Mycorrhizal symbiosis alleviates plant drought stress within and across plant generations via plasticity

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Summary

- Phenotypic plasticity is essential for organisms to adapt to local ecological conditions. Little is known about how mutualistic interactions, such as arbuscular mycorrhizal (AM) symbiosis, mediate plant phenotypic plasticity and to what extent this plasticity may be heritable (i.e. transgenerational effects).

- We tested for plant plasticity within- and across-generations in response to AM symbiosis and varying water availability in a full factorial experiment over two generations, using the perennial apomictic herb *Taraxacum brevicorniculatum*. We examined changes in phenotype, performance, and AM fungal colonization of the offspring throughout plant development.

- AM symbiosis and water availability triggered phenotypic changes during the life cycle of plants. Additionally, both triggered adaptive transgenerational effects, especially detectable during the juvenile stage. Drought stress and absence of AM fungi caused concordant plant phenotypic modifications towards a "stress-coping phenotype" within- and across-generations. Offspring's AM fungal colonization was also affected by the parental environment.

- AM symbiosis can trigger transgenerational effects, including changes in functional traits related to resource-use acquisition and AM fungal colonization of the offspring, in turn affecting the biotic interaction. Thus, transgenerational effects of mycorrhizal symbiosis are not limited to plant fitness, but also improve plants' ability to cope with environmental stress.

Key words:

Adaptation, Arbuscular mycorrhizal symbiosis, drought stress, transgenerational effects, functional traits, AM fungal colonization, phenotypic plasticity

Introduction

Abiotic processes and prevailing biotic interactions select for the best-adapted individuals within and across species (de Bello et al., 2012; Vellend, 2016). The ability of a species to adapt to a particular environment may depend on the pressure of natural selection, heritable genetic variability, but also on its phenotypic plasticity (Price, Qvarnström, & Irwin, 2003). Phenotypic plasticity is the ability of an organism to modify its performance and trait expression in response to the environment without altering the DNA sequence (Price et al., 2003). These changes could operate within the life cycle of the organism subjected to those conditions (named as within-generation plasticity). Additionally, they may be transmitted to the following generations via transgenerational effects (also called across-generation or transgenerational plasticity), meaning that the abiotic and biotic environment experienced by the parental generation influence the phenotype of the offspring (Herman & Sultan, 2011; Jablonka & Raz, 2009). Recent studies suggest that transgenerational effects could play a key role in the adaptive response of organisms to stressors; particularly during juvenile stages when they have been proven essential (Dechaine, Brock, & Weinig, 2015; Lämke & Bäurle, 2017; Latzel, Janeček, Doležal, Klimešová, & Bossdorf, 2014; Puy, P. Carmona, Dvořáková, Latzel, & de Bello, 2020). However, little is known about the relative effect of transgenerational effects triggered by biotic conditions (Alonso, Ramos-Cruz, & Becker, 2019), and even less about how they interact with abiotic factors.

Together with species' adaptations to environmental conditions in a site, biotic interactions are considered key drivers of plant community assembly (de Bello et al., 2012). Among these, positive interactions such as mycorrhizal symbiosis are essential in determining, and potentially expanding, the realized niches of species (Gerz, Guillermo Bueno, Ozinga, Zobel, & Moora, 2018; Peay, 2016; van der Heijden, Wiemken, & Sanders, 2003). Arbuscular mycorrhizal (AM) symbiosis is a widespread mutualistic association between plant roots and fungi from the subphylum Glomeromycotina (Spatafora et al., 2016). This association is considered mutually beneficial, since, in exchange for photosynthetic carbon (Smith & Read, 2008), the AM fungi provide host plants with soil nutrients (mainly phosphates; Smith & Read, 2008), mitigate abiotic stress (e.g., drought) and increase resistance to biotic stress, including pathogens (Pozo, López-Ráez, Azcón-Aguilar, & García-Garrido, 2015; Smith & Read, 2008). Besides the mutual effects of the AM interaction, the occurrence, abundance, activity, and the

final outcome of the interaction (from positive to negative) are known to be affected by multiple factors (Hoeksema et al., 2010; N. C. Johnson, Graham, & Smith, 1997). These factors include intrinsic factors such as the genotype of both partners, or the plant developmental stage or sex (Jones & Smith, 2004; Varga & Kytöviita, 2010), and external factor such as the surrounding plant community (Šmilauer, Košnar, Kotilínek, & Šmilauerová, 2020) or environmental factors like soil nutrient and water availability (Martínez-García, de Dios Miranda, & Pugnaire, 2012; Pozo et al., 2015). Phosphorus, nitrogen or water deficiency in plants generally stimulates AM symbiosis and influences the proportion of AM structures (i.e. arbuscules, vesicles, etc.) (Martínez-García et al., 2012; Pozo et al., 2015). However, it has not been tested whether the environmental conditions previous generations could affect the AM symbiosis of the current generation (De Long et al., 2019).

As enumerated before, the fitness benefits of plants in AM symbiosis are well known (Lu & Koide, 1994; Smith & Read, 2008). However it is unclear whether these benefits partly operates through the phenotypic plasticity induced by the interaction which leads to adaptive changes of plant morphological traits such as modifications in root architecture (Fusconi, 2014; Goh, Veliz Vallejos, Nicotra, & Mathesius, 2013; Nuortila, Kytöviita, & Tuomi, 2004). Moreover, it remains unclear whether AM symbiosis of the parental generation triggers phenotypic changes in the offspring (i.e. transgenerational effects) and whether are beneficial (Koide, 2010; Varga, Vega-Frutis, & Kytöviita, 2013). The transgenerational effects can be due to two non-exclusive mechanisms: by different environmentally-induced heritable epigenetic modifications in the offspring (Lämke & Bäurle, 2017), or by generating differences in seed provisioning, seed nutritional quality or hormonal balance stocked up by the maternal plants in the embryos (Dechaine et al., 2015; Germain, Grainger, Jones, & Gilbert, 2019; Herman & Sultan, 2011). In comparation, transgenerational effects originated by embryo modifications play more significant role during early stages of the development, but they tend to fade away with time (Latzel, Klimešová, Hájek, Gómez, & Šmilauer, 2010). Most of the existing evidence demonstrates that having mycorrhizal parents can be beneficial during the early stages of development of the offspring, i.e. increasing biomass, survival, growth rate, nutrient content, and seed production (Heppell, Shumway, & Koide, 1998; Koide, 2010; Varga et al., 2013). However, when testing for transgenerational effects of AM symbiosis, it has been rarely considered both

mechanisms separated with the exception of Varga et al. (2013) that considered seed mass as covariate. Further, this author has investigated the epigenetic mechanism behind them (i.e. DNA methylation; Varga & Soulsbury, 2017, 2019). Nevertheless, it has not been tested yet whether the transgenerational effects persist further to adult stage, neither influence plant functional traits nor AMF colonization of the offspring. Finally, the relative effect of combined biotic and abiotic drivers on transgenerational effects has been very rarely assessed (González, Dumalasová, Rosenthal, Skuhrovec, & Latzel, 2017; Metz, von Oppen, & Tielbörger, 2015), yet biotic drivers can potentially modulate the effect of environmental stress.

Here, we conducted a two-generation experiment to test for within- and across-generation plant plasticity (i.e. transgenerational effects) in response to AM symbiosis using the perennial apomictic herb *Taraxacum brevicorniculatum*. Further, in order to test whether this plasticity differs under abiotic stress conditions, we included a drought stress treatment. We then tested whether within- and across-generation plant plasticity were adaptive, resulting in an improved ability of the offspring to cope with drought stress. Additionally, we evaluated the persistence of the transgenerational effects throughout offspring development by measuring phenotypic traits, performance, and AM fungal colonization on juvenile and adult offspring.

Materials and Methods

Study material

Taraxacum brevicorniculatum Korol. is an obligate apomictic polycarpic perennial plant (Kirschner, Štěpánek, Černý, De Heer, & van Dijk, 2013). Like most species of the genus *Taraxacum*, it has a wide ecological niche, accepting all types of soils, pH and moisture levels (Luo & Cardina, 2012), and forms an active symbiosis with AM fungi (J. Puy, personal obs.). In this study we used genetically identical seeds collected from a population of plants grown under the same glasshouse conditions for several generations (collected and genetically identified by Kirschner *et al.* 2013). This strategy ensured homogeneous genetic and epigenetic variation in the plant material. Since *T. brevicorniculatum* is an obligate apomictic species, all seeds produced by a plant are effectively clones, thus enabling the study of plasticity within and across generations

(Puy et al., 2018). In other words, all plants in the experiments were genetically identical, and after experiencing different conditions during the parental generation, the offspring only differed in non-genetic (i.e. non-genetic or epigenetic effects) inherited information.

Experimental setup

Parental generation. In order to induce the potential transgenerational effects related to mycorrhizal symbiosis and water availability, we conducted a three-month glasshouse experiment (April-July 2017). We grew 364 genetically identical individuals of *T. brevicorniculatum* in individual pots (7 x 7 x 18 cm), half inoculated with AM fungi (AM) and the other half without (NM). The substrate consisted of 2:1 mixture of sterilized sand and natural soil collected from a mesic meadow 30 km southeast of Tabor, 660 m a.s.l. (Vysočina region, Czech Republic, 49.331N, 15.003E) where *Taraxacum sect. Ruderalia* was present. The natural soil was firstly sieved using a 4 mm mesh sized sieve to remove any stones, macrofauna, or rhizomes from the soil. For the AM treatment the natural soil containing indigenous microbial community was used; whereas for the NM treatment the same soil was sterilized via γ irradiation (>25kGy dose; McNamara, Black, Beresford, & Parekh, 2003). To compensate the loss of soil microbes besides AM fungi due to the sterilization, a microbial wash was also added (Liang et al., 2015). We obtained the microbial wash by blending 5kg of non-sterilized soil in 10 l water and filtering the solution through 20µm pore-size filter paper (Whatman® quantitative filter paper, Grade 41) following van der Heijden *et al.*, (1998), with slight modifications. Gamma-sterilization did not change neither the C nor the N chemical composition of the soil compared to the non-sterilized soil (Fig. S1).

Additionally, these AM and NM treatments were factorially combined with two levels of water availability. Half of the individuals were subjected to cycles of drought stress (Drought stress; W-), while the other half, for creating control conditions, were watered regularly from the bottom ensuring the pot surface was wet (Control; W+). Throughout the entire experiment, plants were watered regularly from the bottom ensuring that the pot surface was wet. The drought stress treatment included watering only when 50% of the individuals had wilted leaves followed by one-week recovery in control conditions. By the end of the experiment, the drought stress treatment comprised

two drought pulses (the first started 12th of May and the second 15th of June) that lasted three weeks plus one-week recovery each.

Prior to the establishment of the experiment, seeds were surface sterilised by immersion in 0.5% sodium hypochlorite solution (commercial bleach) for 20 minutes to avoid inoculation via seeds, and then germinated in Petri dishes. After 10 days of germination, the seedlings were transplanted individually into the pots specified above, with 91 replicates per treatment. After three months we harvested all the plants except 15 plants per treatment that were maintained for four more months in ambient conditions (water control condition) to promote seed production. Then, seeds of each plant were collected, and after measuring the average seed mass per plant, were stored in cold (2-4 ºC).

Offspring experiment. A similar glasshouse experiment to the one described above was repeated the following year (April-August 2018) with the seeds produced by the parental generation. The aim of the offspring experiment was to test for adaptive transgenerational effects of AM symbiosis and water availability on the offspring at their juvenile and the adult stages. We tested this with a full factorial design where the offspring plants from each of the four parental treatments were exposed again to the four possible conditions (AM W+, AM W-, NM W+, NM W-). Thus, the offspring experimental design resulted in 16 combinations: two parental mycorrhizal inoculations (Par. M) x two parental water availability levels (Par. W) x two offspring mycorrhizal inoculations (Off. M) x two offspring water availability levels (Off. W). Since the seed mass of AM parents was on average lower than that of NM parents (Fig. S3, Table S1), and seed provisioning is a potential mechanism of transgenerational effects (Herman & Sultan, 2011), we controlled for it by classifying seeds from all parental treatments into 5 size categories. Then, we took the same number of seeds from each size-group in each parental treatment, resulting in a similar distribution of seed sizes between parental treatments. Thus, the offspring experimental design finally resulted in the 16 combinations x 5 seed size categories x 8 seedlings = 640 pots (Fig. S2)**.**

Plants were harvested at two different developmental stages. Half of the offspring plants were harvested 1.5 months after planting, at their juvenile stage; and the rest of the replicants were harvested five months after planting, at their adult stage. Pots, substrate and watering regime were the same as in the parental experiment to ensure the

most similar conditions. However, the first drought stress pulse of the offspring generation lasted four weeks instead of three (first one started the 25th of April and the second one, the 1st of June) to ensure comparable effects on plants response (i.e. % of plants with wilted leaves). To facilitate the application of the treatments, the replicates were distributed in blocks, placing four replicates of a parental treatment in parallel, one in each offspring treatment (Fig. S2).

Measured traits

In each of the generations we measured plant traits. For each plant in the parental generation, at the time of harvest, we measured survival, total dry biomass (aerial plus root biomass), seed output (i.e. number of seeds) and seed mass. For five randomly chosen plants per treatment, we measured C, N and P content of the seeds, considered to be reliable indicators of seed reserve materials (Toorop et al., 2012). Total C and N content were determined by dry combustion using an elemental analyser (CHNS Elemental Analyzer vario MICRO cube, Elementar Analysensysteme GmbH, Germany). Total P was determined by flow injection analysis. Additionally, several above- and belowground vegetative plant traits were measured. For each plant, two leaves were scanned for leaf area and weighed for fresh mass and dry mass after drying at 60º C (48h). We used these measurements to estimate specific leaf area (SLA; leaf area per dry mass, mm^2/mg) and leaf dry matter content (LDMC; leaf dry mass per leaf fresh mass, mg/mg). In addition, roots were carefully extracted, washed and a subsample of roots (6 cm^2) was scanned at 600 dpi with an Epson Perfection 4990 scanner. From the scans, total root length, average root diameter (mm), and distribution of root length in different diameter classes were determined by using the image analysis software WinRHIZO Pro, 2008 (Regent Instruments Inc., Quebec, Canada). After scanning, the root subsample and the rest of the root system were dried for 48 h at 60 °C and weighed. We used these measurements to estimate specific root length (SRL; root length per dry mass, m/g), and fine roots percentage (root length with a diameter < 0.5mm per total root length). Further, we estimated root biomass allocation (i.e. root mass factor; RMF; root biomass per total biomass, g/g) after drying the remaining radicular part at 60º C (48h).

For each plant in the offspring generation, at the time of the respective harvest (i.e. juvenile and adult offspring harvest), we measured total dry biomass (aerial plus root biomass), and the same above- and belowground vegetative traits as described above. Additionally, we analyzed the content of C, N and P in the leaves of two randomly chosen plants from the juvenile stage and eight plants from the adult stage per treatment, following the methods described above. The root subsamples were stained with Chlorazol Black according to the protocol by Šmilauer, Košnar, Kotilínek, & Šmilauerová (2020). We quantified the AM fungal colonization by measuring the percentage of root length colonized (%RLC) by AM fungal structures (arbuscules, vesicles and hyphae). Magnified intersection method (McGonigle, Miller, Evans, Fairchild, & Swan, 1990) was used with 400 x magnification using a light microscope and observing at least 100 intersections per root sample. We further calculated the arbuscule:vesicle ratio (relative abundance of arbuscules per vesicles), suggested as an indicator of the fungal activity status and the relative cost or benefit of the fungus to the host plant (Braunberger, Miller, & Peterson, 1991; Titus & Lepš, 2000).

Statistical analysis

All analyses were carried out using R v3.2.3 (R Core Team 2016) with α=0.05 as significance level. In the parental generation, the effects of the mycorrhizal inoculation treatments, the parental water availability treatment, and their interaction were analysed by using linear effects models. Plant total biomass was always log-transformed to remove skewness.

In the offspring generation, individuals were grouped into sixteen different treatments (coming from the combination of four factors with two levels each) depending on the parental background and the current conditions. Two of the factors corresponded to parental conditions: mycorrhizal inoculation treatment (Par. M), and water availability treatment (Par. W). The other two factors corresponded to offspring conditions: mycorrhizal inoculation treatment (Off. M) and water availability treatment (Off. W). We analysed the effects of parental and offspring conditions on plant traits of the offspring using linear mixed-effects models (lmer, library lme4), where the four experimental factors (two parental, two offspring conditions) and all their interactions were used as fixed effects, and the experimental blocks as a random effect. Any

significant effect of the parental conditions: either direct (Par. M or Par. W) or in interaction with the offspring conditions (Off. x Par.) is considered a transgenerational effect. We controlled for differences in seed provisioning (Herman & Sultan, 2011) by including seed mass as a covariate (i.e. in the fixed effects part of the model).

For the analysis of the effect of parental and offspring treatments on AM fungal colonization and arbuscule:vesicle ratio of the offspring, we used identical models, but excluding the offspring mycorrhizal inoculation factor (Off. M) from the model due to the lack of AM fungal colonization in the NM plants.

Results

Parental generation

In the parental generation, the drought treatment decreased *T. brevicorniculatum* total plant biomass and survival of only NM plants, with no effect on AM plants (Fig. S3a,b; Table S1). AM fungal inoculation increased plant growth, survival and reproductive investment (i.e. number of seeds per unit plant biomass), with no effect on the total number of seeds produced per plant (Fig. S3, Table S1). Additionally, seeds of AM plants were lighter than the ones of NM plants, but with higher N, P and C contents, although only the latter was significant (Fig. S3, Table S1). Nevertheless, the seed macronutrients stoichiometry, i.e. C:N:P ratios; did not differ between AM and NM plants (Fig. S3, Table S1).

Offspring plant traits

In the juvenile offspring, both offspring conditions (offspring mycorrhizal inoculation treatment, Off. M; and offspring water availability treatment, Off. W) had generally strong effects on most of the measured plant traits, except for SRL and average root diameter that only were affected by the water treatment (Off. W; Fig. 1 and Table S2). In general, plants under drought stress and absence of AM symbiosis had higher RMF, higher LDMC and lower SRL (Fig. 1b,c,f and Table S2).

Additionally, we found transgenerational effects (i.e. where offspring plants were affected by the conditions experienced by their parents; parental mycorrhizal inoculation, Par. M; and water availability treatments, Par. W) in seven out of the nine traits. These effects were either direct or, more commonly, interacting with the offspring conditions (Table S2). From the seven offspring traits, three (total biomass, average root diameter and percentage of fine roots) were affected by both parental conditions (Table S2; Par. M and Par. W), whereas for the other four traits only a single parental condition triggered transgenerational effects. Offspring of parents under water stress (Par. W) had in general lower SRL, whereas offspring of mycorrhizal parents (Par. M) had in general lower RMF, higher LDMC, and higher leaf P content (Fig. 1b,c,d and Table S2). Except for LDMC, the transgenerational effects (Par. M and Par. W) were concordant in the direction of the plastic response to the conditions experienced during their life cycle (Off. M and Off. W). For example, mycorrhizal offspring showed lower RMF, but this reduction was even more pronounced if the parent was mycorrhizal (Fig. 1b and Table S2).

At the adult stage of offspring plants, we did not detect significant transgenerational effects except for LDMC (Fig. 2c and Table S3; Off. W x Par. M), since only the offspring rather than the parental conditions (Off. M and Off. W) were the main drivers of plant plasticity (Fig. 2 and Table S3). The direction of the plasticity in response to offspring conditions (Off. M and Off. W) reversed compared with the juvenile stage, with the exception of leaf P content and total biomass (Fig. 2 and Table S3). For example, offspring with AM fungi and water availability had lower RMF during the juvenile stage, but higher RMF during the adult stage (Fig. 1b and Fig. 2b). Additionally, in the adult stage, more traits responded to the offspring mycorrhizal inoculation treatments (Off. M) compared with the juvenile stage. These additional traits included SRL and percentage of fine roots (Fig. 2f, g and Table S3), which was larger in non-mycorrhizal plants.

Offspring's AM fungal colonization

In the juvenile offspring, water availability (Off. W) generally had a strong effect on AM fungal colonization: drought-stressed plants had lower %RLC, and higher arbuscule:vesicle ratio than control plants (Fig. 3a, b and Table S2).

Additionally, the AM fungal colonization of the offspring was affected by the conditions experienced by their parents. From the two parental treatments (Par. M and Par. W), only the water conditions experienced by the parents influenced the %RLC of the offspring and only in the case of offspring plants under drought stress (Fig. 3a, b and Table S2; Off. W x Par. W). Drought-stressed offspring plants had higher %RLC when their parents had experienced water control conditions (Fig. 3a, b, Table S2 and Fig. S4; Off. W x Par. W). For the arbuscule:vesicle ratio we did not detect significant transgenerational effects.

During the adult stage, we found no significant difference in %RLC between the water availability conditions (Off. W), although there was a higher arbuscule:vesicle ratio in the plants experiencing control water availability (Fig. 3c, d, Table S3 and Fig. S4). Additionally, %RLC of the offspring in the adult stage was affected by the parental mycorrhizal inoculation treatment (Fig. 3c; Table S3; Off. W x Par. M). Offspring of mycorrhizal parents had higher %RLC if drought-stressed, but lower %RLC in water-control conditions (Fig. 3c, Table S3 and Fig. S4).

Discussion

This study expands the knowledge on the importance of AM symbiosis in triggering phenotypic changes in plants during their life cycle that improve their ability to cope with environmental stress and likely increase the species' realized niche. Earlier it was known that mycorrhizal symbiosis influences plant fitness-related performance. Here we show that mycorrhizal symbiosis also affects plant functional traits related to resource use and acquisition strategies. Additionally, we show that these mycorrhizal transgenerational effects on phenotype are adaptive for plant performance. Further, we provide evidence of transgenerational effects on AM fungal colonization. Finally, we found that transgenerational effects could persist through offspring development. Importantly, we show that the transgenerational effects of mycorrhizal symbiosis and water availability could have been transmitted via heritable epigenetic mechanisms, because these effects were not caused by differences in the quality and resources provided in the seed (Herman & Sultan, 2011).

Within-generational plasticity on offspring traits is development–specific

The strong response of plants of *T. brevicorniculatum* to the conditions experienced during their life cycle (mycorrhizal inoculation and water availability) shows the high level of plasticity of this species. However, we found that the response of the performance and phenotype to these conditions differed in juvenile and adult phases, suggesting specific plant plasticity at different developmental stages (Coleman, McConnaughay, & Ackerly, 1994).

As expected, measurements of different fitness-related characteristics suggest that AM symbiosis improved plant performance and mitigated water stress. However, the biomass increase in offspring was even more pronounced in adults than in juveniles (Fig. 2a vs Fig. 1a), probably because during the juvenile stage the cost/benefit ratio of the symbiosis is still high (N. C. Johnson et al., 1997). Mycorrhizal fungal inoculation dramatically increased leaf P content (Fig. 1f and Fig. 2f) at both developmental stages of the offspring plants. This result reflects that, even in drought-stressed conditions, AM fungi were able to mitigate water stress and provide important nutrients, such as P, to the host (Lu & Koide, 1994).

In both developmental stages, mycorrhizal inoculation in combination with water availability induced significant changes in multiple plant phenotypic traits, including both root and aboveground traits (Fusconi, 2014; Goh et al., 2013; Nuortila et al., 2004). During the juvenile stage the effects on trait plasticity triggered by water availability and by the mycorrhizal fungal colonization were additive and in the same direction (Table S2). Similar to findings of Shumway & Koide (1994), plant traits shifted towards a more conservative phenotype under drought stress and in the absence of AM symbiosis (i.e. higher allocation of biomass in the roots; higher leaf dry matter content; and lower specific root length; Fig. 1). A conservative phenotype refers to a conservative resource use and exploitation strategy of the plant, based on the plant economics spectrum framework (Díaz et al., 2016). It is associated with longer lifespan, better resource-use conservation, and generally is the strategy selected in plants from resource-poor environments (Díaz et al., 2016). Thus, the plastic response towards a conservative phenotype could improve *T. brevicorniculatum* ability to cope with drought stress.

During the adult stage, the direction of plasticity changed, and more traits plastically responded to the mycorrhizal fungal inoculation treatments compared with the juvenile stage. Plants increased their specific root length and percentage of fine roots in response to the absence of mycorrhizal fungi, reflecting an adaptive plasticity that improved resource uptake, thereby compensating for the lack of AM symbiosis (Fusconi, 2014; Goh et al., 2013; Pozo et al., 2015). The diversity of plant responses depending on developmental stage could explain why previous studies found a variable effect of mycorrhizal fungi on plant phenotype (D. Johnson, Martin, Cairney, & Anderson, 2012; Nuortila et al., 2004).

Transgenerational effects on offspring performance and phenotype

Most studies have shown that having mycorrhizal parents is beneficial for the performance of the offspring, reflected in higher biomass, survival, growth rate, and seed production (Heppell et al., 1998; Koide, 2010; Varga et al., 2013). However, we did not observe any benefit of having mycorrhizal parents in terms of biomass, nor from growing in the same environmental conditions as the parental generation (Fig. 1a). This contrasting result is probably because we experimentally removed the differences in quality and resources provided in the seed, aiming to identify possible underlying epigenetic mechanisms in transgenerational effects (Herman & Sultan, 2011). Nonetheless, we found that offspring of mycorrhizal parents had higher leaf P content than those of non-mycorrhizal parents (Fig. 1d), suggesting that in addition to directly providing soil nutrients to host plants, mycorrhizal symbiosis increase their offspring's nutrient uptake via epigenetic transgenerational effects. One way to confirm that these effects were epigenetically controlled would be to modify the epigenetic signature of the plants via application of a demethylation agent (Puy et al., 2018). However, it should be first tested whether the demethylation application also affects AM fungi.

Moreover, we found adaptive transgenerational effects in offspring phenotypes and traits linked with the resource use and exploitation strategy of the plant (Díaz et al., 2016). In general, offspring transgenerational plasticity had concordant responses to the within-generational response to treatments. As discussed above, plant traits shifted towards a "stress-coping phenotype" under drought stress and absence of AM symbiosis experienced during their life cycle (i.e. more conservative phenotype:

increased the RMF and decrease SRL). Additionally, offspring became even more conservative when their parents were under those stressful conditions (i.e. drought stress and absence of AM symbiosis; Fig. 1). Thus, the transgenerational effects further reinforced trait plasticity in the same direction, reflecting an adaptive epigenetic "stress memory" that could improve the ability of plants to cope with the predicted environment.

Even though both abiotic and biotic parental environments seemed to trigger transgenerational effects, we found that mycorrhizal inoculation affected plant traits more than the water availability treatments (RMF, LDMC and leaf P content, versus SRL; Fig. 1 and Table S2). Although this may have changed if we had measured another set of phenotypic traits, it is important to note that *T. brevicorniculatum* has a wide ecological niche, accepting all types of moisture conditions (Luo & Cardina, 2012). Consequently, it is likely that water is a less crucial stressor and the transgenerational effects due to water were not evolutionary relevant for this species (Rendina González, Preite, Verhoeven, & Latzel, 2018). Also, it is important to emphasize that we found that the transgenerational effects on traits were expressed early on the ontogeny (Fig. 1), fading away over offspring life development (Fig. 2). This result reinforces the idea of transgenerational effects as an important factor promoting adaptation to repeated ecological conditions, especially during juvenile stages and establishment of communities (Dechaine et al., 2015; Latzel et al., 2014).

Within- and trans-generational effect on AM fungal colonization

As expected, the environmental factors experienced by offspring during their life cycle affected the offspring AM fungal colonization (Martínez-García et al., 2012; Pozo et al., 2015). However, although water deficiency did not stimulate the root AM fungal colonization (%RLC decreased), the proportion of arbuscules:vesicles increased in water-stressed offspring, showing that water deficiency stimulated an increase in the proportion of arbuscules, that are the structures where resource exchange takes place.

We found that AM fungal colonization and AM symbiosis activity could be influenced by the conditions experienced by the parental generation. To our knowledge, this is the first study that shows that epigenetic transgenerational effects influence the

AM fungal colonization. The offspring of parents in water-control conditions had higher AM fungal colonization and proportion of arbuscules, but only if they were under drought stress. While this appears to contradict our initial hypothesis, relative to the total root biomass of the plant (i.e. root biomass x %RLC), offspring from water-stressed and NM parents had more total root colonized and a greater number of arbuscules per individual than offspring from parents under control water conditions because they had larger root systems (see Fig S5). Thus, the result supports that transgenerational effects modify offspring towards the "stress-coping phenotype" stimulating the establishment and activity of the AM symbiosis.

Surprisingly, and contrary to the general pattern found in plant traits, the conditions experienced by the parental generation still influenced AM fungal colonization during the adult stage. This suggests that epigenetic transgenerational effects influence plant–AM fungi relationship, and not only during the establishment and early stages of the symbiosis. Moreover, at the adult stage, %RLC was affected by the parental mycorrhizal status, so that offspring from mycorrhizal parents had higher %RLC under drought stress. These results suggest that mycorrhizal symbiosis could be promoted in the offspring when the parental generation has experienced mycorrhizal symbiosis.

Conclusions

We found transgenerational effects of mycorrhizal symbiosis, in combination with water availability, on offspring performance, phenotype, and root AM fungal colonization. Importantly, we show that transgenerational effects of mycorrhizal symbiosis and water availability were mainly transmitted via heritable epigenetic mechanisms, such as DNA methylation because the effects were not related to larger seed mass or seed resource provisioning (Herman & Sultan, 2011). Drought stress and absence of AM fungi triggered concordant plant phenotypic plasticity (towards a "stress-coping phenotype") both within- and across generations. This reflects an adaptive epigenetic mechanism that promotes rapid adaptation, and probably improves the ability of the species to cope with drought stress. In a context where the importance of individual and intraspecific variation of mycorrhizal plants and fungi in ecosystems is increasingly acknowledged (D. Johnson et al., 2012), our paper adds a new

mechanism of variation that has been ignored so far: transgenerational plasticity. These plastic changes confer competitive advantages to the next generation.

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References:

Alonso, C., Ramos-Cruz, D., & Becker, C. (2019). The role of plant epigenetics in biotic interactions. *New Phytologist*, *221*(2), 731–737. doi: 10.1111/nph.15408

Braunberger, P. G., Miller, M. H., & Peterson, R. L. (1991). Effect of phosphorus nutrition on morphological characteristics of vesicular-arbuscular mycorrhizal colonization of maize. *New Phytologist*, *119*(1), 107–113. doi: 10.1111/j.1469-8137.1991.tb01013.x

Coleman, J. S., McConnaughay, K. D. M., & Ackerly, D. D. (1994). Interpreting phenotypic variation in plants. *Trends in Ecology & Evolution*, *9*(5), 187–191. doi: 10.1016/0169-5347(94)90087-6

de Bello, F., Price, J. N., Münkemüller, T., Liira, J., Zobel, M., Thuiller, W., … Pärtel, M. (2012). Functional species pool framework to test for biotic effects on community assembly. *Ecology*, *93*(10), 2263–2273. doi: 10.1890/11-1394.1

De Long, J. R., Semchenko, M., Pritchard, W. J., Cordero, I., Fry, E. L., Jackson, B. G., … Bardgett, R. D. (2019). Drought soil legacy overrides maternal effects on plant growth. *Functional Ecology*, *00*, 1– 11. doi: 10.1111/1365-2435.13341

Dechaine, J. M., Brock, M. T., & Weinig, C. (2015). Maternal environmental effects of competition influence evolutionary potential in rapeseed (Brassica rapa).

Evolutionary Ecology, *29*(1), 77–91. doi: 10.1007/s10682-014-9735-6

Díaz, S., Kattge, J., Cornelissen, J. H. C., Wright, I. J., Lavorel, S., Dray, S., … Gorné, L. D. (2016). The global spectrum of plant form and function. *Nature*, *529*(7585), 167–171. doi: 10.1038/nature16489

Fusconi, A. (2014). Regulation of root morphogenesis in arbuscular mycorrhizae: What role do fungal exudates, phosphate, sugars and hormones play in lateral root formation? *Annals of Botany*, *113*(1), 19–33. doi: 10.1093/aob/mct258

Germain, R. M., Grainger, T. N., Jones, N. T., & Gilbert, B. (2019). Maternal provisioning is structured by species' competitive neighborhoods. *Oikos*, *128*(1), 45–53. doi: 10.1111/oik.05530

Gerz, M., Guillermo Bueno, C., Ozinga, W. A., Zobel, M., & Moora, M. (2018). Niche differentiation and expansion of plant species are associated with mycorrhizal symbiosis. *Journal of Ecology*, *106*(1), 254–264. doi: 10.1111/1365-2745.12873

Goh, C.-H., Veliz Vallejos, D. F., Nicotra, A. B., & Mathesius, U. (2013). The Impact of Beneficial Plant-Associated Microbes on Plant Phenotypic Plasticity. *Journal of Chemical Ecology*, *39*(7), 826–839. doi: 10.1007/s10886-013-0326-8

González, A. P. R., Dumalasová, V., Rosenthal, J., Skuhrovec, J., & Latzel, V. (2017). The role of transgenerational effects in adaptation of clonal offspring of white clover (Trifolium repens) to drought and herbivory. *Evolutionary Ecology*, *31*(3), 345–361. doi: 10.1007/s10682-016-9844-5

Heppell, K. B., Shumway, D. L., & Koide, R. T. (1998). The effect of mycorrhizal infection of Abutilon theophrasti on competitiveness of offspring. *Functional Ecology*, *12*(2), 171–175. doi: 10.1046/j.1365-2435.1998.00188.x

Herman, J. J., & Sultan, S. E. (2011). Adaptive Transgenerational Plasticity in Plants: Case Studies, Mechanisms, and Implications for Natural Populations. *Frontiers in Plant Science*, *2*, 102. Retrieved from http://journal.frontiersin.org/article/10.3389/fpls.2011.00102/abstract

Hoeksema, J. D., Chaudhary, V. B., Gehring, C. A., Johnson, N. C., Karst, J., Koide, R. T., … Umbanhowar, J. (2010). A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters*, *13*(3), 394–407.

doi: 10.1111/j.1461-0248.2009.01430.x

Jablonka, E., & Raz, G. (2009). Transgenerational Epigenetic Inheritance: Prevalence, Mechanisms, and Implications for the Study of Heredity and Evolution. *The Quarterly Review of Biology*, *84*(2), 131–176. doi: 10.1086/598822

Johnson, D., Martin, F., Cairney, J. W. G., & Anderson, I. C. (2012). The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytologist*, *194*(3), 614–628. doi: 10.1111/j.1469-8137.2012.04087.x

Johnson, N. C., Graham, J. H., & Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, *135*(4), 575–586. doi: 10.1046/j.1469-8137.1997.00729.x

Jones, M. D., & Smith, S. E. (2004). Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? *Canadian Journal of Botany*, *82*(8), 1089–1109. doi: 10.1139/b04-110

Kirschner, J., Štěpánek, J., Černý, T., De Heer, P., & van Dijk, P. J. (2013). Available ex situ germplasm of the potential rubber crop Taraxacum koksaghyz belongs to a poor rubber producer, T. brevicorniculatum (Compositae-Crepidinae). *Genetic Resources and Crop Evolution*, *60*(2), 455–471. doi: 10.1007/s10722-012-9848-0

Koide, R. T. (2010). Mycorrhizal Symbiosis and Plant Reproduction. In *Arbuscular Mycorrhizas: Physiology and Function* (pp. 297–320). doi: 10.1007/978-90-481-9489-6_14

Lämke, J., & Bäurle, I. (2017, December 27). Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. *Genome Biology*, Vol. 18, p. 124. doi: 10.1186/s13059-017-1263-6

Latzel, V., Janeček, Š., Doležal, J., Klimešová, J., & Bossdorf, O. (2014). Adaptive transgenerational plasticity in the perennial Plantago lanceolata. *Oikos*, *123*(1), 41–46. doi: 10.1111/j.1600-0706.2013.00537.x

Latzel, V., Klimešová, J., Hájek, T., Gómez, S., & Šmilauer, P. (2010). Maternal effects alter progeny's response to disturbance and nutrients in two Plantago species. *Oikos*, *119*(11), 1700–1710. doi: 10.1111/j.1600-0706.2010.18737.x

Liang, M., Liu, X., Etienne, R. S., Huang, F., Wang, Y., & Yu, S. (2015). Arbuscular

mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology*, *96*(2), 562–574. doi: 10.1890/14-0871.1

Lu, X., & Koide, R. T. (1994). The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist*, *128*(2), 211–218. Retrieved from https://nph.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-8137.1994.tb04004.x

Luo, J., & Cardina, J. (2012). Germination patterns and implications for invasiveness in three Taraxacum (Asteraceae) species. *Weed Research*, *52*(2), 112–121. doi: 10.1111/j.1365-3180.2011.00898.x

Martínez-García, L. B., de Dios Miranda, J., & Pugnaire, F. I. (2012). Impacts of changing rainfall patterns on mycorrhizal status of a shrub from arid environments. *European Journal of Soil Biology*, *50*, 64–67. doi: 10.1016/J.EJSOBI.2011.12.005

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytologist*, *115*(3), 495–501. doi: 10.1111/j.1469-8137.1990.tb00476.x

McNamara, N. P., Black, H. I. J., Beresford, N. A., & Parekh, N. R. (2003). Effects of acute gamma irradiation on chemical, physical and biological properties of soils. *Applied Soil Ecology*, *24*(2), 117–132. doi: 10.1016/S0929-1393(03)00073-8

Metz, J., von Oppen, J., & Tielbörger, K. (2015). Parental environmental effects due to contrasting watering adapt competitive ability, but not drought tolerance, in offspring of a semi-arid annual Brassicaceae. *Journal of Ecology*, *103*(4), 990–997. doi: 10.1111/1365-2745.12411

Nuortila, C., Kytöviita, M.-M., & Tuomi, J. (2004). Mycorrhizal symbiosis has contrasting effects on fitness components in Campanula rotundifolia. *New Phytologist*, *164*(3), 543–553. doi: 10.1111/j.1469-8137.2004.01195.x

Peay, K. G. (2016). The Mutualistic Niche: Mycorrhizal Symbiosis and Community Dynamics. *Annual Review of Ecology, Evolution, and Systematics*, *47*(1), 143–164. doi: 10.1146/annurev-ecolsys-121415-032100

Pozo, M. J., López-Ráez, J. A., Azcón-Aguilar, C., & García-Garrido, J. M. (2015, March 1). Phytohormones as integrators of environmental signals in the regulation

of mycorrhizal symbioses. *New Phytologist*, Vol. 205, pp. 1431–1436. doi: 10.1111/nph.13252

Price, T. D., Qvarnström, A., & Irwin, D. E. (2003, July 22). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences*, Vol. 270, pp. 1433–1440. doi: 10.1098/rspb.2003.2372

Puy, J., Dvořáková, H., Carmona, C. P., de Bello, F., Hiiesalu, I., & Latzel, V. (2018). Improved demethylation in ecological epigenetic experiments: Testing a simple and harmless foliar demethylation application. *Methods in Ecology and Evolution*, *9*(3), 744–753. doi: 10.1111/2041-210X.12903

Puy, J., P. Carmona, C., Dvořáková, H., Latzel, V., & de Bello, F. (2020). Diversity of parental environments increases phenotypic variation in Arabidopsis populations more than genetic diversity but similarly affects productivity. *Annals of Botany*, In press. doi: 10.1093/aob/mcaa100

Rendina González, A. P., Preite, V., Verhoeven, K. J. F., & Latzel, V. (2018). Transgenerational Effects and Epigenetic Memory in the Clonal Plant Trifolium repens. *Frontiers in Plant Science*, *9*, 1677. doi: 10.3389/fpls.2018.01677

Shumway, D. L., & Koide, R. T. (1994). Reproductive responses to mycorrhizal colonization of Abutilon theophrasti Medic, plants grown for two generations in the field. *New Phytologist*, Vol. 128, pp. 219–224. doi: 10.1111/j.1469-8137.1994.tb04005.x

Šmilauer, P., Košnar, J., Kotilínek, M., & Šmilauerová, M. (2020). Contrasting effects of host identity, plant community, and local species pool on the composition and colonization levels of arbuscular mycorrhizal fungal community in a temperate grassland. *New Phytologist*, *225*(1), 461–473. doi: 10.1111/nph.16112

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis*. Academic Press.

Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., … Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, *108*(5), 1028–1046. doi: 10.3852/16-042

Titus, J. H., & Lepš, J. (2000). The response of arbuscular mycorrhizae to fertilization,

mowing, and removal of dominant species in a diverse oligotrophic wet meadow. *American Journal of Botany*, *87*(3), 392–401. doi: 10.2307/2656635

Toorop, P. E., Campos Cuerva, R., Begg, G. S., Locardi, B., Squire, G. R., & Iannetta, P. P. M. (2012). Co-adaptation of seed dormancy and flowering time in the arable weed Capsella bursa-pastoris (shepherds purse). *Annals of Botany*, *109*(2), 481–489. doi: 10.1093/aob/mcr301

van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., … Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, *396*(6706), 69–72. doi: 10.1038/23932

van der Heijden, M. G. A., Wiemken, A., & Sanders, I. R. (2003). Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. *New Phytologist*, *157*(3), 569–578. doi: 10.1046/j.1469-8137.2003.00688.x

Varga, S., & Kytöviita, M. M. (2010). Mycorrhizal benefit differs among the sexes in a gynodioecious species. *Ecology*, *91*(9), 2583–2593. doi: 10.1890/09-1383.1

Varga, S., & Soulsbury, C. D. (2017). Paternal arbuscular mycorrhizal fungal status affects DNA methylation in seeds. *Biology Letters*, *13*(9), 20170407. doi: 10.1098/rsbl.2017.0407

Varga, S., & Soulsbury, C. D. (2019). Arbuscular mycorrhizal fungi change host plant DNA methylation systemically. *Plant Biology*, *21*(2), 278–283. doi: 10.1111/plb.12917

Varga, S., Vega-Frutis, R., & Kytöviita, M. M. (2013). Transgenerational effects of plant sex and arbuscular mycorrhizal symbiosis. *New Phytologist*, *199*(3), 812–821. doi: 10.1111/nph.12305

Vellend, M. (2016). The Theory of Ecological Communities (MPB-57). In *The Theory of Ecological Communities (MPB-57)*. doi: 10.1515/9781400883790

Supporting information

Fig. S1: N and C content of the substrate (AMF+ and AMF-).

Fig. S2: Schematic representation of the design of the offspring experiment.

Fig. S3: Effect of the treatments on parental generation.

Fig. S4: Effect of the offspring and parental treatments on frequency of AM structures of the juvenile and adult offspring.

Fig. S5: Relative effect of the offspring and parental treatments on total root AM fungal colonization and percentage of arbuscules on juvenile offspring.

Table S1: Summary of the linear mixed-effect model for main and interaction effects of the treatments in the parental generation.

Table S2: Summary of the linear mixed-effect model for main and interaction effects of offspring and parental treatments on juvenile offspring.

Table S3: Summary of the linear mixed-effect model for main and interaction effects of offspring and parental treatments on adult offspring.

Figures:

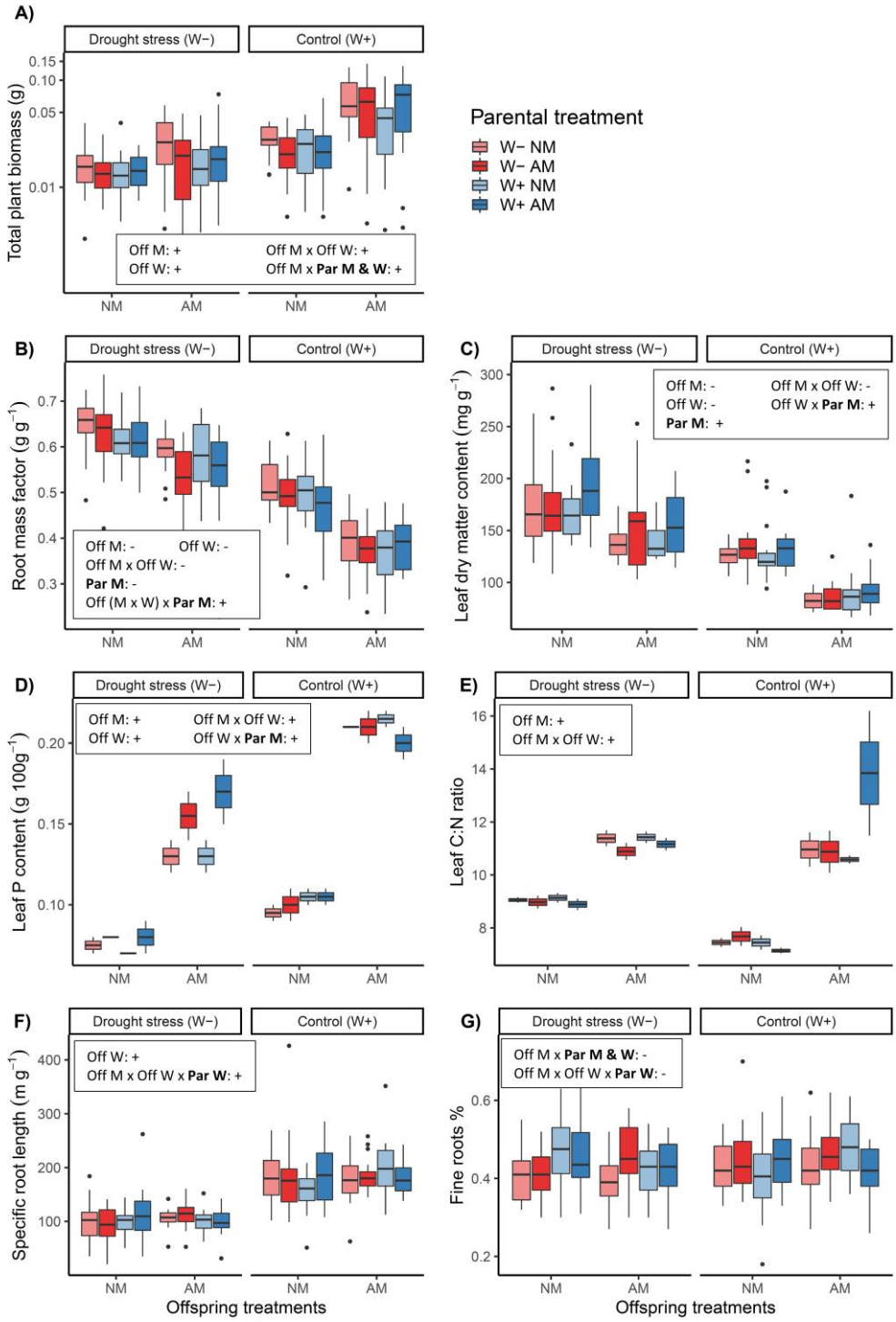

Figure 1: Effect of the offspring and parental treatments on plant phenotype characteristics of the juvenile offspring. a) total plant biomass, b) root mass factor, c) leaf dry matter content, d) leaf P content, e) leaf C:N ratio, f) specific root length and g) fine roots percentage. The significant factors of each model with the directionality of each effect are shown in the boxes. The factors corresponded to the offspring

conditions: mycorrhizal inoculation treatment, Off. M; and water availability treatment Off. W; and the parental conditions (also highlighted in bold face): mycorrhizal inoculation treatment, Par. M; and water availability treatment, Par. W. Colour coding indicates the parental treatments: red - offspring of water-stressed parents, blue - offspring of parents that experienced water control conditions; intense colour - offspring of mycorrhizal parents, light colour - offspring of non-mycorrhizal parents. The bottom and top of the boxes are the 25th and 75th percentiles respectively, the centred band is the median and the whiskers represent 1.5 times the length of the box further from the box limits or the maximum or minimum observation in the absence of outliers.

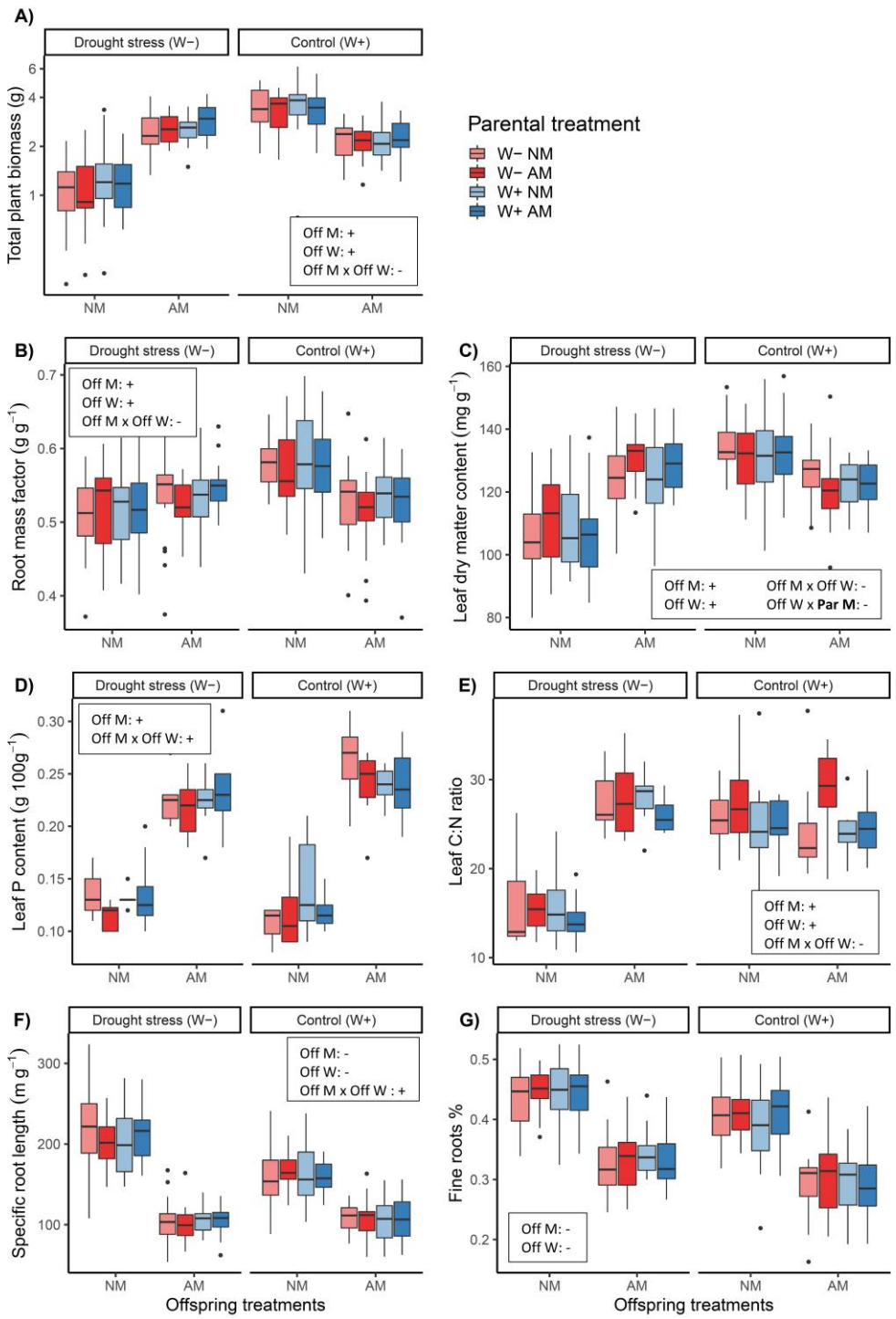

Figure 2: Effect of the offspring and parental treatments on plant phenotype characteristics of the adult offspring. a) total plant biomass, b) root mass factor, c) leaf dry matter content, d) leaf P content, e) leaf C:N ratio, f) specific root length and g) fine roots percentage. The significant factors of each model with the directionality of each effect are shown in the boxes. The factors corresponded to the offspring conditions: mycorrhizal inoculation treatment, Off. M; and water availability treatment Off. W; and

the parental conditions (also highlighted in bold face): mycorrhizal inoculation treatment, Par. M; and water availability treatment, Par. W. Colour coding indicates the parental treatments: red - offspring of water-stressed parents, blue - offspring of parents that experienced water control conditions; intense colour - offspring of mycorrhizal parents, light colour - offspring of non-mycorrhizal parents.

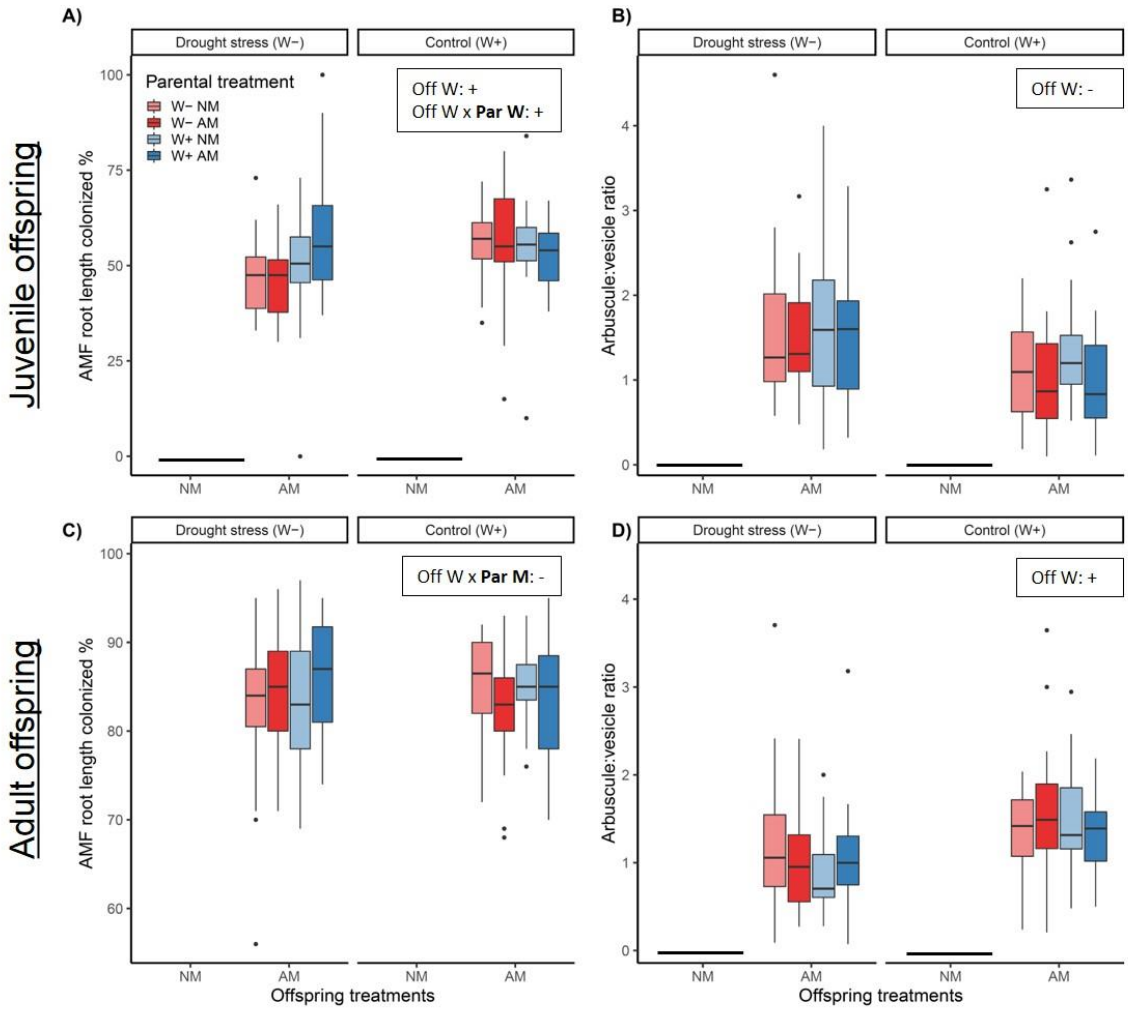

Figure 3: Effect of the offspring and parental treatments on AM fungal root colonisation in juvenile stage (upper row) and adult stage (lower row): a) and c) percentage of root length colonized by AM fungi; b) and d) arbuscule:vesicle ratio. The significant factors of each model with the directionality of each effect are shown in the boxes. The factors corresponded to the offspring conditions: mycorrhizal inoculation treatment, Off. M; and water availability treatment Off. W; and the parental conditions (also highlighted in bold face): mycorrhizal inoculation treatment, Par. M; and water availability treatment, Par. W. Colour coding indicates the parental treatments: red - offspring of water-stressed parents, blue - offspring of parents that experienced water control conditions; intense colour - offspring of mycorrhizal parents, light colour - offspring of non-mycorrhizal parents. The nonmycorrhizal offspring treatment plants were not colonized by AM fungi.