An approach to the mechanisms of use of gypsum crystallization water by plants

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Water is one of the most limiting factors for plant survival in the atypical gypsiferous soils. However, gypsum contains two water molecules in its crystalline structure prone to be used by plants during the driest season (Palacio *et al*, 2014; Palacio *et al*, 2017; de la Puente *et al*, 2021). The mechanisms behind this water up-take process have not been described yet, but it has been suggested that it could involve dissolution processes (Huang *et al*, 2020) due to root exudation or microbial activity. The principal objective of our study was to corroborate the use of this crystallization water by *Helianthemum squamatum,* grown in pots. We also studied root exudation, soil microbial composition and plant physiological parameters (transpiration, stomatal conductance, assimilation rate and water use) of the plants to gain knowledge on the processes related to gypsum crystallization water use.
Plants were subjected to two crossed treatments: a labelling treatment; in which plants were grown in a gypsum soil whose gypsum crystallization water was enriched in deuterium, and, a drought treatment, in which plants were subjected to a progressive decrease in water supply during 23 days. We analysed the isotopic composition of water in the xylem sap of plants and in the soil extracted in a vacuum cryogenic distillation line. Plants physiological status was analysed with an infrared gas analyser and monitoring. Plant water use through the weight of pots during the drought treatment. Phospholipid fatty acid (PLFA) analyses were used to estimate microbial biomass and root exudations were extracted following the method in Teodoro *et al*, (2019) and analysed with UHPLC/Q-TOF-MS.
The isotopic composition of the xylem sap varied between substrate conditions, drought treatments and their interaction. However, values of xylem water of all plants fully aligned with the evaporation line of free water, ruling out the possibility of crystallization water use by these plants. In addition, the labelling treatment of the soil led to a partial sterilization of the soil. Differences between experimental soils also caused different patterns of responses to drought, root exudation and photosynthesis in labelled pots. Drought had a significant effect on certain root exudate production and on the abundance of AMF.
These results call for caution in the interpretation of Bayesian Mixing Models, which should be carefully contrasted with the biplot $\delta^{2}H$-$\delta^{18}O$ of sources and consumers, as this method could lead to the wrong choice of sources used by plants. We compare our results with previous studies on the same species conducted in the field and conclude that the factors that allow the crystallization water uptake by plants during a long drought period in the field were not satisfactorily reproduced in our pot experiment. In particular, the effect negative of both drought and D-labelling on AMF communities could be behind the observed results

Keywords
Crystallization water of gypsum, drought, stable isotopes of water, water sources.