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Amidou Traore

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Magali Nuix ^{a,b,c#}, Guilhem Pages ^{a,b}, Jean-Marie Bonny ^{a,b}, Catherine Pichon-Cochard ^c, Amidou Traore ^{a,b}

^a Université Clermont Auvergne, INRAE, UR QuaPA, 63122, Saint-Genès Champanelle, France

^b INRAE, PROBE research infrastructure, AgroResonance facility, 63122, Saint-Genès-Champanelle, France

^c Université Clermont Auvergne, INRAE, VetAgro Sup, UREP, 63000 Clermont-Ferrand, France

[#] present address : The Institute of Cancer Research 15 Cotswold Road, Sutton, SM2 5NG

Grasslands and forests are the two main terrestrial ecosystems limiting the global warming thanks to their high capability to store carbon [1]. Sap flows are playing a critical role by bringing the water and minerals used for transpiration and photosynthesis (xylem) from the roots to the leaves and transporting the photosynthetic carbons (phloem) to the carbon sinks (e.g., wood, roots, soil). In the context of global warming, a fine understanding of these transport mechanisms is necessary. However, a sensor able to locally probe water content and its movement directly on the plants in their ecosystem (i.e., in situ) does not exist yet. Our objective is to evaluate the capabilities of Mobile Universal Surface Explorer (MOUSE) sensor to measure, in-situ, both water repartition and its flow in roots of herbaceous plants.

Materials and Methods

Monocot (*Dactylis glomerata*, DG) and dicots (*Rumex acetosa*, RA and *Medicago sativa*, MS) cultivated in monoculture in rhizotron model (Fig 1a)

NMR Experiments were performed in climatic chamber (photoperiod : 14 hours, 21/16°C). Ecophysiological parameters : SWC (soil water content, LWP (leaf water potential, Gs (stomatal conductance) were recorded.

Plants were submitted to different hydric conditions :

- Hydrated conditions (HP, 250mL of water twice per day)
- Drought conditions (DP, after 3 weeks without irrigation)
- Rehydrated conditions (RP, 200mL of water three times per day during 3 days) → *R. acetosa*

A Profile NMR-MOUSE® (Mobile Universal Surface Explorer) PM25 (Magritek, Germany) with a ¹H resonance frequency of 13.23 MHz was used. Measurements were performed at the fixed position with the measurement volume (fig. 1c) at the level of the hydration captor in the soil.

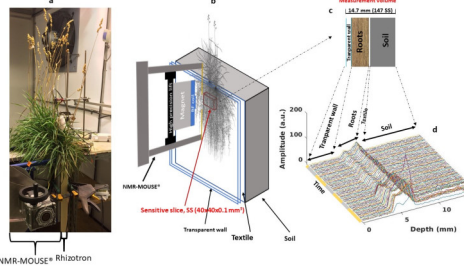


Fig. 1 [2]: (a) Experimental setup in the climatic chamber (case of *D. glomerata*). (b) Schematic representation of the magnet in contact with a rhizotron and of the position of the NMR sensitive slice (red rectangular parallelepiped). (c) Illustration drawing of different structures in the measurement window. (d) 3-day (daytime in yellow and nighttime in gray) profiles, i.e., SI (average of 256 echoes) at each depth, cycle. Soil, roots and transparent wall compartments are clearly revealed in each profile whereas no clear feature can be attributed to the textile

A depth signal intensity (SI) was obtained by summing the 256 echoes acquired in the 100µm-thick sensitive slice (fig 1b) using a CPMG pulse sequence with TE=100µs, TR=2s and 4 scans. Depth profile was then obtained by spanning the 14.7mm of the measurement volume (147 depths and 100µm-step for 35min) thanks to the high precision lift.

Transversal echo decays were also recorded at the position displaying the higher SI in the profile for T₂ measurements, with the following parameters: 2048 echoes, TE=100µs, TR=10s and 8 scans. Multiexponential analysis with NNLS algorithm was used to extract multicomponent T₂ distribution for HP conditions. Whereas a monoexponential fit was done on the average of five transversal decays of highest SI depths in the profiles of HP-DP-RP experiments. Profiles (and T₂ for HP) were continuously acquired during 3 days/nights for HP (Fig.1d) and after hydric stress for DP samples and after rehydration for RP samples.

Results and Discussion

Hydrated conditions

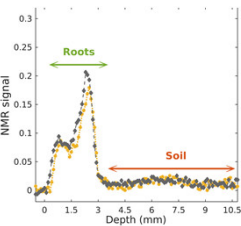


Fig. 2 : Illustration of the MR SI as a function of the depth obtained for DG in a HP during a consecutive night (black diamonds) and day (orange diamonds)

The depth profiles (Fig. 1d and Fig. 2) clearly display a 1D image of the rhizotron sample :

- Transparent wall as a zone with a void signal (no mobile protons)
- Roots as the zone with higher SI
- Soil as a region with lower SI

- Soil and roots zone are contrasted according to their water contents but mainly to their T₂
- The decrease in SI during the day may be attributed to the increase of water temperature (diffusion dephasing)
- The shift of the day's depth profile is attributed to change in magnet temperature [3]

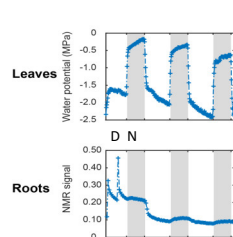


Fig. 3 : Circadian variation of Leaf water potential (LWP) and of the integral of root zone for a DG in a HP

- Plant transpiration resulted in a circadian variation of LWP (Fig. 3 top)
- Circadian variation of root SI integral is attributed to both diffusion (T²) and water flow to satisfy plant transpiration (Fig. 3 bottom)

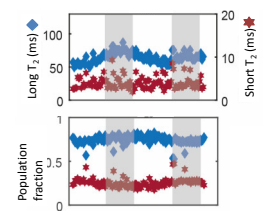


Fig. 4 : Circadian evolution of long and short T₂ along their population fractions measured at the depth of higher SI in the profile for DG in a HP

- Circadian variation of long T₂ (mobile water) is the result of both incoherent (diffusion) and coherent (flow) proton dephasing in the B₀ direction, while the population fractions remained almost constants (Fig. 4)

Drought and rehydrated conditions

Compared to the HP the 3-weeks of hydric stress resulted in :

- A strong decrease in Gs, e.g., 79.7% (DG) and 94% (RA)
- A decrease in SWC, e.g., 52% (MS) and 59% (DG)
- A decrease (far from 0 at night) of LWP : -3.6MPa (DG); -3.85 MPa (RA) and -5.47 MPa (MS)

➔ Loss of 50% of hydraulic conductance ➔ embolism and a huge stress [4]

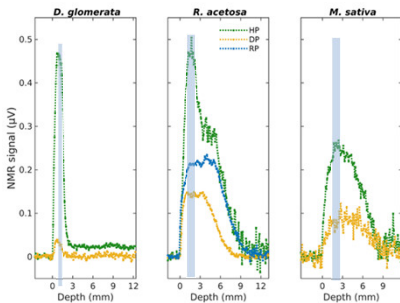


Fig. 5 : Illustration of the MRI signal obtained on (a) *D. glomerata*, (b) *R. acetosa* and *M. sativa* in the well hydrated conditions (HP), in the drought conditions (DP, after 3 weeks without irrigation), and in the rehydrated conditions (RP, 200mL of water three times per day during 3 days). The blue area indicates the depths whose transversal echo decays were averaged before exponential fit for T₂ analysis

- A strong decrease of root area in the depth profiles (Fig. 5) : 93% (DG), 63%(RA) and 58% (MS) compared to HP condition
- The disappearance of circadian variation of the integral of SI of the root area (Figure 6b)

- From the monoexponential fit [A*exp(-t/T₂)] of the average transversal decays of highest SI depths, both signal amplitude A and T₂ decreased from HP to DP. This contrast is exacerbated by combining the two parameters (AT₂). This decrease was attributed to a decrease in both water content and vacuolar compartment in the stele

- The rehydration (RP) is accompanied by an increase of all these parameters as shown in Fig. 6 for RA, e.g., LWP (transpiration recovery) concomitantly with an increase of mobile water (water transport), without yet reaching the HP values in the time of the study



Fig. 6 : (a) LWP for HP (green) DP (yellow) and RP (blue) conditions along with Gs (orange) recorded during the 28 days of experiment. (b) Root NMR area for HP (green), DP (yellow) and RP (blue) conditions. Signal amplitude (A), T₂ and T₂ weighted SI, AT₂ for HP (green), DP (yellow) and RP (blue) conditions

Conclusions

The capabilities of Profile NMR MOUSE to characterize in-situ the state of water in the roots of herbaceous plants in different hydric states is demonstrated

- A circadian variation of root NMR signal is highlighted in both root profiles and T₂, suggesting a potential method for estimating water flow in roots
- Decrease in both NMR signal intensity and T₂ is observed in huge hydric stress along with a disappearance of circadian variation
- A start of transpiration recovery (increase of ecophysiological parameters) and water transport in rehydrated samples is also evidenced by the increase of NMR parameters