- We would like to thank reviewer #2 for his/her constructive comments. The points of concern are addressed below. In the revised draft, changes are highlighted in grey.
- 3 The authors present the results of triple oxygen isotope measurements of plant silica. The aim of this study
- 4 is introducing d17O and d18O of phytoliths as proxy for the relative humidity (RH). The authors conducted
- 5 laboratory experiments with controlled irrigation water composition, temperature and relative humidity.
- 6 Data show that the difference in D170 between irrigation water and phytolith changes with RH. That is
- 7 expected as the kinetic fractionation becomes more important at lower RH. Because kinetic fractionation
- 8 follows a slope 0.516 (and 0.528 was used as reference line for defining D170), resultant D170 values of
- 9 the phytolith change with RH.

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- 10 The authors point out that the weakness of this study is the lack of vapor data. That s true and the study
- 11 would certainly have benefited from such data. The results show that the phytoliths fall on a line about
- 12 parallel to the water evaporation trend typical of leaf water.
 - In figure, the lines should go through all data, including the RH = 100 points. If necessary, draw curves. There is no physical reason why the laws of nature stop operating at RH = 85. It is a continuum; possibly with gradually changing mechanisms above RH = 85. That should not be camouflaged in the figure.
- In agreement with this comment, modifications were made to figure 1 as well as in the text (section 3.1) of the revised draft.
- For readers with a b&w printer only, different symbols would be appropriate to distinguish the different data.
- 21 This was modified in the revised draft.
 - In figure 1, "170-excess" (top, bottom) is not relative to VSMOW. It is, however, reported relative to a reference line with slope (0.528) and intercept (0). Delete "VSMOW". Also, the Δ 180 should not be reported relative to VSMOW; it's a difference between δ values; delete VSMOW here, too.
- This is right. This was modified in the revised draft.
 - Line 386ff: Don't give numbers like 27.948±7.168! Give 28±7. Only report significant number of digits. See also line 405; never give more digits than the uncertainty allows. Change throughout the entire manuscript.
- The precision on $\delta^{17}O$ and $\delta^{18}O$ should indeed be given with only 2 digits. Still, as shown in Landais et al. (2006) for leaf water, the uncertainties on $\delta^{17}O$ and $\delta^{18}O$ are not independent so that the final uncertainty
- 31 on ^{17}O -excess should not be calculated from uncorrelated uncertainties on $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$. This is the reason
- why, there is a need to keep the 3 digits to properly calculate the ¹⁷O-excess. For further clarity 2 digits are
- presented in the text and 3 digits are kept in tables.
- My major criticism on this paper is that it is not evaluated how precise (+/- RH values) the approach is for the reconstruction of the RH.
- In agreement with this comment, this is now discussed in the revised draft (section 4.2):
- 37 "Without taking into account the two outliers, the linear regression between RH-rd0>1 and ¹⁷O-excess_{phyto}
- 38 for a 95% confidence interval can be expressed as follows:
- 39 RH-rd0>1 = 0.14 ± 0.02 (S.E) x 7 O-excess_{phyto} + 100.5 ± 4.7 (S.E) Eq. 2
- 40 where 17 O-excess_{phyto} is expressed in per meg and RH in %, $r^2 = 0.48$, p < 0.001 and S.E. stands for standard
- error. The S.E. of the predicted RH-rd0>1 value is \pm 5.6%. However, the data scattering (fig. 4) call

- for assessing additional parameters that can contribute to changes in ¹⁷O-excess_{Phyto}, beside RH, before using the ¹⁷O-excess_{phyto} for quantitative RH reconstruction."
 - Also, completely missing is a discussion on the heterogeneity of leaf water and the effect on the phytolith composition. Eventually, people will use fossil phytoliths for reconstructing past RH and they will not know from which part of the plant the samples come.

This is now discussed in section 4.2: "In grasses, leaf water is expected to be more prone to evaporative enrichment than stem water, and inside the leaf itself, the heterogeneity of evaporative sites repartition and water movements can lead to a significant heterogeneity in the δ^{18} O signatures of water and phytoliths (Cernusak et al., 2016; Helliker and Ehleringer, 2000; Webb and Longstaffe, 2002). Soil top phytolith assemblages likely record several decades of annual phytolith production and their isotope composition is expected to be an average. This would explain the consistency of the ¹⁷O-excess_{Phyto} data obtained from bulk grass from climate chambers and bulk grasses from natural savannas. However, further investigation on the extent of the heterogeneity of ¹⁷O-excess signature of water and phytoliths in mature grasses would help to clarify the links between water and phytolith signatures and better understand the phytolith proxy."

• After this assessment, come to a decision on whether this proxy works or not (for useful applications). The lack of a quantitative assessment of all uncertainties is a general problem of many proxies. With some corrections and such a quantitative discussion, the manuscript is surely worth being published in Biogeosciences.

We agree with all the points of concern raised above (see answers above). However, as written in the discussion and conclusion sections we would like to emphasize that this is a first step in the assessment and that complementary calibration steps are required to bring us to an accurate quantitative proxy. These assessments, described in the discussion section, are in progress.

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- 92 The triple oxygen isotope composition of phytoliths as a proxy of continental atmospheric
- 93 humidity: insights from climate chamber and climate transect calibrations
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108 Abstract

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109 Continental atmospheric relative humidity (RH) is a key climate-parameter. Combined with 110 atmospheric temperature, it allows us to estimate the concentration of atmospheric water vapor which is one of the main components of the global water cycle and the most important gas 111 112 contributing to the natural greenhouse effect. However, there is a lack of proxies suitable for 113 reconstructing, in a quantitative way, past changes of continental atmospheric humidity. This 114 reduces the possibility to make model-data comparisons necessary for the implementation of 115 climate models. Over the past 10 years, analytical developments have enabled a few laboratories to reach sufficient precision for measuring the triple oxygen isotopes, expressed by the ¹⁷O-excess 116 $(^{17}\text{O-excess} = \ln (\delta^{17}\text{O} + 1) - 0.528 \text{ x ln} (\delta^{18}\text{O} + 1))$, in water, water vapor and minerals. The $^{17}\text{O-excess}$ 117 excess represents an alternative to deuterium-excess for investigating relative humidity conditions 118 119 that prevail during water evaporation. Phytoliths are micrometric amorphous silica particles that 120 form continuously in living plants. Phytolith morphological assemblages from soils and sediments 121 are commonly used as past vegetation and hydrous stress indicators. In the present study, we 122 examine whether changes in atmospheric RH imprint the ¹⁷O-excess of phytoliths in a measurable way and whether this imprint offers a potential for reconstructing past RH. For that purpose, we 123 124 first monitored the ¹⁷O-excess evolution of soil water, grass leaf water and grass phytoliths in 125 response to changes in RH (from 40 to 100 %) in a growth chamber experiment where transpiration reached a steady state. Decreasing RH decreases the ¹⁷O-excess of phytoliths by 4.1 per meg / % 126 127 as a result of kinetic fractionation of the leaf water subject to evaporation. In order to model with 128 accuracy the triple oxygen isotope fractionation in play in plant water and in phytoliths we 129 recommend direct and continuous measurements of the triple isotope composition of water vapor. 130 Then, we measured the ¹⁷O-excess of 57 phytolith assemblages collected from top soils along a RH and vegetation transect in inter-tropical West and Central Africa. Although scattered, the ¹⁷O-excess of phytoliths decreases with RH by 3.4 per meg / %. The similarity of the trends observed in the growth chamber and nature supports that RH is an important control of ¹⁷O-excess of phytoliths in the natural environment. However, other parameters such as changes in the triple isotope composition of the soil water or phytolith origin in the leaf tissue may come into play. Assessment of these parameters through additional growth chambers experiments and field campaigns will bring us closer to an accurate proxy of changes in relative humidity.

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1 Introduction

140 Continental atmospheric relative humidity (RH) is a key climate-parameter. Combined with 141 atmospheric temperature, it allows scientists to estimate the concentration of atmospheric water 142 vapor which is one of the main components of the global water cycle and the most important gas 143 contributing to the natural greenhouse effect (e.g. Held and Soden, 2000; Dessler and Davis, 2010; 144 Chung et al., 2014). However, global climate models (GCMs) have difficulties to properly capture 145 continental humidity conditions (Sherwood et al., 2010; Risi et al., 2012; Fischer and Knutti, 2013). Although tropospheric RH results from a subtle balance between different processes 146 147 (including air mass origins and trajectories, large scale radiative subsidence, evaporation of falling 148 precipitation, detrainment of convective system, evapotranspiration), it is usually depicted as 149 rather constant in GCMs in agreement with thermodynamic coupling between atmospheric water 150 vapor and sea surface temperature (Bony et al., 2006; Stevens et al., 2017). A model-data 151 comparison approach is thus essential to progress on this issue. This approach has to be applicable 152 beyond the instrumental period to make use of past changes in atmospheric water vapor conditions. 153 There are multiple ways to reconstruct past continental temperature and precipitation, for instance from pollen (Bartlein et al., 2010; Herbert and Harrison, 2016; Wahl et al., 2012) or tree ring data 154 155 (Labuhn et al., 2016; Lavergne et al., 2017). However, there is a serious lack of proxies suitable 156 for reconstructing, in a quantitative way, past variations in continental atmospheric RH. Indeed, 157 the stable isotopes of oxygen and hydrogen (δ^{18} O and δ D) of tree rings can be influenced by several 158 parameters other than humidity (precipitation source, temperature). This limits the interpretation 159 of tree ring isotope series in terms of humidity changes to places where variations of these other 160 parameters are well constrained (Grießinger et al., 2016; Wernicke et al., 2015). A promising method relies on the $\delta^{18}O$ and δD of plant biomarkers (e.g. n-alkanes and fatty acids from leaf 161 162 waxes) recovered from soils (or buried soils) and sediments. It allows for an estimate in changes 163 in plant water deuterium-excess (d-excess = δD - 8.0 x $\delta^{18}O$), linked to changes in precipitation 164 sources and RH. This method under development can however be biased by factors other than 165 climatic such as plant functional types and selective degradation of the biomarkers (e.g. Rach et 166 al., 2017; Schwab et al., 2015; Tuthorn et al., 2015).

Phytoliths are micrometric amorphous silica (SiO₂, nH₂O) particles that form continuously in living plants. Silicon is actively absorbed by the roots (Ma and Yamaji, 2006) and is translocated in the plant tissues where it polymerizes inside the cells, in the cell walls and in extracellular spaces

of stems and leaves. Silica polymerization appears to be an active physiological process, which does not only depends on transpiration (Kumar et al., 2017). In grasses, which are well known silica accumulators, silica accounts for several % of dry weight (d.w.) and is mainly located in the stem and leaf epidermis. Phytolith morphological assemblages from soils and sediments are commonly used as past vegetation and hydrous stress indicators (e.g. Aleman et al., 2012; Backwell et al., 2014; Bremond et al., 2005a, 2005b; Contreras et al., 2014; Nogué et al., 2017; Piperno, 2006). The potential of the $\delta^{18}O$ signature of phytoliths ($\delta^{18}O_{Phyto}$) from grasses for paleoclimate reconstruction has been investigated through growth chamber and North American Great Plains calibrations. It has been shown that the $\delta^{18}O_{Phyto}$ of grass stems weakly affected by transpiration correlated with the δ^{18} O signature of soil water (δ^{18} O_{SW}) and the atmospheric temperature, as expected for a polymerization of silica in isotope equilibrium with the plant water (Webb and Longstaffe, 2000, 2002, 2003, 2006). It has also been shown that $\delta^{18}O_{Phyto}$ from grass leaves correlated with RH as expected for an evaporative kinetic isotope enrichment of the leaf water (e.g. Cernusak et al., 2016) imprinted on δ¹⁸O_{Phyto}. However, because grass stem and leaf phytoliths have the same morphology and are mixed in soil and sedimentary samples, these calibrations were not sufficient for using $\delta^{18}O_{Phyto}$ of grassland phytolith assemblages as a paleoclimatic signal. In tropical trees, silica is found in leaves, bark and wood and accounts for a few % d.w. (e.g. Collura and Neumann, 2017). In the wood, silica polymerizes in the secondary xylem supposedly unaffected by transpiration, in the form of Globular granulate phytolith types (Madella et al., 2005; Scurfield et al., 1974; Welle, 1976). These phytoliths make up more than 80% of tropical humid forest and rainforest phytolith assemblages found in soils and sediments (Alexandre et al., 2013; Collura and Neumann, 2017; Scurfield et al., 1974; Welle, 1976). Examination of the $\delta^{18}O_{Phyto}$ of rainforest assemblages showed correlations with the $\delta^{18}O$ of precipitation ($\delta^{18}O_{Pre}$) and the atmospheric temperature (Alexandre et al., 2012). However, in this case, the use of $\delta^{18}O_{Phyto}$ did not further develop because it was applicable only to forested areas and humid climatic periods, which is a major drawback for paleoclimatic reconstructions.

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The triple isotope composition of oxygen in the water molecule represents an alternative for investigating RH conditions prevailing during water evaporation. In the triple isotope system, the mass-dependent fractionation factors between A and B ($^{17}\alpha_{A-B}$ and $^{18}\alpha_{A-B}$) are related by the exponent θ_{A-B} ($^{17}\alpha_{A-B} = ^{18}\alpha_{A-B}^{\theta}$ or $\theta_{A-B} = \ln^{17}\alpha_{A-B}$ / $\ln^{18}\alpha_{A-B}$). The exponent can also be expressed as $\theta_{A-B} = \Delta'^{17}O_{A-B}$ / $\Delta'^{18}O_{A-B}$ with $\Delta'^{17}O_{A-B} = \delta'^{17}O_{A-}\delta'^{17}O_{B}$, $\Delta'^{18}O_{A-B} = \delta'^{18}O_{A-}\delta'^{18}O_{B}$, $\delta'^{17}O = \ln(\delta^{17}O + 1)$ and $\delta'^{18}O = \ln(\delta^{18}O + 1)$. In the $\delta'^{17}O$ vs $\delta'^{18}O$ space, λ_{A-B} represents the slope of the data alignment during a mass-dependent fractionation process between A and B. λ_{A-B} is an empirical way to assess θ_{A-B} (Li et al., 2017). It has been recently estimated that θ equals 0.529 for liquid-vapor equilibrium (θ_{equil} ; Barkan and Luz, 2005) and 0.518 for vapor diffusion in air (Barkan and Luz, 2007). It has additionally been shown that meteoric waters plot along a line with a slope λ of 0.528 \pm 0.001. The departure from the meteoric water line is conventionally called ^{17}O -excess (^{17}O -excess $= \delta'^{17}O$ - 0.528 x $\delta'^{18}O$) (Luz and Barkan, 2010). In case of mass-dependent

fractionation processes, the magnitudes of the ¹⁷O-excess in waters and minerals are very small and measurement of the ¹⁷O-excess, expressed in per meg (10⁻³%) vs VSMOW, requires very high analytical precisions.

211 In the water cycle, the ¹⁷O-excess variations mainly result from diffusion processes, while 212 equilibrium fractionation does not lead to important departure from the meteoric water line. 213 Theoretical and empirical estimations have shown that in contrast to d-excess, and except at very 214 high latitudes, changes in water ¹⁷O-excess are not significantly impacted by temperature (~0.1 per 215 meg / °C; Uemura et al., 2010) and much less sensitive to distillation processes (Angert et al., 216 2004; Barkan and Luz, 2007; Landais et al., 2008; Uemura et al., 2010; Steig et al., 2014). Changes 217 in water ¹⁷O-excess are thus essentially controlled by evaporative kinetic fractionation. The ¹⁷O-218 excess decreases in the evaporating water and increases in the vapor phase when RH decreases at 219 evaporative sites (e.g. sea surface, lake surface, soil surface or leaf surface). Over the last ten 220 years, a few studies used the ¹⁷O-excess of water to interpret ice core archives in climatic terms 221 (Guillevic et al., 2014, Schoeneman et al., 2014; Winkler et al., 2012; Landais et al., 2008, 2012). 222 They supported that ¹⁷O-excess is a marker of RH, sea-ice extent at the moisture source, and air 223 mass mixing (Risi et al., 2010) except at the very high latitudes of East Antarctica where 224 temperature can have a significant influence. The observed variations of ¹⁷O-excess in Greenland 225 ice cores of ~20 per meg maximum were thus interpreted as variations of RH or sea-ice extent at 226 the source region and coincide with variations in the low to mid latitude water cycle as recorded 227 by other proxies (such as CH₄ or δD of CH₄) (Guillevic et al., 2014). An even smaller number of 228 studies measured or attempted to model the ¹⁷O-excess of rainwater at low and temperate latitudes 229 (Affolter et al., 2015; Landais et al., 2010b; Li et al., 2015; Luz and Barkan, 2010; Risi et al., 230 2013). The observed variations in ¹⁷O-excess, partly explained by convective processes and re-231 evaporation of precipitation, were of the order of 30-40 per meg, either during a rainy event or 232 along climatic gradients. Only two studies focused on open surface waters, and showed that 233 variations of the ¹⁷O-excess ranged from tens to hundreds of per meg when the surface water 234 underwent strong evaporative enrichment (Surma et al., 2015; Luz and Barkan, 2010), in 235 agreement with the Craig and Gordon (1965) formulation. The most important variations in ¹⁷O-236 excess occur at the plant-atmosphere interface. In leaf water, variations higher than 200 per meg 237 were encountered (Landais et al., 2006; Li et al., 2017). Difference in ¹⁷O-excess between leaf 238 water subject to evaporation (LW) and stem water (SW) not subject to evaporation, increased with 239 decreasing RH (from 100 to 30 %), as expected for processes dominated by kinetic fractionation. 240 When measuring a sequence of LW-SW couples sampled under different climatic conditions, the slope of the line linking their triple isotope composition and named λ_{transp} , equivalent to θ_{LW-SW} , was 241 242 found to change with RH. This pattern was neither influenced by the plant species nor by the 243 environmental conditions (e.g. atmospheric temperature, soil water conditions) (Landais et al., 244 2006). However opposite trends of λ_{transp} with RH were observed from one study to another 245 (Landais et al., 2006; Li et al., 2017). This discrepancy was attributed to the possibility that steady 246 state is not always reached during sampling and to likely differences in isotope composition of the

- 247 ambient vapor, a parameter of the Craig and Gordon model that is often not measured but estimated
- 248 (Li et al., 2017).
- 249 While ¹⁷O-excess measurements of waters were expanding, analyses of the triple oxygen isotope
- 250 composition of minerals (mostly silicates and carbonates) were also developed, allowing estimate
- 251 of fractionation during polymerization and providing constraints on both temperature and isotope
- 252 composition of the water source (Pack and Herwartz, 2014; Levin et al., 2014; Passey et al., 2014;
- 253 Herwartz et al., 2015; Miller et al., 2015; Sharp et al., 2016). Variations of ¹⁷O-excess of the order
- 254 of tens to hundreds of per meg were reported from one mineral to another. For most of the studies
- 255 cited above, the objective was to discriminate between high and low temperature formation
- 256 processes or to decipher from which type of water the mineral formed (i.e. sea water, hydrothermal
- 257 water, meteoric or surface water). The ¹⁷O-excess of biogenic and sedimentary carbonates was
- 258 also investigated as a potential record of evaporating water sources (Passey et al., 2014). With
- 259 regard to silicate-water fractionation, the relationship between the three oxygen isotopes defined
- 260 by $\theta_{SiO2\text{-water}}$ was estimated between 0.521 and 0.528, increasing logarithmically with temperature
- (Sharp et al., 2016). 261

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- 262 In the present study, in the light of the recent findings cited above, we examined whether changes
- in atmospheric RH imprint the ¹⁷O-excess of phytoliths (¹⁷O-excess_{Phyto}) in a measurable way and 263
- 264 whether this imprint offers a potential for reconstructing past RH. For that purpose, we first
- monitored the ¹⁷O-excess evolution of soil water, grass leaf water and grass phytoliths in response 265
- 266 to changes in RH in a growth chamber experiment. Then, we measured the ¹⁷O-excess_{Phyto} from
- 57 phytolith assemblages collected in soil tops along a RH and vegetation transect in inter-tropical 267
- West and Central Africa. Relationships between ¹⁷O-excess_{Phyto} and RH were looked for and 268
- 269 assessed on the basis of previous quantifications of kinetic isotope enrichment of leaf water and
- 270 equilibrium fractionation between water and silica. Results from the natural sampling were
- 271 compared to the ones from the growth chamber experiment to evaluate the importance of RH in
- 272 controlling ¹⁷O-excess_{Phyto} in natural environment.

2 Materials and methods

2.1 Samples from the growth chamber experiment

- 275 Festuca arundinacea, commonly referred to as tall fescue, is widely distributed globally as forage
- 276 and an invasive grass species (Gibson and Newman, 2001) and can adapt to a wide range of
- 277 conditions. In 2016, F. arundinacea (Callina RAGT Semences) was grown in three chambers
- 278 under three conditions of RH (ca. 40, 60 and 80 %) kept constant using wet air introduction and
- 279 ultrasonic humidifier. We checked that the humidifiers did not lead to any isotope fractionation
- 280 between the water in their reservoirs and the vapor delivered. Temperature and light intensity were
- kept constant at 25 ± 0.6 (SD) °C and 293 ± 14 (SD) mmol / m²/ sec respectively. 281
- 282 In a 35 L tank (53 x 35 x 22 cm), 20 kg of dried commercial potting soil were packed above a 1.6
- 283 cm layer of quartz gravel. A porous cup for water extraction was placed in the soil with its
- 284 extraction tube hermetically extending outside of the tank walls. The soil was irrigated with 10 L

- of the same water as the one used for the humidifier. Four grams of seeds were sown along four
- rows in each tank, resulting in about 6000 seedlings. Each tank was then placed in a chamber and
- was irrigated from a Mariotte bottle (25 L) placed next to it. The Mariotte system was set so that
- a water saturated level of 5 cm remained constant at the base of the tank. The irrigation water was
- supplemented with 105 mg/L of SiO₂ (in the form of SiO₂ K₂O). Ten days after germination, agar-
- agar (polysaccharide agarose) was spread on the soil surface around the seedlings (about 8 cm
- tall), to prevent any evaporation (Alexandre et al., 2016).
- A fourth tank was kept at 100% of RH thanks to the installation of a 20 cm high plexiglass cover,
- in a forth chamber set at 80 % of RH. In this case no agar-agar was added and the vapor around F.
- 294 *arundinacea* came from evaporation and transpiration of the soil water. Otherwise the treatment
- was the same as in the other chambers.
- 296 For each humidity condition, three to four harvests were made at intervals of 10-14 days. The 20-
- 297 25 cm long leaves were cut at two cm above the soil level and weighed. From the first to the fourth
- sampling, the harvested wet leaves increased from 15-20 g (10 days of growth) to 40-60 g (14 days
- of growth). Three to five g of leaves were put in glass gastight vials and kept frozen for bulk leaf
- water extraction. The remaining leaves were dried for phytolith extraction. Forty mL of irrigation
- water from the Mariotte bottle, and of soil water from the porous cup, were kept at 5°C before
- analyses.

- After each harvest, the tanks were left in their chamber of origin but the 40, 60 and 80 % RH
- treatments were rotated between the growth chambers so that the four replicates of a given RH
- treatment would come from at least two different chambers. The 100 % humidity was set up in a
- 306 unique chamber during the entire duration of the experiment. The harvested leaves in this treatment
- were often covered by condensation drops which were blotted between two sheets of wiping paper,
- rapidly after harvesting. The experimental setup details and the harvest list are given in table 1.

2.2 Samples from the natural climate transects

- Fifty-seven top soil samples were collected during several field trips along vegetation and humidity
- transects in Mauritania and Senegal (Bremond et al., 2005b; Lézine, 1988; Pasturel, 2015)
- 312 (Lezine, 1988) Gabon (Lebamba et al., 2009) and Congo (Alexandre et al., 1997) in the saharian,
- sahelian, sudanian, guinean and congolian bioclimatic zones, respectively (White et al., 1983).
- 314 Samplings, phytolith extractions and phytolith morphological assemblages descriptions are given
- in the above-mentioned studies, except for the samples of Gabon from which phytoliths were
- 316 chemically treated and counted in the frame of the present study.
- The sampled site location as well as the associated climatic and oxygen isotope variables are given
- in Table 2. The vegetation overlying the sampled soils was categorized into savanna (Mauritania,
- Senegal), wooded savanna (Senegal), humid forest (Gabon and Congo) and enclosed savanna
- 320 (Gabon). For each sampled site, yearly climate average were calculated from the monthly means
- of temperature, precipitation, RH and diurnal temperature, extracted from the Climate Research
- Unit (CRU) 1961 1990 time series (10' spatial resolution; http://www.cru.uea.ac.uk, Harris et al.,

323 2013, CRU 2.0). Mean Annual Precipitation (MAP), Mean Annual Temperature (MAT) and mean 324 annual RH range from 49 to 2148 mm, 24.3 to 29.8 °C and 40.2 to 82.5 %, respectively. In 325 addition, in order to get a proxy of RH during wet months, likely those of the grass growing season, 326 averaged RH monthly means for months with at least one day with precipitation higher than 0.1 327 mm (RH-rd0>1) was calculated. It ranges from 56.3 to 82.5 %. As maximum transpiration is 328 supposed to be reached around 15:00 UTC we also calculated RH and RH-rd0>1 at 15:00 (RH15 329 and RH15-rd0>1, respectively) according to New et al. (2002) and Kriticos et al. (2012). For each 330 sampling site, estimates of $\delta^{'18}O$ of precipitation for the months with at least one day with precipitation higher than 0.1 mm ($\delta^{'18}O_{Pre-rd0>1}$) were calculated from $\delta^{18}O$ of precipitation 331 extracted from The Online Isotopes in Precipitation Calculator-version OIPC2-2 332 333 (http://www.waterisotopes.org; Bowen and Revenaugh, 2003; Bowen and Wilkinson, 2002; Bowen et al., 2005) and weighted by the amount of precipitation. The estimates range from -1.515 334 to -4.464 ‰. There is currently no data on the ¹⁷O-excess of precipitation (¹⁷O-excess_{Pre}) at these 335 336 sites.

2.3 Phytolith chemical extractions

- Phytoliths from soils were extracted following Crespin et al. (2008) using HCl, H₂O₂, C₆H₅Na₃O₇ and Na₂O₄S3-H₂O at 70 °C, and a ZnBr₂ heavy liquid separation. It has been shown that up to a
- temperature of 70 °C the extraction has no effect on the δ^{18} O (Crespin et al., 2008). We verified
- that it did not have any effect on the ¹⁷O-excess either, using our internal standard MSG extracted
- at 60 and 70°C (Crespin et al., 2008). The obtained ¹⁷O-excess values were similar (-211 and -243
- per meg, respectively) given our reproducibility of ± 34 per meg (see section 2.6.1). Phytoliths
- 344 from Festuca arundinaceae were thus extracted using a high purity protocol with HCl, H₂SO₄,
- 345 H₂O₂, HNO₃, KClO₃ and KOH at 70 °C following Corbineau et al. (2013).

346 **2.4 Phytolith counting**

- Phytolith assemblages from the humidity transects were mounted on microscope slides in Canada
- 348 Balsam, for counting, at a 600X magnification. More than 200 identifiable phytoliths with a
- diameter greater than 5 µm and with a taxonomic significance were counted per sample. Three
- repeated counting gave an error of \pm 3.5 % (SD). Phytoliths were named using the International
- Code for Phytolith Nomenclature 1.0 (Madella et al., 2005) and categorized as Globular granulate
- type produced by the wood (Scurfield et al., 1974; Kondo et al., 1994), palm Globular echinate
- 353 type and grass types comprising Acicular, Bulliform, Elongate psilate, Elongate echinate,
- the state of the s
- Bulliform cells, and Grass Short Cells types. For each sample from the natural transects, the
- phytolith index d/p, a proxy of tree cover density (Alexandre and Bremond, 2009; Bremond et al.,
- 356 2005a), was calculated. It is the ratio of Globular granular phytolith category (Madella et al., 2005)
- formed in the secondary xylem of the dicotyledon (d) wood to the grass short cell phytolith
- category formed in the epidermis of grasses or Poideae (p) (Collura and Neumann, 2017; Scurfield
- et al., 1974; Welle, 1976). Those two categories make up most of the phytolith assemblages
- recovered from inter-tropical soils (Alexandre et al., 1997, 2013; Bremond et al., 2005b, 2005a).

- 361 Phytolith assemblages from the F. arundinacea samples were also mounted and counted. The
- 362 phytolith types were categorized according to their cell of origin in the epidermis into Epidermal
- 363 short cell, Epidermal long cell, Bulliform cell and Hair acicular.

2.5 Leaf and soil water extraction

- 365 Leaf water was extracted using a distillation line. Leaves were introduced in a glass tube connected
- 366 to the distillation line, and frozen through immersion of the glass tube in liquid nitrogen. While
- 367 keeping the sample frozen, the distillation line was pumped to reach a vacuum higher than 5.10⁻²
- 368 mbar. The pumping system was then isolated and the glass sample tube warmed to 80°C.
- 369 Meanwhile, at the other end of the distillation line, a glass collecting tube was immersed in liquid
- 370 nitrogen to trap the extracted water. To avoid condensation, the line between the sample tube and
- 371 the collection tube was heated with a heating wire. The distillation was completed after six hours.
- In order to remove volatiles from the extracted water, a few granules of activated charcoal were 372
- 373 added and the water slowly stirred for 12 h.
- 374 Soil water was extracted using a 31mm porous ceramic cup. Brown or yellow-colored samples
- 375 were filtered at 0.22µm, but remained colored after filtration, indicating the presence of soluble
- 376 compounds.

364

377 2.6 **Isotope analyses**

378 The oxygen isotope results are expressed in the standard δ -notation relative to VSMOW.

379 2.6.1 Phytoliths

- 380 Phytolith samples of 1.6 mg were dehydrated and dehydroxylated under a flow of N₂ (Chapligin
- 381 et al., 2010) and oxygen extraction was performed using the IR Laser-Heating Fluorination
- 382 Technique at CEREGE (Aix-en-Provence, France) (Alexandre et al., 2006, Crespin et al., 2008;
- 383 Suavet et al., 2010). The purified oxygen gas (O₂) was passed through a -114 °C slush to refreeze
- 384 gases interfering with the mass 33 (e.g. NF), potentially produced during the fluorination of
- 385 residual organic N, before being sent to the dual-inlet mass spectrometer (ThermoQuest Finnigan
- 386 Delta Plus). The composition of the reference gas was determined through the analyses of NBS28
- for which isotope composition has been set to $\delta^{18}O=9.60$ %, $\delta^{17}O=4.99$ % and δ^{17} 387
- 388 per meg. During the measurement period, reproducibility (SD) of the analyses of the working
- 389
- quartz standard (Boulangé 2008) against which the isotope composition of the sample gas was
- 390 corrected on a daily basis (3 quartz standards were analysed per day) was $\pm 0.20 \%$, $\pm 0.11 \%$ and
- \pm 22 per meg for δ^{18} O, δ^{17} O and δ^{17} O-excess respectively (n = 63; one run of eight dual inlet 391
- 392 measurements). For every session of measurement, the effectiveness of the entire dehydration and
- 393 IR-Laser-Fluorination-IRMS procedure was checked through the analysis of a working phytolith
- standard (MSG60) with $\delta^{18}O = 36.90 \pm 0.78$ %, $\delta^{17}O = 19.10 \pm 0.40$ % and $\delta^{17}O$ -excess = -215 ± 394
- 395 34 per meg (n = 29). For comparison, the inter-laboratory pooled value for MSG60 is δ^{18} O = 37.0
- 396 ± 0.8 % (Chapligin et al., 2011). Recent measurements of the silicate reference materials UWG-2
- 397 garnet (Valley et al., 1995) and San Carlos (SC) oliving gave the following values: $\delta^{18}O_{\text{HWG-2}} =$
- 5.72 ± 0.12 %, $\delta^{17}O_{UWG-2} = 2.95 \pm 0.06$ %, $\tau^{17}O_{excess}$ $\tau^{17}O_{excess}$ $\tau^{17}O_{excess}$ $\tau^{17}O_{excess}$ $\tau^{17}O_{excess}$ $\tau^{17}O_{excess}$ $\tau^{17}O_{excess}$ 398

- 4.95 ± 0.22 %, $\delta^{17}O_{SC} = 2.56 \pm 0.12$ %, $^{17}O_{excess} = -49 \pm 24$ per meg (n = 3). For comparison,
- silicate analyses presented in Sharp et al. (2016) are normalized to a δ^{18} O value for San Carlos
- Olivine of 5.3 % and a ¹⁷O-excess value of -54 per meg. As previously discussed in Suavet et al.
- 402 (2010), a large scatter is often observed for SC olivine δ^{18} O and δ^{17} O values measured in a given
- laboratory or from a laboratory to another. This is probably attributable to the heterogeneity of the
- analyzed samples. At CEREGE, the internal standard of SC olivine is prepared from a number of
- millimetric crystals with possibly different oxygen isotope composition. The δ^{18} O and δ^{17} O values
- 406 from Suavet et al. (2010), Tanaka and Nakamura (2013) Pack et al. (2016), Sharp et al. (2016) and
- 407 the present study average 5.29 ± 0.23 (1 SD) % and 2.72 ± 0.12 (1 SD) %, respectively.
- Nevertheless, despite the large SD on 18 O and δ^{17} O measurements, the SC olivine 17 O-excess
- appears relatively constant (-71 \pm 23 (1 SD)) per meg.

410 **2.6.2** Leaf water

- Leaf water was analyzed at LSCE (Gif sur Yvette, France) following the procedure previously
- detailed in Landais et al. (2006). In summary, a fluorination line was used to convert water to
- oxygen using CoF₃ heated at 370°C in a helium flow. The oxygen was then trapped in a tube
- 414 immersed in liquid helium before being analyzed by dual inlet IRMS (ThermoQuest Finnigan
- Delta V mass spectrometer) against a reference oxygen gas. All measurements were run against a
- working O₂ standard calibrated against VSMOW. The resulting precisions (2 runs of 16 dual inlet
- 417 measurements) were 0.02 % for both δ^{17} O and δ^{18} O and 5 per meg for 17 O-excess.

418 **2.6.3** Irrigation and soil waters

- 419 Irrigation and soil water were analyzed at the Ecotron of Montpellier (France) with an isotope laser
- analyzer (Picarro L2140i) operated in ¹⁷O-excess mode using an auto-sampler and a high precision
- 421 vaporizer. Each water sample was used to fill three vials randomly dispatched in four groups of
- six samples (three replicates per sample). Each sample group was bracketed by three working
- standards (Giens-1, Iceberg-1 and Eco-1). Ten injections were performed for each vial, and the
- results of the first six injections were discarded to account for memory effects. Following IAEA
- recommendations (IAEA, 2013), each liquid measurement sequence was started with two vials of
- 426 deionized water for instrument conditioning.
- The isotope compositions of each sample group were calibrated using the three interpolated mean
- values obtained for the bracketing working standards (Delattre et al., 2015). All isotope ratios were
- and a normalized on the VSMOW2/SLAP2 scale, with an assigned SLAP2 ¹⁷O-excess value of zero,
- following the recommendations of Schoenemann et al. (2013). The resulting precisions (3
- 431 replicates) were 0.02 ‰, 0.01 ‰ and 10 per meg for δ^{17} O, δ^{18} O and δ^{17} O-excess (n=31).
- 432 The three working standards were also analyzed using the fluorination/IRMS technique used for
- leaf water analyses at LSCE. The ¹⁷O-excess maximum difference was 6.4 per meg, which is lower
- 434 than the analytical precision obtained using the laser spectrometer (Table S1a).
- In order to assess that soluble organic compounds present in some soil water samples did not
- impact the laser analyzer isotope measurements (Martín-Gómez et al., 2015), a representative set

- of colored samples were analyzed with and without the Picarro micro combustion module (MCM)
- set up between the high precision vaporizer and the analyzer inlet. This system was designed to
- partly remove organic volatile compounds using a catalytic process. The obtained isotope
- compositions were not significantly different (Table S1b), suggesting that organic compounds
- were either in low concentration, and/or did not interfere in the spectral window used by the
- analyzer. Therefore, the other soil water samples were analyzed without the MCM.

443 3 Results

444

3.1 Growth chamber experiment

- δ^{18} O and δ^{17} O-excess of the irrigation water (respectively δ^{18} O_{IW} and δ^{17} O-excess_{IW}) average -5.59
- $\pm 0.006\%$ and 26 ± 5 per meg, respectively. $\delta^{18}O$ and $\delta^{17}O$ -excess of the soil water (respectively
- δ^{18} Osw and 17 O-excesssw) average -2.89 \pm 0.19 ‰ and 16 \pm 8 per meg, respectively (table S2).
- The isotope difference is thus significant for $\delta^{'18}$ O, less significant for 17 O-excess, according to
- 449 the analytical error. Although evaporative kinetic fractionation of the top soil water suctioned by
- 450 the porous cup under vacuum cannot be ruled out, isotopic exchanges between the soil water and oxygen-
- bearing phases of the rhizosphere may also have impacted the soil water isotopic composition
- 452 (Bowling et al., 2017; Chen et al., 2016; Oerter et al., 2014; Orlowski et al., 2016). Hereinafter,
- 453 we consider the isotope signatures of the water absorbed by the roots of F. arundinacea to be
- equivalent to the irrigation water that fed the saturation level at the base of the tank. This water
- was reached by the deepest roots, as observed on a cross-section of the soil after the end of the
- experiment, and likely reached the upper roots by capillarity.
- The transpiration of F. arundinacea increases rapidly from 0.03 to 0.6 L / day from 100 to 60 %
- RH and more slowly from 60 to 40 % RH where it reaches 0.61 L / day (averages of the replicates,
- Table 1). In response to decreasing RH, δ^{18} O (table S2) and δ^{17} O-excess (fig. 1a) values of the bulk
- 460 leaf water (δ'¹⁸O_{LW} and ¹⁷O-excess_{LW}) show clear increasing and decreasing trends, respectively.
- The averaged ¹⁸O-enrichment of bulk leaf water relatively to irrigation water (Δ '¹⁸O_{LW-IW})
- increases from 100 to 60 % of RH and seems to be stabilizing from 60 to 40 % RH (fig. 1b; Table
- 463 1). For 100 % RH, the high standard deviations (SD) associated with δ^{18} O_{LW} (table S2), and
- 464 consequently with $\Delta^{'18}O_{LW-IW}$ (Table 1), are due to the very high $\delta^{18}O_{LW}$ value of sample P3-100-
- 465 10-05-16. However, as we do not have any explanation for this high value, this data was not
- excluded from further calculation. The 17 O-excess values associated with the enrichment $\Delta^{'18}$ O_{LW}
- 467 _{IW} (or ¹⁷O-excess_{e I,W-IW} = Δ '¹⁷O _{I,W-IW} 0.528 x Δ '¹⁸O _{I,W-IW}) are scattered for a given RH. The
- averaged value however follows a clear pattern (fig. 1c; table 1): it decreases slowly from 100 to
- 80 % RH (from -88 \pm 48 to -75 \pm 20 per meg,) and more rapidly from 80 to 40% RH where it
- reaches -159 \pm 9 per meg. When the relationship is linearized, the slope of the line between ¹⁷O-
- excess_{e I.W-IW} and 40 to 80 % RH is 2.3 per meg/% (fig. 1f). The raw values of $\theta_{I.W-IW}$ do not show
- any significant trend with RH and average 0.519 ± 0.002 . The slope λ_{LW-IW} of the line linking
- 473 $\Delta^{'17}O_{LW-IW}$ and $\Delta^{'18}O_{LW-IW}$ (table 1) is 0.518.
- The average phytolith content ranges from 1.1 to 0.1% d.w. Silicification of the leaf blade of F.

475 arundinacea increases with increasing transpiration and decreasing humidity (Table 1). Phytolith

476 morphological identification shows that they formed preferentially in the epidermal short cell and

477 to a smaller extent in the epidermal long cells (fig. 2). The proportion of silicified long cells,

- 478 increases with increasing transpiration and decreasing RH (Table 1). Some hair and bulliform cells
- were also silicified, but in much smaller quantities. $\delta^{18}O$ and $\delta^{17}O$ -excess of phytoliths ($\delta^{18}O_{Phyto}$
- and ¹⁷O-excess_{Phyto} respectively) show the same general trends with RH as δ'¹⁸O_{LW} and ¹⁷O-
- 481 excess_{LW} (fig. 1a, table S2).
- The average value of the 18 O-enrichment of phytoliths relative to the bulk leaf water (Δ ' 18 O_{Phyto-}
- 483 LW) increases slowly (from 27.97 ± 6.97 to $28.47 \pm 0.38\%$) when RH decreases from 100 to 80 %
- and more rapidly from 80 to 40% where it reaches 32.32 ± 1.92 % (fig. 1b, Table 1). With regard
- to the enrichment of phytoliths relative to the irrigation water, $\Delta'^{18}O_{Phyto-IW}$ shows the same trend
- with RH as $\Delta^{'18}O_{LW-IW}$ (fig. 1b, table 1). ¹⁷O-excess_{e Phyto-IW} shows the same decreasing trend with
- RH as ¹⁷O-excess_{e LW-IW} (fig. 1c, Table 1). When the relationship is linearized, the slope of the line
- between ¹⁷O-excess_{e Phyto-IW} and 40 to 80 % RH is 4.3 per meg/% (fig. 1f). A Student's t-test
- (relevant when the variance of two data sets are equal; Andrade and Estévez-Pérez, 2014),
- calculated on the ¹⁷O-excess_{e LW-IW} vs RH and ¹⁷O-excess_{e Phyto-IW} vs RH data sets shows that the
- slopes of the lines are not statistically different for a 75% confidence interval. Thus, the link
- between ¹⁷O-excess_{e Phyto-IW} and RH is mainly due to the leaf water ¹⁷O-excess dependency to RH.
- The raw values of $\theta_{Phyto-LW}$ appears constant, averaging 0.52 ± 0.001 (table 1).

494 **3.2 Natural samples**

- Values of $\delta^{'18}O_{Phyto}$ and $^{17}O_{Phyto}$ range respectively from 23.79 to 38.16 % and from -140
- 496 to -290 per meg (table 2). The variations are in the same order of magnitude as for the growth
- chamber experiment. The estimates of $\delta^{18}O_{Pre}$ vary little along the sampled transect (from -4.46 to
- 498 –3.22 ‰). No relationship is observed between $\delta^{'18}O_{phyto}$ or the $^{18}O_{phyto}$ or the phytoliths
- relatively to precipitation ($\Delta'^{18}O_{Phyto-Pre}$) and MAP, MAT or RH (fig. 3, table 2).
- Although scattered, the ¹⁷O-excess_{Phyto} values show a significant positive linear correlation with
- RH (fig. 4), regardless of which RH variable is taken into account. After excluding two outliers,
- the slopes of the correlation lines are 2.1 and 2.2 when RH and RH15 are taken into account, 3.4
- when either RH-rd0>1 or RH15-rd0>1 are considered. The relationship obtained between ¹⁷O-
- excess_{Phyto} and RH-rd0>1 (i.e. RH of the wet months) is the closest to the one obtained between
- 505 ¹⁷O-excess_{phyto} and RH in the growth chambers (fig. 4b). It can be expressed as follows (Eq.3):

506
$$^{17}\text{O-excess}_{\text{phyto}} = 3.4 \text{ x (RH-rd0>1)} - 460$$
 $(r^2 = 0.48; p < 0.48)$

$$(r^2 = 0.48; p < 0.001)$$
 Eq. 1

- where ¹⁷O-excess_{phyto} is expressed in per meg vs VSMOW and RH in %.
- The excluded outliers (Table 3) are RIM1 and C3L4. RIM1 presents a very low ¹⁷O-excess (-305)
- per meg) relative to the ¹⁷O-excess of the samples with close RH-rd0>1, i.e. from 71 to 74 %
- 510 (average of -237 ± 32 per meg for 82-78, 83-116 and 83-115). C3L4 is located next to C4L3 and
- under similar averaged RH but presents a ¹⁷O-excess higher by 133 per meg. RIM1 and C3L4

- 512 show morphological patterns very similar to the other assemblages with the same range of RH.
- 513 Thus, the discrepancies may lie either in the fact that local RH variations may not be reflected in
- 514 RH averaged estimates for 10' ($\approx 185 \text{ km}^2$) or in the particularity of the isotope composition of the
- 515 local soil water (see discussion below).
- 516 The phytolith index d/p ranges from 0.01 to 0.08 in savanna, from 0.14 to 0.49 in wooded savanna,
- 517 from 0.76 to 1.58 in enclosed savanna and from 1.84 to 6.78 in humid forests (Table 2). This
- 518 unambiguous increase of d/p with tree cover density is in agreement with previous calibrations
- 519 performed for the West African area (Bremond et al., 2005b). Interestingly, under high RH
- 520 conditions, humid forest and enclosed savanna that are characterized by a large range of d/p
- represent a small range of ¹⁷O-excess. Conversely, under lower RH conditions, savanna and 521
- 522 wooded savanna that are characterized by a small range of d/p represent a large range of ¹⁷O-excess
- 523 (fig.5). This absence of relationship between ¹⁷O-excess and tree cover density is also mirrored in
- 524 figure 4 where phytolith samples from different vegetation types (i.e. savanna vs wooded savanna
- 525 or humid forests vs enclosed savanna), that have developed under the same RH conditions, have
- 526 the same range of ¹⁷O-excess.

527 Discussion 4

528

4.1 Imprint of changes in atmospheric RH on the ¹⁷O-excess of leaf water

- In the bulk leaf water, the trends observed between $\Delta^{'18}$ O_{LW-IW} or ¹⁷O-excess_{e LW-IW} and RH are in 529
- 530 agreement with an evaporative kinetic fractionation that increases when RH decreases, as expected
- 531 from previous studies on the δ^{18} O or ¹⁷O-excess evolution of leaf water (e.g. Cernusak et al., 2016;
- 532 Landais et al., 2006; Li et al., 2017). The obtained values of θ_{LW-IW} average (0.519) and of λ_{LW-IW}
- 533 (0.518) are respectively close and similar to the value of $\theta_{\rm diff}$ calculated for the diffusion of vapor
- 534 in air (0.518; Barkan and Luz, 2007). As schematically described in Landais et al. (2016), λ_{transp}
- 535 (equivalent to λ_{LW-IW}) represents the interplay among three processes in the leaf boundary layer:
- 536 1) the equilibrium fractionation, which is only temperature-dependent (Majoube, 1971) and drives
- the isotope composition of leaf water along the equilibrium water line ($\theta_{\text{equil}} = 0.529$); 2) the 537
- 538 diffusion transport leading to increasing kinetic fractionation with decreasing relative humidity
- 539 along the diffusion line; 3) the isotope exchange of leaf water with atmospheric water vapor,
- 540 decreasing from turbulent to laminar and molecular leaf boundary layer vapor transport conditions
- 541 (e.g. Buhay et al., 1996). In the case of the growth chamber experiment, the fact that θ_{LW-IW}
- 542 and λ_{LW-IW} are respectively close and similar to θ_{diff} supports that the increasing diffusion of vapor
- in air when RH decreases or transpiration increases is the main process controling the evolution of 543
- 544 ¹⁷O-excess_{LW}. At high humidity (80-100% RH), the kinetic fractionation likely reaches its
- 545 minimum as the diffusion process becomes limited.
- 546 The $\delta^{18}O_{LW}$ is commonly modelled as a function of the isotope composition of absorbed water, the
- isotope composition of water vapor, and RH (Craig and Gordon, 1965). The Craig and Gordon 547
- 548 simple approach overestimates $\delta^{18}O_{LW}$ and different corrections have been proposed to take into
- 549 account the diffusion of the evaporating water back to the leaf lamina and the advection of less

550 evaporated stem water (i.e. the Péclet effect, Buhay et al., 1996; Helliker and Ehleringer, 2000; 551 Roden et al., 2000; Farquhar and Gan, 2003; Farquhar and Cernusak, 2005; Ripullone et al., 2008; 552 Treydte et al., 2014). In the growth chamber experiment, where water availability, relative 553 humidity, and temperature were kept constant, we assume that transpiration rapidly reached a 554 steady state and that the isotope composition of transpired water was the same as that of the 555 irrigation water entering the plant (e.g. Welp et al., 2008). A tentative estimate of the theoretical 556 value of $\Delta'^{18}O_{LW-IW}$, $\Delta'^{17}O_{LW-IW}$ and $\Delta'^{17}O_{LW-IW}$ and $\Delta'^{17}O_{LW-IW}$ was performed using the equations proposed 557 for ¹⁸O-enrichment by Cernusak et al. (2016) (table S3). For calculating the Δ^{17}_{LW-IW} we used for the equilibrium and kinetic fractionations (respectively $^{17}\alpha_{eq}$ and $^{17}\alpha_{k}$ in table S3) $^{17}\alpha_{eq}$ = $^{18}\alpha_{eq}$ $^{0.529}$ 558 and $^{17}\alpha_k = ^{18}\alpha_k$ $^{0.518}$. As expected, the predicted Δ'^{18} O_{LW-IW} values were all higher than the observed 559 values by several \%. Helliker and Ehleringer (2000) proposed, for monocotyledonous species 560 561 characterized by a vertical parallel veinal structure, to use instead of the Craig and Gordon model 562 the Gat and Bowser (1991) equation describing the movement of water through a sequence of pools in series. However this model would further increase the estimates of $\Delta^{'18}O_{I.W-IW}$. The 563 predicted ¹⁷O-excess_e displayed in Table S3 was either higher or lower than the observed ¹⁷O-564 excess_{e LW-IW}. Predicted θ_{LW-IW} increased with RH from 0.521 to 0.529 which is far from the 565 observed values averaging 0.519. The predicted value of 0.529 at 100 % RH reflects pure 566 567 equilibrium in a situation where irrigation water and water vapor are assumed to have similar 568 isotope composition since irrigation water is directly vaporized into the chamber (table S3), 569 without any fractionation. Sensitivity tests show that regardless of the model chosen (Buhay et al., 570 1996; Cernusak et al., 2016; Li et al., 2017), estimations of θ_{LW-JW} are very dependant on the isotope compositions of the water vapor (Li et al., 2017), not measured either in our experiment or in 571 572 previous studies (Landais et al., 2006; Li et al., 2017). In the natural environment, a first order 573 approximation for the isotope composition of water vapor is to consider equilibrium with 574 precipitation. As a result of water-vapor equilibrium fractionation and soil water ¹⁸O-enrichment, 575 this can lead to a water vapor ¹⁸O-depleted by 10-13 ‰ compared to the soil water (Landais et al., 576 2006; Lehmann et al., 2018). In this case the predicted λ_{transp} (equivalent to λ_{LW-SW}) decreases with 577 increasing humidity. Finally, because wrong values of the isotope compositions of the water vapor 578 may affect significantly the calculation of $\Delta'^{18}O_{LW-IW}$, $\Delta'^{17}O$ -excess_{e LW-IW} and θ_{LW-SW} , we call for 579 vapor isotope measurements as a prerequisite to accurately model the leaf water triple oxygen 580 isotope evolution with RH. However, overall, despite the uncertainties on the predicted evolution 581 of λ_{LW-SW} or θ_{LW-SW} with RH, the predicted value of ¹⁷O-excess_{e LW-IW} decreases when RH increases, 582 which is also observed, as well as reflected in the triple isotope composition of phytoliths, as 583 discussed below.

4.2 Imprint of changes in atmospheric RH on the ¹⁷O-excess of phytoliths

584

Polymerization of silica is supposed to occur in isotope equilibrium with the forming-water, and therefore, to be only governed by temperature and the isotope composition of the forming water. Almost a dozen temperature-dependant relationships have been empirically established between the δ^{18} O of quartz, sinters, cherts, diatoms or phytoliths and the δ^{18} O of their forming water

(δ¹⁸O_{PhytoFW}). Although the obtained fractionation coefficients are close (from -0.2 to -0.4 % °C⁻ ¹), the range of fractionation ($\Delta^{18}O_{Phyto-PhytoFW}$) is large (see synthesis in Alexandre et al., 2012). The $\Delta^{18}O_{Phyto-LW}$ values obtained in the frame of the growth chamber experiment (ranging from 27.9 ± 7.2 to $32.3 \pm 2.2\%$) encompass the $\Delta^{18}O_{Phyto-PhytoFW}$ of 31.1% calculated from the Dodd and Sharp (2010) relationship for 25°C. It is lower than the values of 36.4 and 36 ‰ at 25 °C, calculated from Sharp et al. (2016) and Alexandre et al. (2012). Whereas Alexandre et al. (2012) and Sharp et al. (2016) generally estimated the forming-water δ^{18} O values, Dodd and Sharp (2010) measured the the δ^{18} O values of the water samples. The proximity of the obtained range of $\Delta^{18}O_{Phyto-LW}$ values to the $\Delta^{18}O_{Phyto-Phyto FW}$ calculated from Dodd and Sharp (2010) suggests that phytoliths formed in equilibrium with a water of isotope composition close to that of the bulk leaf water. This is additionally supported by the obtained averaged value of $\theta_{Phyto-LW}$ (0.522 \pm 0.001) close to the $\theta_{SiO2\text{-water}}$ equilibrium value of 0.524 calculated for 25 °C from Sharp et al. (2016).

Evolution of the triple isotope composition of bulk leaf water and phytoliths can be illustrated by plotting $\delta'^{17}O$ vs $\delta'^{18}O$, or ^{17}O -excess vs $\delta'^{18}O$ (fig. 6) which is more appropriate to evidence small variations. Figure 6 shows that the leaf water evolved from the irrigation water pool, becomes increasingly subject to kinetic fractionation when RH decreased. This evolution follows a single leaf water line reflecting $\lambda_{LW-IW} = 0.518$ or $\theta = 0.519$ (Table1). Then, if phytoliths polymerized from the bulk leaf waters, at 25°C, according to a constant equilibrium fractionation, their expected isotope signature should follow a line parallel to the leaf water line. This is the case for phytoliths formed at RH higher than 40%. However, the isotope signature of phytoliths formed at 40% RH suggest a forming water more evaporated than the bulk leaf water. The Péclet effect, which is known to scale with transpiration (e.g. Barnard et al., 2007) can explain this discrepancy. Advection of less evaporated stem water may decrease $\delta'^{18}O_{LW}$ and increase ^{17}O -excess_{LW} relative to $\delta'^{18}O$ and ^{17}O -excess of the epidermal water prone to evaporation and from which phytoliths formed. At this point, the data scattering prevents further discussion but the possibility that when RH is low, or when transpiration is high, the phytolith forming-water is different from the bulk leaf water must be investigated in future research developments.

With regard to the natural samples, whereas no relationship was found between $\delta^{'18}O_{phyto}$ and RH, a clear positive linear dependency of ¹⁷O-excess_{phyto} to RH was shown, equivalent to 2.1 per meg / % when the annual RH average was taken into account, or to 3.4 per meg / % when the average of the growing season (RH-rd0>1) was taken into account (fig. 4). These coefficients are close to the slope of the lines obtained for the growth chamber experiment between ¹⁷O-excess_{Phyto}, ¹⁷O-excess_{e LW-IW} and ¹⁷O-excess_{e Phyto-IW} and 80 to 40% RH (fig. 1d, e and f). This consistency represents a major positive step in examining whether changes in atmospheric RH imprint the ¹⁷O-excess of natural phytolith assemblages in a predictable way. Without taking into account the two outliers, the linear regression between RH-rd0>1 and ¹⁷O-excess_{phyto} for a 95% confidence interval can be expressed as follows:

626 RH-rd0>1 = 0.14 ± 0.02 (S.E) x ¹⁷O-excess_{phyto} + 100.5 ± 4.7 (S.E) Eq. 2

where ^{17}O -excess_{phyto} is expressed in per meg and RH in %, r^2 = 0.48, and p < 0.001. S.E. stands for standard error. The S.E. of the predicted RH-rd0>1value is \pm 5.6%. However, the data scattering (fig. 4) call for assessing additional parameters that can contribute to changes in ^{17}O -excess_{phyto}, beside RH, before using the ^{17}O -excess_{phyto} for quantitative RH reconstruction.

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One can expect that the isotope composition of the soil water taken-up by the roots impacts ^{17}O -excess_{Phyto}. In tropical dry and humid areas, evaporative kinetic fractionation can lead to a ^{18}O -enrichment of the soil water of several ‰, in the first dm depth (e.g. Gaj et al., 2016; Liu et al., 2010). Spatial variability in the composition of the rainfall feeding the upper soil water may also intervene. However, the amount-weighted values of $\delta^{'18}\text{O}_{Pre}$ along the sampled transect vary little (Table 2). With regard to ^{17}O -excess, changes in soil water evaporation rather than the small variations expected for ^{17}O -excess_{Pre} (Landais et al., 2010b; Li et al., 2015) should impact the evolution of ^{17}O -excess_{Phyto}, although, here, the lack of measurements only allow for speculation.

The vegetation type and the plant part from which phytoliths come from may also bring some noise to the relationship between ¹⁷O-excess_{phyto} and RH. In grasses, leaf water is expected to be more prone to evaporative enrichment than stem water, and inside the leaf itself, the heterogeneity of evaporative sites repartition and water movements can lead to a significant heterogeneity in the δ^{18} O signatures of water and phytoliths (Cernusak et al., 2016; Helliker and Ehleringer, 2000; Webb and Longstaffe, 2002). Soil top phytolith assemblages likely record several decades of annual phytolith production and their isotope composition is expected to be an average. This would explain the consistency of the ¹⁷O-excess_{Phyto} data obtained from bulk grass from climate chambers and bulk grasses from natural savannas. However, further investigation on the extent of the heterogeneity of ¹⁷O-excess signature of water and phytoliths in mature grasses would help to clarify the links between water and phytolith signatures and better understand the phytolith proxy. In trees, the Globular granulate phytolith is assumed to come from the non-transpiring secondary xylem of the wood. Thus Globular granulate phytoliths should present an isotope signature closer to that of the soil water, or less impacted by kinetic fractionation than grass phytoliths. However, for a given range of RH, samples with significant representations of both phytolith categories (i.e. wooded savanna and enclosed savanna samples with d/p from 0.1 to 1.6) present ¹⁷O-excess values close to the values obtained by samples with very low or very high d/p (figs. 4 and 5). To further assess the significance of the Globular granulate isotope signature, we calculated δ'18O_{PhytoFW} values (Table 2) using the Dodd and Sharp (2010) fractionation factor and compared it to the precipitation-weighted δ'¹⁸O_{Pre-rd0>1} average. For the humid forest assemblages, δ'¹⁸O_{PhytoFW} values are higher than $\delta^{'18}O_{pre\ rd0>1}$ by 4.6 ± 1.5 %. This difference is larger than the range of $^{18}O_{pre\ rd0>1}$ enrichment observed for the upper 10 cm depth of soil water under tropical humid forests (2-3\%): Liu et al., 2008; Stahl et al., 2013), suggesting that evaporative isotope signatures of both soils and leaf water imprinted the Globular granulate phytolith type. This is in line with recent ¹⁸O-labelling experiment showing that the ¹⁸O-enriched oak phloem water may exchange with xylem water under low transpiration rates (Lehmann et al., 2018). Complementary examination of the isotope signature of phytolith assemblages from forests growing under different RH conditions (i.e dry forests, humid forests, rainforests), as well as further investigation of the anatomical origin of the Globular granulate phytolith type are now required to further discuss the meaning of the ¹⁷O-excess signal brought by wooded savanna and tropical forest phytolith assemblages.

Biases due to the calibration methodology may also be responsible for the data scattering. Imperfect adequacy between the space scales recorded by the soil top phytolith assemblages and the RH variables may come into play. Phytolith assemblages represent a mixture of local and wind-transported phytoliths. In the open saharian, sahelian and soudanian zones of West Africa the winter low altitude north-easterly trade winds may transport phytoliths southward, reducing differences between assemblages from different biogeographic zones and increasing differences among assemblages of a given biogeographic zone (Bremond et al., 2005b). Additional samples from other geographic zones are thus needed to increase the robustness of the relationship. With regard to the recorded time scales, the CRU RH 30 years averages are in agreement with the several decades of phytolith production.

5 Conclusion

The present combination of growth chamber and in *situ* transect calibrations lay the groundwork for further examination of the robustness of the ^{17}O -excess_{Phyto} as a proxy of changes in RH. The growth chamber experiment demonstrated that change in RH imprints ^{17}O -excess_{Phyto} (by 4.1 per meg / % between 40 and 80% RH) or the ^{17}O -excess_{e Phyto-IW} (by 4.3 per meg / %, between 40 and 80% RH) through its imprint on ^{17}O -excess_{e LW-IW}. As the isotope composition of the irrigation water was stable, and transpiration likely reached a steady state, the positive correlation between ^{17}O -excess_{LW} and RH was only governed by the kinetic fractionation occurring in the leaf epidermis water subject to evaporation, as supported by the value of $\theta_{\text{LW-IW}}$ of 0.517, close to θ_{diff} .

In order to model the triple oxygen isotope fractionation in play at the soil/plant/atmosphere interface we require direct and continuous measurements of the triple isotope composition of water vapor. Such measurements should develop in the near futur through the use of isotope ratio infrared analyzers (e.g. Berkelhammer et al., 2013; Schmidt et al., 2010). We also suggest to constrain as much as possible the isotope composition of the soil water taken up by the roots. Stem water is usually used as an analogue of soil water when modelling $\delta^{'17}O_{LW}$ and $\delta^{'18}O_{LW}$ (Landais et al., 2006; Li et al., 2017). However, in the stem, water in the phloem that is bidirectional (moves up and down the plant's stem) receives the contribution of evaporating leaf water, and water in the xylem that is unidirectional (moves up the plant's stem) may exchange with phloem waters (Lehmann et al., 2018). Consequently one may expect the isotope composition of stem water to be slightly different than that of soil water (Berkelhammer et al., 2013; Treydte et al., 2014).

When plotting ¹⁷O-excess_{Phyto} vs RH, the samples collected along the West and Central African relative humidity transect define a correlation coefficient ranging from 2.1 to 3.4 per meg / % (depending on the RH variable taken into account) and lay close to the growth chamber ¹⁷O-excess_{Phyto} line. This supports that RH is an important control of ¹⁷O-excess_{Phyto} in natural environment, even if phytolith assemblages come from different vegetation types. However, other parameters such as changes in the triple isotope composition of the soil water, vegetation source

- or imperfect adequation between the space scales recorded by the soil top phytolith assemblages
- and the RH variables may come into play and explain the scattering of ¹⁷O-excess_{Phyto}. Assessment
- of these parameters through additional growth chambers experiments and field campaigns will
- bring us closer to an accurate proxy of changes in relative humidity.

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Table 1. Growth chamber experiment: experimental set-up, phytolith content and morphological characteristics, isotope enrichments $(\Delta^{*}_{A-B} = {}^*\delta^{*}_{a} - {}^*\delta^{*}_{b})$, associated ¹⁷O-excess_e (¹⁷O-excess_e = Δ^{*17} – 0.528 x Δ^{*18}), θ ($\theta = \Delta^{*17}$ / Δ^{*18}) and λ values of phytoliths compared to either leaf water or irrigation water and of leaf water compared to irrigation water. Av : average; n : number of replicates; SD : standard deviation calculated on the replicates; n.v. : no value. Transp. (l/day), Conc. (% d.w.) and LC (%) stands for transpiration expressed in liter/day, phytolith concentration expressed in % of the dry weight and long cell abundance in the phytolith morphological assemblage expressed in % of counted phytoliths with taxonomic significance, respectively. Samples are named according to the climate chamber # they were collected in (e.g. P1, P2), the set relative humidity (e.g. 40, 60) and the date of sampling (e.g. 29-04-16 for dd/mm/yy).

			Expe	rimen	tal set-up			Phytoliths	(Phyto)		Leaf v	vater -irriga	ation water	(LW-IW)	Phy	tolith - leaf	water (Phy	rto-LW)	Phyto	Phytolith -irrigation water (Phyto-IW)					
Duration	Tem p.	SD	ВН	SD	Light	Transp.	Biomass	Sample	Conc.	TC	Δ ^{'18} O	$\Delta^{'17}{ m O}$	¹⁷ O-excess _e	θ	$\Delta^{78}O$	$\Delta^{'17}{ m O}$	¹⁷ O-excess _e	θ	$\Delta^{'18}{ m O}$	$\Delta^{'17}{ m O}$	¹⁷ 0-excess _e	θ			
day	°C		%		mmol/m²/se	c I/day	g		% d.w.	%		‰	per meg			‰	per meg			‰	per meg				
11	25	0.2	41.2	1	278		13	P1-40-29-04-16	5 n.v.		16.238	8.420	-154	0.519	33.776	17.589	-244	0.521	50.013	26.009	-398	0.520			
10	25	0.2	41.3	1.1	278	0.49	21	P10-40-10-05-16	0.8		13.171	6.799	-155	0.516	33.530	17.498	-206	0.522	46.701	24.297	-361	0.520			
11	25	0.4	41.9	1	311	0.69	37	P1-40-20-05-16	0.8	21	16.345	8.460	-170	0.518	29.577	15.401	-216	0.521	45.922	23.861	-385	0.520			
14	25	0.2	41.4	0.9	278	0.65	38	P1-40-03-06-16	1.8		n.v.	n.v.	n.v.	n.v.	32.415	16.874	-241	0.521	n.v.	n.v.	n.v.	n.v.			
					Av.	0.61			1.2		15.251	7.893	-159	0.517	32.324	16.840	-227	0.521	47.545	24.723	-381	0.520			
					SD	0.11			0.6		1.802	0.947	9	0.001	1.925	1.011	19	0.0006	2.172	1.135	19	0.0003			
11	25	0.5	60.2	2.5	311		21	P10-60-29-04-16	n.v.		15.115	7.864	-117	0.520	29.133	15.211	-171	0.522	44.248	23.075	-288	0.521			
11	25	0.2	60.5	1	289	0.57	33	P2-60-10-05-16	0.7		16.885	8.737	-178	0.517	25.877	13.575	-88	0.525	42.761	22.312	-266	0.522			
10	25	0.8	60.2	4.8	311	0.60	48	P10-60-20-05-16	0.8	13	12.014	6.242	-101	0.520	30.254	15.804	-170	0.522	42.268	22.047	-271	0.522			
14	25	0.6	60.3	3.2	311	0.76	60	P10-60-03-06-16	1.3		n.v.	n.v.	n.v.	n.v.	32.915	17.186	-193	0.522	n.v.	n.v.	n.v.	n.v.			
					Av.	0.64			0.9		14.671	7.614	-132	0.519	29.545	15.444	-156	0.523	43.093	22.478	-275	0.522			
		SD		SD	0.10			0.3		2.465	1.266	41	0.001	2.915	1.496	46	0.0012	1.031	0.534	11	0.0001				
11	25	0.2	80.2	2.8	289		24	P2-85-29-04-16	n.v.		7.826	4.067	-65	0.520	28.039	14.668	-136	0.523	35.865	18.736	-201	0.522			
10	25	0.2	81.5	1.3	289	0.28	27	P1-85-10-05-16	0.4		7.957	4.139	-62	0.520	28.276	14.783	-147	0.523	36.233	18.922	-209	0.522			
11	25	0.2	76.6	2.5	278	0.22	27	P2-85-20-05-16	0.6	10	6.679	3.429	-97	0.513	28.668	14.993	-144	0.523	35.347	18.422	-241	0.521			
14	25	0.2	82.5	1.1	289	0.36	37	P2-85-03-06-16	1.0		n.v.	n.v.	n.v.	n.v.	28.888	15.041	-212	0.521	n.v.	n.v.	n.v.	n.v.			
					Av.	0.29			0.7		7.487	3.879	-75	0.518	28.468	14.871	-160	0.522	35.815	18.694	-217	0.522			
					SD	0.07			0.3		0.703	0.391	20	0.004	0.382	0.176	35	0.0012	0.445	0.253	21	0.0007			
11	25		100.0		307	0.03	31	P3-100-10-05-16	0.0		14.681	7.630	-122	0.520	21.325	11.170	-90	0.524	36.006	18.800	-212	0.522			
10	25		100.0		307	0.01		P3-100-20-05-16	0.0	5	7.706	4.014	-54	0.521	27.344	14.284	-153	0.522	35.050	18.299	-208	0.522			
14	25		100.0		307	0.05	21	P3-100-03-06-16	0.2		n.v.	n.v.	n.v.	n.v.	35.233	18.403	-200	0.522	n.v.	n.v.	n.v.	n.v.			
					Av.	0.03			0.1		11.194	5.822	-88	0.520	27.968	14.619	-148	0.523	35.528	18.549	-210	0.522			
<u> </u>					SD	0.02			0.1		4.932	2.557	48	0.001	6.975	3.628	55	0.0008	0.676	0.354	3	0.0000			
								Av.(a)						0.519				0.522				ļ			
								SD (a)						0.002				0.001							
												λ=	0.518							λ=	0.515				

Table 2. Natural West and Central African phytolith samples: coordinates, climatic parameters, calculated phytolith index d/p, measured $\delta^{'18}O_{Phyto}$, $\delta^{'17}O_{Phyto}$, $\delta^{'17}O_{Phyto}$, $\delta^{'18}O_{Pre-rd0>1}$. Average and standard deviation (SD) are given for replicates. MAP: Mean Annual Precipitation; MAT: Mean Annual Temperature; RH: mean annual relative humidity; RH15: RH at 15:00 H UTC; RH-rd0>1: relative humidity average for months with at least one day with precipitation higher than 0.1mm; RH15-rd0>1: RHrd0>1 at 15:00 H UTC. See text for data source and calculation.

	1																
Identifier	Lat	guo	МАР	MAT	H2	RH-rd0>1	RH15	RH15-rd0>1	8 ^{'18} Opre (1)	d/þ	n	δ' ¹⁸ Ο _{Phyto} SD	$\delta^{'17} O_{Phyto}$ SD	¹⁷ O-excess _{Phyto}	SD	8 ¹¹⁸ Ophyto FW	∆ ^{'18} O _{Phyto-Pre} -rd0>1
			mm	°C	%	%	%	%	‰			%	%	per me		%	
C					70	70	70	70	700			700	700	perme	5	700	
Savana RIM 3	21.5	-13.0	52.4	27.3	47.1	61.7	35.4	47.0	-3.220	0.03		33.127	17.218	-243		2.384	36.351
RIM 8	21.0	-12.2	49.1	28.2	44.1	60.5	33.0	45.9	-3.420	0.04		34.813	18.304	-243		4.221	38.239
	20.6	-12.6	68.8	27.6	44.0	58.0	33.0	44.1	-3.829	0.04		28.871	15.088	-268		-1.816	32.707
RIM 11 RIM 10	16.9 16.7	-15.2 -15.2	209.1 227.6	27.3 27.2	45.9 45.7	68.5 68.7	32.5 32.1	52.2 52.1	-4.047 -4.042	0.04		37.506 38.163	19.785 20.094	-211 -256		6.745 7.377	41.561 42.214
S33	16.4	-14.8	270.5	27.7	42.7	57.6	29.7	41.8	-3.861	0.01		35.961	18.939	-225		5.276	39.829
S32	16.3	-15.4	284.4	27.3	46.9	61.6	33.5	46.2	-3.768	0.04		37.297	19.617	-266		6.537	41.072
C4L1	16.1	-14.0	287.7	29.8	40.9	57.1	29.4	42.9	-3.874	0.02	2	34.915 0.368			11	4.609	38.797
S40 S29	16.1 16.1	-13.9 -14.9	329.1 313.0	29.2 27.8	40.6 43.6	56.8 59.1	29.4 30.8	43.0 43.7	-3.969 -3.833	0.06	2	35.385 35.449 0.583	18.592 18.653 0.30	-262 3 -236	0	4.967 4.785	39.363 39.290
82-46	16.0	-16.0	316.4	27.1	53.0	67.5	40.1	54.2	-3.604	0.03	-	33.575	17.654	-228	Ü	2.800	37.185
82-47	16.0	-16.0	316.4	27.1	53.0	67.5	40.1	54.2	-3.604	0.04		36.429	19.169	-247		5.642	40.039
S44	15.8	-13.5	369.1	29.6	40.2	57.2	29.6	44.1	-4.073	0.04	2	36.211 0.593			24	5.863	40.292
C4L3 S54	15.4 15.3	-13.7 -13.0	467.7 443.6	29.6 29.7	41.2 41.3	59.1 60.0	30.3 31.0	45.7 47.2	-4.023 -4.009	0.05	2	33.688 0.312 35.586	17.652 0.17 18.680	5 -290 -282	13	3.345 5.261	37.719 39.603
S58	15.1	-12.8	478.6	29.7	42.0	56.3	31.7	44.3	-4.009	0.05	2	36.161 0.234			21	5.833	40.179
C5L1	15.0	-12.9	583.2	29.7	42.5	57.2	32.1	44.9	-3.972	0.06	3	29.525 0.483	15.500 0.25		7	-0.787	33.505
83-62	14.9	-12.3	515.8	29.7	42.9	58.1	32.6	46.0	-4.097	0.06	2	36.320 0.747	19.095 0.42		36	5.987	40.426
S5 82-79	14.7 14.2	-16.2 -16.1	511.1 669.0	28.1 28.3	53.3 54.2	68.6 70.1	39.2 39.9	53.9 55.2	-3.789 -3.774	0.08	2	24.297 0.115 33.913 0.046			4	-6.312 3.356	28.094 37.694
83-75	14.1	-10.1	736.2	29.1	46.7	63.1	35.6	50.3	-3.774	0.03	2	32.418	16.969	-290		2.000	36.362
82-78	14.1	-16.1	669.0	28.3	55.2	71.0	40.8	56.2	-3.768	0.18		23.789	12.437	-201		-6.785	27.565
S84	13.9	-13.4	775.2	28.9	47.4	64.1	36.1	50.9	-4.040	0.03	2	32.600 0.435			5	2.141	36.648
S118 S88	13.6 13.6	-13.7 -13.6	878.1 880.0	28.6 28.6	49.6 49.4	66.3 66.2	37.7 37.7	52.9 52.9	-4.008 -3.996	0.02		30.007 28.371	15.779 14.900	-188 -189		-0.501 -2.129	34.023 32.375
83-120	13.5	-13.8	934.5	28.5	50.7	67.3	38.7	53.8	-3.984	0.02		31.622	16.570	-262		1.101	35.614
83-122	13.4	-14.9	947.3	28.1	53.6	68.1	39.8	53.7	-3.928	0.03	2	31.240 0.628			9	0.649	35.176
S122	13.3	-13.9	934.5	28.5	52.1	68.1	39.7	53.7	-3.971	0.03		34.379	18.095	-219		3.851	38.350
S93 83-98	13.3 13.1	-13.2 -12.8	1005.3 1067.0		51.6 52.7	68.2 69.3	39.7 40.8	55.1 56.3	-3.925 -4.060	0.08		30.064 29.692	15.787 15.621	-211 -177		-0.435 -0.800	33.989 33.753
S128	13.0	-14.1	1055.1		54.7	70.2	41.7	56.5	-3.765	0.04	2	34.078 0.567			29	3.500	37.843
S.130	12.9	-14.2	1113.9		55.1	70.3	41.8	56.4	-3.961	0.03	3	35.909 0.515			19	5.286	39.870
83-103	12.9	-12.4	1114.0		53.7	70.4	41.8	57.5	-4.329	0.03		30.499	15.855	-249	_	-0.024	34.828
S.138 S136	12.9 12.8	-14.9 -14.7	1127.1 1113.4		56.8 57.4	70.9 71.5	42.4 43.3	56.6 57.4	-4.069 -4.023	0.03	2	35.822 0.5 33.422	18.667 0.3 17.355	-247 -246	5	5.138 2.767	39.891 37.445
	12.7	-12.2	1233.2		54.8	71.4	42.7	58.5	-4.316	0.02	2	31.084	16.149	-264		0.558	35.401
83-115	12.4	-12.3	1301.3		56.5	66.8	44.2	53.8	-4.170	0.01	2	31.524 0.4	16.418 0.2	-226	17	0.887	35.694
Wooded		15.0	405.2	20.0	50.0	67.0	27.2	52.4	2.040	0.16		26.012	10.167	270		C 101	40.763
83-8 S7	14.9 14.8	-15.9 -16.0	485.2 513.6	28.0 28.2	50.9 52.0	67.0 67.9	37.3 38.0	52.4 53.1	-3.948 -4.263	0.16 0.26		36.813 29.491	19.167 15.331	-270 -241		6.181 -1.076	40.762 33.755
83-4	14.7	-16.5	539.7	27.0	57.4	70.9	43.7	57.3	-3.821	0.43		26.127	13.565	-231		-4.665	29.948
82-77	14.6	-16.3	535.5	28.0	53.5	69.0	39.2	54.0	-3.798	0.14	2	35.214 0.8	18.312 0.4	-281	10	4.601	39.012
S91	13.6	-13.4	883.1	28.7	49.4	66.2	37.8	53.0	-3.984	0.40	2	34.512 0.2	18.009 0.1	-213	30	4.013	38.496
C4L8 83-127	13.5	-13.7 -14.1	878.1 1055.1	28.6 28.2	50.7 53.6	67.3 69.3	38.7 40.7	53.8 55.5	-3.984 -3.785	0.13	2	32.302 35.638 0.2	16.850 18.573 0.1	-206 -244	1	1.793 5.054	36.286 39.423
Enclosed		A 71A	1033.1	20.2	55.0	55.5	,	55.5	3.703	5.73		20.000 0.2	20.575 0.1	£77		5.554	33.723
Biendi 1	-2.0	11.1	1839.0		80.9	80.9	67.4	67.4	-3.687	0.76		33.086 0.0	17.233 0.0	-205	20	2.096	36.773
Doubou		10.9	1986.0	25.9	81.2	81.2	67.9	67.9	-3.631	1.58		31.931 0.6	16.665 0.3	-194	8	0.954	35.562
Humid fo 83-151		-16.6	1428.6	26.5	65.8	74.9	51.0	60.8	-3.787	6.78		33.097	17.288	-187		2.221	36.884
S155	12.5	-16.3	1352.6		64.4	74.3	49.4	59.9	-3.777	4.85	2	29.092 0.2	15.180 0.1	-181	4	-1.698	32.869
04-94		13.1	1676.4	24.3	81.4	81.4	65.8	65.8	-4.464	2.44	2	32.638 0.2	17.093 0.1	-140	4	1.371	37.102
04-88	0.3	12.4	1707.0		81.9	81.9	67.1	67.1	-4.458 1 E 1 E	5.45		33.137 0.4	17.345 0.2	-151 105	18	1.977	37.595
04-47 04-66	-0.2 -0.2	12.3 12.5	1724.0 1690.6		82.1 82.0	82.1 82.0	67.4 67.3	67.4 67.3	-1.515 -4.354	3.48 1.84		32.953 0.0 29.959 0.6	17.215 0.0 15.641 0.3	-185 -177	17 24	2.026 -1.040	34.468 34.314
04-65	-0.2	12.6	1690.6		82.0	82.0	67.3	67.3	-4.195	2.19		32.791 0.6	17.158 0.3	-156	25	1.791	36.985
04-118	-0.2	10.5	2148.4	26.4	82.5	82.5	69.2	69.2	-3.556	3.69		31.840 0.4	16.648 0.2	-164	4	0.945	35.396
Dimonik	:-4.1	12.4	1286.6	24.7	80.3	80.3	68.1	68.1	-4.284	5.80		30.928	16.123	-205		-0.275	35.212
Outliers RIM1	16.7	-16.0	216.4	26.7	52.3	72.2	39.4	58.6	-3.857	0.06		38.131	19.828	-305		7.264	41.987
C3L4	15.6	-14.2	362.0	29.3	41.8	59.1	30.3	45.0	-3.968	0.06		25.185 0.2	13.141 0.1	-157	10	-5.211	29.153
<u></u>																	

(1) Amount weighted average for months with at least one day with precipitation>0.1mm

Figure captions

999 1000

- 1001 Figure 1. Growth chamber experiment: a) ¹⁷O-excess vs relative humidity (RH) of irrigation 1002 water (IW), soil water (SW), leaf water (LW) and phytolith (Phyto). Error bars show standard deviation (SD) on the replicates. They are smaller than the symbol when not shown. b) ¹⁸O-1003 1004 enrichment from irrigation water to leaf water (Δ'18O_{LW-IW}), from irrigation water to phytolith $(\Delta^{'18}O_{Phyto-IW})$ and from leaf water to phytolith $(\Delta^{'18}O_{Phyto-LW})$. c) ¹⁷O-excess associated with the 1005 enrichment from irrigation water to leaf water (¹⁷O-excess_{e LW-IW}), from irrigation water to 1006 phytolith (¹⁷O-excess_{e Phyto-IW}), and from leaf water to phytolith (¹⁷O-excess_{e Phyto-LW}). d, e and 1007 f) linear correlations for the 40-80% RH range extracted from a, b and c, respectively. 1008
- Figure 2. Growth chamber experiment: phytolith types extracted from *Festuca arundinaceae* and observed in natural light microscopy: epidermal long cell (LC), epidermal short cell (SC).
- Figure 3. Natural West and Central African transect: $\delta^{'18}$ O of phytoliths ($\delta^{'18}$ O_{Phyto}) *vs* relative humidity RH-rd0>1 (see fig. 4 for explanation). Error bars show standard deviation (SD) on the replicates. When not shown, they are smaller than the symbol.
- Figure 4. Natural West and Central African transect: ¹⁷O-excess *vs* relative humidity (RH) of phytolith assemblages from soil tops collected under savanna, wooded savanna, humid forest and enclosed savanna along a humidity gradient (Table 1). The growth chamber ¹⁷O-excess_{Phyto} *vs* RH correlation line is displayed for comparison. a) RH-Av: yearly average of monthly means; b) RH-rd0>1: yearly average of monthly means for months with at least one day with precipitation higher than 0.1mm; c) RH15: RH at 15:00 H UTC; d) RH15-rd0>1: RH-rd0>1 at 15:00 H UTC.
- Figure 5. Natural West and Central African transect: ¹⁷O-excess of phytoliths (¹⁷O-excess_{Phyto}) vs d/p.
- **Figure 6.** Growth chamber experiment: ¹⁷O-excess vs δ'¹⁸O of irrigation water (IW), soil water 1023 (SW), bulk leaf water (LW) and phytolith (Phyto). Error bars show standard deviation (SD) on 1024 1025 the replicates. The leaf water line (blue) represents how the triple oxygen isotope composition 1026 of the bulk leaf water of Festuca arundinacea evolves from an irrigation water signature to a more evaporated water signature when RH decreases. This evolution follows a slope equivalent 1027 to θ =0.518 in a $\Delta^{'17}$ O vs $\Delta^{'18}$ O space (table 1). Assuming that phytoliths precipitate from the 1028 bulk leaf water, the expected phytolith line (black) should be parallel to the leaf water line as 1029 1030 the equilibrium fractionation between phytolith and leaf water is constant at constant temperature (25°C). In the investigated case this fractionation, represented by the black dotted 1031 line, is equivalent to θ =0.522 (table 1). The isotope signature of phytoliths formed at RH higher 1032 1033 than 40% follow the expected phytolith line. However, the isotope signature of phytoliths 1034 formed at 40% RH suggest a forming water more evaporated than the bulk leaf water.

1036 Figure 1

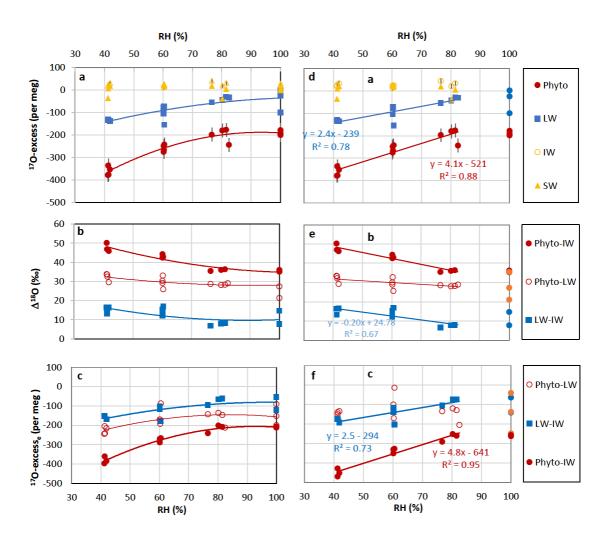
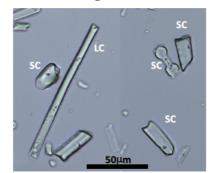


Figure 2



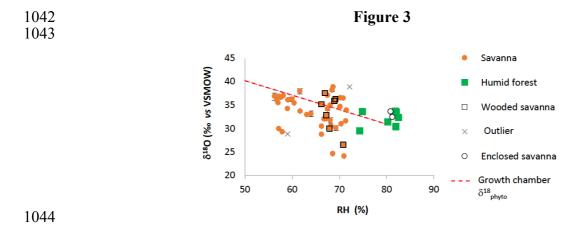
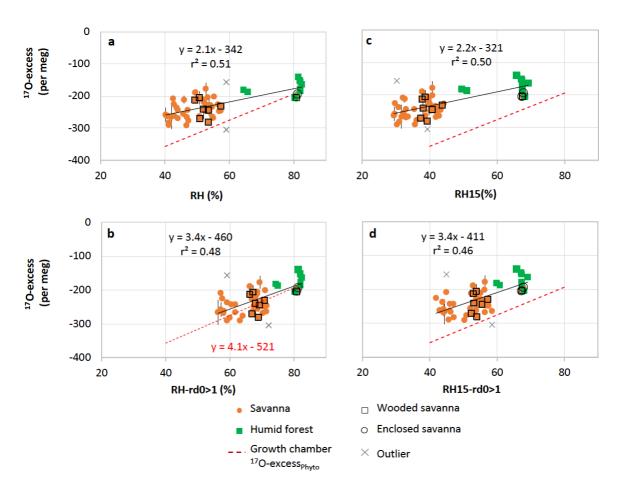


Figure 4 1046



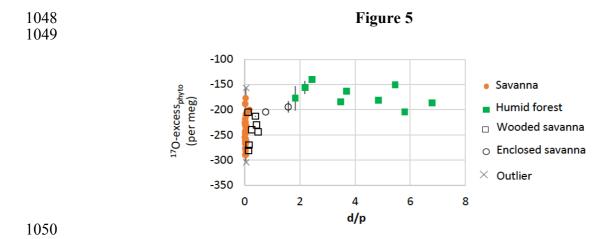


Figure 6 1052

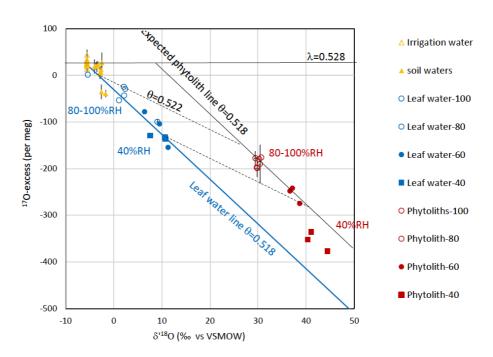


Table S1: a) Measurement of the water laboratory standards with the laser analyzer Picarro L2140i and the isotope ratio mass-spectrometer MAT 253; **b)** Measurements of soil water samples with the isotope laser analyzer (Picarro L2140i) operated in ¹⁷O-excess mode with and without the Picarro micro combustion module (MCM); SD: standard deviation calculated on the replicates.

1059 a)

		aser analy			IRMS		Difference laser analyzer/IRMS								
	Picarro	L2140i (I	Ecotron)	M	AT 253 (LS	CE)									
	δ ¹⁸ O	$\delta^{17}O$	¹⁷ O-excess	$\delta^{18}O$	δ ¹⁷ Ο	¹⁷ O-excess	δ ¹⁸ Ο	δ^{17} O	¹⁷ O-excess						
	‰	‰	per meg	‰	‰	per meg	‰	‰	per meg						
GIENS-1	-0.13	-0.07	1.11	-0.26	-0.14	-5.30	0.13	0.08	6.40						
ECO-1	-5.68	-2.97	29.13	-5.61	-2.94	28.10	-0.07	-0.03	1.04						
ICEBERG-1	-26.88	-14.25	36.09	-27.13	-14.38	35.43	0.24	0.13	0.66						

1061 b)

	WITHOUT	MCM (3 rep	olicates)]		WITH MCN	Л (3 replica										
Sample		δ ¹	⁸ O			δ	²H			δ ¹⁷ (D		¹⁷ O-excess					
		SD		SD		SD		SD		SD		SD		SD				
	%	60	%	00	%	, 00	%	10	%	0	%	00	per	meg	per n	neg		
B3-100-10-05-16	-2.643	0.029	-2.607	0.010	-18.704	0.187	-18.580	0.019	-1.392	0.014	-1.365	0.009	4.1	3.4	12.7	5.8		
B2-60-10-05-16	-3.495	0.014	-3.469	0.023	-23.750	0.082	-23.541	0.073	-1.835	0.010	-1.814	0.019	12.0	6.4	18.7	11.1		
B3-100-03-06-16	-2.799	0.018	-2.766	0.022	-18.868	0.105	-18.894	0.185	-1.462	0.022	-1.457	0.019	16.7	12.7	4.8	7.4		
M1-40-03-06-16	-5.605	0.020	-5.584	0.005	-31.737	0.077	-31.684	0.155	-2.938	0.012	-2.929	0.004	25.7	3.0	23.5	1.7		
B1-85-10-05-16	-2.945	0.038	-2.901	0.010	-20.987	0.018	-20.925	0.050	-1.551	0.045	-1.528	0.008	4.7	25.4	4.8	12.1		
B10-40-10-05-16	-2.726	0.029	-2.697	0.022	-19.891	0.071	-19.594	0.097	-1.434	0.030	-1.416	0.015	6.8	16.2	8.5	10.3		
B1-40-03-06-16	-3.903	0.011	-3.895	0.005	-25.017	0.187	-24.959	0.025	-2.041	0.012	-2.040	0.009	21.6	6.4	18.9	10.7		
-		0.023		0.014		0.104		0.086		0.021		0.012	10.5					

Table S2. Growth chamber experiment: measured $\delta^{18}O$, $\delta^{17}O$ and ^{17}O -excess of irrigation water (IW), soil water, leaf water (LW) and phytoliths. Av: average; n: number of replicates; SD: standard deviation calculated on the replicates; n.v.: no value.

		Irri	gation wate	er				Soil water	,					L	eaf wate	r				Phytoliths					
Sample	δ^{18} O SD	δ^{17} 0	SD n	δ' ¹⁸ Ο	¹⁷ O-excess	δ^{18} O	SD	δ^{17} O SD	n	δ' ¹⁸ 0	¹⁷ O-excess	δ^{18} O	SD	δ^{17} 0	SD	n	8 ¹⁸ 0	¹⁷ O-excess	δ^{18} O SD	δ^{17} O SD	n	δ' ¹⁸ 0 =	O-excess		
	‰	‰		‰	per meg	‰		‰		‰	per meg	‰		‰			‰	per meg	‰	‰		‰ р	er meg		
P1-40-29-04-16	-5.546 0.017	-2.912	0.013 3	-5.562	20	-2.562	0.026	-1.389 0.02	9 3	-2.565	36	10.733	0.106	5.519	0.082	2	10.676	-133	45.454 0.212	23.361 0.152	2	44.451 -3	78 41		
P10-40-10-05-16	-5.594 18.139	-2.933	16.016 3	-5.610	25	-2.697	0.022	-1.416 0.01	5 3	-2.701	9	7.590		3.870		1	7.561	-130	41.947 0.348	21.590 0.199	2	41.091 -3	36 15		
P1-40-20-05-16	-5.580 0.019	-2.917	0.019 3	-5.596	33	-3.658	0.013	-1.913 0.01	3 3	-3.665	20	10.807		5.554		1	10.749	-137	41.150 0.592	21.161 0.291	2	40.326 -3	52 18		
P1-40-03-06-16	n.v.	n.v.		n.v.	n.v.	n.v.		n.v.		n.v.	n.v.	8.530		4.360		1	8.494	-135	41.758	21.451	1	40.909 -3	76		
Av.				-5.589	26					-2.977	21						9.370	-134				41.694 -3	60		
SD				0.025	6					0.600	14						1.596	3				1.867 2	0		
P10-60-29-04-16	-5.564 0.007	-2.929	0.008 3	-5.579	13	-2.504	0.067	-1.296 0.05	7 3	-2.507	27	9.581	0.015	4.942	0.008	2	9.535	-104	39.426 0.528	20.346 0.255	4	38.669 -2	75 23		
P2-60-10-05-16	-5.563 0.001	-2.917	0.016 3	-5.579	24	-3.469	0.023	-1.814 0.01	9 3	-3.475	19	11.370		5.832		1	11.306	-154	37.883 0.340	19.579 0.184	4	37.183 -2	43 4		
P10-60-20-05-16	-5.566 0.021	-2.920	0.027 3	-5.582	23	-3.260	0.028	-1.699 0.00	8 3	-3.266	23	6.453		3.323		1	6.432	-78	37.368 0.504	19.306 0.257	2	36.687 -2	49 4		
P10-60-03-06-16	n.v.	n.v.		n.v.	n.v.	n.v.		n.v.				2.488		1.241		1	2.485	-72	36.034	18.597	1	35.400 -2	65		
Av.				-5.580						-3.083	23						7.440	-102				36.985 -2	158		
SD				0.002						0.509							3.869					1.351 1			
P2-85-29-04-16	-5.594 0.014		0.001 3			-1.667	0.016	-0.920 0.01		-1.668		_	0.067	1.127	0.050		2.217		30.718 0.385	15.920 0.212		30.255 -1			
P1-85-10-05-16	-5.542 22.510					-2.901	0.010	-1.528 0.00		-2.905		2.402		1.238		1	2.399		31.151 0.206			30.675 -1			
P2-85-20-05-16	-5.561 0.014		0.018 3			-3.975	0.018	-2.082 0.01		-3.983	19	1.103		0.528			1.102			15.642 0.036		29.770 -1			
P2-85-03-06-16	n.v.	n.v.		n.v.	n.v.	n.v.			3			0.802		0.391		1	0.802		30.134 0.252	15.552 0.090	2	29.689 -2			
Av.				-5.581						-2.852							1.630					30.098 -1			
SD				0.026						1.158							0.796					0.459 3			
P3-100-10-05-16			0.028 3			-2.607	0.010	-1.365 0.00		-2.611		9.125	1.955	4.707	0.986	2		-100	30.876 0.027	15.992 0.003		30.409 -1			
P3-100-20-05-16		-2.916	6.038 3	-5.588	29	-2.677	0.015	-1.409 0.00	7 3	-2.680		2.121		1.094		1	2.119		29.901 0.148	15.497 0.071	3	29.463 -1			
P3-100-03-06-16	n.v.	n.v.		n.v.	n.v.	n.v.		n.v.		n.v.		-5.382		-2.844		1	-5.396		30.286	15.676	1	29.837 -1			
Av.				-5.593						-2.646							1.935					29.903 -1			
SD	1			0.007						0.049							7.242	53				0.477 1	1		
Av.(a)				-5.586						-2.889															
SD (a)				0.006	5					0.188	8														

(a) Calculated on the raw values.

Table S3. Growth chamber experiment: predicted isotopic enrichment in ^{18}O from irrigation water to leaf water (Δ^{18}_{LW-IW}) after Cernusak et al. (2016; Additional Supporting information). Refer to Cernusak et al. (1996) for symbol and calculations used in the table. Added calculations are displayed in grey columns: Δ^{17}_{LW-IW} and ^{17}O -excess_e were calculated using $^{17}\alpha_{eq} = ^{18}\alpha_{eq}^{0.529}$ and $^{17}\alpha_{k} = ^{18}\alpha_{eq}^{0.518}$, for the equilibrium fractionation and kinetic fractionation, respectively. θ_{LW-IW} was calculated as defined in the text. IW: irrigation water; LW: leaf water (LW).

	Sar	mpling de	etails	Physiolog	gical data		Isotopic data						Calculations																		
																							Predi	icted					Observed	t	
					Boundary	Atm.	Atm.					air vapor	leaf vapor												¹⁷ O-					¹⁷ 0-	
	Air	Leaf		Stomatal	layer	vapor	vapor					pressure-	pressure-				ϵ^* for $\delta^{18}O$	$^{8^*}$ for δ^{17} O							excess _e			∆ ^{'18} LW-	∆ ^{'17} O _{LW-}	excess _e	
Sample	tem.	temp.	Air RH	cond.	cond.	δ ¹⁸ O	δ ¹⁷ O	IW δ ¹⁸ O	IWδ ¹⁷ O	LW δ ¹⁸ O L	.w δ ¹⁷ O	e a	e _i	w_s/w_i	ϵ_k for δ^{18} O	k for δ ¹⁷ O	at leaf temp	at leaf temp	Δ, for δ ¹⁸ O	Δ_v for δ^{17} O	Δ ¹⁸ _{LW-IW}	∆17 LW-IW	Δ ^{'18} LW-IW	∆ ^{'17} LW-IW	LW-IW	O LW-IW	Δ ¹⁸ _{LW-IW}	IW	IW	LW-IW	e _{LW-IW}
	°C	°C	%	mol m ⁻² s ⁻¹	mol m ⁻² s ⁻¹	· %	‰	‰	%	‰	‰	kPa	kPa		%	‰	‰	‰	‰	%	‰		‰		per meg		‰	‰	‰	per meg	
P1-40-29-04-16	25.0	25.0	41.2	0.031	2	-5.55	-2.91	-5.55	-2.91	10.73	5.52	1.31	3.18	0.41	27.860	14.336	9.386	4.954	0.000	0.000	25.922	13,426	25,591	13.336	-176	0.521	16.370	16.238	8.420	-154	0.519
P10-40-10-05-16	25.0	25.0		0.031	2	-5.55 -5.59	-2.91	-5.59		7.59	3.87		3.18	0.41	27.860	14.336	9.386	4.954	0.000	0.000	25.922	13.411	25.564	13.322	-176	0.521			6.799	-154	0.519
	25.0	25.0	-	0.032	2		-2.93					1.31		0.41	27.857	14.334			0.000		25.723	13.324	25.398								
P1-40-20-05-16			-		2	-5.58				10.81	5.55	1.33	3.18				9.386	4.954		0.000	25.723	13.324	25.398	13.236	-174	0.521	16.479	16.345	8.460	-170	0.518
P1-40-03-06-16	25.0	25.0	41.4	0.032	2	n.v. r	n.v.	n.v.	n.v.	8.53	4.36	1.32	3.18	0.41	27.860	14.336	9.386	4.954	n.v.	n.v.											
					_																										
P10-60-29-04-16	25.0	25.0		0.052	2	-5.56	-2.93	-5.56		9.58	4.94	1.92	3.18	0.61	27.770	14.290	9.386	4.954	0.000	0.000	20.458	10.627	20.252	10.571	-122	0.522			7.864	-117	0.520
P2-60-10-05-16	25.0	25.0		0.052	2	-5.56	-2.92	-5.56		11.37	5.83	1.91	3.18	0.60	27.772	14.291	9.386	4.954	0.000	0.000	20.543	10.670	20.335	10.614	-123	0.522			8.737	-178	0.517
P10-60-20-05-16	25.0	25.0		0.052	2	-5.57	-2.92			6.45	3.32	1.92	3.18	0.61	27.770	14.290	9.386	4.954	0.000	0.000	20.458	10.627	20.252	10.571	-122	0.522	12.087	12.014	6.242	-101	0.520
P10-60-03-06-16	25.0	25.0	60.3	0.052	2	n.v. r	n.v.	n.v.	n.v.	2.49	1.24	1.92	3.18	0.60	27.771	14.291	9.386	4.954	n.v.	n.v.											
P2-85-29-04-16	25.0	25.0		0.074	2	-5.59	-2.94	-5.59	-2.94	2.22	1.13	2.55	3.18	0.80	27.680	14.244	9.386	4.954	0.000	0.000	14.918	7.789	14.808	7.758	-60	0.524	7.857	7.826	4.067	-65	0.520
P1-85-10-05-16	25.0	25.0		0.070	2	-5.54	-2.90			2.40	1.24	2.44	3.18	0.77	27.697	14.252	9.386	4.954	0.000	0.000	15.928	8.306	15.802	8.272	-72	0.523	7.989		4.139	-62	0.520
P2-85-20-05-16	25.0	25.0		0.075	2	-5.56	-2.90	-5.56	-2.90	1.10	0.53	2.59	3.18	0.82	27.675	14.241	9.386	4.954	0.000	0.000	14.554	7.602	14.449	7.573	-56	0.524	6.702	6.679	3.429	-97	0.513
P2-85-03-06-16	25.0	25.0	82.5	0.076	2	n.v. r	n.v.	n.v.	n.v.	0.80	0.39	2.62	3.18	0.83	27.670	14.239	9.386	4.954	n.v.	n.v.							1				
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P3-100-10-05-16	25.0		100.0	0.095	2	-5.58	-2.93	-5.58	-2.93	9.13	4.71	3.18	3.18	1.00	27.592	14.199	9.386	4.954	0.000	0.000	9.386	4.954	9.342	4.942	9	0.529			7.630	-122	0.520
P3-100-20-05-16	25.0		100.0	0.095	2	-5.57	-2.92	-5.57	-2.92	2.12	1.09	3.18	3.18	1.00	27.592	14.199	9.386	4.954	0.000	0.000	9.386	4.954	9.342	4.942	9	0.529	7.736	7.706	4.014	-54	0.521
P3-100-03-06-16	25.0	25.0	100.0	0.095	2	n.v. r	1.v.	n.v.	n.v.	-5.38	-2.84	3.18	3.18	1.00	27.592	14.199	9.386	4.954	n.v.	n.v.											

Stomatal conductance: gs ranges from 0.1 to 0.5 in investigated C3 grasses is lower than 0.2 in C4 grasses. Cf Ocheltree et al., 2012. Here gs is calculated according to Liet al., 2017.

Boundary layer cond: 0.2 to 3 in Li et al., 2017