Soil biota impacts on plant access to different water pools in soil

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ABSTRACT

Aims: Soil water availability depends on the capacity of soil pores to hold it via physical forces creating gradient of availability from tightly bound water to highly mobile water. Abiotic factors directly affect the size of these pools and plant access to them. Biotic factors influence plant-soil-water relations and possibly affect soil properties and plant access to different water pools. Thus, our aim was to contrast and assess the effect of biotic and abiotic soil environment on the plant uptake of water from the mobile and bound pool.

Methods: Here, we used an ^{18}O-enriched water approach to trace movement of water from bound and mobile pools into plants with experimentally manipulated soil biotic compositions and soil texture. Comparisons of responses between treatments with intact soil communities

and those excluding larger organisms such as mycorrhizal fungi and microfauna allowed us to estimate the extent these organisms influence plant access to water pools. We assessed these responses in an unmodified soil as well as after dilution of soil with sand, to evaluate whether soil texture might influence biotic effects.

Results: We found that removing larger organisms reduced plant access to bound water by 14 % and decreased exchange of water between the bound and mobile pools from 64 % to 41 % in the soil mixed with sand but not in the unmodified soil.

Conclusions: This novel contribution demonstrates that soil biota can influence plant-soil-water relations and we propose further work to identify the specific soil biota and biophysical mechanisms involved.

KEYWORDS: ^{18}O, isotopically enriched water, plant water source, two water worlds, soil texture, soil biota

INTRODUCTION

Soil water availability depends on the capacity of soil pores to hold it via physical forces. A degree of compartmentalisation of water or, perhaps more accurately, a gradient of availability exists so that separate pools of water can be present in soils: from tightly bound water to highly mobile water (Brooks et al., 2009). While mobile water moves through soil pores that are too large to hold it for very long and is easily drained or is taken up by plants, bound water will be held in pores that are small and will attach strongly to soil particles. During wet conditions, mobile water is expected to flow through the soil and be taken up by plants to a much greater extent than relatively inaccessible bound water. During dry periods when mobile water is not available, only more tightly bound water is available to plants (Brooks et al., 2009; Evaristo et al., 2015). Several studies have since demonstrated that water is not completely compartmentalized and that mixing and degrees of plant access to these two pools happens to different levels (Evaristo et al., 2015; Sprenger et al., 2018; Thielemann et al., 2019; Vargas et al., 2017). The processes leading to such water exchange and resulting plant access to these water pools are poorly understood.

Abiotic soil properties directly influence the distribution of water in soil pore space and thus play an important role in plant access to such reservoirs. Soil texture determines the quantity and size of the pore space, which affects the proportion of bound and mobile pools and possibly the exchange of water between these pools (Adams et al., 2020; Evaristo et al., 2015; Vargas et al., 2017). Fine-textured soils are expected to have a larger bound water pool because aggregations of smaller silt and clay particles form many small pores capable of holding water (Adams et al. 2020). In contrast, sandy soils have poor water retention because their coarser particles do not support aggregation and result in mostly large and few small pores thus limiting the size of the bound water pool (Liu et al., 2020).

The influence of biotic soil properties on water movement between and plant access to mobile and bound water pools has not been assessed, despite the fact that soil biological activity is known to influence plant-soil-water relations and plant behaviours under water stress (Rabbi et al., 2021). For instance, soil biota can directly modify the soil matrix in a variety of ways that affect soil structure, water penetration into soil, movement through soil pore space and water holding capacity (Lehmann et al., 2017). Soil biota contribute to soil aggregation and stabilisation by the production of organic binding compounds such as polysaccharides or by physically binding soil particles e.g. with fungal hyphae (Degens, 1997), thus modifying pore space volume and the area of surfaces where water may be bound. The spatial scale at which these direct effects are observed depends on the soil organisms involved: bacteria mostly contribute to the formation of micro- and macro-aggregates and fungi influence the formation of macro-aggregates (Lehmann et al., 2017). Plant access to water can also be influenced by the presence of fungi (including mycorrhizal fungi) as they can transport water along or inside their hyphae, therefore relocating water from different parts of the soil (Guhr et al., 2015; Plamboeck et al., 2007), possibly including from the bound pool that maybe inaccessible to plants. Micro- and mesofauna also influence aggregation (Lehman 2017) and modify soil pore space as they burrow through soil (Porre et al., 2016).

Beyond these direct effects, soil biota can also indirectly modify the soil matrix via their impact on plant roots, as plants themselves are key drivers of soil structure. Roots can modify the soil structure via the production of exudates or the spatial arrangement and aggregation of soil particles resulting in porous space (Oleghe et al., 2017). A probable pathway for indirect effects is associated with root production, morphology and architecture, responding to changes in nutrient availability due to microbial or faunal activity (de Kroon et al., 2012) or reallocation of resources aboveground due to partnerships with mycorrhizal fungi (Smith & Smith, 2011).

Most of our knowledge on plant water access has been generated by studies of bulk soils due to difficulties studying water movement or compartmentalisation at a very fine spatial scale. Currently, studies tracing isotopically enriched water in controlled environments provide new insights into bound and mobile pools in soils (Adams et al. 2020) and plant use of mobile and bound water (Vargas et al. 2017). In a greenhouse experiment, Vargas et al. (2017) presented an approach for tracing water into plants and the mixing between soil pools by simulating natural changes in soil moisture, with soil drying leading to the establishment of a bound pool of isotopically depleted water, followed by a watering event to establish a mobile pool with isotopically enriched water, and a dry period to trace water exchange between soil pools and identify the source of water accessed by the plant. Using this approach, we aimed to estimate the extent that soil biota can influence water mixing and plant access to these pools. Therefore, we experimentally modified soil communities by removing (or not) larger community members such as arbuscular mycorrhizal fungi, protists and fauna in the presence of *Plantago lanceolata*, a plant species that is highly responsive to changes in its biotic environment (Klironomos & Moutoglis, 1999; Maherali & Klironomos, 2007). We used ^{18}O-enriched water to trace water movement and to differentially label the bound and mobile pools in two soils: an unmodified soil as well as after dilution of that soil with sand to modify soil texture.

Our objective was to contrast the effect of biotic and abiotic soil environment on uptake of water from the mobile and bound pool as well as the mixing between the two, and to assess whether the influence of soil texture varied according to the soil biota present. We hypothesised that modifying soil texture would influence a) the proportion of bound water in soils, b) the extent of the mixing of water between the bound and the mobile pool, and c) plant access to the bound water pool. We expected that soils containing more sand would hold and retain less bound water and exhibit more mixing between pools because coarser particles aggregate less and have fewer small pores. Therefore, we also expected that plants would access less bound water in these soils. We also hypothesised that manipulating the composition of soil biota would modify the responses such that more complex soil communities (i.e., including larger fungi and fauna) and their effects on soil aggregation and water transport would a) increase the bound water pool in soil, b) increase plant access to bound water, and c) increase exchange between mobile and bound pools. Effects of modifying soil communities on plant water uptake were expected to be greater in soil with added sand due to it having a reduced innate capacity to hold bound water and that this would be more strongly evidenced under dry conditions when mobile water is less available.

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127 METHODS

128 For this experiment, we used soil from the Hawkesbury Forest Experiment (HFE; 33◦36 40 S,
129 150◦44 26.5 E; mean annual precipitation ~ 800 mm) near Richmond NSW, Australia,
130 described in Barton et al. (2010). Briefly, the soil is a low fertility, sandy loam with an organic
131 matter content of 0.7 % and low water holding capacity. Prior to use, the soil was sieved using
132 a 3 mm-sieve and dried for two weeks, then sterilised with 50kG gamma irradiation (Steritech,
133 NSW Australia) to eradicate existing soil biota.

134 The experiment used a completely randomised, three-by-two fully factorial design with two
135 levels of soil texture, two levels of inoculation, and two destructive harvest timepoints. Each
136 treatment combination included five replicates for a total of 80 pots.

137 For the soil texture factor we used 1) sterilised HFE soil and 2) sterilised HFE soil diluted with
138 coarse sand (70% soil, 30% sand). This allowed us to isolate the impact of texture while
139 removing all other mineralogical variables such as clay type, which could also affect water
140 availability. Each pot was filled with 700 g of soil or soil-sand mix.

141 To evaluate the effect of the soil community on plant access to bound water, we prepared two
142 inocula that were used to re-establish soil biota in the sterilised soils. Both inocula were derived
143 from soil collected from an arid rangeland ecosystem (29°36'21.2"S, 141°43'01.9"E; mean
144 annual precipitation ~ 250 mm) near Milparinka NSW, Australia. We chose this site because
145 we expected that biota adapted to arid conditions would be more capable of accessing bound
146 water than those adapted to our more mesic site near Richmond. We cultured biota in the soil
147 by planting it with maize for several months. The first inoculum derived from these cultures
148 was applied to all experimental pots in order to control recolonisation of the soil by saprotrophic
149 bacteria and fungi. This inoculum was prepared by wet sieving 250 g of soil and roots from
150 cultures in 1 L of sterilised, PCR-grade water, mixing for 20 min and filtering twice through a
151 20-um sieve, after which 10 mL of the filtrate was added to each pot. The second inoculum
152 derived from these cultures was only applied to half of the pots and consisted of 25 g of soil
153 and roots from cultures. Thus, these pots (hereafter, referred to as 'unfiltered') received
154 inoculum that will have contained soil organisms such as arbuscular mycorrhizal fungi, protists
155 and microinvertebrates that will have been absent or greatly reduced in the other half of the pots
156 (hereafter, referred to as 'size-filtered' or just 'filtered'). Once soil treatments were established,
157 each pot was sown with four seeds of *Plantago lanceolata*, and seedlings were removed after 2

weeks to keep only one individual per pot. Prior to sowing, seeds were sterilised in a 1% sodium hypochlorite solution for five minutes and rinsed with distilled water.

To quantify water access as impacted by texture and biota, we used an approach with ^{18}O-label water (Vargas et al. 2017). A wetting and drying experiment using ^{18}O enriched water was conducted in order to generate differential $\delta^{18}O$ values in more mobile *versus* less mobile water pools (Figure 1; Vargas et al., 2017). Prior to soil inoculation, soils were air dried for 15 days to remove water. Following inoculation, at the time of planting, soil-filled pots were saturated with tap water for 24h to establish a known baseline value of $\delta^{18}O$ in the soil (Fig. 1a). After planting, all pots continued to be irrigated with tap water ($\delta^{18}O$ = 6.1 ‰) once every two days and were fertilised every two weeks with a modified Hoagland solution (Hoagland and Arnon, 1950), reduced by 50% in phosphorus content to promote the establishment of arbuscular mycorrhizal fungal symbiosis with plants (Fig. 1b). The glasshouse temperature was maintained at 23 °C (day) and 20 °C (night) with a 09h:19h photoperiod and a relative humidity at 60% throughout the experiment.

After four months of plant growth, half of the pots were randomly designated as unlabelled (UL) and the other half as isotopically labelled (L). At this time, we stopped watering. Once plants showed signs of wilting, indicating that the most available water had been taken up or had evaporated, UL pots were watered with 50 mL of tap water while L pots were watered with 50 mL of ^{18}O-enriched water (467.4 ‰, Marshall Isotopes LTD, Novachem). This approach was used to produce two distinct isotopic sources of water in the L pots: the remaining tap water present in less available pools (more bound) and the newly added, labelled water (more mobile). These two sources then enabled us to evaluate the proportion of bound water in soils and stems (Fig. 1c). Watering was conducted slowly with a syringe to promote homogeneous distribution and water absorption and to avoid all contact of the plant with the labelling solution.

Half of the pots across all combinations of the other three factors were harvested one day after labelling (the Wet (W) pots). At this point, the soil in labelled pots contained a mix of ^{18}O-enriched mobile water and unlabelled bound water (Fig. 1d). The remaining pots (Dry (D) pots) were harvested once plants showed signs of wilting, four (soil) or six (soil with added sand) days after labelling. At this point, soils contained mainly bound water as the mobile water is present in the plant or lost due to transpiration, evaporation or drainage (Fig. 1e).

During each harvest, plant stems and soil samples were collected and stored immediately at -20 °C. Plant stems were separated from the leaves and peeled down to the cambium layer to remove phloem water, which would contain water taken up prior to labelling, and to keep the xylem water, which would reflect the recently acquired water. Peeled stems were immediately stored in a glass test tube, sealed with an airtight cap held in place using parafilm, and frozen to avoid evaporation. For each pot, soil was gently separated from the roots and homogenised before collecting a sample, which was stored in a sealed glass tube as per the stem samples. In addition, leaves and roots were collected to estimate the aboveground and belowground biomass.

For $\delta^{18}O$ analyses, water was extracted from the soil and stem samples using cryogenic vacuum distillation as described in West et al. (2006), with an extraction time of 1 h before leaving the water to thaw and decant into a sealed vial. Water extracted from soil and stem samples were analysed for ^{18}O at the Carbon, Water and Food Institute (University of Sydney) as described in Loucos et al. (2015). Briefly, water was transferred into previously flushed (with 2 % CO_2) sealed glass vials and left for two days at 25 °C. Equilibrated CO_2 was extracted from the vial to inject into a Tedlar bag containing 1 L of CO_2-free air. The ^{18}O of the equilibrated CO_2 was measured on a Tunable Diode Laser (TGA100A, Campbell Scientific, Logan, UT, USA). Two calibration cylinders with different isotopologue concentrations were used to correct measured sample concentrations, which were then converted to the Vienna Standard Mean Ocean Water scale as in Barbour et al. (2007). Insufficient water was extracted in a few samples reducing the number of replicates in a few treatments (Table S1).

The proportion of bound water present in plant stems and soil samples was calculated using mass balance with the $\delta^{18}O$ of the tap and added ^{18}O-enriched water as end-members as follows:

$$\text{Proportion of bound water} = \boldsymbol{\delta}_{\text{sample}} - \boldsymbol{\delta}_{\text{enriched water}} \,/\, \boldsymbol{\delta}_{\text{tap water}} - \boldsymbol{\delta}_{\text{enriched water}}$$

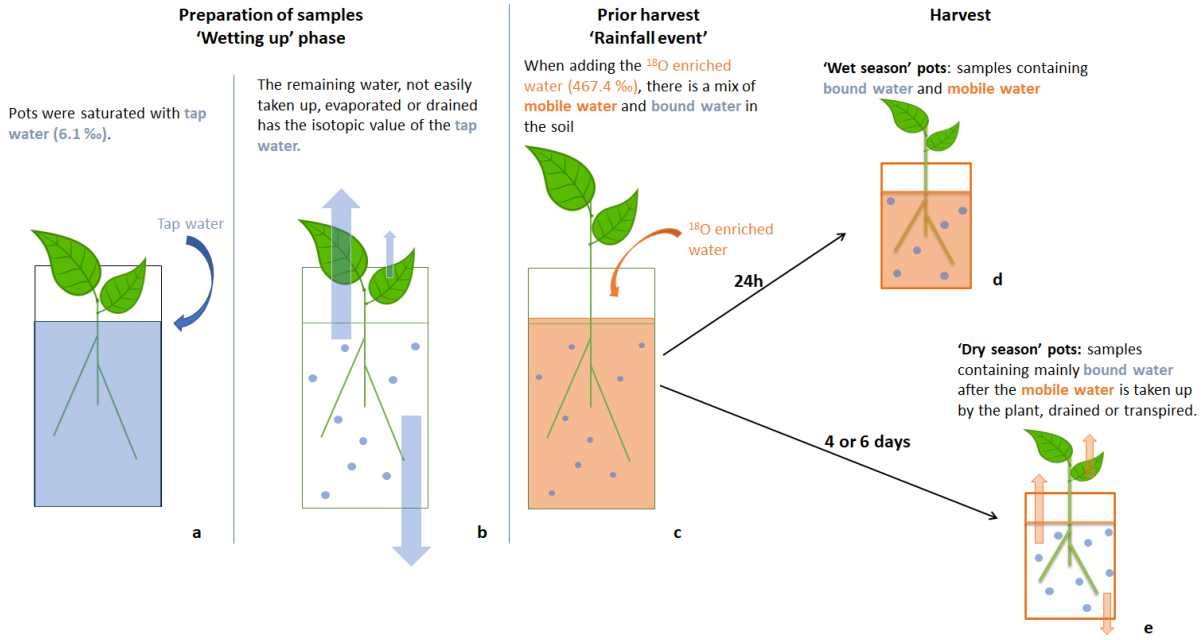
Where $\boldsymbol{\delta}_{\text{sample}}$ is the $\delta^{18}O$ value of the sample, $\boldsymbol{\delta}_{\text{enriched water}}$ is the $\delta^{18}O$ value of the labelled water added to the pots ($\delta^{18}O$ value: 467.4 ‰) and $\boldsymbol{\delta}_{\text{tap water}}$ is the $\delta^{18}O$ value of the unlabelled water ($\delta^{18}O$ value: 6.1‰).

Since water is expected to at least partly mix between pools during uptake, and this may be affected by soil texture, structure and soil moisture, we estimated the proportion of water exchanged between the bound and mobile water pool according to Vargas et al., (2017):

$$\text{Proportion of exchanged water} = (\boldsymbol{\delta}_{\text{LD}} - \boldsymbol{\delta}_{\text{ULD}}) \,/\, (\boldsymbol{\delta}_{\text{LW}} - \boldsymbol{\delta}_{\text{ULW}})$$

Where $\boldsymbol{\delta}_{LW}$ and $\boldsymbol{\delta}_{ULW}$ are the mean $\delta^{18}O$ values of the water extracted from the stems collected in the wet pots (W) in labelled (L) and unlabelled (UL) treatments, respectively; $\boldsymbol{\delta}_{LD}$ and $\boldsymbol{\delta}_{ULD}$ are the mean $\delta^{18}O$ values of the water extracted from the stems collected in the dried pots (D) in labelled (L) and unlabelled (UL) treatments, respectively.

Statistical analyses were performed in R (R version 4.0.4, R Core Team, 2020). Two- or three-way analyses of variance (ANOVA) were conducted as appropriate to evaluate individual and combined interaction effects of variables using 'Anova()' from the 'car' package (Fox & Weisberg, 2011). We identified two potential outliers in the ^{18}O values that inflated variance estimates in the calculated proportion of bound water in stems (see Fig. S1). Removing one of them or both revealed a significant three-way interaction between harvest time, soil texture and biota (Table S2). This result was used as justification for performing separate two-way ANOVAs to evaluate the effects of soil texture and biota within each harvest. In the main text, we report outcomes of the two-way ANOVAs that include these two data points as the analyses that removed them showed the same trends (Table S3). Multiple mean comparisons using Tukey's test were performed to detect differences between groups. A Pearson's correlation test was used to assess the relationship between plant biomass (in leaves or in leaves) and bound water in plant stems using the 'cor.test' function. To test the significance of inoculation and soil effects on water mixing between the mobile and bound pool, we used a bootstrapping method in which observed exchange estimates were compared to 9999 estimates following randomisation of soil and inoculation treatment labels within each level of labelling and harvest.



241

242 Figure 1. ^{18}O-labelled water approach as in Vargas et al. (2017). Wetting and drying using ^{18}O
243 enriched water in order to generate differential δ^{18}O values in more mobile *versus* less mobile
244 water pools.

245

246

247 RESULTS

248 In all treatments and all sample types, δ^{18}O values differed greatly between the labelled and
249 unlabelled samples (Table 1). Soil and stem δ^{18}O in labelled pots were all above 70 ‰ while in
250 unlabelled pots samples reached up to a maximum of 12 ‰. Regardless of the soil texture and
251 biota treatments, values of δ^{18}O in labelled samples were significantly lower in the dry pots
252 (second harvest) than in the wet pots (first harvest) (Table 1 and 2, Fig. S2), meaning that the
253 mobile labelled water was lost via evaporation or transpiration after four or six days and that
254 water remaining and being taken up by plants was mainly unlabelled bound water at this point.

255 Soil δ^{18}O values (‰) tended to be higher in soils mixed with sand than in soils without added
256 sand (Table 1). We also found that soil texture had a significant effect on the proportion of
257 bound water present in soils, with bound water tending to be higher in soils mixed with sand
258 (P-value = 0.05; Table 2, Fig. 2). We found no evidence supporting such effects of soil texture
259 in stem samples (P-value = 0.28; Table 2).

Overall, we found no evidence that soil biota and soil texture influenced the proportion of bound water taken up by plants once mainly bound water remained in dry pots (second harvest), but a marginally nonsignificant trend was observed when mobile water was available in wet pots (first harvest) (Table 3). In soils mixed with sand, we observed that the proportion of bound water in stems tended (P = 0.07, Table 3) to be higher in plants inoculated with the unfiltered soil biota than in pots with the filtered biota (Fig. 2). However, this effect of soil biota was not observed in soils without added sand (Fig. 2). When evaluating these effects in soil, we observed that neither soil texture, soil biota nor their interaction influenced the proportion of water in the tightly bound pool (Table 3).

The soil biota treatment impacted water exchange. In soils mixed with sand, a marginally non-significant trend (P-value = 0.08) was detected showing that filtering the soil biota tended to reduce the proportion of water exchanged between mobile and bound pools from 0.65 to 0.41, meaning that there was more mixing occurring between mobile and bound pools during water uptake when larger soil biota were present (Table 4). This effect was not observed in soils without added sand as we did not observe differences in the proportion of water exchange (both 0.52, Table 4).

The effect of the soil biota on the plant biomass in leaves depended on the soil type ($ANOVA_{interaction\ soil\ *\ inoculum}$; $F\text{-value}_{1,59}$ = 5.32, P-value = 0.02). The unfiltered soil biota inoculum decreased aboveground biomass in soils mixed with sand (Tukey test; t-value= -2.84, P-value = 0.03), but this effect was not observed in soil without added sand (Tukey test; t-value= -2.07, P-value = 0.17). When evaluating such effects on root biomass, we observed that the unfiltered soil biota treatment resulted in a marginally nonsignificant reduction in root biomass (ANOVA; $F\text{-value}_{1,59}$ = 3.26, P-value = 0.08). However, we did not observe any relationship between the proportion of bound water in plants and neither biomass in leaves (Pearson's test; r < -0.01, P-value = 0.99) nor root biomass (Pearson's test; r = -0.11, P-value = 0.54, Fig. S3).

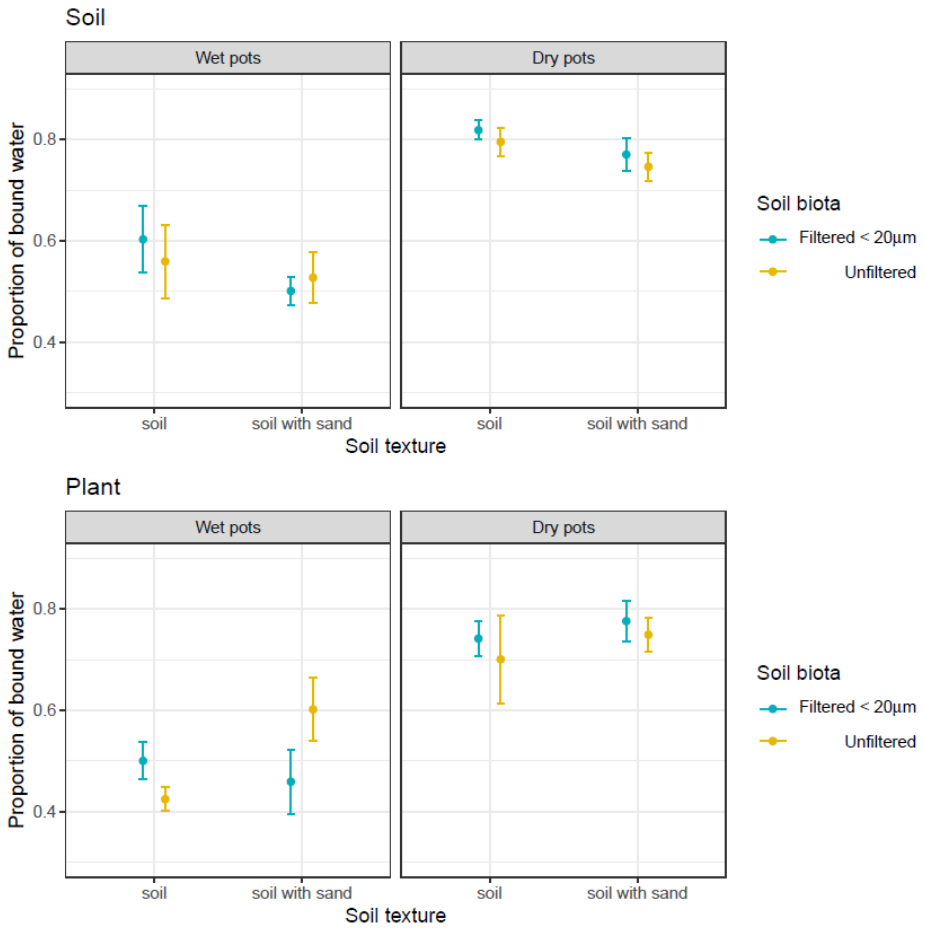


Figure 2. Proportion of bound water in soil (top) and stem samples (bottom) depending on soil type and on soil biota treatment from wet pots (collected at the first harvest) and from dry pots (second harvest).

DISCUSSION:

Overall, we found that the soil biotic influence on plant access to bound water and on water exchange in soil was dependent on soil texture since it was only observed in the soil mixed with sand. In that context, removing larger biota such as arbuscular mycorrhizal fungi, protists and microfauna reduced: 1) plant access to bound water, and 2) the degree of bound / mobile water mixing during plant uptake. This suggests that the effects are due to some direct influence that the biota have on the pools themselves and that is modified depending on the physical properties of the soil in which those pools are formed.

Our initial hypotheses were that the effects of soil biota on plant access to bound water could occur via three non-exclusive pathways: changes in plant traits, modification of the soil

structure (soil pores, aggregation), and water translocation between the different water compartments of the soil. In the context of our study, we did not find support for these effects being indirectly due to the soil biota influencing the plant. Even though the presence of larger soil biota reduced plant biomass, such effects on plants did not translate into effects on uptake of bound water. However, we cannot rule out other factors that we did not assess such as root morphology or architecture. Regarding our second broad hypothesis relating to the soil structure, our data are also limited but the fact that the effect of soil biota was only detected in soil mixed with sand suggests that their contribution could be related to modifications of the soil structure. However, our results showed that the presence of the larger soil biota did not influence the proportion of bound water in soils. Lastly, our results are consistent with the last hypothesis regarding water transport and relocation due to the presence of soil biota. We did observe that the plant's access to bound water increased and so did the mixing between the mobile and the bound pools, suggesting that soil biota may contribute by directly impacting the transfer of water from the tightly bound pool to the plant or by relocating water from the different pools.

Our testing of this approach and resulting observations from a small experiment demonstrate intriguing patterns in the influence of biota on plant water access and their dependency on soil texture. This highlights the need and potential to refine our investigations of mechanisms that regulate water access for plants by stepping away from bulk soil water approaches. Clearly there is a need for work that identifies the relative contributions of the different actors, as well as the roles that they play. In our view, this work falls into three themes.

(1) Direct transfer of bound water by soil biota -- Organisms such as mycorrhizal fungi may play a similar role in bound water transport from particle surfaces to the root-fungal interface as they play in the transport of nutrients. Such movement may occur within or along the surface of fungal hyphae (Mitchell et al., 2010; Plamboeck et al., 2007), which are more capable than roots in penetrating small pores due to their narrow diameter (Allen, 2007) and which already contribute to water movement more generally in soil and into roots (Plamboeck et al., 2007; Ruth et al., 2010). The degree to which this occurs may depend on fungal functional traits as it has been observed that species differ in their ability to enhance water uptake by plants (Marulanda et al., 2003). Thus, fungi adapted to arid conditions may be more capable of accessing bound water and testing the role of fungal traits would contribute to understand regulators of plant access to water. Promising experimental approaches based on the isotopic tracing of water pools include the ability to manipulate hyphal connections between

compartments (e.g., Ruth et al., 2010) where mixing occurs between pools and across which water is transported. Combining this approach with mycorrhizal-defective mutant plants (e.g., Bitterlich et al., 2018) could help resolve the relative importance of access (water bound to soil surfaces in pores), transport (movement from soil surfaces into pore space, between pores and to root surfaces) and exchange (via intraradical fungal structures interacting directly within roots) during the movement and mixing of water from mobile and bound pools.

(2) Soil biotic effects on the size and properties of soil water pools -- As described above, soil biota play important roles during soil aggregation that will have consequences for the sizes and persistence of mobile and bound water pools. Effects on soil aggregation are not entirely microbial in nature since soil fauna also contribute to soil aggregation processes (Maaß et al., 2015). As demonstrated in our study, the relative importance of these effects will depend on a variety of conditions including the starting conditions in terms of soil texture (affecting the capacity of soil organisms to create micro- and macro-aggregates). Other conditions such as the amount and quality of organic matter present in the soil need to be considered because they will affect biomass at the base of the bacterial and fungal soil food webs and turnover during trophic interactions. With so many possible actors and a high degree of context dependency, larger observational and manipulative studies quantifying abundance of biota along soil textural gradients (with or without selective biotic treatments), soil aggregate distributions and the distribution of bound and mobile pools might be a promising approach.

(3) Feedback loops involving soil aggregation, water movement and trophic dynamics -- While biota can influence the distribution and connectivity of pore space in soil, pore size and connectivity can also influence the movement of soil biota through the soil matrix (reviewed in Erktan et al., 2020). For example, nematodes and microarthropods are strongly constrained in their movements by soil structural characteristics (Erktan et al., 2020), while soil protists can modulate their body shape to access soil pores that are out of reach for rigid organisms (Geisen et al., 2018). Their abilities to reach and consume bacteria and fungi in small pores directly impacts the organisms responsible for soil aggregation (Erktan et al., 2020; Klironomos & Kendrick, 1996; Thimm & Larink, 1995). Thus, indirect contributions to the capacity for water to be bound will depend on how restrictive faunal movement and predation is in soil. In our study, even though all pots were reinoculated with bacteria and fungi, it may have been the case that their activities were affected by predation, possibly with counteracting effects. Hence, the lack of effects of inoculation on plant uptake of bound water could be due to the potential benefits being offset by predation if faunal movement is less restricted. Lastly, complementary

368 or facilitative interactions are also possible in that, for example, soil fauna also play a role in
369 the dispersal of fungi (Klironomos & Kendrick, 1996) that likely contribute to mixing between
370 pools and transport of bound water.

371 In conclusion, we used an innovative ^{18}O water labelling approach that made it possible to trace
372 movement of water from bound and mobile pools into plants. Using this approach, we
373 demonstrated that soil biota influences plant access to tightly bound water, and that such effects
374 depend on the soil texture. Here we discussed the potential of using this method to better
375 understand the mechanisms by which soil biota can modify plant access to tightly bound water.
376 Such feedback loops and pathways of influence are difficult to assess, but experimental designs
377 that provide insight into the effects of variation in water availability, plant root traits and biotic
378 populations do exist (e.g. Rabbi et al., 2021) and can be applied in the context of bound and
379 mobile water distributions.

380

Table 1. Soil and stem $\delta^{18}O$ values (average +/- standard error) evaluated from labelled and unlabelled samples according to each soil texture and soil biota treatment collected in 'wet' (first harvest) and 'dry' pots (second harvest). Table indicates whether the samples were watered with enriched water ($\delta^{18}O$ value: 467.4 ‰) or using tap water ($\delta^{18}O$ value: 6.1‰), the harvest timepoint at which samples were collected to obtain pots containing bound and mobile (first harvest) or only bound water (second harvest), the soil texture and soil biota treatment and the corresponding measured $\delta^{18}O$ values from soil and stem samples.

Label	Harvest	Soil texture	Soil biota inoculated	Soil $\delta^{18}O$ values (‰)	Stem $\delta^{18}O$ values (‰)
Labelled with enriched water ($\delta^{18}O$ value: 467.4 ‰)	'Wet pots' (first harvest)	soil	Filtered <20 um	189.3 +/- 30.6	236.6 +/- 17.1
			Unfiltered	209.4 +/- 33.5	261.4 +/- 12.6
		soil with added sand	Filtered <20 um	236.2 +/- 13.2	255.6 +/- 29.4
			Unfiltered	224.2 +/- 22.8	193.4 +/- 20.8
	'Dry pots' (second harvest)	soil	Filtered <20 um	90.0 +/- 8.8	125.5 +/- 16.0
			Unfiltered	100.6 +/- 12.8	144.2 +/- 40.0
		soil with added sand	Filtered <20 um	112.1 +/- 15.3	109.6 +/- 18.9
			Unfiltered	123.3 +/- 12.5	121.9 +/- 15.2
Unlabelled using tap water ($\delta^{18}O$ value: 6.1‰)	'Wet pots' (first harvest)	soil	Filtered <20 um	4.9 +/- 1.4	3.1 +/- 0.9
			Unfiltered	0.9 +/- 2.2	1.3 +/- 0.6
		soil with added sand	Filtered <20 um	-1.6 +/- 0.5	-1.2 +/- 0.6
			Unfiltered	-0.1 +/- 1.5	-1.5 +/- 2.0
	'Dry pots' (second harvest)	soil	Filtered <20 um	4.1 +/- 0.9	4.2 +/- 1.0
			Unfiltered	2.8 +/- 3.1	3.1 +/- 1.7
		soil with added sand	Filtered <20 um	3.8 +/-1.2	3.9 +/- 2.4
			Unfiltered	7.2 +/- 2.6	-1.9 +/- 0.7

393 Table 2. Three-way ANOVA testing the effects of the time of harvest, soil texture and soil biota
394 inoculated on the proportion of bound water from either soil or stem samples.

Samples	Variables	F-value	Degrees of freedom	P-value
Soil	Harvest time	**58.7**	**1, 26**	**< 0.01**
	Soil texture	**4.14**	**1, 26**	**0.05**
	Soil biota	0.3	1, 26	0.58
	Harvest time * Soil texture	0.12	1, 26	0.73
	Soil texture * Soil biota	0.26	1, 26	0.61
	Harvest time * Soil biota	0.07	1, 26	0.79
	Three-way interaction	0.33	1, 26	0.57
Stems	Harvest time	**43.53**	**1, 27**	**< 0.01**
	Soil texture	1.24	1, 27	0.28
	Soil biota	< 0.01	1, 27	0.96
	Harvest time * Soil texture	0.04	1, 27	0.83
	Soil texture * Soil biota	2.13	1, 27	0.16
	Harvest time * Soil biota	0.85	1, 27	0.36
	Three-way interaction	1.82	1, 27	0.19

395

396 Table 3. Two-way ANOVA testing the effects of the soil texture and soil biota inoculated on
397 the proportion of bound water from either soil or stem samples according to the time of harvest.

		Wet samples - first harvest			Dry samples - second harvest		
Sample	Variables	F-value	Degrees of freedom	P-value	F-value	Degrees of freedom	P-value
Soil	Soil texture	1.82	1, 12	0.2	2.99	1, 14	0.11
	Soil biota	0.02	1, 12	0.88	0.71	1, 14	0.41
	Soil texture * Soil biota	0.36	1, 12	0.56	< 0.01	1, 14	0.98
Stems	Soil texture	0.81	1, 14	0.38	0.65	1, 13	0.43
	Soil biota	0.71	1, 14	0.41	0.41	1, 13	0.53
	Soil texture * Soil biota	3.88	1, 14	0.07	0.02	1, 13	0.89

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Table 4. Proportion of water exchange between the bound water and mobile water pool. P-values indicate significance levels when comparing soil biota effects within each soil texture treatment, based on bootstrapping using 9999 iterations.

Soil texture	Soil biota inoculated	Proportion of water exchange	P-value
soil	Filtered <20 um	0.52	0.49
	Unfiltered	0.52	
soil with sand	Filtered <20 um	0.41	0.08
	Unfiltered	0.64	

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AUTHORS CONTRIBUTIONS

All authors contributed to the study conception and design. CD and YC established the experiment and collected the samples. AEB, CD and YC processed samples. CD and JRP performed statistical analyses. CD, YC and JRP interpreted results and wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

Datasets generated during the current study will be archived and accessible on Figshare repository (https://figshare.com/authors/Coline_Deveautour/6952964). Data repository and scripts will be available at https://bitbucket.org/Coline_ Dev/.

421

422 FIGURES AND TABLES

423 Figure 1. ^{18}O-labelled water approach as in Vargas et al. (2017). Wetting and drying using ^{18}O
424 enriched water in order to generate differential $\delta^{18}O$ values in more mobile *versus* less mobile
425 water pools.

426 Figure 2. Proportion of bound water in soil (top) and stem samples (bottom) depending on soil
427 type and on soil biota treatment from wet pots (collected at the first harvest) and from dry pots
428 (second harvest).

429 Figure S1. Proportion of bound water stem samples depending on soil type and on soil biota
430 treatment from wet pots (collected at the first harvest) and from dry pots (second harvest).

431 Figure S2. Water $\delta^{18}O$ values in soil (top) and stem samples (bottom) depending on soil type
432 and on soil biota treatment from wet pots (collected at the first harvest) and from dry pots
433 (second harvest). Figure shows $\delta^{18}O$ values measured in samples that were labelled with
434 enriched water ($\delta^{18}O$ value: 467.4 ‰).

435 Figure S3. Relationship between the proportion of bound water in plants and the leaves biomass
436 (a) or the root biomass (b).

437 Table 1. Soil and stem $\delta^{18}O$ values (average +/- standard error) evaluated from labelled and
438 unlabelled samples according to each soil texture and soil biota treatment collected in 'wet'
439 (first harvest) and 'dry' pots (second harvest). Table indicates whether the samples were
440 watered with enriched water ($\delta^{18}O$ value: 467.4 ‰) or using tap water ($\delta^{18}O$ value: 6.1‰), the
441 harvest timepoint at which samples were collected to obtain pots containing bound and mobile
442 (first harvest) or only bound water (second harvest), the soil texture and soil biota treatment and
443 the corresponding measured $\delta^{18}O$ values from soil and stem samples.

444 Table 2. Three-way ANOVA testing the effects of the time of harvest, soil texture and soil biota
445 inoculated on the proportion of bound water from either soil or stem samples.

446 Table 3. Two-way ANOVA testing the effects of the soil texture and soil biota inoculated on
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449 values indicate significance levels when comparing soil biota effects within each soil texture
450 treatment, based on bootstrapping using 9999 iterations.

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