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Article type : MS - Regular Manuscript

Competition-induced transgenerational plasticity influences competitive interactions and leaf decomposition of offspring

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Received: 27 July 2020

Accepted: 15 October 2020

1 **ORCiD:**

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nph.17037

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Summary

- Phenotypic plasticity, within and across generations (transgenerational plasticity), allows organisms and their progeny to adapt to the environment without modification of the underlying DNA. Recent findings suggest that epigenetic modifications are important mediators of such plasticity. However, empirical studies have, so far, mainly focused on plasticity in response to abiotic factors, overlooking the response to competition.

- We tested for within-generation and transgenerational phenotypic plasticity triggered by plant–plant competition intensity, and tested whether it was mediated via DNA methylation, using the perennial, apomictic herb *Taraxacum brevicorniculatum* in four coordinated experiments. We then tested the consequences of transgenerational plasticity affecting competitive interactions of the offspring and ecosystem processes such as decomposition.

- We found that, by promoting differences in DNA methylation, offspring of plants under stronger competition developed faster and presented more resource-conservative phenotypes. Further, these adjustments associated with less degradable leaves which have the potential to reduce nutrient turnover and might, in turn, favour plants with more conservative traits.

- Greater parental competition enhanced competitive abilities of the offspring by triggering adaptive phenotypic plasticity, and decreased offspring leaf decomposability. Our results suggest that competition-induced transgenerational effects could promote rapid adaptations and species coexistence, and feed back on biodiversity assembly and nutrient cycling.

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Keywords:

Adaptation, Decomposition, DNA methylation, Functional traits, Intraspecific phenotypic variability, Parental effects, Plant competition, Transgenerational epigenetic inheritance

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Introduction

Phenotypic plasticity, referred as the ability of the genotype to modulate its trait expression in response to the environment (Price *et al.*, 2003; Turcotte & Levine, 2016), is considered an important mechanism by which organisms can rapidly adapt to changing ecological conditions (Rottstock *et al.*, 2017; Des Roches *et al.*, 2018; van Moorsel *et al.*, 2019). These phenotypic adjustments could be of highly variable duration, operating within the lifetime of individuals (also referred to as within-generation plasticity) or even be inherited across generations (Turcotte & Levine, 2016). The later, transgenerational phenotypic plasticity – in short, transgenerational plasticity – occurs when the phenotype of progeny is influenced by the environmental conditions experienced by the parents (also referred to as parental or transgenerational effects) (Herman *et al.*, 2014; Turcotte & Levine, 2016). The great majority of existing studies on transgenerational plasticity focus on phenotypic responses to abiotic factors (Galloway & Etterson, 2007; Bej & Basak, 2017; Auge *et al.*, 2017; Puy *et al.*, 2020a) and have generally overlooked the role of biotic interactions (Alonso *et al.*, 2019; Puy *et al.*, 2020b), such as competition between organisms. However these biotic interactions are considered leading factors for controlling species coexistence, biodiversity maintenance and ecosystem functioning (Van der Putten *et al.*, 2013; Kraft *et al.*, 2015; Valladares *et al.*, 2015).

Phenotypic plasticity (within and transgenerational) is driven by different "non-genetic" mechanisms that modify the phenotype without involving changes in the underlying DNA sequence. Among these mechanisms, epigenetic changes such as histone modification, RNA interference, or DNA methylation, have been proposed as the most proximate mediators (Herman & Sultan, 2011; Herman *et al.*, 2014). Epigenetic modifications are known to cause trait variation (Zhang *et al.*, 2013, 2018; Puy *et al.*, 2020a) and to occur in response to environmental factors (González *et al.*, 2016; Bej & Basak, 2017; Richards *et al.*, 2017; Puy *et al.*, 2020a), sometimes mediating an adaptive response to stressors (Galloway & Etterson, 2007; Metz *et al.*, 2015; González *et al.*, 2016). Most importantly, epigenetic modifications are inheritable across generations (Akimoto *et al.*, 2007; Bossdorf *et al.*, 2008; Verhoeven *et al.*, 2010), being key mechanisms of transgenerational effects (Herman *et al.*, 2014; Richards *et al.*, 2017). In particular, DNA methylation – one of the best understood epigenetic mechanisms in ecology and evolution (Akimoto *et al.*, 2007; Richards *et al.*, 2017) – is an excellent mediator for transgenerational

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inheritance (Verhoeven *et al.*, 2010; Herman *et al.*, 2014; Richards *et al.*, 2017) since the methylation of cytosines is inherited through mitosis and meiosis (Niederhuth & Schmitz, 2014).

Functional traits determine organisms' abilities to live in given ecological conditions and coexist with other species (Götzenberger *et al.*, 2012; Kraft *et al.*, 2015). However, organisms are able to functionally adjust their traits to match local conditions, which, in turn, can alter the strength and outcome of ecological interactions (Kraft *et al.*, 2015; Turcotte & Levine, 2016). For example, plant resource-use strategies related to the so-called "plant economic spectrum" are associated with a fundamental trade-off between individuals along a resource-acquisition vs. resource-conservation gradient (Reich, 2014; Díaz *et al.*, 2016). Individuals with acquisitive traits such as faster aboveground growth and "cheaper" and short-lived tissues (i.e. high specific leaf area, SLA; low leaf dry matter content, LDMC; high specific root length, SRL) are assumed to grow best when resources are abundant. In turn, individuals with conservative phenotypes, characterized by higher root biomass allocation and more structural and tougher tissues (i.e. low SLA, high LDMC, low SRL) are usually superior when resources are scarce (Reich, 2014; Díaz *et al.*, 2016; Puy *et al.*, 2020b). When there is an appropriate response towards well-adapted phenotypes, phenotypic plasticity can increase species fitness and promote adaptation. Further, if there is transgenerational inheritance of the response, phenotypic plasticity could even contribute to the adaptation of subsequent generations and promote rapid adaptive evolution of the population (Zhang *et al.*, 2013; van Moorsel *et al.*, 2019).

Besides responding to the environment and biotic interactions, traits also shape the environment organisms live in by affecting ecosystem processes, such as nutrient cycling (Cornelissen & Thompson, 1997; de Bello *et al.*, 2010). This idea has been formalized within the field of functional ecology by the "response–effect" framework (Lavorel & Garnier, 2002) stressing the dual role of traits as being both adaptive and drivers of ecosystem functionality. Thus, the response–effect framework could be theoretically applied also in the case of phenotypic plasticity, because adaptations that help organisms to better cope with their environment can theoretically feed back to the functioning of the ecosystem (Bossdorf *et al.*, 2008; Herman & Sultan, 2011; Richards *et al.*, 2017). However, it remains unclear as to whether or not plasticity (within or across generations) can feed back to key ecosystem functions (Richards *et al.*, 2017; Puy *et al.*, 2020a).

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Here, we tested the existence of transgenerational effects triggered by plant–plant competition, exploring their possible feedback on adaptation and ecosystem functioning. To do so, we performed four coordinated experiments (for one parental generation, two offspring generations, and one decomposition experiment; Fig. 1) using genetically identical individuals of *Taraxacum brevicorniculatum* Korol. Specifically, we tested whether (1) plant–plant competitive interactions triggered phenotypic plasticity towards more conservative strategies, not only within generations but also across generations (transgenerational plasticity). We then analyzed whether (2) phenotypic plasticity was mediated by epigenetic mechanisms, explicitly DNA methylation. And finally, (3) we explored the extent to which transgenerational plasticity feeds back to competitive interactions, contributes to adaptation, and affects decomposition.

Materials and methods

Study material

Taraxacum brevicorniculatum Korol. is an obligate apomictic, polycarpic perennial species (Kirschner *et al.*, 2013), ecologically similar to any other *Taraxacum sect. Ruderalia*. The genetically identical seeds used in this study were collected from a greenhouse-grown population of plants experiencing equal 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. We ran four experiments using *T. brevicorniculatum*: a parental generation, two offspring generations, and a decomposition experiment (Fig. 1). Since *T. brevicorniculatum* is an obligate apomictic species, all plants in all experiments were genetically identical, and after experiencing different competition levels during the parental generations, the offspring only differed in non-genetic information they inherited. Thus, any differences in the offspring generation were due to transgenerational effects induced by competition in parental generation that did not involve changes in the DNA sequence (i.e. non-genetic or epigenetic effects).

Experimental set-up

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Parental generation. To induce competition-related transgenerational effects, we conducted a two-month greenhouse-pot experiment (mid-May–mid-July 2015) where genetically identical individuals of *T. brevicorniculatum* were grown with or without competition until flowering. For pots with competition we planted one individual of the focal species surrounded by six other individuals. The six surrounding individuals could be either monospecific (i.e. only one species from either *T. brevicorniculatum* itself or ten other different species, replicated eight times per combination; see Table S1) or a mixture of six different species (eight different combinations, replicated five times, see Table S1). This resulted in 19 competition levels. Further, a no-competition treatment (replicated eight times) was performed, where only the focal *T. brevicorniculatum* individual was planted in the pot; this gave a total of 20 different competition levels. All combinations were planted after germinating the seeds separately in Petri dishes and then transplanting the seedlings into round pots with a volume of 2 l filled with a 1:1 mixture of sand and commercial soil. Throughout the entire experiment, plants were watered regularly from the bottom ensuring that the pot surface was wet.

We estimated the intensity of the competition experienced by the focal *T. brevicorniculatum* with the relative interaction intensity (RII) index, which reflects the effect of competition by comparing the aboveground biomass observed when growing with competitors with the biomass achieved growing in the absence of interaction, following the formula outlined in Armas *et al.* (2004). The more negative the RII value is, the stronger the reduction in biomass experienced by the focal plant is, relative to the biomass without competition. Consequently, in subsequent experiments, we used the average RII across all pots from each of the 20 competition treatments of the parental experiment to express the competition intensities experienced by the parental generation as a continuous variable (see Table S1).

At the end of the parental generation experiment, seeds of each focal plant were collected. After measuring the average seed mass per competition level, seeds were stored in the cold (2–4ºC).

Offspring experiment 1. Demethylation in juvenile offspring. The aims of this experiment were to test for transgenerational effects on the performance of juvenile offspring, and to test whether these effects were transmitted via DNA methylation. For this purpose, we used seeds coming from individuals that experienced monospecific competition during the previous

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competition experiment. Seedlings from these seeds were grown individually, and without competition, in a growth chamber until they reached the juvenile stage. Plants were grown with a 12 h (20°C) / 12 h (10°C) light/darkness-and-temperature regime and watered regularly. From each monospecific parental competition level, we established 20 pots (7 x 7 cm square-shaped and 18 cm depth), and for half of them we altered the epigenetic status by DNA-demethylation with 5-azacytidine (5-azaC). Experimental demethylation is a well-established method by which epigenetic marks (heritable or not) are removed; this allows us to test whether or not phenotypic traits have been mediated by epigenetic mechanisms (Richards *et al.*, 2017; Puy *et al.*, 2018; Alonso *et al.*, 2019).

To measure germination, six seeds were placed in each pot, and after 11 days, when all the pots contained at least one individual with a true leaf (i.e. excluding cotyledons), the emerged seedlings were thinned until only the biggest one remained in each pot. At the same time (after 11 days), we started to apply the demethylation treatment, which involved spraying a 50 μM aqueous solution of 5-azaC onto the leaves daily for six weeks (following Puy *et al.* 2018). To remove any potential effect of non-uniform growing conditions from our design, we distributed the replicates in 10 blocks, each of them including two replicates of each of the 11 monospecific competition levels, one with and one without the demethylation treatment. Thus, the final design comprised 10 blocks x 11 competition levels x 2 demethylation treatments = 220 plants in total. The position of the replicates for each competition level was randomized between the blocks but maintained between demethylation treatments within blocks. Sand was used as the potting substrate in all cases to facilitate root extraction during the harvest.

Offspring experiment 2. Competition experiment with adult offspring. The aim of this experiment was to test for transgenerational effects on the offspring during their adult stage. In this case, offspring undergo similar or distinct competition intensity than their parents. We consider transgenerational effects to be adaptive when offspring living under the same conditions as their parents perform better in those conditions (e.g. higher biomass) than plants with a different origin. In this experiment, seeds from six of the 20 parental competition levels were selected to attain a manageable experimental size – see below. The six levels included the following: two intense competition levels (one from the monospecific and another from the mixture combination), two weak competition levels (one from the monospecific and another from the mixture combination), intraspecific competition, and no competition (see Table S1). To do so, after germinating the seeds

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in Petri dishes, we transplanted and grew the offspring under the six competition levels experienced by the parental generation using a full factorial design. This design considered all six competition levels (6 parental competition levels x 6 offspring competition levels = 36 combinations). Following the same experimental set-up as in the parental generation, we conducted a two-month greenhouse-pot experiment (mid-May–mid-July 2016) where 12 replicates per parental-and-offspring-condition combination were randomly placed in the greenhouse, giving a total of 432 pots. The pots, substrate and watering regime were the same as in the parental experiment to ensure as similar conditions as possible.

Decomposition experiment. We aimed to test whether effects of transgenerational plasticity extend the life of offspring individuals, affecting the decomposability of their leaves and litter-senescent material. For this purpose, we incubated five replicates per treatment of fresh leaves from offspring experiment 2 and, as a reference, one replicate of senescent material. The plant material was collected during the harvest of offspring experiment 2 and oven-dried at 60ºC. The samples were incubated in 18 x 18 cm nylon bags with a 1 mm mesh on the bottom and a 4 mm mesh on the top to avoid loss of litter material and, at the same time, allow macrofauna access to the litter. Each litterbag contained 0.36 g of biomass. The litterbags were placed in a purpose-built outdoor incubation bed, located in an open area of the botanical garden of the Institute of Botany in Třeboň, Czech Republic (N 49°00′ 20″, E 14°46′25″). To maintain homogeneous microenvironmental conditions, the incubation bed was cleaned from vegetation and covered with sand. For the same reason, the litterbags were covered with 1 cm of sand. Extra samples of all the treatments were incubated and checked every two weeks to monitor the speed of the decomposition and to terminate the experiment when the samples reached a minimum of 50% biomass loss on average (Pérez-Harguindeguy *et al.*, 2013). Incubation started on 19th September and was terminated on 21st October when the samples had lost ca. 65% of biomass.

Measured variables

Parental generation. At the time of harvest, we measured seed output (i.e. number of seeds), total dry biomass (radicular and aerial) per plant, and aboveground vegetative traits (Pérez-Harguindeguy *et al.*, 2013). For each focal plant, two leaves were collected, scanned, and then weighed firstly by fresh mass and secondly by dry mass after drying at 60ºC (48 h). We used these

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measurements to estimate specific leaf area (SLA; leaf area per unit dry mass, mm^2/mg) and leaf dry matter content (LDMC; the ratio of leaf dry mass to leaf fresh mass, mg/mg). As mentioned above, the intensity of the competition experienced by the focal individual was estimated using the RII index based on the aboveground biomass. Using other indicators to measure RII (e.g. total biomass or seed production) gave similar results since they are highly correlated (0.97 and 0.79 Pearson's coefficient respectively). We transformed the 20 competition levels into a continuous variable reflecting the competition gradient by assigning to each level the average RII of the focal plants at the respective competitive level (see Table S1). This allowed us to characterize each plant in the offspring experiments by a "parental competition" RII.

Offspring experiment 1. Demethylation in juvenile offspring. The number of germinated seeds per pot was counted five times (4, 6, 8, 10 and 11 days after sowing, always before applying the demethylation treatment). Total germination percentage was calculated as the final cumulative germination of the six sown seeds. We also calculated T_{50} (i.e. the time at which half of the total germination percentage was reached in each pot, following Coolbear *et al.* (1984)). Every fourth day, starting four days after the beginning of the demethylation treatment until the end of the experiment (six weeks), we measured the maximum diameter of the rosette (cm) and the total number of leaves. We used this information to estimate growth rates for the plants; for this, in each pot, we regressed the diameter of the rosette and number of leaves against time (in days) using linear and Poisson regressions, respectively. We used the slopes of these regressions in each pot as indicators of the growth rates in these two parameters, with greater slopes indicating faster growth.

Epigenetic parental effects are likely to fade away with time (Dechaine *et al.*, 2015). We checked this by estimating the growth rates described above several times in each pot; the first growth rates were estimated considering only the first four measurements (i.e. 4, 8,12 and 16 days after the beginning of the demethylation treatment), and then we estimated the growth rate for each day on which a new measurement was taken (each time including the measurements up to that time). Thus, in total, since we measured every four days, from the first 16 days until the 42nd day, we had seven measurements of growth rate.

At the end of the experiment, plants were harvested and above- and belowground vegetative traits and total biomass were measured (Pérez-Harguindeguy *et al.*, 2013). For each

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plant, SLA and LDMC were measured. In addition, roots were carefully extracted by digging up the whole root system, washing it, scanning it, and weighing it as both fresh mass and then dry mass after drying at 60ºC (48 h). Total root length, average root diameter (mm), and distribution of root length in different diameter classes were determined using the image analysis software WinRHIZO Pro, 2008 (Regent Instruments Inc., Quebec, Canada). We used these measurements to estimate specific root length (SRL; root length per unit dry mass, m/g), root dry matter content (RDMC; the ratio of root dry mass to root fresh mass, mg/mg) and percentage of fine roots (ratio of root length with a diameter < 0.5mm divided by the total root length). Further, we estimated the root mass factor (RMF; ratio of root biomass to total biomass, g/g) after drying the remaining aerial plant parts at 60ºC (48 h).

Offspring experiment 2. Competition experiment with adult offspring. Total plant biomass and reproductive investment (i.e. number of seeds per unit plant biomass) were measured at the time of harvest, as for the parental generation. In addition, for each plant we measured SLA, LDMC, SRL, fine root percentage and RMF, following the protocols described above (Pérez-Harguindeguy *et al.*, 2013). Additionally, for five replicates per parental and offspring condition we measured C, N and P content of leaves, as well as storage-carbohydrate content of taproots. Total C and N concentrations 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 (FIA), and storage-carbohydrate content was measured using a total starch assay procedure (Megazyme, Bray, Ireland) following the amyloglucosidase/alpha-amylase method.

Decomposition experiment. Biomass loss was calculated as the difference between initial *vs.* remaining biomass. Given that the samples were difficult to separate from the sand, the remaining biomass was measured after burning the samples in a specifically designed oven at 575ºC for four hours. Thus, the remaining biomass after decomposition was calculated as the difference between the initial weight before burning and the final weight after ashes were removed with only inorganic material remaining.

Statistical analysis

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All analyses were carried out using R v3.2.3 (R Core team, 2016) with $\alpha = 0.05$ as the significance threshold. Because parental competition might generate differences in seed provisioning of the offspring that could mask other transgenerational effects on its performance and phenotype (Herman & Sultan, 2011; Dechaine *et al.*, 2015; Germain *et al.*, 2019), we included seed mass as a covariate in all analyses when its effect was close to significant ($P < 0.10$).

Offspring experiment 1. Demethylation in juvenile offspring. We tested the effect of the parental competition (RII computed from the parental experiment) on offspring germination (T_{50} and germination percentage) and growth rate (rosette diameter increase rate and leaf production rate, both measured every four days from the first 16 days until the 42nd day). We also examined the parental competition effect on offspring functional traits, considering individual traits and all traits combined. The latter was approached via a principal component analysis (PCA) combining the different traits, performed in order to reduce the multi-trait space to a single main axis, as in Kraft *et al.* (2014). Additionally, in order to test whether the parental effects on the offspring parameters depended on epigenetic modifications, we checked the effect of the demethylation treatment (demethylated vs. control) and its interaction with parental competition. This last comparison was not performed for the germination-related indices since the demethylation treatment had not been applied by that stage. We fitted a mixed-effects model where parental competition, demethylation treatment and the interaction were used as fixed factors, and seed mass included as a covariable. The experimental blocks were used as a random factor. Since, for some parameters, the interaction term was close to significant (i.e. below $P = 0.10$), suggesting there is some different effect of competition depending on demethylation, we decided to also split the data between demethylated and non-demethylated individuals to examine the potential different effect of parental competition within the demethylation treatments separately.

Offspring experiment 2 and decomposition experiment. The parental and offspring competition values were assigned based on the RII value measured in the parental competition experiment (see above, i.e. average of the treatment level RII values). In other words, we assigned a competition strength value to each of the competition levels (no matter whether they are from the parental or offspring generation) based on the RII measured in the parental generation. For example, an offspring plant coming from a parental plant that competed with *Leontodon* in the parental experiment would have a parental competition value equal to -0.59 (Table S1). If this offspring plant grows with the same competitor in offspring experiment 2, the (expected) offspring

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competition value would be the same (RII = -0.59; Table S1), since is the RII was measured in the parental experiment. However, if the offspring plant in question grows with a different competitor in offspring experiment 2 (e.g. competing with *Plantago media)*, the (expected) offspring competition value would be the RII value of the corresponding competition level measured in the parental experiment (e.g. RII of *Plantago media* = -0.24; Table S1).

The effect of parental and offspring competition on plant traits (single traits and also a PCA combination) was analysed using mixed-effects models with parental and offspring competition and their interaction (when close to significant; $p < 0.10$) as fixed factors, and taking into consideration seed mass as a covariable. The location of the individual in the greenhouse was used as a random factor to account for potential effects of spatial heterogeneity. Likewise, the effect of parental and offspring competition on leaf and litter decomposition was analysed using linear regression models with the same fixed factors and covariables as the mixed models, but without the random factor (since there were no blocks in the decomposition experiment).

Results

Parental generation

The analysis of the parental generation experiment showed that all 20 different competition levels used in the experiment were detrimental to the biomass of *T. brevicorniculatum* (i.e. negative RII; Table S1). Further, we observed within-generation phenotypic plasticity towards a more conservative phenotype (i.e. higher LDMC and RMF) that was linearly related to competition strength (Fig. S1).

Offspring experiment 1. Demethylation in juvenile offspring.

We found that juvenile offspring coming from parents experiencing more intense competition had faster germination (i.e. lower T_{50}; $F = 6.76$, $df = 208$, $P = 0.010$; Fig. 2a, Table S2a) but without differences in the overall germination percentage (z value = -0.008, $P = 0.994$; Table S2a), and faster growth (measured as leaf creation rate; $F = 8.35$, $df = 206$, $P = 0.004$; Table S2a).

The competition experienced by parents also affected the phenotypic characteristics of the offspring, and it did so rather differently in demethylated and non-demethylated populations

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(competition: demethylation interaction; F = 2.83, df = 206, P = 0.09; Table S2a) which meant the competition effect within each demethylation treatment required assessing. In the PCA based on the ensemble of traits measured, the first axis absorbed 46% of the variation and reflected the resource-use strategy gradient between individuals: from more positive PCA values reflecting plants with a conservative strategy (higher LDMC, RMF and root diameter) to more negative values for individuals with a more acquisitive strategy (higher SLA, SRL and percentage of fine roots; Fig. S2). When using the PCA scores, we observed that offspring acquired a more conservative phenotype with stronger parental competition, particularly when non demethylated (F = 4.05, df = 98.02, P = 0.047; Fig. 2c, Table S2b). However, when we removed the epigenetic signature of the individuals by application of a demethylation agent (Puy *et al.*, 2018), the effect of parental competition disappeared (phenotype: F = 0.19, df = 96.90, P = 0.663; Fig. 2c, Table S2b), suggesting that it was controlled epigenetically via DNA methylation.

On the other hand, the demethylation treatment did not modify plant growth (i.e. neither directly, nor by interacting with the parental competition; Table S2a). However, when assessing the competition effect within each demethylation treatment, we found that the offspring from parents experiencing more intense competition grew faster only when non-demethylated (F = 7.42, df = 98.09, P = 0.008; Fig. 2b, Table S2b). When offspring were demethylated we did not find that response (F = 1.90, df = 98.08, P = 0.172; Fig. 2b, Table S2b). This result also suggests that the parental competition probably induced differences in DNA methylation patterns.

Offspring experiment 2. Competition experiment with adult offspring.

Offspring functional traits were strongly affected by the offspring competitive environment and towards more resource-conservative phenotypes in response to stronger competition (Fig. 3, Table S3). Additionally, for some of the traits (SLA, RMF, storage-carbohydrate allocation, seed mass and seed production per unit of biomass, Fig. 3, Table S3) we found that transgenerational effects further reinforced the conservative phenotype when the offspring came from parents that had experienced strong competition. These transgenerational effects were either concordant with the plastic response to the offspring competition environment (e.g. lower SLA, high RMF; Fig. 3a, 3b, Table S3), or operated regardless of the offspring conditions (e.g. allocating more storage carbohydrates; Fig. 3d, Table S3). Offspring from parents that suffered no or little competition became smaller when growing with strong competition, whereas the offspring from parents under

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strong competition showed the opposite pattern, becoming taller when they had a competitive environment (Fig. 3c, 3f, Table S3).

Decomposition experiment

We showed that increasing levels of both offspring ($F = 24.03$, $P < 0.001$) and parental competition ($F = 8.32$, $P = 0.004$) resulted in reduced leaf decomposition rates (Fig. 4, Table S4), consistent with the shift in more conservative traits shown above. The effect of parental competition on decomposition was mediated by changes in the leaf traits that regulate these processes; decomposition rates were positively correlated with SLA and leaf P content, and negatively with LDMC and leaf C:N content ratio (Fig. S3). The litter decomposed following the same decomposition pattern as fresh leaves (Table S4, Fig. S4).

Discussion

To the best of our knowledge this study provides the first empirical evidence for the importance of parental competition affecting competition and functioning of the following generations via transgenerational plasticity. We found that stronger competition triggered within-generation phenotypic modifications towards a more competitive, resource-conservative phenotype. We found that the offspring from plants under stronger competition also had more resource-conservative phenotypes and faster development, even when they were not in a highly competitive environment. Further, we have shown that these transgenerational effects are most likely controlled by DNA-methylation mechanisms. Via a leaf decomposition experiment, we found that stronger parental competition results in less decomposable leaves, showing that the transgenerational effects could affect ecosystem processes.

Several studies have shown the importance of trait plasticity for the assembly and functioning of populations and communities (Price *et al.*, 2003; Rottstock *et al.*, 2017; Des Roches *et al.*, 2018; Puy *et al.*, 2020a). Although faster growth of cheaper tissues (i.e. susceptible to rapid tissue loss) could be expected in plants to counterbalance competition for light, intraspecific adjustments towards more conservative phenotypes have frequently been found in response to plant–plant competitive interactions (Kraft *et al.*, 2015; Carmona *et al.*, 2019). In our case, during the parental generation we found the same pattern of within-generation plasticity, where stronger

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competition triggered trait modifications towards a more conservative phenotype (i.e. higher LDMC and RMF; Fig. S1). This adjustment can increase a plant's ability to cope with stress, and it can lead to adaptation when the competitive hierarchy is dominated by more conservative-strategy phenotypes (as in Kraft *et al.*, 2015), which promote coexistence by reducing trait hierarchies and competition intensity (Carmona *et al.*, 2019). We then hypothesized that if these phenotypic changes were passed on to the offspring through transgenerational effects, this could in turn modify the competitive interactions in the next generation. This is the first work reporting that competitive interactions trigger transgenerational plasticity, which affects not only the early performance of the offspring, but also their adult life stage and ecosystem processes.

We found that juvenile offspring coming from parents experiencing more intense competition achieved greater competitive performance and advantage via benefits including faster germination and faster growth (Afonso *et al.*, 2014; Larson *et al.*, 2020). Further, the offspring from parents under intense competition displayed a more conservative resource-use phenotype (i.e. higher LDMC, RMF and root diameter), maintaining the same pattern as the parental generation (Fig. S1). Parental competition may affect offspring performance and phenotype through two main mechanisms: by generating differences in seed provisioning or quality stocked up by the maternal plants in the embryos, or by epigenetic variation mechanisms (Herman & Sultan, 2011; Dechaine *et al.*, 2015; Metz *et al.*, 2015; Germain *et al.*, 2019). In our study, stronger parental competition produced smaller seeds. However, the effects of parental competition remained significant even after including seed mass as a covariate. This suggests that embryo modifications were not the only mechanism driving our observed transgenerational effects and points to other mechanisms such as heritable epigenetic modifications or hormonal balance in embryos (Herman & Sultan, 2011; Rottstock *et al.*, 2017). Also, even though any parental effects are likely to fade away with time (Dechaine *et al.*, 2015; Puy *et al.*, 2020b), the effects associated with differences in seed mass seem to fade away faster (Latzel *et al.*, 2010). Meanwhile the effect of seed mass on growth rate lasted until the 24th day (i.e. 35-day-old plants) and the transgenerational effects persisted until the end of the experiment (Fig. S5). In our case, when we applied the demethylation agent that removed the epigenetic signature of the plants (Puy *et al.*, 2018, 2020a), the differences in performance and phenotype of the individuals disappeared. This strongly suggests that the observed adaptive transgenerational effects were controlled epigenetically, and at least partially enabled by DNA methylation. However, a detailed molecular study of the plant material would be needed to completely confirm the importance of the role of

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DNA methylation as a driver of the observed transgenerational effects and future experiments should take this into account.

We found that the transgenerational effects also persisted in the adult stage. At that stage, transgenerational effects further reinforced a conservative phenotype when the offspring came from parents experiencing strong competition (Fig 3a–b). We consider transgenerational plasticity to be adaptive because, although we did not observe better performance of the offspring that re-experienced the exact condition as their parents in terms of biomass (Fig. 3f), offspring grew taller when they were in the same competitive environment as their parents (Fig. 3c). Altogether, these results confirm broad phenotypic modification due to parental coexistence conditions that are maintained in the offspring generation. Although transgenerational plasticity was far from being negligible, it seemed to be less strong than within-generation plasticity. Therefore, although transgenerational plasticity acts like an adaptive "stress memory" that improves the ability of the offspring to cope with the predicted environment, within-generation plasticity could override it allowing progeny to respond more accurately to their own environmental cues (Auge *et al.*, 2017). At the same time, it should be noted that concordant within- and across-generation responses could act in synergy, driving progeny phenotypes to a distant optimum and, as long as the selective environment persists (i.e. so that the environment experienced by the progeny matches with that of the parents), this could accelerate adaptation to the environment (Herman *et al.*, 2014; Auge *et al.*, 2017). In this way, plant–plant biotic interactions are presumably predictable (i.e. parental environment is a good predictor of the offspring environment in space or time); thus, the adaptive value of the plasticity in response to these interactions is reinforced (Herman *et al.*, 2014; Burgess & Marshall, 2014; Metz *et al.*, 2015).

Finally, we found evidence that transgenerational effects are not only triggered by, but also shape the environment by affecting ecosystem processes, as expected from the response–effect framework (Lavorel & Garnier, 2002). Specifically, we showed a clear example whereby transgenerational effects can extend on a larger scale and affect the "afterlives" of the individuals by affecting leaf decomposition. Increasing levels of offspring and parental competition resulted in more conservative leaf traits (like LDMC and leaf C:N), which are related to more structural and more slowly degrading organic matter in leaves that takes longer to be returned to the soil (Cornelissen & Thompson, 1997). Interestingly, slower degradation might in turn favour those plants with a more resource-use-conservative phenotype, which have lower rates of nutrient

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uptake, subsequently affecting plant–plant competitive interactions (Van der Putten *et al.*, 2013; Semchenko *et al.*, 2017). This opens a new field of research on the potential positive plant–soil feedback triggered by plant–plant competition.

In a context where the importance of intraspecific variability for populations and communities is increasingly acknowledged, our study provides strong evidence of how heritable epigenetic phenotypic adjustments can have relevant and diverse ecological consequences for both coexistence and ecosystem functioning. For example, our study adds transgenerational plasticity as both a consequence and a driver of coexistence between species (Kraft *et al.*, 2015; Turcotte & Levine, 2016; Carmona *et al.*, 2019), and suggests possible implications of transgenerational plasticity on rapid adaptation and nutrient cycling (Van der Putten *et al.*, 2013; Semchenko *et al.*, 2017). Of course, since our study is only the first proof of concept of the relevance of the response–effect framework in the context of transgenerational plasticity, further investigation is certainly needed. In this sense, the ecological relevance and realism of our study might need expanding since we only used one plant genotype under experimentally controlled environments. More realistic studies are needed to understand the relevance of transgenerational plasticity in response to different biotic interactions. Such studies could involve, for example, examining to what degree there are heritable adjustments maintained across more generations, or exploring whether those adjustments are found both experimentally and in the field across many different species in natural populations.

Acknowledgements

We thank T. Galland and I. Hiiesalu for assistance, P. Macek and J. Mackova for performing the chemical analysis, and R.B. Davis for English revision. The study was financially supported by a Czech Science Foundation grant (GACR 20-00871S). C.P.C. was supported by the Estonian Research Council (project PSG293) and the European Union through the European Regional Development Fund (Centre of Excellence EcolChange).

Author contributions

J.P., F.d.B., C.P.C., V.L. and N.G.M. designed the research; J.P., N.G.M., C.P.C. and H.D. performed the experiments; J.P., F.d.B. and C.P.C. analysed the data; J.P. wrote the main

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manuscript. All authors contributed substantially to revisions and gave final approval for publication.

Data availability

The data that support the findings of this study is available on Figshare repository with the identifier https://doi.org/10.6084/m9.figshare.13116920 (Puy *et al*., 2020c).

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Supporting information

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607 **Table S1**: Summary of the competitive strength associated with each of the 20 different
608 competition levels measured during the parental experiment.

609 **Table S2**: Summary of the linear mixed-effect models for several plant parameters of offspring
610 experiment.

611 **Table S3**: Summary of the linear mixed-effect models for several plant traits of offspring
612 experiment 2.

613 **Table S4**: Summary of the linear models for decomposition in leaves and litter-senescence
614 material of the decomposition experiment.

615 **Fig. S1**: Effect of competition on trait plasticity of the parental generation.

616 **Fig. S2**: Principal component analysis (PCA) of morphological traits of the offspring generation
617 (offspring experiment 1).

618 **Fig. S3**: Correlation between pairs of traits measured in offspring experiment 2 and the
619 decomposition rate of the decomposition experiment.

620 **Fig. S4**: Effect of offspring and parental competition on the litter-senescence decomposability of
621 the offspring.

622 **Fig. S5**: Effect of parental competition on leaf production rate of the offspring experiment 1 at
623 different times.

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624 **Figures:**

625

626 **Figure 1**: Schematic representation of the experiments conducted. A) Parental generation
627 experiment (two-month greenhouse-pot experiment; n = 364) where genetically identical
628 individuals of *T. brevicorniculatum* were grown in competition with 20 different combinations of
629 neighbours that differ in their competitive ability; thus *T. brevicorniculatum* individuals
630 experienced a gradient of competition until flowering. Seeds were collected and two offspring
631 experiments were carried out with them. B) Offspring experiment 1 (one-month growth-chamber
632 pot-experiment; n = 220) where the progeny from different parental origins were all grown under
633 common conditions and the epigenetic status of half of them was altered via DNA-demethylation.
634 C) Offspring experiment 2 (two-month greenhouse-pot experiment; n = 432) where the progeny
635 from each of the parental competition were grown in all possible competitive conditions in a full
636 factorial design. D) Decomposition experiment: leaves and litter (n = 199 & 36) from the second
637 offspring experiment were incubated for a month to analyse their decomposability.

Figure 2: Effect of the competition experienced by the parents on different offspring parameters (Offspring experiment 1. Demethylation in juvenile offspring): a) germination, b) growth rate over 42 days for the control treatment (top row) and demethylated treatment (bottom row), and c) multi-trait variation for the control treatment (top row) and demethylated treatment (bottom row). The different colours of the points, from blue to red tones, represent the gradient of competition experienced by the parents from low to high. The significance values of the fixed factors included in each model are shown in the boxes. Significant relationships with parental competition are represented with solid lines, while non-significant ones are represented with dashed lines.

638

Figure 3: Effect of offspring and parental competition on different adult phenotype characteristics of the offspring (Offspring experiment 2. Competition experiment with adult offspring): a) specific leaf area, b) root mass factor, c) vegetative height, d) root storage-carbohydrate content, e) seed mass and f) total dry biomass. The different colours of the points, from blue to red tones, represent the gradient of competition experienced by the parents from low to high. The significance values of the fixed factors included in each model are shown in the boxes. When the

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effect of the parental competition was significant, the graphs include coloured lines representing trait variation of the offspring that experienced the strongest (red) and the lightest (blue) parental competition. If not, just the average line is represented in black.

639

Figure 4: Effect of offspring and parental competition on the leaf decomposability of the offspring (Decomposition experiment). The different colours of the points, from blue to red tones, represent the gradient of competition experienced by the parents from low to high. The significance values of the fixed factors included in the model are shown in the box. Since the effect of the parental competition was significant, the coloured lines represent the decomposition of offspring that experienced the strongest (red), and the lightest (blue) parental competition.

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A)

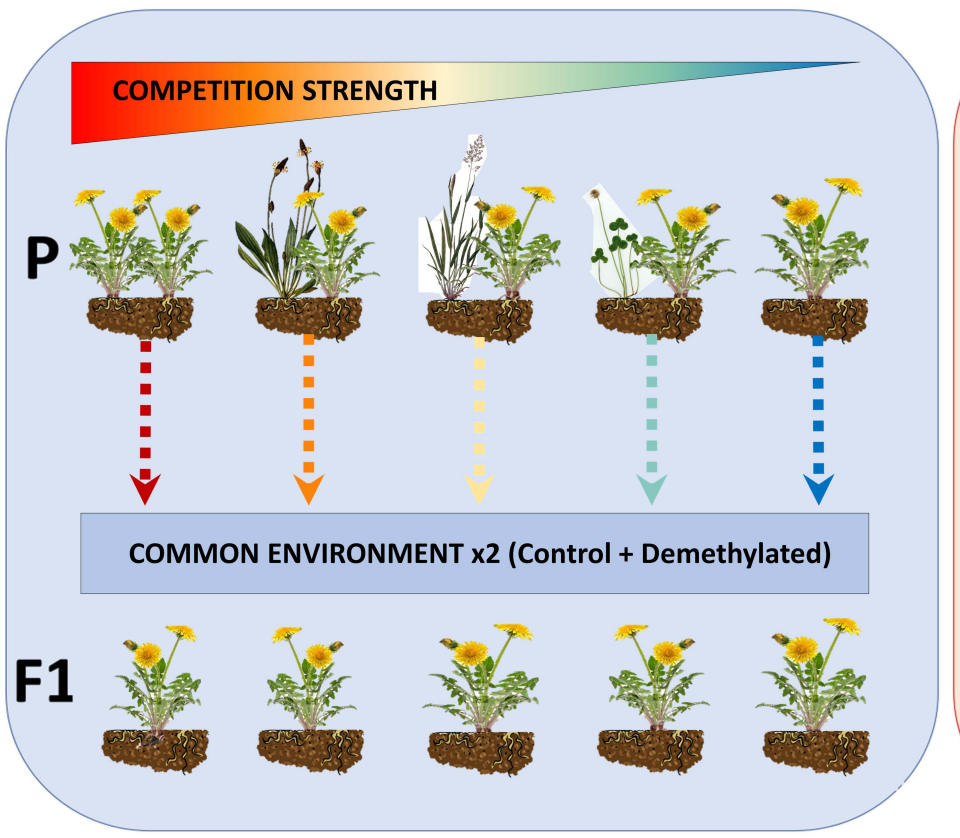
Parental generation

Taraxacum brevicorniculatum

- Apomictic species
- Genetically uniform start material

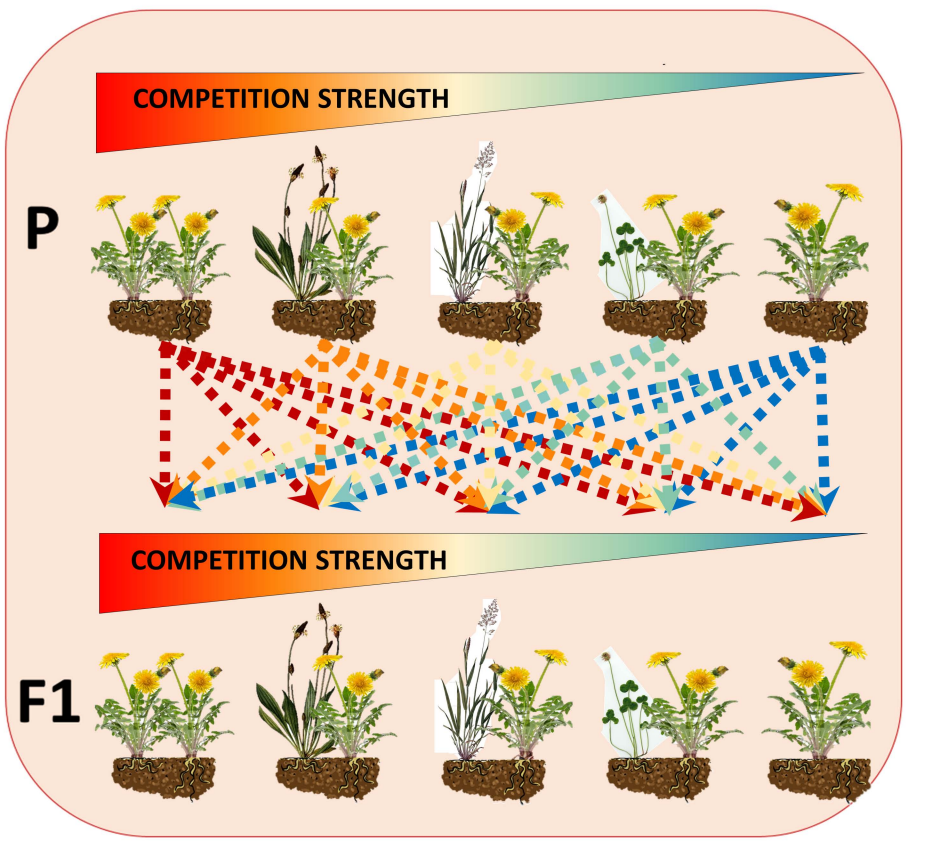
B)

Offspring Experiment 1



C)

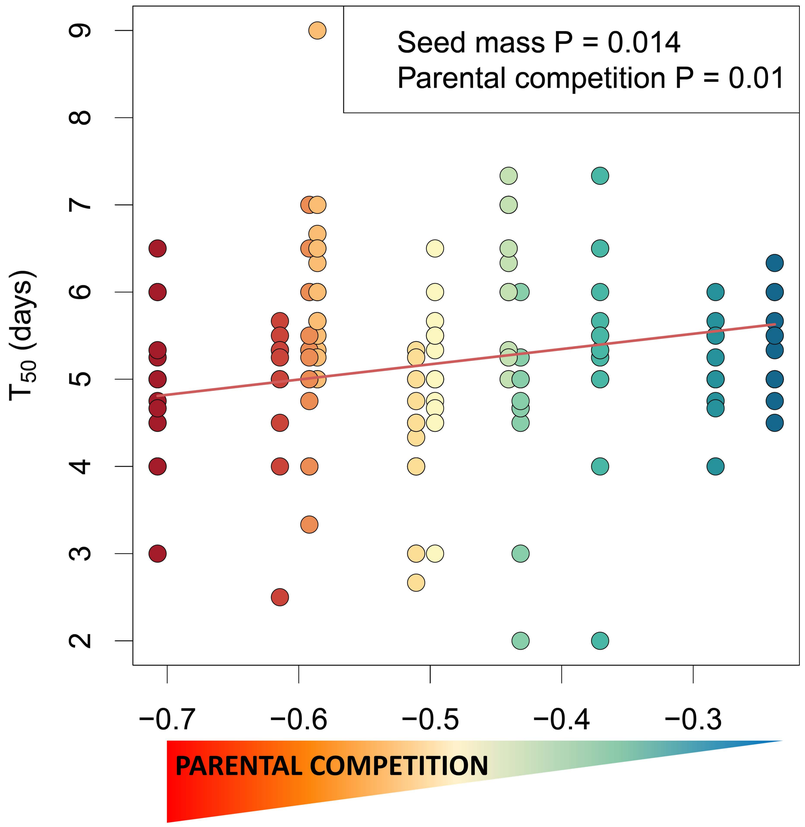
Offspring Experiment 2



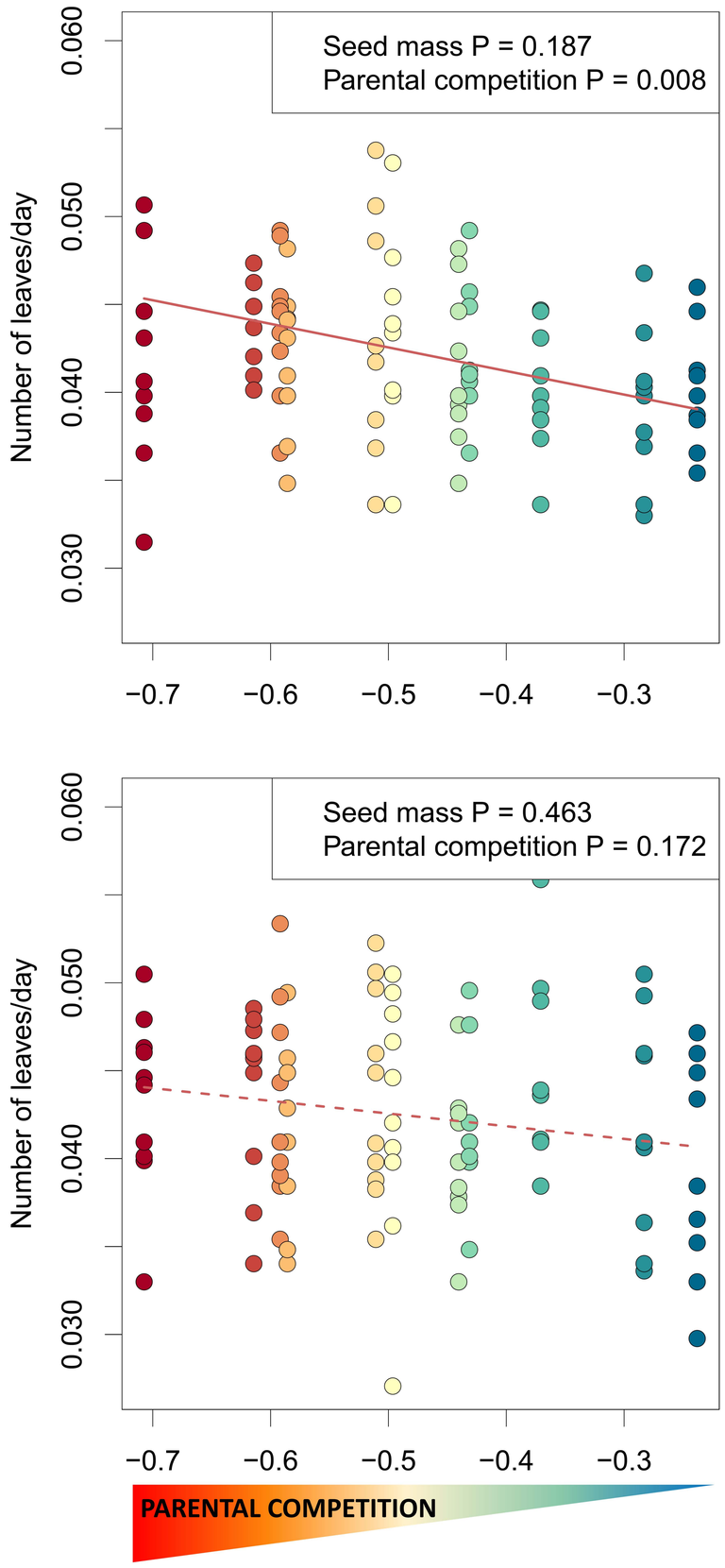
D)

Decomposition Experiment

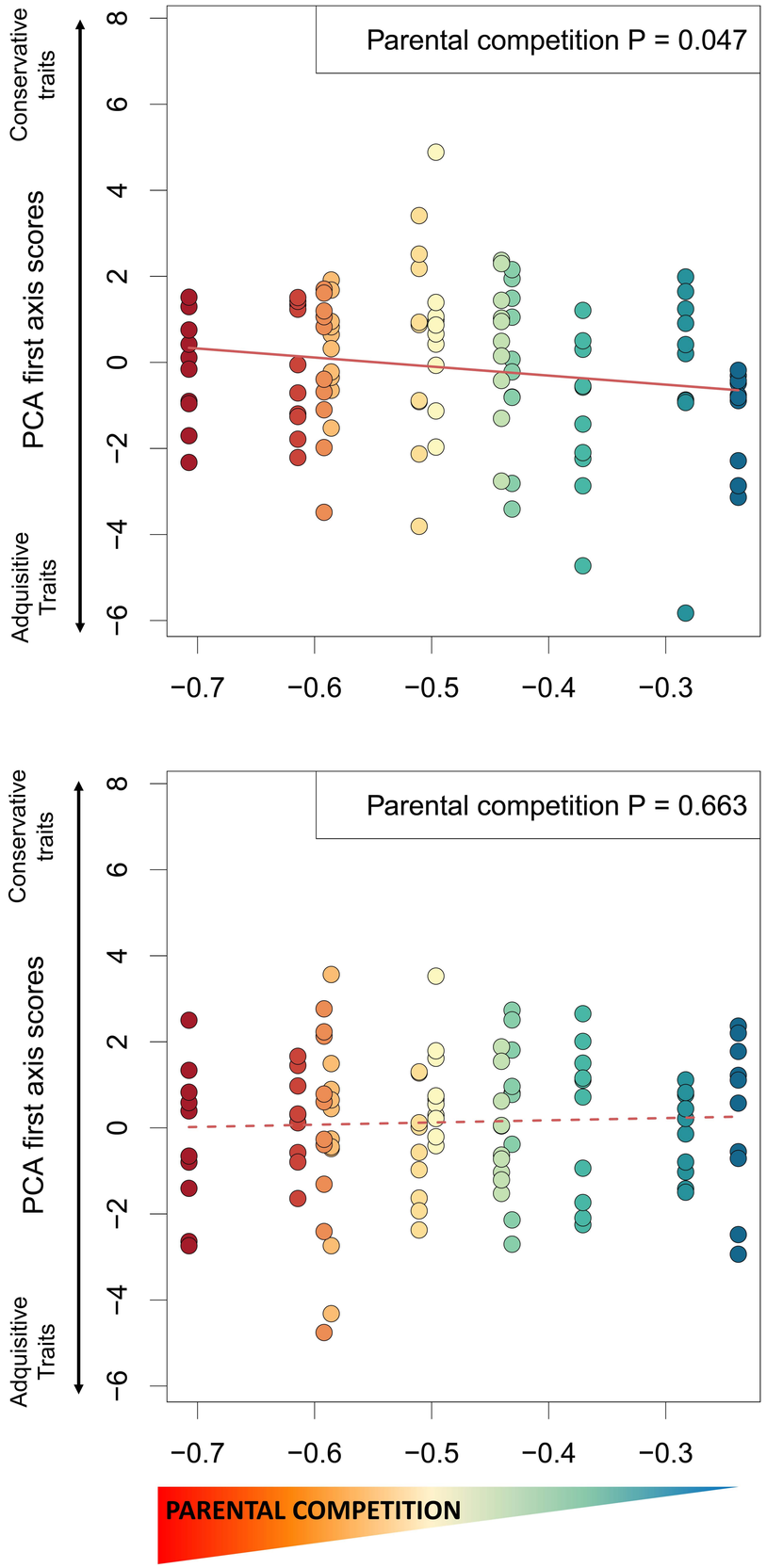
A) Germination



Growth rate



C) Phenotype



A)

Specific leaf area ($mm^2\ mg^{-1}$)

Offspring competiton: P < 0.001
Seed mass: P = 0.02
Parental competition: P = 0.012

− Parental competition

+ Parental competition

B)

Root mass factor ($g\ g^{-1}$)

Offspring competiton: P < 0.001
Seed mass: P = 0.016
Parental competition: P = 0.032

+ Parental competition

− Parental competition

C)

Height (cm)

Offspring competiton: P = 0.025
Seed mass: P = 0.244
Parental competition: P = 0.747
Par:Off competition: P = 0.026

+ Parental competition

− Parental competition

D)

Root storage carbohydrates ($mg\ g^{-1}$)

Offspring competiton: P < 0.001
Seed mass: P = 0.001
Parental competition: P = 0.012
Par:Off competition: P < 0.001

+ Parental competition

− Parental competition

E)

Seed mass (mg)

Offspring competiton: P = 0.001
Seed mass: P = 0.452
Parental competition: P = 0.008
Par:Off competition: P = 0.073

− Parental competition

+ Parental competition

F)

Total biomass (g)

Offspring competiton: P < 0.001
Seed mass: P = 0.449
Parental competition: P = 0.946

OFFSPRING COMPETITION

OFFSPRING COMPETITION

Decomposition