

Research

Effects of intrinsic precipitation-predictability on root traits, allocation strategies and the selective regimes acting on them

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Oikos

2022: e07970

doi: 10.1111/oik.07970

Subject Editor: Eric Seabloom

Editor-in-Chief: Dries Bonte

Accepted 22 October 2021



Climate change affects means, variances and the intrinsic predictability of the climate. However, experimental tests of how changes in intrinsic climatic predictability affects plant traits, allocation strategies and the selective regime acting on them are scarce, as well as evidence for the importance of root functional traits to cope with climatic uncertainty. Here, we experimentally manipulated intrinsic daily and inter-seasonal precipitation-predictabilities and tested their effect on root traits, root allocation strategies, the selective regime acting on them, and transgenerational root responses, using a four-year field experiment and *Onobrychis viciifolia*. More predictable precipitation led to lower root biomass and a lower overall plant performance, and to higher allocation to roots and higher within-root allocation (i.e. allocation to root branching and maximum rooting depth relative to allocation to roots). Differences in intrinsic daily and inter-seasonal predictability induced differences in the strength of selection acting on the studied traits, but did not affect the type of the selective regime, nor the transgenerational responses. The results indicate that higher predictability constrained a plant's performance, while plants were able to cope with lower predictability. Absence of transgenerational responses in root traits with respect to the predictability treatments, points to slow or no inter-generational changes of root traits in unpredictable habitats. Thus, adjustments in root allocation strategies and changes therein might be key to deal with increasing climatic uncertainty.

Keywords: allocation strategy, allometry, environmental predictability, intrinsic daily and inter-seasonal precipitation predictability, maximum rooting depth, *Onobrychis viciifolia*, plant performance, root branching, selection gradients

Introduction

Climatic variability has increased over the last decades, and projections anticipate an increased frequency of extreme events (Stocker et al. 2013). This leads to lower intrinsic predictability of the environment and to higher uncertainty of the available

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resources (Karl et al. 1995, Matías et al. 2011, Mora et al. 2013). Phenotypic plasticity may buffer negative effects arising due to changing conditions (Reich et al. 2003), and if environmental changes persist, evolutionary adaptation to the new conditions may happen (Ghalambor et al. 2007). It has been suggested that the intrinsic predictability (i.e. the degree of temporal autocorrelation) of the conditions themselves, importantly affects the speed of adaptation. For instance, lower intrinsic predictability of climatic conditions may impose weaker selection, which may result in slower evolutionary responses (Kingsolver and Buckley 2015), and it may also lead to the evolution of higher phenotypic plasticity (Reed et al. 2010, Hallsson and Björklund 2012, Wennersten and Forsman 2012). In contrast, higher intrinsic predictability may impose stronger selection (Cohen and Levin 1991, Caro et al. 2016) and lead to reduced phenotypic plasticity (Gomez-Mestre and Jovani 2013). However, the predictions are not consistent and knowledge about whether and how intrinsic climatic predictability affects the selective regimes are scarce (Baum and Larson 1991, Travis 2001, Botero and Rubenstein 2012), as well as experimental studies testing how organisms deal with changes in environmental predictability.

While rapid responses to climate change (i.e. responses within the same generation) include dispersal, migration and acclimatization, plants may primarily respond by acclimating to new conditions through changes of their allocation strategies, particularly by changing allocation strategies affecting resource uptake (Schmid 1992, Bardgett et al. 2014, Weemstra et al. 2016). For example, when water becomes more limiting, plants may allocate resources to the production of deeper roots in order to access soil moisture present in deeper soil layers (Balachowski and Volaire 2018) and even moderately stressful conditions may lead to profound ecological changes (Hajek and Knapp 2022). Such changes may be subjected to tradeoffs (Reich 2014), where higher allocation to a given plant structure may lead to lower allocation to another plant structure. Similarly, tradeoffs among current and future reproduction may exist in perennial species, potentially explaining why they may allocate more resources to roots in order to survive, rather than aiming at high current reproduction (Siddique et al. 1990, Perrin et al. 1996, White et al. 2013, Friedman and Rubin 2015). Allocation strategies are generally measured using ratios (e.g. the proportion of mass invested into roots) and differences in a given ratio may be achieved with a single allometric strategy (Müller et al. 2000), given that differences in environmental conditions may affect plant size and thereby allometry, i.e. the quantitative relationship between growth and allocation (Weiner 2004). For example, while in small plants allocation to roots is generally highest, big plants may allocate more resources to leaves. Consequently, environmentally induced slow plant growth, which leads to small plants at time x , may lead to high root ratios; and environmentally induced fast plant growth, which leads to big plants at time x , may lead to lower root ratios. This reflects a single strategy rather than different strategies in how plants cope with differences in environmental conditions (Müller et al. 2000, Weiner 2004).

Assessing whether differences in climatic predictability affect root traits, root allocation strategies and selective regimes acting on both is important to improve our understanding of whether plants may cope with the anticipated climatic change. Nevertheless, most studies investigating effects of climate change on plant traits, allocation strategies and selective regimes focused on changes in environmental means (Emmett et al. 2004, Donelson et al. 2012, Anderson and Gezon 2015). In addition, they usually do not test whether observed changes are due to changes in allocation strategy or differences in allometric growth. Very little is known about how plants will deal with changes in environmental predictability (for exceptions, Parepa et al. 2013, Liu and van Kleunen 2017, March-Salas et al. 2019, 2021), and experimental studies manipulating the intrinsic predictability are needed.

Here, we experimentally tested how differences in the intrinsic precipitation-predictability affect roots, whose functions include water acquisition, nutrient uptake, anchorage and storage of resources (Bardgett et al. 2014). More specifically, we tested how more (M) and less (L) predictable intrinsic precipitation-predictability affect root traits (root biomass, number of lateral roots and maximal root length), allocation to roots (allocation to roots relative to total allocation), within-root allocation strategies (allocation to root branching and maximum rooting depth relative to allocation to roots), and whether potential differences can be explained by allometric growth rather than differences in allocation. Moreover, we tested to which selective regimes these functional root traits are exposed, and whether treatment-effects in these traits may lead to higher fitness. To this end, we used as a model species the common sainfoin *Onobrychis viciifolia* (Fabaceae), a perennial forb whose root system consists of a taproot with a few first order and numerous higher order lateral roots (Atkinson et al. 2014), and fine root hairs (Fig. 1) (Osmont et al. 2007, Mohajer et al. 2012). We manipulated the intrinsic predictability of precipitation, because it may affect the intrinsic predictability of water availability, and because it has changed over the last decades in many territories (Karl and Trenberth 2003, Stocker et al. 2013). Moreover, precipitation is one of the main drivers of plant growth (Peñuelas et al. 2007), and changes therein can drive variation in natural selection (Siepielski et al. 2017).

Intrinsic environmental predictability has different temporal dimensions (March-Salas et al. 2019). It can refer to the regularity in the timing and magnitude of environmental fluctuations over a short time scale (e.g. autocorrelation among daily or weekly measures), or it can refer to fluctuations over larger time scales (e.g. autocorrelation among seasonal or annual measures) (March-Salas et al. 2019). It is generally believed that lower short-term predictability rapidly induces changes in phenotypic traits and negatively affects reproduction (Dewar and Richard 2007). Differences in the predictability of seasonality can alter plant responses and species diversity (Tonkin et al. 2017, March-Salas et al. 2019, but see Novoplansky et al. 1994), and theory predicts that high transgenerational predictability is required for rapid adaptation (Mousseau and



Figure 1. Root diversity in *Onobrychis viciifolia*. Examples of different root architectures are shown. From left to right: roots with decreasing numbers of lateral roots. The scale bar represents 50 mm.

Fox 1998, Reed et al. 2010, Botero et al. 2015). Therefore, we tested whether and how plants respond to differences in daily and inter-seasonal precipitation-predictability by applying a two-factorial design. Differences in intrinsic inter-seasonal precipitation-predictability were simulated by exposing plants to more and less predictable precipitation between seasons (between spring and summer; i.e. between early and late plant growth). Thus, plants were exposed to higher inter-seasonal predictability (MM, LL) or lower inter-seasonal predictability (ML, LM), or in other words, to a higher or lower autocorrelation of precipitation between early and late growth. We further tested whether differences in precipitation-predictability impose differences in the strength and type (e.g. directional, stabilizing or disruptive selection) of the selective regime acting on a particular trait, and whether the observed plant responses let to higher reproductive success. Moreover, we exposed three generations of descendants (G_1 , G_2 and G_3) to the same treatment combination experienced by their mothers (e.g. seeds of LL mothers were exposed to LL), to test whether in presence of high inter-generational predictability, fast transgenerational responses to the different predictability regimes exists.

We tested the following main questions: 1) how do functional root traits respond to higher and lower daily and inter-seasonal precipitation-predictability? 2) Do daily and inter-seasonal precipitation-predictability affect the selective regime acting on functional root traits? 3) Are the observed responses in functional root traits optimizing reproductive success? 4) Are differences in daily and inter-seasonal precipitation-predictability inducing rapid transgenerational responses in functional root traits?

Material and methods

Experimental setup

At the experimental field station ‘El Boalar’ (42°33’N, 0°37’W, 705 m a.s.l.; IPE-CSIC, Jaca, Huesca, Spain) seeds

of *Onobrychis viciifolia* (Fabaceae) were sown during four consecutive years (2012–2015). A single seed lot originating from the same year and site was used for the entire experiment and seeds of this lot are hereafter referred to as the ancestral generation (G_0). The seed lot of origin was obtained in 2011 from a farm located in Castillo de Lerés (23 km from the experimental station), which exhibits more precipitation (on average, ~340 mm more per year) but otherwise a similar climate as the field site (‘El Boalar’). *Onobrychis viciifolia* naturally grows at the field site and also in more and less humid conditions than those prevailing at the field site (Supporting information). It exhibits a high nutritional value, and, thanks to the symbiosis with Rhizobia, it possesses the ability to fix atmospheric nitrogen (Prévost et al. 1987). Therefore, it is used as fodder and as green manure (Mora-Ortiz and Smith 2018). *Onobrychis viciifolia* is a perennial forb that can reproduce in the first year of life, flowering happens from spring to summer, and seeds are produced from early summer onwards.

For this experiment, 16 open-air plots of 7.2 m² were established in 16 different enclosures (March-Salas and Fitze 2019a). Each enclosure was surrounded by metal walls and covered with a mesh (mesh width: 1.6 × 1.6 cm) to protect the plants against aboveground and belowground vertebrate herbivores and birds, but still provided access for pollinators. Each plot was additionally surrounded by a mosquito mesh (30 cm aboveground and 10 cm belowground) to prevent slug predation. In each year and plot, we manually ploughed, loosened and homogenised the top 30 cm of the soil before sowing. All weeds, roots and visible seeds were removed to avoid competition between experimental and non-experimental plant species, and the ground was smoothed. In each plot, 28 planting positions, separated by 40 cm and aligned in a quadratic grid, were marked. This standardized design minimized competition among experimental plants (Liu et al. 2008), and it avoided contact between root systems, since the distance between plants (40 cm) was higher than twice the maximum root-system radius measured over

the entire duration of the experiment (maximal root-system radius: 16.55 cm; average root-system radius = 6.25 ± 0.16 SE cm).

Sowing procedure

In April, randomly selected seeds of *O. viciifolia* were sown at a depth of 2 cm. In 2012, G_0 seeds were sown in 28 planting positions and from 2013 to 2015 in seven positions per plot. G_0 seeds were planted in different years to understand whether the precipitation-predictability effects were similar across years (Supporting information). In each of the years, the same number of seeds was sown in each plot and treatment. To make sure that the randomly selected subsamples were representative for the entire seed lot, we tested, prior to sowing, whether there were significant differences in the average and variance of seed mass between seeds of the subsample and the remaining seeds, and among seeds used in different years, plots and experimental treatments. No significant differences were found (all $p \geq 0.1$, including all interactions). From 2013 to 2015, descendent seeds (G_n) were sown in 21 positions per plot to test for transgenerational responses. Seeds produced by G_0 are hereafter referred to as G_1 , those produced by G_1 as G_2 , and those produced by G_2 as G_3 . G_1 were sown in 2013, G_2 in 2014, and G_3 in 2015 in the same treatment combination as their mother (Supporting information) (March-Salas et al. 2019). While descendants were potentially able to exhibit a transgenerational response, by experimental design, ancestors were unable to do a transgenerational response with respect to the experimental conditions (Supporting information). Thus, differences among ancestors planted in different years represent differences owing to variation among years, while differences between ancestors and descendants growing in the same plot and year represent transgenerational responses (Supporting information) (March-Salas and Fitze 2019b, March-Salas et al. 2019).

Precipitation-predictability treatment

Plots were subjected to two different temporal scales of intrinsic precipitation-predictability (i.e. two different levels of temporal autocorrelation of precipitation) (Ashander et al. 2016, Pennekamp et al. 2019): intra- and inter-seasonal predictability.

First, we simulated differences in intrinsic intra-seasonal predictability by varying the daily precipitation-predictability using an automatic irrigation system with four sprinklers per enclosure, one sprinkler in each corner, and providing homogenous precipitation in the whole enclosure (San-Jose et al. 2014). More ('M') or less ('L') predictable precipitation was applied to eight outdoor plots each. The field site's (natural) precipitation-predictability was manipulated by providing M- and L-plots with supplemental precipitation at regular and random intervals, respectively. M-plots were irrigated 14 times per week, each irrigation event lasted 5 min and irrigation happened at regular time-intervals. L-plots were also irrigated 14 times per week, each irrigation

event lasted 5 min, and irrigation happened at randomly chosen time points (see the Supporting information for more details). Each plot (and treatment) obtained the same number of precipitation events and the same amount of total (natural precipitation + supplemental precipitation) precipitation per week. Irrigation happened from sowing to the end of the plant's annual life-cycle (for approximately 4.25 month) (March-Salas et al. 2019), which can last in some natural populations until September (Badoux 1964, Hayot Carbonero et al. 2011). Each irrigation event provided 1.3 mm m^{-2} of supplemental precipitation, which sums up to approximately 330.2 mm of supplemental precipitation per year. Together with the natural precipitation, the total precipitation obtained corresponds to the natural precipitation conditions from where the seeds originated. During 2013–2015, precipitation falling at the field station ('El Boalar') exhibited no seasonality (March-Salas et al. 2019), the average annual precipitation was 916 mm (2012–2015), and maximum annual precipitation registered at the field station in the last 10 years was 1265 mm m^{-2} . The intrinsic predictability of the total precipitation (natural precipitation + supplemental precipitation) estimated by permutation entropy (Pennekamp et al. 2019) was 0.77 in the M-plots, which was equal to the predictability of the natural precipitation of the study site. In L-plots, permutation entropy was 0.86, and thus, the intrinsic precipitation-predictability was 11.2% lower than that of the M-plots (March-Salas et al. 2019). At the field site, average monthly temperatures raise from February to July/August (maximum average monthly temperature was $19.74^\circ\text{C} \pm 0.5$ SE in August – mean of the four experimental years) (March-Salas et al. 2019), and drying up of the plants can occur from June to August when the average monthly temperatures are greater than 16°C (Supporting information). The experimental precipitation-design assured completion of the experimental plants' annual life-cycle and it thus represents a conservative measure of the effects of intrinsic precipitation-predictability, since all experimental plants were able to complete their life-cycle. Moreover, in each plot, the total precipitation (natural precipitation + supplemental precipitation) was within the natural range registered during the last 10 years at the study site. The minimum of the natural daily precipitation at the study site was identical to the minimum of the daily total precipitation in the plots. The maximum of the daily total precipitation did not exceed the daily maximum of the natural precipitation registered at the study site during the growing season, and the maximum of the variance in the simulated daily precipitation measured per week did neither exceed the maximum variance in the natural daily precipitation measured per week at the study site (mixed model ANOVA with Natural versus Natural precipitation + irrigation as a fixed factor and year as random factor: $F_{1,62} < 0.001$, $p \geq 0.98$) (March-Salas and Fitze 2019a, March-Salas et al. 2019).

Second, inter-seasonal predictability was manipulated by either exposing plots during the late growth period (hereafter referred to as 'late treatment', i.e. during summer) to the same or to the other treatment as during the early growth

period (during spring). This resulted in a two-factorial design with four treatment combinations of daily precipitation: 1) more predictable during early and late growth (MM), 2) less predictable in both periods (LL), 3) more predictable during early and less predictable during late growth (ML) and 4) less predictable during early and more predictable during late growth (LM). LL and MM exhibit higher inter-seasonal predictability, and LM and ML lower inter-seasonal predictability, or, in other words, a higher or lower autocorrelation of precipitation between early (spring) and late growth (summer). In ML and LM, treatments were changed in the middle of the phenological cycle (i.e. when the first flower buds appeared), in late June.

Data collection

At the end of the growing season (i.e. when all fruits of a given plant were ripe), the above-ground and root parts of all plants were individually harvested. The complete root system was carefully dug out from the soil with the help of cutting blades (Ostonen et al. 2007). To this end, we applied cylindrical digging within a perimeter of 40 cm from the stem. Complete extraction was possible, because the maximum root length measured over all experimental plants and years was 35.80 cm (average root length = 16.81 ± 0.15 SE cm), because each year the top 30 cm of soil of each plot was manually ploughed, loosened and all roots removed before sowing (above), and because all emerging non-experimental seedlings were removed every week. None of the root-systems exceeded the digging perimeter, and no interactions among root systems existed. In the lab, the roots were washed to remove the substrate adhered to the root system (Fig. 1). For each plant, the longest root was determined (Fig. 1) and its length was measured as an estimate of maximum rooting depth. The number of first and higher (second, third and so on) order lateral roots (all root branches ≥ 2 cm; note that all root hairs were shorter than 2 cm) was counted and hereafter referred to as ‘number of lateral roots’. Plant parts were dried at 50°C for four days to measure the dry weight of above-ground and root biomass (in g). To test whether treatments affected the allocation to the roots and the within-root allocation, the following traits were calculated: 1) root weight ratio (RWR; root biomass/total biomass); 2) relative root branching (number of lateral roots/root biomass); and 3) the relative maximum rooting depth (maximum root length/root biomass).

Statistical analysis

All statistical analyses were run in R ver. 3.5.0 (<www.r-project.org>). Treatment effects on root traits and allocation strategies across the four years were analysed using linear mixed-effect models (LMM) implemented in the lme4 package and the lmer function (Bates et al. 2015). Early treatment (less predictable versus more predictable), late treatment (less predictable versus more predictable), year (2012, 2013, 2014, 2015) and their two- and three-way interactions were modelled as fixed parameters (Table 1). Since plants are

nested in plots and years (fully nested design) ‘plot_year’ (a parameter containing the plot $\times$ year interaction) was modelled as random factor, as described in Bates (2010). To test whether existing differences may be explained by allometric growth, we first tested whether the expression of the root traits is size-dependent by regressing the number of lateral roots and maximum root length on plant size (i.e. total biomass). In these analyses, a linear relationship, and the absence of non-linear relationships will point to the absence of allometric growth. The presence of significant non-linear relationships will show that allometric growth exists and absence of treatment $\times$ plant size interactions will indicate that treatment affects plant size rather than allocation strategies (Müller et al. 2000), which points to a single strategy rather than difference in how plants cope with a given treatment (Müller et al. 2000, Weiner 2004). In contrast, significant treatment $\times$ plant size interactions (simple and/or quadratic effect) in the presence of allometric growth will show that plants exhibit different allometric strategies (Müller et al. 2000).

The selective regime (the type and the strength of natural selection) to which root traits and allocation strategies are exposed was analysed using selection gradients (Lande and Arnold 1983). As a performance proxy, we used total biomass, because it is positively related with seed number (Younginger et al. 2017) (in this study: $F_{1,588} = 115.16$, $p < 0.001$, $\beta = 0.4$), and because it integrates investment in current and future reproduction (for further justification, see the Supporting information). Consequently, we tested for differences in reproduction and not for differences in survival based on root traits and allocation strategies. Prior to the analyses, total biomass and all functional root traits were $\ln$ -transformed and standardized to obtain standardized selection gradients (Lande and Arnold 1983). Thereafter, LMMs were applied in which total biomass was the response variable, early treatment, late treatment and year were modelled as fixed factors, and the functional root trait of interest was modelled as a co-variate. To account for non-independence of plants measured in the same year and plot, plot_year was included as random factor. To test for directional, stabilizing or disruptive selection, linear and quadratic terms of the functional root trait of interest were included. The full model also contained all two- and three-way interactions between treatments and the co-variables.

To test whether descendants exhibited a transgenerational response in functional root traits with respect to the imposed treatments, ancestors (G_0) and descendants (G_n) of the same plot and year were compared during 2013, 2014 and 2015 (Supporting information). LMMs were run with generation (ancestral versus descendants), early treatment, late treatment, year and their interactions as fixed parameters, and ID of the matriline (i.e. ID of the G_0 ancestor; Supporting information) and plot_year as random factors. In these analyses, a significant generation $\times$ treatment interaction shows that ancestors and descendants respond differently to the precipitation treatments, which points to a transgenerational response (March-Salas et al. 2019).

Table 1. Experimental precipitation-predictability effects on root traits and allocation strategies. Response variables are root biomass, number (N) of lateral roots, maximum root length; root weight ratio (RWR); relative maximum rooting depth (i.e. maximum root length/root biomass), and relative root branching (i.e. number of lateral roots/root biomass). Significant treatment ('Early' and 'Late' refer to early and late treatment), year and interactive effects including treatment are shown. p-values of significant parameters are italicised. Effect size (Cohen's d) and its 90% confidence interval are given for each parameter. For significant interactions the number of the figure showing the effect is given. In all analyses, the sample size was 590.

Response variable	Parameter	χ^2	df	p-value	Cohen's d	90% CI	Figure
Root biomass ^f	Early	7.07	1	<i>0.008</i>	0.84	[0.03, 0.30]	
	Late	1.61	1	0.204	0.46	[0.00, 0.18]	
	Year	15.59	3	<i>0.001</i>	1.12	[0.06, 0.38]	
	Early × Late	3.03	1	<i>0.042</i>	0.84	[0.00, 0.18]	Fig. 2a
	Early × Year	8.97	3	<i>0.029</i>	0.87	[0.00, 0.26]	Fig. 3a
	Late × Year	8.05	3	<i>0.044</i>	0.81	[0.00, 0.25]	Supporting information
N lateral roots ⁺	Early	0.69	1	0.406	0.29	[0.00, 0.12]	
	Late	2.16	1	0.141	0.20	[0.00, 0.10]	
	Year	109.54	3	<i><0.001</i>	2.92	[0.55, 0.76]	
	Early × Late	3.59	1	0.058	0.55	[0.00, 0.21]	
	Late × Year	9.81	3	<i>0.020</i>	0.87	[0.01, 0.29]	Fig. 3b
	Early × Year	10.60	3	<i>0.014</i>	0.91	[0.02, 0.30]	Fig. 3c
Maximum root length [/]	Early	4.62	1	<i>0.032</i>	0.67	[0.01, 0.25]	
	Late	3.17	1	0.075	0.55	[0.00, 0.20]	
	Year	6.10	3	0.107	0.70	[0.00, 0.23]	
	Early × Year	10.60	3	<i>0.014</i>	0.91	[0.02, 0.30]	Fig. 3c
	Late × Year	8.52	3	<i>0.036</i>	0.81	[0.00, 0.27]	Fig. 3d
	Year	6.65	3	0.084	0.70	[0.00, 0.22]	
Root weight ratio [#]	Early	7.74	1	<i>0.005</i>	0.84	[0.03, 0.30]	
	Late	4.40	1	<i>0.036</i>	0.59	[0.00, 0.23]	
	Year	80.82	3	<i><0.001</i>	2.55	[0.47, 0.71]	
	Early × Year	11.66	3	<i>0.005</i>	0.77	[0.00, 0.26]	Fig. 3e
	Late × Year	8.09	3	<i>0.044</i>	0.81	[0.00, 0.27]	Fig. 3f
	Year	6.65	3	0.084	0.70	[0.00, 0.22]	
Relative root branching ⁺	Early	7.74	1	<i>0.005</i>	0.84	[0.03, 0.30]	
	Late	4.40	1	<i>0.036</i>	0.59	[0.00, 0.23]	
	Year	80.82	3	<i><0.001</i>	2.55	[0.47, 0.71]	
	Early × Year	11.66	3	<i>0.005</i>	0.77	[0.00, 0.26]	Fig. 3e
	Late × Year	8.09	3	<i>0.044</i>	0.81	[0.00, 0.27]	Fig. 3f
	Year	6.65	3	0.084	0.70	[0.00, 0.22]	
Relative max. rooting depth ⁺	Early	6.05	1	<i>0.014</i>	0.77	[0.02, 0.28]	
	Late	0.73	1	0.391	0.41	[0.00, 0.16]	
	Year	25.16	3	<i><0.001</i>	1.44	[0.14, 0.48]	
	Early × Late	5.37	1	<i>0.020</i>	0.70	[0.01, 0.26]	Fig. 2b
	Early × Year	7.18	3	0.066	0.77	[0.00, 0.26]	
	Late × Year	8.09	3	<i>0.044</i>	0.81	[0.00, 0.27]	Fig. 3f

Transformations: ^{*}^0.2; ^f^0.25; ⁺^0.3; [/]^0.4; [†]log.

In all statistical analyses, the most parsimonious model was determined using stepwise backward elimination of non-significant terms ($p > 0.1$). Post hoc tests (lsmeans package; Lenth 2016) were applied using Tukey's HSD test, whenever there were significant main effects or interactions with factors containing more than two levels. If three-way interactions between a co-variate, the early and the late precipitation treatment were significant, we used the *lstrends* function to test for significant differences between slopes. For all Gaussian models, the assumption of normality and homoscedasticity of the residuals was tested. If the normality assumption was not met, response variables were transformed (see transformations used in Table 1). In the presence of heteroscedasticity, and if transformation did not result in homoscedasticity, weighted least square regression was applied.

Results

Effects of precipitation predictability on functional root traits

Root biomass of *Onobrychis viciifolia* was affected by three significant two-way interactions: early × late treatment,

early treatment × year and late treatment × year (Table 1, Fig. 2a, 3a, Supporting information). The early × late treatment interactions shows that roots of plants growing in MM were lighter than those of the other treatment combinations (Fig. 2a), and the early treatment × year interaction shows that in 2014, but not in the other years, root biomass was significantly lower in M than in L (Fig. 3a).

The number of lateral roots was affected by a significant late treatment × year interaction (Table 1, Fig. 3b). In 2012, plants in L had significantly fewer lateral roots than those in M and in the other years no significant differences among treatments existed (Fig. 3b). The number of lateral roots significantly increased with total biomass ($\chi_1^2 = 29.40$, $p < 0.001$) and no quadratic relationship existed ($\chi_1^2 = 0.207$, $p = 0.649$), pointing to the absence of allometric growth.

Maximum root length was significantly affected by two two-way interactions: early treatment × year and late treatment × year (Table 1, Fig. 3c–d), both indicating that in 2014 maximum root length was significantly shorter in M compared to L, and no significant differences existed in the other years (Fig. 3c–d). In the more and in the less predictable early and late treatment, there existed significant quadratic

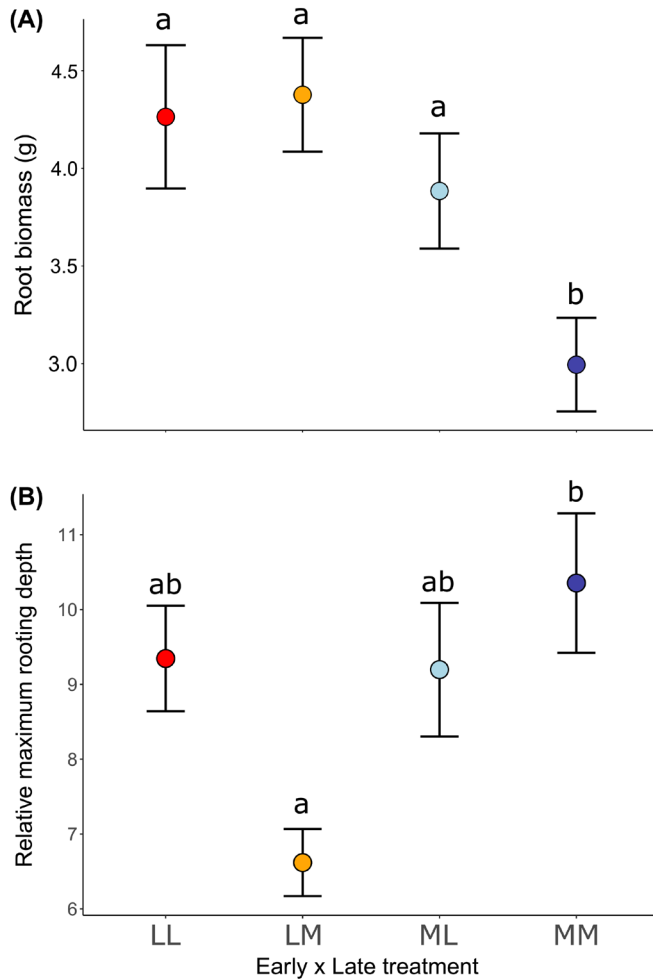


Figure 2. Significant early treatment $\times$ late treatment interaction on root biomass. Shown are means $\pm$ SE of (A) the significant early treatment $\times$ late treatment interaction on root biomass and (B) the significant early treatment $\times$ late treatment interaction on relative maximum rooting depth. Significant post hoc contrasts (Tukey's HSD test) are indicated with different letters.

relationships between Maximum root length and total biomass and the quadratic slopes significantly differed among treatments (interaction between early treatment and total biomass: $\chi^2_1 = 4.029$, $p = 0.045$ (Supporting information); interaction between late treatment and total biomass: $\chi^2_1 = 14.277$, $p < 0.001$ (Supporting information)), pointing to significant differences in allometric growth between treatments.

Root weight ratio (RWR) was significantly higher in the late M treatment (Table 1; average RWR in M: 0.178 ± 0.078 , in L: 0.155 ± 0.071), and the early treatment was not significant ($\chi^2_1 = 1.75$, $p = 0.186$).

Relative root branching was significantly affected by an early treatment $\times$ year interaction (Table 1). In 2014, significantly more branches per root mass existed in the more compared to the less predictable early treatment ($p < 0.001$; Fig. 3e) and relatively more root branches existed in late M compared to late L (relative root branching in late M: 4.721 ± 0.342 , in L: 4.178 ± 0.381 ; Table 1).

Relative maximum rooting depth was significantly affected by an early treatment $\times$ late treatment and a late treatment $\times$ year interaction (Table 1, Fig. 2b, 3f). Relative maximum rooting depth was significantly higher in MM compared to LM (Fig. 2b) and LL and ML did not significantly differ from the other treatment groups. The late treatment $\times$ year interaction shows, that in L, relative maximum rooting depth was significantly higher in 2012 than in the other years (all $p < 0.001$), while no significant differences among years existed in M (all $p \geq 0.12$, Fig. 3f).

Effect of precipitation predictability on the selection gradients of the measured root traits and allocation strategies

The selection gradient of the number of lateral roots was significantly affected by early treatment (Table 2). In L, the relationship between performance and the number of lateral roots was slightly convex, and it was slightly concave in M (Fig. 4a); but overall, both relationships were positive, i.e. more lateral roots led to higher performance over the entire range of lateral root numbers. The two selection gradients did not cross, and in plants exposed to M, the predicted performance was worse over the entire range of the number of lateral roots (Fig. 4a). Similarly, there existed a significant interaction between early and late treatment in the selection gradient of maximum root length (Table 2) and in all treatment combinations, longer roots led to better performance (Fig. 4b). The interaction unravelled that in MM and LL, the maximum root length was significantly ($p < 0.05$), but only slightly steeper compared to ML and LM (Fig. 4b).

The selection gradient of RWR significantly differed between levels of the late treatment (Table 2). In both treatment levels, the relationship between performance and RWR was concave (Fig. 5a), and the optimum performance was reached at higher RWRs (in L: optimal RWR of standardized variables = 0.123; back-transformed optimal RWR in Fig. 5a optimal RWR = 5.542) in the less compared to the more predictable treatment (in M: optimal RWR of standardized variables = 0.095; back-transformed optimal RWR in Fig. 5a optimal RWR = 4.497). The selection gradient of relative root branching and relative maximum rooting depth was affected by an interaction between early and late treatment (Table 2). In MM and LL, the selection gradient of relative root branching was significantly steeper than in LM (Fig. 5b, $p < 0.05$), and the selection gradient in MM of relative maximum rooting depth was significantly steeper than in the other treatment combinations (Fig. 5c, LL, LM and ML compared to MM $p < 0.05$). Moreover, the selection gradient in ML of relative maximum rooting depth was significantly weaker than that in LL (Fig. 5c, $p = 0.046$). In summary, higher relative root branching and higher relative maximum rooting depth led to lower performance in all treatment combinations, and the significant treatment effects reflect differences in the performance loss per unit of additional allocation (Fig. 5b–c).

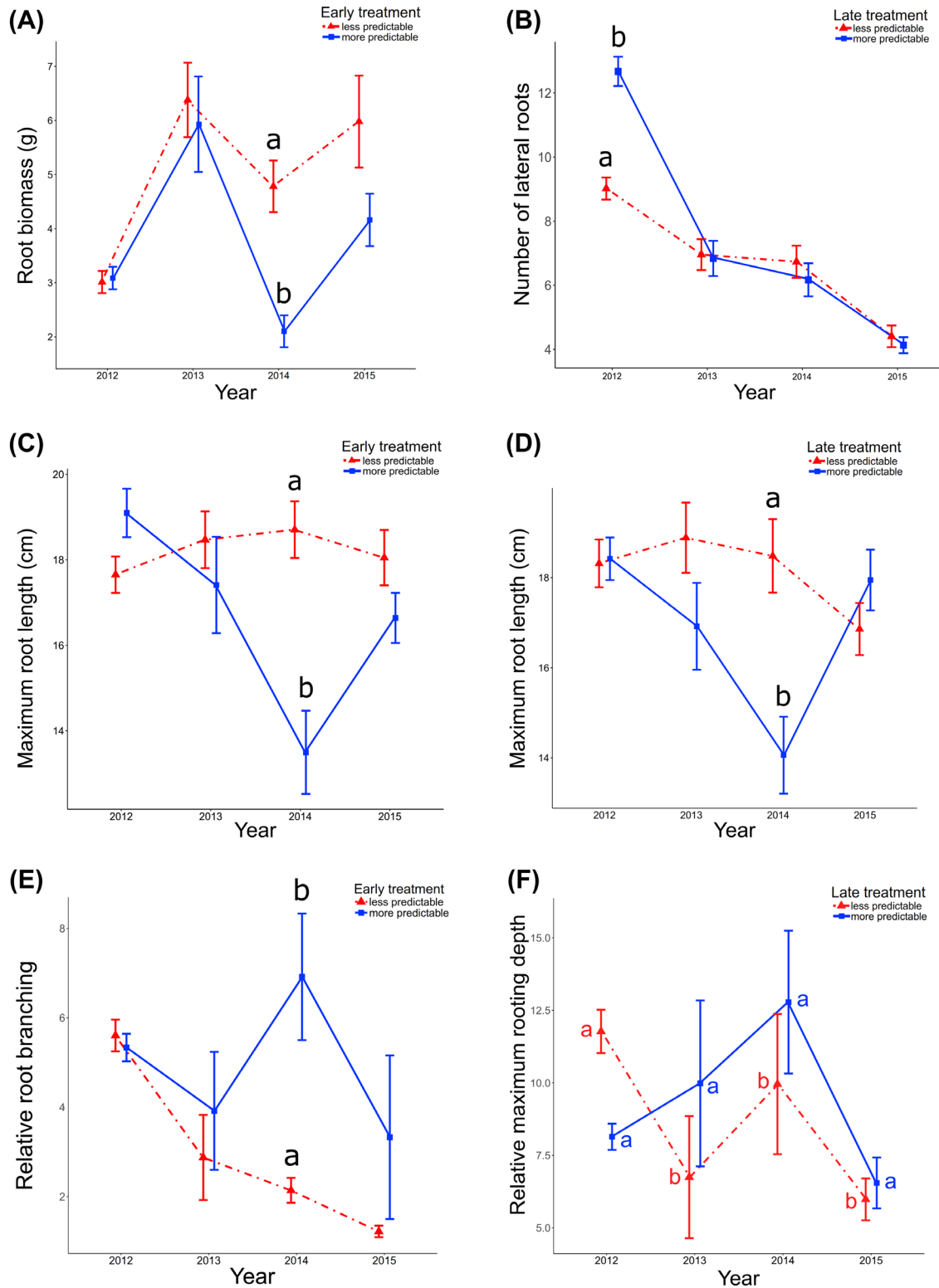


Figure 3. Significant treatment $\times$ year interactions on different root traits and allocation strategies. (A) Interaction between early treatment and year on root biomass. (B) Interaction between late treatment, and year on number of lateral roots. (C) Interaction between early treatment and year on maximum root length. (D) Interaction between late treatment and year on maximum root length. (E) Interaction between early treatment and year on relative root branching. (F) Interaction between late treatment and year on relative maximum rooting depth. Means $\pm$ SE are shown. Significant within-year post hoc contrasts among treatment combinations are indicated with black letters. In (F), significant within treatment level contrasts between years are indicated with differently coloured letters. Letter colours correspond to treatment levels.

Transgenerational responses

In the three years that ancestor and descendant generations were grown, no significant differences existed among ancestors and descendants in any of the functional root traits (for all functional root traits: $p \geq 0.1$ of the main effect, and all interactions including generation and treatment). The power to detect a treatment effect in descendants of the effect size observed in other traits (Fig. 2d, f of March-Salas et al. 2019) was 99%.

Discussion

How differences in environmental predictability affect organisms is of high interest given the current and projected future reductions in climatic predictability. However, few experimental studies have addressed effects of intrinsic predictability and, to our knowledge, none investigated the consequences of different time scales of predictability on functional root traits. Here we tested in a four-year field experiment the effects of subtle, non-catastrophic differences in daily and inter-seasonal predictability on functional root traits. Our study provides new insights into how root traits respond to changes in precipitation-predictability, whether and how differences in precipitation-predictability affect the selective regime to which functional root traits are exposed, whether treatment-induced changes in functional root traits are optimizing the plant's reproduction, and whether transgenerational responses in functional root traits exist with respect to differences in intrinsic precipitation-predictability.

Effects of precipitation-predictability on functional root traits

All functional root traits were significantly affected by differences in precipitation-predictability (Table 1), and four traits exhibited year-independent treatment effects (in root biomass: early $\times$ late treatment interaction; in root weight ratio: late treatment; in relative root branching: late treatment; and in relative maximum rooting depth: early $\times$ late treatment interaction). Overall years, MM plants exhibited consistently lower root biomass compared to the other treatment combinations (Fig. 2a), the allocation to roots was higher in the more compared to the less predictable late treatment (Table 1), roots exhibited relatively more branching in late M compared to late L, and the relative maximum rooting depth was smaller in LM compared to MM (Fig. 2b). Moreover, all but one root trait exhibited significant interactions with year (Table 1). In 2014, root biomass and maximum root length were significantly smaller under more predictable conditions (Fig. 3a, c, d), and allocation to root branching was significantly higher in the more predictable compared to the less predictable early treatment (Fig. 3e). Lower root biomass and shorter roots (i.e. shorter maximum root length in 2014) are associated with lower nutrient and carbon capture and lower carbon sequestration to the soil (Pierret et al. 2016). Moreover, shorter roots allow to access smaller soil volumes (Atkinson et al. 2014, Pierret et al. 2016) and thus less nutrients and water (Balachowski and Volaire 2018), and they may not reach the more constant humidity at deeper soil layers (Osmont et al. 2007). The effects of more predictable

Table 2. Experimental precipitation-predictability effects on the selection gradients of the root traits and allocation strategies. All dependent and independent variables were ln-transformed and standardized prior to analyses, and main effects of functional root traits and significant two- and three-way interactions between treatments and linear or quadratic functional root traits are shown. Significant p-values are shown in italics. N=590 in all analyses.

Parameter	χ^2	df	p-value
N lateral roots	5.81	1	<i>0.016</i>
N lateral roots $\times$ Early	5.66	1	<i>0.010</i>
N lateral roots ²	0.10	1	0.754
N lateral roots ² $\times$ Early	4.55	1	<i>0.039</i>
Maximum root length	241.92	1	< <i>0.001</i>
Maximum root length $\times$ Early	0.68	1	0.408
Maximum root length $\times$ Late	1.399	1	0.237
Maximum root length $\times$ Early $\times$ Late	5.81	1	<i>0.016</i>
Root weight ratio	16.57	1	< <i>0.001</i>
Root weight ratio ²	24.51	1	< <i>0.001</i>
Root weight ratio ² $\times$ Late	3.82	1	<i>0.048</i>
Relative root branching	516.25	1	< <i>0.001</i>
Relative root branching $\times$ Early	1.40	1	0.236
Relative root branching $\times$ Late	3.43	1	0.075
Relative root branching $\times$ Early $\times$ Late	3.93	1	<i>0.016</i>
Relative maximum rooting depth	1184.14	1	< <i>0.001</i>
Relative maximum rooting depth $\times$ Early	0.38	1	0.539
Relative maximum rooting depth $\times$ Late	10.60	1	<i>0.001</i>
Relative maximum rooting depth $\times$ Early $\times$ Late	5.41	1	<i>0.019</i>

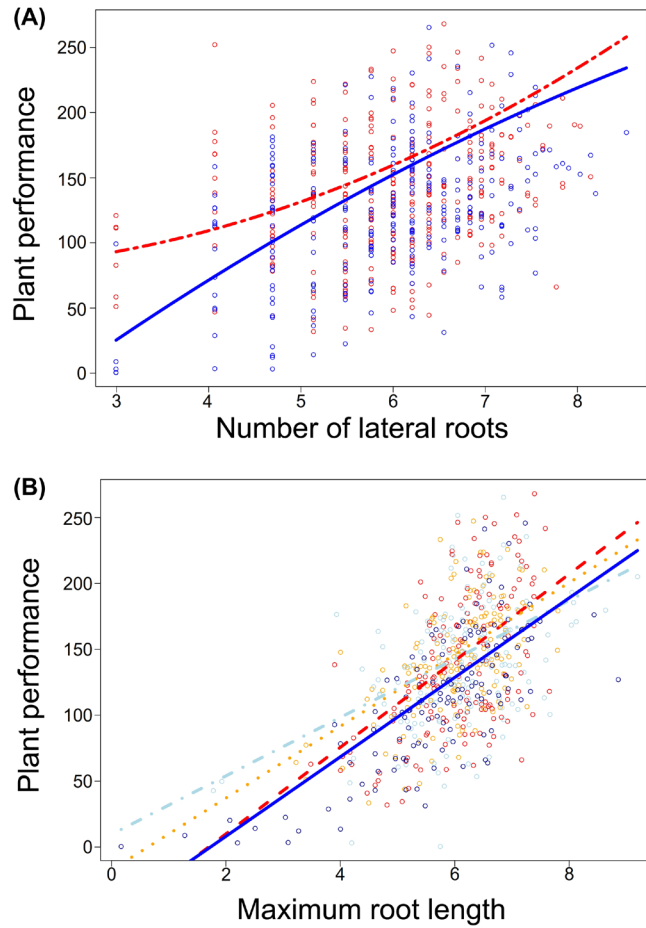


Figure 4. Treatment effects on the selection gradient of the number of lateral roots (A) and maximum root length (B). Model predictions of significant treatment effects on the selection gradient are shown (Table 2): early treatment (A), and early $\times$ late treatment (B). Colours correspond to different treatment combinations: red (LL in (B), and L in (A)), orange (LM), light blue (ML) and dark blue (MM in (B), and M in (A)).

precipitation on maximum root length during late growth (Fig. 3c–d), when ambient temperatures are 10–20°C higher than in spring (March-Salas et al. 2019; Supporting information) and drying out of the top soil layers is more likely (Supporting information), suggest that plants in the more predictable treatment had a disadvantage in 2014, and to a lower extent also in 2013. Moreover, higher allocation to roots by plants exposed to more predictable late precipitation (i.e. the year-independent late treatment effect on RWR, Table 1) indicates that plants in the M and L treatment had different allocation strategies. But despite the higher allocation, in 2014, root biomass and maximal root length were smaller in the more predictable treatment. Similarly, the year-independent investment in root branching and maximum root length were highest in M and MM (Table 1, Fig. 2b), but nevertheless the root biomass was smallest in MM (Table 1, Fig. 2a). This points to constraints imposed on plants exposed to more predictable precipitation that could not be overcome by changing the root allocation strategy. This hypothesis is strengthened by the absence of treatment differences in the number of lateral roots in 2013–2015 (Fig. 3b), absence of

allometric relationships in the number of lateral roots, and different allometric relationships in maximum rooting depth (Supporting information, Müller et al. 2000).

These results contrast with studies showing that lower environmental predictability negatively affects life-history traits and population trends (Parmesan et al. 2000, Masó et al. 2019), suggesting that different organisms exhibit different responses. This is in line with the findings of studies on the predictability of nutrient availability, which showed that different plant species reacted differently (Liu and van Kleunen 2017).

Significant differences among treatments also existed in 2012 in the number of lateral roots and the allocation to rooting depth compared to the other years (Fig. 3b, f), and no differences existed in root biomass, maximum root length and allocation to root branching (Fig. 3a, c–e). The lower number of lateral roots of plants exposed to L during late growth (Fig. 3b), together with the higher allocation to maximum rooting depth of plants exposed to L during late growth in 2012 (compared to 2013–2015; Fig. 3f), indicates that in 2012 plants were exposed to a tradeoff (Fig. 6). In L plants

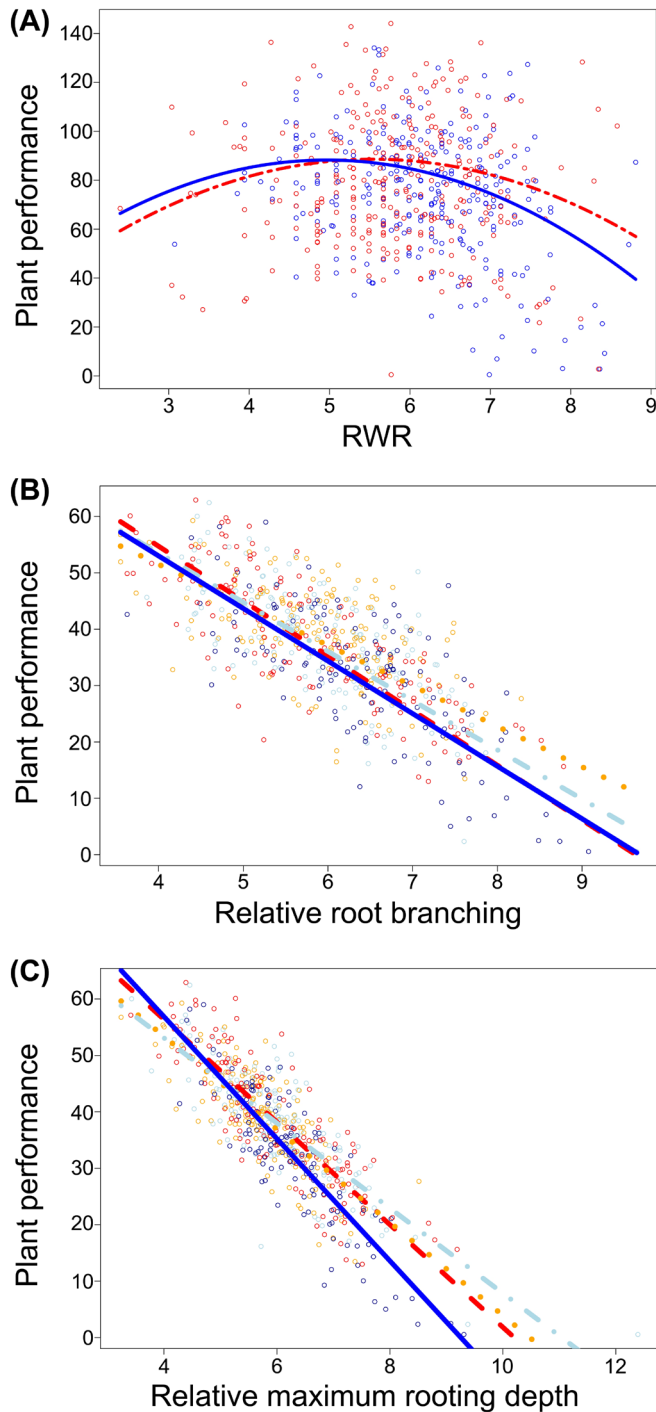


Figure 5. Treatment effects on the selection gradients of the root allocation strategies (root weight ratio [RWR], relative root branching and relative maximum rooting depth) per predictability level. Model predictions of significant terms including interactions with treatments are shown (Table 2): late treatment effect on the selection gradient of RWR (A), early $\times$ late treatment effect on the selection gradient of relative root branching (B), and early $\times$ late treatment effect on the selection gradient of relative maximum rooting depth (C; Table 2). Colours correspond to different treatment combinations: red (LL in (B) and (C), and L in (A)), orange (LM), light blue (ML) and dark blue (MM in (B) and (C), and M in (A)).

maximum rooting depth was bigger but less lateral roots were produced, which may be explained by the fact that 2012 was the driest summer (March-Salas et al. 2019) during which in L the top soil layer may have dried out most due to the low predictability of the experimental precipitation. In contrast, M plants produced more lateral roots but shorter maximum rooting depth, potentially because in M the experimental precipitation more regularly provided water to the top soil layers. The absence of significant differences between treatments in root biomass, maximum root length and allocation to root branching in 2012 (Fig. 3a, c–e), suggest that the changes in allocation observed in plants exposed to L allowed them to better cope with less predictable precipitation. This is in line with the higher survival of plants exposed to L compared to M in 2012 (March-Salas et al. 2019b). These results indicate that *O. viciifolia* rapidly altered its strategy to exploit temporally unpredictable resources (Wright et al. 2004, Franch-Gras et al. 2017, March-Salas and Perterra 2020), in order to maintain or increase its performance. This is in agreement with bed-hedging strategies that protect organisms against unexpected changes (Franch-Gras et al. 2017).

The significant early $\times$ late treatment interactions (Table 1) suggest that roots may have been affected by changes in inter-seasonal predictability. However, root biomass was higher under less predictable daily precipitation during early and/or late growth (i.e. in LL, LM and ML; Fig. 2a) and the relative maximum rooting depth differed between LM and MM (Fig. 2b). Thus, there was no evidence that higher (i.e. MM and LL) and lower (ML and LM) inter-seasonal predictability per se affects the allocation to roots, within-root allocation or the expression of root traits.

Selective regime to which root traits and allocation strategies are exposed

Precipitation-predictability affected the strength of the natural selection acting on all measured functional root traits (Table 2), which is in line with the observation that selective regimes can be predicted by precipitation factors and by the North Atlantic Oscillation (NAO) (Siepielski et al. 2017). All root traits (number of lateral roots and maximum root length) and all within-root allocation strategies (allocation to root branching and allocation to rooting depth) were exposed to directional selection (Fig. 4a–b, 5b–c). Thus, precipitation-predictability did not affect the direction and the type (e.g. directional versus stabilizing or disruptive selection) of the selective regime. This implies that plants of all treatment combinations should adopt the same strategy to increment performance. In contrast, allocation to roots (RWR) was exposed to a selective regime imposing stabilizing selection (Fig. 5a). In the less predictable late treatment, optimal RWR was reached at higher RWR values (i.e. optimal allocation to roots was 2.78% higher) compared to the more predictable late treatment (Fig. 5a), and there were no differences in the strength of selection imposed by precipitation-predictability (i.e. the curves of both treatments exhibit the same curvature). Thus, plants exposed to less predictable precipitation need to

allocate more resources to the roots to reach the optimum, and only in the narrow segment between the two optima, should plants of the two treatment groups exhibit different tactics to reach their optima. In all but one of the other functional root traits, the selective regime was strongest (steepest slope) under more predictable daily (Fig. 4a) and/or more predictable inter-seasonal precipitation (Fig. 5b–c). These findings are in line with theoretical predictions that more predictable climates and environments will provide stronger selection (Cohen and Levin 1991, Kingsolver and Buckley 2015, Caro et al. 2016), and it shows that this prediction is valid over short and long time-scales of predictability.

Performance consequences of differential allocation to root traits

The analyses of the selective regimes allow to understand which strategies optimize plant performance, and whether differences in root traits and root allocation may improve reproduction. A trait or allocation change that leads to higher performance reflects an advantage (Lande and Arnold 1983), and a change that leads to a performance decrease would point to constraints, e.g. imposed by changes in precipitation-predictability (Chevin 2013). Average RWR in late L was closer to the optimal RWR (distance from optima = 0.032) than in M (distance from optima = 0.123; Fig. 5a). In both treatments, average RWR was on the right of the respective optimum, and no differences existed in the curvature of the selection gradient. Thus, the RWR in late L led to higher performance compared to late M (Fig. 5a). This shows that under less predictable precipitation, plants better adjusted their

root allocation to the unpredictable soil resources than plants exposed to M (von Wangenheim et al. 2020). The observed root responses are in line with high plasticity (defined as environmentally initiated phenotypic change, without the need of underlying genetic variation, Levis and Pfennig 2016) of root traits allowing for rapid adjustment to different environmental conditions in order to increase their performance (Hodge 2004, Bardgett et al. 2014, von Wangenheim et al. 2020). The observed root responses are most likely species-specific, since they usually depend on the roots' architecture and size (Morris et al. 2017).

Furthermore, in this study, we also tested for transgenerational responses in the measured root functional traits and for differences in transgenerational responses among treatments. According to the selection gradients and depending on the trait, the trans-generational response might be strongest in plants exposed to more or to less predictable precipitation, given that the selection gradient (the slope) was steepest in maximum root length and relative root branching when exposed to less predictable early and late precipitation (see LL [red lines and dots] in Fig. 4B, 5B), and in relative maximum rooting depth when exposed to more predictable early and late precipitation (see MM [dark blue line and dots] in Fig. 5C). Nevertheless, in none of the treatments and for none of the functional root traits, we found a significant transgenerational response, despite the high statistical power with which trans-generational responses of the effect size observed in phenology and reproductive traits could have been detected (March-Salas et al. 2019). This suggests that the simulated differences in precipitation-predictability may not lead to fast and differential transgenerational responses (e.g. epigenetic changes,

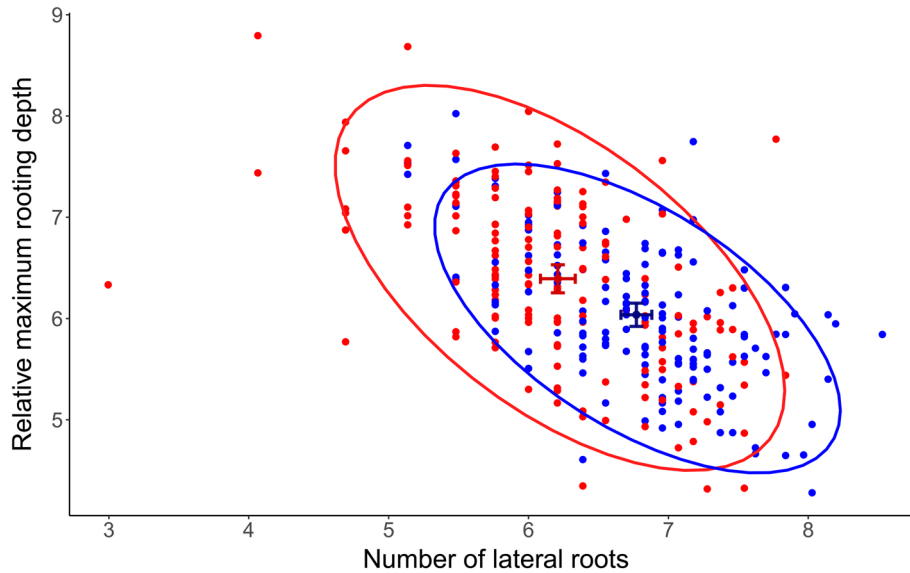


Figure 6. Tradeoff between investment into rooting depth and the production of lateral roots in 2012. The correlation between the two variables was negative (Pearson correlation: estimate $\pm$ SE = -0.592 ± 0.066 ; $r = -0.590$, $t = -9.031$, $p < 0.001$). Colours correspond to different treatments: red, less predictable precipitation during late growth, and dark blue more predictable precipitation during late growth. Plotted ellipses correspond to 95% confidence ellipses drawn for each treatment (Fox and Weisberg 2011). Dots reflect data points and their colour indicates the treatment level. The mean point and the vertical and horizontal standard errors are represented with coloured crosses for each treatment level.

maternal and paternal effects, etc., Yin et al. 2019) in functional root traits of *O. viciifolia*, which may be an advantage if plants are exposed to low inter-annual predictability (Dewar and Richard 2007). It also suggests that the ability of *O. viciifolia* to survive in rather dry climates, the length of its root system, and its perennial lifestyle may limit transgenerational responses in roots. In contrast to absence of precipitation-predictability-induced transgenerational effects on functional root traits, transgenerational responses with respect to precipitation-predictability existed in flowering time, probability of seed production and number of produced seeds, and thus in reproductive traits which are more closely related to fitness than the measured functional root traits (March-Salas et al. 2019). This suggests that *O. viciifolia* may prioritize transgenerational responses in traits directly related to reproduction over responses in traits that are less strongly related to reproduction and that may exhibit high plasticity. This difference is in line with tradeoffs between allocation to reproduction and roots (Hermans et al. 2006, Reich 2014), and it suggests that transgenerational responses may be more likely or manifest faster in reproductive traits than in functional root traits.

Conclusions

Our study on *O. viciifolia* experimentally demonstrates that functional root traits rapidly responded to differences in precipitation-predictability. The consistent rapid root responses observed under less predictable precipitation led to higher performance, which could not be explained by pure allometric growth (Müller et al. 2000, Weiner 2004). This shows that a species' immediate response to environmental changes does not only depend on average conditions, but also on the intrinsic predictability of the environmental conditions (Stenseth et al. 2002, Jackson et al. 2009, Lawson et al. 2015). In contrast to the expectations, lower predictability did not constrain plant performance, most likely thanks to the rapid responses observed in different functional root traits of the plants exposed to less predictable precipitation. Moreover, more predictable precipitation appeared to impose constraints, and led to lower performance. This suggests that thanks to allocation changes and differential expression of root traits, at least some plant species may be able to cope with projected reductions of precipitation-predictability (Reed et al. 2010). The absence of transgenerational effects in functional root traits further suggests that inter-generational changes in functional root traits may take longer than those observed in traits directly related to reproduction or that bigger and more constant effects are required to trigger transgenerational responses. This highlights the complexity of predicting long-term plant responses and ecosystem responses to ongoing changes in precipitation patterns (Griffin-Nolan et al. 2021).

The observed changes in functional root traits may have important consequences at different hierarchical levels, since they may not only affect a single plant, but even plant communities and ecosystem processes by means of inter-specific root-root interactions and root-soil interactions (Hodge

2004, Bardgett et al. 2014, Gherardi and Sala 2015). This implies that belowground investment strategies might be key for a deeper understanding of whether and how plants will deal with increasing climatic uncertainty.

Acknowledgements – We thank Guillermo Mercé, Blanca Santamaría, Diana Íñigo and Miguel Moreno for practical assistance, and María Urieta and Helena Clavero for technical assistance. We also thank Federico Fillat, Luis Villar, Gemma Palomar, Paloma Mas and Guillem Masó for their productive inputs.

Funding – Funding was provided by the Spanish Ministry of Economy and Competitiveness (CGL2012-32459, CGL2016-76918 AEI/FEDER, UE to PSF) and the Swiss National Foundation (PPOOP3_128375, PP00P3_152929/1 to PSF). MMS was supported by a PhD grant (BES-2013-062910) financed by the Spanish Ministry of Economy and Competitiveness.

Conflict of interest – The authors declare no competing interests.

Author contributions

Martí March-Salas: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal). **Mark van Kleunen:** Investigation (equal); Supervision (equal); Writing – review and editing (equal). **Patrick S. Fitze:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from digital.csic.es at <<https://digital.csic.es/handle/10261/253837>> (March-Salas and Fitze 2021).

Supporting information

The supporting information associated with this article is available from the online version.

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