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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, BD showed much more volatilised matter (VM) during pyrolysis, indicating an increased amount of SOM. 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 labile carbon 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

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proportions increased at the expense of 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 fertilized 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 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). These correlations do not causally explain which SOC constituents that form the majority of SOM are mineralized. 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 effects on the reproduction of the SOM level 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.

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

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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 loamy sand texture overlying bedrock of till. The top soil (0-30 cm) has an organic carbon content of 1.16% (standard deviation (SD) = 0.1, *n* = 3, measured with CN-analyser ”vario MAX”, Elementar, Hanau, Germany), pH of 7.4 (SD = 0.9, *n* = 3, measured in H_2O with pH meter ”CX-401”, Elmetron, Zabrze, Poland) and bulk density of 1.51 g cm^{-3} (SD = 0.08, *n* = 3, measured on 250 cm^3 soil cores). 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. The previous crops were winter wheat (*Triticum aestivum* L.) followed by maize.

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 (personal communication, 2014), a mineral fertiliser equivalent of 70% of total N in the biogas digestates (229 kg N ha^{-1}) was assumed. The biogas 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 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 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 m³ 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 (44 g mol^{-1}), p the air pressure (Pa), V the chamber volume (m^3), R the gas constant (8.31 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 to discard 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 at 5 – 15 cm depth with soil sample rings (V = 250 cm³) in a triangular arrangement between the three collars for gas sampling (see 2.3) 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 Pyrolysis-field ionization mass spectrometry (Py-FIMS), about 5 milligrams of the 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 were 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 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 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).

3 Results

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

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 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 Ionisation 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). 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

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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, 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 signal intensities 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.

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).

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The latter was derived from Table 2 by Eq. (2).

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

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 BR in the field (Odlare et al., 2014). On the contrary, in a pot experiment with maize by (Bachmann et al., 2011), the soil N content was higher under application of biogas digestate compared to application of mineral fertiliser. Since the latter and the present study were rather short-termed (weeks and months, respectively), the C- and N-contents obtained may not be representative for long-term effects of mineral fertiliser vs. biogas digestates.

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, (Schulten et al., 1997) 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 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 broken up aggregates by tillage (Calderon and Jackson, 2002; Ellert and Janzen, 1999; Reicosky et al., 1997). It is commonly suggested that a few hours afterwards, waning of this physical outgassing is accompanied by an increased soil respiration due to a better substrate supply for microorganisms from disrupted aggregates as well as increased soil aeration (Schulten et al., 1997; Grandy and Robertson, 2007). The amounts of the observed fluxes are well in accordance with the findings of previous studies, e. g., (Rochette and Angers, 1999) and can be explained both by the magnitude of the disturbance, i.e. soil comminution, and the fertilisation history of the soil (Schulten et al., 1997).

The smaller relative efflux from BD compared to MF and CL after tillage is remarkable since before tillage the CO_2 fluxes in BD were of the same magnitude as those in MF and exceeded those in CL (Fig. 2). This becomes particularly evident when one considers the relation of cumulated CO_2 fluxes between the treatments before (19 October) and after tillage (24 – 29 October) (Fig. 3). Before tillage, the ratio of cumulated CO_2 fluxes in CL : MF : BD was 1 : 1.27 : 1.21 and changed to 1 : 1.21 : 0.71 after tillage. The relatively lower CO_2 efflux from BD after tillage may

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have different reasons. On the one hand, the organic matter originating from the digestates is likely less available to soil microorganisms, i. e. more "recalcitrant", since the most labile C has been consumed already in the biogas reactor (Thomsen et al., 2013; Möller, 2015; Wentzel et al., 2015). 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. On the other hand, 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.

4.3 Pyrolysis-Field Ionisation 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 impact of tillage in the treatments MF and BD.

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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, J. A. J. 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). This assumption is supported by the decreasing shares of carbohydrates in MF.

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 a lignin decomposition faster than that of the total SOM (Leinweber et al., 2008b; Rasse et al., 2006; Thevenot et al., 2010). (Kalbitz

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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. Our data from the BD treatment supports this suggestion. Furthermore, the built up of heterocyclic N-containing compounds might also imply a relative shortage of available carbohydrates during the microbial transformation (Follett, R. F. and Schimel, D. S., 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 ($p > 0.5$) 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).

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

In BD, the cumulated CO_2 efflux and the amounts of sterols were negatively correlated (Fig. 6). This supports the suggestion of (Heumann et al., 2011) and (Heumann et al., 2013) that sterols may have an inhibitory effect on microorganisms of the N cycle. Furthermore, (Negassa et al., 2011) reported a significant inhibition of the urease activity with increasing sterol proportions in agro-industrial byproducts. Since microbial activity can affect heterotrophic soil respiration (Ryan and Law, 2005), it is likely that increased amounts of sterols as they are typically found in biogas digestates (Leinweber, 2015, unpublished Py-FIMS data) delay the decomposition and, thus, may slow down soil respiration. However, since the amounts of sterols decreased significantly after tillage in DB (Table 2), the actual sterol contribution to reduced CO_2-efflux in BD relative to the other treatments cannot be ascertained by the present data set. In light of the contradicting observation of increased labile N after tillage in BD, inhibitory effects of sterols as reported in the above publications may be more pronounced in undisturbed soils.

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

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Table 1. Means and standard deviations of soil organic carbon (C), nitrogen (N), C/N ratio, hot-water extractable carbon (HWC) and nitrogen (HWN) and HWC/HWN ratio before and after tillage. Different letters in each column indicate significant differences ($p < 0.05$) in means. Additionally, significant changes within a treatment (BD, biogas digestate; MF, mineral fertiliser; CL, 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
	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}**
BD	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}**
	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}
MF	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}
	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}
CL	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}

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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 ($p < 0.1$) differences 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

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

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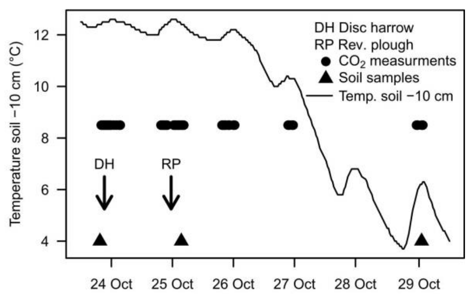

Figure 1. Timeline of the soil sampling and the CO_2 measurements in relation to the tillage events. Additionally, soil temperature in 10 cm depth is plotted, recorded every 30 minutes with an automated meteorological station (DALOS 535, F&C, Gülzow, Germany).

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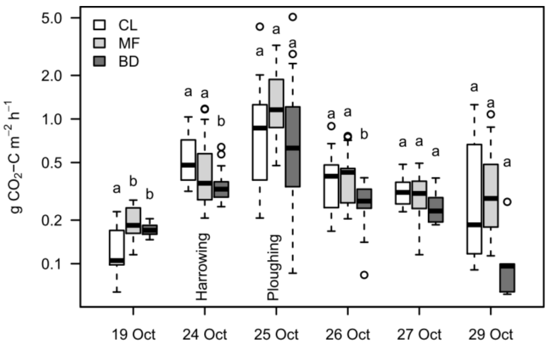

Figure 2. Soil CO_2 efflux around the days of tillage (harrowing up to 10 cm depth and ploughing up to 30 cm depth). Note that for the days of tillage (24 and 25 October) only the fluxes after tillage are included in order to get a better attribution of the tillage effect. Different letters indicate significant differences (p < 0.05) in mean fluxes of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) per each measurement day.

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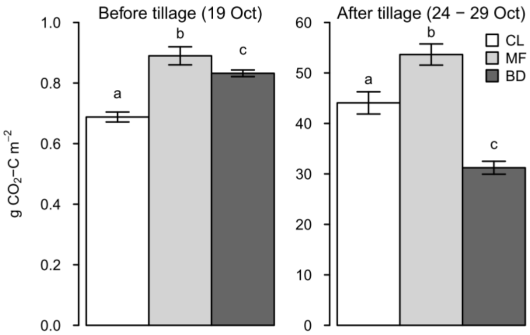

Figure 3. Cumulated soil CO_2 effluxes of a day before (19 October) and the period (24 – 29 October) after tillage. Different letters indicate significant differences in means of the cumulated fluxes of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before and after, respectively. Error bars represent the standard deviation of interpolation by bootstrapping after 250 iteration runs.

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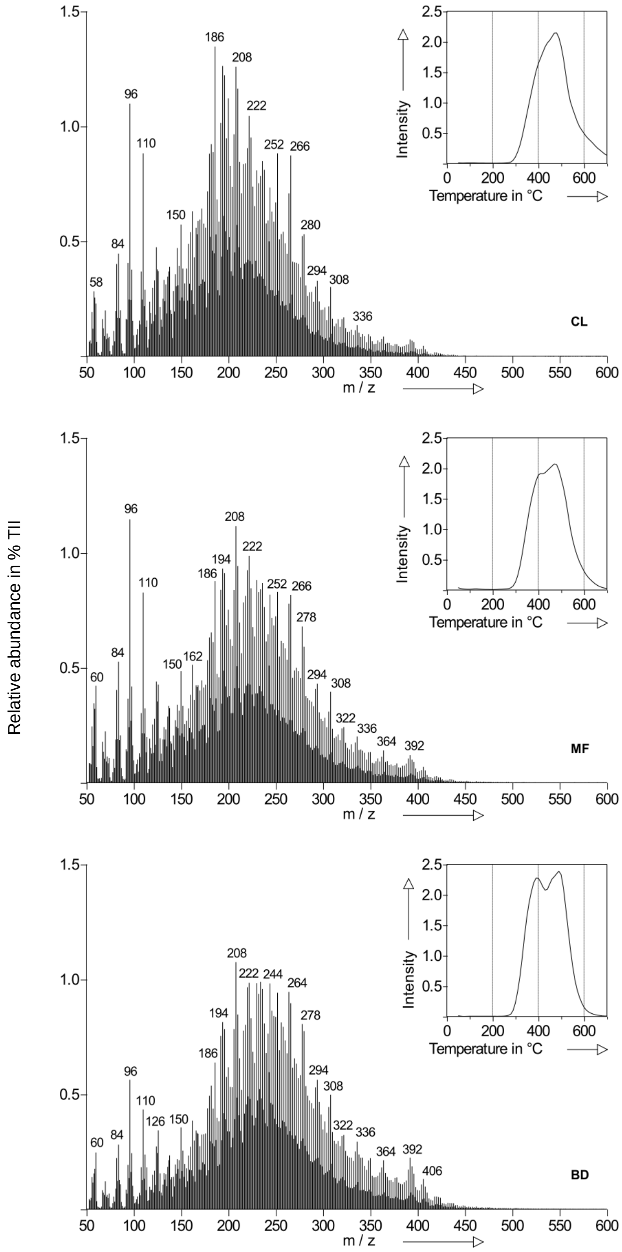

Figure 4. Thermograms of total ion intensity (TII, inserts upper right) and summed pyrolysis-field ionisation mass spectra of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before tillage.

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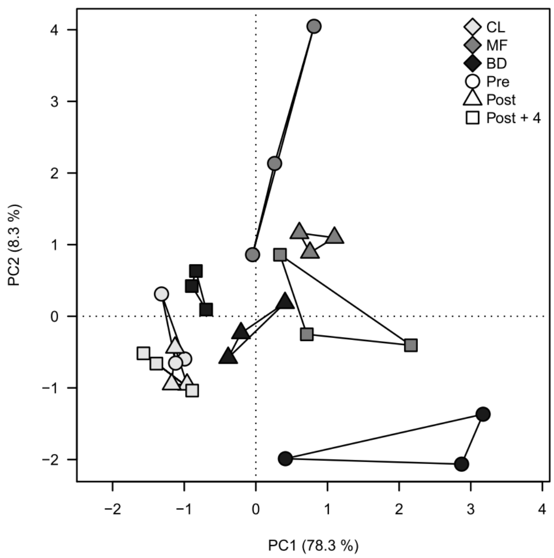

Figure 5. Principal component analysis of mass signals with significant differences according to Wilks' λ. from the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) of the different sampling times (pre-tillage, post-tillage and post-tillage + 4 days). Treatments and sampling times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

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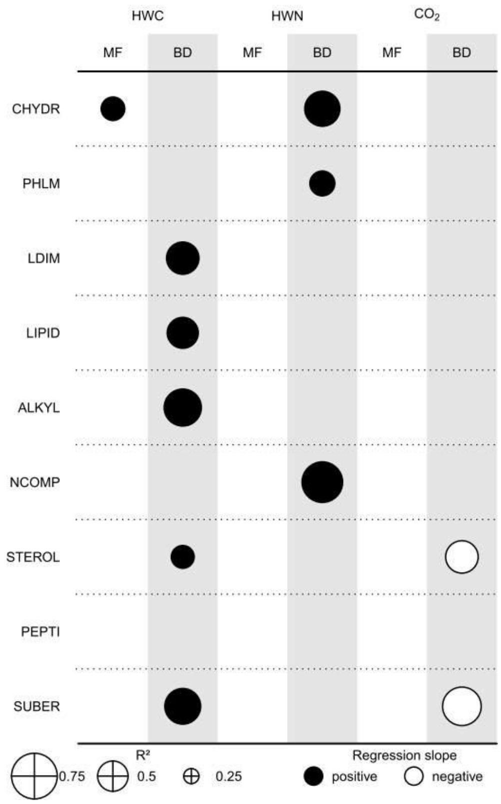
797 

798

799 Figure 6. Significant ($p < 0.05$) linear correlations between absolute signal counts of the
800 compound classes and hot-water extractable carbon (HWC), hot-water extractable nitrogen
801 (HWN) and soil respiration (CO_2), respectively, with the corresponding coefficients of
802 determination (R^2) and direction of regression slopes, derived from the three soil sampling
803 dates.