Dear Editor:
Below are our point-by-point responses to Ref. #1 (page 1 – 6) and to Ref. #2 (page 7 – 9) and the tracked manuscript showing all our changes (page 10 – end).

Karl Auerswald

Referee #1
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 $\varepsilon_S$ and $\varepsilon_a$ to $\varepsilon_{S/U}$, $\varepsilon_{T/U}$ and $^{18}$O and $^2$H were modified to $^{18/16}$O and $^{2/1}$H.


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

“$^2$H and $^{18}$O 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.


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 high-productivity 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/cm$^3$ and 2.65 g/cm$^3$, 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 % ± 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 % ± 13 %) did not reach again the same water content as fresh silage (128 % ± 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):

$$\varepsilon_{E/T} = \frac{(F^{18}_E - F)}{F - 1} \quad (7)$$
Where $\alpha_{E/T}$ 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 $\alpha$ 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 $^2$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.”
Dear referee. Thank you for your comments. We carefully considered all suggestions. Explanations how we modified the manuscript are given in red below.

In this work, Chen et al. present an experimental study aimed to quantify isotopic fractionation in water adsorbed to organic matter. The work is timely, particularly considering the increasing interest on understanding isotopic fractionation of water within the soil (see e.g. Tang Feng 2001; Brooks et al. 2010). In previous studies, the role of mineral adsorption and hydration have been explored. However, till now a study on the effect of water adsorption by organic matter was lacking, despite this may also play a significant role in soil processes. In this regard, the present work provides robust experimental evidence of a process with strong implications in different fields, from surface hydrology to the study of plant water uptake.

Specific comments

Experiments

The methodology of the experiments is generally well described. My main concern is about the calculation of the solid:water ratio. Is it the apparent volume, as typically done for soils? Then, to what extent the porosity of the material could affect the results? For example, depending on how we handle the medical cotton, we can easily modify its volume. Would this change in “solid volume” affect the relationship in the same way as a change in hydration?

The calculation of solid: water ratio is based on the solid volume, which is not the apparent volume. The porosity of the material will not affect this ratio. To make it clearer to the readers, we emphasize that the volume calculation has nothing to do with bulk volume:

“The solid volume (exclusive voids) can be calculated by knowing the weight and the particle density of the organic matters (casein: 1.43 g/cm$^3$ (Paul and Raj, 1997); silage, hay, litter, filter paper, cotton and flour: 1.5 g/cm$^3$ (Yoshida, et al., 2006)).”

The experiments describe the effect of water-vapour adsorption, but I wonder whether these results could be extrapolable to adsorption processes in the liquid phase, e.g. along the apoplastic water transport in plants. If this were the case, adsorption might explain the fractionation of water between source and xylem water that has been described for some xerophytic and halophytic species (see e.g. Ellsworth Williams 2007). A definitive mechanistic explanation for this process is still lacking and, interestingly, the effect is significant for hydrogen, but not for oxygen. Although right now rather speculative, this topic might deserve consideration for future work.

We added the discussion about this:

“The surface effect may also play a role in the fractionation between source water and xylem water that has been described for some xerophytic and halophytic species (e.g. Ellsworth and Williams 2007) for which an explanation is presently missing.”

Application case

This section is particularly relevant, as a first attempt to validate the findings of the laboratory experiments. However, the way the data is presented could be improved, and the results deserve more attention in the discussion section.

Firstly, I would expand the methods section, describing the sampling protocol and, in particular, stating clearly that these values correspond to distilled water from the original soil sampling (i.e. nothing to do with the equilibration experiments).

We expand the methods section and showed our aims of the soil sampling in grassland:

“Soil at 7 cm and 20 cm depths and rain water were sampled at the grassland in Grünschwaige Experimental Station, Germany (48°23’N, 11°50’E, pasture #8 in Schnyder et al. (2006); 8.3 % organic matter, 30 % clay, 22 % sand) at biweekly intervals during the growing season (April to November) from 2006 to 2012 and at weekly intervals during the winter season.
(October to February) in 2015/2016. Soil sampling was always carried out on dry days at midday (between 11 a.m. and 16 p.m.). Two replicates of soil samples were collected on each sampling date. 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.”

The authors mention that they did not consider sand for their volume calculations, however, as for the rest of materials, they did not explain how they actually determined the ratio solid:water volume. Is it the apparent volume (i.e. including pores), or an estimate of the solid volume?. In the second case, how was this calculated/estimated? The methodology is likely to be based on standard techniques in soil science, but it is worth to mention them explicitly, particularly to help other researchers to validate the models with their own field data.

The volume of solids, in general, was calculated excluding pores. The volumetric solid:water ratio of soil was estimated by the weight and solid density of the organic matter and minerals without sand. We added the calculation of volumetric solid:water:

“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/cm$^3$ and 2.65 g/cm$^3$, respectively; Chesworth, 2008).”

On the other hand, the discussion of the application case could be expanded by considering whether the observed relationship can be used to correct field data, and under which conditions. For example, it is worth to discuss why the upper soil seems to fit better with the solid:water ratio than the 20 cm layer. Potentially, this could be related to differences in organic matter content: was this actually the case?

There was a small difference in organic matter content between both depths (the soil was arable about 10 yr before sampling) but we do not expand on this because we do not use this information.

We modified the results part:

“The deviation between the winter season data and the local meteoric water line correlated significantly ($p < 0.001$) with the solid:water ratio for 7 cm depth but not for 20 cm depth, which varied less in water content. For both depths, the data moved closer to the local meteoric water line when the influence of confined water was removed by applying the general regression with solid:water ratio from Fig. 2 (Fig. 6b). The mean deviation for $^{2}H$ changed from -8.1 ‰ to 1.0 ‰ for both depths due to this correction.”

On the other hand, the authors apparently pooled together organic matter with clay as "porous" material in their calculations. However, if clay and organic matter do not behave in the same way, this might explain the differences between upper and lower soil layers. Since the soils tested have about 30% of clay, I would try to compare these results with the expected effects of clay minerals, e.g. as in Meissner et al. (2013).

Meissner et al. used a different experimental setup, in which they did not equilibrate sample water with unconfined water but they compared extracted water with the water added. With this approach they measured the influence of exchange on the
isotopic composition of the extracted water. This is a different process than in our case. Hence we did not compare with the results by Meißner et al. (2013). But we compared our results with the results by Oerter et al. (2014):

“In our experiments we had only examined organic materials while the soil in our application case also contained minerals. Given the “paradoxical effect” (Drost-Hansen, 1978) and that we had not found any effect of the nature of the organic materials on the surface effect, the simplest assumption was that there is also no large difference between organic and mineral surfaces regarding the isotope effect. This seemed reasonable because pure clay with 30 % water content (equivalent to 0.8 solid:water content) as used by Oerter et al. (2014) created -0.4‰ oxygen isotopic fractionation on average. This was close to the predicted apparent isotopic fractionation (-0.7‰) for the same solid:water ratio for organic materials. Oerter et al. (2014), however, also manipulated the composition of the solutes, which are known to affect fractionation and which do not allow direct comparison.”

Technical corrections

The experiments were designed to test the effect of water-vapour capillary absorption. Adding “vapour” (...water vapour adsorbed...) to the title may help the reader to quickly understand the experimental setup. Actually “vapour adsorption” is not appropriate because most of our materials contained quite a lot of liquid water. We only used a saturated atmosphere for the exchange between unconfined water and material water in order to be able to measure both separately. To improve the title, we changed it:

“²H and ¹⁸O depletion of water close to organic surfaces”.

Section 2.8 Modelling, and Figures 2–4. In the modelling section, the authors included in the equations the water:solid ratios, whereas in the figures the ratio solid:water is used. I guess that for most readers the water:solid ratio would be more intuitive, so I would suggest to use it also in the figures.

Yes, there were inconsistencies between the figures and equation in terms of solid:water ratio. It should always read “solid:water ratio”.

We agree that the water:solid ratio is much more common but our prediction was that εₐ should be related linearly to the volumetric solid:water ratio for the total adsorbed water. A linear equation is easier to fit (e.g., by linear regression) and it would be difficult for the reader to see whether the data really follow an inverse relation and not a deviating curvilinear relationship while it is rather easy to judge a linear relationship.

Similarly I would replace the term "enrichment" by "offset" or "deviation", which is neutral.

We replaced the term “enrichment” by “isotopic fractionation” throughout the manuscript according to your suggestion; we follow strictly Coplen (2011) now.


The way the data is presented in Fig.6 is somewhat inconsistent: whereas individual winter values are represented as symbols, the rest of data is presented indirectly with the fitted regression line. I would suggest to present all the data in the same form, ideally as individual dots with their corresponding regression line.

We made it more consistent: all the values were shown as markers in this figure. However, the summer values (which previously were shown only as lines) are small markers because our arguments are based on the winter data and the summer data just shown for comparison. And we do not present all regressions in the figure (they would be hard to distinguish because they overlap).
Isotopic offset between unconfined water and water adsorbed to organic matter in equilibrium $^2$H and $^{18}$O depletion of water close to organic surfaces

Guo Chen, Karl Auerswald, Hans Schnyder

Lehrstuhl für Grünlandlehre, Technische Universität München, Alte Akademie 12, Freising-Weihenstephan 85354, Germany.

Correspondence to: Karl Auerswald (auerswald@wzw.tum.de)

Abstract. Hydrophilic surfaces influence the structure of water close to them and may thus affect the isotope composition of water. Such an effect should be relevant and detectable for materials with large surface areas and low water contents. The relationship between the volumetric solid:water ratio and the isotopic fractionation enrichment of heavy isotopes in between adsorbed water compared with unconfined water was investigated for the materials silage, hay, organic soil (litter), filter paper, cotton, casein and flour. Each of these materials was equilibrated via the gas phase with unconfined water of known isotopic composition to quantify the isotopic difference between adsorbed water and unconfined water. Across all materials, enrichment-isotopic fractionation of the adsorbed water was significant (Confidence interval at 95 % level did not include 0 $p < 0.05$) and negative (on average $-0.91 \pm 0.22$ ‰ for $^{16}$O and $-20.6 \pm 2.4$ ‰ for $^2$H at an average solid:water ratio of 0.9). The observed enrichment-isotopic fractionation was not caused by solutes, volatiles or old water because the enrichment fractionation did not disappear for washed or oven dried silage, the enrichment isotopic fractionation was also found in filter paper and cotton, and the enrichment fractionation was independent of the isotopic composition of the unconfined water. Enrichment–Isotopic fractionation became linearly more negative with increasing volumetric solid:water ratio and even exceeded $-4$ ‰ for $^{18}$O and $-44$ ‰ for $^2$H. This enrichment–fractionation behavior could be modeled by assuming two water layers: a thin layer that is in direct contact and influenced by the surface of the solid and a second layer of varying thickness depending on the total moisture content that is in equilibrium with the surrounding vapor. When we applied the model to soil water under grassland, the soil water extracted from 7 cm and 20 cm depth was significantly closer to local meteoric water than without correction for the surface effect. This study has major implications for the interpretation of the isotopic composition of water extracted from organic matter, especially when the volumetric solid:water ratio is larger than 0.5 or for processes occurring at the solid-water interface.

Key-words: discrimination isotopic fractionation; protein; cellulose; surface effect; O-18; H-2

1 Introduction

The $^{18}$O and $^2$H isotope composition of water reflects climate and many processes within the water cycle (Bowen, 2010; Gat, 1996). Changes in the isotope composition of water can either result from the mixing of water with differing isotopic composition or from the change in isotopic composition by fractionation, especially between vapor and liquid. Such-The vapor/liquid fractionation can is not only be affected by temperature but also by ion hydration (Kakiuchi, 2007). In aqueous solutions, ions change the activities of the isotopologues of water (H$_2$O, HDO, and H$_2^{18}$O) due to their hydration. This, in turn, causes the isotopic fractionation between aqueous solutions and water vapor to differ from the fractionation between pure water and vapor (Kakiuchi, 2007; Stewart and Friedman, 1975). Similar to salt, the surface of hydrophilic materials also interacts with water molecules creating a two-dimensional ice like water layer near the surface and a three dimensional liquid
layer far from the surface (Asay and Kim, 2005; Miranda et al., 1998). Additionally, adsorption may cause an energetic difference between water molecules at the surface of solids and the bulk water molecules (Richard et al., 2007). These structural and energetic differences may cause a difference in isotopic composition between these two layers of water. If existent, such a surface effect should be strongest in materials with large specific surface area and with low water content. There are some indirect hints from studies of plant water uptake from soil, which show that mobile water differs isotopically from immobile water (Brooks et al., 2010; Evaristo et al., 2015; Tang and Feng, 2001) but to the best of our knowledge, such a surface effect has only been directly studied for clay (Oerter et al., 2014) and silica surfaces (Richard et al., 2007). It is not known how large the effect is for organic matter, which are associated with practically all mineral surfaces in the critical zone or form major constituents of other surfaces in the biosphere (Chorover et al., 2007; Nordt et al., 2012; Vazquez-Ortega et al., 2014).

A surface effect may be detected by establishing an equilibrium between water adsorbed to a material and air vapor created by unconfined water with known isotope composition in a closed chamber. If there is no surface effect, then the $^{18}$O and $^{2}$H isotope composition of the adsorbed water and unconfined water should be identical after equilibration. This is because the isotope composition of water under steady conditions is determined by the isotope composition of the water vapor, air humidity, equilibrium fractionation and kinetic fractionation (Helliker and Griffiths, 2007; Welhan and Fritz, 1977). All of these parameters are identical for adsorbed water and unconfined water when they both share the same atmosphere in a closed chamber for a sufficiently long enough time, as is the case in a closed chamber.

We examined the hypothesis that the surfaces of organic materials influence the isotopic composition of adsorbed water and we choose materials of broad relevance. Silage, the product after anaerobic fermentation of fresh forage, is an important feedstuff which also delivers water to the animal and thus influences the body water composition (Kohn, 1996; Soest, 1994; Wilkinson, 2005) and animal products like milk. Hay in particular for example, has a particularly low water content. Organic horizons at the soil surface provide the interface where most vapor and water flows have to pass (Haverd and Cuntz, 2010). More materials like filter paper, cotton, casein-protein powder, and wheat flour were included to identify whether the chemical identity causes or influences the effect. Finally we had to exclude that the effect resulted from artifacts like old water or volatiles and solutes interfering with the isotope measurements (Martín-Gómez et al., 2015; Schmidt et al., 2012; Schultz et al., 2011; West et al., 2011). Silage, which likely is a source of volatiles and solutes in rather large amounts (e.g., lactic acid, acetic acid, propionic acid, ethanol, and propanol; Porter and Murray, 2001), was also pretreated by washing and heating to remove potentially interfering substances. Water of contrasting isotope composition was used to identify any old water. Finally, we derived a simple prediction model for the effect and demonstrated its versatility in an application case with environmental samples.

2 Materials and Methods

We performed three equilibration experiments. Each equilibration experiment involved the exposure of samples to water vapor which originated from unconfined water, followed by cryogenic water extraction from samples and isotope composition measurement. We use $\delta^{18}$O and $\delta^{2}$H to describe the isotope composition of oxygen ($^{18}$O) and hydrogen ($^{2}$H) in water (with $\delta^{18}$O or $\delta^{2}$H = $R_{\text{sample}}$/$R_{\text{standard}}$−1, where $R_{\text{sample}}$ and $R_{\text{standard}}$ denote the ratio of the abundances of heavy and light isotopes in samples following the international SMOW standard).

2.1 Preparation of samples

The materials comprised fresh silage, oven dried silage, washed silage, hay, fibric and hemic litter, filter paper, cotton, casein and wheat flour. Silage was also oven-dried to remove all volatiles and it was also 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 commercial casein powder to represent proteins.

The silage and hay were obtained from a farm near Freising and were cut in pieces (4 cm to 8 cm). The silage was stored in a -18 °C deep freezer while the hay was kept in a dark and dry place before use. The hemic and fibric horizons were gathered from a conifer forest near Freising (Germany) from a Haplic Podzol (according to IUSS Working Group WRB, 2014) area and stored in air tight bags in a refrigerator until use. In order to create a relative big range of water content, half of the litter samples were oven dried (16 h for 100 °C) before the equilibration experiment. Filter paper (Rotilabo®-round filters, type 11A, Germany), made of 100 % cellulose, and bleached medical cotton (Paul Hartmann AG, Germany) were prewetted by spraying because the initially dry filter paper and cotton hardly adsorbed any humidity from air. 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. 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.

### 2.2 Unconfined water

Five isotopically distinct, unconfined waters were used. We term them very heavy, heavy, tap, light and very light waters according to their relative ranking of δ^{18}O and δ^{2}H. These waters were produced from deionized water (δ^{18}O = -10 ‰, δ^{2}H = -70 ‰) by means of a rotary evaporator. Very heavy, heavy, light and very light waters had δ^{18}O values of 15, 2, -15 and -22 ‰, and δ^{2}H values of 125, 21, -113 and -160 ‰ respectively with slight deviations between individual experiments.

### 2.3 Set-up of the equilibration procedure

The different materials were individually placed in closed chambers (glass exsiccator vessels with a volume of approximate 20 L with drying agent removed) to equilibrate with unconfined water (Fig. 1). In a preliminary experiment, the effectiveness of the chambers’ air seal was verified by flushing the containers with N₂ followed by monitoring the concentration of CO₂ and water vapour inside the vessels. The concentrations after closing the chamber remained constant, which indicated that leaks were negligible. In another preliminary experiment we assessed the development of humidity in the chamber. The humidity reached 100 % within 20 min (half-life 1.8 min) after we put 200 mL of water at bottom of the chamber (Fig. 1), closed it and started the recycling pump (Laboport, Germany). All equilibration experiments lasted for 100 h. Sun et al. (2014) have shown that even for moist samples equilibration is relatively fast (half-life 20 h). 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 after equilibration and material water because the condensate will also be equilibrated.

In each experiment, 200 mL of (unconfined) water was placed in a glass bowl (15 cm in diameter) on the bottom of the chamber and dishes containing the material samples under focus (about 3 g fresh matter per dish) were placed on a perforated sill in the chamber. We flushed the chamber with nitrogen gas to remove the air vapor and the oxygen to prevent
2.4 Experiment A: Influence of materials

This experiment focused on the enrichment fractionation between water in different materials and unconfined water after equilibration. Dishes containing oven dried silage, hay, oven dried and fresh hemic litter, oven dried and fresh fibric litter, filter paper, bleached medical cotton, casein powder, or flour were all placed in different chambers for equilibration with unconfined water to avoid interference of volatiles in different materials. Eight samples for each material that differed in solid:water ratio were put in one chamber. Some materials (i.e., litter, filter paper, silage) were replicated in different experiments. The maximum number of samples for one material (silage) was 72. Flour and casein were powders and prone to form dust during vacuum water extraction. To prohibit this, the opening of vials containing flour and casein powder was covered by parafilm with tiny holes.

2.5 Experiment B: Influence of isotopic composition in unconfined water

This experiment aimed to find evidence that the enrichment-isotopic fractionation was independent of the isotopic composition of the unconfined water. This independence will also prove that the enrichment-isotopic fractionation cannot be caused by old water within the materials due to insufficient equilibration. Eight samples of oven dried silage in each case were placed into chambers to equilibrate with five different unconfined waters.

2.6 Experiment C: Pretreatment of silage

This experiment investigated the influence of volatiles on the isotope measurement and it assessed the effect of silage solutes on isotopic fractionation between silage water and vapor.

Fresh silage was divided into three groups (8 samples each): The first group did not undergo any pretreatment. For the second group, about 20 g of silage was immersed in 7 L of deionized water for about 2 min, stirred during immersion, then taken out using a colander and flushed with distilled water. After that we squeezed the silage by hand until no water drained off. This washing process was repeated three times. Finally, we reduced the water content of the washed silage by drying at 80 °C for 40 min. For the third group, silage was oven dried for 16 h at 100 °C to remove water and organic volatiles. These three groups (we call them fresh silage, washed silage and oven dried silage, respectively, thereafter) were placed in individual chambers and equilibrated with tap water for 100 h.

2.7 Statistics
For statistical evaluation we report two-sided 95\% limits of confidence (abbreviated CL) to separate between treatments and OLS (Ordinary Least Squares) regression to describe relations between two variables. Measured values were fitted to expected relations by minimizing the root mean squared error (RMSE). Statistical requirements (normal distribution) were met in all cases. Significance, even if not explicitly stated, always refers to \( p < 0.05 \).

2.8 Modelling

Conceptually, we assumed water to be part of one of two pools, which are arranged in a shell-like structure around the solid: an inner shell (or layer) which is in immediate contact or close to the surface of the solid and an outer layer that differs in thickness depending on the moisture content or solid:water ratio of the sample. Assuming that the outer layer has the same isotopic composition as the unconfined water once equilibrium was attained and that the inner layer has an isotopic composition that is influenced by the solid, the isotope composition of total adsorbed water (\( \delta_T \)) was defined as:

\[
\delta_T = f_O \times \delta_U + (1 - f_O) \times \delta_S
\]

where \( f_O \) is the fraction of water in the outer layer isotopically identical to the unconfined water, \( \delta_U \) and \( \delta_S \) are the isotope compositions of unconfined water and water influenced by the surface.

We defined enrichment–isotopic fractionation (\( \varepsilon \)) between \( \delta_S \) and \( \delta_U \) as

\[
\varepsilon_{\delta_S\delta_U} = (\delta_S - \delta_U)/(1000 + \delta_U) \times 1000
\]

Combining eq. (1) and (2) leads to:

\[
\delta_T = \frac{1000 \times \varepsilon_{\delta_S\delta_U} \times \delta_U + \delta_S - \delta_S \times f_O}{1000} \quad (3)
\]

\[
\delta_T = (1000 + \varepsilon_{\delta_S\delta_U} \times f_O)/(1000 \times \delta_U + \varepsilon_{\delta_S\delta_U} \times f_O) \quad (3)
\]

From this it follows that the apparent enrichment–isotopic fractionation (\( \varepsilon_a \)) between the total water in the material and unconfined water is given as:

\[
\varepsilon_{\delta_{\text{total}}\delta_S} = (\delta_T - \delta_U)/(1000 + \delta_U) \times 1000 = (1 - f_O) \times \varepsilon_{\delta_S\delta_U} \times f_I \times \varepsilon_{\delta_{\text{total}}\delta_S} \quad (4)
\]

The fraction constituted by the inner layer \( f_I \) in eq. (4) can be replaced by the ratio between \( R_I \), the volumetric ratio of water:solid, and \( R_T \), the volumetric water:solid:water ratio of total adsorbed water:

\[
\varepsilon_{\delta_{\text{total}}\delta_S} = \varepsilon_{\delta_{\text{total}}\delta_S} \times R_I/R_T \quad (5).
\]

Assuming that the size of the inner layer \( R_I \) as well as \( \varepsilon_{\delta_{\text{total}}\delta_S} \) are constant for a certain material, \( \varepsilon_{\delta_{\text{total}}\delta_S} \) should be related linearly to the inverse of \( R_T \), which is the volumetric solid:water ratio for the total adsorbed water. The solid volume (not the bulk volume exclusive voids) can be calculated by knowing the weight and the particle density of the organic matters (casein; 1.43 g/cm\(^3\) (Paul and Raj, 1997); silage, hay, litter, filter paper, cotton and flour; 1.5 g/cm\(^3\) (Yoshida, et al., 2006)).

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

\[
\varepsilon_{\text{predicted}} = (F^{\delta_S} - F) A F^{-1} \quad (6)
\]

Where \( \varepsilon_{\text{predicted}} \) 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 \( \alpha \) stands for isotope fractionation factor (1.0059 and 1.0366 for \(^{2}H\) and \(^{18}O\) at 80 °C extraction temperature, respectively).

2.9 Application case

Soil at 7 cm and 20 cm depths and rain water were sampled at the grassland in Grünschwaige Experimental Station, Germany (48°23’N, 11°50’E, pasture #8 in Schnyder et al. (2006); 8.3 \% organic matter, 30 \% clay, 22 \% sand) following the MIBA protocol (Moisture Isotopes in Biosphere and Atmosphere) at weekly–biweekly intervals during the vegetation growing period–season (April to November) from 2006 to 2012 and at weekly intervals during the winter–cold season (October to February) in 2015/2016. Soil sampling was always carried out on dry days at midday (between 11 a.m. and 16
p.m.). Two replicates of soil samples were collected on each sampling date. The data were used (i) to analyze 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) without sand.

Further, the winter data, effects of soil evaporation from the vegetation covered soil, can be excluded. We verify if the offset can be corrected by accounting for the volumetric solid:water ratio of the soil according to our model. To this end, the sand content of the soil was not considered in the calculation of the solid:water ratio given that the contribution of sand to water storage is marginal (Waleczak et al., 2002) and sand grains usually are coated by clay, sequioxides, organic matter and biofilms and do not directly interact with water (Bisdom et al., 1993; Bolster et al., 2001).

3 Results

3.1 Experiment A: Influence of materials

The apparent enrichment-isotopic fractionation (sensu eq. 4) of δ¹⁸/O and δ²/H was negative and significant (p < 0.05) for all materials, except for ¹⁸/O with filter paper and cotton and for ²/H in a few samples of cotton. The volumetric solid:water ratios differed between materials but also between different samples within the materials providing a wide range. δ¹⁸/O and δ²/H apparent enrichment-isotopic fractionation decreased significantly with volumetric solid:water ratio over the range of materials. The decrease was also significant for the different samples within each material (Fig. 2).

3.2 Experiment B: Influence of isotopic composition in unconfined water

The isotope composition of absorbed water correlated closely with the unconfined water due to the wide range compared to the measurement errors (R² = 0.9990 and 0.9989 for ¹⁸/O and ²/H, respectively; Table 1). However, the regressions showed that the intercept differed significantly (p < 0.05) from zero and the slope from one, which indicated that the isotope composition of absorbed water was significantly different from that of unconfined water.

Equation (3) predicted a linear relation between δ₂/H and δ₁₈/O similar to the linear regressions shown in Table 1. Different to a regression, however, the slope and the intercept of eq. (3) are not independent but depend on ε₁₈/O × f₀. To account for this dependency, the slope and the intercept of the linear equations were estimated by adjusting ε₁₈/O × f₀ in eq. (3) to minimize RMSE, while fitting the measured δ₂/H and δ₁₈/O values. The optimal fits lead to:

\[
\delta_{18O} = \frac{1000 \times 1.23}{1000} \times \delta_{2H} - 1.23 \\
\delta_{2H} = \frac{1000 \times 22.6}{1000} \times \delta_{18O} - 22.6 
\]

(6)

\[
\delta^{18}O_{T} = \frac{(1000 - 1.23) \times 1000 \cdot \delta^{18}O \cdot 1.23}{1000} - 1.23 \\
\delta^{2}H_{T} = \frac{(1000 - 22.6) \times 1000 \cdot \delta^{18}O \cdot 22.6}{1000} - 22.6 
\]

(67)

The R² between the predictions resulting from the two-layer model and the measurement were similar to that of the linear regression (R² = 0.9990 for ¹⁸/O and 0.9989 for ²/H), although the model has one degree of freedom less than the
regression. The resulting optimal \( e_{\text{app}} \times f_0 \) values were -1.23 \( \% \)o for \(^{18}\)O and -22.6 \( \% \)o for \(^{2}^\text{H}\) meaning that the effect was 18 times stronger for \(^{2}^\text{H}\) than for \(^{18}\)O.

Equation (5) predicted that the apparent enrichment isotopic fractionation changes linearly with the solid:water ratio. This relation was highly significant \( (p < 0.01) \) also in the case when waters with very differently isotopic composition were used \( (R^2: 0.7589 \text{ and } 0.8599 \text{ for } ^{18}\text{O} \text{ and } ^{2}^\text{H}, \text{ respectively}; \text{ Fig. } 3) \). These relations were identical for very heavy, heavy, tap, light and very light water.

3.3 Experiment C: Pretreatment of silage

There was no significant difference between mean gravimetric water contents (based on dry matter) of washed silage (153 \( \% \) \pm 33 \( \% \)) and fresh silage (128 \( \% \) \pm 10 \( \% \)) after 100 h equilibration. 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 \( \% \)). The apparent enrichment isotopic fractionation of washed silage, oven dried silage and fresh silage all decreased with the solid:water ratio (Fig. 4), as already noted in the experiment with different materials (Fig. 2) or in investigations with unconfined waters of different isotopic composition (Fig. 3). Washing and oven drying should have removed most solutes and volatiles respectively and thus have created a large variation in the amount of solutes and volatiles among the treatments. Still, the relationship between enrichment-apparent isotopic fractionation of all three types of silage and solid:water ratio followed the same line and the areas overlapped each other for the three types of silage (Fig. 4). This implied that neither the volatiles, which possibly could have adulterated the measurements, nor the solutes, which possibly could have influenced water activity in the silage, were the reason of enrichment isotopic fractionation. The different treatments, however, separated along the common line due to their differences in water content, which again corroborated the prediction that the apparent enrichment isotopic fractionation should linearly change with solid:water ratio.

3.4 Combining experiments A, B and C

When combining all experiments with different materials, different pretreatments and different unconfined waters, apparent enrichments isotopic fractionation covered a wide range of about 5 \( \% \)o for \(^{18}\text{O} \) and 46 \( \% \)o for \(^{2}^\text{H} \) (Fig. 5). Even within the same materials, the range was up to 2.5 \( \% \)o for \(^{18}\text{O} \) and 25 \( \% \)o for \(^{2}^\text{H} \). Apparent enrichments isotopic fractionation within materials linearly decreased with the volumetric solid:water ratio.

The enrichments isotopic fractionations predicted for Rayleigh fractionation fell far apart the observed enrichments isotopic fractionations (Fig. 5). The average deviation between the expected and the observed \(^{2}^\text{H} \) enrichment isotopic fractionation was about 15 \( \% \). Furthermore, the slope of the relation between the enrichment fractionation of \(^{2}^\text{H} \) and \(^{18}\text{O} \) was significantly steeper \( (p < 0.05) \) for the observed enrichment than the slope predicted for a Rayleigh process. Additionally, the average \(^{2}^\text{H} \) fractionation of the materials \( i \) 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, which means that if the isotopic fractionation was caused by incompletely extraction an unrealistically small fraction of water would have to be extracted (far below 0.8) to cause the same enrichment isotopic fractionation of \(^{2}^\text{H} \) as observed for most of the samples.

3.5 Application

For the growing season, soil water at 20 cm depth and 7 cm depth showed a distinct deviation from the local meteoric water line (mean deviation for \(^{2}^\text{H}\): -8.1 \( \% \)) with a slope almost identical to that of the meteoric water line (regression lines in Fig. 6, top panels). An identical mismatch was detected for the winter season (markers in Fig. 6, top panels) for which confounding effects of evaporation are minimal and summer season.
The deviation between the winter season data and the local meteoric water line correlated significantly ($p < 0.001$) with the solid:water ratio for 7 cm depth but not for 20 cm depth, which varied less in water content. For both depths, the data moved closer to the local meteoric water line when the influence of confined water was removed by applying the general regression with solid:water ratio from Fig. 2 (Fig. 6, bottom panel). The mean deviation for $^{2}H$ changed from -8.1 \% to 1.0 \% for both depths due to this correction.

4 Discussion

The extraction of water from solid-water mixtures can be biased by incomplete extraction (Araguás-Araguás et al., 1995) or by the exchange of hydrogen or oxygen from the soil material with water molecules (Meißner et al., 2014). Here we add another confounding effect, which is the inhomogeneous isotopic composition of water above a solid surface. In the following we will discuss (1) whether the observed effect can be due to measuring errors or other reasons than the proposed surface effect, (2) what could be possible reasons of the surface effect, (3) which fields of application will this surface effect likely be important, and (4) which further work related to the surface effect may follow.

4.1 Excluding other mechanisms than the proposed surface effect

The study provided clear evidence that the water adsorbed by organic surfaces differed from what would be expected from the isotopic composition of the source unconfined water and it showed that this deviation became larger with decreasing water content. Alternative mechanisms leading to an isotopic offset-fractionation other than the proposed surface effect could be (A) volatiles adulterating the measurements; (B) solutes influencing the isotopic composition of adsorbed water; (C) insufficient equilibration time; (D) incomplete extraction of water; (E) metabolically produced water from microorganisms adhering to the materials; (F) exchange of hydrogen and oxygen between the organic matter and the adsorbed water.

(A) The surface effect was largest for flour and casein that do not produce volatiles. Also the filter paper and cotton, which contain no volatiles, had the decreasing trend between apparent enrichment-isotopic fractionation and solid:water ratio (Fig. 2). Even for silage the influence of volatiles was not evident because washed or oven-dried silage, which should have lost all their volatiles, behaved identical to fresh silage. Also the error in water content caused by not accounting for volatile losses was negligible. Using the correction function by Porter and Murray (2001) to calculate the true water content from the loss of weight, moves the respective data points of silage in Fig. 4 only invisibly (about 0.03 L/L towards right side).

(B) Solutes in water can influence the isotopic fractionation between water and vapor because the energy stage of water molecules bound in the primary hydration sphere of cations and anions differs from that of the remaining bulk water molecules (Kakiuchi, 2007). This effect has been shown for many salts (e.g., KCl, NaCl, Na$_2$SO$_4$ and ZnSO$_4$). The strength of this effect varies between different ions and may be small (Kakiuchi, 2007; Sofer and Gat, 1975; Stewart and Friedman, 1975). NaCl even does not have an measurable effect on $^{18}$O (O’Neil and Truesdel, 1991). Most of the solutes in our materials were organics for which the effect is unknown. However, this effect must have been small as the washed silage did not show a different pattern in enrichment-isotopic fractionation compared to fresh silage (Fig. 4). Also the filter paper of analytical grade and bleached cotton that both should not carry any solutes did not show a different pattern.

(C) Insufficient time for equilibration may especially be relevant for silage and litter, which had the highest initial water content. For silage we could show that the apparent enrichment-isotopic fractionation was independent of the isotopic composition in the unconfined water (Experiment B) despite the wide range of differently labelled unconfined waters (range for $^{18}$O: 32 \%, range for $^{2}H$: 285 \%). However, any old water would have led to a separation in the apparent enrichment isotopic fractionation. In contrast, our results were in accordance with the general rule that isotopic enrichment-fractionation is independent of the isotope composition of the source, which is also underlying eq. (4) and (5). Furthermore, all our experiments used deionized water prepared from tap water, except for the experiment with labelled waters for which we can
exclude the existence of old water. Our deionized water was similar in isotopic composition to silage water and soil water. The mean $\delta^{18}O$ of our water was $\sim 10\%$ while the mean for 52 fresh silage samples analyzed by Sun et al. (2014) was $-11\%$ (SD 3\%). A small fraction of old water thus cannot cause the large observed effects.

(D) An incomplete extraction should cause a large error at low moisture content, similar to the general relation between solid:water ratio and enrichment isotopic fractionation that we have observed (Fig. 5). However, the predicted enrichment isotopic fractionation by incomplete extraction based on a Rayleigh fractionation fell far apart from the observed enrichment isotopic fractionation (Fig. 5). In addition, no significant weight difference before and after oven drying of the samples was observed after vacuum extraction. Incomplete extraction is thus an unlikely explanation.

(E) Kreuzer-Martin et al. (2005) found that 10 \% of the total water extracted from Escherichia coli cells during the log-phase of growth was generated by metabolism from atmospheric oxygen. Thus, intracellular water was distinguishable from extracellular water in $\delta^{18}O$. We flushed the chambers with nitrogen gas before equilibration to reduce availability of atmospheric oxygen and minimize microbial growth. For materials like silage dried at 100 °C or filter paper, any significant microbial growth is unlikely. Furthermore, isotopic adulteration caused by microorganisms should have caused $^{18}O$ and $^2H$ deviations in the opposite direction for the very heavy and the very light labeled experiments akin to the experiments by Kreuzer-Martin et al. (2005). In contrast to this $^{18}O$ and $^2H$ were always depleted in our experiments regardless of the isotope composition of unconfined water.

(F) 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. Thus, the exchange and the subsequent equilibration with the unconfined water will happen within 100 h. Thus the $H$-exchange happened within our experiment time (100 h), which will not influence the final equilibration because the exchange has already stopped after 100 h of exposure in the chamber. Furthermore, an exchange of hydrogen would not explain the observed offset in $^{18}O$. Some literatures also reported that there was a fractionation between carbonate oxygen and water $O$xygen (Savin and Hsieh, 1998; Zeebe, 2009); however, there was no or small amount of carbonate in our materials. Especially for the casein and flour, the $O$ isotopic fractionation still existed although no carbonate contained although our samples did not contain any carbonate.

4.2 Possible reason for the surface effect

The enrichment–isotopic fractionations became more negative with increasing solid:water ratio and they followed the predictions of eq. 5. This implied that similar enrichment–isotopic fractionations existed in different materials and that the simple two-layer model sufficiently described the experimental values. Abundant evidence exists that the properties of water change close to a surface (Anderson and Low, 1957; Goldsmith and Muir, 1960; Miranda et al., 1998). A hydrogen-bonded ice-like network of water grows up as the relative humidity increases. Above 60\% relative humidity, the liquid water configuration grows on top of the ice-like layer (Asay and Kim, 2005). This transition from a two-dimensional ice-like water to a three-dimensional water-like layer has been already been shown in several cases (Kendall and Martin, 2005). As we used 100\% relative humidity in our chamber, both layers should have been present.

The anomalies of water close to a surface appear not to be particularly affected by the detailed chemical nature of the solid substrates with which the water is in contact. This is referred to as the “paradoxical effect” and is tentatively interpreted in terms of an energy delocalization phenomenon. 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). This agrees with our observation that the difference
between materials was small compared to the large variation of the effect caused by a varying solid-water ratio. The small differences between materials that appear in Fig. 2 may hence only be an effect due to differences between the different materials in their specific surface area per volume of solid but not due to their chemical nature. The water content of oven dried silage (81 % ± 13 %) did not reach again the same water content as fresh silage (128 % ± 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).

In accordance with our study, Richard et al. (2007) found that water adsorbed in porous silica tubes was depleted in $^2$H compared to unconfined water and depletion increased with decreasing water quantity as a result of the interplay of molecular vibrational frequencies and intermolecular H-bonding. This mostly depends on the difference in zero-point energy between the $^{16}$H-$^{17}$H bonds, which is compressed at the transition between the bulk liquid and the confined liquid influenced by the surface (Richard et al., 2007). Our data show, that the effect is much larger for $^2$H than for $^{18}$O and it practically disappears for $^{18}$O when the solid:water ratio decreases below 0.5 (Fig. 5). This may explain why the effect has been previously described for $^2$H but not for $^{18}$O. 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.

4.3 Fields of application

In our experiments we had only examined organic materials while the soil in our application case also contained minerals. Given the “paradoxical effect” (Drost-Hansen, 1978) and that we had not found any effect of the nature of the organic materials on the surface effect, the simplest assumption was that there is also no large difference between organic and mineral surfaces regarding the isotope effect. This seemed reasonable because pure clay with 30 % water content (equivalent to 0.8 solid:water content) as used by Oerter et al. (2014) created -0.4 ‰ oxygen isotopic fractionation on average. This was close to the predicted apparent isotopic fractionation (-0.7 ‰) for the same solid:water ratio for organic materials. Oerter et al. (2014), however, also manipulated the composition of the solutes, which are known to affect fractionation and which do not allow direct comparison.

The isotopic composition of water in porous samples is usually determined by extracting all water in order to avoid any shift caused by Rayleigh fractionation. Hence, the inner layer close to the surface and the outer layer will be mixed. 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).

For many processes, especially in the transport of liquid water (e.g., groundwater recharge, stream flow discharge, water uptake by plants) only the outer, mobile layer will be relevant. The extraction of total water will then give a biased estimate of the mobile water. In accordance with our hypothesis, Brooks et al. (2010) even suggested two different soil water worlds to explain their data (mobile water and tightly bound water), which were not identical in terms of isotope composition. They also measured soil water collected in low-tension lysimeters, which represents mobile water, and bulk soil water extracted cryogenically. Bulk soil water was always more depleted in heavy isotopes than lysimeter water collected at the same depth, which was in line with the isotopic fractionation direction observed in our soil case. Tang and Feng (2001) also found isotopic differences between mobile and immobile water in soil and explained this by incomplete replacement of soil water by rainwater. Our laboratory experiments aimed to exclude such an effect. In our application case we also found a consistent
offset between rain water and soil water that cannot result from incomplete replacement of old rain water in soil with new rain water because soil water had an offset from the meteoric water line. Such an offset has been shown for many locations around the world (Brooks et al., 2010; Evaristo et al., 2015), which challenges the assumption in land surface models that plants and streams derive their water from a single, well mixed subsurface water reservoir. Additionally, the surface effect may also play a role in the fractionation between source water and xylem water that has been described for some xerophytic and halophytic species (e.g. Ellsworth and Williams 2007) for which an explanation is presently missing.

In other cases, which focus on the liquid-solid interface, only the water of the inner layer, which is influenced by the surface effect, will be relevant. For example, in studies of cell wall formation or degradation, the total water should be a biased estimate of the isotopic composition near the cell wall. Due to the change in apparent enrichment isotopic fractionation with water content, the total cell water will change just by a variation in vacuole volume even if the isotopic composition near the cell wall and in the vacuole remain unchanged. 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.

4.4 Further work
Solid:water ratio is clearly not the best parameter to describe the two-layer model. The relation should be influenced by specific surface area and by wettability. Hence, the water volume per wetted surface area would likely be a better parameter. For instance, when we wet the filter paper inhomogeneously, we got random results because the average solid:water ratio neither reflected the situation of the wet spots nor that of the dry spots. Also the increasing scatter for solid:water ratios >1.5 (Fig. 5) likely resulted from an inhomogeneous water distribution in these rather dry samples that may have left some parts of the sample completely dry and thus underestimated the water content of other parts. Still, our model was easy to apply and it worked sufficiently for the wide variety of materials examined. More materials varying in hygroscopic/hydrophobic behavior and in surface area should be included to better understand the rule behind the variation of enrichment isotopic fractionation and to expand the model.

5 Conclusions
There was an abundance of evidence to suggest that the surface effect influenced the enrichment isotopic fractionation between water adsorbed by organic matter and unconfined water. Many hypothetical reasons for an erroneous enrichment isotopic fractionation could be excluded. The variation of apparent enrichment isotopic fractionation with water content was well described by a simple, easy to apply two-layer model. This enrichment isotopic fractionation should not be neglected when the surface area is huge and the water content is low. The surface effect will become especially relevant for processes happening at the liquid-surface interface like the growth or degradation of the organic materials.

Author contribution
G.C. and K.A. designed the experiments and analyzed the data. G.C. carried out the experiments and wrote a first draft. All authors developed and approved the manuscript.

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References


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Table 1: Regressions between water adsorbed by silage (δ₁₈O) and unconfined water (δ₂H) for five types of water (very heavy, heavy, tap, light and very light water) based on equation δ₁₈O = slope × δ₂H + intercept; n = 40; values in parenthesis denote the 95% confidence level.

<table>
<thead>
<tr>
<th></th>
<th>δ₁₈O</th>
<th>δ₂H</th>
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<tbody>
<tr>
<td>Intercept</td>
<td>-1.30 (± 0.14)</td>
<td>-22.9 (± 1.1)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.987 (± 0.010)</td>
<td>0.968 (± 0.011)</td>
</tr>
<tr>
<td>R²</td>
<td>0.9990</td>
<td>0.9989</td>
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</tbody>
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Fig 1: Experimental set-up with an exsiccator vessel as the equilibration chamber. P: recycling pump ensuring air mixing and air movement within the chamber; U: unconfined water filled in the bottom part of the chamber; S: samples placed on top of the perforated middle plate. The arrows indicate the direction of air flow. Vaseline was used as sealant between the lid and the vessel.
Fig. 2: Relationship between volumetric solid:water ratio and apparent enrichment-isotopic fractionation of (a) $^{18}\text{O}$ and (b) $^2\text{H}$ between unconfined water and total water adsorbed by different materials. Taken together, the regressions are $y = -0.906x$ ($R^2 = 0.6789; N = 96$) for the enrichment-isotopic fractionation of $^{18}\text{O}$ and $y = -17.75x$ ($R^2 = 0.8355$) for the enrichment-isotopic fractionation of $^2\text{H}$.
Fig. 3: Relationship between volumetric solid:water ratio and apparent (a) $^{18}$O and (b) $^2$H enrichment isotopic fractionation of total water absorbed by silage compared to unconfined waters with different isotopic composition. The lines show the best fit (see eq. 26).
\[ \Delta \] washed sludge
\[ \square \] oven dried sludge
\[ \bigcirc \] fresh sludge
\[ \bigtriangleup \] Fig. 2 + 3

\( \delta^{18}O \) enrichment (‰)

\( \delta^2H \) enrichment (‰)

Solid:water ratio (L/L)
Fig. 4: Relationship between volumetric solid:water ratio and the apparent isotopic enrichment fractionation of (a) $^{18}\text{O}$ and (b) $^{2}H$ between unconfined water and total water adsorbed by silage with different pretreatments ($N = 8$ each). The data of Fig. 2 and Fig. 3 (both oven dried silage, $N = 32$) are provided for comparison.
Fig. 5: Apparent isotopic enrichment-fractionation ($^{2}H$ versus $^{18}O$) of extracted water as observed in all experiments (markers indicate three groups of solid:water ratios) and enrichment-fractionation as expected from Rayleigh fractionation (line; numbers denote the fraction of extracted water).
Fig. 6: Isotope composition of measured total soil water (top) and estimated unconfined water (bottom) in soil at 7 cm depth (circles, N = 26) and 20 cm depth (crosses, N = 26) during the winter season. The bold solid line denotes the local meteoric water line (N = 79; \(y = (8.0 \pm 0.2)x + (10 \pm 2)\), \(R^2 = 0.99\)). The thin solid line and the dashed line indicate the range and the linear regressions for soil water at 7 cm (N = 84; \(y = (7.7 \pm 0.5)x - (0 \pm 4)\), \(R^2 = 0.92\)) and 20 cm depth (N = 85; \(y = (7.4 \pm 0.5)x - (5 \pm 3)\), \(R^2 = 0.92\)) during the growing season.

Fig. 6: Isotope composition of soil water at 7 cm and 20 cm depth (winter season: N = 26; growing season: N = 48). (a) Measured total soil water. (b) Estimated unconfined water. The solid line denotes the local meteoric water line (N = 79; \(y = (8.0 \pm 0.2)x + (10 \pm 2)\); \(R^2 = 0.99\)).