Dear referee. Thank you for your comments. We carefully considered all suggestions. Explanations how we modified the manuscript are given in red below.

My main comments are that the authors spend most of the discussion on refuting other explanations for these effects (which is good), but they do not spend much time discussing why these effects are relevant and in what instances. I think the discussion of the natural test case of soils needs to be better discussed and "rounded out" to include how these findings may be used in practice (i.e. What is the use of this effect in casein or flour?). In the abstract, "major implications" are claimed, but they are not ever really discussed. For example, could this effect explain observations of isotopic differences between mobile and immobile water in soils (i.e. Brooks et al., 2010, NatureGeosci, DOI: 10.1038/NGEO722)?

We reorganized the discussion part and added more relevance in this part and discussed under which condition the findings can be used. We also compared the results of Brooks et al. (2010) with our results.

The figures could be improved by denoting each by a specific identifier (e.g. "A" or "B" etc). Figure 6 is relatively unclear to me, and could be improved by a more informative caption.

We denoted each figure by letters according to your suggestion and added the letter to the corresponding caption. The caption in Fig. 6 was considerably simplified (about 50% shorter).

The use of "enrichment" to describe the isotope effect throughout is problematic. Enrichment is used when the isotope effect is both positive and negative.

I suggest a more neutral term that is also more descriptive, such as "positive (or negative) isotope effect" to describe the direction of change.

Now we use "isotopic fractionation" throughout the manuscript according to the recommendation of Coplen (2011). In order to follow strictly these recommendations we also changed ε_S and ε_a to $\varepsilon_{S/U}$, $\varepsilon_{T/U}$ and ¹⁸O and ²H were modified to ^{18/16}O and ^{2/1}H.

Coplen, T. B.: Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results, Rapid Commun. Mass Spectrom., 25, 2538-2560, doi: 10.1002/rcm.5129, 2011.

Specific comments by line number:

1. Needs a more specific, or informative title.

We changed the title:

"Isotopic fractionation between unconfined water and water adsorbed in equilibrium to organic matter of biological materials and soils" to

"²H and ¹⁸O depletion of water close to organic surfaces"

13. Avoid the term enrichment. The d values of the water either got higher or lower, or remain unchanged.We use 'isotopic fractionation' throughout now (see comment above)

14. Define significant

We added *p* values in many cases and additionally wrote in the M&M section "Significance, even if not explicitly stated, always refers to p < 0.05".

26. modify "discrimination" to something more descriptive and specific to this paper. We modified it to isotopic fractionation.

31. "Such fractionation can be affected by ion hydration." Needs revising and a reference to support it.We added a reference and improve the sentence. It now reads:"The vapor/liquid fractionation is not only affected by temperature but also by ion hydration (Kakiuchi, 2007)."

36. "Additionally, adsorption, may cause an energetic difference between water molecules at the surface of solids and the bulk water molecules." – Needs a reference
We refer to Richard et al. (2007) now.

Richard et al. 2007 Experimental study of D/H isotopic fractionation factor of water adsorbed on porous silica tubes, Geochim. Acta, 71.

51. Please define "silage".

We defined silage here. Now it reads:

Silage, the product after anaerobic fermentation of fresh forage, is likely the most important feedstuff in highproductivity ruminant husbandry, which also delivers water to the animal and thus influences the body water composition.

54. Please define "casein powder".

54. What kind (grain type, i.e. wheat?) of flour?

69 - 70. Please briefly define "fibric" and "hemic" rather than sending the reader somewhere else to find a definition that is important to understanding this paper.

Now we explain better the properties of the substances and the rationale behind their selection. In order to make the rationale better visible, we have combined the information, which previously was distributed in the Introduction and in Materials and Methods in one paragraph in M&M. This paragraph reads:

"The materials comprised fresh silage, oven dried silage, washed silage, hay, fibric and hemic litter, filter paper, cotton, casein and wheat flour. Silage was oven-dried to remove all volatiles and it was washed to remove all solutes. Fibric litter is slightly decomposed organic material on top of the mineral soil derived from plant litter, thus more decomposed than silage but partly still resembling the structure of plant organs. Hemic litter is strongly decomposed organic material of low fiber content, which has lost the structure of the plant litter but which contains dark brown soluble substances that dye the water extract (Schoeneberger et al., 2012). More pure materials were included to

identify whether the chemical identity causes or influences the effect. We used filter paper and cotton to represent pure cellulose, the most common plant material, commercial wheat flour to represent less pure carbohydrates including branched carbohydrates and casein powder to represent proteins."

Further, we provide the brand name of the casein powder in the following paragraph.

78. Why were these materials "slightly dried" before the experiment?

We now explain the reason like this in manuscript:

"Both materials were then slightly oven dried for different times (ranging from 0 to 60 min) at 50°C before the equilibration experiment to achieve a water content comparable to that of fresh silage and to create a water content gradient."

79 -80. How do you know these constituent amounts? Did you do an analysis for this?

We got the information from the product instruction. Now it reads:

"According to the product information, the casein powder (My Supps GmbH, Germany) contained 90 % natural casein and a small amount of carbohydrates while the commercial wheat flour contained 70.9 % carbohydrates, most of which was starch."

80. Why was casein and flour only dried at 50C for 6 hr rather than at 100C and overnight?

The drying was not a necessary step for these materials. The information was mistakenly introduced here. It was deleted now to avoid confusion.

99 – 100. Was there condensation inside the pump? How do you know?

To be honest, we did not explicitly record whether there was any condensation inside the pump. However, in our experiment we only focused on the final equilibrium after 100 h of exposure, which means that even if there was condensation of water in pump, it will not influence the final equilibrium between the vapor in chamber and dish water (or material water). We improved our explanation:

"A preliminary experiment with silage showed no significant isotope difference (p > 0.05 for both H and O) in silage water between 60 and 100 h of equilibration, which implied that 100 h of equilibration were sufficient to achieve equilibrium conditions. Equilibrium conditions also imply that even if there had been condensation within the atmosphere-circulation system, it will not influence the isotope relation between dish water and material water because the condensate will also be equilibrated."

104. Was there any evaporative enrichment on the water dish after 100 hr of evaporation?

The maximum evaporation is given by the volume of our vessel, temperature and atmospheric pressure. For ambient temperature and atmospheric pressure, the air within the vessel contains about 0.6 g of water. Hence total evaporation was less than 0.3 % of the liquid water within the vessel. We did not examine whether there was any enrichment due to this small evaporative loss because the unconfined water changed its isotopic composition anyway due to the equilibration with the sample water. Measurement errors (e.g. the amount of water added as unconfined

water and as sample moisture; isotopic composition of initial and final unconfined water, isotopic composition of sample moisture) were much larger than the expected change due to evaporation. Even this evaporation is irrelevant because we measured the isotopic composition of the unconfined water after the experiment, which included any change due to evaporation that happened after closure of the vessel (100% humidity is reached within 20 min). We modified our description:

"During equilibration the unconfined water underwent changes due to the increase of humidity within the chamber (less than 0.3 % of the total water within the chamber) and exchange with the varying amount of sample water (up to 10 % of the total water). To determine its isotopic composition when in equilibrium with the sample water, we sampled 1 mL unconfined water at the end of equilibration, and also subjected it cryogenic vacuum distillation before measurement."

136. What is OLS?

We deleted the unnecessary abbreviation and write "ordinary least squares" now.

163. "MIBA Protocol" doesn't mean anything to me. Again, please briefly describe your methods and definitions without making the reader go look somewhere else to understand what you did.

We deleted the MIBA protocol and just describe the sampling protocol because the IAEA has deleted this program from their homepage.

168 "Further, the winter data, effects of soil evaporation from the vegetation covered soil, can be excluded." Please justify and explain why you make this assumption.

We apologize. This sentence was a stub. We modified the entire paragraph. See below.

171-172. "and sand grains usually are coated by clay, sequioxides, organic matter and biofilms and do not directly interact with water" This seems problematic: If the sand grains are coated with a fractionating substance, how can you neglect them in your analysis? Please address this.

We modified the entire paragraph:

"The data were used (i) to examine if there was an offset between soil water and rain water and (ii) whether the offset can be corrected by accounting for the solid:water ratio according to our model. In order to exclude that the offset is caused by soil evaporation, we only use winter season data. During the winter season, evaporation demand was low (average actual evaporation 0.5 mm/d while average precipitation was 1.9 mm/d; German Weather Service, 2016) and evaporation demand should be entirely met by transpiration and intercepted water due to the complete grass cover. Growing season data are only shown for comparison. We had developed the relation between the volumetric solid:water ratio and the isotopic offset only for organic materials. These materials differed from the soil in so far as they did not contain minerals. Especially for sand it can be expected that it practically does not absorb water due to its small surface area. Hence, we considered the sand to be inert and did not consider it in the volumetric solid:water ratio, which in consequence was calculated from (volume of dry soil excluding sand) / soil

moisture volume. The volume of dry soil excluding sand was calculated by dividing its dry weight by particle density of the organic and mineral components (1.5 g/cm³ and 2.65 g/cm³, respectively; Chesworth, 2008)."

184. Again, define "significantly". We defined it as p < 0.05.

200-201. "The water content of oven dried silage did not reach again the same water content as fresh silage but was significantly lower ($81 \% \pm 13 \%$)." This seems important, please address this result.

We explained the possible reasons and added a reference in the discussion part:

"The water content of oven dried silage (81 % \pm 13 %) did not reach again the same water content as fresh silage (128 % \pm 10 %) but was significantly lower, which may be because oven drying changes the surface roughness and other structural properties of silage (Tabibi and Hollenbeck, 1984)."

216-222. Please expand this to include exactly how you calculated the Raleigh fractionation line in Fig. 5.

We explained the Rayleigh fractionation in the M&M section and gave a reference there:

"In order to exclude that incomplete extraction caused isotopic fractionation, we compared the observed isotopic fractionation with predictions based on Rayleigh equation (Araguás-Araguás et al., 1995):

 $\varepsilon_{\rm E/T} = \left(F^{1/\alpha} - F\right) / (F - 1) \tag{7}$

Where $\varepsilon_{E/T}$ is the predicted isotopic fractionation between the incompletely extracted water (subscript E) and total water (T). *F* stands for fraction of water remaining in the material after the extraction and α stands for isotope fractionation factor (1.0059 and 1.0366 for H and O at 80 °C extraction temperature, respectively)."

220-222. "Additionally, an unrealistically small fraction of water would have to be extracted (far below 0.8) to cause the same enrichment of 2 H as observed for most of the samples." Please explain this more.

We modified the text:

"Additionally, the average $^{2/1}$ H fractionation of the materials was -20.6 ‰. This net fractionation could be expected for a Rayleigh process if only 80 % of the water would have been extracted while 20 % remained in the sample. This, however, was not the case because subsequent oven-drying did not cause further weight loss."

227: How where the evaporation numbers calculated?

This information was now moved to the M&M section where we provide a reference for the data.

284-289. I do not understand how this explanation rules out exchange of hydrogen and oxygen exchange.

We modified this part to make it clearer:

"Hydrogen bound to oxygen and nitrogen in many organic materials like bitumen, cellulose, chitin, collagen, keratin or wood may exchange isotopically with ambient water hydrogen (Bowen et al., 2005; Schimmelmann, 1991). At room temperature, this isotopic exchange occurs rapidly in water and an exchange with vapor is even several orders of magnitude faster (Bowen et al., 2005; Schimmelmann et al., 1993). Such an exchange would influence the adsorbed water but it would also influence the unconfined water, which is in equilibrium with the adsorbed water but it could not influence the fractionation between both. The same would apply for an exchange between carbonate oxygen and water oxygen (Savin and Hsieh, 1998; Zeebe, 2009) but our samples did not contain any carbonate."

301. Again, you need to explain terms that you invoke, i.e. what is "energy delocalization phenomenon"?

We explain the terms now:

This was taken from the references (as we had indicated). Our original sentence was: "This is referred to as the "paradoxical effect" and is tentatively interpreted in terms of an energy delocalization phenomenon (Drost-Hansen, 1978)."

We changed this to become better comprehensible to:

"This is referred to as the "paradoxical effect", which describes that – independent of the nature of the surface – water close to a solid interface is characterized by long-range ordering including high-pressure ice polymorphs of low energy (Drost-Hansen, 1978)."

305-311. Oerter et al., 2014 (J. Hydrology) discussed possible reasons for these effects near clay surfaces in some detail. That work should be referenced here.

We added the possible reasons proposed by Oerter et al. (2014) here:

"Oerter et al. (2014) investigated water adsorbed to clay and also found isotopic fractionation. They explained this by the negatively charged clay surface, which increases the ionic strength in the solution close to the clay surface. Ions are known to cause fraction in their hydration sphere (Kakiuchi, 2007; Stewart and Friedman, 1975). This mechanism could also be active in our samples although the surface charge of most of our samples (e.g., cellulose) is much smaller than surface charge of clays, and washing, which should have removed most of the solutes, did not remove the fractionation."

316. What is the volume ratio of the inner perturbed water to the total bulk water? I assume small, so why would it show up in bulk water extractions?

We could only speculate about this because our measurements do not allow quantifying this. Hence we did not add anything to our manuscript. However, the effect could still be large even if little water is affected in the case when fractionation is large. Furthermore, the surface itself can be large. For clay minerals, for which the surface is better defined and better known, the surface is often in the range of 500 m²/g. For a rather high solid:water ratio of 1:1 this would mean that 1 mL of water is spread on 500 m², which could cause a large effect even when fractionation is small. It is thus not unlikely that the perturbed water contributes a significant share to the total water. We write it now:

"We could not estimate the thickness of inner layer for our experimental materials. The high-pressure ice polymorphs near surfaces may be one tenth of a micrometer in thickness (Drost-Hansen, 1978) but other effects at the surface-water interface like effects on solute composition extend to a scale of tens of micrometers and in extreme cases up to 0.25 millimeters (Zheng and Pollack, 2003)."

323-331. In the abstract you claim "major implications" for these results but you do not really discuss any. What are some of the bigger implications of these results?

We added more implications involved in many fields in discussion part: We added comparisons with the results in other studies (such as Oerter, Brooks) and discussed the possible reason for the fractionation between source water and xylem water in halophytic species. We also added more implications in the application part (such as the measurement of exchangeable hydrogen):

"Another example is the determination of fraction of exchangeable hydrogen in organic tissues, which is needed to trace the origin of animals (such as the protein in hair, Bowen et al., 2005). This is usually determined by exposing the tissue to vapor in equilibrium with either heavy or light water similar to our experiments. The surface effect may thus also play a role for the exchangeable hydrogen."