly suggested that a
349 few hours afterwards, waning of this physical outgassing is accompanied by an
350 increased soil respiration due to a better substrate supply for microorganisms from
351 disrupted aggregates as well as increased soil aeration (Grandy and Robertson, 2007).
352 The amounts of the observed fluxes are well in accordance with the findings of previous
353 studies (e. g., Rochette and Angers, 1999) and can be explained both by the magnitude
354 of the disturbance, i.e. soil comminution, and the fertilisation history of the soil (Fiedler
355 et al., 2015).

356 The smaller relative efflux from BD compared to MF and CL after tillage is remarkable
357 since before tillage the CO_2 fluxes in BD were of the same magnitude as those in MF
358 and exceeded those in CL (Fig. 2). This becomes particularly evident when we consider
359 the relation of cumulated CO_2 fluxes between the treatments before (19 October) and
360 after tillage (24 – 29 October) (Fig. 3). The relatively lower CO_2 efflux from BD after
361 tillage may have different reasons. On the one hand, C originating from the digestates is
362 likely less available to soil microorganisms compared to undigested organic matter, i. e.
363 more 'recalcitrant', since the most labile C is generally consumed in the biogas reactor
364 (Möller, 2015). On the other hand, even a single application of organic amendment can
365 increase aggregate stability (Grandy et al., 2002). Therefore, the resilience against
366 disruption by tillage might be promoted, leading to a better physical protection of labile
367 soil C not contained within digestates. As a consequence, the effect of increased CO_2
368 efflux after tillage as observed in CL and MF may have been substantially reduced by a
369 relative shortage of labile substrate for soil respiration in BD.

370 ### 4.3 Pyrolysis-Field Ionization Mass Spectrometry and synthesis

371 Generally, the Py-FIMS basic data and mass spectra (Fig. 4) and the proportions of
372 compound classes (Table 2) confirm published data from this method for Luvisols in
373 terms of relatively high proportions of lignin monomers, phenols and alkylaromatics
374 (Leinweber et al., 2009). Lignin monomers and phenols might be collectively attributed
375 to residues of the just harvested maize. Indeed, Gregorich et al. (1996) found that these
376 are important components of maize leaves and roots as well as of the light fraction of
377 the soil under this crop. Overall, the Py-FIMS data indicate differences in SOM

378 composition between the fertilization treatments and a pronounced impact of tillage in
379 the treatments MF and BD (Fig. 5).

380 In the spectra of samples from BD, the additional peak at 390° C in the TII-thermogram
381 (Fig. 4) can be attributed mainly to phenols and lignin monomers which likely
382 originated from primary organic matter residues since this relatively low volatilization
383 temperature indicates labile and fairly undecomposed organic matter (Sleutel et al.,
384 2011). It is reasonable to refer this organic matter to residues from the application of
385 BD. VM as well as TII, which are indicators of SOM content (Sorge et al., 1993), were
386 larger in BD than in MF and CL before tillage (Table 2). This suggests a tendency to
387 increased SOM content through application of BD. The compound classes of BD
388 revealed the largest proportions of lignin dimers, lipids, sterols, suberin and free fatty
389 acids at the expense of carbohydrates, heterocyclic N-containing compounds and
390 peptides before tillage (Table 2). Such a SOM composition most likely reflects the
391 cattle manure and plant residues of the biogas feedstock and their relative depletions
392 (amides and polysaccharides) or enrichments (lignins and long-chain aliphatic
393 compounds) during anaerobic fermentation (Möller, 2015; van Bochove et al., 1996).
394 The pronounced tillage effect in this treatment, obvious from the increased relative
395 signal intensities of carbohydrates, phenols and lignin monomers, alkylaromatics,
396 heterocyclic N-containing compounds and peptides at the expense of lignin dimers,
397 lipids, sterols and free fatty acids following tillage (Table 2), suggests the
398 decomposition of lignin and the new formation of carbohydrates and peptides. This is in
399 line with reports of lignin decomposition faster than that of the total SOM (Leinweber et
400 al., 2008; Thevenot et al., 2010). Kalbitz et al. (2003) suggested that lignin-derived
401 moieties and lipids are utilised by microorganisms at low initial availability of
402 carbohydrates, accompanied by an accumulation of the resulting microbial metabolites
403 like carbohydrates and peptides. Recently, Rinkes et al. (2016) also found that
404 decomposers may break down lignin to acquire C for their metabolism in the absence of
405 available labile C. This suggestion is supported on the one hand by the effect of specific
406 lignins on soil CO_2 efflux (Table 3) since CO_2 is an indicator for microbial
407 decomposition activity (Kuzyakov, 2006). On the other hand, a relative increase of the
408 signals for *m/z* 125, 167, 185 and 203 was observed in the BD treatment (data not

409 shown) which are assigned to the bacterial cell wall products N-acetylmuramic acid and
410 N-acetylmuramyl-L-alanyl-D-isoglutamine (Bahr and Schulten, 1983). Furthermore, the
411 build-up of heterocyclic N-containing compounds might also imply a relative shortage
412 of available carbohydrates since a reduced C availability during the microbial
413 transformation of N is suggested to promote formation of heterocyclic N instead of N
414 immobilisation (Follett and Schimel, 1989; Gillespie et al., 2014; Schulten and
415 Hempfling, 1992). The increased proportion of lipids at the expense of carbohydrates
416 and peptides in MF likely results from increased heterotrophic respiration of labile
417 substrates driven by enhanced microbial activity after tillage (La Scala et al., 2008;
418 Zakharova et al., 2014). Decreasing proportions of carbohydrates and decreasing
419 relative signal intensities of *m/z* 17 and 18 (data not shown), which are assigned to
420 ammonia and ammonium, also point to a microbial immobilisation in MF (Mengel,
421 1996). Accordingly, this two m/z were also selected by the PLSR as explanatory signals
422 for CO_2 efflux (Table 3). The minor changes in SOM compounds in CL might be a
423 consequence of the wider HWC/HWN ratio compared to MF and BD since it indicates a
424 lower availability of labile N for microbial utilisation (Mengel, 1996). However, the
425 total C/N ratios were not critical for microbial activity (Table 1) (Kuzyakov et al.,
426 2000).

427 A significant ($p > 0.05$) and positive correlation was observed between HWC and
428 carbohydrates in MF. This linkage was previously described by Leinweber et al. (1995)
429 and attributed to microbial biomass (Ghani et al., 2003) and labile soil C (Sparling et
430 al., 1998). In contrast, this correlation was not apparent in BD. This corroborates the
431 assumption that microorganisms in BD may have been short in available labile C.
432 Interestingly, HWN correlated positively with carbohydrates in BD. Since the major
433 part of carbohydrates in soils originates from microorganisms and their residues
434 (Gunina and Kuzyakov, 2015), this may suggest a metabolic coupling between
435 carbohydrates and HWN because many N-cycling processes are mediated microbially
436 (Isobe and Ohte, 2014).

437 Increased amounts of sterols are typically found in biogas digestates (Leinweber, 2016,
438 unpublished Py-FIMS data). In BD, the cumulated CO_2 efflux and the amount of sterols
439 was negatively correlated. This supports the suggestion of Heumann et al. (2011, 2013)

that sterols may have an inhibitory effect on microorganisms of the N cycle and, thus, may slow down soil respiration. However, since the amounts of sterols decreased significantly after tillage in BD (Table 3), the actual sterol contribution to reduced CO_2-efflux in BD relative to the other treatments cannot be ascertained by the present data set.

Our data and analyses suggest a short-term induction of enhanced microbial N-turnover by tillage in soils amended with biogas digestates; possible co-occurring with the decomposition of lignin as C source due to a relative shortage of carbohydrates. This is supported by the results of each of the used methods, i.e., (i) HWN as an indicator for labile N increased, (ii) lignins, ammonia and ammonium were discriminated as explanatory variables for cumulated CO_2 efflux by PLSR and (iii) Py-FIMS data point at an increase of N-containing compounds along with decomposition of lignins and formation of carbohydrates and peptides.

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 relative shortage of labile N as indicated by the higher HWC/HWN-ratio which possibly prevented an enhanced microbial activity.

4.4 Limitations

Although the relatively small sampling areas around the bases in each treatment plot might suggest a ‘pseudo-replication’ in soil sampling, we have evidence suggesting a very high spatial variability in the soil, which alleviates this problem: in a master thesis on spatial variability, Jacobs (2014) revealed that N_2O fluxes from the soil of the study site show very high small-scale variability well below the meter scale. Therefore, we assume ‘real’, i.e., independent replicates, though the comparison *between* the treatments should be done carefully because of possibly rather small differences. Due to the, thus, potentially lowered influence of spatial variability, our sampling design might have biased our results towards the detection of even small temporal changes *within* the treatments. Because we are mainly interested in the impact of tillage, this limitation is

not interfering with our findings.

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 of the investigated soil 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 amendments, especially with the relatively new biogas digestates.

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696 Table 1. Means and standard deviations of soil organic carbon (C), nitrogen (N), C/N ratio, hot-water extractable carbon (HWC) and
697 nitrogen (HWN) and HWC/HWN ratio before (Pre), after (Post) and four days after tillage (Post + 4). Different letters in each column
698 within treatments (BD, biogas digestate; MF, mineral fertiliser; CL, control) indicate significant differences (Tukey's HSD test, $p < 0.05$)
699 in means.

Treatment	Date	C (mg g^{-1})	N (mg g^{-1})	C/N	HWC (mg g^{-1})	HWN (mg g^{-1})	HWC/HWN
	Pre	8.4 ± 0.1	0.9 ± 0.0	9.0 ± 0.1	0.44 ± 0.02	0.05 ± 0.00 [a]	8.5 ± 0.1 [a]
BD	Post	8.5 ± 0.1	1.0 ± 0.0	8.8 ± 0.3	0.44 ± 0.03	0.07 ± 0.01 [b]	6.1 ± 0.4 [b]
	Post + 4	8.4 ± 0.6	1.0 ± 0.0	8.7 ± 0.0	0.40 ± 0.02	0.07 ± 0.01 [b]	6.0 ± 0.4 [b]
	Pre	8.7 ± 0.3	1.0 ± 0.0	8.5 ± 0.2	0.44 ± 0.05	0.08 ± 0.00	5.9 ± 0.8
MF	Post	8.4 ± 0.3	1.0 ± 0.0	8.5 ± 0.1	0.42 ± 0.04	0.09 ± 0.02	4.9 ± 0.7
	Post + 4	8.6 ± 0.2	1.0 ± 0.0	8.5 ± 0.1	0.31 ± 0.14	0.06 ± 0.01	5.0 ± 0.8
	Pre	8.5 ± 0.2	1.0 ± 0.0	8.8 ± 0.2	0.50 ± 0.10	0.06 ± 0.02	8.9 ± 1.3
CL	Post	8.6 ± 0.2	1.0 ± 0.0	8.8 ± 0.0	0.48 ± 0.04	0.05 ± 0.01	8.8 ± 0.8
	Post + 4	8.5 ± 0.0	1.0 ± 0.0	8.7 ± 0.1	0.40 ± 0.03	0.04 ± 0.00	9.6 ± 0.3

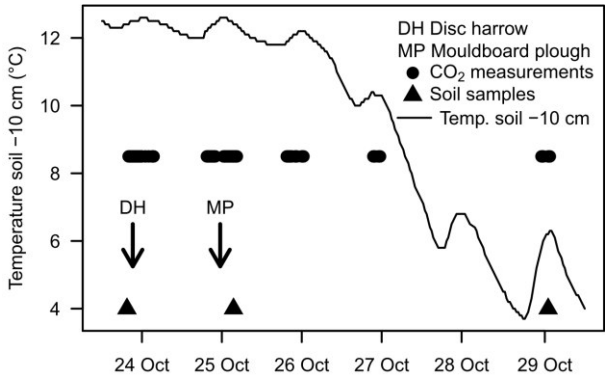
Table 2. Total ion intensity (TII), percentage of matter volatilised in pyrolysis (VM), and relative contribution of soil organic matter compound classes to the TII as detected by Py-FIMS in the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) close before (Pre), after (Post) and four days after tillage (Post + 4) with standard deviations. Different letters in a column within 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
BD	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]
	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]
MF	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
	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
CL	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
	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

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

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

m/z	Molecule/compound class
17/18	Ammonia/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 ($C_{19:1}$, $C_{19:0}$, $C_{22:2}$, $C_{28:3}$, $C_{28:0}$)



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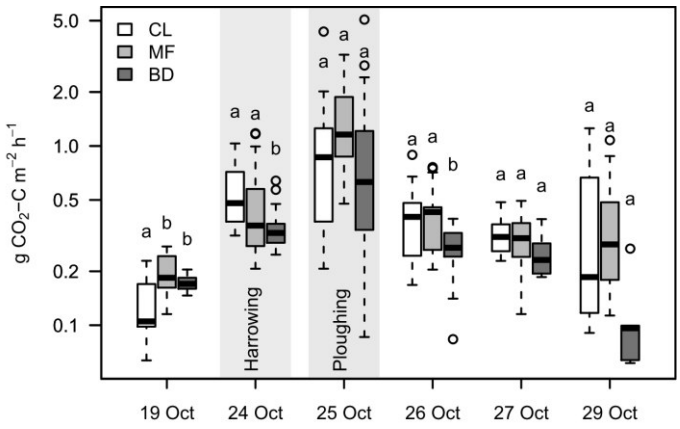
712

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

716



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718

719 Figure 2. Soil CO_2 efflux and time of tillage operations (harrowing down to 10 cm depth and
720 ploughing down to 30 cm depth). Note that for the days of tillage (24 and 25 October) only
721 the fluxes after tillage (distinguished by light grey backgrounds) are included in order to get a
722 better attribution of the tillage effect. Different letters indicate significant differences
723 (Tukey's HSD test, $p < 0.05$) in mean fluxes of the treatments (CL, control; MF, mineral
724 fertiliser; BD, biogas digestate) for each measurement day.

725

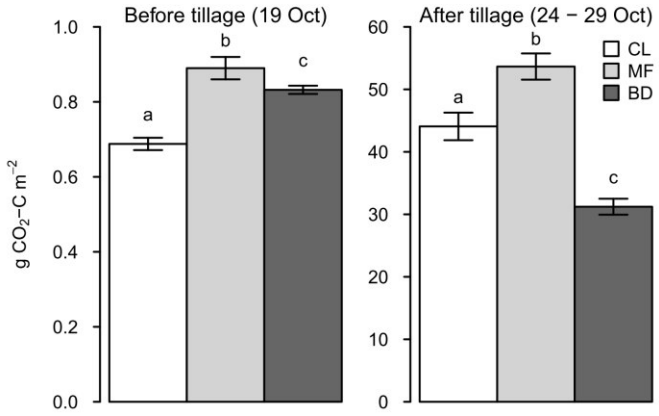
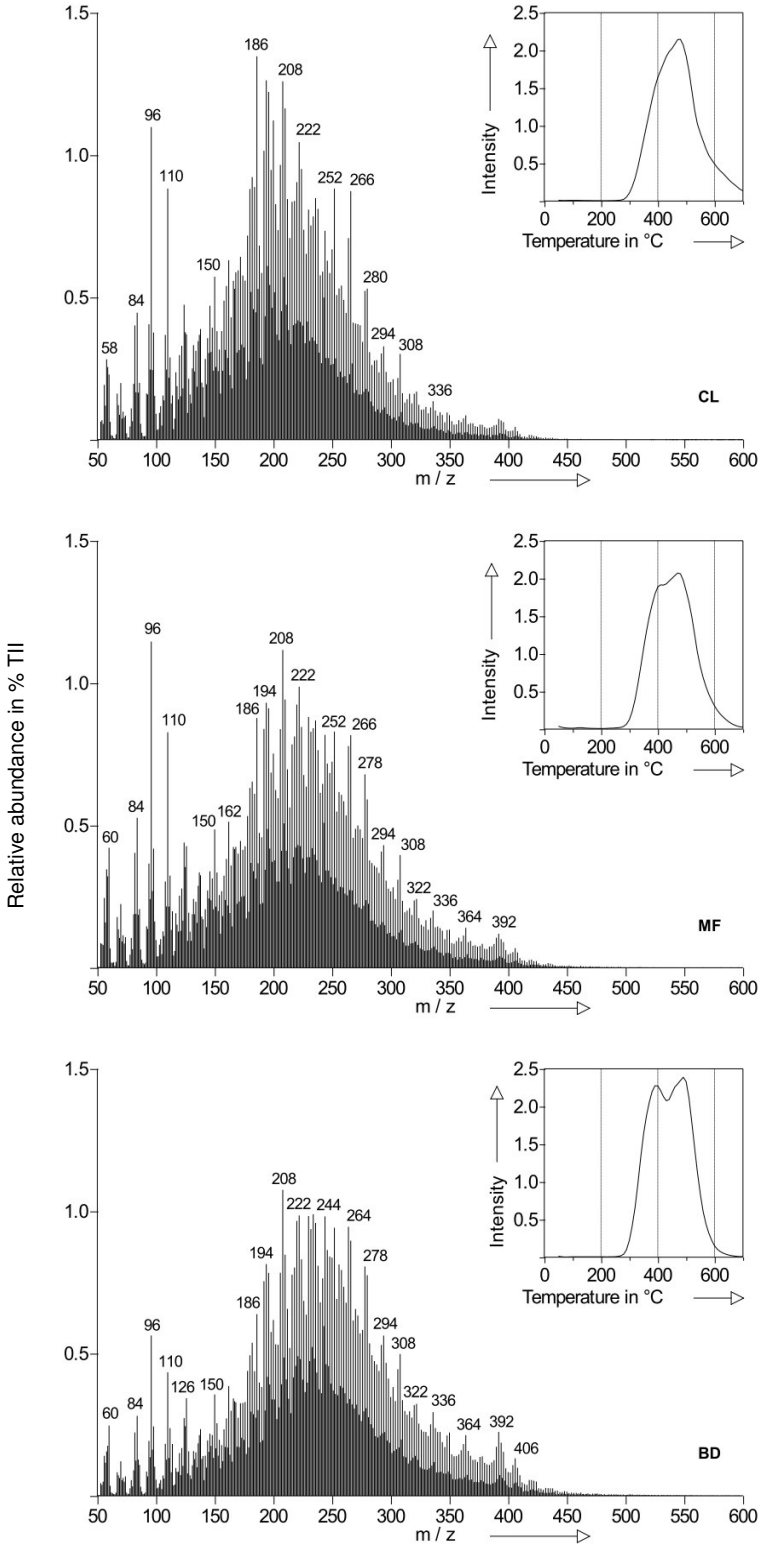
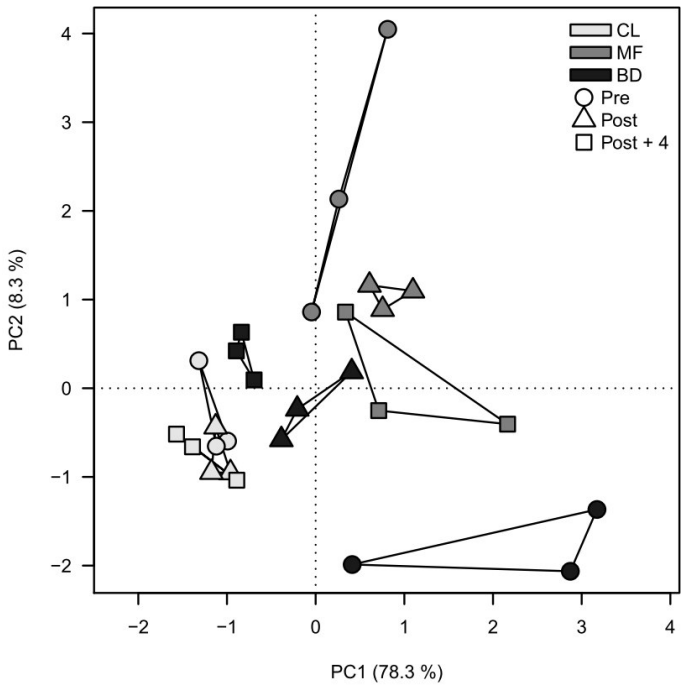


Figure 3. Cumulated soil CO_2 effluxes on a day before (19 October, between 7 a.m. and 1 p.m.) and the period (24 October, 7 a.m. – 29 October, 1 p.m.) tillage. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$) in means of the cumulated fluxes of the treatments (CL, control;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



734

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



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739

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

746