

Dear Prof. Steven Sleutel,

Thank you for your comments – they are much appreciated because they acknowledge our efforts. Given below, we will enter your suggestions.

Beside them, we additionally described the exclusion of an outlier from the hot-water data in L 235-237: ‘One of the replicates in MF exhibited exceptionally low HWC and HWN values. According to Dixon’s Q-test, these values were outlier (one-third and half, respectively, as high as for the other replicates in MF) and thus excluded from further analysis.’

One suggestion for the data-analysis: to maximally explore linkages between (shifts in) the  $m/z$  datasets by tillage and (shifts in) CO<sub>2</sub> efflux, advantage could have been taken from newer statistical techniques like partial least squares regression. This would have resulted in principal components that maximally explain variation in CO<sub>2</sub> efflux (the dependent) and not in a set of components aimed at describing variation in the  $m/z$  data.

We seized your suggestion and applied PLSR to discriminate explanatory mass signals with regard to CO<sub>2</sub>. For visualisation, we used a PCA on these obtained masses, again:

‘Partial least squares regression (PLSR) was used for discrimination (Barker and Rayens, 2003) to maximally explore linkages between shifts in the  $m/z$  data by tillage and shifts in CO<sub>2</sub> efflux. PLSR models were built by using 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, thus in their predictive performance. Based on the performance index suggested by Bauwe et al. (2015), the 500 “best” models were extracted and, finally, the mass signals which were utilised more than 50 times in the latter models were chosen.’ (220-230).

The outcomes are presented in the results and the discussion section:

‘The discrimination of relative mass signals with PLSR to explain cumulated CO<sub>2</sub> efflux revealed mainly functional groups from ketones and amides, peptides, carbohydrates as well as lignins and fatty acids (Table 3)’ (308-311)

Table 3. Results of iterative partial least square regression for cumulated CO<sub>2</sub> efflux as dependent variable and  $m/z$  data of all treatments and sampling times as explaining variables.

| <i>m/z</i>                       | Molecule/compound class  |
|----------------------------------|--|
| 17/18                            | Ammonium   |
| 31                               | $[M+H]^+$ of formaldehyde  |
| 34                               | H <sub>2</sub> S   |
| 43                               | C <sub>2</sub> H <sub>3</sub> O from ketones/amides and C <sub>3</sub> H <sub>7</sub> propyl                     |
| 46                               | Formic acid  |
| 55                               | C <sub>3</sub> H <sub>3</sub> O from ketones/amides  |
| 57                               | C <sub>3</sub> H <sub>5</sub> O from ketones/amides and C <sub>4</sub> H <sub>9</sub> butyl                      |
| 73                               | Propanamide  |
| 83                               | C <sub>5</sub> H <sub>9</sub> N from peptides  |
| 85                               | C <sub>4</sub> H <sub>5</sub> O <sub>2</sub> 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 <sub>19:1</sub> , C <sub>19:0</sub> , C <sub>22:2</sub> , C <sub>28:3</sub> , C <sub>28:0</sub> ) |

‘Accordingly, these two *m/z* [17 and 18] were also selected by the PLSR as explanatory signals for CO<sub>2</sub> efflux.’ (425 -426)

‘This suggestion is supported on the one hand by the effect of specific lignins on soil CO<sub>2</sub> efflux (Tab. 3) since CO<sub>2</sub> is an indicator for microbial decomposition activity (Kuzyakov, 2006).’ (442-444)

L 19-20 ‘Before tillage, BD showed much more volatilised matter (VM) during pyrolysis, indicating an increased amount of SOM.’ Not really mention worthy in an abstract

We agree because there were no significant differences in means.

42 suggest to replace by ‘readily biodegradable OM’ instead of ‘labile organic matter’, an ambiguous concept

We changed the respective phrase to 'readily biodegradable (hereinafter referred to as "labile") organic matter' (now L 41-42) and also replaced 'labile' in the abstract (24).

52 'which SOC constituents that form the majority of SOM are mineralized' reads strangely, please rephrase

Rephrased to 'Admittedly, SOC accounts for the majority of SOM, but these correlations do not causally explain which SOC constituents are mineralised.' (51-53)

56 better: 'long-term stability in soil is still under debate. . .'

changed accordingly (55-56)

2.1 please provide sand, silt & clay percentages

sand, silt and clay percentages added (97)

115 so is this then a mouldboard plough?

It's a special type of mouldboard plough, yes. We stated the respective phrase more precisely to 'reversible mouldboard plough' (122) & also in L 256

143 all between the ( ) appear to be too much detail

We think that it is important to enable readers to comprehend the formula directly; therefore we discarded only specific values and would like to keep at least the respective units (144-147).

161 'see 2.3', is written in 2.3, so where is this reference pointing at?

The reference is pointing at 2.2; changed accordingly (168).

The purpose of measuring HWC and HWN should be better motivated in the introduction/M&M, when readers are mainly convinced that the whole intent of the present study was to understand which OM building blocks are preferentially degraded by consequences of tillage soil disturbance.

The purpose of measuring HWC and HWN is now elucidated in the introduction after the outline about Py-FIMS: 'Hot-water extraction is a relatively simple method to release labile SOM and to estimate how much of soil C and N can be easily utilised by microorganisms (Leinweber et al., 1995). These labile pools have been suggested to be an important indicator of short-term changes in SOM quality due to soil management (Haynes, 2005). Furthermore, a significant proportion of hot water-extracted organic matter originates from microbial biomass. Thus, this approach is a potential

indicator for changes in microbial biomass or activity (Sparling et al., 1998), which may reflect sources of CO<sub>2</sub> efflux following tillage. ' (80-87)

178 The reference is here part of your sentence and should not be expressed as (Leinweber et al., 2013) Leinweber et al. (2013 Correct these mistakes in annotation throughout the text, several other examples follow later on in your text, e.g. 57 Möller et al. (2015), 309, 320. . .)

Corrected accordingly.

Table 1 Designation of significant differences by the superscript letters is not clear. Is this the outcome of an ANOVA on all 9 treatment/date combinations? Seems that way, but be clearer in the caption text

This is the outcome of Tukey's HSD, added to the affected caption texts.

Fig 3 Not clear what time intervals these cumulative CO<sub>2</sub> emissions represent. Should be indicated.

The intervals are now indicated: 'Cumulated soil CO<sub>2</sub> 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.) after tillage.'

254 Indicate here in the text also the direction of 'differences' in the abundance of m/z 55, 60, etc.

Direction of differences explained now: 'marker signals for carbohydrates and peptides (e.g., m/z 58, 60, 84, 69, 110, 126 and 162) were lower.' (275)

282 presumably the authors are mentioning increases in the TII proportion of marker peaks for carbohydrates?

We intended to mention changes in the proportions of compound classes – 'relative signal intensities' now corrected to 'relative proportions'. (303)

Fig 6 Why were correlations with the CL treatment's CO<sub>2</sub> efflux, HWC and HWN not displayed?

There were no significant correlations in treatment CL – a corresponding phrase is added to the caption, now.

308 'BD'

Corrected (333)

350-353 A strange explanation: in the BD plots you also expect labile 'physically protected C' not contained within digestate to be equally present as in the MF plots, next to of course labile C in the amended digestate. So lifting of bio-availability of previously 'entrapped' labile OM should equally

have occurred in the BD and MF treatments, equally contributing to a short-term CO<sub>2</sub> efflux. So I propose to reformulate this section. The microbial use efficiency theory which follows looks much more plausible. But then again, the HWC/HWN ratio was in fact much lower in all MF samples, and yet CO<sub>2</sub> efflux was higher. So in the end I think part 4.2 should end with an acknowledgement that the described mechanisms do not well explain why CO<sub>2</sub> efflux was lower after tillage in the BD when compared to the MF plots.

We found that ‘even a single application of organic amendment can increase aggregate stability (Grandy et al., 2002) and thus the resilience against disruption by tillage might be promoted, leading to a better physical protection of labile soil C not contained within digestates.’ (375-378)

&

‘But in fact, the HWC/HWN ratio of BD after tillage was not lower than that of MF, so, in conclusion, the above described mechanisms do not well explain why the CO<sub>2</sub> efflux was lower after tillage in BD when compared to MF.’ (393-395)

410 build-up

Corrected (448)

411 The link between trends of carbohydrates and heterocyclic N is not clear. This statement requires further explanation.

We added ‘since a reduced C availability during the microbial transformation of N is suggested to promote formation of heterocyclic N instead of N immobilisation’ (449-451).

401-409 Reads like a plausible explanation for the observed shifts in SOM biochemical composition. Can trends of individual *m/z* indicative for carbohydrates or peptides confirm that the short-term build-up of these substances is indeed through production of microbial biomass and metabolites?

We found an increase for muramic acid: ‘This suggestion is supported on the one hand by the effect of specific lignins on soil CO<sub>2</sub> efflux (Tab. 3) since CO<sub>2</sub> is an indicator for microbial decomposition activity (Kuznyakov, 2006). On the other hand, a relative increase of the signals for *m/z* 125, 167, 185 and 203 was observed in the BD treatment (data not shown) which are assigned to the bacterial cell wall products N-acetylmuramic acid and N-acetylmuramyl-L-alanyl-D-isoglutamine (Bahr and Schulten, 1983)’ (added L 442-448).

771 suggest ‘...and time of tillage operations...’

accepted (831)

780 indicate significance level

Implemented (834 & 842)