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Interactive comment on "Short-term effects of fertilization on dissolved organic matter (DOM) in soil leachate" *by* Alexandra Tiefenbacher et al.

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We would like to thank the anonymous reviewer for her/his critical comments, which will help to improve the quality of this manuscript. Our response follows the outline of the comments given by the reviewer.

General comments: Referee: In this manuscript, the results of a leaching experiment using undisturbed soil columns were presented. The authors aimed to reveal the effects of mineral and organic fertilization on amounts and composition of DOM in dependence on soil texture. There are many published results available in the literature showing quite contrasting results related to this topic. I do not see that the results of the present study increase our knowledge about effects of fertilization or soil texture on

DOM leaching. The use of only two different soils does not allow general conclusions.

Answer: Most studies evaluating the effects of fertilization on dissolved organic carbon (DOC) concentrations and dissolved organic matter (DOM) composition have been based on the analysis of water extractable soil organic matter (WEOM) in disturbed soil samples (De Troyer et al., 2011; Gao et al., 2016; Gonet and Debska, 2011; Lei et al., 2017; Seifert et al., 2016; Tye and Lapworth, 2016; Wang et al., 2016; Xu et al., 2018; Zhang et al., 2019). However, due to the destructive sampling, the WEOM method may overestimate the immobile fraction of organic matter, which is permanently or temporarily sorbed onto soil particles (Guigue et al., 2015). Deviations between the WEOM content and the actually leached DOC concentrations have been already demonstrated with zero tension lysimeters by Lu et al. (2013). In addition, the lack of a standardised method to acquire WEOM is challenging. "This is crucial since WEOM is affected by several factors, such as the composition of the extractant (deionised water or diluted salt solution), soil pre-treatment (fresh or air-dried), soil solution ratios, and the contact time (De Feudis et al., 2017)- direct quote L356- 358". We have addressed this issue in L 83- 88 and L355- 366 of our manuscript, respectively.

To overcome these problems, we decided to use undisturbed soil samples in a controlled scientific laboratory experiment with microlysimeters. The complexity of the experimental setup limited the number of possible soils/replicates. However, we are convinced that using undisturbed soil samples in a laboratory experiment provided a more realistic picture of the soil leaching processes, while the controlled conditions in the laboratory facilitated the understanding of the underlying mechanisms. Because of the limited number of soils that could be tested, we decided to focus on two soils differing in soil texture, as literature has shown that soil texture is a driving factor for leaching (Autio et al., 2016). We selected silt loam, because it is one of the most dominating textures in European agriculture (Ballabio et al., 2016), and compared this with a loamy sand because of its different soil physical properties.

A number of studies assessed the effects of fertilizer on organic matter cycling and organic carbon leaching either with mineral (Fröberg et al., 2013; Liang et al., 2016; Lu et al., 2013; McDowell et al., 2004; Sjöberg et al., 2003; Tian et al., 2017) or organic fertilizers (Adams et al., 2005; Lloyd et al., 2012; Long et al., 2015). However, only few studies compared the effects of both mineral and organic fertilizers on organic carbon leaching directly in the same experiment (Gonet and Debska, 2011; Manna et al., 2005; Xu et al., 2018). Our study demonstrates that organic and inorganic fertilizer show partly similar and partly deviating effects on the leaching due to the provision of either nutrients alone or nutrients plus organic matter. We consider this a crucial information for further soil leaching studies. Thus, we think that our study will contribute significantly to the mechanistic understanding of DOC leaching from undisturbed soils due to the four-factorial design of different soil textures and different fertilizer types, even though the number of tested soils is restricted. However, the comments of both reviewers have shown us that we need to stress the significance of our study and the knowledge gain for the scientific community more clearly in a revised version. The study can certainly not (and was never intended) to deliver generally (or widely) applicable results on DOC leaching rates or amounts due to the restricted number of specific tested soils, as the reviewer rightly point out. However, the 2x2 factorial design, with different soil types and different fertilizer types tested in undisturbed soils under controlled experimental lab conditions very well facilitates general conclusions about mechanisms behind the interaction of soil texture and fertilizer type, which provides valuable information for further research and is applicable to other scientific studies. Our results may encourage other colleagues to focus more on soil processes in undisturbed soils and on the interactive effects of the different drivers for soil leaching to improve our current knowledge and enable sustainable management of soils in agricultural landscapes.

Referee: In addition to the limited contribution of the study to increase our knowledge about dynamics of DOM in agricultural soils and their controls, the experimental approach is not convincing. First, the authors compared effects of fertilization in dependence on soil texture using two soils sampled at different seasons. One soil was samples in early spring whereas the other soil was sampled in fall. Taking into account

the strong seasonality of DOM dynamics in soils (amounts and composition) it is very difficult to compare the results of the two soils.

Answer: While acknowledging the concerns of the reviewer about the restricted number of soils and the different sampling times, we do not agree to the argument of “limited contribution of the study to increase our knowledge” nor can we agree that “the experimental approach is not convincing”. Certainly, any scientific experiment would gain in significance if the number of treatments, replicates, or influencing factors is increased. However, as stated above, not many studies have so far looked into the interactive effects of soil texture and fertilizer type in undisturbed soils under controlled lab conditions; thus, we regard our study as a valid contribution to the general knowledge of fertilization effects on soil leaching of organic carbon quantity and quality. Regarding the comments about the seasonality, we agree that the different sampling times may have an influence on the results. Due to the comprehensive experimental set-up (and sampling schedule), we had to perform the experiments with the two soils separately in consecutive order. This has unavoidably led to some seasonal differences between the samplings, e.g. with regard to microbial activities. This is why we compared the fertilizer treatments with the untreated control of the same soil, but not primarily across soils. However, we tried to minimize eventual seasonal effects by several means: In order to reduce the effects of in-field conditions, the soil cores were acclimated under laboratory conditions 7 days before the start of the experiment. The treatments were compared with a control to account for differences in seasons or soil properties. In addition, we tried to facilitate maximum comparable management preconditions of the soils sampled, a factor which may cause a much higher variability than seasonal effects mentioned by the reviewer. Both soils had been treated with mineral fertilizer (NAC 27- calcium ammonium nitrate) latest 5 months before the sampling. In both cases, crops had been removed between 1 to 3 months prior to the experiment (one spring harvest, one autumn harvest), the soils had been chiselled, and the seedbeds had been prepared exactly 3 days before the sampling. Regarding the soil types, we selected two soils common for this region (the silt loam is also one of the most dominating textures in European agriculture (Ballabio et al., 2016) with clearly different soil physical properties.

Referee: Second, the authors did not give any information about the used pig slurry, e.g. DOC concentration, DOM composition. Therefore, it is impossible to assess effects of organic fertilization if amounts and composition of added organic matter is not known. In addition, the studied two soils differ in pH (1 unit). A higher pH should be related to a higher solubility and could superimpose assumed effects of fertilization and soil texture.

Answer: We would like to thank the reviewer for her/ his excellent idea. In a revised version of this manuscript we will include DOC concentration and composition as well as nutrient concentration of the used organic fertilizer, pig slurry. Indeed, soil pH affects the solubility and transportation of DOM through the soil as also other soil properties, such as texture or soil chemistry, do in nature. Our study design is intended to reflect the real situation in the field as best as possible (for this region), including the natural differences in the properties of the tested soils. We consider the inclusion of this variability an important factor in the design to elucidate general mechanisms as were found in our study, such as e.g. a potentially enhanced OM mineralization through nutrients or the interaction of DOC transport vs internal OC processing. However, we will explicitly address the influence of pH in the discussion in a revised version as suggested by the reviewer.

Proposed adaption: As suggested by the reviewer, we will add chemical characteristics of the used organic manure. Additionally, we will explicitly emphasize the impact of soil pH in the discussion in the revised version of the manuscript.

Detailed comments

Referee: Line 25: The authors did not determine molecular size – the methods they used might indicate some changes occurring during the experiment.

Answer: Information about molecular sizes were obtained via absorbance spectra and the most commonly used indices SUVA and E2:E3 (see L191-192). While this certainly does not yield the information gained by Fourier transform ion cyclone resonance mass spectrometry (FT−ICR MS; Li et al., 2019), it nevertheless is an established surrogate parameter for molecular size, which is widely used in the literature (e.g. (Avneri-Katz et al., 2017; Awad et al., 2015; D'Andrilli et al., 2019; Fleck et al., 2014; Frey et al., 2016; Graeber et al., 2015; Heinz et al., 2015; Inamdar et al., 2012; Li and Hur, 2017; Olshansky et al., 2018; Singh et al., 2018; Weishaar et al., 2003; Xian et al., 2018; Xu and Guo, 2017). In fact, our study actually showed a change in SUVA and E2:E3 during the experiments, indicating changes in the molecule sizes (e.g. L296-298; L309-311).

Referee: Line 64 :The term stabilization is used in two different aspects; (i) solubility / desorption and(ii) decreasing microbial decay

Answer: Original: "Stabilization of DOM against leaching depends on the chemical nature and physical properties of sites available to interact with the ions and molecules present in soil solution (Baldock and Skjemstad, 2000; Silveira, 2005)." Proposed modification We agree that this term is ambiguous, and we will change it in a revised version as follows: "The retention of DOM against leaching depends on the chemical nature and physical properties of sites available to interact with the ions and molecules present in soil solution (Baldock and Skjemstad, 2000; Silveira, 2005)."

Referee: Line 93: Justification of the hypothesis is needed.

Answer: Original: "Pore water of coarser textured loamy sand will exhibit higher DOC concentrations than finer textured silt loam soil." Proposed modification: Since soil texture is a strong predictor of DOC leaching (Autio et al., 2016), we assume that the coarser textured loamy sand soil is more prone to leaching than the finer textured silty loam soil, resulting in a rapid increase of DOC in the leachate. Due to shorter retention times and, thus, probably lower microbial decomposition, we expect that the leached DOM of the loamy sand soil will consist of a higher share of humic compounds.

Referee: Line 109: The combination of a loamy sand, 0.8% of organic C and a Haplic Chernozem is not a very common one.

Answer: We agree. However, the second soil was chosen because of its contrasting soil physical properties to the silt loam and because we had detailed information about the agricultural preconditions there, which were highly comparable to the second sampling site. The silt loam was selected, because it is one of the most dominating textures in European agriculture (Ballabio et al., 2016). In a revised version of the manuscript, we will reassess the soil type to avoid misunderstandings.

Referee: Line 185: The authors did not describe any correction of the fluorescence spectra, e.g. for inner filter effects.

Answer: We agree that this information needs to be added to a revised version, as it is crucial for assessing the quality of our data. Actually, extensive pre-processing of the DOM dataset was done with the "staRdom" package built for R (Pucher et al., 2019). Pre-processing of the dataset included: blank subtraction, inner-filter effect correction and the removal and interpolation of Rayleigh and Raman scattering of first and second order. In addition, the outcomings of the PARAFAC- analysis were evaluated with a split-half analysis and the obtained model was evaluated using correlations between the component (Murphy et al., 2013).

Referee: In their figures, the authors mixed up soil type and soil texture. Answer: Proposed Modification: We will exclude the term "soil type".

Referee: Although the topic of this study might be of high interest for the readership of SOIL, I cannot recommend publishing because of the indicated weaknesses and the limited new knowledge they provided. The authors might think about the main story they want to tell. Then they might consider re-writing the manuscript as a short communication taking the limited character of their study into account.

Answer: We respectfully disagree with the reviewer on this comment for the reasons

detailed above. To summarize: Our extensive literature research showed that fertilizer-induced changes in DOM composition and DOC leaching are still poorly explained. The data available on leaching of organic carbon in undisturbed soil columns under controlled conditions is sparse and to the best of our knowledge, there is no study investigating the immediate response of DOM leaching to organic and mineral fertilization using undisturbed soil cores. Thus, the results of this study will increase our knowledge on the behaviour and characteristics of organic carbon movement in soils.

We may add, that the experiment has been carried out to comply with scientific requirements: we performed extensive PARAFAC modelling and statistical analysis to evaluate fertilizer- induced changes in DOM composition of the soil leachate throughout the whole experiment. The precision and accuracy of the measurements were monitored continuously with four replicates per treatment. The overall, maximum allowed relative standard deviation between replicates was set to 5%. In addition, changes in DOM composition were compared with non-fertilized soil cores constantly, to avoid misinterpretation of the measurements.

Nevertheless, we are prepared to modify our manuscript in response to this comment. We will reorganize the manuscript to enhance the clarity and the focus of our work towards the effects of fertilization. We will also display half-split analysis and the outcomings of the PARAFAC in figures. We thank the reviewer for his/her comments, which will certainly help to strengthen this manuscript.

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