



## Could conservation tillage increase the resistance to drought in Mediterranean faba bean crops?

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### ABSTRACT

Climate change, especially in Mediterranean countries, might have a detrimental effect on agro-ecosystems and agricultural production, as well as on the price, quantity and quality of the products. This study assesses the potential impact of drought predictions on a faba bean crop, and evaluates the adoption of conservation tillage practices to reduce this effect. For this purpose, a rainfall exclusion experiment was carried out in a Mediterranean rotation in Southwest Spain, where a long-term tillage experiment was running since 2008. Soil water storage (SWS), crop productivity and mineral nutrition, plant ecophysiology and biomass quality, as well as the colonization of roots by arbuscular mycorrhizal fungi (AMF) and symbiotic *Rhizobium* bacteria were evaluated over one growth cycle to study the combined effect of different tillage systems - non-tillage (NT), reduced tillage (RT) and traditional tillage (TT)- and rainfall reduction. Rainfall reduction had a negative impact on faba bean germination on the TT system, and on its aboveground biomass across the three tillage systems, but did not affect grain production. The percentage of biomass yield reduction by rainfall exclusion was 30% for NT, 50% for RT and 20% for TT. In the case of RT, low yields were influenced by a high incidence of weeds under rainfall reduction. The negative effect of water exclusion was especially evident on photosynthesis rates and stomatal conductance across all tillage types, while leaf water potential was affected by drought only in the TT system. These ecophysiological indicators were positively associated to some variables of arbuscular mycorrhizal colonization and *Rhizobium* nodulation in roots. Changes in the patterns of symbiotic interactions in response to drought depended on the tillage type. We conclude that, despite no effect on soil water storage, in the conservation tillage systems the increase in the mycorrhizal colonization in roots, particularly under NT, could be beneficial for plants to face drought stress. However, in a scenario of reduced rainfall it will be necessary to invest more resources in weed control under RT.

### 1. Introduction

Climate models predict changes in precipitation patterns with an increasing risk of droughts (IPCC, 2021), especially in Mediterranean countries. With no doubt, climate change is the main social and environmental challenge of the XXI century. In this regard, agriculture is the economic sector most exposed to changes in climate patterns, with more or less impacts depending on the region, the specific crop and the type of management (Rosenzweig et al., 2014; Valverde et al., 2015). Therefore, climate change might have a cascading effect on agro-ecosystems and agricultural production, as well as on the price, quantity and quality of the products (EEA, 2019).

In this expected scenario of reduced precipitation, management of Mediterranean rainfed agriculture is particularly challenging. These agricultural systems rely on rainwater inputs and on soil water retention capacity. Thus, actions to mitigate the impacts of future droughts in these systems should be aimed at increasing the infiltration and retention of rainwater in the soil, as well as at reducing its losses through evaporation. All these measures are based on favouring the porosity and stability of the soil aggregates, and on reducing the impact of water erosion; for this, the addition of organic matter and the mechanical treatment of the soil play a fundamental role (Bot and Benites, 2005; Lal, 2004).

Among mechanical procedures, conservation tillage is one of the

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measures proposed to increase soil resistance to drought in arid and semi-arid climates (Bot and Benites, 2005). Conservation agriculture aims to reduce mechanical soil perturbation to a minimum, by suppressing mouldboard ploughing, or applying only shallow perturbations of less than 25% of the soil surface (FAO, 2011). In Mediterranean soils, often characterized by a low organic matter content, the main positive effect of conservation tillage is the increase in the amount of fresh organic residues incorporated into the soil (Panettieri et al., 2013; Hontoria et al., 2016). This in turn favours the formation of aggregates (Panettieri et al., 2013), increasing water and nutrient retention capacity (Ramzan et al., 2019; Chandrasekhar et al., 2019).

The effects of conservation tillage on soil commonly translate into some plant performance indices. Many studies have shown that conservation tillage has positive effects on soil water usage and crop yield (Sun et al., 2018; Mairghany et al., 2019; Obia et al., 2020). Some of these works have shown an amelioration of plant water stress in soils under conservation tillage, as indicated by stomatal opening and/or leaf water potential, resulting in an improvement on plant photosynthetic capacity leading to greater crop yields (Liu et al., 2019; Sher et al., 2021; Mukherjee et al., 2022). These positive effects of conservation tillage on crop water dynamics have been recently reported to be very important in semiarid areas (Zhang et al., 2022).

Some of the positive effects of conservation tillage on plant water status could be mediated by its effects on soil biota. It is well known that conservation tillage can promote fungal abundance in soil in comparison to intensive tillage, due to a lower mechanical disruption of fungal networks (Zhang et al., 2014). In particular, tillage can have profound effects on the abundance of mycorrhizal fungi (Såle et al., 2015). This effect of tillage on mycorrhizal community, however, seems to be soil and crop-species specific (Hannula et al., 2021). In any case, the maintenance of plant-mycorrhizal associations could be vital in scenarios of reduced rainfall, given the important role of these fungi for water acquisition by plants under conditions of soil water deficit (Khalvati et al., 2005).

The studied crop, faba bean (*Vicia faba* L.) is the sixth most produced grain legume worldwide. Like other grain legumes, faba bean and its symbiotic rhizobacteria fix atmospheric nitrogen in a wide range of conditions. Therefore, this crop is important for its contribution to residual nitrogen in crop rotation and its potential in green manuring (Muktadir et al., 2020). However, sustainability of this crop could be compromised by climate change predictions, as it is more sensitive to drought than other field crops. Indeed, drought stress has large effects on faba bean metabolism and photosynthesis (Mansour et al., 2021).

The objective of this study was to assess the potential impact of drought predictions on the productivity of a faba bean crop, and to evaluate whether this effect can be mitigated by the adoption of conservation tillage practices. We hypothesized that these practices (reduced tillage and no tillage) could have a positive effect on the resistance of crop productivity to reduced rainfall. We expected that this effect could be related to the improvement in soil water holding capacity, therefore it would be indicated by some plant ecophysiological indices (stomatal conductance, photosynthesis and leaf water potential) as well as by crop productivity. We also expected that the improved plant water status would be related to a higher colonization on plant roots by mycorrhizal fungi under conservation tillage. To test these hypotheses a rainfall exclusion experiment was carried out in a typical wheat/legume Mediterranean rotation in SW Spain, where a long-term tillage experiment was running since 2008. Rainout shelters excluding around ca. 30% of water inputs to soils were installed in this tillage experiment, and a faba bean crop was established. Crop productivity, plant ecophysiology and biomass quality was evaluated over one growth cycle, as well as the effect of tillage and rainfall reduction on the colonization of roots by arbuscular mycorrhizal fungi (AMF) and by symbiotic *Rhizobium* bacteria.

## 2. Materials and methods

### 2.1. Experimental area and description of experimental design

The experiment was established at the agriculture experimental farm “La Hampa” of the “Instituto de Recursos Naturales y Agrobiología de Sevilla” (IRNAS-CSIC), located at Coria del Río (Seville, SW Spain). The soil texture is sandy clay loam, and it is classified as Typic Xerofluvent (Soil Survey Staff, 2014). The most relevant characteristics of the soil before the establishment of the drought experiment are reported in Table S1.

The climate is Mediterranean, with mild rainy winters and hot and dry summers. Annual average precipitation (1971–2012) is 485 mm and annual average temperature is 17.5 °C. Weather conditions during the study were recorded with a meteorological station located at the experimental farm (Fig. S1).

The tillage experiment started in 2008 in 9 plots of 6 m × 33.5 m, allocated to three different tillage treatments (traditional tillage (TT), reduced tillage (RT) and no-tillage (NT)) in a completely randomized experimental design with three replicates per treatment (Panettieri et al., 2020). TT consists of mouldboard ploughing with soil inversion (~25–30 cm deep) and two chisel passes at 25 cm depth (0.57 m separation between chisels) followed by a disc harrowing of 12 cm depth; in the RT treatment, tillage operation is reduced to only one chisel operation at 25 cm depth followed by a disc harrowing of 5 cm depth. In the NT treatment no operations are conducted and sowing is done by direct drilling. At least 30% of the soil surface under NT and RT is covered by crop residues from previous crop, while 50% of crop residues from TT are left onto soil surface after harvest (which typically occurs in late Spring-early Summer, depending on the crop), remaining on the soils over the Summer and been buried with the ploughing in Autumn. Tillage operations are conducted in early Autumn before seed sowing. Sowing dates depend on the crop type within the rotation and also on weather conditions. Mouldboarding is used to control weeds in the TT plots, while in the NT treatment spraying with pre-emergence glyphosate and pendimethalin at a rate of 4 L ha<sup>-1</sup> is applied. In 2015 and 2016 a harrow crossed-pass (15 cm) was conducted in all treatments, including the NT to ameliorate the observed soil compaction (López-Garrido et al., 2014).

A *Triticum aestivum* L. – *Helianthus annuus* L. – *Pisum arvense* L. crop rotation was established at the start of the experiment in 2008. In the sunflower and pea seasons plots were not fertilized, while in the wheat season a compound N-P-K fertilizer was applied (15 N– 15 P<sub>2</sub>O<sub>5</sub>–15 K<sub>2</sub>O; 60 kg N ha<sup>-1</sup>, 26.4 P kg ha<sup>-1</sup>, 49.8 K kg ha<sup>-1</sup>). Since the 2013–2014 season, *Helianthus annuus* L. was suppressed from the rotation, and in the legume seasons *Pisum arvense* L. was alternated with *Vicia faba* L.

At the beginning of October 2020, a rainfall exclusion experiment was established using rainout shelters. These shelters covered a soil surface of 6.25 m<sup>2</sup> and are made of light scaffolding and bands of transparent acrylic that exclude approximately 30% of rainfall inputs, in agreement with the climate change projections for the Mediterranean region for the end of the century (IPCC, 2021). This design has been proved to achieve rainfall exclusion while minimally affecting other environmental variables (Yahdjian and Sala, 2002). The shelters are light and can be moved if needed to conduct tillage and other agronomic tasks. At each tillage plot (9 plots in total, 3 per tillage system) two rainout shelters were established (exclusion treatment). Likewise, at each tillage plot two surfaces of equal size (2.5 m × 2.5 m) were delimited on the ground, subject to environmental precipitation conditions, which have been considered as climatic control treatment (control treatment). The total number of experimental plots is 36 (3 tillage systems × 2 rainfall levels × 6 replicates). In a subsample of 3 replicates per tillage system and rainfall levels soil moisture across the 0–40 cm depth was periodically recorded with a FDR probe (Delta-T Devices) in fibre glass tubes installed at the centre of each plot.

Tillage operation described for NT, RT and TT were performed at the

beginning of November 2020. The crop, *Vicia faba* L., PROTHABAT 69 variety was sown at the end of November with a density of  $\sim 200$  kg of seeds  $\text{ha}^{-1}$ .

## 2.2. Plant germination, biomass and analysis

Seed germination was measured 23 days after sowing by counting all the germinated seedlings inside the rainout shelters and in the control plots.

In the early May, when faba bean plants were at maturity and had already produced beans, aboveground biomass was estimated at each plot. For this, plant biomass was collected within two circles  $0.50 \text{ m}^2$  per plot. These samples included both *V. faba* plants and weeds, which were further separated. Faba bean fruits were further separated from stems. Plant material (faba bean fruits and shoots, as well as weeds) was weighed fresh, and subsequently dried at  $60^\circ\text{C}$  for 48 h. Dried weights of each plant fraction were also recorded. Finally, fruits were peeled to obtain the production of beans.

## 2.3. Ecophysiological measurements

In the middle of April 2021, coinciding with the peak of phenological plant development, measurements of gas exchange were conducted with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) to determine maximum rates of photosynthesis ( $A_{N, \text{max}}$ ) and stomatal conductance ( $g_{s, \text{max}}$ ). Gas exchange was measured between 10:00 and 13:00 am on healthy, fully developed young leaves in a selection of 24 plots (4 plots  $\times$  3 tillage systems  $\times$  2 rainfall levels), measuring three plants per plots. Measurement conditions were set to  $350 \mu\text{mol air s}^{-1}$ ,  $430 \mu\text{mol CO}_2 \text{ air mol}^{-1}$ , with a PAR of  $1500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ .

A Scholander-type pressure chamber (PMS Instrument Company, Albany, Oregon, USA) was used to measure leaf water potential. For these measurements one leaf per plant and two plants per plot were selected. Fully developed leaves from the outer part of the plant canopy were selected for these measurements. The sampled leaves were stored in closed plastic bags with humid filter paper and kept in a portable cooler until they were measured in the laboratory (Rodríguez-Dominguez et al., 2022).

## 2.4. Mycorrhizal root colonization and nodule formation

In early April the colonization of faba bean roots by arbuscular mycorrhizal fungi (AMF) and the nodulation by *Rhizobium* was evaluated in 18 out of the 36 plots (3 plots  $\times$  3 tillage systems  $\times$  2 rainfall levels). At each of these 18 plots three complete plants were uprooted. Total number of nodules in each root sample was counted (Ramoneda et al., 2021). A subsample of secondary roots (with diameter less than 2 mm) was obtained from each sample to quantify mycorrhizal variables. We followed the staining method developed by Vierheilig et al. (1998). The root material was hot digested with KOH 10% (w/v) until roots were discolored and further stained with 0,05% blue ink (Pelikan 4001) in vinegar. They were further cut into 1-cm fragments and examined under a microscope (Olympus BX40). The following mycorrhizal parameters were calculated according to Trouvelot et al. (1986): intensity of colonization by arbuscular mycorrhizal fungi in the root system (M %), frequency of mycorrhizal fragments (F %), intensity of the colonization of mycorrhizal fragments (m %), vesicles abundance (v %, structures used for storing lipids and other elements) and arbuscule abundance (a %, where carbon and nutrient exchange occurs; Smith and Read, 2008) in mycorrhizal fragments.

## 2.5. Plant traits and chemical analyses

In the same sampling described above, shoots and roots of the three chosen plants from each plot were fresh weighed, then washed with distilled water and then dried at  $60^\circ\text{C}$  during at least 48 h. In these

plants shoots and roots were dried separately and weighed to estimate Root Mass Fraction (RMF) and the shoot:root ratio.

Dried leaves were ground and passed through a 1 mm stainless-steel sieve. C, N and their isotopic ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) were measured by combusting leaf subsamples at  $1020^\circ\text{C}$  using a continuous flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, DE). Another leaf subsample was calcinated at  $450^\circ\text{C}$  during 2 h. Subsequently, ash content was calculated. Ashes were hot solubilized with concentrated HCl and distilled water, and then filtered (Whatman 2). In this extract P, K, Ca, Mg, Na Fe, Cu, Mn and Zn were determined (C.I.I.T. D.F., 1969). P was determined according to Murphy and Riley (1962), K and Na by atomic emission spectroscopy, and Ca, Mg and micronutrients by atomic absorption spectroscopy (Perkin Elmer, AAnalyst 100).

## 2.6. Data analysis

Soil water storage (SWS, mm) within the root-zone depth (0–40 cm) was determined from periodical soil water content measurements through the soil profile ( $\theta$ ), using the trapezoidal rule (Jia et al., 2013) according to the following equation:

$$\text{SWC} = 50 \cdot \theta_1 + \sum_{i=1}^3 50 \cdot (\theta_i + \theta_{i+1})$$

Where  $\theta_i$  is volumetric moisture ( $\text{cm}^3 \text{ cm}^{-3}$ ) in the  $i$ -th soil depth (1: 10 cm; 2: 20 cm; 3: 30 cm; 4: 40 cm); the soil depth interval is 10 cm.

Linear mixed models were applied to test for the effect of tillage and rainfall exclusion (as well as their interactions) on soil water storage, including the plot as a random term to account for repeated measurements, using the nlme package in R. For each sampling date differences in soil water storage between control and rainfall exclusion plots were calculated, and similar models were applied to evaluate whether water storage responded differently to drought among tillage treatments.

Linear mixed models were also applied to test for differences in plant variables due to the experimental treatments (rainfall and tillage). When homogeneity of variance was not met, a variance coefficient was introduced in the model to account for heteroscedasticity among different factor levels, using the gls function of the nlme package (Zuur et al., 2009). Validation of the model assumptions was done by exploration of model residuals. Relationships among plant variables were explored by Pearson's bivariate correlations.

## 3. Results

To study the effect of water reduction and tillage on the faba bean crop, we studied first the soil water storage and different aspects of the plant that can affect crop yield (germination, photosynthesis, AMF and *Rhizobium* nodulation, and nutritional status of the plant) and can help to understand and improve the knowledge about these processes.

### 3.1. Effect of rainfall exclusion on soil water storage (SWS)

The rainfall exclusion treatment had a significant effect ( $F = 5.87$ ;  $p = 0.035$ ) on SWS (0–40 cm depth) across the three tillage systems (Fig. 1). Tillage type did not have a significant effect on SWS ( $F = 2.42$ ;  $p = 0.139$ ). Maximal differences between control and exclusion plots were observed in March 2021, when the exclusion treatment led to a reduction of SWS of 31, 25 and 14 mm in the TT, RT and NT treatments on average, respectively (Fig. S2). These values represented reductions of 21%, 20% and 10% of SWS in control plots in the TT, RT and NT, respectively.

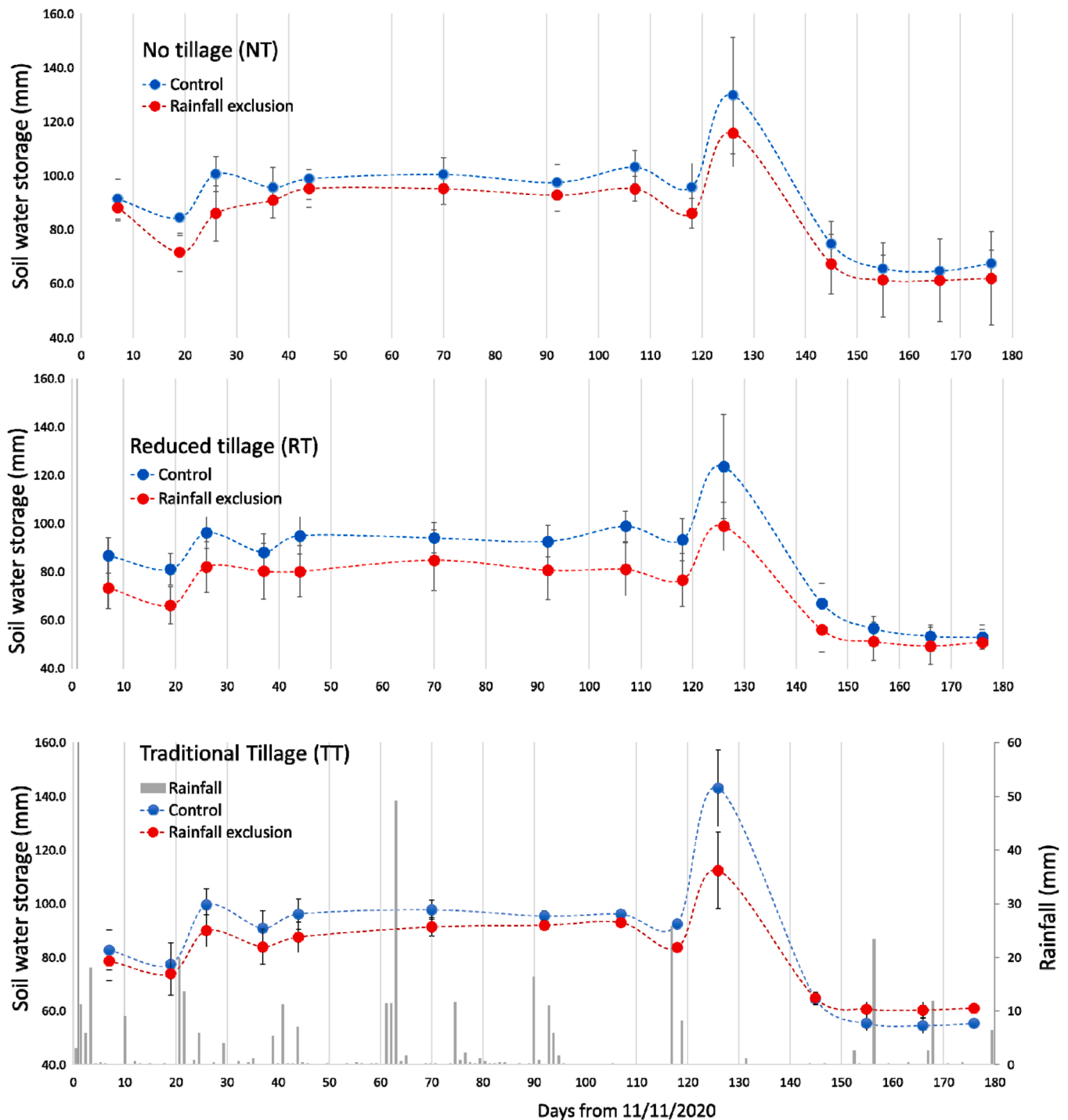


Fig. 1. Differences in soil water storage in the 0–40 cm layer under different tillage and rainfall conditions from sowing to harvest in 2020 and 2021. Vertical bars represent the standard deviation. In the upper part of the graph, precipitation for from sowing to harvest is shown.

### 3.2. Germination of faba bean seeds

Rainfall exclusion had a marginally significant influence ( $F = 3.38$ ;  $p = 0.076$ ) on seed germination and this parameter tended to be lower in the rainfall exclusion plots. Tillage did not influence germination ( $F = 0.57$ ;  $p = 0.568$ ). However, there was a significant rainfall  $\times$  tillage interaction, so that drought led to a significant reduction in germination in the TT treatment, but not in the other tillage systems (Fig. 2a). Although significant differences considering only tillage were not observed, the low number of germinated seeds in the NT was remarkable (on average, 170 seeds in the control plots), in comparison to TT (on

average, 222 germinated seeds in the control plots).

### 3.3. Crop yield and weed production

The effect of the rainfall reduction on crop yield was evident, showing a significantly lower production in the plants growing under the exclusion treatment ( $F = 9.36$ ,  $p = 0.003$ ) (Fig. 2b). However, when considering also the tillage effect, differences between control and exclusion in crop biomass were only significant in RT. The percentage of crop yield reduction by rainfall exclusion was 30% for NT, 50% for RT and 20% for TT. Therefore, crop yield showed the highest sensitivity to

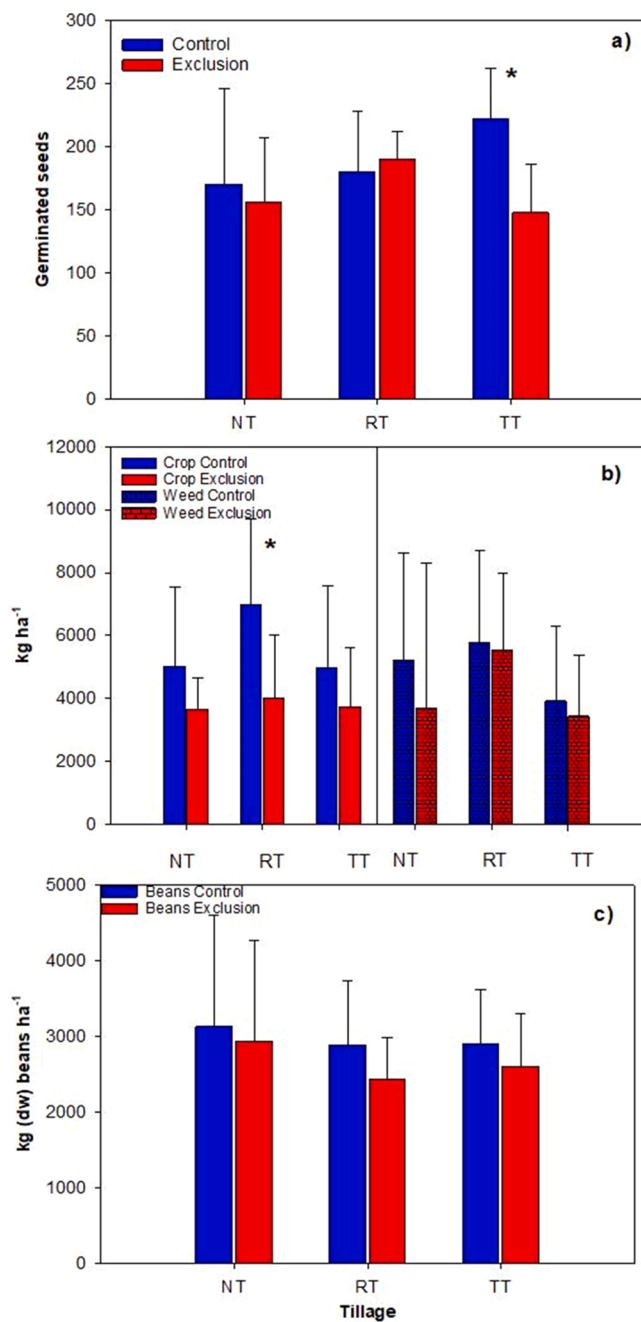


Fig. 2. a) Number of germinated seeds, b) biomass of crop and weed production and c) production of beans (grain) in the three tillage systems and in control and exclusion of rain plots (mean and SD). The asterisk (\*) indicates significant differences between the control and exclusion per tillage system.

drought in the RT system.

The yield of beans (grain) was not affected by either tillage type or rainfall reduction (Fig. 2c). This indicates that this organ was less affected by the rainfall exclusion than total aboveground biomass. In this case the highest bean production was observed in NT (mean values 3039 kg ha<sup>-1</sup>), followed by RT (2710 kg ha<sup>-1</sup>) and TT (2488 kg ha<sup>-1</sup>).

Rainfall reduction tended to reduce weed production, although differences were not significant. The highest weed incidence was found for RT (showing a high variability), followed by NT especially in control soils (Fig. 2b). Interestingly, the incidence of weeds was high in the RT treatment even in the rainfall exclusion plots.

### 3.4. Crop ecophysiological measurements

Water content was measured in different parts of the plants (Table S2). Results indicated similar water content in leaves and stems (around 0.80%) and a higher content in fruits (0.85%). In general, no differences were found due to the tillage system or the contributions of rain. However, when consider data for each tillage system separately significant differences were observed for RT ( $F = 4.650$ ,  $p = 0.039$ ), with plants growing under the rainfall exclusion treatment having a lower leaf water content. Moreover, RMF was significantly increased by rainfall reduction in the three tillage systems ( $F = 8.852$ ,  $p = 0.012$ ; Fig. S3).

#### 3.4.1. Measurements of gas exchange in leaves: photosynthesis and stomatal conductance

The effect of rainfall reduction was clearly observed in photosynthesis (AN) and stomatal conductance (gs), showing significantly higher values in control plants than in plants under rainfall exclusion ( $F = 80.41$ ,  $p < 0.001$  for AN, and  $F = 54.75$ ,  $p < 0.001$  for gs; Fig. 3a, b). The effect of rainfall exclusion was also evident at each tillage system separately ( $p < 0.001$  for NT and RT, and  $p < 0.01$  for TT).

If only the control treatment is considered, plants presented significantly higher photosynthetic values in the NT and RT systems than in TT ( $F = 4.317$ ,  $p = 0.022$ ). In general, for these parameters it was evident the positive effect of conservation tillage, without rainfall restrictions.

#### 3.4.2. Leaf Water potential

Leaf water potential was significantly higher in all plants growing in control soils than in the exclusion treatment ( $F = 4.933$ ,  $p = 0.032$ ). When considering the tillage systems separately, NT and RT minimized the effect of the rainfall reduction, showing smaller differences among exclusion and control plots than TT (Fig. 3c). Indeed, the effect of rainfall reduction was much more evident in TT, with significant differences ( $F = 2.84$ ,  $p = 0.007$ ) between control and rainfall exclusion plants. In NT and RT there were not significant differences due to rainfall exclusion. When only considering the tillage factor, there was no effect of tillage type on water potential, showing similar average values among treatments ( $-1.29$ ,  $-1.30$  and  $-1.25$  MPa for NT, RT and TT, respectively).

### 3.5. AMF colonization and Rhizobium nodulation in roots

Tillage and rainfall had some influence on the incidence of mycorrhizal colonization in roots, although data dispersion was relatively high. The intensity of mycorrhizal colonization (M, %) was significantly influenced by tillage ( $F = 7.02$ ,  $p = 0.009$ ), and was higher in the NT and the RT treatment, especially in the plants under rainfall exclusion (Fig. 4a). Tillage had also a marginally significant effect on m % ( $F = 3.61$ ,  $p = 0.059$ ). These mycorrhizal colonization parameters (M % and m %) tended to be higher in the plants under rainfall exclusion than in control plots, especially in NT and RT treatments. In contrast, in the TT these variables tended to decrease under drought. Indeed, for the frequency of mycorrhizal infection (F %) there was a marginally significant rainfall × tillage interaction ( $F = 2.98$ ,  $p = 0.088$ ; Fig. 4b). Vesicle and arbuscule abundance was not significantly influenced by rainfall exclusion (Table S3).

The abundance of *Rhizobium* nodules followed an opposite trend to that of mycorrhizal colonization. In fact, there were negative correlations between the abundance of nodules and some of the parameters of mycorrhizal colonization (for example,  $r = 0.59$ ,  $p < 0.001$  between M % and the number of nodules per plant). The number of nodules per root mass was significantly affected by tillage ( $F = 5.98$ ;  $p = 0.031$ ), with a significant interaction between tillage and rainfall ( $F = 7.1811$ ,  $p = 0.0089$ ), so that nodulation responded differently to drought depending on the tillage system. In the RT and NT systems nodulation tended to decrease in the rainfall exclusion treatment, while in TT it

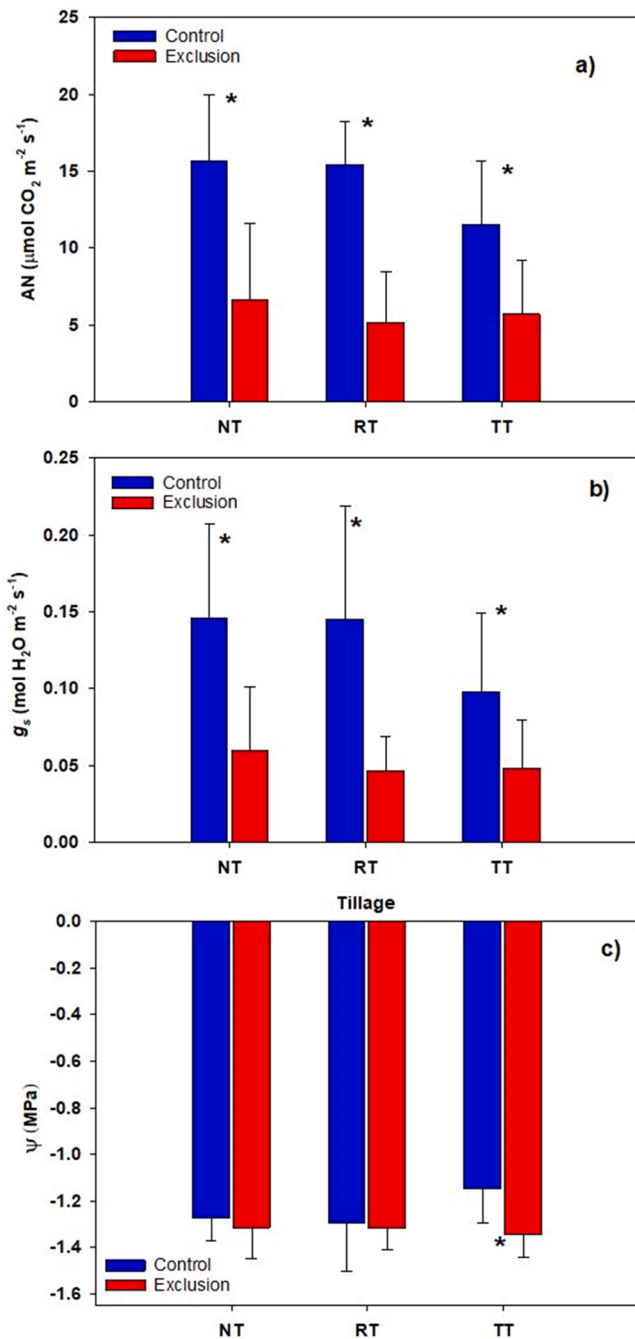


Fig. 3. a) maximum rates of photosynthesis (AN, max), b) stomatal conductance ( $g_s$ , max) and c) leaf water potential in the three tillage systems and in control and exclusion of rain plots (mean and SD). The asterisk (\*) indicates significant differences between the control and exclusion per tillage system.

increased (Fig. 4c).

In summary, considering only the tillage factor, for all these root-microbe interaction traits, the tendency was NT>RT>TT, except for *Rhizobium* nodules, which followed the opposite trend (TT>RT>NT).

When parameters of root symbiosis in the plants at each plot were compared to the ecophysiological status of these plants, some interesting patterns were observed. The abundance of AMF arbuscules in roots was significantly and positively related to water potential ( $r = 0.69$ ,  $p < 0.01$ ) and leaf  $\delta^{13}\text{C}$  ( $r = 0.49$ ;  $p = 0.039$ ), while the abundance of *Rhizobium* nodules was associated to net photosynthesis rates ( $r = 0.55$ ,  $p < 0.05$ ) and stomatal conductance ( $r = 0.66$ ,  $p < 0.001$ ).

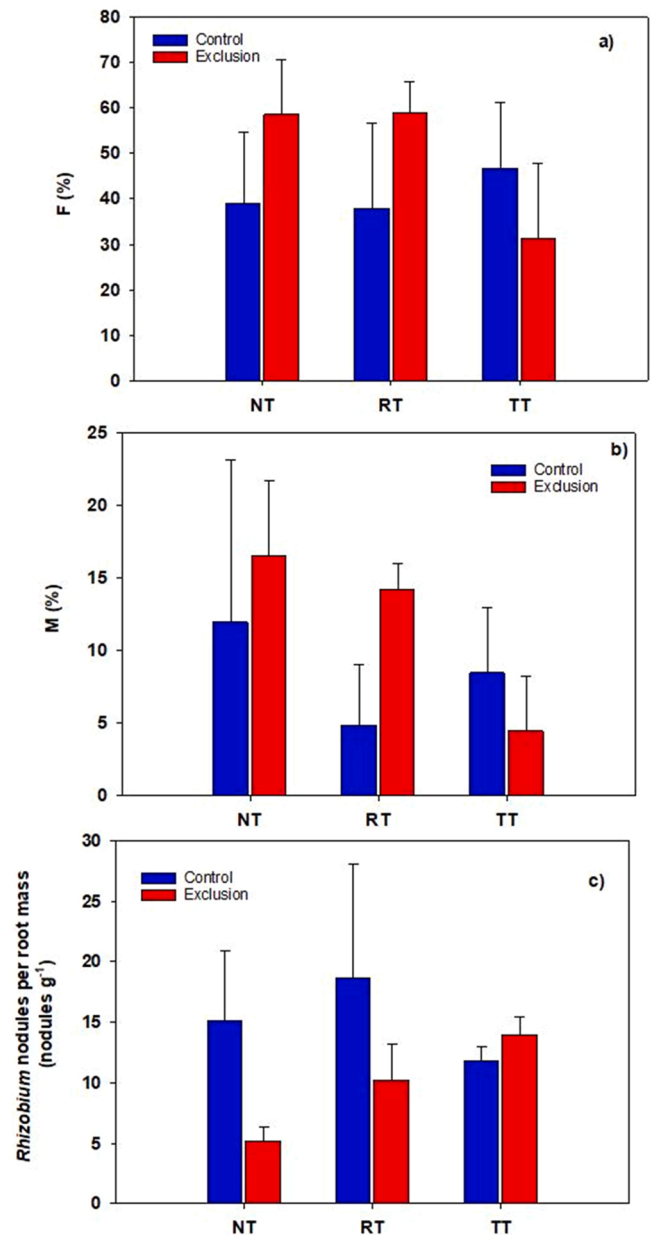


Fig. 4. Intensity of the mycorrhizal colonization in the root system (F %, a), frequency of mycorrhizae in the root system (M %, b), and number of *Rhizobium* nodules per root dry mass (c) in the different treatments.

### 3.6. Plant chemical traits

Isotopic C signatures in *V. faba* leaves were not influenced by rainfall exclusion or tillage type.  $\delta^{15}\text{N}$  values presented a high variability and did not follow any clear trend regarding the rainfall exclusion at each of the tillage types studied, although these values always differed between control and rainfall exclusion plants, especially in NT. In this tillage system drought changed the sign of the  $\delta^{15}\text{N}$  values, being slightly negative in the control plants and positive in the rainfall exclusion treatment (Fig. 5).

Concentrations of macro (C, N, Ca, K, Mg, Na and P) and micro-nutrients (Fe, Cu, Mn and Zn) in leaves were analysed. The effect of rainfall reduction was only significant for Ca among all the studied macronutrients (Table 1). For this element, concentrations in leaves were always higher in control plants, especially at TT (2.4 times higher in control plants). Calcium concentrations were significantly and positively related to the abundance of *Rhizobium* nodules ( $r = 0.59$ ,

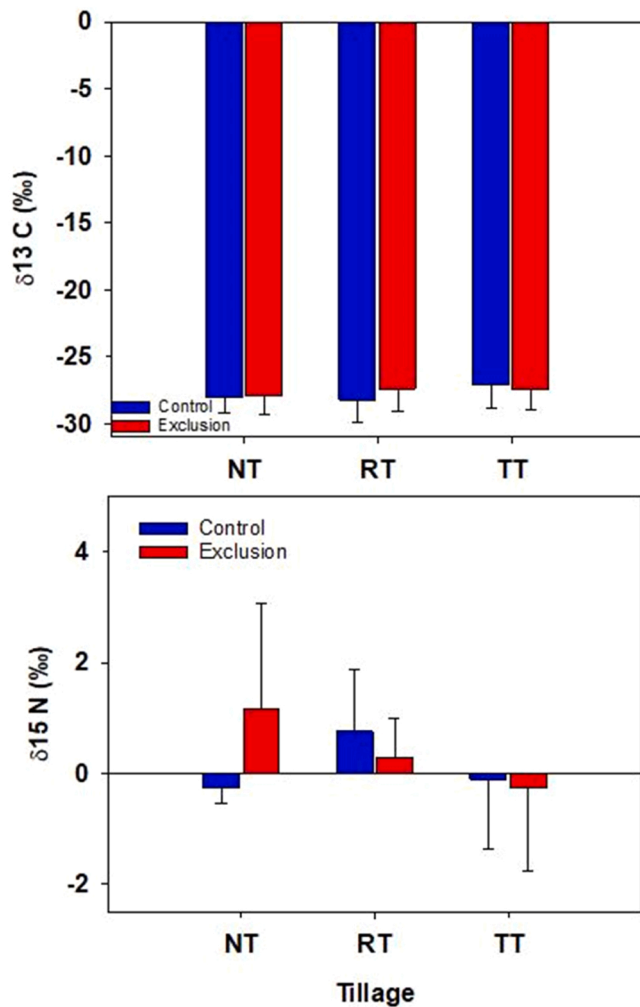


Fig. 5. Isotopes composition in leaves ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in the three tillage systems and in control and exclusion of rain plots (mean and SD).

$p < 0.05$ ), and closely related to photosynthesis rates ( $r = 0.74$ ,  $p < 0.001$ ), stomatal conductance ( $r = 0.82$ ,  $p < 0.004$ ) and leaf water potential ( $r = 0.65$ ,  $p < 0.01$ ; Fig. S4).

Regarding micronutrients, significant differences due to tillage system were observed for Fe, Mn and Zn. For these elements concentrations were always higher in the leaves of plants growing in the NT treatment,

Table 1

Concentrations of macro and micronutrients in *Vicia faba* leaves according to the two treatments, tillage and rainfall conditions (mean values and (SD). Results of the two-way ANOVA (F and p values) with the effects of the treatments Rainfall and tillage are included. Significant effects ( $p < 0.05$ ) are marked in bold.

| Tillage  | Rainfall | C                   | N              | Ca             | K              | Mg               | Na               | P                | Cu             | Fe            | Mn             | Zn             |
|----------|----------|---------------------|----------------|----------------|----------------|------------------|------------------|------------------|----------------|---------------|----------------|----------------|
|          |          | %                   |                |                |                |                  |                  |                  |                |               |                |                |
|          |          | mg kg <sup>-1</sup> |                |                |                |                  |                  |                  |                |               |                |                |
| NT       | EXC      | 40.6<br>(4.82)      | 4.64<br>(0.47) | 0.43<br>(0.10) | 0.73<br>(0.11) | 0.122<br>(0.009) | 0.084<br>(0.044) | 0.092<br>(0.011) | 5.00<br>(2.02) | 156<br>(35.8) | 22.3<br>(4.50) | 5.22<br>(1.75) |
|          | C        | 41.8<br>(7.28)      | 5.12<br>(0.77) | 0.65<br>(0.27) | 0.79<br>(0.22) | 0.127<br>(0.003) | 0.105<br>(0.072) | 0.100<br>(0.069) | 5.07<br>(0.30) | 168<br>(32.0) | 22.5<br>(2.48) | 4.6<br>(0.28)  |
| RT       | EXC      | 42.5<br>(9.93)      | 4.82<br>(1.06) | 0.41<br>(0.01) | 0.73<br>(0.11) | 0.133<br>(0.022) | 0.092<br>(0.052) | 0.100<br>(0.027) | 4.29<br>(0.28) | 148<br>(1.21) | 21.2<br>(0.96) | 3.8<br>(0.75)  |
|          | C        | 44.0<br>(2.82)      | 4.20<br>(1.25) | 0.70<br>(0.24) | 0.60<br>(0.19) | 0.125<br>(0.004) | 0.116<br>(0.026) | 0.088<br>(0.017) | 3.60<br>(0.85) | 142<br>(25.5) | 19.1<br>(1.90) | 3.30<br>(0.33) |
| TT       | EXC      | 40.7<br>(3.44)      | 4.41<br>(0.37) | 0.28<br>(0.10) | 0.43<br>(0.17) | 0.085<br>(0.021) | 0.168<br>(0.004) | 0.097<br>(0.043) | 3.39<br>(0.56) | 105<br>(19.3) | 15.3<br>(2.70) | 2.94<br>(0.69) |
|          | C        | 44.5<br>(6.63)      | 5.34<br>(0.17) | 0.66<br>(0.27) | 0.64<br>(0.15) | 0.118<br>(0.017) | 0.098<br>(0.026) | 0.084<br>(0.013) | 3.93<br>(0.39) | 135<br>(6.01) | 19.7<br>(2.47) | 3.26<br>(0.67) |
| Rainfall | F        | <b>0.524</b>        | <b>0.514</b>   | <b>13.689</b>  | <b>0.332</b>   | <b>1.997</b>     | <b>0.178</b>     | <b>0.151</b>     | <b>0.003</b>   | <b>1.162</b>  | <b>0.425</b>   | <b>0.397</b>   |
|          | p        | <b>0.483</b>        | <b>0.487</b>   | <b>0.003</b>   | <b>0.575</b>   | <b>0.183</b>     | <b>0.680</b>     | <b>0.705</b>     | <b>0.961</b>   | <b>0.302</b>  | <b>0.527</b>   | <b>0.540</b>   |
| Tillage  | F        | <b>0.159</b>        | <b>0.442</b>   | <b>1.187</b>   | <b>2.811</b>   | <b>6.028</b>     | <b>1.323</b>     | <b>1.001</b>     | <b>3.463</b>   | <b>4.775</b>  | <b>4.984</b>   | <b>6.786</b>   |
|          | p        | <b>0.855</b>        | <b>0.653</b>   | <b>0.339</b>   | <b>0.100</b>   | <b>0.015</b>     | <b>0.303</b>     | <b>0.394</b>     | <b>0.065</b>   | <b>0.030</b>  | <b>0.027</b>   | <b>0.011</b>   |

followed by RT and TT, and were positively correlated to the parameters of mycorrhizal colonization (Fig. S4).

#### 4. Discussion

##### 4.1. Effect of tillage on soil water storage in a reduced rainfall scenario

Land management practices can have effects on soil water storage. Several studies have showed that SWS increases with the use of conservation tillage systems, particularly in arid sites and/or in periods of increased water deficit (Lampurlanés et al., 2016; Zhang et al., 2022). This effect is commonly related to an increase in rainfall infiltration and to an improvement of soil structure and organic matter content due to the reduction of tillage, which enhances soil water retention capacity (Álvarez-Fuentes et al., 2008; Plaza-Bonilla et al., 2013).

In our study, conservation tillage did not have a clear effect on SWS, at least within the 0–40 cm depth. Despite the tillage experiment is running since 2008, SWC profiles in the control plots, exposed to ambient rainfall, were similar across tillage types. Previous works at the same experiment showed that after 4 years of tillage treatments soil water content was actually higher in the NT soils than in the TT soils over the growing season, likely due to its higher soil organic matter content (López-Garrido et al., 2014). In addition, another study conducted at the site comparing RT and TT found that after 3 years of tillage treatments the hydraulic conductivity of the soil surface layer was significantly higher in the RT than in the TT, related to the existence of preferential paths created by an increase in the earthworm population in the RT treatment (Moreno et al., 1997). This resulted in a higher replenishment of soil water storage in the RT system, particularly in the driest year. Moreover, a study conducted before the start of the rainfall exclusion experiment showed that NT and RT had slightly higher soil organic C contents than TT, especially just after tillage operations (Panettieri et al., 2020). For all these reasons, we expected a positive effect of NT or RT application on soil water storage.

The lack of positive effects of conservation tillage on SWS might be due to an increase in soil compaction under conservation tillage, especially in the NT treatment, which has been previously reported to affect crop production at the site (López-Garrido et al., 2014). That work showed that 4 years after the start of the experiment penetration resistance in the top 10 cm under NT was extremely high, ca. 10 and 15 times higher than that in RT and TT, respectively. Indeed, in all the treatments included NT, a harrow crossed-pass (15 cm) was done in November 2015 and in November 2016 to reduce the compaction and deterioration of physical properties observed after years of direct drilling. This compaction of the topsoil could impair rainfall infiltration in the NT soils. Thus, it is possible that in our experiment NT and RT had

counterbalanced effects on different soil physical properties after 12 years of treatment, resulting in no net change in soil water storage in comparison to TT. Other works with Mediterranean soils have also showed that, depending on specific soil properties, NT can lead to soil compaction that affects plant growth, resulting even in a reduction in SWS in comparison to TT (López et al., 1996). Given that soil compaction under NT is a frequent fact, and given its potential effects on soil water infiltration, specific monitoring and management of soil physical properties is needed in no-till systems, especially in a context of predicted reductions in rainfall.

Despite NT did not clearly increase SWS there were other evidences of a lower impact of drought on the NT system in comparison to RT or TT, such as the more reduced impacts of rainfall exclusion on germination, on crop yield and on leaf water potential in the NT treatment (discussed below). For RT, the acute decrease in SWS in the rainfall exclusion treatment could be related to greater water consumption by weeds in comparison to NT (discussed below).

#### 4.2. Effects of tillage and drought on germination and crop yield

Water is a major limiting factor for seedling establishment, especially in areas characterized by dry climate seasons (Cochrane et al., 2015). This factor explains the results of lower germination rates in the exclusion treatment, which affected the TT system. Moreover, the mechanical resistance imposed by soil crusts is directly dependent on its water content (Souty and Rode, 1993), which affects the cracking characteristics of the crust, and hence the seedling emergence process (Aubertot et al., 2002). In the presence of sufficient soil moisture, germinating seedlings degrade this mechanical barrier, which enables crack penetration and emergence. In the case of RT, similar germination rates were observed at both rainfall treatments. Regarding NT, it is known that larger soil aggregates, likely more abundant in the soils under this tillage type, act as a mechanical obstacle to impede seedling emergence from soil. Although the number of germinated seeds in this tillage system was slightly lower than that in the conventional tillage differences were not statistically significant, contrasting with previous results showing a clear reduction of germination rates under NT due to soil compaction (López-Garrido et al., 2014).

The studied crop, *Vicia faba*, is considered to be very sensitive to water stress in comparison to other grain legumes (Muktadir et al., 2020). This fact can explain the clear effects of rainfall reduction on crop yield (especially in the RT system) and on plant physiological status across the three tillage types. Similar results were found in a meta-analysis study considering 44 years, which reported marked reductions of faba bean yield due to reduction of water availability (Daryanto et al., 2015).

When considering only the effect of tillage, we did not find significant differences among tillage treatments. As mentioned above, in the experimental site crop production in the NT system has been often influenced by the spatial variability of soil compaction (López-Garrido et al., 2014; Panettieri et al., 2020). Four years after the start of the tillage experiment wheat production was decreased by more than a 25% in the NT system in comparison to RT or TT (López-Garrido et al., 2014), while after 10 years of the implementation of the tillage trial, RT tended to show the highest production with increases of 10% and 19% compared to TT and NT, respectively (Panettieri et al., 2020). After 12 years yield was not impaired by no-tillage; this is in agreement with results from a meta-analysis of tillage experiments showing that crop yields decline the first years after application of NT, but matches production in the TT after 3–10 years (Pittelkow et al., 2015). It is also worthy to note that the time period covered by this study was especially dry in Southern Spain (annual rainfall of 365 mm for the year 2021 in comparison to the interannual average of 495 mm). Thus, it is also possible that the particularly dry conditions recorded at the site over the growing season have enhanced the potential benefits of conservation tillage on crop yields, as previously observed at the site (Moreno et al.,

1997). Several authors have reported a clear improvement of yield under conservation tillage (Sher et al., 2021; Sun et al., 2018), especially in semiarid areas (Zhang et al., 2022).

In the RT treatment crop production showed a high sensitivity to rainfall reduction. This was very likely related to the high incidence of weeds under this tillage, both in the control and the rainfall exclusion treatments. Weeds are one of the major constraints for crop production (Milberg and Hallgren, 2004), and a high incidence of weeds under RT in comparison to other tillage systems has already been reported (Hofmeijer et al., 2019). In RT weed biomass was not reduced by rainfall exclusion, which means that faba bean plants had to cope with a more intense competition for scarce water resources with weeds. Weed production has not been specifically studied before in this long-term tillage study, and therefore we have not data to infer the influence of water inputs on weed incidence among the different tillage systems or across years with different rainfall inputs. It is possible that the low water inputs over the study period (November 2020–May 2021) resulted in decreases in the germination of the faba bean seeds in comparison to wetter years, which could have promoted a greater incidence of weed growth. Indeed, the drought treatment had a negative effect on seed germination in the TT tillage. Because of the lower tillage intensity in RT compared to TT, weed abundance could have increased in RT due to higher seedling recruitment in the upper soil layers and the difficulty to wreck the rhizomes of perennial weeds (Gruber et al., 2009). The lower weed production in TT is related to the mouldboard ploughing before seeding, while in the NT treatment weed emergence is constrained by the initial application on herbicides. Possibly, if herbicide would have been also applied to RT the impact of drought would have been lower in this tillage system. In fact, Hofmeijer et al. (2019) concluded that RT system can produce similar or higher yields than TT if weed management is improved and good nutrient supply is assured. The interaction between weeds and the main crop under different water inputs deserves further attention; although this study reports data from only one growing season the results suggest that in a scenario of reduced rainfall the higher competition for water between crop plants and weeds will force the farmers to invest more resources in weed control.

Competition with weeds for nutrients and water might have affected bean (grain) production. However, we did not find a clear reduction of grain yield due to drought. These results are indicative of the lower effect of water reduction at the grain level, at least in this study. Finally, it is interesting to note that the highest productions of grain were observed in the NT system, despite the lower germination rates in these soils.

#### 4.3. Plant ecophysiological responses

Although the faba bean originates from a semi-arid region, this species has relatively higher water requirements than other typical Mediterranean crops. Under moderate drought stress conditions, plants reduce their stomatal conductance ( $g_s$ ) and as a consequence photosynthesis is inhibited because of the reduced supply of  $CO_2$  to the intercellular space (Singh and Reddy, 2011). This direct relationship between both measures explains their positive and significant correlation in our study ( $r: 0.979, p < 0.001$ ). A significant reduction in AN and  $g_s$  under drought stress had been reported earlier in crops including faba beans (Abid et al., 2017). Regarding the tillage effect, similar values of both measurements across tillage types were obtained in the rainfall exclusion treatment. However, under control rainfall conditions, plants in TT showed significantly lower values than those in the NT and RT systems. Several studies have also found that the benefits of conservation tillage on soil water content or nutrient availability result in an improvement of plant photosynthetic capacity, leading to greater crop yields (Liu et al., 2019; Sher et al., 2021; Mukherjee et al., 2022).

Moreover, water stress usually induces a decrease in leaf water potential (Osakabe et al., 2014). In accordance with the results obtained for  $g_s$  and AN, water potential was also significantly affected by the reduction of rainfall. Plants in the rainfall exclusion treatment also



showed higher RMF, which is a common response to soil water limitation, so that the capacity of plants to explore soil for water is enhanced (Poorter et al., 2012).

Some effect of tillage on water potential was also observed. Conservation tillage systems, NT and RT, showed the lowest differences on water potential between control and rainfall exclusion plants, indicating the positive effect of both tillage types for ameliorating plant water stress. Similarly, Peng et al. (2019) found positive effects of conservation tillage on leaf water potential. The negative effect of rainfall exclusion on these physiological values, as well as the lower photosynthetic rates in the TT compared to conservation tillage systems, can help to explain the results obtained for crop production.

Owing to its sensitivity towards environmental constraints, as soil water content, isotopic C and N signatures in leaves are widely used to assess the effects of changing climatic condition on plant ecophysiology. However, Peuke et al. (2006) showed that  $^{13}\text{C}$  was more affected by environmental factors, while  $^{15}\text{N}$  is more affected by genetic factors. In particular, under drought conditions stomatal opening limits the renewal of  $\text{CO}_2$  for photosynthesis, and therefore  $^{13}\text{C}$  increases in C3 plants (Araus et al., 1997). Moreover, Warren et al. (2001) concluded that  $\delta^{13}\text{C}$  may be a useful indicator of water availability or drought stress, but only in seasonally dry climates. Thus, we expected some  $^{13}\text{C}$  enrichment in the leaves, as frequently reported in other drought experiments (Andresen et al., 2018). However, we did not find a clear pattern of  $^{13}\text{C}$  variation between rainfall treatments. Likewise, the  $^{15}\text{N}$  signature did not show a clear response to drought, as also found by Robinson et al. (2000).

Interestingly, in plants under NT drought changed the sign of the  $\delta^{15}\text{N}$  values, being slightly negative in the control plants and positive in the rainfall exclusion treatment (Fig. 5). This could be related to a shift in the patterns of microbial symbiosis in the roots of the plants due to drought, which were especially clear in the NT system. In the NT system drought provoked an increase in the frequency and intensity of AMF colonization, and a decrease in the nodulation with nitrogen-fixing *Rhizobium* bacteria (discussed below). Soil mineral N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) is usually enriched in the heavy isotope compared to atmospheric  $\text{N}_2$  (Boddey et al., 2000). Therefore, the  $^{15}\text{N}$  enrichment in the plants under rainfall exclusion could be related to a larger contribution to plant nutrition of soil mineral N than of N from biological fixation.

#### 4.4. Patterns of microbial symbiosis in roots and relationships with plant responses to drought

As we expected, there was a trend for a higher incidence of mycorrhizal colonization in the soils under conservation tillage, especially in the NT treatment. Most of the parameters of colonization by AMF (F, M, m and v) tended to be higher in NT and RT in comparison to TT, with a significant effect of tillage in some variables (M and m). Several studies have shown that mycorrhizal colonization is negatively affected by tillage (Kabir, 2005; Schalamuk et al., 2004). This general behavior is directly related to the mechanical disturbance imposed by tillage that may affect the extension of the extra-radical mycelium and therefore the possibility of associating with the plants (Alguacil et al., 2014; Oehl and Koch, 2018). Tillage therefore may reduce soil mycorrhizal infectivity and AM root colonization at the early stages of crop growth. De la Cruz-Ortiz et al. (2020) also found that conservation tillage systems promote mycorrhizal colonization of crop plants in comparison to traditional tillage systems.

The greater potential of AMF infectivity in soils under conservation tillage could be beneficial for plants to face drought stress. Indeed, in the NT and RT systems rainfall reduction led to an increase in the incidence of mycorrhizal colonization in roots; probably under water stress plants allocated more C belowground to enhance their capacity to explore soil for water resources, as suggested by the increase in RMF, which includes the association with AMF. Symbiosis with AMF improves plant tissue hydration and physiology under drought stress, due to a range of

mechanisms that can include the uptake of water through the fungal hyphae and transfer to the host plant, the improvement of plant osmotic adjustment and root hydraulic conductivity, or the regulation of plant hormonal balance (reviewed in Ruiz-Lozano et al., 2012). In our study, NT and RT showed the lowest differences on water potential between control and rainfall exclusion plants, which could be related to the higher levels of symbiosis with AMF, in comparison to TT. Moreover, water potential was positively related to the abundance of AMF arbuscules in the roots. In the TT system such increase in mycorrhizal colonization under drought stress was not observed, likely due to the more limited AMF infectivity of these soils.

In the case of *Rhizobium* nodules, we found opposite results compared to mycorrhization. In the NT and RT systems drought tended to reduce the number of nodules in the roots, while the opposite was observed in TT. Indeed, the incidence of AMF colonization and nodulation in roots were negatively correlated. It is possible that *Rhizobium* bacteria and AMF compete for plant C in the roots, and in soils where AMF abundance is high, such as in conservation tillage soils, fungi can have an advantage over bacteria under water deficit. Previous studies have shown that, under water deficit, modifications of the bacterial environment can cause the decline in N fixation and a reduction in the number of nodules in faba beans (Muktadir et al., 2020). Moreover, different works have shown that fungal diversity is less sensitive to seasonal changes in moisture, temperature and plant activity than bacterial diversity (Yuste et al., 2011).

The present study shows that interaction with symbiotic organisms at the root level is highly correlated to some plant physiological indices. Besides the positive association between AMF arbuscules, leaf water potential and  $\delta^{13}\text{C}$ , we found positive relationships between the abundance of *Rhizobium* nodules and net photosynthesis rates and stomatal conductance. We also found different adjustments in the patterns of symbiotic interactions in response to drought, depending on the tillage type. Put together, our results reinforce the idea that plant-associated bacteria and fungi can improve stress resistance and help plants to cope with the negative impacts of drought through the induction of various mechanisms, which involve plant biochemical and physiological changes (de Vries et al., 2020; Poudel et al., 2021). These results are particularly novel and interesting, as they suggest that the influence of tillage in the response of crops to reduced water inputs is more related to its impact on soil biotic communities than to its effects on soil water storage.

#### 4.5. Plant nutritional status

Finally, the nutritional level of the crop was also taken into account. Main results indicated that macronutrients were not affected by rainfall exclusion. In fact, Muktadir et al. (2020) in a review about *Vicia faba* crops reported that although droughts caused yield reductions on this crop, this effect did not alter the mineral nutritional quality. However, in this study we found certain effect on Ca due to the rainfall exclusion. Drought stress can decrease the nutrient uptake by roots and its translocation from roots to shoots. Calcium is only transported through xylem vessels, being a relatively immobile element in the phloem (Marschner, 1974). Because of this, leaf Ca concentrations can be high in plants with high transpiration rates; indeed, its uptake is affected under drought conditions by the limited availability of water (Naem et al., 2018), and in some species leaf Ca has been proposed as a proxy for leaf transpiration rates (Urban et al., 2012). In fact, in our work there was a close correlation between leaf Ca, stomatal conductance and leaf water potential.

Regarding the effect of tillage, the nutritional status of the plants tended to be better in soils under conservation tillage, especially for micronutrients. No-tillage practices with no disturbance in soil increase soil organic matter and soil nutrients (da Silva et al., 2021). As mentioned above, in our site RT and NT produced an increase in the organic matter content in the soil, and NT also an increment in soil N

(Panettieri et al., 2020). This improvement in soil quality, together with the higher incidence of mycorrhizal colonization in conservation tillage soils, can explain that plants presented significantly higher photosynthetic values in the NT and RT systems than in TT, when only control plots were considered.

## 5. Conclusions

Despite NT and RT did not provoked a clear increase in soil water storage capacity, there were some evidences that suggest that crops under NT and RT were less affected by rainfall reduction: 1) germination rates were less affected by rainfall reduction in NT and RT than in TT; 2) leaf water potential was more affected by drought in TT than in NT or RT. However, the high incidence of weeds in the RT treatment resulted in a greater reduction of aboveground biomass due to drought in this tillage system. This suggests that in a scenario of reduced rainfall the higher competition for water between crop plants and weeds will force the farmers to invest more resources in weed control. Grain production, however, was not affected by drought in any tillage system.

Our work also showed the close relationships between the intensity of microbial symbiosis in roots and some plant physiological indices. We also found different adjustments in the patterns of symbiotic interactions in response to drought, depending on the tillage type. The greater potential of AMF infectivity in soils under conservation tillage could be beneficial for plants to face drought stress, in particular in the NT systems. Due to these effects on plant-microbe interactions, the adoption of conservation tillage practices (RT and NT) could be beneficial for plant physiology in a context of reduced precipitations, if proper weed management is assured.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2023.108449](https://doi.org/10.1016/j.agee.2023.108449).

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