Differential effect of climate on structural and biochemical plant traits

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ABSTRACT

Exploring patterns and causes of intraspecific trait variation is crucial for a better understanding of the effects of climate change on plant populations and ecosystems. However, our current understanding of the intraspecific trait variation is mainly based on structural (morphological) traits, and we have limited knowledge on patterns and causes of variation in biochemical traits (e.g., leaf pigments) which are also crucial for plant adaptation. As a result, we also do not know how similar the climatic effects on structural versus biochemical traits are.

Using plant traits from 110 genotypes representing 11 populations of *Festuca rubra* grown in 4 different climates, we studied trait covariation among structural traits (linked to fitness, resource use, gas exchange and reproduction) and biochemical traits (linked to photosynthesis, photoprotection and oxidative stress). We also disentangled the relative role of climate of origin and cultivation climate on structural versus biochemical traits and tested for adaptive plasticity in the traits.

We found that 1) biochemical traits did not covary with structural traits and represent *'photoharvesting – photoprotection'* strategy dimension of functional variation; 2) the interactive

effects of climate of origin and cultivation were more pronounced for biochemical than structural traits. 3) Trait plasticity was affected by the climate of origin (precipitation and temperature as well as their interaction); 4) *F. rubra* showed both adaptive as well as mal-adaptive plasticity and adaptiveness depends upon trait type, cultivation climate and climate of origin.

Overall, our results suggest that structural and biochemical plant traits respond differentially to climate and response of one group of traits thus cannot be predicted from the other. The responses are also strongly determined by interactions between the climate of origin and cultivation. More studies on variation in biochemical traits, their correspondence to other traits and their variation with climate are thus needed.

Key Words: climate change effects; adaptive plasticity; clonal plant; adaptation strategies; intraspecific trait variation; variance decomposition

INTRODUCTION

Plant traits determine various plant functions, define ecological strategies, describe responses to environmental change and also the effects of plants on ecosystem functioning (Westoby et al. 2002, McGill et al. 2006, Violle et al. 2007, Enquist et al. 2015). Therefore, establishing links between functional traits and environmental factors is crucial for understanding how plant populations and communities respond to climate change.

Traits vary both within and between species, however, our trait-based understanding is mainly based on interspecific trait variability and only recently, ecologists have focused on the implications and consequences of intraspecific trait variation (ITV) (Bolnick et al. 2011, Siefert et al. 2015, Westerband et al. 2021). Lately, ITV has been suggested to be equally important as interspecific variation for understanding, predicting, and managing large ecological processes (Bennett et al. 2016, Martin et al. 2017, Henn et al. 2018, Mitchell et al. 2020, Westerband et al. 2021).

ITV arises from both genetic differences and phenotypic plasticity (Matesanz et al. 2012). Intraspecific variation in traits due to genetic differences is achieved very slowly by the process of natural selection while plasticity allows for rapid responses. Many studies elucidated genetic versus plastic responses in various plant traits in various species (Blanquart et al. 2013, Münzbergová et al. 2017, Datta et al. 2017, Manzanedo et al. 2019, Kosová et al. 2022). However, most of these studies deal only with structural traits (i.e., morphological traits e.g., specific leaf area, plant height, plant biomass etc.). Variation in biochemical traits, such as contents of leaf pigments, is an important mechanism through which plants can increase mean fitness in a variable environment (Taiz et al. 2015), but variation in such traits is much less studied. Unlike structural traits, biochemical traits are easy to construct and thus easy to modify

with changing conditions within a growing season and could thus be more plastic than the structural traits.

Biochemical traits are closely related to the physiological activity of plants under variable environments (Taiz et al. 2015) and should be closely linked to structural traits as all the structures in plants are formed as a result of biochemical processes (e.g., high content of photosynthetic pigments should lead to increased plant biomass). The available ecological studies exploring biochemical traits study them separately of structural traits e.g. (Alonso-Amelot et al. 2007, Martin et al. 2007). Due to this, we do not know if the genetic versus plastic responses differ between structural and biochemical traits. Identifying specific plant functions that are more genetically controlled or are more plastic, is crucial for our ability to understand and predict species responses to the ongoing climate change more accurately (Henn et al. 2018, Pérez-Ramos et al. 2019).

Phenotypic plasticity is considered a major mechanism of response to environmental variability (Valladares et al. 2006, Nicotra et al. 2010, Henn et al. 2018). Despite a range of studies on the plasticity of various traits, we know little if the plasticity is adaptive. Plasticity is advantageous only if it increases plant fitness of an individual in a new environment (Nicotra et al. 2010). In such a case, it can be considered as adaptive (van Kleunen and Fisher 2001, Enquist et al. 2015). Recently, Enquist et al. (2015) suggested that we can determine whether phenotypic plasticity is adaptive by exploring if the trait changes toward its optimum in a specific environment (i.e., trait values in the home environment). If the traits of plants grown in a foreign climate converge towards traits of plants which are at home in that climate, the trait shift is considered adaptive. However, only very few studies have aCtempted to explore this so far (Henn et al. 2018, Bush et al. 2021).

The extent and adaptive nature of plasticity may be limited by genetic basis as well as by new environmental conditions (Valladares et al. 2006). For instance, plants from one climate of origin may be more plastic and plastic responses may be stronger to one environmental factor than to another (Münzbergová et al. 2017). Similarly, plants from a specific climate of origin may show adaptive plasticity in some climates but not in others. However, such knowledge is still largely lacking. Therefore, we also need to elucidate if the adaptive or mal-adaptive plasticity is consistent among populations from different climates of origin shifted to different novel climates. We also need to test if adaptive plasticity is consistent among different trait types (e.g., structural versus biochemical traits). This will enable us to better understand the adaptive potential of plant populations under climate change.

Both at intraspecific and interspecific levels, traits do not vary independently but covary with other traits (Westoby et al. 2002, Fajardo and Piper 2011, Walker et al. 2017, Yang et al. 2019). The covariation among traits is due to multiple traits having common functional roles or developmental pathways, or due to genetic linkages and pleiotropy (Armbruster and Schwaegerle 1996). Examining the trait covariation enhances understanding of plant ecological strategies and fundamental tradeoffs (e.g., acquisitive – conservative resource use strategy) and can also help to improve the prediction of one set of traits from others (e.g., root morphology from leaf morphology) (Westoby et al. 2002, Hu et al. 2019, Yang et al. 2019). However, we don't know about trait covariation patterns (and plant adaptation strategies) at biochemical levels and if the structural and biochemical traits covary in the same dimensions.

Considering the above gaps, we used plant genotypes sampled from 11 populations of *Festuca rubra* occurring in contrasting climates (climates of origin) and grown in 4 different

growth chambers (cultivation climates) and measured wide range of biochemical and structural traits of the plants to address the following questions:

1. What are the major dimensions of variation in structural and biochemical traits (trait co-variation)?
2. Do the relative role of climate of origin and cultivation climate and their interactions in trait variation differ among structural and biochemical traits?
3. What is the magnitude of variation in structural and biochemical traits in plants from different climate of origin in response to contrasting cultivation climates?
4. Is the plasticity in both structural and biochemical traits adaptive, and does the adaptiveness depend upon climate of origin and cultivation climate?

We hypothesized that 1) some of the structural traits co-vary with biochemical traits (e.g., plant biomass and content of photosynthetic pigments), because plant structural traits are the result of processes occurring at biochemical levels; 2) biochemical traits should be more plastic (and less genetically controlled) than the structural traits and thus less affected by the climate of origin than structural traits because of their flexible nature and ease in construction; 3) both structural and biochemical traits show adaptive plasticity, but because flexible nature and ease of construction, biochemical traits show a higher degree of adaptive plasticity.

METHODOLOGY

Study species and sampled localities for plant material

In the experiment, we used *Festuca rubra* ssp. *rubra*, a widespread perennial grass species occurring in the temperate grasslands in Europe. This species can reproduce both by seeds as well as clonally by rhizomes. The experimental plants of this species were collected in

2014 from 12 natural localities representing factorially crossed precipitation and temperature gradients corresponding to the SeedClim Grid (Meineri et al. 2014, Tingstad et al. 2015) in Western Norway. This grid represents an independent combination of three levels of mean summer temperature (4 warmest months, 6.5 °C, 8.5 °C and 10.5 °C) with four levels of annual precipitation (600, 1300, 2000 and 2700 mm).

The plant material used in the current study has been collected for previous studies, that have set up the experiments and provided data also for this study (Münzbergová et al. 2017). From each locality, at least 40 plants of *F. rubra* (at least 1 m apart) were collected in the growing season of 2014 and transported to the experimental garden of the Institute of Botany, Czech Academy of Sciences in Průhonice, Czech Republic (49°59′38.972″N, 14°33′57.637″E) and planted into pots. To ensure that each of the collected plants corresponded to a single genotype, the plants were reduced to a single ramet and planted into 16 × 16 × 16 cm pots in 1:2 soil:sand ratio in August 2014. Later, the plants were checked by flow cytometry and confirmed that all belonged to the hexaploid cytotype of *F. rubra* ssp. *rubra* (Šurinová et al. 2019). In one of the localities (i.e., 6.5 °C temperature and 1300 mm precipitation), *F. rubra* ssp. *rubra* was absent. So, finally, we worked with plants from only 11 localities.

In November 2014, 25 genotypes from each locality were transferred to a greenhouse with temperature between 5 and 10 °C. In the greenhouse, the genotypes were multiplied (for details see (Münzbergová et al. 2017)). At the end of February 2015, four ramets of each of the 25 genotypes from all the 11 localities were planted in separate pots, resulting in a total of 1100 ramets. One ramet of each genotype has been shifted to one of each 4 different cultivation climates (see next paragraph) so that each genotype is present in each cultivation climate.

Cultivation climates

The experimental plants were grown in 4 different growth chambers (Votch 1014) under conditions simulating four extreme localities in the SeedClim grid (wettest/driest combined with warmest/coldest) for the spring to summer climate in the field. The temperature and moisture in each of the growth chambers followed the course as in the simulated natural locality (for details, see Münzbergová et al., 2017). The moisture level in the growth chambers was monitored using TMS dataloggers (Wild et al. 2019) (three inside each chamber) and then adding the necessary amount of water to mimic the soil moisture of the natural localities (also monitored using the TMS dataloggers). Briefly, in the climate chambers with dry conditions, plants were watered with about 20 ml of tap water per plant, applied to the trays if the soil moisture was lower than 15%. In the wet regime, plants were cultivated under full soil saturation with ~1.5 cm water level in the tray. By manipulating the soil moisture in the growth chambers, soil moisture at localities with a certain precipitation level was mimicked. Thus, the moisture conditions in the growth chamber were referred to as precipitation values of the simulated localities. Day length and radiation levels inside the growth chambers were also controlled in a manner to mimic the conditions of the original localities (Münzbergová et al. 2017).

Due to the limitation of resources to take measurements of the biochemical traits from all the grown plants (see below), only 10 genotypes per locality were used for the purpose of this study. The other genotypes along with those considered in this study were used in other studies exploring the growth, fitness, and physiological traits of the plants (Münzbergová et al. 2017, Stojanova et al. 2018, Kosová et al. 2022, Thakur and Münzbergová 2022). The dataset used in this study comprised of samples collected from 440 plants (i.e., 11 localities × 10 genotypes × 4 cultivation climates = 440 individuals).

Plant trait measurements

From each genotype in each of the cultivation climate, we measured 11 biochemical traits and 9 structural (i.e., morphological) traits. The biochemical traits were represented by the content of antheraxanthin, β-carotene, chlorophyll a, chlorophyll b, phenolic substances, lutein, neoxanthin, superoxide dismutase (SOD), violaxanthin and zeaxanthin and xanthophyll cycle de-epoxidation state (DEPS). These biochemical traits have been estimated specifically for this study. The considered biochemical traits are related to plant photosynthesis, photoprotection and oxidative stress response (Table 1).

Structural traits included aboveground biomass, plant height, rhizome weight, root weight, stomatal density, stomatal size, and number of ramets. Additionally, specific leaf area (SLA) was measured from the 10 genotypes originating from each of the four most extreme climates of the climatic grid. All these structural traits have been also used in our previous studies to address totally different hypotheses (Münzbergová et al. 2017, Kosová et al. 2022, Thakur and Münzbergová 2022). The considered structural traits are linked to plant resource use, competitive ability, fitness, growth and gas exchange (Table 1).

During the experiment, in mid-June, the biomass of the plants has been cut at 3 cm to simulate biomass removal during regular management in the field sites. This biomass was used for trait measurements as described below. The number of ramets was counted and the length (cm) of the longest ramet (hereafter referred to as plant height) was measured at the end of August 2015 and the plants were subsequently harvested, sorted into aboveground parts, roots and rhizomes, dried to a constant mass at 60 °C and weighed.

The remaining traits were measured during biomass removal in mid-June 2015. Stomatal density and stomatal size (length) were measured on epidermal imprints generated using nail polish (Gitz & Baker, 2009). The impressions were mounted on microscope slides with transparent adhesive tape and stomata were counted from three different regions on a leaf (each 500 × 500μm). From each of these three areas, three stomata were randomly chosen, and their length was measured, resulting in nine measured stomata per sample. SLA was estimated as a ratio of leaf area (mm^2) to dry mass (mg). Leaf area was calculated on scanned folded leaves (as *Festuca* leaves are naturally folded and are often hard to unfold) multiplied by two to acquire the real area of the leaves.

The analyses of chlorophylls and carotenoids followed the protocol described in (Münzbergová and Haisel 2019). The freshly sampled leaves (collected from the plants in mid-June 2015) were frozen in liquid nitrogen, lyophilized for 24 h and stored in a freezer (-80 °C) until the analyses. The contents of chlorophyll (chlorophyll a and chlorophyll b) and carotenoids (b-carotene, lutein, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin) in all the samples were analyzed by High-performance liquid chromatography (HPLC; ECOM, Prague, Czech Republic) using a reversed-phase column (Watrex Nucleosil 120-5-C18, 5 mm particle size, 125 x 4 mm, ECOM, Prague, Czech Republic). Prior to HPLC injection, pigments were extracted from the leaves with acetone. In HPLC, elution was carried out for 25 min using a gradient solvent system acetonitrile/methanol/water (80:12:6) followed by methanol:ethylacetate (9:1), the gradient was run at the time of 2–5 min. The flow rate was 1 ml min^{-1} and the detection wavelength was 445 nm. Each sample was analyzed twice, and the results were averaged. The contents of all the pigments were expressed as mg/g of dry weight. The de-epoxidation state (DEPS) was derived from xanthophyll pigments following (Pospíšilová et al. 2000).

Total content of phenolic compounds was quantified spectrophotometrically from 80% methanolic extract of dried leaves using modified Folin-Ciocalteu method (Folin and Ciocalteu 1927). The absorbance was measured in 1 cm cuvette at 765 nm. The total phenolic compound concentration in the analyzed extract was expressed in gallic acid equivalents.

SOD was extracted from frozen leaves homogenized with mortar and pestle in 2.5 ml buffer (0.1 M Tris-HCl, 1 mM dithiothreitol, 1 mM Na2EDTA, 1% Triton X-100, 5 mM ascorbic acid, pH 7.8) and a small amount of PVP (polyvinylpyrrolidone). Samples were incubated on ice in the dark for 30 min and centrifuged (16,000 g, 10 min, 4 °C). Pellets were discarded, and supernatants were divided into Eppendorf tubes, frozen in liquid nitrogen, and stored at -70 °C (Lubovska et al., 2014). Later, supernatants were used for gel electrophoresis and SOD was stained on the gel (Fridovich 1986). These stained gels were scanned and densitograms were created and analyzed in the ImageJ program (Schneider et al. 2012). The relative activity of total SOD was estimated as the sum of intensities of bands expressed as peak areas.

Data analysis

All statistical analyses were performed using R version 4.0.5 (R Development Core Team 2019). Our data were having some missing values (less than 1 % for any trait). Therefore, prior to analysis, missing values in the data were imputed using the 'missMDA' package (Josse and Husson 2016). This imputation of missing values was important specifically for doing multivariate analyses. SLA was estimated only for genotypes from 4 localities (corners of SeedClim grid), and therefore it was only used in analyses involving traits from genotypes from

only these localities and not used in any of the following analyses involving genotypes from all the localities.

Trait covariation analysis and dependence of traits on climate

We used principal component analysis (PCA) [‘vegan’ package (Oksanen et al. 2019)] to characterize trait covariation patterns and to identify the major dimensions of functional variation. The number of principal components to retain after PCA was determined on the basis of randomization-based procedure (Peres-Neto et al. 2005) using rndLambdaF function of ‘PCDimension’ package (Wang et al. 2018). To analyze the dependence of traits on temperature and precipitation of original and cultivation climate and their interaction, we used redundancy analysis (RDA, vegan package) and tested their effects using permutation tests. In this analysis, all the variables and their interactions had significant effect on traits, but some had only very low explanatory power. Therefore, in the final model, we only retained the variables which were significant and explained at least 1% variation in the data. A table showing the change in R^2 upon addition of the given predictor to the model along with significance is shown in Supplementary Table 1.

Relative role of different factors in variation of structural versus biochemical traits

To investigate the relative role of each factor (temperature and precipitation of origin and cultivation climate and their interactions) in trait variation, we determined what fraction of the total variance was explained by each factor independently. This was done for each trait individually by performing variance decomposition analysis using linear mixed-effects model in ‘lme4’ package. In these models, the intercept was the only fixed effect and all the factors (i.e.,

temperature and precipitation of origin and cultivation climate) and their interactions, as well as genotype, were entered as random effects. The variance attributable to each factor was then expressed as a percentage of the total variance. Such models for partitioning the total variance into various components have been widely used (Messier et al. 2017, Isaac et al. 2017, Anderegg et al. 2018, Henn et al. 2018). Before analysis, the traits with non-normal distributions were transformed appropriately (log or cube root).

The variance partitioning results obtained from the analyses above were then used to test if the effect of climate of origin, cultivation climate and interactions between the climate of origin and cultivation differ among trait types. The variance due to climate of origin was taken as the sum of variation explained by original temperature, original precipitation and their interaction (Otemp, Oprec and Otemp × Oprec in Supplementary Table 1), due to the cultivation climate as variance explained by cultivation temperature, cultivation precipitation and their interaction (Ctemp, Cprec and Ctemp × Cprec in Supplementary Table 1). The variance due to all the remaining interactions represents the variance due to the interaction of climate of origin and climate of cultivation. The significant effect of trait type on explained variance was analysed using the Mann-Whitney test with explained variance as a response variable.

We also estimated the plasticity of each trait for each genotype across all the four cultivation climates as

Plasticity = (maximum value – minimum value) / maximum value

These plasticity values were then used to test the effect of climate of origin on trait plasticity. The relationship between the climate of origin and plasticity of all the traits was analyzed using RDA with plasticity values as the response variable and climate of origin (temperature and precipitation and their interaction) as explanatory variables.

Trait adaptive potential

To estimate trait adaptive potential, we first performed a PCA analysis of the standardized trait values separately for biochemical and structural traits and expressed the position of each genotype (in each growth chamber separately) as its PC scores. For structural traits, we used scores of the first 3 axes and for biochemical traits, we used scores of the first 2 axes. The number of PCs to be retained was again determined based on randomization-based method as mentioned earlier. In structural traits, the first three axes of the PCA explained 32%, 18% and 15% of the total variance, respectively. For biochemical traits, the first two axes explained 65% and 13% of the total variance, respectively. These scores later served as summarised biochemical and structural traits. We then tested the effects of climate of origin and cultivation climate and their interaction on these scores using linear mixed-effects models with genotype as a random factor in 'lme4' package (Bates et al. 2015) in R. For model statistics see supplementary Table 1. This test has only been done for the 4 extreme populations corresponding to the climates simulated in the growth chambers and thus also included SLA values.

Based on these models, we calculated marginal means of the summarised traits for plants of each origin in each cultivation climate. We then estimated standardized effect size among pairs of means and tested which pairs of means differed significantly from each other. Both marginal means and standardized effect sizes were estimated using 'emmeans' package (Searle et al. 2022) in R. We used this information to make inferences if traits converged or diverged or none of the two in response to cultivation climate. For example, let us consider two climates A and B. In case of convergence, when plants originating from A will be grown in B climate their

traits will be more similar to plants of origin B grown in B climate than the traits of plants originating in A and grown in A. Divergence means the opposite. Therefore, in case of trait convergence among plants (from different climates of origin) grown in a common climate, there will be significant shift in the estimated marginal mean of plants grown in a foreign climate towards that of plants that are grown in their home climate (decreased standardized effect-size). According to Enquist et al. (2015), phenotypic plasticity may be adaptive if it results in the shift of trait values toward an optimum that is adaptive for a specific environment, i.e., a trait can be considered as adaptative if we detect trait convergence, while trait divergence indicates mal-adaptation.

RESULTS

Trait covariation

The PCA showed that the studied traits are not independent but show covariation patterns. Our analysis revealed 5 independent dimensions of trait variation and structural traits varied independently of biochemical traits. The first 5 axes of the PCA accounted for 66% of the total trait variation (Table 2 and Supplementary Figure 1). The PC 1 alone explained more variation than the other 4 axes combined and was dominated mainly by biochemical traits related to photosynthetic machinery (photosynthetic traits as opposed to photoprotective traits). Root weight also varied along PC1 but orthogonally to biochemical traits (Figure 1). The remaining PC axes represented traits related to plant fitness and biomass allocation (PC2), gas exchange (PC3), low wavelength light harvesting (PC4) and plant size (PC5) (details in Table 2 and Supplementary Figure 1).

Effect of original and cultivation climate on traits

The RDA analysis showed that climatic factors have a significant effect on traits (Supplementary Table 2A) and explained 37% of their total variation. The first two axes jointly explained 31 % of the variation with axis 1 explaining 8 times more than axis 2. Precipitation of cultivation climate (Cprec) was the key variable along with the interaction of Cprec with precipitation of origin (Oprec) and temperature of cultivation conditions (Ctemp) associated with axis 1 (Figure 2). Ctemp along with Cprec × Ctemp were the variables associated with axis 2. Traits related to photosynthetic machinery increased towards wetter cultivation climate, whereas the traits related to photoprotection and biomass of belowground parts tend to have higher values in plants cultivated in drier climates. The key variable that tends to increase with increasing temperature of cultivation climate were plant height and aboveground biomass.

Climate change [difference between precipitation and temperature of cultivation and original climate)] had a significant effect on traits. In the RDA with change in precipitation and temperature as predictors, 24% of the total variance in traits was explained (Figure 3). Axis 1 explained a lot more (10×) variation than axis 2. Change in precipitation was associated with axis 1 and change in temperature was associated with axis 2. Interaction between change in precipitation and change in temperature was not associated with any of the first two axes. From the RDA plot, it is evident that photoprotective pigments and allocation to belowground parts (roots and rhizomes) increased while photo-harvesting pigments decreased in plants shifted to drier conditions (Figure 3). Plant height increased in plants shifted to warmer conditions.

Drivers of trait variation in structural versus biochemical traits

The relative role of cultivation climate and its interactions with the climate of origin on traits was specific to trait type ($P<0.05$ in the Mann-Whitney test). The variation in structural traits was significantly higher due to the interaction of climate of origin with cultivation climate but was significantly lower due to cultivation climate (Figure 4). However, the effect of trait type on variance explained by the climate of origin was non-significant.

Genotypes from different climates of origin differed in their plasticity. In the RDA, a total of 20 % of the variability in the plasticity of traits was explained by the climate of origin. Both the precipitation and temperature of origin as well as their interaction significantly affected trait plasticity ($P<0.05$). Biochemical traits (except phenol and SOD) of plants originating from wet or wet and warm climate (axis 1) tend to be more plastic than biochemical traits of plants originating from drier climates (Figure 5).

Trait adaptive potential

In structural traits, the overall tendency was to converge (i.e., the traits showed adaptive potential). PC1 which was loaded with plant fitness traits (i.e., number of ramets and plant biomass traits) showed the highest degree of convergence (Table 3, Supplementary Figure 2) while PC2 scores (representing gas exchange traits) mainly showed no change. The results based on PC3 (representing plant size) also showed either convergence or no change. Biochemical traits representing PC1 (photoharvesting and photoprotection related traits) tend to diverge except convergence in cold and wet cultivation climates (Table 3, Supplementary Figure 3) while those representing PC2 (related to low wavelength light harvesting) tend to converge. Overall, these results suggest that *F. rubra* shows adaptive plasticity as well as mal-adaptive plasticity in

response to different cultivation climates. However, adaptive plasticity depends upon trait type, cultivation climate and climate of origin.

DISCUSSION

Independent covariation of biochemical traits and *photoharvesting – photoprotection* strategy dimension

One of the key findings (contrary to our expectation) of trait covariation analysis was independent variation of structural and biochemical traits in the multivariate trait space. The possible reason for the observed patterns (i.e., for decoupling in trait variation among structural and biochemical traits) could be that plants have distinct strategies at biochemical and structural levels and differential effects of climatic factors on morphological traits than on biochemical traits. This lack of covariation among biochemical and structural traits limits our ability to predict biochemical plant functions from morphological plant functions or *vice-versa.* Our results, therefore, highlight the importance of incorporating biochemical plant traits (which are largely ignored) in ecological studies to better understand plant functioning in response to environmental change.

The second key finding emanating from covariation analysis was covariation between photosynthesis and photoprotective biochemical traits along the first axis. At the positive end of this axis, there were individuals with high photoprotective and low photosynthetic pigments, a syndrome that can be considered as the '*photoharvesting – photoprotection*' strategy dimension of functional variation. This strategy dimension is inferred following Wright et al (2002) who proposed the famous leaf economics spectrum ('*conservative-exploitative'* resource use strategy based on the leaf trait covariation).

The strategy that we propose represents trade-off among leaf biochemical traits related to photosynthetic machinery and is characterized by an increase in photoharvesting pigments with a decrease in photoprotective pigments or *vice versa*. Higher amounts of photoprotective pigments are important in high light conditions for the protection of photosynthetic machinery (Ramírez-Valiente et al. 2015, Munné-Bosch et al. 2016) and high amounts of photoharvesting pigments are important in low light conditions to make maximum use of available solar radiation. In this study, all genotypes were grown under the same light conditions in each cultivation climate simulating the original light conditions in Norway, hence, light is not the direct driving factor here. Variation in leaf pigments in this study may be determined by temperature and precipitation (of both origin and cultivation climates) in addition to light. For instance, plants growing in higher temperatures and drier conditions have higher chance of photodamage (Taiz et al. 2015, Szymańska et al. 2017) and hence higher amounts of photoprotective pigments are needed even if light is unchanged.

Another trade-off evident in our analysis was between stomatal size and stomatal number. This corroborated with earlier studies (Lawson and Blatt 2014, Kardiman and Raebild 2018). Trade-off between stomatal size and number is linked to plant gas exchange strategy. Plants with large and few stomata tend to have higher water-use efficiency, but are compromised with reduced photosynthetic rate, compared with plants that have many but smaller stomata. The higher gas exchange efficiency of smaller but higher number of stomata per unit area is because they are easy to regulate (Franks and Beerling 2009, Taiz et al. 2015). Considering this, higher number of smaller stomata per unit area may be important in drier and warmer climates for efficiently using water to fix carbon. In this study, stomatal size and plant aboveground biomass also covaried closely in same direction suggesting a close link between stomatal size and

biomass production. However, currently much less is known about linkage between biomass and stomatal traits. Smaller and greater number of stomata cause loss of excessive water through transpiration leading to sub-optimal gas exchange as well as lost opportunities for photosynthesis (Lawson and Blatt 2014) and could be the possible reason for lower plant biomass (when smaller but dense stomata are present).

Climate change effects on structural and biochemical traits

Temperature is often considered more important than precipitation in climate change context (Pepin et al. 2015, Bjorkman et al. 2018, Vitasse et al. 2018), however, our results suggested that change in precipitation (cultivation – original climate) had stronger effect on plant traits than change in temperature. Additionally, structural traits are the most used to understand climate change impacts (Valverde-Barrantes et al. 2017, Bjorkman et al. 2018, Henn et al. 2018, Yang et al. 2019, Shen et al. 2019) [biochemical in case of a few crop or experimental species (Renaut et al. 2005, Lei et al. 2006, Hekneby et al. 2006, Pradhan et al. 2019)], and the effects were stronger in biochemical traits. Especially, photosynthesis related traits were the most affected by climate change (cultivation – original climate) suggesting that the most important process leading to primary production in the Biosphere is the most sensitive to climate change. The greater effect of change in precipitation on photosynthesis related traits could be because photosynthesis is closely related to gas exchange through stomata and the stomatal opening and closing depends upon water availability (Taiz et al. 2015, Kardiman and Raebild 2018). Plant height and plant aboveground biomass increased and root mass decreased with increase in temperature of cultivation conditions. This can be attributed to lower rate of photosynthesis (carbon shortage) or direct thermal restriction of tissue formation (Hoch et al. 2002). The

possible reason for decreased allocation to root mass in warmer and wetter cultivation climates could be linked to increased resource availability [water and nutrient availability in soil, because of increased mineralization] (Pugnaire et al. 2019). The increased root mass was also linked to decrease in precipitation of cultivation. This is the expected result as more roots are needed to meet the water demand in drier climates. Overall, these findings highlight the importance of studying change in precipitation and incorporating biochemical traits in studies to better understand climate change impacts on plants.

Interactive effects of climate of origin and cultivation climate are stronger on biochemical traits

High intraspecific trait variation is considered important in population persistence (Jung et al. 2010, Bolnick et al. 2011) and can be achieved through genetic differentiation and/or plasticity (Matesanz et al. 2012). We expected structural traits to be more genetically differentiated than biochemical traits. In contrast to this, we found that biochemical traits were more strongly affected by interaction of climate of origin and cultivation climate (i.e., genetic differentiation in plasticity) than structural traits. This implies that individuals from some plant populations will be better able to persist under novel climates than others. Genetic controls over trait variability in plants in both structural (Münzbergová et al. 2017, Laitinen and Nikoloski 2019) and biochemical traits (Benomar et al. 2016, Kreyling et al. 2019) has been already reported. However, evidence for higher genetic differentiation in plasticity in biochemical traits than structural traits is not reported previously up to the best of our knowledge. We theorize that the biochemical traits are directly downstream of gene expression, enable quick response and are also easy to construct, and therefore, could have higher genetic differentiation in plasticity. On

the other hand, the structural traits are one more step downstream than biochemical traits (i.e., structures are product of biochemicals), require more time and lot of energy, thereby leading to lower genetic differentiation in plasticity in these traits.

Plants originating from climates with higher precipitation or a combination of higher temperature and precipitation, tend to have higher plasticity in biochemical traits compared to structural traits. The possible reason is that plants originating from drier and warmer climates always tend to have high levels of photoprotective pigments (because of higher risk of photodamage) (Ramírez-Valiente et al. 2015) causing lower plasticity. While in climates with non-limiting conditions, there is no such requirement and plants show more plastic responses. However, this needs to be validated using gene regulation studies. The other possible reason could be attributed to intraspecific trait convergence at stressful climates due to which plants originating from more limiting climates should have more similar trait values (i.e., lower plasticity). However, why it is more evident in biochemical traits is still an open question.

Adaptiveness of plasticity depends upon climate (origin and cultivation) and trait type

Adaptive plasticity may facilitate or even speed up the process of adaptive evolution (Ghalambor et al. 2007). We did find evidence for adaptive as well as mal-adaptive plasticity in this study. However, we also report that, structural traits showed adaptive plasticity while biochemical traits largely showed mal-adaptive plasticity. These findings are in line with studies of (van Kleunen and Fisher 2001, Henn et al. 2018, Kreyling et al. 2019, Laitinen and Nikoloski 2019) suggesting that adaptive as well as mal-adaptive responses in plasticity might be quite common. Our findings suggest that the adaptiveness is also dependent upon the type of plant function studied. The possible reason could be that when plants are growing outside their home

climates (i.e., non-optimal climates), they are under stress (Lichtenthaler 1998) which is causing divergence in biochemical traits. We also believe that greater genetic differentiation in plasticity in biochemical traits (see Figure 4) might also be limiting adaptive responses in biochemical traits.

Another important question is, if plants are showing adaptive plasticity in climates that are predicted for the future. In the context of climate change scenarios of the studied populations (i.e., in Norway), warming and increased precipitation are predicted (Hanssen-Bauer et al. 2017). By looking into our findings (related to adaptive plasticity in populations originating from cold and dry climates and cultivated in warm and wet climates), the species is likely to show adaptive plasticity in structural traits and will likely show mal-adaptive plasticity in photosynthesis related traits. Even if the climate change will be in the other direction (i.e., warmer and drier), the adaptive responses of populations will remain the same.

This combination of adaptive plasticity in structural and mal-adaptive plasticity in biochemical traits will likely lead to incomplete overall adaptive responses in plants (Ghalambor et al. 2007) and may have negative consequences to some extent on plant population persistence in the future (Lande 2009, Chevin et al. 2010). Furthermore, plasticity is also affected by climate of origin (Münzbergová et al. 2017), which possibly have resulted in plants from different climates of origin showing adaptive plasticity in some cultivation climates and neutral response or non-adaptive in others. Considering this, some populations are expected to adapt better than others to climate change through mechanism of adaptive plasticity. The ability of this species to adapt to the novel climate is also supported by Münzbergová et al. (2021), demonstrating that *Festuca rubra* (i.e., the same model species in the same study system as used here) is capable of real rapid evolution by selection of genotypes best adapted to novel conditions.

CONCLUSIONS

We conclude that the structural and biochemical plant traits respond differentially to climate. Biochemical traits also represent an important but independent plant strategy linked to plant photosynthetic machinery i.e., *'photoharvesting – photoprotection'* strategy dimension of functional variation. The responses of each trait type to climatic factors are complex and interactive effects of climate of origin and cultivation are very important (different populations will respond in a different way to novel climates). The traits showed adaptive as well as mal-adaptive plasticity, however, adaptiveness also depends upon traits under consideration along with climate (of origin and cultivation). Therefore, studying biochemical traits is important to better understand climate change effects on plants and predict the ability of plants to persist under climate change.

ACKNOWLEDGEMENTS: We thank the POPEKOL discussion group for useful comments on the manuscript draft. The study was supported by the project GACR 19-00522S and institutional research projects RVO 67985939 and MSMT. We also thank Vigdis Vandvik and her team for granting access to the SEEDCLIM climate grid from where the initial material had been obtained. DT also acknowledges "European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101038052" for providing research fellowship.

AUTHOR CONTRIBUTIONS: DT and ZM conceptualised the study. ZM supervised all the work including experiments and secured funding to carry out all the experimental work. DT

analysed the data with extensive comments and suggestions from ZM. VH, RS, HS, DH and NW collected the experimental data. DT wrote the draft of the paper with extensive suggestions, inputs and editing from ZM. TD helped in some of the data analysis and provided comments during manuscript preparation. All the authors commented on and approved the final version of the manuscript.

CONFLICT OF INTEREST: Nothing to declare

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Figures and tables

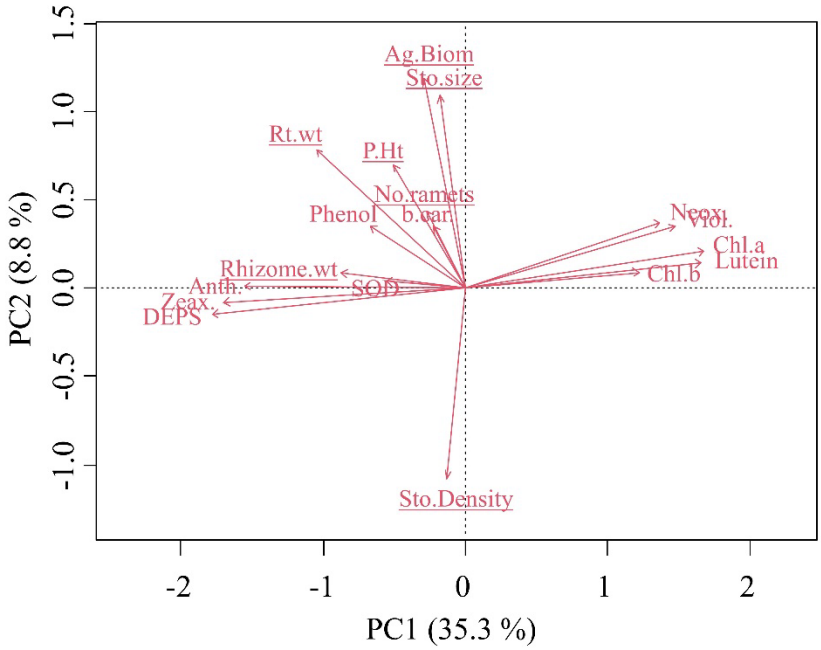


Figure 1: Principal component (PC) showing covariation patterns of studied functional traits. Structural traits are underlined. Trait abbreviations are as: Ag.Biom = Aboveground biomass; P.Ht = Plant height; Rhizome.wt = Rhizome weight; Rt.wt = Root weight; Sto.Density = Stomatal density; Sto.Size = Stomatal size; No.Ramets = Number of ramets; Anth. = Antheraxanthin; b-car = β-carotene Chl.a = Chlorophyll a; Chl.b = Chlorophyll b; DEPS = xanthophyll cycle de-epoxidation state; Neox. = Neoxanthin; SOD = Superoxide dismutase; Viox. = Violaxanthin; Zeax. = Zeaxanthin; Phenol = phenolic compounds.

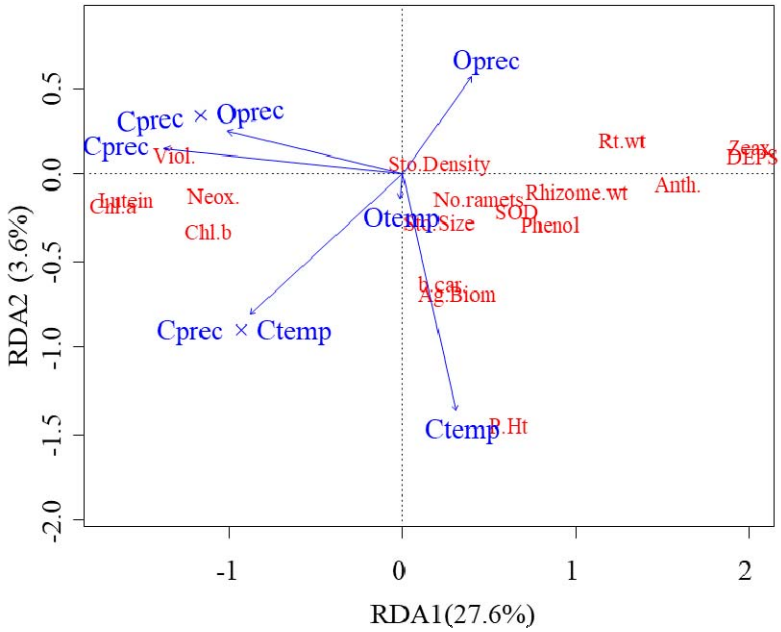


Figure 2: RDA plot representing the effects of cultivation climate and climate of origin on various traits. The names of structural trait are underlined. Ag.Biom = Aboveground biomass; P.Ht = Plant height (cm); Rhizome.wt = Rhizome weight ; Rt.wt = Root weight; Sto.Density = Stomatal density; Sto.Size = Stomatal size; No.Ramets = Number of ramets; Anth. = Antheraxanthin; b-car = β-carotene; Chl.a = Chlorophyll a; Chl.b = Chlorophyll b; DEPS = xanthophyll cycle de-epoxidation state; Neox. = Neoxanthin; SOD = Superoxide dismutase; Viox. = Violaxanthin; Zeax. = Zeaxanthin; Phenol = phenolic compounds. Oprec and Otemp represent precipitation and temperature of climate of origin; Cprec and Ctemp represent precipitation and temperature of cultivation climate.

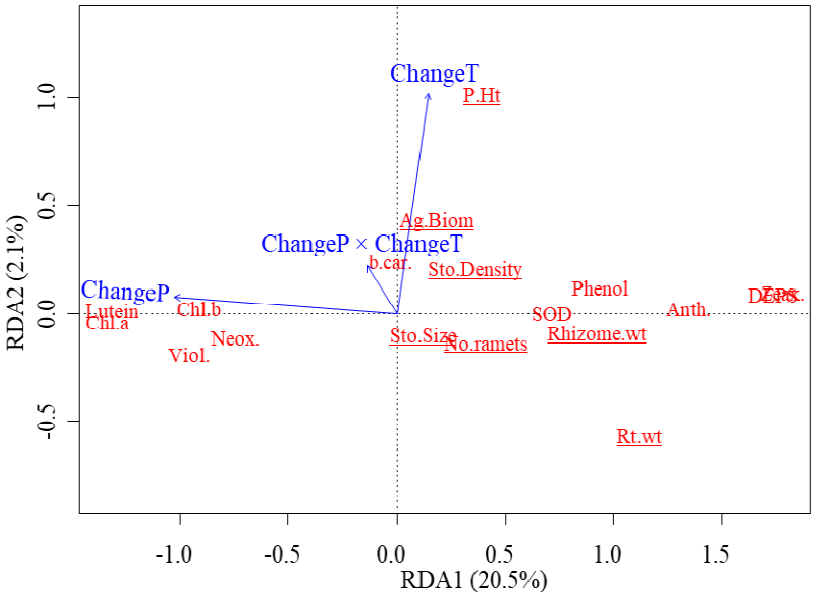


Figure 3: RDA plot representing effect of climate change (cultivation - origin) on various traits. The names of structural trait are underlined. Ag.Biom = Aboveground biomass; P.Ht = Plant height (cm); Rhizome.wt = Rhizome weight; Rt.wt = Root weight ; Sto.Density = Stomatal density; Sto.Size = Stomatal size; No.Ramets = Number of ramets; Anth. = Antheraxanthin; b-car = β-carotene; Chl.a = Chlorophyll a; Chl.b = Chlorophyll b; DEPS = xanthophyll cycle de-epoxidation state; Neox. = Neoxanthin; SOD = Superoxide dismutase; Viox. = Violaxanthin; Zeax. = Zeaxanthin; Phenol = phenolic compounds. ChangeP and ChangeT represent difference in precipitation and moisture between origin and cultivation climate (cultivation-origin).

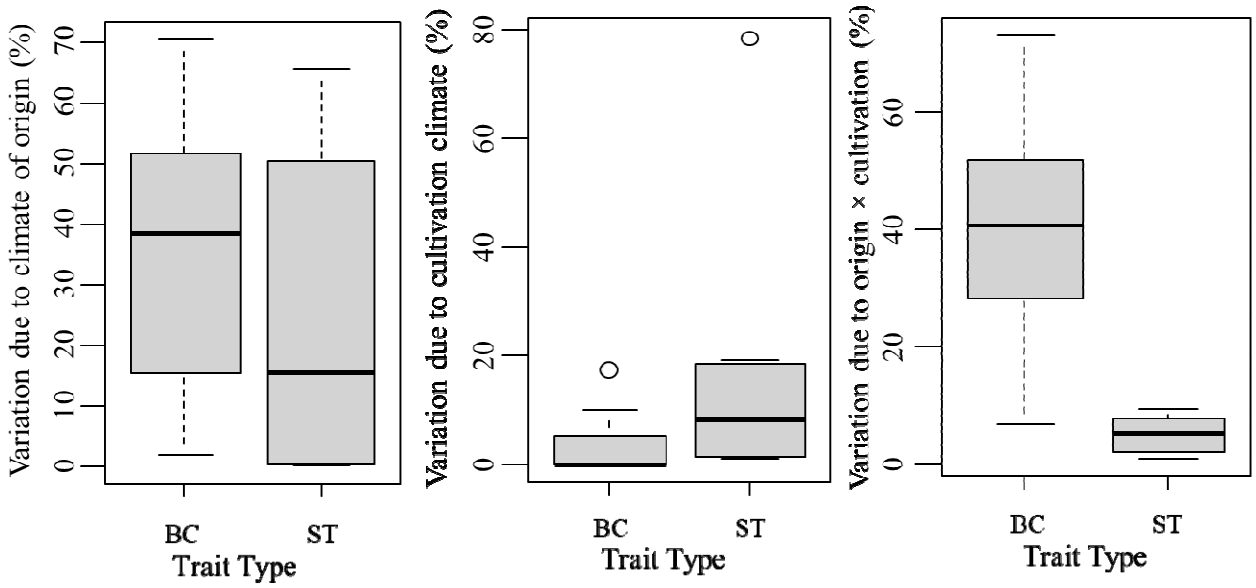


Figure 4: Boxplots showing the fraction of total variation explained by climate of origin, cultivation climate and their interaction in biochemical (BC) versus structural (ST) traits. Dark horizontal lines in the plots represent the median value and the grey part in each boxplot represent the interquartile range.

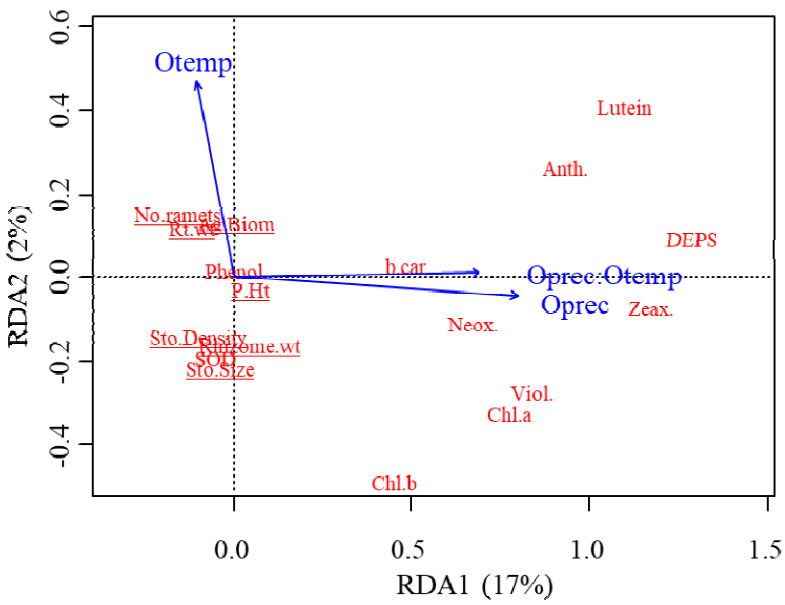


Figure 5: RDA plot representing the effect of climate of plant origin on plasticity in plant traits. The names of structural trait are underlined. Ag.Biom = Aboveground biomass; P.Ht = Plant height (cm); Rhizome.wt = Rhizome weight; Rt.wt = Root weight; Sto.Density = Stomatal density; Sto.Size = Stomatal size; No.Ramets = Number of ramets; Anth. = Antheraxanthin; b-car = β-carotene; Chl.a = Chlorophyll a; Chl.b = Chlorophyll b; DEPS = xanthophyll cycle de-epoxidation state; Neox. = Neoxanthin; Phenol = phenolic compounds; SOD = Superoxide dismutase; Viox. = Violaxanthin; Zeax. = Zeaxanthin. Oprec and Otemp represent precipitation and temperature of climate of origin.

Table 1: List of traits used in this study and their significance. Trait names that are underlined represent structural traits and the remaining represent biochemical traits

Trait	**Significance**
Aboveground biomass	Biomass allocation to above ground resource acquisition, is related to plant size and is also indicator of plant fitness
Plant height	Plant competitive ability for light
Rhizome weight	Investment into clonal reproduction
Root weight	Biomass allocation to soil resource acquisition
Specific leaf area	Per unit leaf construction cost; related to plant resource use strategy; indicator of plant growth rate
Stomatal density & Stomatal size	Gas exchange and allow plants to adjust their stomatal pore area in response to the surrounding environment.
Number of ramets	Measure of plant fitness and related to plant resource use strategy
Chlorophyll *a* & *b*	Key photosynthetic pigments in plants. Chlorophyll *a* absorb both high (~662 nm) and low (~430nm) wavelength light and chlorophyll *b* mainly absorbs low wavelength (~453 nm) light
β-carotene	Carotenoids are responsible for the quenching of light and protection of cells from damages caused by light and superoxide radicales
Xanthophylls (Antheraxanthin, Neoxanthin, Violaxanthin, Zeaxanthin and Lutein)	Accessory light-harvesting pigments and plays important role in the protection of photosynthetic machinery from the potentially toxic effects of light
Xanthophyll cycle de-epoxidation state	De-epoxidation state provides information on xanthophyll cycle activity and the degree of violaxanthin to zeaxanthin conversion
Phenolic compounds	Protect against UV radiation by scavenging the reactive oxygen species generated due to light stress.
Superoxide dismutase	It forms the first line of defence against oxidative stresses in plants and plays vital role is scavenging the reactive oxygen species produced during metabolic processes and under stress conditions.

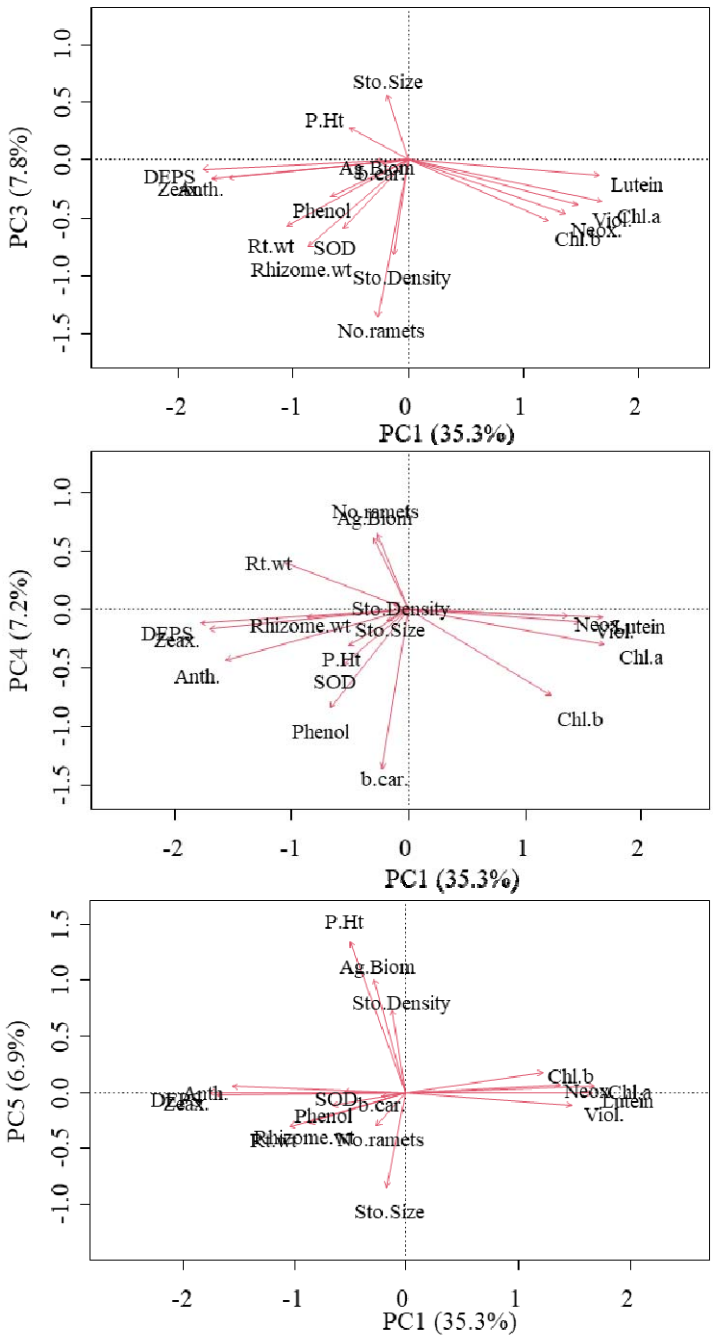
Table 2: Trait loading, eigenvalues and percentage of trait variation explained by the first five principal components (PCs).

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Aboveground biomass	-0.34	**1.40**	-0.01	0.72	**1.18**
Plant height	-0.59	0.83	0.33	-0.36	**1.59**
Rhizome weight	**-1.02**	0.10	-0.88	-0.07	-0.32
Root weight	**-1.23**	0.92	-0.67	0.47	-0.36
Stomatal density	-0.15	**-1.27**	-0.96	0.06	0.87
Stomatal size	-0.21	**1.28**	0.66	-0.10	**-1.00**
Number of ramets	-0.32	0.51	**-1.58**	0.77	-0.35
Antheraxanthin	**-1.83**	0.01	-0.18	-0.50	0.07
β-carotene	-0.26	0.41	-0.04	**-1.59**	-0.04
Chlorophyll *a*	**1.97**	0.25	-0.42	-0.34	0.07
Chlorophyll *b*	**1.43**	0.11	-0.62	-0.87	0.22
Xanthophyll cycle de-epoxidation state	**-2.09**	-0.17	-0.09	-0.14	0.00
Phenolic compounds	-0.79	0.41	-0.37	-0.97	-0.14
Lutein	**1.95**	0.17	-0.15	-0.08	-0.01
Neoxanthin	**1.61**	0.44	-0.54	-0.06	0.08
SOD	-0.66	0.05	-0.70	-0.55	0.01
Violaxanthin	**1.73**	0.41	-0.45	-0.12	-0.14
Zeaxanthin	**-2.00**	-0.10	-0.18	-0.19	-0.03
Explained variation (%)	***35.3***	***8.8***	***7.8***	***7.2***	***6.9***

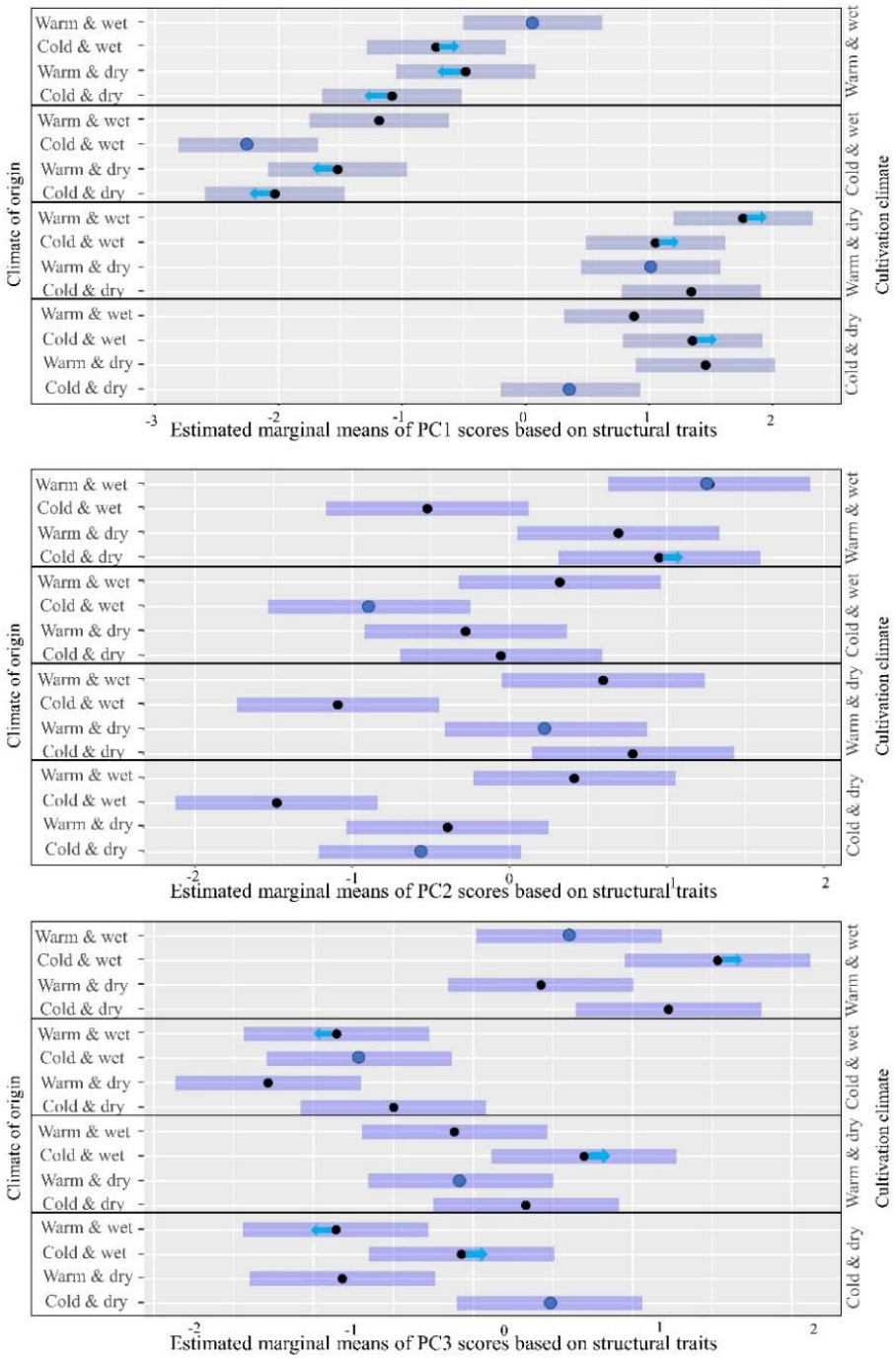
Loadings > 1 in magnitude are in bold

Table 3: Trait convergence or divergence in different populations of *Festuca rubra* when grown in different foreign climates. Inferences were made based upon the significant differences between estimated marginal means between plants in climate of origin (home) and cultivation (foreign) climate. Convergence refers to significant shift in estimated marginal mean (based on PCA scores generated using traits) of plants grown in a cultivation (foreign) climate towards that of plants in climate of origin (home). Conv. = convergence; Div. = divergence; None = neither convergence nor divergence. Cells with '-' represent those plants were in their home climates.

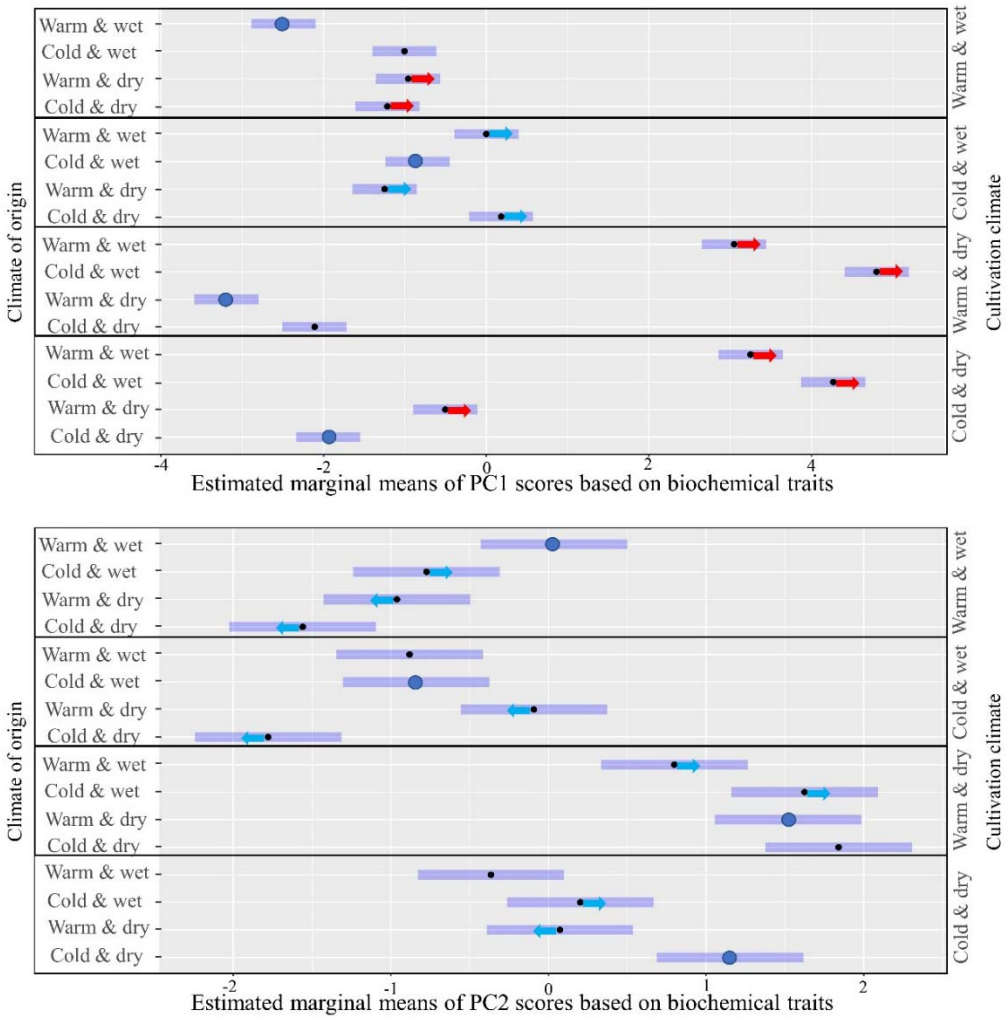
		Cultivation climate			
		Cold and dry	Warm and dry	Cold and wet	Warm and wet
		Structural traits (PC1)			
Climate of Origin	Cold and dry	-	None	Conv.	Conv.
	Warm and dry	None	-	Conv.	Conv.
	Cold and wet	Conv.	Conv.	-	Conv.
	Warm and wet	None	Conv.	None	-
		Structural traits (PC2)			
	Cold and dry	-	None	None	None
	Warm and dry	None	-	None	None
	Cold and wet	None	None	-	None
	Warm and wet	Conv.	None	None	-
		Structural traits (PC3)			
	Cold and dry	-	None	Conv.	None
	Warm and dry	None	-	None	None
	Cold and wet	Conv.	Conv.	-	Conv.
	Warm and wet	Conv.	None	None	-
		Biochemical traits (PC1)			
	Cold and dry	-	None	Conv.	Div.
	Warm and dry	Div.	-	Conv.	Div.
	Cold and wet	Div.	Div.	-	None
	Warm and wet	Div.	Div.	Conv.	-
		Biochemical traits (PC2)			
	Cold and dry	-	None	Conv.	Conv.
	Warm and dry	Conv.	-	Conv.	Conv.
	Cold and wet	Conv.	Conv.	-	Conv.
	Warm and wet	None	Conv.	None	-



Supplementary Figure 1: Principal component (PC) patterns of studied functional traits. The axes 3 to 5 are plotted against axis 1. Trait abbreviations are as: Ag.Biom = Aboveground biomass; P.Ht = Plant height; Rhizome.wt = Rhizome weight ; Rt.wt = Root weight ; Sto.Density = Stomatal density; Sto.Size = Stomatal size; No.Ramets = Number of ramets; Anth. = Antheraxanthin; b-car = β-carotene Chl.a = Chlorophyll a; Chl.b = Chlorophyll b; DEPS = xanthophyll cycle de-epoxidation state; Neox. = Neoxanthin; SOD = Superoxide dismutase; Viox. = Violaxanthin; Zeax. = Zeaxanthin; Phenol = phenolic compounds.



Supplementary Figure 2: Estimated marginal means of PC axis 1 to 3 (based on trait values of structural traits) for different populations cultivated in contrasting climates. The shaded areas are 95% confidence intervals. Means in large blue dots are for populations grown in home climate. The arrows on shaded areas represent direction of shift in traits (from home climate) along with convergence (blue arrows).



Supplementary Figure 3: Estimated marginal means of PCA axis 1 and 2 scores (based on trait values of biochemical traits) for different populations cultivated in contrasting climates. The shaded areas are 95% confidence intervals. Means in large blue dots are for populations grown in home climate. The arrows on shaded areas represent direction of shift in traits (from home climate) along with convergence (blue arrows) and divergence (red arrows).

Supplementary Table 1: Mixed effects model statistics showing the effect of climate of origin and cultivation climate on PC scores based structural and biochemical traits separately. Significant effects are shown in bold. Oprec and Otemp represent precipitation and temperature of climate of origin; Cprec and Ctemp represent precipitation and temperature of cultivation climate.

Predictor	**Structural traits**						**Biochemical traits**			
	PC1		PC2		PC3		PC1		PC2	
	F value	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**
Otemp	8.30	**0.007**	14.15	**<0.001**	10.98	**0.002**	628	**<0.001**	91.6	**<0.001**
Oprec	1.90	0.177	3.27	0.079	0.06	0.812	1.6	0.207	0.3	0.614
Ctemp	31.24	**<0.001**	23.1	**<0.001**	115.3	**<0.001**	628	**<0.001**	91.6	**<0.001**
Cprec	308.2	**<0.001**	6.07	**0.015**	1.03	0.313	25.5	**<0.001**	13.6	**<0.001**
Otemp:Oprec	0.02	0.881	23.9	**<0.001**	0.12	0.735	0.6	0.425	0.4	0.542
Otemp:Ctemp	0.17	0.683	0.10	0.749	0.01	0.942	628.1	**<0.001**	91.6	**<0.001**
Otemp:Cprec	3.43	0.067	0.31	0.577	0.56	0.457	26.3	**<0.001**	13.6	**<0.001**
Oprec:Ctemp	0.63	0.431	2.88	0.093	1.89	0.172	7.4	**0.008**	5.1	**0.026**
Oprec:Cprec	0.02	0.884	0.15	0.695	1.26	0.265	24.5	**<0.001**	5.7	**0.019**
Ctemp:Cprec	11.97	**<0.001**	0.38	0.540	34.10	**<0.001**	30.7	**<0.001**	5.9	**0.017**
Otemp:Oprec:Ctemp	4.54	0.035	0.88	0.351	3.88	0.052	0	0.939	2.4	0.126
Otemp:Oprec:Cprec	1.46	0.230	0.14	0.704	0.22	0.641	19.5	**<0.001**	4.7	**0.032**
Otemp:Ctemp:Cprec	0.00	0.963	1.48	0.227	3.76	0.055	53.7	**<0.001**	14.3	**<0.001**
Oprec:Ctemp:Cprec	0.49	0.487	0.38	0.539	0.56	0.454	10.4	**0.002**	9.9	**0.002**
Otemp:Oprec:Ctemp:Cprec	8.01	**0.006**	0.01	0.940	0.03	0.872	79.8	**<0.001**	16.1	**<0.001**