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KEYWORDS

Stony soil, rock fragments, water absorption, hydrological processes, water stress, stomatal conductivity, drought

ABSTRACT

Background and aims Soils are composed of both fine and coarse materials. Coarse material (> 2 mm) is considered to be inert and is usually discarded in models of plant water balance, even though it affects soil properties. No studies have yet attempted to assess whether rock fragments may act as a water reservoir for plants.

Methods Cuttings of *Populus euramericana* were planted in 5-L pots containing reconstituted soil made up of fine earth (silty clay loam texture) and either limestone or inert (quartz) pebbles (rock fragments 2-5 cm) at 0, 20, and 40% volume in a cross-factorial experiment. Two drought periods were applied and the growth, evapotranspiration, water stress status by stomatal conductance, and water content of the two soil phases (fine earth and pebbles) were monitored.

Results First, pebbles can contain water, and ignoring this water induced and underestimations of the soil available water content by respectively 11% and 30% for the treatment with 20% and 40% limestone pebbles. Second, the plants grown on limestone pebbles were up to 70% less stressed than the plants grown on inert pebbles during drought. Third, stomatal conductance, a water stress indicator, was correlated with the water content of both the fine earth and the limestone pebbles.

Conclusion These results demonstrate that limestone rock fragments can retain available water and act as a reservoir during drought periods.

ABBREVIATIONS

AWC: available water capacity

ET: evapotranspiration

WUE: water use efficiency

G: stomatal conductivity

INTRODUCTION

Stony soils, or skeletal soils, are defined as soils that contain more than 35% (or 40% in some authors) of rock fragments, that is particles larger than 2 mm, in volume (FAO 2006; IUSS Working Group WRB 2006; Soil Survey Staff 2010). In Western Europe, stony soils cover approximately 30% of the land (Soil Map of the European Community), while they cover 60% in the Mediterranean area (Poesen and Lavee 1994) where some skeletal soils can reach very high rock fragment contents.

Rock fragments affect soil physical properties such as soil bulk density, porosity, soil erosion, thermal properties and hydrological processes (Poesen and Lavee, 1994). The importance of their influence depends on several factors, including the proportion of rock fragments, their location in the soil and their nature (Hlaváčiková et al. 2016; Zhang et al. 2016). For example, Gras and Monnier (1963) showed that flint provides the lowest available water content, being close to zero. On the other hand, the available water capacity (AWC) of limestone rock fragments can be nearly 30% and is extremely high for chalk. These rock fragments constitute a significant part of the water holding capacity in some soils. In the same way, Tetegan et al. (2011) determined the various water storage capacity of rock fragments depending on their sedimentary lithology and proposed a pedotransfer function to estimate this capacity. Mi et al. (2016) showed the significant effects of rock fragments on plant water consumption, biomass, growth and water-use efficiency under different water conditions in sandy loamy soils. The recent work of Parajuli et al. (2017) compared experimental data with numerical simulations to obtain more accurate averaging schemes to estimate the water retention curve and to develop predictive models for the unsaturated hydraulic conductivity of stony soils.

Surprisingly, most researchers analyze only the fine earth (fraction < 2 mm), while the coarse fraction (fraction > 2 mm, the rock fragments) is discarded by sieving in the first phase of sample preparation. Consequently, discarding the coarse fraction or only taking coarse fraction volume into account can lead to interpretive errors in the results, such as an over- or under-estimation of available water capacity or available water content for plants (Coile 1953; Cousin et al. 2003; Ugolini et al. 1998).

Few studies on water transfer from rock fragments to fine earth point out a suspected benefits to plants (Ballif 1980; Gras and Monnier 1963). In a recent review, Zhang et al. (2016) concluded their section on the rock-root interface by stating that “soil hydrological processes occurring at the rock-root interface are poorly characterized […] and that further investigations are required.” Tetegan et al. (2015a) demonstrated that water exchanges between rock fragments and fine earth do indeed exist and deduced theoretical exchanges between rock fragments and plants during soil desiccation. However, they did not assess the role of water in rock fragments on plant growth and drought resistance. However, one could hypothesize that the water contained in porous rock fragments may help the plant resist drought. To test this hypothesis, we designed a greenhouse experiment based on monitoring both soil water content and certain biometric parameters of poplar cuttings. To assess whether rock fragments may act as water reservoirs for poplar cuttings, we compared the plants’ grown on fine earth mixed with either porous limestone pebbles or nonporous quartz pebbles. We aimed to do paired comparison between the treatments with limestone porous pebbles and the nonporous quartz pebbles. First, we characterized the amount of water that limestone and quartz pebbles can retain. Second, in addition to standard biometric measurements, we monitored leaf stomatal conductance (G), which reflects stomatal opening and the resistance to water-vapor transfer from the leaf to the atmosphere (Beadle et al. 1993).We then used G to characterize plant water stress during the experiment with soil desiccation periods and plant responses to weather variables and water stress. Third, we compared the changes in stomatal conductance with the available water content of the fine earth and pebbles.

MATERIALS AND METHODS

Constitution of stony soils and treatments

Reconstituted soils were set up with fine earth (soil particle diameter < 2 mm) mixed with different proportions of pebbles (rock fragment diameter 2 cm to 5 cm): 0, 20 and 40% in volume (Fig. 1). The rock fragments were either limestone or nonporous pebbles of pure quartz. Limestone pebbles were chosen for their physical characteristics, as they are porous and can potentially act as a water reservoir, while quartz pebbles were chosen as a hydraulically inert material, as they act as a physical substitute for the coarse soil fraction. Both fine earth and limestone pebbles were collected from the Ap horizon of a Calcaric Cambisol (IUSS Working Group WRB 2006) containing more than 30% rock fragments in the Beauce region of central France at the Villamblain site (48°01'N, 1°551'E). The fine earth had a silty clay loam texture (according to the USDA textural triangle: 34% silt, 61% clay and 5% sand, Suppl. 1). A volume of approximately 300 dm^3 was collected to be representative of the whole Ap horizon. Among the rock fragments, only the pebble fraction (2–5 cm) was selected. In the laboratory, the pebbles were brushed under water to remove all soil particles adhering to the surface. The reconstituted soils were carefully created by hand with air-dried sieved fine earth (<2 mm) and air-dried pebbles placed in 3-L pots (15 cm high with a top diameter of 19.4 cm). The bottom of the pots under the geotextile had 13 holes of 1 cm in diameter, which allowed for correct irrigation by capillarity. We had previously calculated the amount of pebbles and fine earth (in grams) necessary to fill up the pots for each treatment according to the bulk density and proportion of pebbles and the bulk density of the fine earth (fixed at 1.1 g.cm^{-3}). To fill the pots, we first put down a 3-cm layer of fine earth, then pebbles and fine earth were added in successive layers. Five treatments were established: 0% pebbles (pure fine earth), 20% limestone pebbles, 40% limestone pebbles, 20% quartz pebbles and 40% quartz pebbles (Fig. 1). The treatments with 20% pebbles represented a significant amount of the rock fragments, and the treatments with 40% pebbles corresponded to the maximum amount of rock fragments that reconstituted soils could contain. We filled ten pots per treatment, making a total of fifty pots, for the plant biometric measurements. Fifteen additional pots for each of the following treatments - 0% pebbles, 20% limestone pebbles, 40% limestone pebbles and 40% limestone pebbles without plants (for a total of sixty extra pots) were added to the experimental design to monitor soil water content during the first desiccation period. The results are presented in Tetegan et al. (2015a). To monitor soil water dynamics, pots were sampled and deconstructed to measure the water content on both the fine earth and the pebbles; these pots could therefore not be used for biological measurements. In all, 110 pots were used in the experiment.

A 10-cm woody-stem cutting of *Populus euramericana (Dode) Guinier cv. 'Robusta'* was planted in each pot (mean diameter 0.71 ± 0.12 cm) and grown in a greenhouse under semi-controlled conditions (the glass of the greenhouse was painted white to prevent extreme temperatures and insolation, and extra artificial light was added to have a constant daylight period to 16 hours.day^{-1}. The temperature was maintained at 22 °C ± 2 °C) for the entire experiment.

Water characteristics of pebbles and reconstituted stony soils

The water retention of both the fine earth and the pebbles was determined by pressure plate experiments (Richards 1956) following the Klute (1986) method. The available water capacity (AWC_i, $cm^3.cm^{-3}$) was calculated for each phase (i) based on the difference between the water content at field capacity (θ_{pF2} measured at the matrix potential of – 10 kPa or pF2) and the water content at the permanent wilting point ($\theta_{pF4.2}$ measured at the matrix potential of – 1585 KPa or pF4.2), as shown in equation (1):

$$AWC_i = \theta_{pF2} - \theta_{pF4.2} \qquad (1)$$

The total AWC ($cm^3.cm^{-3}$) for each treatment was calculated according to equation (2):

$$AWC = \omega AWC_{FE} + (1-\omega)AWC_{RF} \qquad (2)$$

where ω represents the volume proportion of fine earth, AWC_{FE} represents the AWC of the fine earth and AWC_{RF} represents the AWC of the rock fragments.

Water regime during the experiment

All the pots were watered at the beginning of the experiment by capillary rise until maximum soil water holding capacity, then irrigation was controlled to maintain the water content at 40% of total AWC, which corresponds to moderate water stress. To do this, we first waited until the soil in the pots had reached the desired 40% water content level, then we manually watered each pot roughly every two days to maintained the same level. The quantity of water added was equal to the water lost through evapotranspiration, which we measured by weighing the pots. As plant growth between two days could be considered negligible (less than one gram) compared to several hundred grams of water loss, we attributed mass loss to the water lossed via evapotranspiration.

After two months of growth, all the pots were again watered to saturation by capillary rise for ten days. The end of this rewatering period corresponded to the start of our drought experiment (day 0), which was designed to measure plant water stress during soil drying periods. The "first desiccation period" lasted 13 days (starting from day 0); no water was supplied to the pots during this time. From day 13 to 19, the pots were once again watered by capillarity rise. Water stress was then applied a second time during the “second desiccation period”, from day 19 to the end of the experiment (day 34); the pots were not watered during this period.

Soil water content dynamics

Soil samples were collected at five dates during the first desiccation period for the pots from three treatments: pure fine earth and fine earth + 20% and 40% limestone pebbles. At each sampling date (2, 4, 6, 9 and 13 days after saturation), three pots for each treatment were used to measure the gravimetric water content for pebbles (see Tetegan et al., 2015, for more details). The equivalent volumetric water content of each phase was calculated with the median value of the bulk density of the respective phase (1.1 $g.cm^{-3}$ and 2.02 $g.cm^{-3}$ for the fine earth and the pebbles, respectively). The dynamics of the soil water content in the fine earth and the pebbles during the desiccation period are presented and discussed in Tetegan et al. (2015a). In the present study, we used the water content in both the fine earth and limestone pebble phases during the first desiccation period to study the relationship with leaf stomatal conductance, a plant water stress indicator.

Plant measurements

Leaf stomatal conductance G is a measure of the rate of gaseous exchange (water vapor flux, in $mmol.m^2.s^{-1}$) through the leaf stomata as determined by the degree of stomatal aperture (and therefore the physical resistance to the movement of gases between the air and the interior of the leaf). For a given plant, G is a function of the degree of stomata opening: stomata gradually close as drought becomes more severe allowing lower conductance, and consequently indicating that photosynthesis and transpiration rates are lower, and water stress higher. We did not used the predawn leaf water potential to t its destructive drawback and the small number of leaves.

We used a leaf porometer (SC-1 leaf Porometer, Decagone Devices Inc.) to measure leaf stomatal conductance between 12 and 2 pm on 21 dates over a period of 32 days. These figures indicated the level of plant water stress during the desiccation periods. For the 21 dates of measurements throughout the experiment, one leaf from each of ten plants per treatment was monitored. A fully illuminated mature leaf was chosen, usually the third leaf from the top, and identified with a ring. As our plants were growing in the same atmospheric conditions (in a controlled greenhouse environment and in a completely random design), a higher G indicated lower soil water stress.

Plant height was measured throughout the three months of the experiment: four times before and four times during the drought period.

After three months, the experiment ended, and biometric parameters were measured (height, number of leaves and basal diameter). The plants were collected and separated into below- and above-ground parts. The latter were further separated into stems and leaves. All the leaves were counted, some were also selected to measure maximum width and surface area with a scanner connected to WinFOLIA™ software. Finally, all the parts of the plant were dried at 40 °C until a constant weight was obtained (approximately three days) and weighed to determine biomass (dry weight). The leaves used for surface area measurements were weighed separately to obtain an allometric relationship between leaf width and total leaf area. Similarly, the total height of the stem of each plant was measured and the stem was weighed (oven dried at 65°C until constant weight) to obtain an allometric relation for each plant between stem height and dry mass. The increase in biomass between two dates during the experiment was calculated using this height-biomass allometry for each individual plant at the end of the experiment (mean = 0.117 g per cm of growth, SD = 0.025, min = 0.061, max =

0.180). Based on the growth in height of each plant during the desiccation period, we could estimate the increase in biomass, and calculate the WUE (by dividing the growth in biomass by the evapotranspiration, $g.mm^{-1}$).

During the first desiccation period, water loss, expressed in g, was measured by weighing the pots every two days. Evapotranspiration was calculated from mass loss and expressed in mm. In addition, some specific calculations were performed for the first desiccation period: evapotranspiration rate (which is water loss divided by leaf surface and by the number of days in the period, $mm.day^{-1}.cm^{-2}$ leaf area) and water use efficiency (WUE, growth in biomass divided by the evapotranspiration, $g.mm^{-1}$).

Statistical analysis

Comparisons of treatments were tested using an ANOVA, and when differences were significant ($p<0.05$), a pairwise comparison test was performed (Tukey's HSD).

All statistical tests were performed with Statgraphics Centurion XVI.

RESULTS

Water content in pebbles

The mean water content in limestone pebbles at field capacity was 0.2 $cm^3.cm^{-3}$, (water potential equal to −10 KPa), or 10% in mass for a mean pebble bulk density of 2.02 $g.\ cm^{-3}$ in our case, and reached 0.13 $cm^3.cm^{-3}$ at the permanent wilting point (matrix potential of –1585 KPa). While limestone can retain water and presents an AWC of 0.076 $cm^3.cm^{-3}$ (7.6%vol), quartz pebbles, on the other hand, cannot retain more than 1% after saturation. We confirmed that quartz pebbles are hydraulically inert and could be used in our experiment to replicate the physical effects of the coarse fraction in reconstituted soils. The mean water content in the fine earth at field capacity was 0.40 $cm^3.cm^{-3}$ and reached 0.23 $cm^3.cm^{-3}$ at the permanent wilting point, leading to an AWC of 0.17 $cm^3.cm^{-3}$. Based on the AWC of each material (fine earth, limestone or quartz pebbles) and their proportion in the reconstituted soils, we calculated the AWC in each treatment (Table 1). The AWC of the five treatments ranged from 0.10 to 0.17 $cm^3.cm^{-3}$ and was in the following ascending order: 0%, 20% limestone, 40% limestone, 20% quartz and 40% quartz (Table 1). Treatments with 20% and 40% limestone pebbles showed a soil available water content of 11% and 30% higher than the corresponding treatments with quartz pebbles. Therefore, if limestone is considered inert, AWC would be considered equal to that of quartz, and an underestimation of 10 and 23% would be made.

Evapotranspiration

The dynamics of evapotranspiration (ET) during the first desiccation period showed two phases (Fig. 2). The first period, from day 2 to day 7 after saturation, corresponded to steady and rather fast ET, with a mean of 3 $mm.day^{-1}$ (from 2.5 to 3.8, depending on the treatment). From day 8 to day 13, ET slowed down to half the value of the first period, with a mean of 1.3 $mm.day^{-1}$ (from 0.8 to 1.6, depending on the treatment). For the whole experiment, the lowest water loss (Table 2) was observed for the treatments with 40% pebbles, values were intermediate for the treatments with 20% pebbles and the highest was observed for the treatment with fine earth only. Differences between the two types of pebbles were significant only when we compared the treatments with 20% pebbles (Table 2, Suppl 2): plants grown in limestone pebbles evapotranspired 15% more water compared to plants grown in quartz pebbles.

Moreover, similar results were observed for water use efficiency (WUE): plants grown on fine earth showed a WUE 1.6 times higher than those grown on 40% quartz pebbles; WUE values measured for plants grown in the 20% limestone pots or the 20% quartz pots did not differ.

Plant water stress

Measurements of plant water stress via stomatal conductance (G) over the course of the experiment showed four periods (Fig. 3a, Table 4). In the first six days (Period I, day 0 to day 6 after saturation with no irrigation), plants showed high conductance, ranging from 150 to 250 $mmol.m^{-2}.s^{-1}$ (mean of all treatments > 170 $mmol.m^{-2}.s^{-1}$), indicating that there was enough water in all the pots. On the first day measurements were taken, a high variability among pots was observed in all treatments, and furthermore there were no significant differences among treatments. Variations in the first two days were due to the weather: regularly passing clouds blocked the sunlight thus reducing gaseous exchanges for some plants during the measurements. Overall during the first six days, only a slight difference was observed: the 20% quartz treatment had a higher G than did the 40% limestone treatment ($p=0.01$, Table 4).

The second period (Period II, days 7 to 13) corresponded to a water stress period with conductance decreasing over time in all the pots. During this period , plants grown in 20% limestone or quartz pebbles had similar stomatal conductance (Fig. 4b). At 40%, plants grown in limestone pebbles showed a higher stomatal conductance compared to plants grown in quartz pebbles (Fig. 4c).

The third period corresponded to rewatering (Period III, days 14 to 19), where stomatal conductance increased for all the plants. The plants grown on 40% quartz seemed to recover more slowly, with the lowest stomatal conductance (40 ± 8 $mmol.m^{-2}.s^{-1}$) at day 16 (Fig. 3). At the end of this period on day 19, all the plants had similar foliar stomatal conductance (mean 162 ± 17 $mmol.m^{-2}.s^{-1}$); however, this level was still much lower than on day 2 and was equivalent to the level at the beginning of the first stress period (day 6, mean = 174 ±33 $mmol.m^{-2}.s^{-1}$).

The fourth and last period corresponded to the second desiccation period (rewatering stopped on day 19; Period IV, days 19 to 34). Conductance ranged from 149 to 192 $mmol/m^2/s$ (mean of all treatments 162 $mmol.m^{-2}.s^{-1}$) and showed a regular and similar decrease in stomatal conductance for all the plants.

In addition to measuring stomatal conductance, we looked for a relationship between conductance and soil water content during the first desiccation period. We found that, whatever the treatment, only the fine earth phase reached water content above 0.2 $cm^3.cm^{-3}$, corresponding to the first two days with no irrigation and the plants showed a high steady G value, indicating no water limitation for the plants (Fig. 4 a). Below 0.2 $cm^3.cm^{-3}$, G decreased linearly with SWC both in the fine earth and pebbles phases ($p<0.0001$). The linear relationship between G and water content in the pebble phase was similar to the one in the fine earth phase (Fig. 4 b, that is the slopes were not significantly different whatever the treatment, $p= 0.82$). This indicates that the water stored in the pebbles decreased concomitantly with the water in the fine earth, and was also related to plant water stress (G). Therefore, during the first desiccation period, limestone pebbles contained as much available water as the fine earth.

Plant biometric measurements

At the end of the experiment, we observed differences in plant height, diameter, number of leaves, foliar surface area, biomass and evapotranspiration (Table 3). Most of the characteristics of plant growth (height, number of leaves, total biomass and diameter) were negatively correlated with the proportion of pebbles in the pot. The highest biometric parameters were recorded for the pots without any pebbles; plants grown with 20% and 40%

pebbles had heights of 0.78and0.6, respectively, and diameters of 0.85 and 0.73 times that of plants grown on fine earth only.

For the pots containing rock fragments, the plants with fewer pebbles grew best. For instance, plants grown with 40% pebbles had half the biomass of those grown on fine earth regardless of the type of pebbles, while the biomass of the plants grown with 20% pebbles was reduced by only approximately one third. In addition, the type of pebbles (limestone or quartz) affected some other plant biometric parameters in pots containing 20% pebbles; for example, biomass and root to shoot ratio was higher for limestone pebbles than it was for quartz pebbles. However, for plants in pots containing 40% pebbles, most of the biometric values were the same for limestone and quartz pebbles (Table 3).

DISCUSSION

Estimating soil available water capacity (AWC) and available water content in stony soils is difficult, and most often, rock fragments are considered to be inert. They are sometimes completely ignored or their volume is simply subtracted from the fine earth volume. In our experiment, the mean maximal AWC of limestone pebbles was 0.20 $cm^3.cm^{-3}$ (or 10% in mass for a mean pebble bulk density of 2.02 $g.cm^{-3}$ in our case). Most importantly, for the treatments with 20% and 40% limestone pebbles, we showed that neglecting the pebble water content leads to a respective underestimation of 10% and 23% of the soil available water content. Therefore, ignoring the pebble water content underestimated the soil available water capacity by more than 5% for every 10% of pebbles present in the soil. Likewise, several studies have shown that rock fragments can account for a significant part of the soil water reserves in stony soils (Gras and Monnier 1963; Poesen and Lavee 1994; Tetegan et al. 2015b). Coutadeur et al. (2000) and Cousin et al. (2003) showed that the available water of agricultural soils can be underestimated by 8-34% if the hydrological properties of rock fragments are neglected, or overestimated by 22-39% if their volume is not considered. Nevertheless, as in our experiment, the AWC of a soil horizon usually decreases when the rock fragment content increases (Baetens et al. 2009; Cousin et al. 2003; Poesen and Bunte 1996), as the available water content of a rock fragment is rarely higher than that of fine earth.

Nevertheless, the capacity of rock fragments to retain water does not necessarily mean that this water is available for plants. Water availability in chalky soils was discussed in the 1970's (Ballif 1980; Burnham and Mutter 1993; Gras and Monnier 1963), but the authors concluded that such soils were unusual and that their results could not be applied to other soils; furthermore, they never showed that plants could absorb water from the rock fragments. In our experiment, two observations suggest that water stored in porous limestone rock fragments is available and is used by plants: (i) plants grown with porous limestone pebbles compared to nonporous quart rock pebbles exhibited less water stress, and (ii) we found a the linear relationship between soil water content (both for pebbles and pure fine earth) and plant water stress (G). We discuss these two points in the paragraphs below. Our work was based on paired comparison between the treatments with limestone porous pebbles and the nonporous quartz pebbles. We did not compare treatments of different proportion of pebbles, which could not have been relevant knowing the differences in number of leaves and leaf area.

First, we compared the water stress, in terms of the leaf stomatal conductance (G), of the plants grown on stony soils composed of porous limestone or nonporous quartz pebbles during a desiccation period. We showed that plants grown on limestone are less stressed than plants grown on quartz pebbles. This observation is even more significant when the proportion of rock fragments increases. At 20% pebbles, and although biomass and evapotranspiration (water loss) were higher for limestone (Table 2, 3 and 4), leaf stomatal conductance of the plants grown on limestone was similar to that of plants grown on quartz pebbles. At 40% pebbles, neither evapotranspiration nor any of the biometric measurements were different between the limestone and quartz treatments, but leaf stomatal conductance was much reduced with limestone. In addition, at 40% pebbles, the leaf stomatal conductance of the plants grown on quartz pebbles rapidly fell below 50 $mmol.m^{-2}.s^{-1}$, while the leaf stomatal conductance of the plants grown on limestone pebbles decreased regularly (Fig. 4). Therefore, for both percentages of pebbles, the plants grown with limestone rock fragments coped better with water shortage than the plants grown on inert quartz pebbles. These results demonstrate that plants grown with limestone rock fragments accessed more water, which resulted in fewer signs of water stress (lower leaf stomatal conductance).

Second, we showed that during soil desiccation, stomatal conductance (G) was linearly correlated with water content both in the fine earth and in the pebbles. As conductance is linked to water availability, these results demonstrate that the plants did have access to the water retained in the pebbles. Furthermore, the slope of the linear regressions were similar for fine earth and limestone pebbles (Fig. 4b), suggesting that the water stored in the rock fragments was as available as the water in the fine earth, and was absorbed simultaneously by the plants.

Therefore, our experiment is one of the first to demonstrate that plants benefit from the water contained in porous rock fragments (limestone) during desiccation compared to inert fragments (quartz). Several authors have stated that plants access water in the pores of pebbles either directly or after a transfer to the fine earth (Coile 1953; Gras and Monnier 1963; Tetegan et al. 2011). The direct access hypothesis is supported by our experiment; indeed, we observed the development of many fine roots around and even in the pores of the limestone pebbles (Picture 1), which could directly absorb the water present in the pores. We also measured a higher root/shoot ratio for plants grown on 20% limestone pebbles compared to those grown on quartz pebbles, reinforcing this hypothesis (Table 3). Nevertheless, the root/shoot ratio was not significantly different for the treatments with 40% pebbles, probably because root prospection was limited by the high density of pebbles and water stress appeared early (low soil available water capacity). Similarly, Ingelmo et al. (1994) found field evidence that in stony Mediterranean soils, vegetation can grow quite well due to the abundance of rootlets in the vicinity of cobbles, stones and boulders. Du et al. (2017) also showed that rock fragments promoted root growth in an alpine steppe. The indirect access hypothesis is also possible, as Tetegan et al. (2015a) demonstrated that water could theoretically move from the stony phase to the fine soil phase for water potentials lower than −10 kPa. In addition, the pebble water content started to decrease at the same time plants presented signs of water stress: a decrease in pebble water content (i) with leaf conductance as shown in Fig 4b, and (ii) from day 6 in Fig. 2b in Tetegan et al. 2015a, suggesting that the water from the pebble pores of was used by the plants only during drought.

In our experiment, all the plants grown on pebbles, regardless of the type and proportion of pebbles, had less available water (Table 2). Indeed, compared to fine earth only, AWC was reduced by 11 and 22% for the limestone treatments, and by 20 and 40% for the quartz treatments, for 20 and 40% of rock fragments, respectively. Meanwhile, we observed that total plant biomass was negatively related to the proportion of pebbles in the soil (Table 3). Even if other factors could have been involved, available water must have been the main limiting resource in our experiment, with lower AWC resulting in lower growth in the rock fragment treatments. Indeed, poplar is known to be a water-demanding species; furthermore, due to the type of soil and the duration of our experiment, nutrients were not a limiting factor. Nevertheless, in the field, the effect of rock fragments on plant growth or crop productivity can be either positive or negative depending on several factors such as the typology of the fine earth, soil depth, and water regime (Gras 1994). Even if a decrease in productivity has often been reported with increased stoniness (Babalola and Lal 1977), some studies or observations have shown that certain stony agricultural soils can actually improve crop yield (Kosmas et al. 1994). For instance, Danalatos et al. (1995) reported an increase in production five to ten times higher on stony soils of shale-sandstone formation compared to marl soils, which are free of rock fragments, due to a reduction in evaporation. Poesen and Lavee (1994) thoroughly reviewed the different physical roles rock fragments play in hydrological processes.

All of these studies focused on the effects of rock fragments on soil physical processes and did not address the use of water by plants or plant physiological processes. In our study, we showed, as expected, that our poplar plants grew better on soil composed of fine earth only compared to stony soils, due to higher AWC. We also showed that during drought, plants accessed the water stored in porous rock fragments, thus reducing their water stress compared to plants growing on nonporous rock fragments. During the rewatering period, plants on 0% pebbles recovered faster, suggesting a faster reloading of water in the pots with fine earth only, followed by limestone and then quartz pebbles. Then, in contrast to the first desiccation period, the plants immediately entered a water-stressed period after watering was stopped (decrease in leaf stomatal conductance), and no differences in stomatal conductance were observed among treatments, suggesting that mainly (or only) the fine earth was reloaded with water, not the pebbles. This is supported by the level of stomatal conductance recorded just after the rewatering period (day 19), which was equivalent to the level at the beginning of the stress period during the first drought (day 6). Rewatering by capillary rise lasted five days, with half the duration applied before the first desiccation period and the soil was drier before the second rewatering period than before the first, suggesting that rewatering time was probably insufficient to fill the porosity in the pebbles. Consequently, the stony phase probably did not reach saturation, and the porous pebbles could not be reloaded. For water potentials near saturation, water could theoretically move from the fine phase to the stony phase of the substrate (Tetegan et al. 2015a).

Our experiment showed that rock fragments can not only retain water but also make it available for plants, thus acting as reservoirs during drought periods for plants. Nevertheless, after drought, the soil substrate probably must be reloaded with water to full field capacity in order to reload the pores of the rock fragments and be beneficial to plants in successive drought events.

CONCLUSION

We showed that rock fragments contained in cultivated soils are not inert material and can significantly contribute to plant growth. Limestone rock fragments can retain up to 20% of the water in their pores and participate significantly in the total soil available water capacity. Our experiment demonstrated that stomatal conductance, used as an indicator of plant water stress, was correlated not only with the water content of the fine earth but also with that of the limestone pebbles. In addition, by comparing nonporous and porous pebbles, our results showed that plants can use the water retained in the pores of certain rock fragments, lowering plant water stress during a drought event. Plants can therefore benefit during desiccation periods from the water retained in the pores of rock fragments if the soil has been saturated before drought. All these results indicate that the water in the pores of rock fragments acts as a reservoir of extractable water which is useful during medium- to high-severity drought periods.

Our work highlights the importance of taking into account the coarse soil fraction in soil-plant functioning.

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Fig. 1:

Schematic procedure for making up the reconstituted soils for the 5 treatments with fine earth and either limestone or quartz pebbles in proportions of 0%, 20% and 40%.

Fig. 2:

Water loss (g) during the first desiccation period for the five treatments varying in type (limestone or quartz) and percentage of pebbles (0, 20, 40%) (vertical bars show standard deviations).

Fig. 3:

Leaf stomatal conductance (G) for plants grown under different soil treatments (A) throughout the drought experiment. Different periods are separated by vertical dashed lines and are indicated with roman letters. Periods I and II correspond to the first drought period which started after saturation (day 0) and lasted until day 13 without watering. Period III was a rewatering period (days 13 to 17) and was followed by the second drought period (Period IV, day 17 to the end of the experiment). Graphs B and C focus on Period I (days 8 to 13) for plants with 20% and 40% pebbles, respectively. Standard errors are indicated by error bars and significant differences between treatments are indicated by stars (*: $0.05<p<0.01$; **: $0.01<p<0.001$; ***: $p<0.001$).

Fig. 4:

Relationship between leaf stomatal conductance and soil water content (SWC) in fine earth and in the pebble phases: (a) during the first drought period and (b) the linear relationship when SWC fell below 0.2 $cm^3.cm^{-3}$.

Fig. 5:

Root system in a limestone pebble treatments after 3 months of growth. On the right, part of the root system still attached to pebbles after washing away the fine earth. On the left, a root growing in the pore of a pebble.

Suppl. 1:

Physico-chemical characteristics of the soil (fine earth from 0-32 cm)

Suppl. 2:

Cumulative evapotranspiration (mm) at the end of the first desiccation period, divided into two sub-periods (2-7 days and 8-13 days), for the five treatments varying in type (limestone or quartz) and percentage of pebbles (0, 20, 40%) (vertical bars are standard deviations). Different letters indicate significant differences (ANOVA, $p<0,00001$).

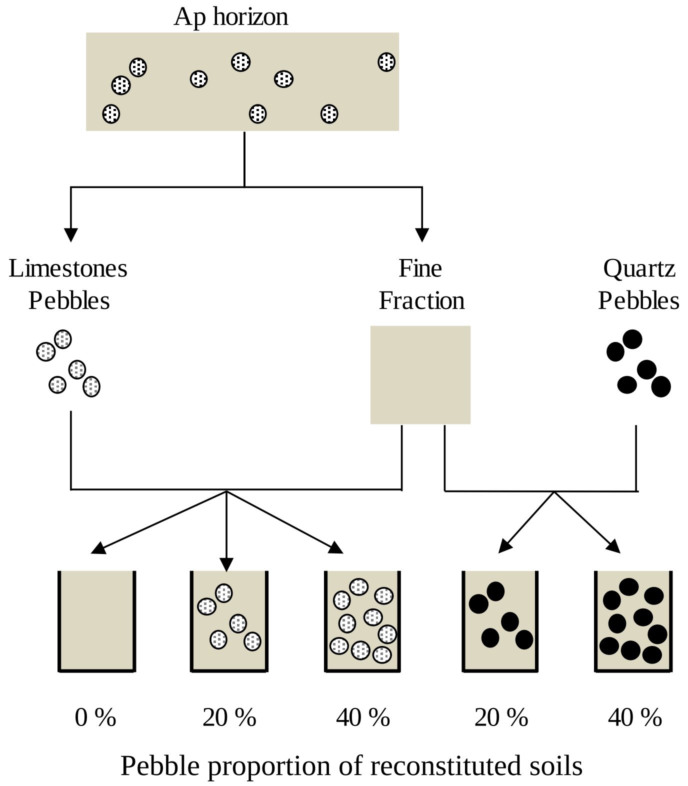
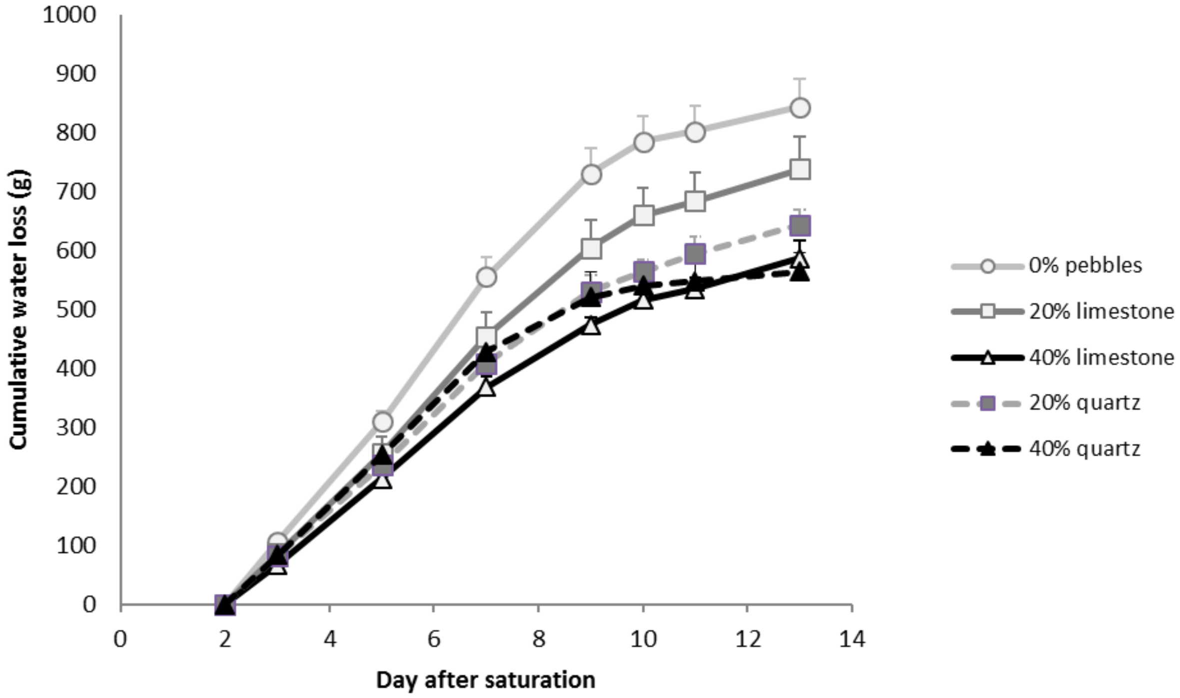

Ap horizon
Limestones Pebbles
Fine Fraction
Quartz Pebbles
0 %
20 %
40 %
20 %
40 %
Pebble proportion of reconstituted soils

Fig. 1



Cumulative water loss (g)
Day after saturation
0% pebbles
20% limestone
40% limestone
20% quartz
40% quartz

Fig. 2

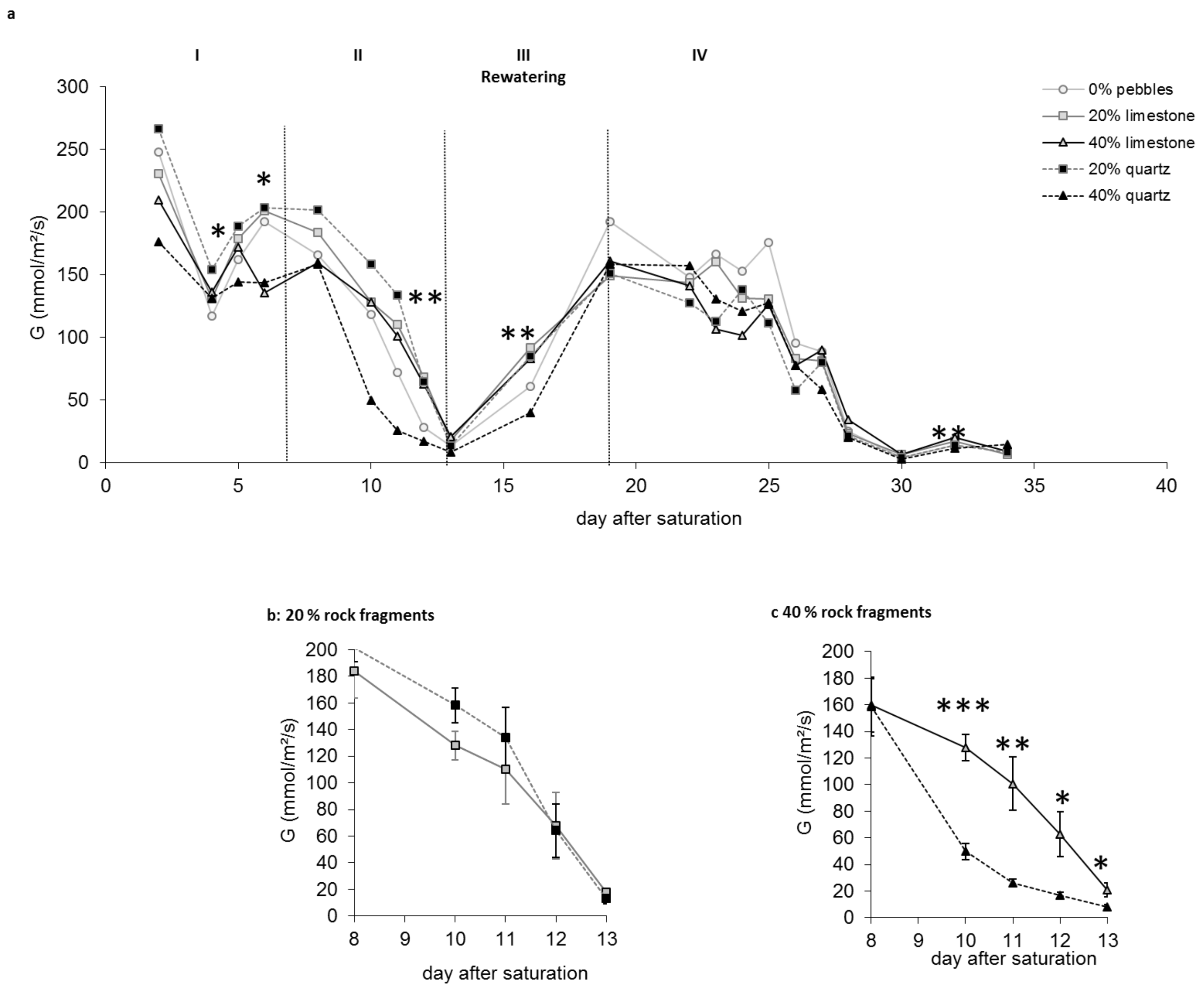
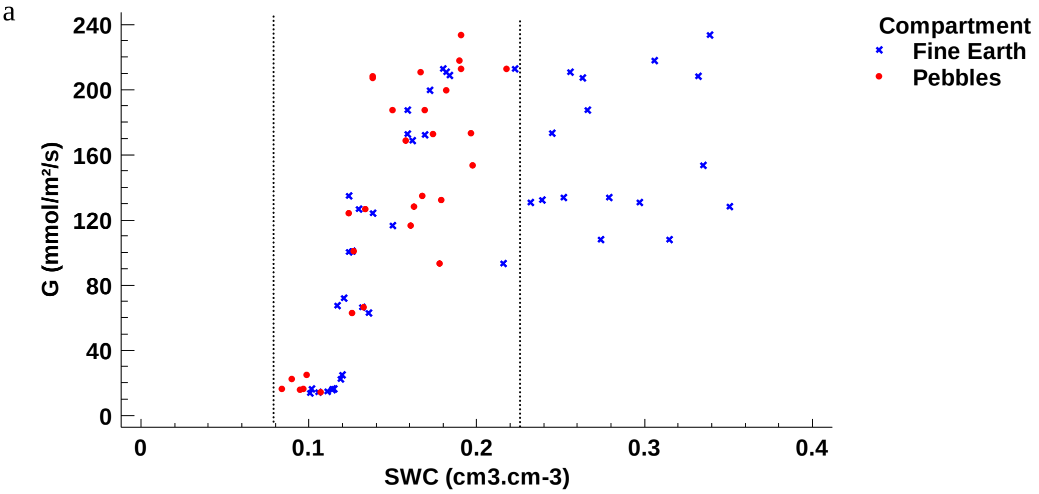

a
I
II
III
Rewatering
IV
0% pebbles
20% limestone
40% limestone
20% quartz
40% quartz
G (mmol/m²/s)
day after saturation
b: 20 % rock fragments
G (mmol/m²/s)
day after saturation
c 40 % rock fragments
G (mmol/m²/s)
day after saturation

Fig. 3


a
240
200
160
120
80
40
0
G (mmol/m²/s)
0
0.1
0.2
0.3
0.4
SWC (cm3.cm-3)
Compartment
Fine Earth
Pebbles

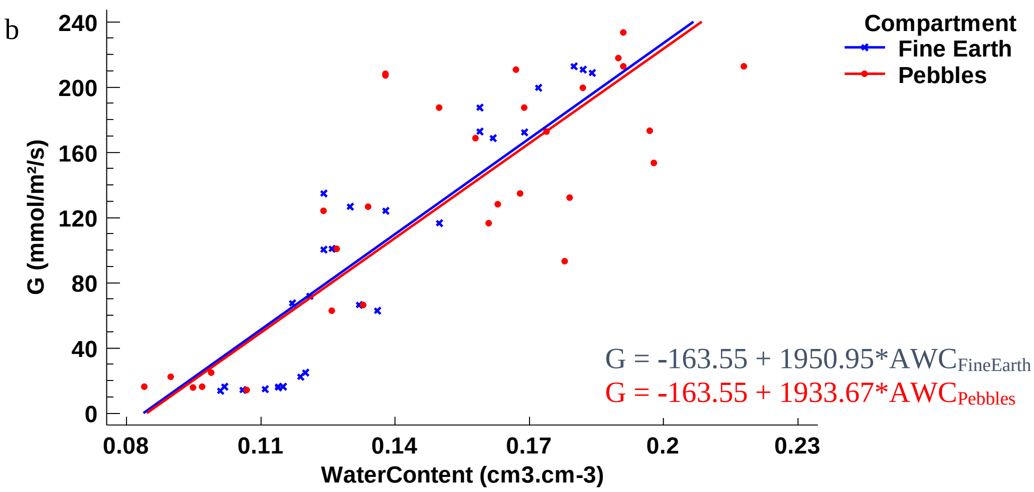

b
240
200
160
120
80
40
0
G (mmol/m²/s)
0.08
0.11
0.14
0.17
0.2
0.23
WaterContent (cm3.cm-3)
Compartment
Fine Earth
Pebbles
G = -163.55 + 1950.95*AWC_FineEarth
G = -163.55 + 1933.67*AWC_Pebbles

Fig. 4

Table 1: Available water capacity (cm^3.cm^{-3}) of the 5 treatments of reconstituted soils (fine earth + rock fragments). In brackets, the negative difference in % of treatments with pebbles compared to the treatment with fine earth only (0% pebbles), and the positive difference in % of the treatment with porous limestone pebbles compared to nonporous quartz pebbles.

	Proportion of pebbles				
Type of pebbles	0%	20%		40%	
	0.170				
Limestone		0.151	*(-11%, +11%)*	0.133	*(-22%, +30%)*
Quartz		0.136	*(-20%)*	0.102	*(-40%)*

Table 2: Physiological measurements (mean ±SD) during the first desiccation period of plants grown under different types (limestone or quartz) and percentage of rock fragments (0, 20, 40%). Different letters indicate significant differences between modalities (and results of the statistical test).

Treatment	evapotranspiration rate (mm/day/cm² leaf area)			WUE (g/mm)			Water loss (g)		
0% pebbles	1.5	±0,2	a	1.14	±0.095	a	843	±47	a
20% limestone	1.8	±0,2	ab	0.97	±0.081	ab	738	±56	b
40% limestone	2.5	±0,2	b	0.73	±0.133	ab	587	±30	cd
20% quartz	1.8	±0,2	ab	0.87	±0.101	ab	643	±26	c
40% quartz	1.9	±0,2	ab	0.71	±0.082	b	564	±31	d
ANOVA p-value	*0.0003*			*0.03*			*<0,00001*		

Table 3: Biometric measurements (mean ±SD) after three months of growth of plants grown under different types (limestone or quartz) and percentage of rock fragments (0, 20, 40 %). Results of the statistical test (ANOVA) are given for ANOVA1 testing differences between modalities, and result of the post-hoc test presented as different letters indicating significant differences between modalities, and for ANOVA2, testing the factor %pebbles.

Treatment	Height (cm)	Number of leaves	Foliar surface (cm^2)	Total Biomass (g)	Root/shoot ratio	Diameter (cm)
0% pebbles	42.6 ±2,4 a	24.1 ±2,6 a	514 ±79 a	7.2 ±0,7 a	0.22 ±0,03 ab	0.65 ±0,06 a
20% limestone	34.4 ±2,7 b	23.8 ±2,5 a	386 ±68 b	5.7 ±0,8 b	0.28 ±0,06 a	0.56 ±0,06 b
40% limestone	27.3 ±1,8 c	20.7 ±3,5 b	226 ±48 d	3.4 ±0,4 d	0.28 ±0,03 a	0.48 ±0,04 c
20% quartz	32 ±2,0 b	23.3 ±2,7 ab	345 ±69 bc	4.3 ±0,3 c	0.19 ±0,05 b	0.55 ±0,03 b
40% quartz	26.9 ±4,6 c	21.1 ±2,5 ab	268 ±38 cd	3.4 ±1,0 cd	0.24 ±0,07 ab	0.47 ±0,07 c
ANOVA1 p-value	*6.73E-16*	*0.0003*	*2.16E-10*	*1.58E-11*	*0.0022*	*1.50E-08*
ANOVA2 p-value	*<0.00001*	*0.0011*	*ns*	*0.0036*	*ns*	*<0.00001*

Table 4: Leaf stomatal conductance (G, mmol/m²/s, mean ±SD) for plants grown under different treatment of soils over two periods of drought after saturation of substrate (day 0), and after rewatering (day 13 to 17). Different letters indicate significant differences between modalities (and results of the statistical test).

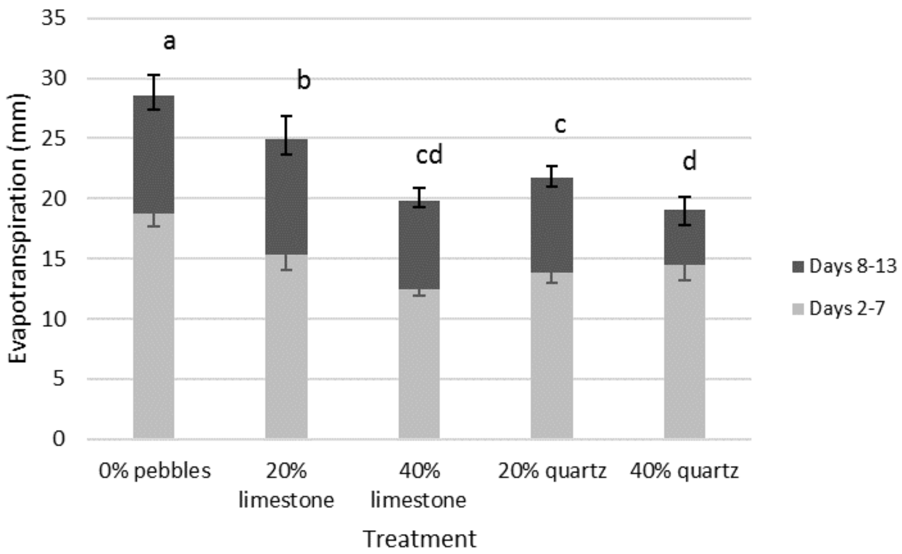
	Day after saturation										
Modality	**2**	**4**	**5**	**6**	**8**	**10**	**11**	**12**	**13**	**16**	**19**
0% pebbles	248 ± 94 a	117 ± 21 a	162 ± 21 a	192 ± 33 ab	166 ± 33 a	118 ± 36 a	72 ± 33 ab	28 ± 9 a	13.2 ± 4 a	61 ± 25 ab	192 ± 39 a
20% limestone	231 ± 85 a	130 ± 22 ab	179 ± 23 a	201 ± 32 ab	184 ± 49 a	128 ± 28 a	110 ± 63 b	68 ± 61 a	17.7 ± 8 a	92 ± 36 b	149 ± 12 a
40% limestone	210 ± 78 a	136 ± 22 ab	172 ± 26 a	135 ± 53 a	160 ± 49 a	128 ± 25 a	101 ± 49 ab	63 ± 42 a	20.8 ± 13 a	83 ± 12 b	161 ± 16 a
20% quartz	266 ± 97 a	154 ± 21 b	189 ± 19 a	203 ± 27 b	202 ± 26 a	158 ± 32 a	134 ± 56 b	64 ± 49 a	13.0 ± 9 a	85 ± 23 b	151 ± 29 a
40% quartz	177 ± 45 a	131 ± 8 ab	144 ± 46 a	144 ± 46 ab	159 ± 54 a	50 ± 14 b	26 ± 7 a	17 ± 5 a	8.2 ± 4 a	40 ± 8 a	158 ± 39 a
ANOVA p-value	*0.390*	*0.046*	*0.117*	*0.010*	*0.389*	*<0.0001*	*0.005*	*0.106*	*0.128*	*0.003*	*0.518*

	Day after saturation									
Modality	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**30**	**32**	**34**
0% pebbles	148 ± 26 a	166 ± 40 a	153 ± 11 a	176 ± 27 a	95 ± 33 a	89 ± 43 a	25 ± 24 ab	4 ± 7 a	14 ± 4 ab	
20% limestone	144 ± 28 a	160 ± 34 a	131 ± 40 a	131 ± 58 a	83 ± 33 a	81 ± 41 a	23 ± 9 ab	6 ± 3 a	17 ± 4 ab	7 ± 5 a
40% limestone	141 ± 22 a	107 ± 52 a	102 ± 41 a	126 ± 34 a	78 ± 24 a	90 ± 26 a	34 ± 16 b	6 ± 5 a	20 ± 5 b	9 ± 1 a
20% quartz	128 ± 36 a	112 ± 38 a	138 ± 19 a	111 ± 31 a	58 ± 21 a	80 ± 48 a	21 ± 14 ab	4 ± 4 a	14 ± 3 ab	8 ± 0 a
40% quartz	157 ± 29 a	130 ± 35 a	120 ± 26 a	127 ± 36 a	78 ± 33 a	58 ± 15 a	20 ± 8 a	3 ± 5 a	11 ± 2 a	15 ± 3 a
ANOVA p-value	*0.103*	*0.052*	*0.071*	*0.080*	*0.284*	*0.591*	*0.531*	*0.142*	*0.0072*	*0.224*

Fig. 5 : Root system in a limestone pebble modalities after 3 months of growth. On the right, part of the root system still attached to pebbles after washing away the fine earth. On the left, a root developed in the pore of the pebble.

Suppl. 1: Physico-chemical characteristics of the soil (fine earth from 0-32 cm)

Characteristics			Unit
Texture			
	Clay	33.8	$g.kg^{-1}$
	Fine silt	30.5	$g.kg^{-1}$
	Coarse silt	30.6	$g.kg^{-1}$
	Fine Sand	2.6	$g.kg^{-1}$
	Coarse Sand	2.5	$g.kg^{-1}$
CaCO3		36	$g.kg^{-1}$
Carbone	C	18.1	$g.kg^{-1}$
Nitrogen total	N	1.51	$g.kg^{-1}$
pH		8.32	
Exchangeable cations			
	Ca	9.03	$g.kg^{-1}$
	Mg	0.152	$g.kg^{-1}$
	K	0.241	$g.kg^{-1}$
CEC Metson		18.8	$cmol^{+}. kg^{-1}$


Evapotranspiration (mm)
35
30
25
20
15
10
5
0
a
b
cd
c
d
0% pebbles
20% limestone
40% limestone
20% quartz
40% quartz
Treatment
Days 8-13
Days 2-7

Suppl. 2