Diversity of parental environments increases phenotypic variation in *Arabidopsis* populations more than genetic diversity but similarly affects productivity

Javier Puy*[1,2], Carlos P. Carmona[3], Hana Dvořáková[1], Vít Latzel[2], Francesco de Bello[4]

[1]Department of Botany, Faculty of Sciences, University of South Bohemia, Na Zlaté Stoce 1, 370 05, České Budějovice, Czech Republic; [2]Institute of Botany, Czech Academy of Sciences, Zámek 1, 252 43 Průhonice, Czech Republic; [3]Institute of Ecology and Earth Sciences, Department of Botany, University of Tartu, Lai Street 40, 51005, Tartu, Estonia; [4]CIDE-CSIC, C. Naquera Km 4.5, Montcada, Valencia, 46113, Spain

**For correspondence. E-mail puy.javi@gmail.com*

- **Background and Aims.** The observed positive diversity effect on ecosystem functioning has been scarcely assessed in terms of intraspecific trait variability within populations. Intraspecific phenotypic variability could stem both from underlying genetic diversity and plasticity in response to environmental cues. The latter might derive from modifications on plant's epigenome and potentially last multiple generations in response to previous environmental conditions. We disentangled experimentally the role of genetic diversity and diversity of parental environments on population productivity, resistance against environmental fluctuations and intraspecific phenotypic variation.

- **Methods.** A greenhouse-experiment was conducted where different types of populations of *Arabidopsis thaliana* were established: one population type with differing levels of genetic diversity and another type, genetically identical, but with varying diversity levels of parental environments (parents grown in same or different environments). The latter population type was further combined, or not, with experimental demethylation to reduce the potential epigenetic diversity produced by the diversity of parental environments. Further, all populations were each grown under different environmental conditions (control, fertilization and waterlogging). Mortality, productivity and trait variability were measured in each population.

- **Key Results.** Parental environments triggered phenotypic modifications on the offspring, which translated into more functionally diverse populations when offspring from parents grown under different conditions were brought together in mixtures. In general, the increase of neither genetic diversity nor diversity of parental environments had a remarkable effect on productivity or resistance against environmental

fluctuations. However, when the epigenetic variation was reduced via demethylation, mixtures were less productive than monocultures (i.e. negative net diversity effect), caused by the reduction of phenotypic differences between different parental origins.

- **Conclusions.** Diversity of environmental parental origins within a population could ameliorate the negative effect of competition between coexisting individuals by increasing intraspecific phenotypic variation. Diversity of parental environments could thus have comparable effects to genetic diversity. Disentangling the effect of genetic and diversity of parental environments appears as an important step in understanding the effect of intraspecific trait variability on coexistence and ecosystem functioning.

Key words: Arabidopsis thaliana, competition, DNA methylation, epigenetic diversity, functional traits, genetic diversity, intraspecific phenotypic variability, parental effects, productivity, transgenerational effects.

INTRODUCTION

Positive relationships between biodiversity and ecosystem functioning have been demonstrated repeatedly in many observational and experimental studies. This body of research has found that higher diversity is generally associated with higher productivity, stability and resistance to disturbances/stresses (Balvanera *et al.* 2006; Marquard *et al.* 2009). Most of the research has commonly measured biodiversity at the community level as interspecific diversity, in terms of both taxonomic or functional trait diversity (Marquard *et al.* 2009; Hector *et al.* 2010). In contrast, the effect of intraspecific diversity within populations has been generally overlooked. However, intraspecific diversity effects on population and ecosystem functioning can be of comparable magnitude to those of interspecific diversity (Crutsinger *et al.* 2006; Hughes *et al.* 2008; Liancourt *et al.* 2015), and phenotypic variation within species is sometimes as large as that observed among species (Hughes *et al.* 2008; Messier *et al.* 2010; Albert 2015; Siefert *et al.* 2015).

Existing evidence suggests that intraspecific diversity has a positive effect on productivity and resistance to disturbances/stresses of populations (Bolnick *et al.* 2011; Latzel *et al.* 2013; Zuppinger-Dingley *et al.* 2014). These effects have been generally interpreted as the result of contrasting phenotype and diversity in functional traits between coexisting individuals, which should lead to decreased competition and enhanced complementarity in resource use (altough this hypothesis has been mostly tested in studies comparing different species, e.g. Reich *et al.* 2001; Fridley 2001, 2003; Díaz *et al.* 2007; Zuppinger-Dingley *et al.* 2014; Gross *et al.* 2017). Moreover, intraspecific trait variation has been most often attributed to genetic variation. As a result, experiments have mostly manipulated the genetic diversity of the populations by modifying the number of genotypes of the populations (Zhu *et al.* 2000; Booth and Grime 2003; Reusch *et al.* 2005; Crutsinger *et al.* 2006; Hughes *et al.* 2008; Kotowska *et al.* 2010; Cook-Patton *et al.* 2016). However,

intraspecific trait differences, and thus functional trait diversity, can also be generated by phenotypic plasticity in response to environmental cues. Furthermore this effect might not occur only within the life cycle of an organism but also across generations through transgenerational plasticity (Turcotte and Levine 2016).

Phenotypic plasticity is generally defined as the variation in trait expression observed in a genotype that is influenced by the environment (Sultan and Stearns 2005; Bradshaw 2006), that allows the organism to respond to environmental fluctuations. Such environmental variation could promote phenotypic changes on the organism undergoing those conditions (referred as within-generation plasticity) and even be heritable, meaning that the environmental conditions experienced by the parents modify progenies' phenotype (also referred as parental or transgenerational effects). These "non-genetic" mechanisms of phenotypic plasticity (within or across generations) may result from underlying epigenetic modifications (Richards *et al.* 2010; Herman and Sultan 2011). Epigenetic modifications are known to occur in response to environmental factors (Herman and Sultan 2016; Richards *et al.* 2017), to cause trait variation (Cubas *et al.* 1999; Latzel *et al.* 2012; Zhang *et al.* 2013, 2018) and sometimes to be transmitted to following generations (Akimoto *et al.* 2007; Bossdorf *et al.* 2008; Verhoeven *et al.* 2010) being a key mechanisms of transgenerational effects (Herrera *et al.* 2012; Herman *et al.* 2014). Epigenetic variation could be enabled by various mechanisms that modify the expression of the DNA without modifying its underlying sequence (e.g. histone modification, RNA interference and DNA methylation). Among them, although probably is not the most significant one, DNA methylation is the most studied and best understood mechanisms in ecology and evolution (Akimoto *et al.* 2007; Bossdorf *et al.* 2010).

The seminal work by Latzel *et al.* (2013) showed that epigenetic diversity can increase the productivity and stability of plant populations. Nevertheless, Latzel *et al.*

manipulated the epigenetic diversity by creating populations of differing number of epigenetic recombinant inbred lines (epiRILs) of *Arabidopsis thaliana* L. with highly variable DNA methylation, originated by artificial crossings of Columbia wild type and mutants with decreased genome-wide DNA methylation. This approach, while useful, cannot be fully and realistically compared to the epigenetic variation found in natural populations, e.g. induced by differences in parental environments. A way forward is to create experimental mixtures of organisms whose parents experienced different environmental conditions. This approach can be further combined with new techniques of experimental demethylation of the populations that, by reducing the epigenetic variation of the individuals, could level-off the parental effects of different environmental imprints within a population (Puy *et al.* 2018). Comparing non-demethylated vs. demethylated populations, would allow disentangling the relative effect of the epigenetic variation from the overall parental effect and testing the effect of environmentally induced epigenetic diversity on phenotypic variability and the possible consequences on ecosystem functions.

The positive effect of diversity on ecosystem functions, such productivity (also called positive "net diversity effect") can be easily quantified by comparing how mixtures perform better than monocultures. Specifically, existing approaches (e.g. Loreau and Hector 2001) compare performance of individuals in a mixture to their 'expected' performances when growing in monocultures. For example, in a mixture of different genotypes, each genotype is compared to the biomass observed when grown alone (monoculture). Any positive net diversity effect (observed biomass in mixture higher than expected from monocultures) could be driven by two not mutually exclusive mechanisms: "selection" and "complementarity" (Loreau and Hector 2001; Marquard *et al.* 2009; Tobner *et al.* 2016). Selection operates when a specific competitively superior individual is dominant in mixtures and drives disproportionately the functioning of the community (Loreau and Hector 2001). By contrast,

complementarity takes place when niche differences between coexisting individuals result in more efficient use of resources by the community and better functioning (Loreau and Hector 2001). Because fitness and niche differences can be related to underlying plant functional traits, both mechanisms can be also approximated from a trait-based perspective (Cadotte 2017). When selection is the main mechanism, we should expect to observe a dominance of particular traits and/or trait values associated with the competitive/fitness advantage (e.g. tall stature). On the other hand, diversity in traits values (functional diversity) related to resource foraging between individuals of the community would reflect that complementarity is the main mechanism driving the positive effect of diversity (Loreau and Hector 2001; Cadotte 2017). While an increase in functional diversity should enhance complementarity effects phenotypic plasticity could either decrease or increase selection effects (Roscher et al. 2015).

Here, we summarize the results of a two-generation experiment on *A. thaliana* to test the role of genetic diversity and diversity of parental environments (i.e. populations consisting of individuals from parents that experienced diverse environments), on 1) affecting plant assemblage and productivity and resistance against environmental fluctuations of plant populations, and 2) creating intraspecific phenotypic variation. By experimental alteration of DNA methylation status of a subset of the populations we also aim to determine whether the effect diversity of parental environments could be based on epigenetic diversity. Further, we control the role of within-generational plasticity on modulating those effects. We hypothesize that, compared to the effect of genetic diversity, the effect of environmental diversity on population functioning will be weaker but still important; and that within-generational plasticity can partially compensate for both trans-generational and genetic diversity effects. Also, we test, for the first time, the relative effect of the two potential biodiversity mechanisms, complementarity and selection effects (Loreau and Hector 2001; Marquard *et al.* 2009; Tobner *et al.* 2016), as potentially caused by either genetic or environmental

diversity. Finally, we test whether traits (average and diversity of trait values in the populations) explain these diversity effects.

MATERIALS AND METHODS

Plant material

Four different genotypes of *A. thaliana* were selected and provided by the Nottingham Arabidopsis Stock Centre (NASC): Col-1, Gue-0, Mer-6 and Vav-0. Because of the natural predominance of this species for self-fertilization, the genotypes are genetically adapted to specific environments and show unique ecologies (Alonso-Blanco and Koornneef 2000). The Col-1 genotype was chosen because of its widespread use in genetic studies. The other three genotypes were selected from populations of the Iberian peninsula with differing moisture and fertility preferences due to their selection history and phenotypic variation (Picó *et al.* 2008; Méndez-Vigo *et al.* 2013): Gue-0 from north and oceanic influenced part of the peninsula, and Mer-6 and Vav-0 from southern and more continental part (NASC). Mer-6 and Vav-0 habitats differed in their fertilization influence: while the first population was located on a sandy area, the second was on a farm (NASC).

To ensure sufficient seed stock of the genotypes and evening out possible unknown transgenerational effects of previous cultivation in NASC, we first grew all the genotypes in controlled conditions for one prior generation in populations of conspecifics of the same genotype (to maintain homogeneous genetic variation). Each population (one population per genotype) was established by transplanting 30 seedlings of the same genotype in 9 cm square plant pots with a volume of 0,5 l filled with a 2:1 mixture of sand and commercial soil, creating dense populations (similar to Latzel *et al.*, 2013) with realistic population structure, allowing interactions between individuals. The seedlings were previously stratified for one-week at 4ºC and germinated in sterilized potting mix. The 30 individuals within a pot were

placed in a regular distribution, covering the whole surface with 6 columns x 5 rows of plants with equal space between individuals.

First generation: Environmental imprinting

We ran the first generation where, following the methodology described above, we stablished three population per genotype from the Iberian Peninsula (Gue-0, Mer-6 and Vav-0) and we grew all of them in identical control conditions to avoid any epigenetic variation in the offspring material. At the same time, nine populations of the Col-1 genotype were also created and grown under three different conditions (control, similar to Gue-0, Mer-6 and Vav-0, plus fertilization and waterlogging, with 3 populations each) to trigger transgenerational effects in the offspring and potentially generate offspring material with heritable epigenetic variation. The control conditions consisted on watering by sub-irrigation only when needed (i.e. once/twice per week). The fertilization treatment comprised the same watering regime as the control treatment but with an addition of fertilizer (KRISTALON; NPK 15-5-30+3Mg+5S) at the concentration of 300ppm in each watering day. The waterlogging treatment consisted in constantly watered plants by sub-irrigation, ensuring constant wet surface of the soil. All the treatments started 7 days after the transplanting to ensure the good establishment of the populations and lasted until all the plants produced seeds. At the end of the parental generation, seeds of each population were collected.

Second generation: Diversity experiment

The seeds collected from the first generation were stratified for one-week at 4º C, and then germinated in sterilized potting mix. With this offspring generation, we run a one-month diversity experiment where we stablished two types of populations of *A. thaliana* by, as in the previous generations, transplanting 30 seedlings in each pot. The populations could include either seedlings form the same or different genotype (genetic diversity), or from the same or

different parental environmental conditions (diversity of parental environments). Transplanting allowed us to spatially arrange the populations maximizing interactions between dissimilar individuals in mixtures (Supplementary data Fig. S1) and to know their "origin" (i.e. referring to either genotypes in genetic diverse mixtures, or parental environments in the mixtures of diverse parental environments).

The first type of populations included varying levels of *genetic diversity* achieved by sowing individuals from one genotype (monocultures), and mixtures of the three genotypes (Mer-6, Gue-0, Var-0). Monocultures were replicated five times, and the mixtures replicated 15 times.

The second type of populations included varying levels of *diversity of parental environments* achieved by sowing individuals from single genotype (Col-1), but either from one parental origin (i.e. experienced one specific environmental condition; monocultures) or mixing individuals coming from the three parental environments (control, fertilization and waterlogging). In this case, monocultures were replicated seven times, and the mixtures replicated 21 times. Besides triggering epigenetic heritable modifications, the parental environmental inductions could trigger transgenerational effects through other mechanisms like generating differences in seed quality and hormonal balance (Herman and Sultan 2011). We tested whether diversity of parental environments was indeed based on *epigenetic diversity* by modifying the epigenetic variation of the plants and suppressing their epigenetic-parental status (Puy *et al.* 2018). Thus, another set of populations with the same amount of pots (3 monocultures replicated 7 times and mixtures replicated 21 times) were demethylated via daily application of a 5-azacitidine onto the leaves (following the methodology of Puy *et al.* 2018). With this approach we intended to create population types with reduced epigenetic diversity compared to populations without demethylation.

All the combinations mentioned above were replicated 3 times, to grow the populations under three different environmental conditions (control, waterlogging and fertilization; described in the previous section). Thus, the final set-up finally comprised (5+7+7) x 3 monocultures and 15+21+21 mixtures x 3 environmental conditions = 342 experimental populations and 10,260 individuals of *A. thaliana*. Pot size, substrate, population density and the environmental conditions were the same as in the previous generations to ensure the most similar conditions.

Measured traits

At the time of harvest, 30 days after the establishment of the second generation, we measured survival and total dry biomass (radicular and aerial) of the 18 individuals closest to the edges in each pot, and survival and individual total dry biomass for each of the 12 central plants, as a measure of fitness. Additionally, in order to characterize niche differences between individuals with a manageable effort, we measured one key functional trait: specific leaf area (SLA; leaf area per dry mass, mm^2/mg). SLA is a trait that relate to various functions, including resource foraging strategy of the plant and competitive ability since it correlates with potential relative growth and photosynthetic rate (Roscher *et al.* 2012; Kraft *et al.* 2014, 2015; Carmona *et al.* 2019). We estimated the SLA by scanning the area of one to three leaves per plant and weighing the dry mass (after drying at 60º C for 48h). In mixtures we sampled the 12 central individuals, thus sampling four individuals of each of the three different origins (i.e. three genotypes in genetic diverse mixtures, or three parental environments in the mixtures of diverse parental environments). To keep consistent sampling effort, we sampled 4 individuals within monocultures. Sampling was done by choosing individuals situated in fixed positions to avoid subjective election from the researcher.

We calculated the average value and coefficient of variation (CV) of the traits (biomass and SLA) per population (i.e. of the whole pot/population), considering CV as a measure of functional trait diversity within a population (Albert *et al.* 2010). Within mixtures, we also estimated the average mean and CV separately per origin (e.g. a mean and CV per genotype).

With the plant biomass data we also calculated the net diversity effect on productivity and its additive components, selection and complementarity effects, following the well-known additive partitioning formula by Loreau & Hector (2001). The net diversity effect for a given mixture is the difference between the observed productivity (i.e. total population's dry biomass) and the expected productivity, which is the productivity of the different origins that comprise the population in the corresponding monoculture. In our case, for example, the net diversity effect of a mixture with three genotypes grown in fertilized conditions is given by the difference between the observed productivity in that mixture (sum of the biomass of the three genotypes in the fertilized mixture, i.e. whole pot/population biomass) and the expected productivity from the same type of monoculture (the average productivity of the three genotypes in their fertilized monoculture). Due to knowing the position of the different "origins" within mixtures, and the dedicated effort of measuring productivity of each individual separately, it was possible to apply the formula Loreau & Hector (2001) that decomposed the net diversity effect into the effects due selection and complementarity (for further information and formulas please see Loreau & Hector 2001).

Statistical analysis

Phenotypic variation across origins First, focusing only on the monocultures we assessed plasticity in population response to environmental conditions experienced in the second generation (control, waterlogging, fertilization), visualized by the reaction norms (Supplementary data Fig. S2). We specifically tested for differences in the reaction norms across different origins (i.e. three genotypes: Gue-0, Mer-6, Vav-0 and three parental environments for Col-1, i.e. from parents that experienced one of the three treatments in the first generation: waterlogging, fertilization and control). The test was done for variability in mortality, total biomass and average and CV of SLA by using linear regression models. In the model we tested the effect of environmental conditions experienced in the second generation (control, waterlogging, fertilization) and the origins of the population (genotype and parental environments), plus their interaction. Whenever we found a significant effect of the origin, a post-hoc Tuckey test was performed to see which ones differed significantly.

Diversity vs. monocultures We tested the effects of environmental conditions (control, waterlogging, fertilization), diversity (monocultures vs mixtures), and their interaction, on *Arabidopsis* populations' total mortality, total biomass and average and variation in SLA values, using linear regression models. To do so, we analysed the populations with varying levels of *genetic diversity* (Table 1b) separately from the populations with varying levels of *diversity of parental environments* (Table 1a), given the lack of overlapping genotypes. Additionally, for the latter test, in order to test whether the diversity of environmental exposures in the parental generation was inducing epigenetic diversity, we included in the models the effect of the demethylation treatment (non-demethylated vs demethylated) and all possible interactions with environmental conditions and diversity level in parental environments. When interactions were significant, the effect of diversity was tested within the other treatments.

Biodiversity effects Within mixtures, the response of the biodiversity effects (net diversity effects, selection and complementary) to the environmental conditions in the second generation were tested with separate linear models for populations with varying levels of genetic diversity (Table 2b), and for varying levels of diversity of parental environments (Table 2a), as for the test above. For the test using parental environments, the demethylation treatment (non-demethylated vs demethylated) and the interaction with the environment experienced in the second generations were included as fixed factors (Table 2a). When the interaction was significant, the effect of the source of diversity was tested within different environments.

Traits and biodiversity effects To see whether biodiversity effects (net diversity effect and its components: selection and complementarity effect) were related to population's traits parameters in mixtures we used linear regression models. These models included average and CV of SLA and the source of diversity (genetic, environmental and demethylated populations), the environmental conditions in the second generation and their interactions as explanatory variables (Supplementary data Table S1a). When the interactions were significant, the effect of the trait on biodiversity effects was tested within the different treatments separately (Table 3).

All analyses were carried out using R v3.2.3 (R Core Team 2016) with α=0.05 as significance level.

RESULTS

Genotypic variation and phenotypic plasticity

We found that the four genotypes used in the experiments, when grown in monocultures, had similar phenotypes. The only exception to this was found for the Col-1 genotype, which had higher SLA and slightly higher mortality than the genotypes of the Iberian peninsula (Gue-0, Mer-6 and Vav-0; Supplementary data Fig. S2). The differences were even more pronounced when the parents of the Col-1 genotype were grown in waterlogging conditions (Supplementary data Fig. S2). Also, Gue-0 was more productive than the other genotypes (Supplementary data Fig. S2).

Importantly, the four genotypes had similar response to the environmental conditions (control, fertilization, and waterlogging; Supplementary data Fig. S2). The reaction norms (i.e. patterns of phenotypic variability in the second generation) of the different genotypes were generally similar, so they showed a similar plasticity pattern. Specifically, the waterlogging treatment caused a general decrease of the productivity, a decrease in the average values of SLA, and an increase of trait variability (i.e. CV of SLA) in all genotypes of *Arabidopsis* populations considered and irrespective of the parental environments for Col-1 genotype (Table 1a, b; Supplementary data Fig. S2).

Diversity vs. monocultures.

We found no remarkable effects of diversity on *Arabidopsis* populations' mortality (i.e. mixtures were similar to monocultures) nor any effect of different environmental conditions experienced in the second generation (Table 1a, 1b; Supplementary data Fig. S3). However, the demethylated populations showed lower mortality in comparation with the non-demethylated (Table 1a; Supplementary data Fig. S3).

Similarly, we detected no overall significant effect of diversity on productivity neither due to genetic diversity nor diversity of parental environments (Table 1a, 1b; Fig. 1). Total biomass was similar between mixtures and monocultures. However, mixtures of demethylated populations had lower biomass than the demethylated monocultures (Fig. 1). Under fertilization, demethylated mixtures produced significantly lower biomass compared with monocultures (Fig. 1). Also, we detected overall higher biomass of demethylated populations compared to the non-demethylated (Table 1a; Fig. 1).

Genetic diversity and diversity of parental environments increased phenotypic variability (i.e. mixtures had higher CV of SLA than monocultures; Table 1a, 1b), although only the latter did so significantly (i.e. mixtures with diverse parental environments; Fig. 2). In contrast, in the demethylated populations this effect disappeared, and phenotypic variability decreased in mixtures (Fig. 2). The higher CV of SLA of mixtures was explained by the differences between origins of the mixtures and not because the different origins increased their CV of SLA from monocultures to mixtures (Supplementary data Fig. S4). This result suggests that the phenotypic variability of the mixtures was explained by heritable epigenetic effects with virtually no within-generation plasticity involved.

In general, we found no differences in SLA values between monocultures and mixtures with the exception of the genetically diverse mixtures in the fertilization treatment with significant lower SLA than the respective monoculture. Also, demethylated populations had in general higher SLA than the populations with manipulated parental diversity (Fig. 3). As we found with the CV of SLA, the average SLA of the different origins were not changing between monoculture and mixture (Supplementary data Fig. S5), suggesting no within-generation phenotypic plasticity triggered by the population structure.

Biodiversity effects

In accordance with the previous results on populations' biomass, we found that net diversity effect and its components (selection and complementarity on genetic and parental diverse populations) did not significantly vary across environmental conditions in the second generation, nor they are different from zero (Table 2a, 2b; Fig. 4). However, we found differences in biodiversity effects between the demethylated and non-demethylated mixtures, although they were specific for each environmental condition (Table 2a). Specifically we found a negative net effect in the demethylated population under fertilization (Table 2a; Fig. 4a), which was mostly explained by a negative complementarity effect (Fig. 4c), meaning that the three different origins that comprise the population (i.e. coming from the three different parental environments) were similarly less productive in mixtures than expected from monocultures (Supplementary data Fig. S6). Although the effect of selection compared with complementarity was relatively small (± 3 mg vs. ± 50 mg; Fig. 4b and 4c), we found more positive selection effect of the non-demethylated mixtures compared with the demethylated ones in the fertilization and the waterlogging treatment, although only in the latter was significant (Table 2a, Fig 2b). This difference did not contribute generating any differences in the overall net effect.

Functional traits and biodiversity effects

When we use traits to explain the net diversity effect and its two components, we found a general significant effect of average SLA on increasing net effect and complementarity, plus an effect of CVSLA dependent of the environmental conditions and source of diversity (i.e. interaction E x S x CVSLA; Supplementary data Table S1a). In order to characterize these effects correctly, we explore the relationship within the populations under the same environmental condition (i.e. waterlogging, fertilization and control in the

second generation). We found that traits could explain the diversity effects only in waterlogging and control treatments (Supplementary data Table S1b). In mixtures undergoing waterlogging, a positive net effect (i.e. more productive mixtures beyond the predicted by the monocultures) was characterized by populations with low CVSLA and high values of SLA (Supplementary data Table S1b). In control conditions the effect of CVSLA and SLA was dependent of the source of diversity (i.e. interaction S x CVSLA/SLA; Supplementary data Table S1b), so we further segmented the treatments. By doing that, in control conditions, we found a positive effect of SLA in the selection effect of mixtures with diverse parental environments, and a positive effect of CVSLA on net effect and complementarity in demethylated mixtures (Table 3).

DISCUSSION

This study expands the knowledge on the role of genetic diversity and diversity of parental environments on intraspecific trait variation as a potential mechanism of coexistence and functioning of communities. Specifically, this is the first empirical test of the effect of both genetic diversity and diversity of parental environments on productivity and resistance to environmental fluctuations of populations. In general, we found no direct effect of diversity on productivity and resistance to environmental fluctuations in neither genetic diverse populations nor populations which experienced different parental environments. However, in demethylated populations we found a negative effect of diversity on productivity. This result has two important implications. First, it suggests that the environmentally induced transgenerational effects were controlled epigenetically via DNA cytosine methylation. Second, that when the epigenetic diversity is removed, the competition within the population can increase because of decreased phenotypic variability. Thus, populations composed by individuals from different parental environments seems to ameliorate the negative effects of intraspecific competition on the productivity of *Arabidopsis* populations, and to have similar

effects on productivity as genetic diversity. The higher intraspecific trait variation found in the epigenetic diverse mixtures compared to the demethylated ones further suggests that the reduction of competition could be partially caused by an increase of niche differences between parental origins.

We show that the waterlogging treatment was the most stressful condition for the populations of *Arabidopsis*. Although it did not cause higher mortality, it decreased biomass production by more than 50% compared to control conditions. However, we did not find that diversity (neither genetic nor environmental) increased population resistance against environmental fluctuations. Probably, the strong decrease in the size of the individuals in the waterlogging treatment have decreased the direct interaction between individuals, thus, decreasing potential biotic effects in species coexistence. At the same time, especially stronger diversity effects in populations undergoing the fertilization treatment compared to the other environmental conditions could be caused by the greater size of the individuals (Fig 1 and 2), which likely increased the intensity of the interactions. This stronger diversity effects when competition and interactions are stronger, for example under fertilized conditions, is actually expected by theoretical studies and it has been repeatedly shown in several diversity experiments (Reich *et al.* 2001; Fridley 2003). The relatively low selection effect founded in our results could be promoted by the low mortality, thus competition exclusion, occurred in the experiment.

We show that the three environmental conditions (control, fertilization and waterlogging) imprinted on the Col-1 genotype in the first generation triggered phenotypic differences between their offspring (i.e. transgenerational effects; Supplementary data Fig. S1). These effects, when offspring from different parental environments were brought together in mixtures, translated into more functional diverse populations (i.e. higher CVSLA). Moreover, the higher CV of SLA of the mixtures was due to differences between

parental origins and not to an increase of the variation within origins from monocultures to mixtures (Supplementary data Fig. S4). These results suggest that transgenerational, rather than within-generational plasticity, was driving the intraspecific trait variation of the mixtures, possibly promoting niche and resource partitioning and potentially increasing complementarity. To the best of our knowledge this is the first experiment clearly demonstrating an increase in intraspecific variability of populations composed by individuals from parents grown in different environments, with clear implications for coexistence and ecosystem functioning.

Interestingly, when we applied the demethylation treatment that removed (at least partially) the parental-epigenetic signature of the individuals, the functional diversity of the mixtures disappeared. The lack of phenotypic variation of the demethylated mixtures strongly suggests that the diversity of parental environments was based on epigenetic variation and partially controlled via DNA cytosine methylation. However, it is also possible that DNA damage from the use of the demethylation agent could have happened (as found in Liu *et al.* 2015). However, our findings in which we observed overall biomass increase and lower mortality of demethylated populations suggest that this does not seem to be the case. We suggest that detailed molecular study of the plant material can complement this type of experiments in the future.

By increasing divergence in traits between individuals, we expected reduced competition between such individuals, enhancing niche segregation and complementarity on the environmentally/epigenetic diverse populations, and thus promoting a positive diversity net effect on productivity or resistance to environmental fluctuations (Fridley 2001, 2003). However, we found neither positive nor negative diversity effects, although with similar effect than genetic diversity. On the contrary, in demethylated mixtures, where we removed the epigenetic diversity and its relative functional diversity, we found a negative diversity

effect, due to a negative complementarity effect, only statistically significant under fertilization. Demethylation, by evening out the epigenetic trait differences, could have reduced niche differences between origins and increased the competition intensity between origins. In that case, we would have expected the biomass produced in demethylated mixtures to be similar to the demethylated monocultures. However, we found higher average biomass of the different origins in monocultures than in mixtures, meaning that the competition was less intense among conspecifics of the same origin than between origins. This result indicates that other mechanism of origin-specific cooperative behaviour could have been also involved in reducing the competition between conspecifics of the same origin (also found in Semchenko *et al.*, 2014). In any case, diversity of parental environments seems to ameliorate the negative effect of competition between different origins.

Although we expected genetically diverse populations to have the highest phenotypic variability related to resource foraging compared to the rest of populations, we found lower functional diversity in the mixtures than the monocultures. It is surprising that the mixture of the three selected genotypes with contrasting evolutionary history — which are described to differ in phenotypic traits like root system and flowering period (Picó *et al.* 2008; Méndez-Vigo *et al.* 2013) — did not differ in SLA which is a trait related to resource foraging strategy of the plant. The absence of prominent morphological variability in SLA of these genotypes and with significant lower values compared to Col-1 genotype could also suggests that low SLA is a selected plant life-history trait that contributed to their success under Mediterranean climates (Wright *et al.* 2005; Blonder *et al.* 2015). Nevertheless, not finding a negative diversity effect in these populations (like we found in demethylated populations), indicates that, indeed, there should be some functional differences between genotypes.

When we tested the relative importance of trait values and variation for explaining the biodiversity effects, we found that average values of SLA rather than trait variance (i.e.

CVSLA) of the populations drove the productivity of the mixtures. In general, we found that net diversity effects were higher when mixtures contained individuals with high SLA (i.e. higher average SLA) and were also associated with lower variation in SLA (i.e. negative standard coefficients of CVSLA; Table 3). This means that populations of individuals with high SLA produced more biomass than more diverse assemblages with lower SLA. Although it is possible that measuring SLA alone could have been insufficient for characterizing organisms' niche differences (Kraft *et al.* 2015; Kunstler *et al.* 2016; Cadotte 2017), this result suggests that the increase in productivity of populations was not driven only by niche differences, and that average traits provided better explanation for it (Kunstler *et al.* 2016). This does not surprise because, besides being a trait related to resource foraging strategy of the plant (i.e. niche segregation), SLA is a hierarchical trait linked to the fitness or competitive ability of the individuals (Roscher *et al.* 2012; Kraft *et al.* 2014, 2015; Carmona *et al.* 2019). Species tend to be more productive when having higher SLA (Kraft *et al.* 2014; Madani *et al.* 2018). Interestingly, only in demethylated populations, in contrast to the other populations, we found that niche differences (i.e. CVSLA) were more positively driving the productivity, in some cases even with a stronger effect than the one of the SLA (Table 3). This suggests that when the phenotypic variation is low, and consequently the competitive intensity high, the importance of intraspecific trait variation and niche differentiation on productivity becomes more important.

CONCLUSIONS

Our study unravels the fraction of intraspecific phenotypic diversity that can be enabled by transgenerational effects, likely via environmentally induced epigenetic changes. Because transgenerational effects are common and very likely ubiquitous in plants, we suggest that more efforts should be devoted to testing its ecological and evolutionary relevance and consequences. Specifically, we compared the effect diversity of environmentally induced transgenerational effects and genetic diversity on productivity and resistance against environmental fluctuations. This aspect might be of particular importance in facing rapid environmental change since transgenerational effects have been increasingly recognized as determinants of successful adaptation of plants to novel climate (e.g. Herman and Sultan 2011; Latzel *et al.* 2014). Thus, epigenetically driven diversity can provide plant populations with another mechanism in coping with novel climate and maintaining the functioning.

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Captions to Figures

Figure 1: Productivity of experimental populations of *Arabidopsis*: monocultures vs. mixtures. Each column corresponds to the differing source of diversity: genetic, diversity of parental environments and demethylated; and each column to the different environmental conditions waterlogging, fertilization and control. The direction and magnitude of the effect of increasing diversity in each experimental treatment are shown in the boxes. Asterisks in the environmental conditions indicates the treatment significantly different from the others. Asterisks in the demethylated populations indicate significant overall effect of the demethylation treatment (i.e. par env. div vs. demethylated).

Figure 2: Functional diversity measured as coefficient of variation of SLA within each experimental population of *Arabidopsis*: monocultures vs. mixtures. Each column corresponds to the differing source of diversity: genetic, diversity of parental environments and demethylated; and each column to the different environmental conditions waterlogging, fertilization and control. The direction and magnitude of the effect of increasing diversity in each experimental treatment are shown in the boxes. Asterisks in the demethylated populations indicate significant overall effect of the demethylation treatment (i.e. par env. div vs. demethylated).

Figure 3: Average SLA of each experimental population of *Arabidopsis*: monocultures vs. mixtures. Each column corresponds to the differing source of diversity: genetic, diversity of parental environments and demethylated; and each column to the different environmental conditions waterlogging, fertilization and control. The direction and magnitude of the effect of increasing diversity in each experimental treatment are shown in the boxes. Asterisks in the environmental conditions indicates the treatment significantly different from the others.

Asterisks in the demethylated populations indicate significant overall effect of the demethylation treatment (i.e. par env. div vs. demethylated).

Figure 4: A) Net biodiversity effect, and its two components B) selection effect and C) complementarity effect in the mixtures of *Arabidopsis* with differing source of diversity: genetic, diversity of parental environments, and absent (demethylated). Each column corresponds to the different environmental conditions: waterlogging, fertilization and control. Asterisks indicate significant differences of the mixtures compared with environmentally diverse mixtures.

Table 1: Effects of environmental conditions, diversity, and demethylation treatment, and their interactions on *Arabidopsis* populations' mortality, total productivity, and variation and average SLA, testing separately the effect on A) populations with varying levels of diversity of parental environments, i.e. par. environmental and demethylated; and on B) populations with varying levels of genetic diversity. Result of the full factorial linear models including all fixed factors. In bold the significant effects of the factors.

A) Par. Env. and Demethylated		Mortality			Total biomass			CV of SLA			Average SLA		
	d.f.	MS	F ratio	P value	MS	F ratio	P value	MS	F ratio	P value	MS	F ratio	P value
Env. condition	2	0.76	0.26	0.77	94281	147.39	**<0.01**	0.429	63.51	**<0.01**	45356	179.48	**<0.01**
Diversity	1	2.28	0.79	0.38	622	0.97	0.32	0.100	14.80	**<0.01**	290	1.23	0.27
Demethylation	1	16.25	5.6	**0.02**	3656	5.71	**0.02**	0.000	0.05	0.83	2439	10.33	**<0.01**
E x Div	2	4.33	1.49	0.23	127	0.20	0.82	0.066	9.70	**<0.01**	535	2.27	0.11
E x Dem	2	1.53	0.53	0.59	545	0.85	0.43	0.001	0.10	0.9	889	3.77	**0.02**
Div x Dem	1	0.68	0.93	0.34	1608	2.51	0.11	0.008	1.18	0.27	26	0.11	0.74
E x Div x Dem	2	2.2	0.76	0.47	1481	2.31	0.10	0.005	0.69	0.50	48	0.20	0.81
Residuals	233*	2.9			640			0.006			236		

E = Environmental condition; Div = Diversity; Dem = Demethylation treatment; d.f. = degrees of freedom; MS = means square; F = variance ratio; P = error probability.
*For mortality d.f.= 240

B) Genetic

	d.f.	Mortality			Total biomass			CV of SLA			Average SLA		
		MS	F ratio	P value	MS	F ratio	P value	MS	F ratio	P value	MS	F ratio	P value
Env. condition	2	0.13	0.98	0.38	38933	87.33	**<0.01**	0.017	2.43	0.09	13980	147.60	**<0.01**
Diversity	1	0.27	2.03	0.16	283	0.64	0.43	0.022	3.29	0.07	164	1.73	0.19
E x Div	2	0.04	0.33	0.72	4	0.01	0.99	0.001	0.14	0.87	353	3.72	**<0.03**
Residuals	82*	0.14			446			0.007			95		

E = Environmental condition; Div = Diversity; d.f. = degrees of freedom; MS = means square; F = variance ratio; P = error probability. *For mortality and for total biomass d.f.= 84

Table 2: Effects of environmental conditions and demethylation treatment, and their interactions on diversity net effect, selection effect and complementarity effect on *Arabidopsis* populations' biomass, testing separately the effect on A) mixtures with varying levels of diversity of parental environments, Par. environmental and Demethylated; and on B) mixtures with varying levels of genetic diversity. Result of the full factorial linear models including all fixed factors. In bold the significant effects of the factors.

A) Par. Env. and Demethylated

		Net effect			Selection effect			Complementarity effect		
	d.f.	MS	F ratio	P value	MS	F ratio	P value	MS	F ratio	P value
Env. condition	2	548	0.92	0.40	17.50	6.82	**<0.01**	741	1.28	0.28
Demethylation	1	3196	5.37	**0.02**	7.05	2.75	0.10	2903	5.01	**0.03**
E x Dem	2	3040	5.10	**<0.01**	10.26	4.00	**0.02**	3088	5.32	**<0.01**
Residuals	115	595			2.56			580		

B) Genetic

		Net effect			Selection effect			Complementarity effect		
	d.f.	MS	F ratio	P value	MS	F ratio	P value	MS	F ratio	P value
Env. condition	2	20	0.06	0.94	0.94	0.17	0.84	29	0.09	0.92
Residuals	42	345			5.40			331		

Table 3: Influence of average SLA and CVSLA on diversity net effect, and its additive components: selection and complementarity effect on *Arabidopsis* mixtures. Regression standardized coefficients of each trait predictors of the linear models made in each experimental treatment separately (i.e. mixtures with different source of diversity and under different environmental condition separately). In bold the significant effects of the factors.

Environmental condition	Trait predictor	Source of diversity								
		Genetic			Par. Environmental			Demethylated		
		Net	Sel	Comp	Net	Sel	Comp	Net	Sel	Comp
Waterlogging	SLA	28.00	-0.93	**28.93**[a]	12.76	0.26	12.49	**13.87***	0.88	**12.99***
	CVSLA	-4.47	0.47	-4.95	-3.22	0.05	-3.27	1.24	0.65	0.59
Fertilization	SLA	-6.79	**-2.57**[a]	4.22	10.02	-0.14	10.16	18.15	0.22	17.93
	CVSLA	4.37	-0.91	5.28	-19.52	-0.33	-19.19	2.49	-0.65	3.14
Control	SLA	17.57	-2.81	20.28	**21.29**[a]	**2.69***	18.60	9.00	-0.29	9.29
	CVSLA	-11.45	-1.19	-10.26	-5.88	-1.08	-4.81	**29.88****	-0.20	**30.08****

Net = Net diversity effect; Sel = Selection effect; Comp = Complementarity. Significance: [a] ($0.05 \leq P < 0.1$); * ($0.01 \leq P < 0.05$); ** ($P < 0.01$)

Figure 1

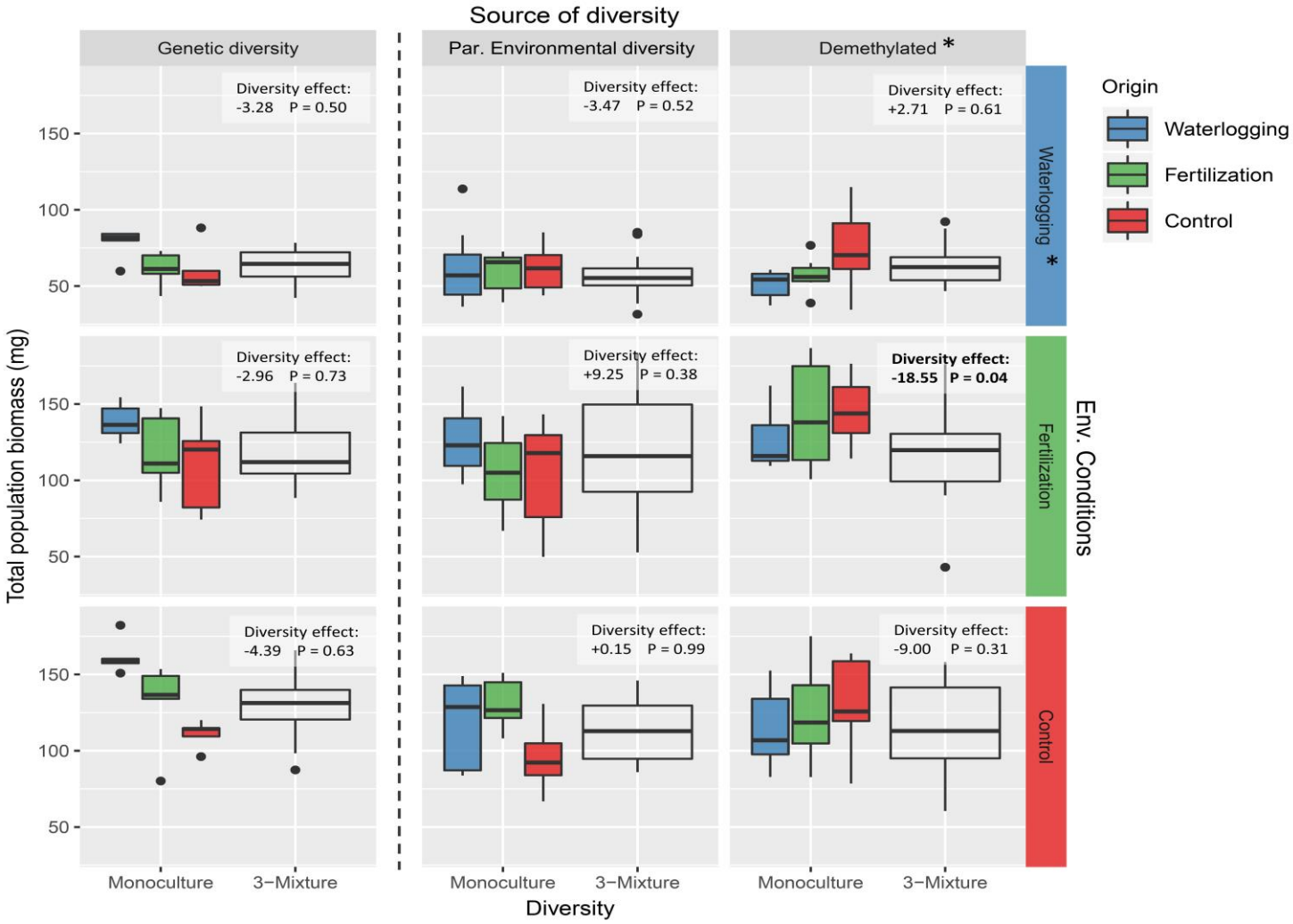

Figure 2

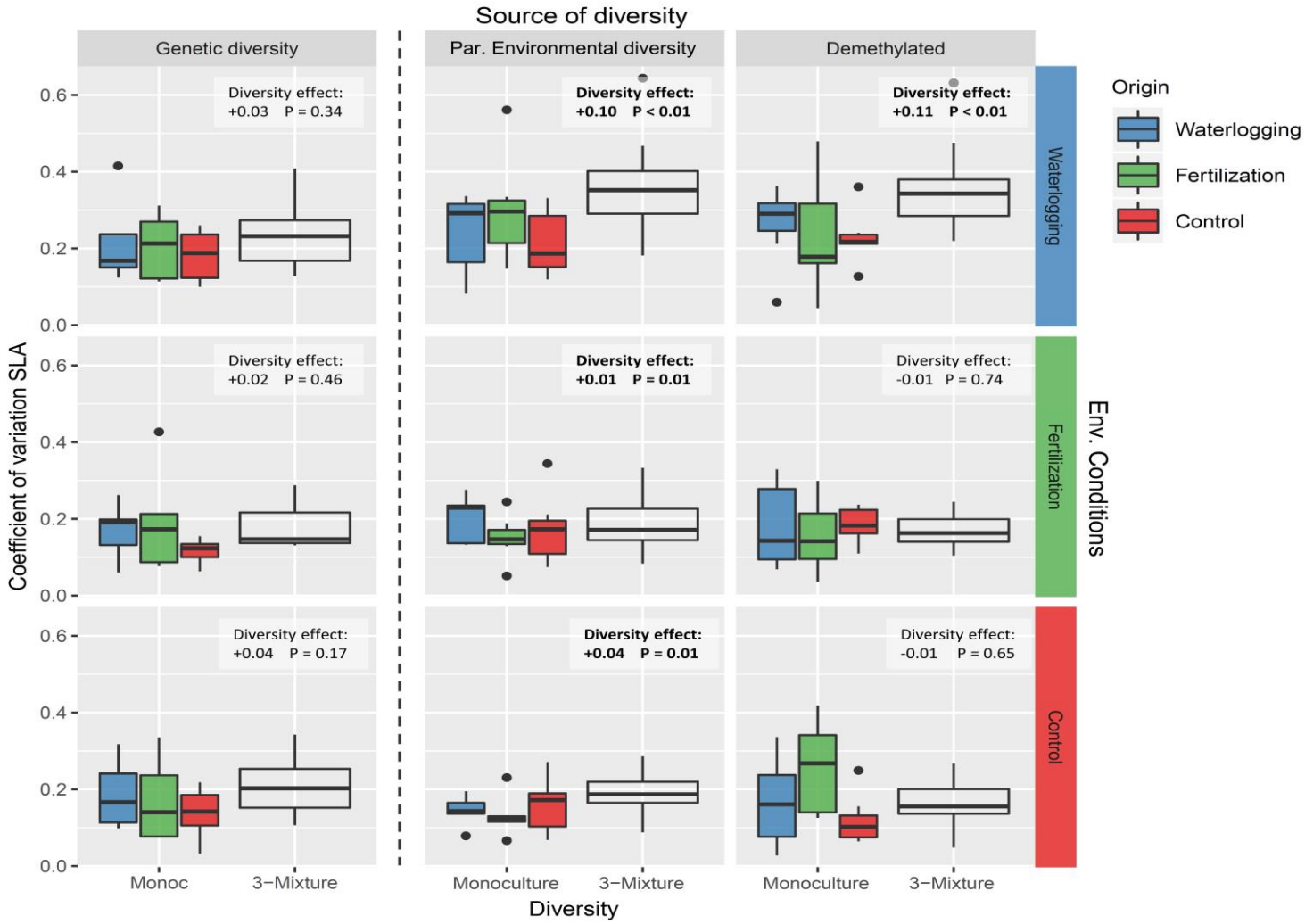

Figure 3

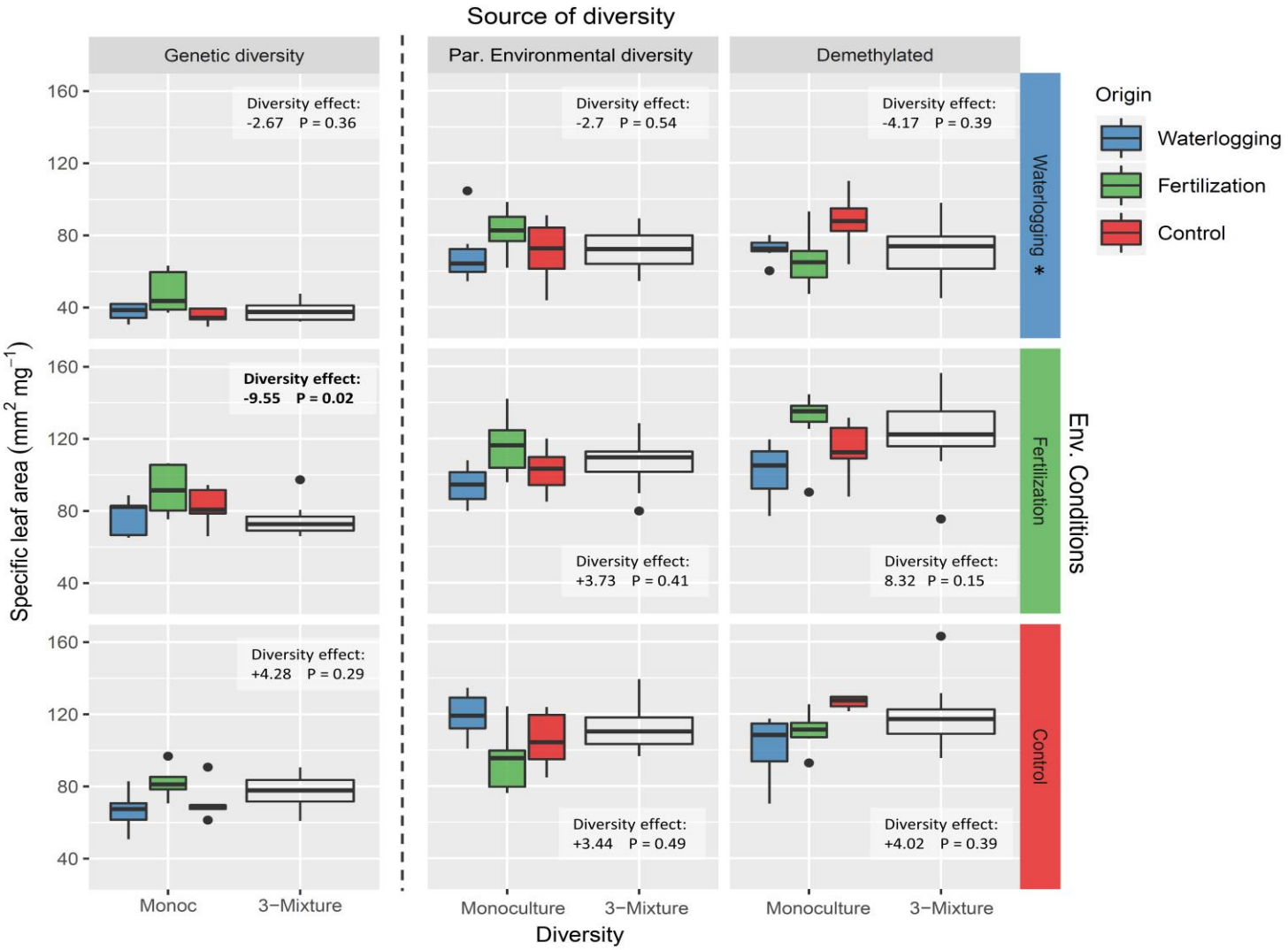

Figure 4