Ecological drivers at the warm edge of species' distribution

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Erstbetreuer/in: Prof. Dr. Yvonne Willi

Zweitbetreuer/in: Prof. Dr. Klaus Schläppi

Externe/r Experte/in: Prof. Dr. Oliver Boßdorf

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Summary

Why are species not evenly distributed all over the globe but are restricted to certain areas? This question has fascinated biologists for a long time. By studying ecology and evolution, biologists try to understand the limits in species geographic distribution and the restriction of adaptation to environmental stresses. Past research has shown that the low latitudinal range edges are often characterised by abiotic environmental factors. The aim of my thesis was to systematically study two pivotal environmental factors, temperature and precipitation, of the model species, *Arabidopsis lyrata*. In a transplant experiment, I showed that the increase in temperature and variability of precipitation patterns, caused by climate change, negatively affect plant performance. Specifically, germination and flowering success, as well as survival rates were reduced. A greenhouse experiment focusing on the effects of heat, drought, and their combination was conducted to study the adaptation strategies to the different stresses. Even though exposure to heat or drought alone did not affect survival rates much, the combination of both stresses caused a high mortality rate. Populations originating at the warm range edge displayed a slightly better performance and plants had a higher specific leaf area and root-to-shoot ratio. However, plant performance is also dependent on the interaction with the rhizosphere microbiome. Therefore, I analysed how different plant traits correlate with microorganisms under different environmental conditions. I compared the correlations of plant traits with different primary root exudate compounds and rhizosphere bacteria and fungi under different watering treatments. The composition of root exudates and bacteria changed significantly between a moist and a drought treatment. Essentially, more high positive correlations were found under drought, confirming the importance of plant-microbe interactions under stress conditions. In summary, my thesis emphasises the threat of global warming to population persistence at the warm range edge, even if populations originating at the warm edge are adapted to a certain degree. With ongoing climate change, the range optima are likely to shift northwards, despite the beneficial interactions in the rhizosphere.

General Introduction

All living organisms are constantly exposed to changing environmental conditions to which they must adapt (Hoffmann and Parsons 1997; Hofmann and Todgham 2010; Kristensen et al. 2020). These conditions can change on different scales – from daily over seasonal and yearly, to climatic periods ranging from ice ages to warm periods. Due to climate change, however, conditions are changing at an extreme speed posing enormous challenges for the entire living environment (Jump and Peñuelas 2005; Gauthier et al. 2015). This can range from overall raising temperatures across the globe to a more variable precipitation pattern in space and time (IPCC 2023). Plants as stationary organisms are less mobile than most animals and thus can often not move fast enough to stay with suitable conditions, which forces them to adapt to the changing conditions (Jump and Peñuelas 2005; Corlett and Westcott 2013). The aim of my work is to analyse the different adaptation strategies of the model plant *Arabidopsis lyrata*. In this introduction, I am going to discuss three different aspects that are relevant for my thesis: first, causes of stress at species distribution limits, second, adaption strategies by modulated plant growth and third, interactions of plants with microorganisms in the soil.

Limitations to species distribution

Many plant species are restricted by geographical boundaries, which are often defined by environmental gradients (Lennon et al. 1997; Gaston 2009). Among these factors climate defines the distribution limits of a species rather well, as plants grow best under suitable conditions (Hargreaves et al. 2014; Lee-Yaw et al. 2016; Normand et al. 2009). However, not all species are able to follow rapid environmental changes over a small geographic range change fast enough, and many have a higher local extinction rate at the warm end of distribution (Rumpf et al. 2019). In addition, climate change may amplify the intensity and frequency of single and combined stress, such as heat or drought. Even though, under climate warming, species distribution may be more limited by adaptation limits, the rear edge is often less studied

than the leading edge of species ranges (Cahill et al. 2014; Willi and Van Buskirk 2022). Thus, it is important to investigate plant growth and adaptation under conditions common to climate change to understand the impact of future climate warming on natural populations, especially at the more vulnerable warm range edge.

Plants‘ strategies of adaptation

Plants have evolved various ways of coping with stressors such as heat and drought. Studying the responses to either stressor is complex and it is difficult to dissociate them from each other, as they often occur together and influence each other in nature. Some adaptation strategies can be very well combined, while others cause contradictory responses. For example under both, heat or drought, a change in phenology to escape the stress is observed and deeper and wider root systems are favoured to maintain water supply (Natarajan and Kuehny 2008; Franks 2011; Taylor et al. 2019; Dinneny 2019). However, coping strategies can also go into opposite directions. For example, plants prevent overheating under heat stress by opening stomata and increased transpiration (Crawford et al. 2012; Deva et al. 2020; Sadok et al. 2021). But during drought, stomata are closed and transpiration is reduced to prevent water loss (Verslues and Juenger 2011; Tardieu 2013). The combination of these stressors can lead to a conflicting response, for example in the opening or closing of stomata to increase or decrease transpiration, and can be detrimental for plant performance (Rizhsky et al. 2002, 2004; Marchin et al. 2022).

Interaction with soil microbiota via exudates

Even though the geographic range of a species can often be explained by abiotic climate models alone, biotic interactions may be of added relevance (Sexton et al. 2009; Godsoe et al. 2017). Next to abiotic factors, causes of retreat can also include the biotic factors, or interactions between both (Cahill et al. 2014; Paquette and Hargreaves 2021). Biotic factors include negative pressure by competition, predation, parasitism and pathogens, but also positive effects

of mutualism (Eckert et al. 2008; Sexton et al. 2009; Pigot and Tobias 2013; Godsoe et al. 2017). For example, the interaction between plants and soil microorganisms can buffer the impact of harsh weather conditions, such as extreme drought, on plant performance (Classen et al. 2015; Fitzpatrick et al. 2019; Karlowsky et al. 2018a; Redman et al. 2011; Rodriguez et al. 2008; Rolli et al. 2015). Bacteria and fungi in the rhizosphere can reduce abiotic stress experienced by plants by positively influencing the water and nutrient uptake (Meddich et al. 2015; Almario et al. 2017; Hiruma et al. 2018; Tang et al. 2022), and by increasing the surface area of water uptake and root adhered soil thus reducing evaporation and soil drying (Alami et al. 2000; Chenu 1993; Dudman 1977; Roberson and Firestone 1992; Rosenzweig et al. 2012; Sutherland 2001). One mechanism that is particularly important in dry conditions is the increased availability of nutrients to plants via reduced soil pH due to organic acid excretion (Franche et al. 2009; Vassilev et al. 2012; Bista et al. 2018). In return, plants keep conditions habitable for microbes by creating enzymatic hotspots via root exudation, increasing the abundance of beneficial microorganisms (Zhang et al. 2023). Root exudates consist of a large proportion of primary metabolites such as sugar, organic aids, amino acids and, to a lower degree, of secondary metabolites (Badri and Vivanco 2009; Naveed et al. 2017; Sasse et al. 2018). Similar to microorganisms, root exudates can directly increase plant performance under stress conditions, by increasing nutrient uptake or reducing the drying speed of the rhizosphere (Passioura 1988; McCully 1999; Carminati et al. 2009, 2016; Ahmed et al. 2014; Karlowsky et al. 2018b; Wu et al. 2018; Canarini et al. 2019; Benard et al. 2019).

Study system

Study organism in this thesis is *Arabidopsis lyrata*. 5 million years ago, *A. lyrata* separated from the genetically well-studied sister species *A. thaliana* (Koch et al. 2000). *Arabidopsis lyrata* is a small, mainly outcrossing and short-lived perennial plant, facilitating experimental studies. It grows in disturbed and dry habitats like sand dunes along lake shores or in rocky

outcrops (Al-Shehbaz and O'Kane 2002; Mable et al. 2005). This species is widely distributed and occurs circumpolar, with the three subspecies *A. lyrata* spp. *kamchatica*, *A. lyrata* spp. *petraea*, and *A. lyrata* spp. *lyrata* (Koch et al. 2001; Al-Shehbaz and O'Kane 2002; Schmickl et al. 2010).

In my thesis, I used *A. lyrata* spp. *lyrata* as a model system, hereafter referred to as *A. lyrata*. This subspecies is mainly restricted to eastern and mid-western regions of the United States of America and southern Canada (Fig. 1). For this species, niche modelling and transplant experiments demonstrated that low latitudinal range limits overlap with niche limits. Critical parameters for the occurrence of *A. lyrata* plants are especially the minimum temperature in early spring, the amount of precipitation in the wettest quarter, and the evapotranspiration rate (Lee-Yaw et al. 2018). A transplant experiment across the entire south-north gradient of *A. lyrata*'s range indicated that climate change has shifted the spatial layout of climatically suitable areas towards the north (Sánchez-Castro et al. 2024), as more extreme weather events occur in the US, including heavy rainfall events, severe droughts and heatwaves, or increased frequencies of storms and hurricanes (USGCRP 2023).

Overview of the 3 chapters

The aim of **chapter 1** is to investigate the effects of different aspects of weather on plant performance at the warm range edge. This chapter presents and discusses the results of a common garden experiment, where plants of populations from different geographic origins were grown along a transect across the low latitudinal range edge. Different phenological important events were recorded during 1.5 years to find which aspects of weather, temperature or precipitation, has the biggest impact on plant performance.

Chapter 2 describes a greenhouse experiment, which included the same populations as chapter 1. Heat and drought stress as found at the warm range edge, were applied and plant growth and performance were measured to identify different adaptation strategies. The aim of

this study is to disentangle in a controlled setup the effects of heat, drought and their interaction on plant growth and to investigate what happens if one versus both stressors are applied to a plant.

The last study, **chapter 3**, zooms into drought responses and the interplay of plants with the microorganisms in the rhizosphere. The aim is to study different phenotypic plant traits, root exudate composition, as well as rhizosphere bacteria and fungi. With a holistic approach, we were able to directly compare correlation networks between different watering regimes.

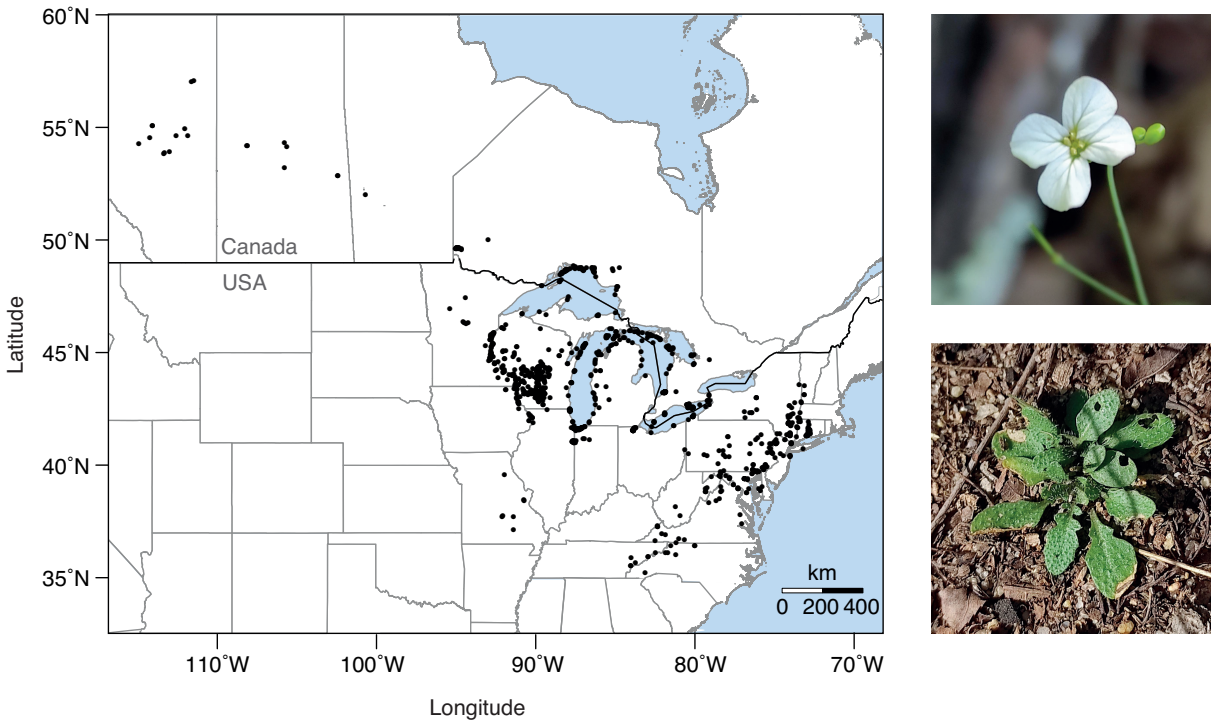

Fig. 1: Distribution of the model species *A. lyrata* spp. *lyrata*
Left side: Map of USA and Canada with known occurrences of *A. lyrata* spp. *lyrata* as black dots. Blue areas indicate the ocean or big lakes, while black lines divide the different states within the countries.
Right side: Images of *A. lyrata* spp. *lyrata* of a population at the south-eastern edge of distribution taken in May 2022 by the author. The top image shows a blossom with a closed bud and in the background an unripened fruit. The bottom image shows the plant‘s rosette with low competition, but some herbivore damage.

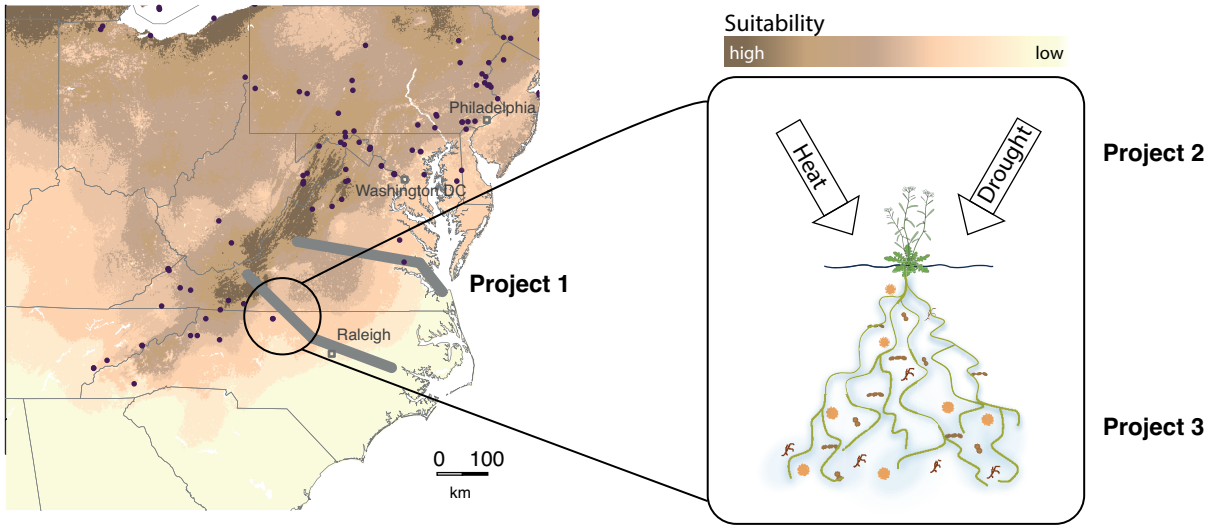

Fig. 2: Graphical overview of the three thesis chapters.
Left side: Map of the south-eastern edge of *A. lyratas'* distribution. Colours in the map are based on the suitability reported in Lee-Yaw et al (2018), with darker shades indicating a higher suitability. Occurrences of *A. lyrata* are reported with black dots. Project 1 focused on two transects across the distribution limits (thick grey lines).
Right side: Project 2 focused on historical precipitation and temperature conditions of the low latitudinal range edge, and project 3 focused on the response of plant-microbe interactions under drought conditions.

References

Ahmed MA, Kroener E, Holz M, Zarebanadkouki M, Carminati A, 2014. Mucilage exudation facilitates root water uptake in dry soils. Funct. Plant Biol. 41, 1129–1137. doi.org/10.1071/FP13330

Al-Shehbaz IA, O'Kane SL, 2002. Taxonomy and phylogeny of *Arabidopsis* (Brassicaceae). Arab. B. 1, e0001. doi.org/10.1199/tab.0001

Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G, Bucher M, 2017. Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. Proc. Natl. Acad. Sci. U. S. A. 114, E9403–E9412. doi.org/10.1073/pnas.1710455114

Aston MJ, Lawlor DW, 1979. The relationship between transpiration, root water uptake, and leaf water potential. J. Exp. Bot. 30, 169–181.

Badri D, Vivanco J, 2009. Regulation and function of root exudates. Plant. Cell Environ. 32, 666–681. doi.org/10.1111/j.1365-3040.2008.01926.x

Balachowski JA, Bristiel PM, Volaire FA, 2016. Summer dormancy, drought survival and functional resource acquisition strategies in California perennial grasses. Ann. Bot. 118, 357–368. doi.org/10.1093/aob/mcw109

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D, 2006. Potent Induction of *Arabidopsis thaliana* flowering by elevated growth temperature. PLOS Genet. 2, e106. doi.org/10.1371/journal.pgen.0020106

Basu S, V R, Kumar A, Pereira A, 2016. Plant adaptation to drought stress. F1000Research 5, 1554. doi.org/10.12688/f1000research.7678.1

Benard P, Zarebanadkouki M, Brax M, Kaltenbach R, Jerjen I, Marone F, Couradeau E, Felde VJMNL, Kaestner A, Carminati A, 2019. Microhydrological niches in soils: how mucilage and EPS alter the biophysical properties of the rhizosphere and other biological hotspots. Vadose Zo. J. 18, 180211. doi.org/10.2136/vzj2018.12.0211

Bista DR, Heckathorn SA, Jayawardena DM, Mishra S, Boldt JK, 2018. Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and -tolerant grasses. Plants. doi.org/10.3390/plants7020028

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, Hua X, Karanewsky CJ, Ryu HY, Sbeglia GC, Spagnolo F, Waldron JB, Wiens JJ, 2014. Causes of warm-edge range limits: Systematic review, proximate factors and implications for climate change. J. Biogeogr. 41, 429–442. doi.org/10.1111/jbi.12231

Canarini A, Kaiser C, Merchant A, Richter A, Wanek W, 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front. Plant Sci. doi.org/10.3389/fpls.2019.00157

Carminati A, Vetterlein D, Weller U, Vogel H-J, Oswald SE, 2009. When roots lose contact. Vadose Zo. J. 8, 805. doi.org/10.2136/vzj2008.0147

Carminati A, Zarebanadkouki M, Kroener E, Ahmed MA, Holz M, 2016. Biophysical rhizosphere processes affecting root water uptake. Ann. Bot. 118. doi.org/10.1093/aob/mcw113

Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA, Moorhead LC, Patterson CM, 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6, art130. doi.org/10.1890/ES15-00217.1

Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA, 2012. High temperature exposure increases plant cooling capacity. Curr. Biol. 22, R396–R397. doi.org/10.1016/j.cub.2012.03.044

Deva CR, Urban MO, Challinor AJ, Falloon P, Svitákova L, 2020. Enhanced leaf cooling is a pathway to heat tolerance in common bean. Front. Plant Sci. 11. doi.org/10.3389/fpls.2020.00019

Dinneny JR, 2019. Developmental responses to water and salinity in root systems. Annu. Rev. Cell Dev. Biol. 35, 239–257. doi.org/10.1146/annurev-cellbio-100617-062949

Eckert CG, Samis KE, Lougheed SC, 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. Mol. Ecol. doi.org/10.1111/j.1365-294X.2007.03659.x

Fitzpatrick CR, Mustafa Z, Viliunas J, 2019. Soil microbes alter plant fitness under competition and drought. J. Evol. Biol. 32, 438–450. doi.org/10.1111/jeb.13426

Franche C, Lindström K, Elmerich C, 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321, 35–59. doi.org/10.1007/s11104-008-9833-8

Franks SJ, 2011. Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. New Phytol. 190, 249–257. doi.org/10.1111/j.1469-8137.2010.03603.x

Gaston KJ, 2009. Geographic range limits: Achieving synthesis. Proc. R. Soc. B Biol. Sci. 276, 1395–1406. doi.org/10.1098/rspb.2008.1480

Gauthier S, Bernier P, Kuuluvainen T, Shvidenk AZ, Schepaschenko DG, 2015. Boreal forest health and global change. Science 349, 819–822. doi.org/10.1126/science.aaa9092

Giri A, Heckathorn S, Mishra S, Krause C, 2017. Heat stress decreases levels of nutrient-uptake and -assimilation proteins in tomato roots. Plants 6. doi.org/10.3390/plants6010006

Godsoe W, Holland NJ, Cosner C, Kendall BE, Brett A, Jankowski J, Holt RD, 2017. Interspecific interactions and range limits: contrasts among interaction types. Theor. Ecol. 10, 167–179. doi.org/10.1007/s12080-016-0319-7

Hiruma K, Kobae Y, Toju H, 2018. Beneficial associations between Brassicaceae plants and fungal endophytes under nutrient-limiting conditions: evolutionary origins and host–symbiont molecular mechanisms. Curr. Opin. Plant Biol. 44, 145–154. doi.org/10.1016/j.pbi.2018.04.009

Hoffmann AA, Parsons PA, 1997. Extreme environmental change and evolution.

Hofmann GE, Todgham AE, 2010. Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment. Annu. Rev. Physiol. 72, 127–145. doi.org/10.1146/annurev-physiol-021909-135900

Intergovernmental Panel on Climate Change (IPCC), 2023. Climate Change 2021 – The physical science basis: Working group I contribution to the sixth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press.

Jump AS, Peñuelas J, 2005. Running to stand still: Adaptation and the response of plants to rapid climate change. Ecol. Lett. 8, 1010–1020. doi.org/10.1111/j.1461-0248.2005.00796.x

Karlowsky S, Angela A, Ingrisch J, Hasibeder R, Lange M, Lavorel S, Bahn M, Gleixner G, 2018a. Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. J. Ecol. 106, 1230—1243. doi.org/10.1111/1365-2745.12910

Karlowsky S, Augusti A, Ingrisch J, Akanda MKU, Bahn M, Gleixner G, 2018b. Drought-Induced Accumulation of Root Exudates Supports Post-drought Recovery of Microbes in Mountain Grassland. Front. Plant Sci. 9, 1593. doi.org/10.3389/fpls.2018.01593

Koch M, Haubold B, Mitchell-Olds T, 2001. Molecular systematics of the Brassicaceae: evidence from coding plastidic matK and nuclear Chs sequences. Am. J. Bot. 88, 534–544. doi.org/10.2307/2657117

Koch MA, Haubold B, Mitchell-Olds T, 2000. Comparative Evolutionary Analysis of Chalcone Synthase and Alcohol Dehydrogenase Loci in *Arabidopsis*, *Arabis*, and Related Genera (Brassicaceae). Mol. Biol. Evol. 17, 1483–1498. doi.org/10.1093/oxfordjournals.molbev.a026248

Kristensen TN, Ketola T, Kronholm I, 2020. Adaptation to environmental stress at different timescales. Ann. N. Y. Acad. Sci. 1476, 5–22. doi.org/10.1111/nyas.13974

Lee-Yaw JA, Fracassetti M, Willi Y, 2018. Environmental marginality and geographic range limits: a case study with *Arabidopsis lyrata* ssp. *lyrata*. Ecography (Cop.). 41, 622–634. doi.org/10.1111/ecog.02869

Lennon JJ, Turner JRG., Connell D, 1997. A metapopulation model of species boundaries. Oikos 78, 486–502. doi.org/10.2307/3545610

Mable BK, Dart AVR, Berardo C Di, Witham L, 2005. Breakdown of self-incompatibility in the perennial *Arabidopsis lyrata* (Brassicaceae) and its genetic consequences. Evolution (N. Y). 59, 1437–1448.

Marchin RM, Backes D, Ossola A, Leishman MR, Tjoelker MG, Ellsworth DS, 2022. Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species. Glob. Chang. Biol. 28, 1133–1146. doi.org/10.1111/gcb.15976

McCully ME, 1999. Roots in soil: Unearthing the complexities of roots and their rhizospheres. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 695–718. doi.org/10.1146/annurev.arplant.50.1.695

Meddich A, Jaiti F, Bourzik W, El Asli A, Hafidi M, 2015. Use of mycorrhizal fungi as a strategy for improving the drought tolerance in date palm (*Phoenix dactylifera*). Sci. Hortic. (Amsterdam). 192, 468–474. doi.org/10.1016/j.scienta.2015.06.024

Natarajan S, Kuehny JS, 2008. Morphological, physiological, and anatomical characteristics associated with heat preconditioning and heat tolerance in *Salvia splendens*. J. Am. Soc. Hortic. Sci. J. Amer. Soc. Hort. Sci. 133, 527–534. doi.org/10.21273/JASHS.133.4.527

Naveed M, Brown LK, Raffan AC, George TS, Bengough AG, Roose T, Sinclair I, Koebernick N, Cooper L, Hackett CA, Hallett PD, 2017. Plant exudates may stabilize or weaken soil depending on species, origin and time. Eur. J. Soil Sci. 68, 806–816. doi.org/10.1111/ejss.12487

Paquette A, Hargreaves AL, 2021. Biotic interactions are more often important at species' warm versus cool range edges. Ecol. Lett. 24, 2427–2438. doi.org/10.1111/ele.13864

Parker J, 1949. Effects of variations in the root-leaf ratio on transpiration rate. Plant Physiol. 24, 739–743. doi.org/10.1104/pp.24.4.739

Passioura JB, 1988. Water transport in and to roots. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 245–265. doi.org/10.1146/annurev.pp.39.060188.001333

Pigot AL, Tobias JA, 2013. Species interactions constrain geographic range expansion over evolutionary time. Ecol. Lett. 16, 330–338. doi.org/10.1111/ele.12043

Redman RS, Kim YO, Woodward CJDA, Greer C, Espino L, Doty SL, Rodriguez RJ, 2011. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6, e14823. doi.org/10.1371/journal.pone.0014823

Rizhsky L, Liang H, Mittler R, 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol. 130, 1143–1151. doi.org/10.1104/pp.006858

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R, 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. Plant Physiol. 134, 1683–1696. doi.org/10.1104/pp.103.033431

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS, 2008. Stress tolerance in plants via habitat-adapted symbiosis. ISME J. 2, 404–416. doi.org/10.1038/ismej.2007.106

Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, Gandolfi C, Casati E, Previtali F, Gerbino R, Pierotti Cei F, Borin S, Sorlini C, Zocchi G, Daffonchio D, 2015. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environ. Microbiol. 17, 316–331. doi.org/10.1111/1462-2920.12439

Sadok W, Lopez JR, Smith, KP, 2021. Transpiration increases under high-temperature stress: Potential mechanisms, trade-offs and prospects for crop resilience in a warming world. Plant. Cell Environ. 44, 2102–2116. doi.org/10.1111/pce.13970

Sánchez-Castro D, Perrier A, Willi Y, 2022. Reduced climate adaptation at range edges in North American *Arabidopsis lyrata*. Glob. Ecol. Biogeogr. 31, 1066–1077. doi.org/10.1111/geb.13483

Sasse J, Martinoia E, Northen T, 2018. Feed your friends: Do plant exudates shape the root microbiome? Trends Plant Sci. 23, 25–41. doi.org/10.1016/j.tplants.2017.09.003

Schmickl R, Jørgensen MH, Brysting AK, Koch MA, 2010. The evolutionary history of the *Arabidopsis lyrata* complex: A hybrid in the amphi-Beringian area closes a large distribution gap and builds up a genetic barrier. BMC Evol. Biol. 10. doi.org/10.1186/1471-2148-10-98

Sexton JP, McIntyre PJ, Angert A., Rice KJ, 2009. Evolution and Ecology of Species Range Limits. Annu. Rev. Ecol. Evol. Syst. 40, 415–436. doi.org/10.1146/annurev.ecolsys.110308.120317

Tang H. Hassan MU, Feng L, Nawaz M, Shah AN, Qari SH, Liu Y, Miao J, 2022. The critical role of arbuscular mycorrhizal fungi to improve drought tolerance and nitrogen use efficiency in crops. Front. Plant Sci. doi.org/10.3389/fpls.2022.919166

Tardieu F, 2013. Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Front. Physiol. 4. doi.org/10.3389/fphys.2013.00017

Taylor MA, Cooper MD, Schmitt J, 2019. Phenological and fitness responses to climate warming depend upon genotype and competitive neighbourhood in *Arabidopsis thaliana*. Funct. Ecol. 33, 308–322. doi.org/10.1111/1365-2435.13262

USGCRP (2023) Fifth National Climate Assessment. In: Crimmins AR, Avery CW, Easterling DR, et al. (eds) Global Climate Change Impacts in the United States. Cambridge University Press, Washington, DC, USA. doi.org/10.7930/NCA5.2023

Vassilev N, Eichler-Löbermann B, Vassileva M, 2012. Stress-tolerant P-solubilizing microorganisms. Appl. Microbiol. Biotechnol. 95, 851–859. doi.org/10.1007/s00253-012-4224-8

Verslues PE, Juenger TE, 2011. Drought, metabolites, and *Arabidopsis* natural variation: A promising combination for understanding adaptation to water-limited environments. Curr. Opin. Plant Biol. 14, 240–245. doi.org/10.1016/j.pbi.2011.04.006

Willi Y, Van Buskirk J, 2022. A review on trade-offs at the warm and cold ends of geographical distributions. Philos. Trans. R. Soc. B Biol. Sci. 377, 20210022. doi.org/10.1098/rstb.2021.0022

Wu L, Kobayashi Y, Wasaki J, Koyama H, 2018. Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil Sci. Plant Nutr. 64, 697–704. doi.org/10.1080/00380768.2018.1537093

Zhang X, Bilyera N, Fan L, Duddek P, Ahmed MA, Carminati A, Kaestner A, Dippold MA, Spielvogel S, Razavi BS, 2023. The spatial distribution of rhizosphere microbial activities under drought: water availability is more important than root-hair-controlled exudation. New Phytol. 237, 780–792. doi.org/doi.org/10.1111/nph.18409

Chapter 1

Beyond the edge: The effect of climate on life stage transitions and mortality at the warm end of a species' distribution

Judith R. Schepers, Jessica Heblack, Yvonne Willi

Affiliations:

Department of Environmental Sciences, University of Basel, 4056 Basel, Switzerland

Email:

judith.schepers@unibas.ch, jessica.heblack@unibas.ch, yvonne.willi@unibas.ch

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Abstract

The distribution of many temperate species has been shrinking at the low-latitude range edge due to climate warming, indicating the importance of climate in setting warm-edge range limits. Though, it is unclear what the most detrimental aspect of climate is. We conducted a transplant experiment on two transects crossing the low-latitude range edge of *Arabidopsis lyrata* and studied the aspects of climate that made seeds germinate, plants flower, or plants die. Across sites, germination was positively affected by precipitation in the week before, while flowering was negatively correlated with cool temperatures three to six weeks before and warmer temperatures in the week before flowering. Mortality was significantly enhanced by warm temperatures or heavy precipitation. With recent climate change, average temperature and precipitation had generally increased in the focal area, but at the same time, phases with low precipitation had increased. Based on the findings, both increased rainfall, but also phases of particularly low precipitation may be the reason for *A. lyrata'*s distribution limit at the southern range edge. It is likely that these stressors combined with heat interact in their effect on causing low population persistence in general and even more so under climate warming.

Keywords: *Arabidopsis lyrata*, climate warming, common garden experiment, local adaptation, range limit

Introduction

Within its geographic range, a species typically encounters abiotic and biotic conditions under which it can persist, which meet its niche requirements (Holt and Keitt 2000; Hargreaves et al. 2014). To understand why persistence stops at some point in space, we need to know the range-limiting factors and how they change in the area of range limits (Angert et al. 2018; Lee-Yaw et al. 2018; Willi and Van Buskirk 2019). The most parsimonious predictor of constraining the evolution of niche expansion is a strong change in conditions over a short distance. This makes the change in range-limiting factors intriguing as a steep selection gradient (Kirkpatrick and Barton 1997; Polechová 2018). However, many species are restricted to geographical boundaries that appear as if conditions change gradually, such as over latitude or gradients of heat and dryness (Lennon et al. 1997; Gaston 2009). To shed light on this conundrum, we studied the range-limiting climatic conditions of a species at the warm end of distribution and how they changed at the range limit. We focused on the warm end because it is where species retractions have been frequently reported under global warming (Chen et al. 2011; Rumpf et al. 2019).

Evolutionary theory has come up with many hypotheses on the causes of range limits (reviewed in Sexton et al. 2009). Similarly, there has been an important body of theory on the ability of populations to adapt to marginal conditions (reviewed in Kawecki 2008). This work aligns with one of many predictions, namely that if selection gradients are steep or change to be steep, adaptation along the gradient fails. When range limits are caused by niche limits, the evolution of the niche in the direction needed is not possible due to a high number of selective deaths. Consequently, range limits are established. To study range limits in this eco-evolutionary context, we need to know precisely what the niche- and range-determining environmental gradients are (Willi and Van Buskirk 2019).

Climate has been shown to predict the distribution limits of many species rather well (Normand et al. 2009; Hargreaves et al. 2014; Lee-Yaw et al. 2016; Paquette and Hargreaves

2021). However, some challenges need to be overcome when studying the effects of climatic factors impeding organisms. Climate has many aspects; for example, temperatures vary on a daily basis and over the season, which has different effects on different organs or across life stages (Körner and Hiltbrunner 2018). It has been demonstrated that plants are especially vulnerable to being harmed by extreme weather at critical life stages such as germination and flowering (Hedhly 2011; Waterworth et al. 2015; Ali and Elozeiri 2017)). But there are also studies pointing to average climatic conditions at range edges being responsible for why species stop occurring (Sanchez-Castro et al. 2024).

With climate change, general warming has been recorded, resulting in a temporal shift in phenology. The shift in phenology has been observed across life stages: warmer temperatures cause plants to germinate, bud, or bloom earlier in the year. As these are critical life stages, when frosts occur at these times, it can cause significant damage to plants (Root et al. 2003; Gu et al. 2008; Lenz et al. 2013; Iler et al. 2019). Another change has been more variable weather, with more heat events and heat combined with drought (Easterling et al. 2000; IPCC 2023). Heat events have been shown to result in reduced biomass production and reproduction success and, beyond a certain tipping point, to increased plant die-off (Siebers et al. 2017; Zhao et al. 2017; Lohani et al. 2020). However, heat combined with drought has been shown to be particularly harmful due to the increased vapour pressure deficit (Eamus et al. 2013). For herbs like *Arabidopsis lyrata* to trees, it was reported that the combination of heat and drought increased mortality drastically (Allen et al. 2015; Adams et al. 2017; Schepers et al. 2024).

The goal of this study was to assess the environmental factors that constrain the distribution of a plant species at its low-latitude boundary. Our study organisms was the North American *Arabidopsis lyrata* subsp. *lyrata* at its south-eastern range limit. Niche modelling and a large-scale transplant experiment across the latitudinal range had shown that the southern range edge was primarily constrained by unsuitable climate conditions, especially limited by too high temperatures in early spring (Vergeer and Kunin 2013; Lee-Yaw et al. 2018; Sánchez-

Castro et al. 2022, 2024). In this study, we attempted to go further by identifying the conditions that affect vital life-stage transitions, germination, flowering, and mortality. Plants of a genetically and phenotypically diverse population were sown at six common garden sites across the southern range limits on transects in North Carolina and Virginia in autumn. Life stage transitions were recorded and related to local weather conditions. More specifically, the questions were split into: (1) Does the transect and position across the transect influence plant performance? (2) What is the shape of the climatic gradient across the range edge? (3) What is the climate at different life stages across a transect? (4) Which aspect of weather influences the transition into the next life stage?

Methods

Experimental design

The geographic distribution of *Arabidopsis lyrata* spans from Missouri and North Carolina in the south (USA) to southern Alberta (CAN) and up-state New York (USA) in the north. The species is a short-lived perennial plant, producing basal rosettes with flower stems and flowers that are predominantly cross-pollinated. For the experiment, we selected three sites along two transects based on their position relative to the range edge, with one site being within the range, one around the range edge, and one beyond the range edge. Furthermore, they were picked depending on the predicted suitability of sites in the recent past based on niche modelling with climate data (Table 1, Lee-Yaw et al. 2018). More specifically, the sites on each transect had a suitability that was high (>0.5), medium (0.5-0.1), or low (<0.1), from inside to outside of the range (Fig. 1). Transects had a total distance of 320 km in the north and 360 km in the south.

We used seeds from mainly one population from the southerly centre of distribution, apart from some seeds from two northern and two southern range edge populations. The edge populations were used to confirm that the central population was indeed within the responses seen across the species' range. Seeds had been propagated for one generation in the greenhouse

before sowing at the transplant sites. The collection of seeds in the field included 600 plants of the central population in Saugatuck Dunes State Park (MI, USA) and a few dozen plants of the edge populations (see Schepers et al. 2024 for details about populations). One plant per family was raised in the greenhouse and then crossed in pairs to receive full-sib families. Hand-pollinations were done on emasculated flowers. In total, full-sibships were available: 266 for the central population and 3 for each edge population. In autumn of 2021, sowing in the field started. At each site, 72 multi-pot trays with 38 pots with open bottoms each were filled with a soil-sand mixture of 2:1 (Schepers et al. 2024; Heblack et al. 2024). Trays were split into 8 spatial blocks, and one seed per seed family was sown in each of them in a random position (with two random layouts, one with a seed family replica at the tray's edge and the other on the inner). Due to heavy rainfall after sowing at one of the sites, on the northern transect at the range edge, half of the trays were resown after one week, and a trench was dug around the experimental site to prevent a recurrence of flooding.

Blocks with the 9 trays were placed together into 2.7 x 0.6 m big trenches that were filled with the same soil-sand mixture. Between these trench blocks and around the outer blocks, walkways were covered with weed barriers to reduce the growth of local plants. Around the sites, a 0.5 m chicken wire fence was installed to keep animals away. If animal pressure was high, a 2 m mesh fence was added. Trays were watered carefully and covered by a plastic tunnel to keep moisture high. For four weeks, plants were sprayed daily with water to ensure higher germination rates. After two weeks, the plastic covers were replaced with mesh nets for another four weeks to have a gradual change to local weather conditions, which they were exposed to during the rest of the experiment.

Germination was checked first every second day, later weekly and biweekly during winter. Flowering onset was checked weekly, documenting the flowering date and inflorescences were harvested, measured, and weighed after six weeks. If fruits turned ripe before the time of harvest, they were collected and weighed to avoid spreading of seeds. To

capture changes, photographs were taken once per week and monthly during winter (SONY, DSC-H300, Minato City, Tokyo, Japan). Survival was recorded after winter each year to record winter survival, and longevity was reconstructed using the mean time between two pictures. If no pictures were taken over a period longer than three months, the plants that died in that period were excluded from survival analysis due to inaccuracy. The experiment ended in April 2023, when all plants were harvested, above-ground biomass was weighed, and the two biggest leaves as well as root length outside of the pots were measured.

Climate data

At each of the sites, microclimate was recorded with iButtons (iButtonLink Technology, Whitewater, WI, USA) for air temperature and humidity (5 cm, 0.5 m, and 2 m) and soil temperature (2 cm inside pots). Furthermore, weather data from September 2021 to May 2023 was downloaded from "weatherstem.com" for Blacksburg, VA, Norfolk, VA, and Greenville, NC, at an hourly resolution. The rain rate in inch was summed over each day per day. Data for Durham, NC, was from "wrcc.dri.edu" and for Lexington, VA, from "ncdc.noaa.gov", both at daily resolution. Data from the York River State Park weather station CBVTCMET was downloaded from "cdmo.baruch.sc.edu" at 15 minute resolution. The daily total precipitation was translated into mm, and the minimum, maximum, and mean temperatures were translated from °F to °C for all sites. Weekly summaries were calculated for each site separately. Missing microclimate temperature data measurements were reconstructed in R 4.2.1 using the local climate station data by predicting missing values based on linear regression (R-Core-Team, 2021). Models of minimum, mean, and maximum temperatures had an adjusted R^2 of 0.92, 0.92, and 0.78, respectively (Fig. S2).

Long-term climate data at the research sites were obtained from WorldClim v1.4 (Hijmans et al. 2005), v2.1 (Fick and Hijmans 2017), and CRU-TS 4.06 (Harris et al. 2020) downscaled with WorldClim v2.1. We downloaded monthly precipitation as well as monthly

minimum and maximum temperature of each year for the periods of 1960-1990 and 1970-2000 and calculated the mean across the years for each time period in R version 3.6.1. Further, we obtained the precipitation of the wettest quarter (BIO16). For 2000-2021, we loaded the monthly precipitation, minimum temperature, and maximum temperature. We calculated the average maximum precipitation per month across the years and then the precipitation of the wettest quarter (P_{wet}). Lastly, we calculated the minimum temperature in March and April (spring T_{min}), as well as mean temperature and precipitation in April to June (growing season T_{mean} and P_{mean}), and averaged across years using the dplyr and raster packages (Hijmans 2022; Wickham et al. 2023a). For the first two periods, the resolution was 30 seconds; for 2000-2021, the resolution was 2.5 minutes. Raster plots (Figs. 1, S3) were produced with the R packages sp version 1.0-5 and sf version 1.0-5 (Pebesma and Bivand 2005; Pebesma 2018).

Analysis

All analyses were conducted using the packages dplyr v1.1.2 (Wickham et al. 2023a) and tidy v1.3.0 (Wickham et al. 2023c), and plots were generated with the ggplot2 package v3.4.2 (Wickham 2016) in R 4.2.1. In order to determine the responsiveness of plants from the central population in comparison to the edge populations, we tested for population differences. The variables included days until germination, flowering, and death, as well as proportion of germinating and flowering plants and survival rate in the first year of the experiment. Day until germination is defined as the duration from sowing to germination, while days until flowering or death are relative to the germination date. The selected type of model either assumed a binomial distribution for binary data or a Poisson distribution for continuous data. The generalised linear models included population identity as a fixed effect and site as well as tray number nested within block as random effects. Pairwise posthoc comparisons were performed with the games-howell test in *emmeans* (Table 1; Lenth et al. 2018). Further analyses were done on the southerly-central population only.

In the main analyses, we first tested if the transect and position across the transect influenced plant performance in the central population with generalised linear models. The performance traits included germination success, time to germination, longevity, survival after the first winter, and how often a plant flowered (lme4 package v1.1-35.1; Bates et al. 2015). The models included transect and position in the transect as fixed effects, and block and tray nested within block as random effects. Germination success and winter survival (survival until April of the first year) were binary variables. Models of flowering and survival only included plants that had germinated. The model outputs were evaluated by type-3 testing (car v3.1-1; Fox and Weisberg 2019).

Second, we investigated the shape of the climatic gradient across the range edge. Based on temperature and precipitation at the six sites, we calculated the climate during different life stages. Third, the weather at the transition from one life stage to another was calculated at weekly resolution for the minimum temperature (T_{min}), maximum temperature (T_{max}), mean temperature (T_{mean}), and total precipitation (P). The transitions considered were germination, flowering, and survival.

Fourth, we tested how weather affected life stage transitions. For each type of transition, we generated a long-format dataset, which included the week, aspects of the weather, and whether the transition did not happen or whether it happened, the latter being the last row for each plant. The fixed effects were the different aspects of the weather, namely weekly P, T_{mean}, T_{min}-T_{mean} (ΔT_{min}), and T_{max}-T_{mean} (ΔT_{max}), depicting general microclimatic conditions and extreme temperature events. P and T_{mean} were centred to zero (scales package; Wickham et al. 2023b). P was divided by ten to account for the fact that temperature and precipitation have very different ranges. Conditions as far back as one week back for weather, six weeks back for flowering, and two weeks back for death were considered. As T_{mean} of the different weeks was highly correlated, only the one week before the stage transitions was included (Table S4). For flowering and survival, the model additionally included the age (weeks after germination) and

its interaction with weather, as the age might influence transition. Death was analysed for winter and summer separately, by only including weeks that had a scaled T_{mean} across four weeks above or below the threshold of zero. This allowed us to investigate the effect of climate on mortality for each season individually. To account for the effect of high and low precipitation on mortality, P^2 was added as a fixed effect. We applied binomial models, with experimental site and plant identity as random effects (glmmTMB package version 1.1.8; Brooks et al. 2017). The results are shown with dotwhisker plots of the dotwhisker package version 0.7.4 (Fig. 6; Solt and Hu 2021).

Results

We compared the performance of plants of the southernly-central population with that of the peripheral populations to see whether it was representative of the species (Fig. 2). Even though phenology was often different between the central and the edge populations (Table 1), the southerly-central population (solid green line) had germination, flowering, and death events after time spans comparable with those observed in the peripheral populations (Fig. 2A). This indicates that all populations have phenological reactions to climate that are comparable. Additionally, the central population had rates of germination, flowering, and survival in the first year that were that were intermediate to those of edge populations (Table 1, Fig. 2B). The high percentages in flowering rates of some edge populations might have been caused by the comparably small sample sizes of the edge populations. Overall, the results revealed that the southerly-central population is representative of the species.

In the first main analysis, we tested whether the transect and position along the transect affected plant performance. Indeed, transect, position, and their interactions differed strongly in regard to phenology and performance. Seeds of the northern transect germinated earlier and had, in general, a higher germination success, especially at the beyond-edge site, N_{out} (Table 2, Fig. 3). Additionally, plants across the northern transect had a higher chance of surviving the

first winter and had, in general, a more similar phenology across the positions compared to those of the southern transect. Further, across the southern transect and at sites outside of the range, plants flowered more often than across the northern transect or at sites inside the range (Table 2, Fig. 3).

Second, as transect and position within transect significantly influenced the performance of *A. lyrata*, we investigated the shape of climatic gradients across the range edge in more detail and how the sites differed in their respective climate conditions. This analysis focused on weather data from 1960 to 2021 to show which climate the plants had adapted to in the past, also including the time when seeds were collected (2007-2014). As described earlier, spring T_{min} and P_{wet} were the main factors influencing the suitability of *A. lyrata* across its distribution, thus we focused on these factors. T_{min} in early spring and T_{mean} during growing season increased by over 2 °C from inside to edge and edge to outside the range, respectively, in each of the three time-ranges (Table 3, Fig. S3). Additionally, from 1960-1990 to 2000-2021, the T_{min} in spring increased by approx. 2 °C and T_{mean} during growing season by approx. 1 °C. P_{wet} increased by 50 mm from inside to edge and 20-90 mm from edge to outside in 1960-1990, with higher precipitation at the southern transect. Overall, P_{wet} and P_{mean} during growing season increased over the last decades by 30-50 mm, or approx. 10 mm, still having the wettest sides outside of the range (Table 3, Fig. S3).

Third, we focused on the weather during life stage transitions. Not only the overall weather conditions seemed important, but especially those during sensitive stages of a plant's life, such as germination or flowering. Thus, we studied the weather at different life stages across each transect (Table 4, Fig. 5). During germination and death events, T_{mean} increased from inside to outside of the range at both transects, but not during flowering events. Generally during germination and death events, ΔT_{max} decreased and ΔT_{min} increased from inside to outside of the species range. However, we found no clear trend for the ΔT_{max} and ΔT_{min} during

flowering events. While precipitation differed significantly between transects and positions within transects, no coherent trend along the transect was found.

Fourth, we determined the aspects of the weather influencing transitions, germination, flowering, and mortality. Most germination was observed between October and December, with a peak right after sowing in October and November of the first year, while most plants flowered in the early summer of their first year, and they mostly died within a few weeks after germination or before summer (Fig. 4). A plot of the effect size shows which component of the weather had a significant influence on the transition of the plant life stage, with the x-axis being the estimated effect (Fig. 7, Table S3). For germination, a higher temperature ΔT_{max} in the week of germination had a positive effect, and a higher P had a negative effect. However, in the week prior to germination, the exact opposite effects were found, with a positive effect of lower temperatures and higher precipitation. Overall, a high P and ΔT_{min} in the six weeks before flowering had a negative effect on plant flowering. As the interaction effect of ΔT_{min} and plant age was especially high three to six weeks before flowering, the negative effect of ΔT_{min} was higher on older plants (Table S3). However, a high T_{mean} in the two weeks before flowering had a positive effect on triggering flowering onset, with a stronger effect on younger plants. Plant mortality was analysed for summer and winter separately by the scaled moving four-week T_{mean} average, as here different factors might be important. In both seasons, a high P was linked with a high mortality the week after, while in winter, a low P was also linked with a higher mortality after two weeks. In the colder season, P^2 was significantly positive, indicating increased mortality at high and low precipitation levels. Additionally in both seasons, a high T_{mean} had a negative effect on survival. Only in the summer was a highly negative effect of ΔT_{min} on survival within one week observed. The effect of P in both seasons, and of T_{mean} and ΔT_{min} in summer the week before a peak in mortality, was especially strong on younger plants.

In summary, we showed that the performance of a plant is different along a transect across the range edge, with surprisingly higher performance outside of the current range. This

performance was strongly influenced by the local weather conditions, which varied considerably between sites all over the year, but especially during the sensitive life stages.

Discussion

The growth and performance of a plant are highly influenced by its abiotic conditions (Suzuki et al. 2014). Precipitation and especially temperature have been shown to have a high impact on the suitability of a location, particularly regarding the growth of a species (Moles et al. 2014; Tao et al. 2017; Lee-Yaw et al. 2018). But the importance of each of the specific weather conditions on the transition of life stages is not well studied. This motivated us to investigate the effect of different weather variables on the performance of the plant species *A. lyrata* across the known low latitudinal range edge.

It has been reported before that the phenology of peripheral populations and central population differs due to climatic differences (Sheth and Angert 2015; DeMarche et al. 2019; Zettlemoyer and Peterson 2021; Estarague et al. 2022). We could confirm these deviations, as our analysis showed some variation among populations. However, the differences of the edge populations compared to the central population were often insignificant, especially for the relevant parameters such as the establishment success and survival rates (Table 1). Even though phenology often differed significantly from the edge populations, as indicated by the density curves, the populations did overlap mostly in the high peaks (Fig. 2A). We observed east-west differences between populations, with a higher percentage of germinating plants in the western cluster, while north vs. south populations showed similar trends (Fig. 2B). This pattern can be explained by the known split of *A. lyrata* into an eastern and a western cluster, originating from different glacial refugia (Willi and Määttänen 2010; Griffin and Willi 2014; Willi et al. 2018). In summary, we conclude that a high number of replicates of the central population are representative for the variation among populations of all origins. This population experiences

many different microclimatic conditions in its natural habitat, due to the growth on an active sand dune (Willi et al. 2018; Willi and Van Buskirk 2019).

The study included two transects that crossed from inside of the known range towards outside of the range. Analysis showed a significant difference in plant growth between those sites (Table 2, Fig. 3). The northern transect had earlier germination days and had a higher survival rate until the first spring, while plants at the southern transect flowered more often, thus had a higher reproductive output. This indicates that, in general, the conditions at the northern transect were better for plant establishment, but the few plants that germinated and survived at the southern transect were very well adapted to the weather conditions and could grow and reproduce exceptionally well. This could hint at a trade-off between 1) investing in fast growth and life cycle to complete sensitive life stages before the environmental stress becomes too great, and 2) slower growth and phenology, risking death but potentially achieving higher reproduction (Willi and Van Buskirk 2022; Grossman 2023; Heblack et al. 2024). Even though, differences between both transects in weather during phenology events often weren't significantly different, the interaction of site and position was significant, showing that the specific site conditions are essential, particularly P and ΔT_{min} (Table 4). We observed a much greater variation in the southern transect than the northern transect, both between and within sites. Plants "in" the range germinated and flowered later than "outside" the range (Fig. 3). A higher germination rate in spring rather than autumn might be caused by an earlier and more intense winter close to the Appalachians (Fig. 4). Interestingly, only "at the edge" and "outside" positions, plants germinated in the second spring at both transects, even though more ungerminated seeds should have been present inside of the range. The first flowering plants were observed outside of the range (Fig. 4), which is likely linked to an earlier start of spring that allowed earlier bolting (Abu-Asab et al. 2001; Ellwood et al. 2013). This allowed plants to flower more than once in the same season, but also increased the risk of being exposed to late frost events (Beaubien and Hamann 2011).

Climate can explain the distribution limits of species quite well (Normand et al. 2009; Hargreaves et al. 2014; Lee-Yaw et al. 2016). Evidence for the importance of climate is available from the observed changes in species distributions to higher latitudes or higher elevations in the last couple of decades due to global warming (Hickling et al. 2005; Parmesan 2006; Parmesan and Yohe 2003; Root et al. 2003). The global temperature on Earth is increasing (IPCC 2023) and also in the United States, an increase in average temperature and precipitation have been measured over the last few decades. However, the pattern of change in space and time is very heterogeneous. More extreme weather events occur, including heavy rainfall events, severe droughts and heatwaves, as well as increased frequencies of storms and hurricanes (USGCRP 2023). The effect of climate change is especially significant at species-range edges (Cahill et al. 2014). Not all species are able to adapt to climate change fast enough, and examples have reported heightened local extinction rates at the warm end of distribution (e.g. Rumpf et al. 2019). Models have shown that temperature and precipitation are the main factors limiting the latitudinal distribution of *A. lyrata*, thus we compared the local climate at the different sites during the time of the experiment (Lee-Yaw et al. 2018). The temperature during the growing season along both transects increased by 2° C from inside to outside of the range (Table 3, Fig. S3). As the temperature has continuously increased over the last decades, the average and extreme temperatures may exceed the optimal growth conditions of *A. lyrata*. Additionally, precipitation has slightly increased over the last few decades. Precipitation is often a limiting factor during germination and growth, while an accumulation of water due to heavy rainfall events and therefore high saturation can lead to reduced plant performance. *A. lyrata* originally grows under well-drained conditions, such as sand dunes, rocky outcrops, sandy or rocky riverbanks, and shorelines (Sánchez-Castro et al. 2024), which can mean that the growth in less-drained soil is not always optimal.

The differences in local climates were not only found during the whole experiment's duration, but we also observed that conditions specifically differed during germination,

flowering, and death (Table 4, Fig. 5). The weather conditions during phenological events of a plant differed among positions within a transect, but not so much between the two transects. Germination was mainly influenced by a high ΔT_{max} and a low P in the week of germination. This means plants were more likely to germinate if higher temperatures occurred and the precipitation was not too high. As plants need to be well-watered during germination, they received regular additional water during the first few weeks of the experiment. This is shown by the low but significant positive effect of precipitation on germination the week prior to germination (Table S3, Fig. 6). Especially within the range, precipitation was low during the seed establishment (Fig. 5), which caused lower germination success (Fig. 3A). However, with too much rain from heavy rainfall events, sites were flooded, and high germination rates were only observed on the moist soil of the following week. Additionally, plants only had a short time span to germinate and establish at sites inside the range, as winter started early close to the mountains and cold stress is known to reduce germination and seedling establishment (Jame and Cutforth 2004). The high germination rates close to the cold winter can be seen by the positive effect of ΔT_{max} on germination (Fig. 6). The strong variation of P and ΔT_{max} at the different positions along the transects might therefore explain the observed differences in germination rates among sites (Table 4). Flowering onset was mainly influenced by a high T_{mean} one to two weeks before flowering and a lower ΔT_{min} three to six weeks before flowering. This means that more plants flowered if it was generally rather warm and less extreme cold events occurred before hand. Explanations can be that after bolting, cold temperatures have a negative effect on successful flowering and reproduction (as reviewed in Hassan et al. 2021). Supporting this theory is a negative interaction between T_{mean} and plant age, which results in a greater beneficial effect of T_{mean} on younger plants since early flowering plants, right after winter, are younger.

The transition to death was mostly influenced by a high P in the week before death was recorded (Fig. 6). This is probably linked to young plants being flooded after a heavy rainfall

event. This is confirmed by the negative interaction term of age and P in both seasons (Table S3). Even though high precipitation before germination was advantageous, several studies have shown that optimal germination conditions can be unsuitable for seedling survival (Qi and Redmann 1993; Lloret et al. 2004). However, a significant effect of drought on plant survival was also found. Mortality increased within two weeks after a drought period (Fig. 6). The negative effect of long-term drought on plant growth and performance has already been discussed in many studies (Engelbrecht and Kursar 2003; He and Dijkstra 2014; Schepers et al. 2024). The drought effect might have been overshadowed by extreme flooding events, which is confirmed by the positive effect of the quadratic precipitation term of the model output. Additionally to the effect of precipitation, a too warm T_{mean} was linked with a higher mortality the week after (Fig. 6). During the warm season, this can be caused by overall too warm temperatures. In winter, the negative effect of warmer temperatures could be linked to a snowmelt and the plant being exposed to frost (Bannister et al. 2005; Buma et al. 2017). As mean temperatures have been rising across the last centuries (Table 2), this could have an increasing effect on plant survival at the warm range edge.

Conclusion

In summary, our findings emphasise that especially extreme events, which have already started to occur more frequently, limit plant establishment and affect all important life stages, including germination, flowering, and survival. With the transect setup, we could show that formerly suitable areas now lead to low plant performance due to climate change, increasing temperatures, and more variable precipitation patterns. Overall, we could see that high precipitation, typically heavy rainfall events, had a negative effect, hindering germination, flowering and survival. As precipitation has increased over the last decades, both in the wettest quarter and during the growing season (April-June), this could limit the establishment and persistence of *A. lyrata* at the southern range edge (Fig. S3A). Additionally, the time series data

allowed us to disentangle the effects and showed the long-term negative effect of cold temperatures on survival, while too high temperatures reduced survival in a shorter response time.

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Author contribution

YW, JH and JS designed the study. YW collected seeds in the field, and JH propagated seeds. JH and JS performed the experiment. JS did the analysis and wrote the manuscript, with inputs by YW. All authors read and agreed on the manuscript.

References

Abu-Asab MS, Peterson PM, Shetler SG, Orli SS (2001) Earlier plant flowering in spring as a response to global warming in the Washington, DC, area. Biodivers Conserv 10:597–612. doi.org/10.1023/A:1016667125469

Adams HD, Barron-Gafford GA, Minor RL, et al (2017) Temperature response surfaces for mortality risk of tree species with future drought. Environ Res Lett 12:11. doi.org/10.1088/1748-9326/aa93be

Ali AS, Elozeiri AA (2017) Metabolic processes during seed germination. Adv seed Biol 2017:141–166. doi.org/10.5772/intechopen.70653

Allen CD, Breshears DD, McDowell NG (2015) On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6(8):129. doi.org/10.1890/ES15-00203.1

Anderson JT, Wadgymar SM (2020) Climate change disrupts local adaptation and favours upslope migration. Ecol Lett 23:181–192. doi.org/10.1111/ele.13427

Angert AL, Bayly M, Sheth SN, Paul JR (2018) Testing range-limit hypotheses using range-wide habitat suitability and occupancy for the scarlet monkeyflower (*Erythranthe cardinalis*). Am Nat 191:E76–E89. doi.org/10.1086/695984

Bannister P, Maegli T, Dickinson KJM, et al (2005) Will loss of snow cover during climatic warming expose New Zealand alpine plants to increased frost damage? Oecologia 144:245–256. doi.org/10.1007/s00442-005-0087-3

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. doi.org/10.18637/jss.v067.i01

Beaubien E, Hamann A (2011) Spring flowering response to climate change between 1936 and 2006 in Alberta, Canada. Bioscience 61:514–524. doi.org/10.1525/bio.2011.61.7.6

Brooks ME, Kasper K, van Benthem JK, et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9:378–400. doi.org/10.32614/RJ-2017-066

Buma B, Hennon PE, Harrington CA, et al (2017) Emerging climate-driven disturbance processes: Widespread mortality associated with snow-to-rain transitions across 10° of latitude and half the range of a climate-threatened conifer. Glob Chang Biol 23:2903–2914. doi.org/10.1111/gcb.13555

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, et al (2014) Causes of warm-edge range limits: Systematic review, proximate factors and implications for climate change. J Biogeogr 41:429–442. doi.org/10.1111/jbi.12231

Chen IC, Hill JK, Ohlemüller R, et al (2011) Rapid range shifts of species associated with high levels of climate warming. Science (80) 333:1024–1026. doi.org/10.1126/science.1206432

DeMarche ML, Doak DF, Morris WF (2019) Incorporating local adaptation into forecasts of species' distribution and abundance under climate change. Glob Chang Biol 25:775–793. doi.org/10.1111/gcb.14562

Eamus D, Boulain N, Cleverly J, Breshears DD (2013) Global change-type drought-induced tree mortality: vapor pressure deficit is more important than temperature per se in causing decline in tree health. Ecol Evol 3:2711–2729. doi.org/10.1002/ece3.664

Easterling DR, Meehl GA, Parmesan C, et al (2000) Climate extremes: Observations, modeling, and impacts. Science 289:2068–2074. doi.org/10.1126/science.289.5487.2068

Ellwood ER, Temple SA, Primack RB, et al (2013) Record-breaking early flowering in the eastern United States. PLoS One 8:e53788. doi.org/10.1371/journal.pone.0053788

Engelbrecht BMJ, Kursar TA (2003) Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. Oecologia 136:383–393. doi.org/10.1007/s00442-003-1290-8

Estarague A, Vasseur F, Sartori K, et al (2022) Into the range: A latitudinal gradient or a center-margins differentiation of ecological strategies in *Arabidopsis thaliana*? Ann Bot 129:343–356. doi.org/10.1093/aob/mcab149

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. Int J Climatol 37:4302–4315. doi.org/10.1002/joc.5086

Fox J, Weisberg S (2019) An R companion to applied regression. Third edition. Sage, Thousand Oaks CA. socialsciences.mcmaster.ca/jfox/Books/Companion/

Gaston KJ (2009) Geographic range limits: Achieving synthesis. Proc R Soc B Biol Sci 276:1395–1406. doi.org/10.1098/rspb.2008.1480

Griffin PC, Willi Y (2014) Evolutionary shifts to self-fertilisation restricted to geographic range margins in North American *Arabidopsis lyrata*. Ecol Lett 17:484–490. doi.org/10.1111/ele.12248

Grossman JJ (2023) Phenological physiology: seasonal patterns of plant stress tolerance in a changing climate. New Phytol 237:1508–1524. doi.org/10.1111/nph.18617

Gu L, Hanson PJ, Post W Mac, et al (2008) The 2007 eastern US spring freeze: Increased cold damage in a warming world? Bioscience 58:253–262. doi.org/10.1641/b580311

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: The rear edge matters. Ecol Lett 8:461–467. doi.org/10.1111/j.1461-0248.2005.00739.x

Hargreaves AL, Samis KE, Eckert CG (2014) Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. Am Nat 183:157–173. doi.org/10.1086/674525

Harris I, Osborn TJ, Jones P, Lister D (2020) Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. Sci Data 7:109. doi.org/10.1038/s41597-020-0453-3

Hassan MA, Xiang C, Farooq M, et al (2021) Cold stress in wheat: Plant acclimation responses and management atrategies. Front Plant Sci 12. doi.org/10.3389/fpls.2021.676884

He M, Dijkstra FA (2014) Drought effect on plant nitrogen and phosphorus: A meta-analysis. New Phytol 204:924–931. doi.org/10.1111/nph.12952

Heblack J, Schepers JR, Willi Y (2024) Reduced evolutionary potential under heat and drought stress at the southern range edge of North American *Arabidopsis lyrata*. J Evol Biol voae045. doi.org/10.1093/jeb/voae045

Hedhly A (2011) Sensitivity of flowering plant gametophytes to temperature fluctuations. Environ Exp Bot 74:9–16. doi.org/10.1016/j.envexpbot.2011.03.016

Hickling R, Roy DB, Hill JK, Thomas CD (2005) A northward shift of range margins in British Odonata. Glob Chang Biol 11:502–506. doi.org/10.1111/j.1365-2486.2005.00904.x

Hijmans RJ (2022) raster: geographic data analysis and modeling. https://rspatial.org/raster/

Hijmans RJ, Cameron SE, Parra JL, et al (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978. doi.org/10.1002/joc.1276

Holt RD, Keitt TH (2000) Alternative causes for range limits: a metapopulation perspective. Ecol Lett 3:41–47. doi.org/10.1046/j.1461-0248.2000.00116.x

Iler AM, Compagnoni A, Inouye DW, et al (2019) Reproductive losses due to climate change-induced earlier flowering are not the primary threat to plant population viability in a perennial herb. J Ecol. doi.org/10.1111/1365-2745.13146

Intergovernmental Panel on Climate Change (IPCC), 2023. Climate Change 2021 – The physical science basis: Working group I contribution to the sixth assessment report of the intergovernmental panel on climate change. Cambridge Univ Press Press. doi.org/10.1017/9781009157896

Jame YW, Cutforth HW (2004) Simulating the effects of temperature and seeding depth on germination and emergence of spring wheat. Agric For Meteorol 124:207–218. doi.org/10.1016/j.agrformet.2004.01.012

Kawecki TJ (2008) Adaptation to marginal habitats. Annu Rev Ecol Evol Syst 39:321–342. doi.org/10.1146/annurev.ecolsys.38.091206.095622

Keith DA, Keith DA, Akçakaya HR, et al (2008) Predicting extinction risks under climate change: Coupling stochastic population models with dynamic bioclimatic habitat models. Biol Lett. doi.org/10.1098/rsbl.2008.0049

Kirkpatrick M, Barton NH (1997) Evolution of a species' range. Am Nat 150:1–23. doi.org/10.1086/286054.

Körner C, Hiltbrunner E (2018) The 90 ways to describe plant temperature. Perspect. Plant Ecol. Evol. Syst. 30:16–21. doi.org/10.1016/j.ppees.2017.04.004

Lee-Yaw JA, Fracassetti M, Willi Y (2018) Environmental marginality and geographic range limits: A case study with *Arabidopsis lyrata* ssp. *lyrata*. Ecography (Cop) 41:622–634. doi.org/10.1111/ecog.02869

Lee-Yaw JA, Kharouba HM, Bontrager M, et al (2016) A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. Ecol Lett 19:710–722. doi.org/10.1111/ele.12604

Lennon JJ, Turner JRG, Connell D (1997) A metapopulation model of species boundaries. Oikos 78:486–502. doi.org/10.2307/3545610

Lenth R, Singmann H, Love J, et al (2018) Package Emmeans. cran.r-project.org/package=emmeans

Lenz A, Hoch G, Vitasse Y, Körner C (2013) European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients. New Phytol 200:1166–1175. doi.org/10.1111/nph.12452

Lloret F, Peñuelas J, Estiarte M (2004) Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Glob Chang Biol 10:248–258. doi.org/10.1111/j.1365-2486.2004.00725.x

Lohani N, Singh MB, Bhalla PL (2020) High temperature susceptibility of sexual reproduction in crop plants. J Exp Bot 71:555–568. doi.org/10.1093/jxb/erz426

Moles AT, Perkins SE, Laffan SW, et al (2014) Which is a better predictor of plant traits: Temperature or precipitation? J Veg Sci 25:1167–1180. doi.org/10.1111/jvs.12190

Normand S, Treier U, Randin C, et al (2009) Importance of abiotic stress as a range-limit determinant for European plants: Insights from species responses to climatic gradients. Glob Ecol Biogeogr 18:437–449. doi.org/10.1111/j.1466-8238.2009.00451.x

Paquette A, Hargreaves AL (2021) Biotic interactions are more often important at species' warm versus cool range edges. Ecol Lett 24:2427–2438. doi.org/10.1111/ele.13864

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669. doi.org/10.1146/annurev.ecolsys.37.091305.110100

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42. doi.org/10.1038/nature01286

Polechová J (2018) Is the sky the limit? On the expansion threshold of a species' range. PLOS Biol 16:e2005372. doi.org/10.1371/journal.pbio.2005372

Qi MQ, Redmann RE (1993) Seed germination and seedling survival of C3 and C4 grasses under water stress. J Arid Environ 24:277–285. doi.org/10.1006/jare.1993.1024

R-Core-Team (2021) R: A language and environment for statistical computing. www.r-project.org

Rinnan DS (2018) Population persistence in the face of climate change and competition: A battle on two fronts. Ecol Modell 385:78–88. doi.org/10.1016/j.ecolmodel.2018.07.004

Root TL, Price JT, Hall KR, et al (2003) Fingerprints of global warming on wild animals and plants. Nature 421:57–60. doi.org/10.1038/nature01333

Rumpf SB, Hülber K, Wessely J, et al (2019) Extinction debts and colonization credits of non-forest plants in the European Alps. Nat Commun 10:1–9. doi.org/10.1038/s41467-019-12343-x

Sánchez-Castro D, Patsiou T-S, Perrier A, et al (2024) Uncovering the cause of breakup between species' range limits and niche limits under climate warming. J Biogeogr: doi.org/10.1111/jbi.14796

Sánchez-Castro D, Perrier A, Willi Y (2022) Reduced climate adaptation at range edges in North American *Arabidopsis lyrata*. Glob Ecol Biogeogr 31:1066–1077. doi.org/10.1111/geb.13483

Schepers JR, Heblack J, Willi Y (2024) Negative interaction effect of heat and drought stress at the warm end of species distribution. Oecologia 204:173-185. doi.org/10.1007/s00442-023-05497-5

Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and Ecology of Species Range Limits. Annu Rev Ecol Evol Syst 40:415–436. doi.org/10.1146/annurev.ecolsys.110308.120317

Sheth SN, Angert AL (2015) Artificial selection reveals high genetic variation in phenology at the trailing edge of a species range. Am Nat 187:182–193. doi.org/10.1086/684440

Siebers MH, Slattery RA, Yendrek CR, et al (2017) Simulated heat waves during maize reproductive stages alter reproductive growth but have no lasting effect when applied during vegetative stages. Agric Ecosyst Environ 240:162–170. doi.org/10.1016/j.agee.2016.11.008

Solt F, Hu Y (2021) dotwhisker: Dot-and-Whisker Plots of Regression Results. cran.r-project.org/package=dotwhisker

Suzuki N, Rivero RM, Shulaev V, et al (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43. doi.org/10.1111/nph.12797

Tao Z, Wang H, Liu Y, et al (2017) Phenological response of different vegetation types to temperature and precipitation variations in northern China during 1982–2012. Int J Remote Sens 38:3236–3252. doi.org/10.1080/01431161.2017.1292070

USGCRP (2023) Fifth National Climate Assessment. In: Crimmins AR, Avery CW, Easterling DR, et al. (eds) Global Climate Change Impacts in the United States. Cambridge University Press, Washington, DC, USA. doi.org/10.7930/NCA5.2023

Vergeer P, Kunin WE (2013) Adaptation at range margins: Common garden trials and the performance of *Arabidopsis lyrata* across its northwestern European range. New Phytol 197:989–1001. doi.org/10.1111/nph.12060

Waterworth WM, Bray CM, West CE (2015) The importance of safeguarding genome integrity in germination and seed longevity. J Exp Bot 66:3549–3558. doi.org/10.1093/jxb/erv080

Wickham H (2016) ggplot2: Elegant graphics for data analysis. ggplot2.tidyverse.org

Wickham H, François R, Henry L, et al (2023a) dplyr: A grammar of data manipulation. dplyr.tidyverse.org

Wickham H, Pedersen T, Seidel D (2023b) scales: Scale functions for visualization. scales.r-lib.org

Wickham H, Vaughan D, Girlich M (2023c) tidyr: Tidy messy data. tidyr.tidyverse.org

Willi Y, Fracassetti M, Zoller S, Buskirk J Van (2018) Accumulation of mutational load at the edges of a species range. Mol Biol Evol 35:781–791. doi.org/10.1093/molbev/msy003

Willi Y, Määttänen K (2010) Evolutionary dynamics of mating system shifts in *Arabidopsis lyrata*. J Evol Biol 23:2123–2131. doi.org/10.1111/j.1420-9101.2010.02073.x

Willi Y, Van Buskirk J (2019) A practical guide to the study of distribution limits. Am Nat 193:773–785. doi.org/10.1086/703172

Willi Y, Van Buskirk J (2022) A review on trade-offs at the warm and cold ends of geographical distributions. Philos Trans R Soc B Biol Sci 377:20210022. doi.org/10.1098/rstb.2021.0022

Zettlemoyer MA, Peterson ML (2021) Does phenological plasticity help or hinder range shifts under climate change? Front. Ecol. Evol. 9. doi.org/10.3389/fevo.2021.689192

Zhao C, Liu B, Piao SL, et al (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA 114:9326–9331. doi.org/10.1073/pnas.1701762114

Tables

Table 1: Results of mixes effect models comparing plant performance of edge populations with the central population

The table gives estimates and P-values of the post-hoc test of generalized linear models for continuous data (phenology and times flowering) and binary data (winter survival, germination and flowering success), when comparing edge populations with the central population. Significant differences (P-value <0.05) are indicated in bold.

Population	Germination Success	Germination Time	Flowering Success	Flowering Time	Times Flowering	Winter Survival	Longevity
NW	**-0.46 (<0.01)**	**0.15 (<0.01)**	-0.03 (1)	0.03 (0.52)	0.06 (1)	-0.05 (1)	**-0.22 (<0.01)**
NE	**0.33 (0.05)**	**-0.31 (<0.01)**	0.58 (0.17)	**0.42 (<0.01)**	**0.83 (<0.01)**	**0.99 (<0.01)**	**0.23 (<0.01)**
SW	**1.18 (<0.01)**	**-0.68 (<0.01)**	1.44 (0.1)	-0.05 (0.93)	0.94 (0.21)	0.61 (0.55)	**0.69 (<0.01)**
SE	-0.3 (0.1)	**0.27 (<0.01)**	**-1.07 (<0.01)**	-0.03 (0.36)	**-1.09 (<0.01)**	-0.24 (0.83)	**-0.06 (<0.01)**

Table 2: Results of mixed-effect models testing the effect of position of the common garden site along transects on plant phenology and performance.
Effect of transect and position within transect on different performance and growth parameters. Germination success and winter survival were binary data. Days to germination started from sowing and days to flowering started with germination. Times flowering described how often a plant flowered. Random effects were block and tray nested within block. For binary predictive variables a generalized linear mixed model was used and for continuous data a linear mixed effect model. Significant differences (P-value <0.05) are indicated in bold.

	Coefficient Estimates						P-value		
Response Variable	Intercept	Transect South	Position Edge	Position Out	Transect South: Position Edge	Transect South: Position Out	Transect	Position	Transect: Position
Germination Success	-1.4	-0.4	1.4	2.2	0.51	-1.2	**<0.01**	**<0.01**	**<0.01**
Germination Time	36	59	1.8	0.5	61	-65	**<0.01**	**<0.01**	**<0.01**
Times Flowering	0.02	0.00	0.03	0.08	-0.02	0.36	**<0.01**	**<0.01**	**<0.01**
Longevity	47	-27	29	92	-26	41	**<0.01**	**<0.01**	**<0.01**
Winter Survival	-1.9	-1.0	1.0	3.2	-1.4	-1.0	**<0.01**	**<0.01**	**<0.01**

Table 3: Overview of characteristics of experimental sites.
Position at the transect and suitability (based on 1990 data), as well as mean minimum temperature in March and April in °C and precipitation in the wettest quarter in mm of the time periods of 1960-1990, 1970-2000 and 2000-2021. Weather data is coloured based on the range of spring temperature (<4, 4-5, 6-8, >8) and precipitation of the wettest quarter (<300, 300-350, 350-400, >400), temperature in growing season (<17, 17, 18, 19, 20, >20) and precipitation in growing season (<90, 90-100, 100-110, >110). Corresponding figures are shown in the Fig. S4.

			$T_{min\ Spring}$			P_{wet}			$T_{mean\ April\text{-}June}$			$P_{April\text{-}June}$		
Site	Closest City	Suitability	1960-1990	1970-2000	2000-2021	1960-1990	1970-2000	2000-2021	1960-1990	1970-2000	2000-2021	1960-1990	1970-2000	2000-2021
N_{in}	Lexington, VA	0.548	2.45	2.45	4.45	282	292	320	17	17	18	83	88	101
N_{edge}	Williamburg, VA	0.124	4.85	5.00	7.26	334	351	389	18	19	19	92	87	105
N_{out}	Norfolk, VA	0.014	6.50	6.85	9.05	357	356	403	19	19	20	94	87	105
S_{in}	Blackburg, VA	0.517	2.05	2.15	3.72	274	280	302	16	16	17	80	84	96
S_{edge}	Durham, NC	0.233	4.70	4.90	6.97	325	317	362	19	19	20	95	93	105
S_{out}	Greenville, NC	0.011	6.30	6.55	8.54	415	405	455	20	20	21	109	99	119

Table 4: Results of generalized linear models testing if the weather during stage transitions is different between transects and position within transect. Mean, minimum and maximum temperature (T_{mean}, ΔT_{min}, ΔT_{max}) and precipitation (P) of weeks when a plant germinated, flowered for the first time or died. The differences are plotted in Fig. 5. P-values below 0.05 are indicated in bold.

		Coefficient Estimates						P-value			
Trait	Weather	Intercept	Position Edge	Position Beyond	Transect South	Position Edge: Transect South	Position Beyond: Transect South	Intercept	Position	Transect	Position: Transect
Germination	P	6.7	-3.0	1.7	12.1	-10.9	-14.2	**0.00**	**0.00**	**0.00**	**0.00**
	T_{Mean}	6.7	2.8	7.4	-0.17	3.7	-1.7	**0.00**	**0.00**	0.37	**0.00**
	ΔT_{Min}	-12	0.68	3.2	-0.40	0.71	-1.4	**0.00**	**0.00**	**0.01**	**0.00**
	ΔT_{Max}	15	-2.5	-3.3	0.1	0.2	-0.38	**0.00**	**0.00**	0.38	**0.00**
Flowering	P	17	-3.0	7.3	1.2	2.3	-9.1	**0.01**	**0.01**	0.89	**0.03**
	T_{Mean}	15	4.7	5.1	-0.65	-2.9	-2.5	**0.00**	**0.00**	0.75	0.42
	ΔT_{Min}	-12	1.2	2.7	1.0	-2.4	-4.0	**0.00**	**0.00**	0.26	**0.00**
	ΔT_{Max}	12	-0.29	-0.11	1.1	-1.3	-0.52	**0.00**	0.91	0.32	0.39
Death	P	11	-0.89	9.4	2.6	0.05	-13.2	**0.00**	**0.00**	0.11	**0.00**
	T_{Mean}	5.7	4.8	13.6	2.0	-1.4	-9.8	**0.00**	**0.00**	**0.00**	**0.00**
	ΔT_{Min}	-12	1.9	4.2	0.5	-0.8	-4.0	**0.00**	**0.00**	**0.01**	**0.00**
	ΔT_{Max}	16	-2.7	-4.2	-1.1	1.0	1.9	**0.00**	**0.00**	**0.00**	**0.00**

Figures

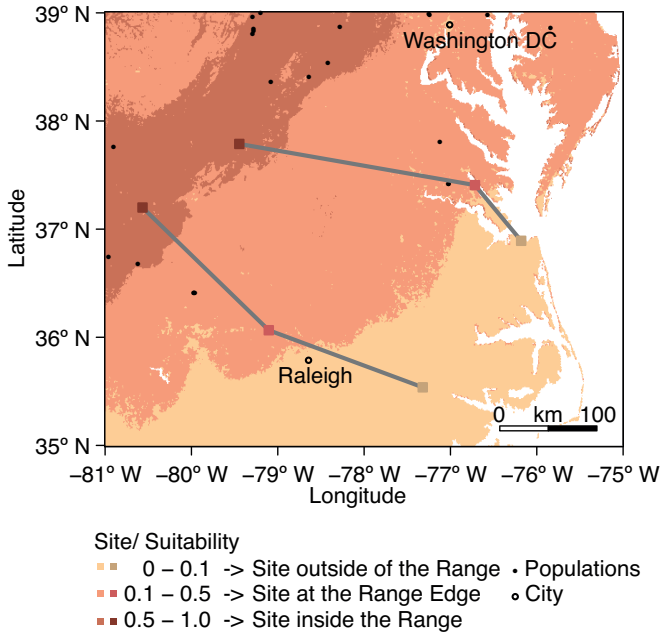

Figure 1: Map of the southern *A. lyrata* range edge with the populations (black dots) and the sites of the common garden experiment (coloured squares). The coloured background shows the suitability based on a prior suitability study (Lee-Yaw et al. 2018) with brown indicating high a suitability (>0.5), salmon a medium suitability (0.5-0.1) and light brown a low suitability (0.1-0).

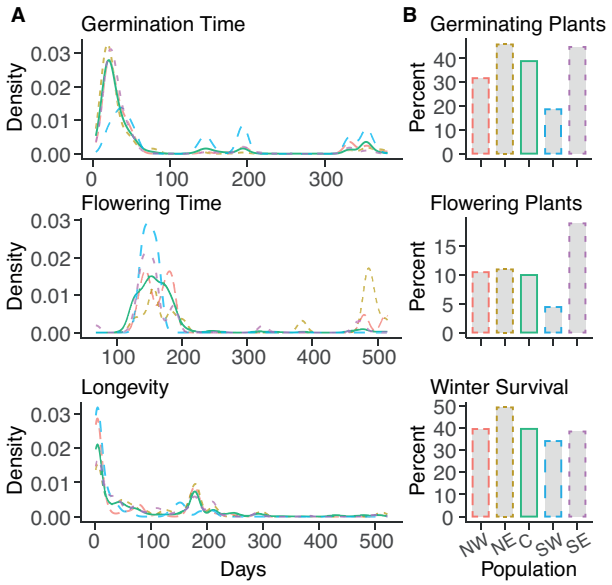

Figure 2: Differences between populations from different areas of the *A. lyrata* distribution.
A) Density plots of germination, flowering and survival time: the figure shows the time in days on the x-axis and on the y-axis the fraction of plants that germinated, flowered or died at that time. The bin width is seven days and the line type and colour indicate the populations as explained in B.
B) Bar plots give the percentage of plants that germinated, flowered and survived until the first or second year across the five populations. Line type and colour indicate the different populations North-East (NE), North-West (NW), Central (C), South-West (SW), and South-East (SE). Plant flowering and survival only include plants that germinated.

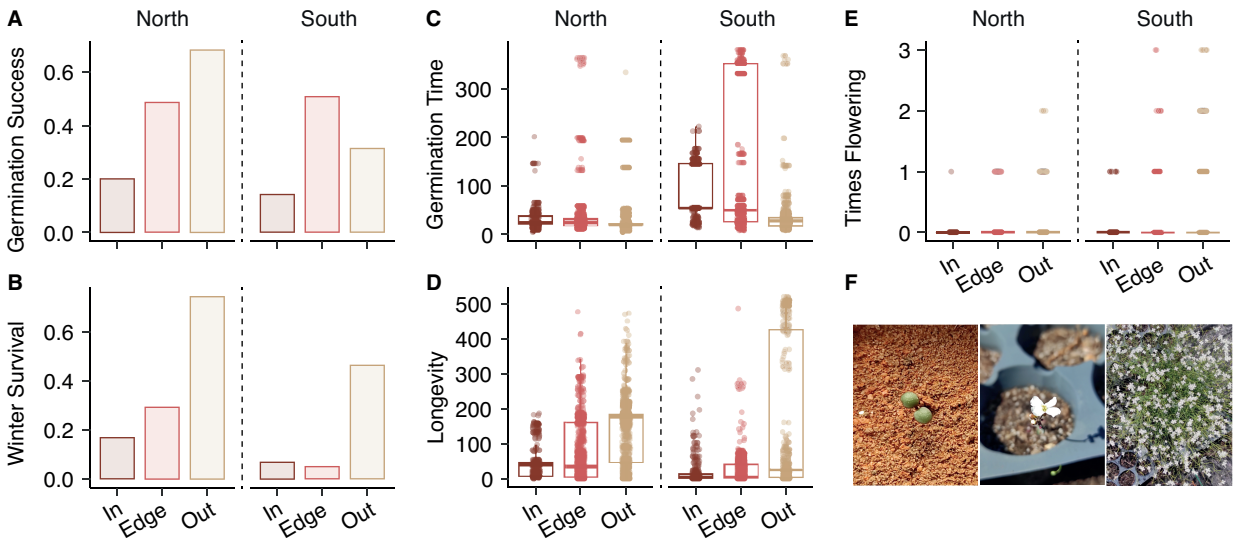

Figure 3: Differences in plant performance across transects and positions within transects of the central population.
A) Bar plots show Germination success of all seeds and the days it took from sowing to germination and B) fraction of plants that germinated and died before the first summer of the experiment. In distribution plots (C-E), each observation is indicated as a coloured dot and boxes give the 1.5 inter-quartile range with medians as thick lines. These are C) days from sowing to germination, D) longevity in days after germination and E) how often a single plant flowered. F) shows images of a newly germinated plant, a plant flowering in the first year with only one blossom and in the second year with many flowering stems.

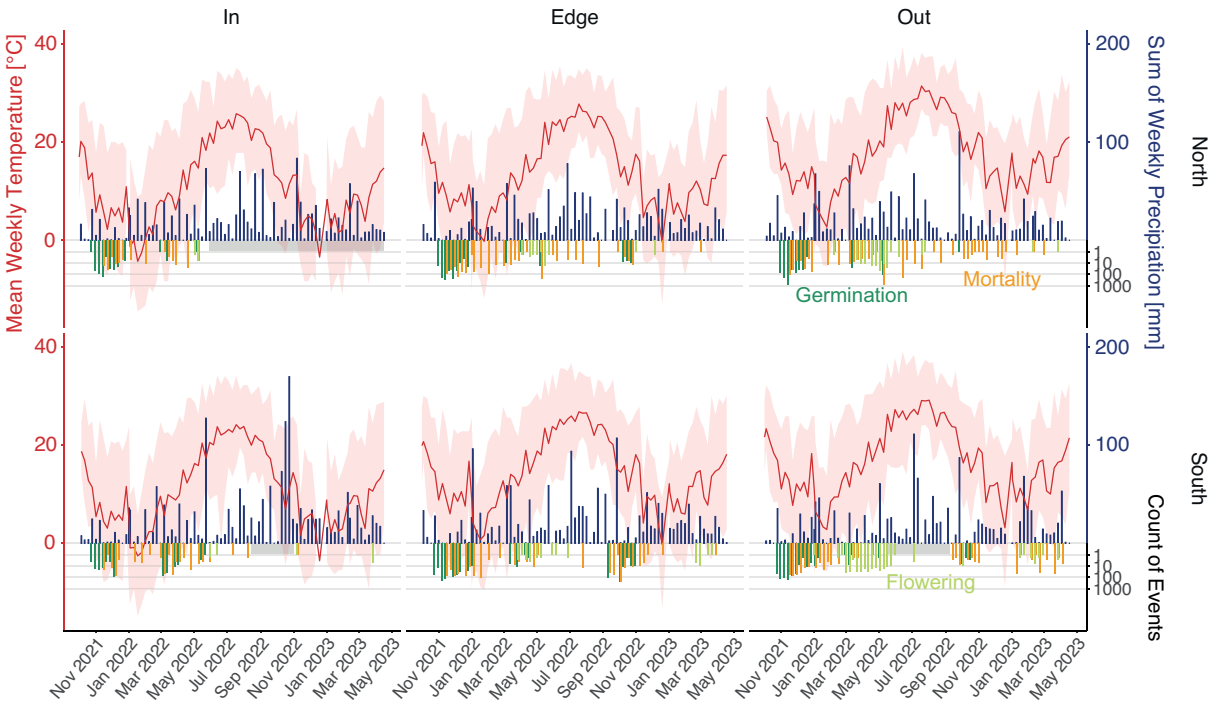

Figure 4: Weather at sites during the experiment with mean weekly records of temperature (red, with minimum and maximum indicated by red shaded areas) and cumulative precipitation in blue (mm). Below the weather data is on a $\log_{10}$ transformed scale in dark green the count of new germinations per week, in light green the sum of newly flowering plants per week and in orange the sum of plants that died per week, all of the central population. Light grey areas indicate missing photos to determine death dates.

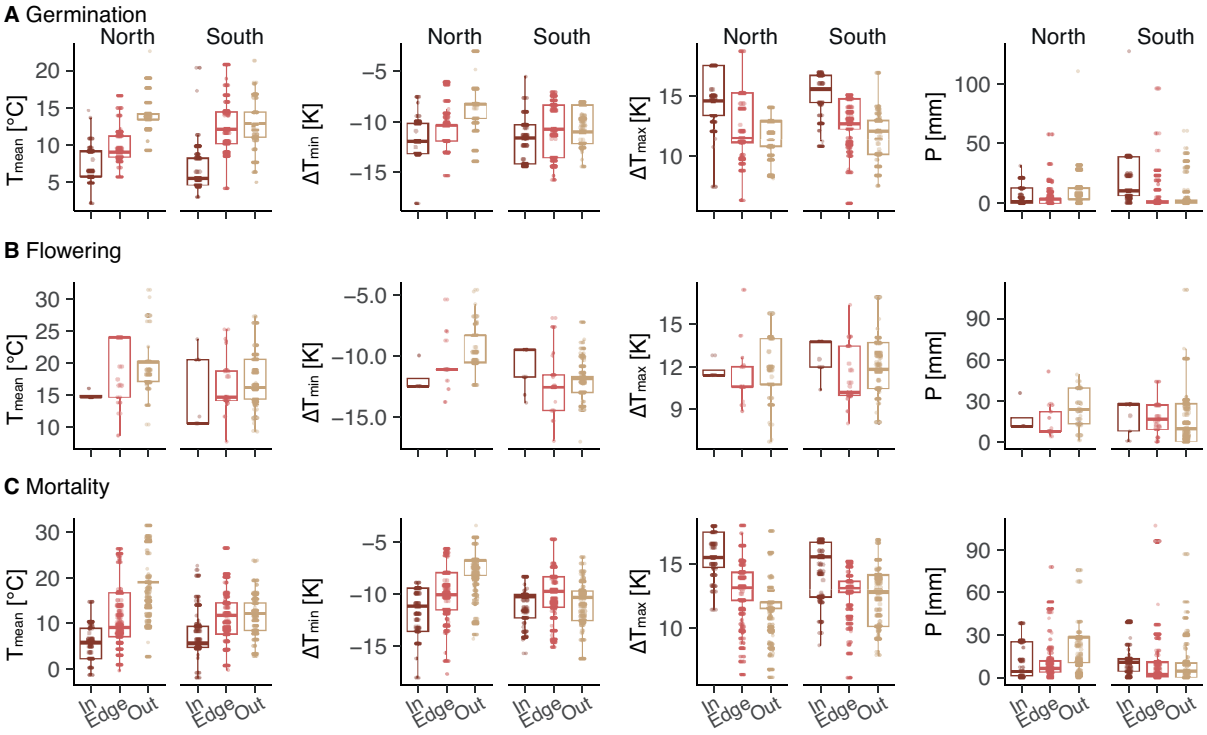

Figure 5: Overview of characteristics of experimental sites during the experiment.
Mean temperature (T_{mean}, in ˚C), difference from mean to minimum temperature (ΔT_{min} in K) and mean to maximum temperature (ΔT_{max} in K) and precipitation (P in mm) of weeks, when plants transitioned from A) seed to germination, B) growing to flowering, or C) from alive to dead. Each observation is indicated as a coloured dot and boxes give the 1.5 inter-quartile range with medians as thick lines.

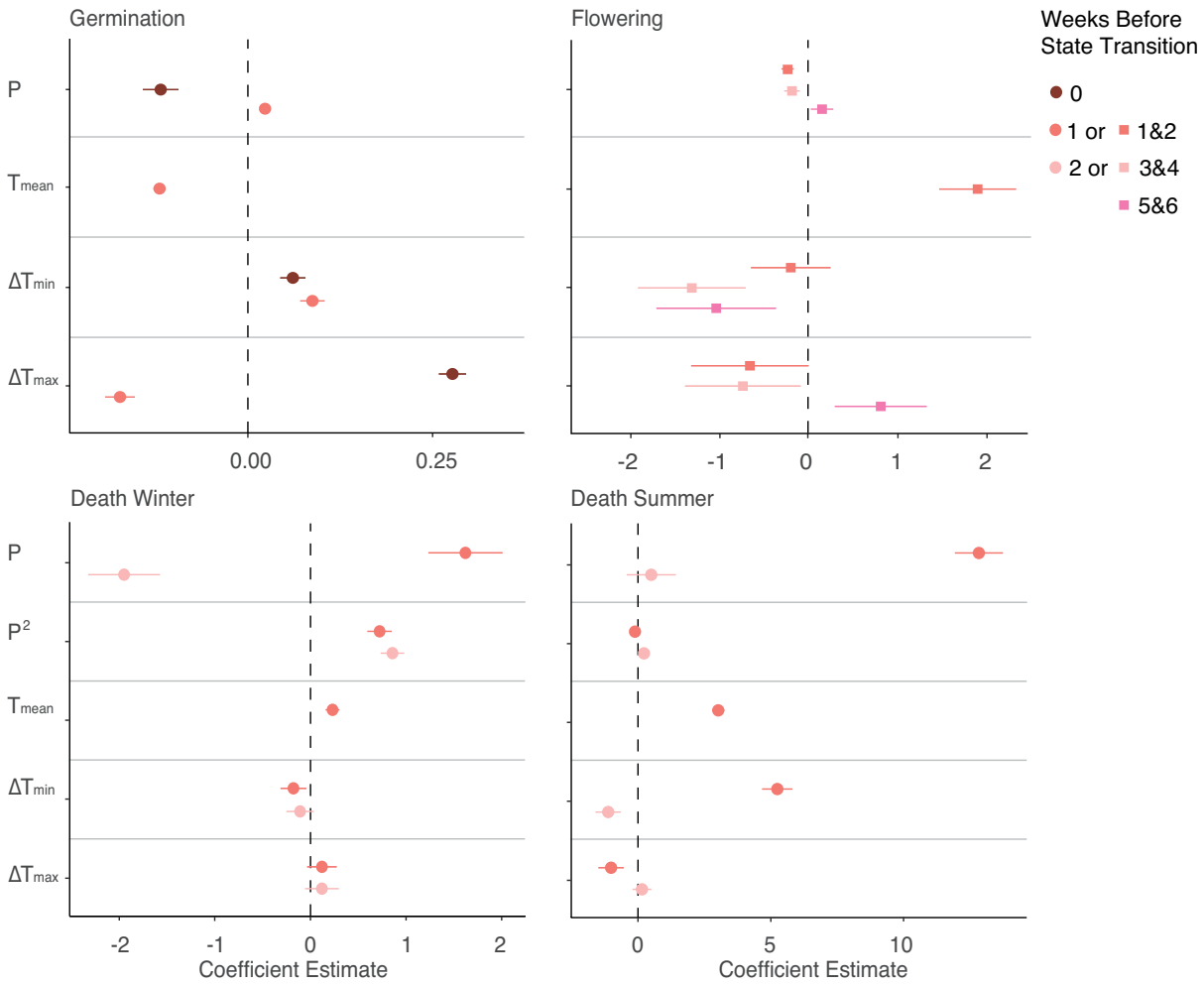

Figure 6: Influence of weather on transition to a new life stage in a dot-whisker-plot.
The effect sizes of mixed effect models including all important weeks prior to the stage transition for each considered life stages. Dots reflect the model estimates and horizontal lines the corresponding 95% confidence interval. The further a point is from the dashed line, the higher is the effect and the smaller the whiskers are, the higher is the accuracy.

Chapter 2

Negative interaction effect of heat and drought stress at the warm end of species distribution

Judith R. Schepers*, Jessica Heblack, and Yvonne Willi

* Correspondence

Affiliations:

Department of Environmental Sciences, University of Basel, 4056 Basel, Switzerland

E-mail:

judith.schepers@unibas.ch, jessica.heblack@unibas.ch, yvonne.willi@unibas.ch

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Abstract

Geographic range limits of species are often a reflection of their ecological niche limits. In many organisms, important niche limits that coincide with distribution limits are warm and warm-dry conditions. We investigated the effects of heat and drought, as they can occur at the warm end of distribution. In a greenhouse experiment, we raised North American *Arabidopsis lyrata* from the centre of its distribution as well as from low- and high-latitude limits under average and extreme conditions. We assessed plant growth and development, as well as leaf and root functional traits, and tested for a decline in performance and selection acting on growth, leaf, and root traits. Drought and heat, when applied alone, lowered plant performance, while combined stress caused synergistically negative effects. Plants from high latitudes did not survive under combined stress, whereas plants originating from central and low latitudes had low to moderate survival, indicating divergent adaptation. Traits positively associated with survival under drought, with or without heat, were delayed and slowed growth, though plastic responses in these traits were generally antagonistic to the direction of selection. In line, higher tolerance of stress in southern populations did not involve aspects of growth but rather a higher root-to-shoot ratio and thinner leaves. In conclusion, combined heat and drought, as can occur at southern range edges and presumably more so under global change, seriously impede the long-term persistence of *A. lyrata*, even though they impose selection and populations may adapt, though under likely interference by considerable maladaptive plasticity.

Keywords: adaptation, drought stress, heat stress, phenotypic selection, warm range limit

Introduction

Across the globe, temperatures have been increasing and precipitation has become more variable, with more droughts or extreme rain (IPCC 2021). In turn, warming has been linked to the retreat of some species from the warm limits of their distribution (Parmesan 2006; Cahill et al. 2014; Sánchez-Salguero et al. 2017; Rumpf et al. 2018). Causes of retreat can include the direct effect of abiotic stressors, biotic stressors, or interactions among them (Cahill et al. 2014; Paquette and Hargreaves 2021). Populations often evolve particular strategies to cope with one type of stressor over their evolutionary histories, which can interfere with strategies for coping with more extreme stress or other stressors (Fry 2003; Ågren and Schemske 2012; Santos del Blanco et al. 2013; Willi and Van Buskirk 2022). For example, it was shown that combined stressors, such as heat and drought, can act to amplify negative effects (Craufurd and Peacock 1993; Savin and Nicolas 1996; Dreesen et al. 2012; Zandalinas and Mittler 2022). Consequently, if we aim to understand why species fail to cope with extreme conditions at the warm end of species distributions, stressors need to be studied both individually and in combination (Suzuki et al. 2014).

Plants have evolved various ways of coping with heat, which have been studied in regards to the genes involved, the physiology, morphology, and development (Berry and Bjorkman 1980; Bita and Gerats 2013; Zhao et al. 2021; Sher et al. 2022; Yadav et al. 2022). In many species, a general strategy of coping with heat is leaf cooling through increased transpiration (Crawford et al. 2012; Deva et al. 2020; Sadok et al. 2021). Increased transpiration is achieved by a longer stomatal opening and higher stomatal conductance (Marchin et al. 2022). Such cooling requires a continuous supply of water, which is ensured, for example, by deep roots, an extensive and complex root system, or by a high root-to-shoot ratio (Parker 1949; Aston and Lawlor 1979; Natarajan and Kuehny 2008; Giri et al. 2017). Strategies affecting morphology are generally targeted at decreasing surface area to reduce the area of water loss by thick stems and leaves, short internode lengths, or smaller leaves (Vile et al. 2012; Stewart

et al. 2016; Leigh et al. 2017). Coping with heat may also include a faster phenology, such as early flowering to escape the heat during critical life stages (e.g., in *Arabidopsis thaliana*, Balasubramanian et al. 2006; Taylor et al. 2019). Additionally, leaf pigments can play an important role during heat and high irradiation as paler leaves with less chlorophyll help maintain energy balance and lower the risk of overheating (Kume 2017; Genesio et al. 2020), while carotenoids can dissipate excess energy and thereby protect the chlorophyll apparatus (Kumar et al. 2020).

Plants have evolved also various strategies to cope with drought (Murtaza et al. 2016), which sometimes differ substantially from those of coping with heat (Zhang and Sonnewald 2017). Under drought conditions, an immediate reduction of water-loss is achieved by the closure of the stomata; this ensures that the leaf water potential does not drop to critical levels and that plant metabolic processes are maintained (Verslues and Juenger 2011; Tardieu 2013). In combination with increased water uptake from the soil, the plant can thus maintain the physiological water balance (Rodrigues et al. 2019). Increased water uptake during a short period of drought is achieved by a wider and deeper root system (Dinneny 2019). In addition to longer roots, smaller leaves are a common response of plants growing under drought conditions, leading to an increased root-to-shoot ratio and reduced leaf surface area per dry weight (lower specific leaf area, SLA) (Matsui and Singh 2003; Dovrat et al. 2019). Another adjustment to a dry climate is accelerated reproductive development (Franks et al. 2007). Further strategies related to escape include a shorter growth period, earlier germination, or dormancy during extreme events (Basu et al. 2016; Franks 2011; Verslues and Juenger 2011; Tardieu 2013; Balachowski et al. 2016).

Combined heat and drought may be particularly challenging for plants. Marchin et al. (2022) reported for broadleaf evergreens that stomata closure is of advantage during drought, as it can maintain a high water potential of the leaves, but it can lead to overheating of leaves under heat. Conflicting responses to heat and drought were also reported for *A. thaliana* (cv

Columbia) and *Nicotiana tabacum* (Rizhsky et al. 2002, 2004). While plants responded to heat by increased photosynthesis and respiration, they responded to drought by reducing both processes. Under combined heat and drought, plants increased respiration but reduced photosynthesis, leading to senescence. Also in *A. thaliana*, high temperatures and the combination of heat and water deficiency accelerated reproductive development, while water deficiency alone delayed reproduction (Vile et al. 2012). The different responses to heat, drought and both stressors in combination confirm the need to investigate single and combined stressors to reveal the conflicts among strategies that impede their fitness benefits, particularly in the face of global warming.

The response to climatic stress often depends on the climate history of populations and can therefore vary greatly within species (Lexer et al. 2003). Indeed, local climate has been linked with adaptive differences among populations in several studies (e.g., Richardson et al. 2014; Estarague et al. 2022; Sánchez-Castro et al. 2022). Adaptive differences may be expressed under stress, but also when plants grow under ideal climatic growth conditions. In the canopy species *Corymbia calophylla* and in *A. thaliana*, plants originating from hot and/or dry areas differed in trait expression even under benign conditions; they had lower SLA, higher leaf dry matter content (LDMC), or smaller leaf area (May et al. 2017; Ahrens et al. 2020). Another aspect of climate adaptation is that within species or closely related species, there may be differences in how it is achieved. For example European *A. lyrata* subsp. *petraea* of southern range edges was shown to flower earlier and have a higher flowering propensity (Riihimäki and Savolainen 2004), while in North American *A. lyrata* subsp. *lyrata*, plants from northern latitudes have faster reproductive development (Paccard et al. 2014).

The aim of this study was to test whether heat, drought and combined stress had similar effects on growth, leaf and root functional traits, whether populations responded differently depending on their climate of origin, and whether plastic changes were in the direction favoured by selection. The study organism was the North American *Arabidopsis lyrata* spp. *lyrata*

(hereafter *A. lyrata*). Environmental niche modelling had revealed that the range limits of *A. lyrata* in the south and the north were associated with climate niche limits, with minimum temperature in early spring being the most niche- and range-limiting factor (Lee-Yaw et al. 2018). But with climate change, temperature and precipitation have changed across the distribution area of *A. lyrata*, resulting in reduced environmental suitability at the southern distribution limit (Online Resource 1 Fig. S1, Online Resource 2 Table S1). We analysed the stress responses of five populations, one from the range centre and two each from the warm and cold ends of the species' distribution (Figs. 1, Online Resource 1 S1, Online Resource 2 Tables S1, S2). Plants were grown in the greenhouse under four distinct temperature and watering conditions, based on average or higher temperature and average or lower precipitation as they occur at the low-latitude range edge during the growing season (Online Resource 2 Table S1). We addressed the following questions: (1) Do heat, drought, and heat-drought differ in how they affect growth, leaf and root functional traits, and do responses vary among populations and seed families within populations? (2) What is the difference in trait expression in populations from the southern edge as compared to central and northern populations? Are trait differences between these groups of populations the same as the plastic changes? And (3) how does selection act on traits? Does selection in the different environments align with plastic changes?

Materials and methods

Plant material

Arabidopsis lyrata subsp. *lyrata* is native to the eastern and mid-western United States and south-eastern Canada, and it is locally restricted to substrates with little water-holding capacity, sand and rocky outcrops (Koch et al. 2001; Al-Shehbaz and O'Kane 2002; Schmickl et al. 2010). Seeds were collected from five *A. lyrata* populations (Fig. 1): a genetically highly diverse one from the centre of the range (**C**) (Wos and Willi 2018), and four from the edges, in the north-east (**NE**), north-west (**NW**), south-east (**SE**), and south-west (**SW**, details in Table

S2). Collections were performed between 2007 to 2014, and seeds of field-collected plants were propagated together during one generation in the greenhouse by performing crosses within unique pairs of plants of the same population. For this experiment we considered three pairs of plants for range-edge populations and 120 pairs for the central population; the latter population was used for selection analysis and therefore included many more plants.

Climate data

Climate data at the sites of the five populations were obtained from WorldClim v1.4 (Hijmans et al. 2005), v2.1 (Fick and Hijmans 2017) and CRU-TS 4.06 (Harris et al. 2020) downscaled with WorldClim v2.1. We downloaded monthly average temperature (T_{mean}), maximum temperature (T_{max}), precipitation (P) and precipitation during the driest month (P_{min}, Bio14) for the periods of 1960-1990 and 1970-2000. For 2000-2018, we used the monthly minimum temperature (T_{min}), maximum temperature (T_{max}), and precipitation (P), and calculated monthly average temperature (T_{mean}) and precipitation of the driest month (P_{min}). For T_{mean}, T_{max} and P of the three time periods, we calculated averages for the months of April to June and June to August (using the dplyr and raster packages; Hijmans 2022; Wickham et al. 2022). For the first two periods, the resolution was 30 seconds, for 2000-2018, the resolution was 2.5 minutes. Plots and all statistics were done with R (R-Core-Team 2021). Raster plots (Figs. 1, Online Resource 1 S1) were produced with the R packages sp and sf (Pebesma and Bivand 2005; Pebesma 2018).

Experimental design

Offspring plants were grown under four climatic conditions in a two-by-two factorial design, with average or high temperature, and average or low precipitation as occurs at the two warm-end populations (**SE** and **SW**) (Online Resource 1 Fig. S1). We assumed that plants would germinate during fall or early spring and grow and develop thereafter. To imitate average conditions, values close to mean temperature and precipitation for April to June were chosen

(data in Online Resource 2 Table S1). For the heat treatment, temperature was set close to the mean of June to August. For the drought treatment, precipitation of the driest month for the two sites was taken.

For each treatment combination, five blocks were set up, each with one replicate seed per cross (edge populations were only represented in three blocks). Seeds were placed into 54-multipot trays within a block, filled with a sand-peat mixture of 2:1. Only every second pot of a tray was used to prevent plants from growing into each other and to facilitate image analysis. Seeds were stratified for twelve days at 4 °C in climate chambers at 70% humidity (ClimeCab 1400, KÄLTE 3000 AG, Landquart, Switzerland) and then transferred to four greenhouse chambers (temperature of 18 °C). During stratification and germination, plants were covered with mesh nets to maintain high humidity. To ensure a gradual change between stratification and experimental conditions, day length was increased from 8 h by 1 h every 3-4 days until the day length was 16 h, with a light intensity of 200 μM s^{-1} m^{-2}. During the transition phase, day temperature was 20 °C and night temperature was 18 °C, and plants were watered daily by spraying from above. After seven days, when approximately 75% of the plants had germinated, the mesh nets were removed. After an additional 14 days, when about 80% of germinated plants were at the four-leaf stage, stress treatments began.

Two of the four greenhouse chambers (University of Basel greenhouse) were set to have cold temperatures, and two to have warm temperatures. Each chamber of a particular temperature regime contained either two or three spatial blocks of multi-pot trays with plants of both watering treatments. Based on climate data from the two southern sites (Online Resource 2 Table S1), we set the low-temperature regime to an average of 20.6 °C: 22 °C during the day, a one-hour heat peak of 25 °C at noon, and night temperature at 18 °C for 8 h. The high-temperature regime had an average of 25.2 °C: 27 °C during the day, a heat peak of 30 °C at noon, and a night temperature of 23 °C. The high precipitation/watering regime was initially 8.4 ml of water every second day, corresponding to 100 mm m^{-2} of monthly precipitation. The

low-precipitation treatment was 5 ml of water every second day corresponding to 65 mm m^{-2} of monthly precipitation. Due to sudden early dieback in the dry treatment because the soil in the small pots dried out quickly, watering was increased by 20%, to 10 ml and 6 ml in the high and the low precipitation regimes, respectively; in nature, soil bodies where *A. lyrata* grows are typically deeper and less likely to dry out as rapidly. In all chambers, air humidity was set to 70%. Trays were randomized twice per week (within blocks, and block position in the paired chambers), and fertilizer was given every four weeks (0.2% Wuxal universal fertilizer, Westland Schweiz GmbH, Dielsdorf, Switzerland). Additionally, after 14 weeks, an insecticide (1.5% Kendo gold, Westland Schweiz GmbH) was applied once a week to protect the plants from insect infestations.

Trait assessment

Performance. After stratification, every day for two weeks we recorded the day of germination, when the cotyledons became visible. Afterwards, pots were examined every second to third day for further germination, death (all leaves brown and dry), bolting (visible flowering stem), flowering (first flower), revival of plants (green leaves), and infestation. This approach resulted in data on days to germination, survival, longevity (days until death or harvest), and flowering propensity.

Growth traits. We monitored the growth of rosettes by taking images twice a week starting with germination. Images were taken per multiport tray with a 12 MP Panasonic DMC-FS10 digital camera (Kadoma, Japan) with ISO 100 and -2/3 exposure in a photo box that was placed over individual trays. Imaging stopped when 40% of plants from the control treatment had bolted. Additional images were taken before harvest. Images were analysed by an adapted script of Exposito-Alonso et al. (2019). From each image, two new images were produced, one retaining pixels in the range of green and the other in the range of red. The two images were then merged, the sum of pixels counted for each pot and time point, and the value transformed

into mm^2. For each plant, seven growth models were explored (linear, exponential, power, two- and three-parameter logistic, von Bertalanffy, and Gompertz) to fit the size data over time. Of these, the three-parameter logistic model – together with the more complex Gompertz model – was the best supported across plants and treatments. From the three-parameter logistic model we extracted the asymptote (maximum rosette size [mm^2], *size*), the time to the inflection point (time to fastest growth [days], x_{mid}), and the slope at the inflection point (*growth rate*). The script is accessible at github.com/heblackj/image_analysis.

Leaf and root functional traits. We stopped the experiment one month after 40% of the plants of the control group had started flowering. All plants were separated into four components, if present: inflorescences, dead leaves, living leaves, and roots. Leaves and roots were washed to remove attached soil and dried with a paper towel to remove excess water. The fresh weight of inflorescences, living leaves, and roots was taken. Then the material was dried separately for 48 h in an oven at 60 °C. We calculated the specific leaf area (*SLA*, size [mm^2] per dry weight of all leaves [mg], excluding dead leaves), the leaf dry matter content (*LDMC*, dry weight leaves [mg] per wet weight leaves [g], excluding dead leaves), and the root-to-shoot ratio (*root:shoot*; dry weight roots per dry weight all leaves and inflorescences). The range of trait values per treatment and family are presented in Online Resource 2 Table S3.

Statistical analysis

To approach normality of the dependent variables, we log_{10}-transformed growth rate, root:shoot ratio, SLA, and LDMC. An initial analysis of variance was performed to reveal the effects of days to germination, block, and tray within block on variables (Anova in car package; Fox and Weisberg 2019). If considerable variance was explained, variables were corrected for the specific effects. Furthermore, we looked into trait dependencies by correlating all traits within the central population at the level of the plant for each treatment separately (Fig. 3, rcorr in Hmisc; Harrell 2022) and performed a principal component analysis for each treatment (Online Resource 1 Fig. S2, factoextra package; Kassambara and Mundt 2020).

In the main analysis, we tested for the effect of temperature, watering and the interaction term on aspects of performance and functional traits using linear mixed effects models for continuous data or generalized linear models for binary data (lmerTest package; Kuznetsova et al. 2017). The random effects included population and family nested within population, but the precise structure was set based on model selection. The models that were compared by Akaike information criterion (AIC) varied from: including intercept, slope on temperature, slope on watering, and all covariances for population and family nested within population, to including intercepts only (results in Online Resource 2 Table S4). For each dependent variable the best model was chosen for final analysis. The random effects were evaluated by likelihood ratio testing (Table 1; lrtest in the lmtest package; Zeileis and Hothorn 2002). Differences in plant performance and traits between low- (SE, SW) and high-latitude populations (NE, NW, C) were tested by Wilcoxon rank sum tests (Table 2).

We conducted univariate and multivariate phenotypic selection analyses on the growth and functional traits of the central population with generalized linear models (de Jong 1995; Scheiner and Callahan 1999; Callaway et al. 2003). Trait data was standardized (mean = 0, deviation = 1) within treatment, and models were run for each treatment separately. An exception was the combined heat and drought treatment. As we lacked data on SLA, LDMC and root:shoot ratio of the many plants that had died in this treatment, we replaced values; we calculated family means for these traits under drought or heat treatment, averaged those values over the two treatments, and used this trait data instead in the selection analysis of the combined stress treatment. In models including single traits, we first evaluated the inclusion of both the linear and quadratic term by AIC (Table 3). As the inclusion of the quadratic term was rarely better, the multivariate models were built by only including linear terms (packages mcglm and htmcglm; Bonat 2018; de Freitas et al. 2022). As fitness variables, we used the propensity to flower for the control treatment, survival for single stress treatments, and longevity for the combined stress treatment.

Results

Climate change

For the five populations studied, the climate had shifted between the periods of 1960-1990 and 2000-2018 (Online Resource 1 Fig. S1, Online Resource 2 Table S1). The change in mean temperature for the growing season of April to June and the summer months of June to August had increased by 0.4 ºC and 0.6 ºC, respectively. Change varied considerably among sites, e.g. for the summer means from +0.1 ºC to +1.1 ºC. At the same time, mean precipitation during April to June and June to August increased by 11 mm and 8.6 mm, respectively, again with some variability among sites. However, precipitation during the driest month of the year, which tends to be in late winter at the southern edge of *A. lyrata*, had declined by 14.5 mm. Under the conditions chosen in the experiment, we simulated average spring compared to summer temperature at the southern edge, and average spring precipitation compared to dry conditions, assuming that such extreme events may become more likely under global warming already during spring, when plants grow and start flowering.

Heat and drought stress

The treatments, temperature and watering, had strong additive and interaction effets (Table 1). Heat and drought lowered survival, and both stressors combined lowered survival even further (Fig. 2A). Longevity and the propensity to flower generally followed this pattern. The variable of longevity had low values and high variability in the treatment with combined stress (Online Resource 2 Table S3). For treatments with low temperatures, there was considerable flowering, and plants showed a lower propensity to flower under dry compared to control conditions (Fig. 2B).

Patterns for plant size were similar to those for survival. Maximum plant size was negatively affected by drought and – as a trend – by heat, and under combined heat and drought, their negative effect was exacerbated (Fig. 2C, Table 1). In turn, time to mid-size was shorter

under single stress and interacted to be much shorter under combined stress (Fig. 2D). Furthermore, maximal growth rate was higher under heat and lower under drought, though the interaction term was again positive, indicating highly accelerated growth rates under combined heat and drought (Fig. 2E). LDMC decreased and the root:shoot ratio increased under single stress, indicating more water relative to dry weight in leaves and more relative investment into roots (Figs. 2G, H). However, the interaction term was not significant for the two traits. For SLA, only the interaction term was significant, indicating that plants had thinner leaves under combined heat and drought (Fig. 2F).

Populations did not differ significantly in traits across treaments nor in response to drought or heat stress, except in the root:shoot ratio (Table 1). All other significant random effects involved families or how families reacted to heat and watering. Nevertheless, some trends of population differences could be detected based on contrasts between the southern and the more northerly populations, including the central population (Table 2). Survival was similar among populations across treatment combinations except for combined heat and drought; in that treatment, southern populations tended to perform better, indicating some adaptation to extreme heat combined with drought (Fig. 3A). Other traits that differed between the southern and all other populations were SLA and the root:shoot ratio. Plants of southern populations had higher SLA, particularly under combined heat and drought (Fig. 3B), as well as higher root:shoot ratios, and the ratio increased more under single stress (Fig. 3C).

Correlations among traits were investigated for patterns within treatments by considering plants of the central population only (Figs. 4, Online Resource 1 S2). A few correlations were rather consistent across treatments, such as the negative correlation between maximal growth rate and both asymptotic plant size and time to mid-size, and the positive correlation between time to mid-size and plant size. There were two additional, consistently negative correlations both involving the root:shoot ratio, with plant size and LDMC.

Traits under selection

Lastly, we investigated the traits under phenotypic selection under the different treatments (Table 3). Only the diverse central population was included in this analysis, as it covered most of the variation in traits of the edge populations. Under heat alone, no evidence for a trait under selection could be found, neither in the univariate nor in the multivariate selection analyses. Under drought, high x_{mid}/late vegetative growth and a low growth rate were selectively favoured, though this was only found under univariate selection. Under combined heat and drought stress, we found evidence for positive linear selection favouring late maximal growth (univariate selection only), slow growth, large final size, and small SLA (multivariate selection only). Finally, under control conditions, we found evidence for positive linear selection favouring larger size (univariate selection only), higher SLA, higher LDMC, and lower root:shoot ratio.

Discussion

Populations from the southern edge of the distribution of *A. lyrata* are affected by climate change, warmer average temperatures and more variable precipitation (Online Resource 2 Table S1). In our experimental study, we found that an increase in temperature and lower precipitation/watering had a negative effect on plant survival, and combined stress had a worse than additive effect on survival (Fig. 2A). Parallel findings were revealed for vegetative growth. Under single stress, plants had fast growth earlier and reached or tended to reach a smaller final size, while under combined stress, fastest growth happened even earlier and final size was smaller than if stressors had acted additively (Figs. 2C, D). Moreover, southern populations had a higher survival under combined stress compared to northern populations, indicating some adaptation to such extreme climatic conditions. We discuss these and further results below in regard to strategies for coping with climatic extremes and conflicts among strategies under variable climatic extremes at the low latitudinal edge.

Single stressors, heat or drought, lowered survival to a similar extent, though other aspects of performance differed. Size was reduced more under drought, but hardly any plants flowered under heat (Fig. 2B, C). The combination of heat and drought was then particularly devastating for plant survival, as stressors interacted in a synergistic manner. *Arabidopsis lyrata* must regularly experience very hot and dry conditions where it occurs. The species thrives in relatively open vegetation, on active sand dunes and on rocks with little vegetation cover, which heat up on sunny days. Furthermore, sandy soils typically have little water-holding capacity, and rocky outcrops have hardly any, except for cracks that may be filled with organic substrate. Given these features of the habitat, one would assume that the species can cope with both stressors, but apparently not when they co-occur as in our pot-design experiment. The result is in line with many studies showing that stressors multiply in their effect on plant performance (Mittler 2006; Zhang and Sonnewald 2017; Zandalinas and Mittler 2022).

We observed a number of plastic responses to heat, drought, and combined stress along the slow-fast continuum that did not seem adaptive. Plants exposed to heat or drought had the fastest growth early, a higher maximal growth under heat, and they reached a smaller final size (Figs. 2C-E, Table 1). This pattern of earlier and faster growth together with reduced size was strengthened under combined stress. Therefore, results suggest that *A. lyrata* generally responds to heat and/or drought by a strategy of escape in time (Levitt 1980; Ludlow and Muchow 1990) that seems to come at the cost of small size, in line with the concept of the slow-fast continuum (Reich 2014). The study of phenotypic selection indicated that these induced responses in vegetative growth were not adaptive or even maladaptive, with selection favouring opposite trait responses (Table 3). Under drought and combined heat and drought, selection tended to favour late and slow growth. Furthermore, under combined heat and drought, selection favoured large size. A reason could be that the plastic responses evolved in environments of short stress exposure, whereas the one applied in our study lasted longer and might have possibly favoured adaptations increasing climate tolerance (or resistance). Divergence between strategies of

escape and tolerance have often been reported in response to drought stress. While early growth can be a drought escape or avoidance strategy with a short life cycle, plants with a tolerance strategy commonly grow more slowly under long-term drought stress and over a longer period of time, and thus live longer (Franks 2011; Tardieu 2012; Bouzid et al. 2019; Csilléry et al. 2020; Burnette and Eckhart 2021).

Small size need not necessarily be a cost of early and rapid growth but could be beneficial under heat and drought. Under heat, small leaves rather than large ones are more likely to maintain a low leaf temperature by higher transpiration (Vile et al. 2012; Stewart et al. 2016; Saini et al. 2022). Under drought, small leaf size can be beneficial as water loss is lower (Lin et al. 2017). Such benefits may have also partially existed in our experiment, as under heat or drought alone we found no sign of positive selection for larger size (Table 3). Moreover, small size seems largely a cost of early and fast growth. Phenotypic correlation analysis on the central population supported that the three traits of time to fastest growth, maximal growth rate and final plant size, were strongly integrated in each of the four treatment combinations used in our study, with the strongest found under combined stress (Fig. 4). Therefore, while small size may be of some advantage under single stress, it is a serious cost to early and rapid growth under combined stress.

We also observed plastic responses in leaf and root functional traits. Plants had a higher root:shoot ratio and more water in leaves (lower LDMC) under single stress and thinner leaves (higher SLA) under combined stress (Table 1). Morphological adaptations to maintain a high water potential under stress are typically achieved by increased root systems, reduced vegetative growth or reduced stomatal transpiration loss, e.g. by thicker leaves (Sicher et al. 2012; Maggio et al. 2018; Seleiman et al. 2021). Alternatively, tolerance strategies are associated with maintaining hydrostatic pressure, by osmotic adjustments, and cavitation resistance (Delzon 2015; Blum 2017). Except for thinner leaves being disfavoured under combined heat and drought (in multivariate selection analysis only), none of the three leaf and

root functional traits were found to be under selection under single or combined stress while they were under control conditions. Under control conditions, a high root:shoot ratio was negatively selected against, indicating costs. Furthermore, thin leaves (higher SLA) with a high dry matter content (higher LDMC) – potentially photosynthetically highly active – were favoured. Plants seem to adjust plastically in response to stress mainly by trait expression away from what is favoured under benign conditions.

However, southern populations, which had the highest survival under combined heat and drought, differed exactly in leaf and root functional traits. The two northern populations had no survival under combined stress, the central population, represented by many more plants in the experiment, had some survival, and the two southern-range-edge populations had considerable survival (Fig. 3A, Table 2). The southern populations seem to have been pre-exposed to similar stress conditions in the past and adapted to them. Therefore, traits that we found divergent between southern and more northern populations can indicate the traits of adaptation (Estarague et al. 2022). Southern populations differed in the expression of a higher root:shoot ratio, especially under stress (Fig. 3C). This response of low-latitude populations in the root system should allow the cooling by transpiration while maintaining the leaf water potential and photosynthesis (Stewart et al. 2016; Berny Mier y Teran et al. 2019; Csilléry et al. 2020; Marchin et al. 2022). Furthermore, under combined heat and drought, plants mainly from a southern population had thinner leaves (higher SLA, Fig. 3B, Table 2). This latter finding is hardly an adaptation, however, as thicker leaves were shown to be better at heat buffering and low water loss by evaporation (Wright et al. 2005; Leigh et al. 2012; H. Zhou et al. 2020), leaving the root:shoot ratio as the most likely candidate.

In fact, the combination of results of the different analyses suggests some important differences in the root:shoot ratio between southern and northern populations. At a first glance, the presumably adaptive differences between the southern and northern populations are in line with induced responses by stress – higher root:shoot ratio under single stress and higher SLA

under combined stress (Table 1), but with selection not found to act on these traits (Table 3). However, a high root:shoot ratio can be achieved by either investing less in shoots or investing more in roots. The plastic response of an increased root:shoot ratio under single stress may have been the result of smaller plant size and lower investment in shoots, which was neither disfavoured nor favoured by selection in those environments. In line with this, thin leaves, as found under combined heat and drought, may indicate less investment in above-ground structures as compared to roots (Wright et al. 2005; de Castro et al. 2019), which was not an adaptation but actually disfavoured in that environment (under negative selection in multivariate selection analysis). It is important to emphasize that these results were found with a focus on the central population. Southern populations are probably different in that they had a high root:shoot ratio owing to a higher investment in root structures and that is why they performed better under stress. Evidence in favour of this is their higher root:shoot ratio, particularly under stress, that is not paralleled with a lower investment in above-ground plant size (Table 2). The results clearly indicate the need to study the evolutionary potential of root traits in the context of southern range limits and climate change (Zhou et al. 2019; Taseski et al. 2021).

Conclusion

We studied replicate *A. lyrata* populations from across its distribution for their ability to cope with single stress, heat or drought as well as combined heat and drought as can be expected at the southern range edge under global warming. Our results led to two main conclusions for the species. First, the combination of heat and drought reduces plant survival more than predicted by the additive effects of heat and drought. Second, while plants from the north cannot persist under such conditions, plants originating from the southern end of the range have some survival, indicating the potential for adaptation. Selection analysis with a focus on the central population suggested that plastic responses to heat and drought followed a strategy of escape, which was

not favoured under any of the stress environments. In line with this, the higher stress tolerance of the southern populations did not involve adjustments on the slow-fast continuum but was probably achieved by a higher allocation into roots as compared to shoots.

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Author contributions

YW, JH and JS designed the study. YW collected seeds in the field, and JH propagated seeds. JH and JS performed the experiment. JS did the analysis and wrote the manuscript, with inputs by YW. All authors read and agreed on the manuscript.

Availability of data and material

The datal is available on figshare (https://doi.org/10.6084/m9.figshare.23104481).

References

Ågren J, Schemske DW (2012) Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. New Phytol 194:1112–1122. doi.org/10.1111/j.1469-8137.2012.04112.x

Ahrens CW, Andrew ME, Mazanec RA, et al (2020) Plant functional traits differ in adaptability and are predicted to be differentially affected by climate change. Ecol Evol 10:232–248. doi.org/10.1002/ece3.5890

Al-Shehbaz IA, O'Kane SL (2002) Taxonomy and phylogeny of *Arabidopsis* (Brassicaceae). Arab B 1:1–22. doi.org/10.1199/tab.0001

Aston MJ, Lawlor DW (1979) The relationship between transpiration, root water uptake, and leaf water potential. J Exp Bot 30:169–181

Balachowski JA, Bristiel PM, Volaire FA (2016) Summer dormancy, drought survival and functional resource acquisition strategies in California perennial grasses. Ann Bot 118:357–368. doi.org/10.1093/aob/mcw109

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent Induction of *Arabidopsis thaliana* flowering by elevated growth temperature. PLOS Genet 2:e106 doi.org/10.1371/journal.pgen.0020106

Basu S, V R, Kumar A, Pereira A (2016) Plant adaptation to drought stress. F1000Research 5:1554. doi.org/10.12688/f1000research.7678.1

Berny Mier y Teran JC, Konzen ER, Medina V, et al (2019) Root and shoot variation in relation to potential intermittent drought adaptation of Mesoamerican wild common bean (*Phaseolus vulgaris* L.). Ann Bot 124:917–932. doi.org/10.1093/aob/mcy221

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. Annu Rev Plant Physiol 31:491–543. doi.org/10.1146/annurev.pp.31.060180.002423

Bita C, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:273. doi.org/10.3389/fpls.2013.00273

Blum A (2017) Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ 40:4–10. doi.org/10.1111/pce.12800

Bonat WH (2018) Multiple response variables regression models in R: the mcglm package. J Stat Softw 84:1–30. doi.org/10.18637/jss.v084.i04

Bouzid M, He F, Schmitz G, et al (2019) *Arabidopsis* species deploy distinct strategies to cope with drought stress. Ann Bot 124:27–40. doi.org/10.1093/aob/mcy237

Burnette TE, Eckhart VM (2021) Evolutionary divergence of potential drought adaptations between two subspecies of an annual plant: Are trait combinations facilitated, independent, or constrained? Am J Bot 108:309–319. doi.org/10.1002/ajb2.1607

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, et al (2014) Causes of warm-edge range limits: systematic review, proximate factors and implications for climate change. J Biogeogr 41:429–442. doi.org/10.1111/jbi.12231

Callaway RM, Pennings SC, Richards CL (2003) Phenotypic plasticity and interactions among plants. Ecol 84:1115–1128. doi.org/10.1890/0012-9658(2003)084[1115:PPAIAP]2.0.CO;2

Craufurd PQ, Peacock JM (1993) Effect of heat and drought stress on sorghum (*Sorghum bicolor*). II. Grain yield. Exp Agric 29:77–86. doi.org/10.1017/S0014479700020421

Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA (2012) High temperature exposure increases plant cooling capacity. Curr Biol 22:R396–R397. doi.org/10.1016/j.cub.2012.03.044

Csilléry K, Buchmann N, Fady B (2020) Adaptation to drought is coupled with slow growth, but independent from phenology in marginal silver fir (*Abies alba Mill.*) populations. Evol Appl 13:2357–2376. doi.org/10.1111/eva.13029

de Castro JN, Muller C, Almeida GM, Costa AC (2019) Physiological tolerance to drought under high temperature in soybean cultivars. Aust J Crop Sci 13:976–987. doi.org/10.21475/ajcs.19.13.06.p1767

de Freitas LAC, de Oliveira Carlos L, Campos ACL, Bonat WH (2022) Hypothesis tests for multiple responses regression: effect of probiotics on addiction and binge eating disorder. doi.org/10.48550/arXiv.2208.00027

de Jong G (1995) Phenotypic plasticity as a product of selection in a variable environment. Am Nat 145:493–512

Delzon S (2015) New insight into leaf drought tolerance. Funct Ecol 29:1247–1249. doi.org/10.1111/1365-2435.12500

Deva CR, Urban MO, Challinor AJ, et al (2020) Enhanced leaf cooling is a pathway to heat tolerance in common bean. Front Plant Sci 11. doi.org/10.3389/fpls.2020.00019

Dinneny JR (2019) Developmental responses to water and salinity in root systems. Annu Rev Cell Dev Biol 35:239–257. doi.org/10.1146/annurev-cellbio-100617-062949

Dovrat G, Meron E, Shachak M, et al (2019) Plant size is related to biomass partitioning and stress resistance in water-limited annual plant communities. J Arid Environ 165:1–9. doi.org/10.1016/j.jaridenv.2019.04.006

Dreesen FE, De Boeck HJ, Janssens IA, Nijs I (2012) Summer heat and drought extremes trigger unexpected changes in productivity of a temperate annual/biannual plant community. Environ Exp Bot 79:21–30. doi.org/10.1016/j.envexpbot.2012.01.005

Estarague A, Vasseur F, Sartori K, et al (2022) Into the range: a latitudinal gradient or a center-margins differentiation of ecological strategies in *Arabidopsis thaliana*? Ann Bot 129:343–356. doi.org/10.1093/aob/mcab149

Exposito-Alonso M, Vasseur F, Ding W, et al (2018) Genomic basis and evolutionary potential for extreme drought adaptation in *Arabidopsis thaliana*. Nat Ecol Evo, 2:352–358. doi.org/10.1038/s41559-017-0423-0

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. Int J Climatol 37:4302–4315. doi.org/10.1002/joc.5086

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks CA. socialsciences.mcmaster.ca/jfox/Books/Companion/

Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. New Phytol 190:249–257. doi.org/10.1111/j.1469-8137.2010.03603.x

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proc Natl Acad Sci 104:1278–1282. doi.org/10.1073/pnas.0608379104

Fry JD (2003) Detecting ecological trade-offs using selection experiments. Ecology 84:1672–1678. doi.org/10.1890/0012-9658(2003)084[1672:DETUSE]2.0.CO;2

Genesio L, Bassi R, Miglietta F (2020) Plants with less chlorophyll: a global change perspective. Glob Chang Biol 27:959–967. doi.org/10.1111/gcb.15470

Giri A, Heckathorn S, Mishra S, Krause C (2017) Heat stress decreases levels of nutrient-uptake and -assimilation proteins in tomato roots. Plants 6:6. doi.org/10.3390/plants6010006

Harrell Jr F (2022) Hmisc: Harrell Miscellaneous. R package version 5.1-0, hbiostat.org/R/Hmisc

Harris I, Osborn TJ, Jones P, Lister D (2020) Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. Sci Data 7:109. doi.org/10.1038/s41597-020-0453-3

Hijmans RJ (2022) Raster: geographic data analysis and modeling. R package version 3.6-20, rspatial.org/raster/

Hijmans RJ, Cameron SE, Parra JL, et al (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978. doi.org/10.1002/joc.1276

Intergovernmental Panel on Climate Change (IPCC) (2023). Climate Change 2021 – The physical science basis: Working group I contribution to the sixth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press.

Kassambara A, Mundt F (2020) Factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7.999, CRAN.R-project.org/package=factoextra

Koch M, Haubold B, Mitchell-Olds T (2001) Molecular systematics of the Brassicaceae: evidence from coding plastidic *mat*K and nuclear *Chs* sequences. Am J Bot 88:534–544. doi.org/10.2307/2657117

Kumar P, Yadav S, Singh MP (2020) Possible involvement of xanthophyll cycle pigments in heat tolerance of chickpea (*Cicer arietinum* L.). Physiol Mol Biol Plants 26:1773–1785. doi.org/10.1007/s12298-020-00870-7

Kume A (2017) Importance of the green color, absorption gradient, and spectral absorption of chloroplasts for the radiative energy balance of leaves. J Plant Res 130:501–514. doi.org/10.1007/s10265-017-0910-z

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26. doi.org/10.18637/jss.v082.i13

Lee-Yaw JA, Fracassetti M, Willi Y (2018) Environmental marginality and geographic range limits: a case study with *Arabidopsis lyrata* ssp. *lyrata*. Ecography (Cop) 41:622–634. doi.org/10.1111/ecog.02869

Leigh A, Sevanto S, Ball MC, et al (2012) Do thick leaves avoid thermal damage in critically low wind speeds? New Phytol 194:477–487. doi.org/10.1111/j.1469-8137.2012.04058.x

Leigh A, Sevanto S, Close JD, Nicotra AB (2017) The influence of leaf size and shape on leaf thermal dynamics: does theory hold up under natural conditions? Plant Cell Environ 40:237–248. doi.org/10.1111/pce.12857

Levitt J (1980) Responses of plants to environmental stress, Volume 1: chilling, freezing, and high temperature stresses, 2nd edn. Academic Press, New York

Lexer C, Randell RA, Rieseberg LH (2003) Experimental hybridization as a tool for studying selection in the wild. Ecology 84:1688–1699. doi.org/10.1890/0012-9658(2003)084[1688:EHAATF]2.0.CO;2

Lin H, Chen Y, Zhang H, et al (2017) Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat. Funct Ecol 31:2202–2211. doi.org/10.1111/1365-2435.12923

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron 43:107–153. doi.org/10.1016/S0065-2113(08)60477-0

Maggio A, Bressan RA, Zhao Y, et al (2018) It's hard to avoid avoidance: uncoupling the evolutionary connection between plant growth, productivity and stress "tolerance". Int. J. Mol. Sci. 19:11. doi.org/10.3390/ijms19113671

Marchin RM, Backes D, Ossola A, et al (2022) Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species. Glob Chang Biol 28:1133–1146. doi.org/10.1111/gcb.15976

Matsui T, Singh BB (2003) Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp Agric 39:29–38. doi.org/10.1017/S0014479703001108

May R-L, Warner S, Wingler A (2017) Classification of intra-specific variation in plant functional strategies reveals adaptation to climate. Ann Bot 119:1343–1352. doi.org/10.1093/aob/mcx031

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19. doi.org/10.1016/j.tplants.2005.11.002

Murtaza G, Rasool F, Habib R, et al (2016) A review of morphological, physiological and biochemical responses of plants under drought stress conditions. Imp J Interdiscip Res 2:1600–1606

Natarajan S, Kuehny JS (2008) Morphological, physiological, and anatomical characteristics associated with heat preconditioning and heat tolerance in *Salvia splendens*. J Amer Soc Hort Sci 133:527–534. doi.org/10.21273/JASHS.133.4.527

Paccard A, Fruleux A, Willi Y (2014) Latitudinal trait variation and responses to drought in *Arabidopsis lyrata*. Oecologia 175:577–587. doi.org/10.1007/s00442-014-2932-8

Paquette A, Hargreaves AL (2021) Biotic interactions are more often important at species' warm versus cool range edges. Ecol Lett 24:2427–2438. doi.org/10.1111/ele.13864

Parker J (1949) Effects of variations in the root-leaf ratio on transpiration rate. Plant Physiol 24:739–743. doi.org/10.1104/pp.24.4.739

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669. doi.org/10.1146/annurev.ecolsys.37.091305.110100

Pebesma E (2018) Simple features for R: standardized support for spatial vector data. R J 10: 439-446. doi.org/10.32614/RJ-2018-009

Pebesma E, Bivand R (2005) Classes and methods for spatial data in R. R News 5:9–13

R-Core-Team (2021) R: A language and environment for statistical computing, Vienna. www.R-project.org

Reich PB (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol 102:275–301. doi.org/10.1111/1365-2745.12211

Richardson JL, Urban MC, Bolnick DI, Skelly DK (2014) Microgeographic adaptation and the spatial scale of evolution. Trends Ecol Evol 29:165–176. doi.org/10.1016/j.tree.2014.01.002

Riihimäki M, Savolainen O (2004) Environmental and genetic effects on flowering differences between northern and southern populations of *Arabidopsis lyrata* (Brassicaceae). Am J Bot 91:1036–1045. doi.org/10.3732/ajb.91.7.1036

Rizhsky L, Liang H, Mittler R (2002) The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol 130:1143–1151. doi.org/10.1104/pp.006858

Rizhsky L, Liang H, Shuman J, et al (2004) When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. Plant Physiol 134:1683–1696. doi.org/10.1104/pp.103.033431

Rodrigues J, Inzé D, Nelissen H, Saibo NJM (2019) Source–sink regulation in crops under water deficit. Trends Plant Sci 24:652–663. doi.org/10.1016/j.tplants.2019.04.005

Rumpf S, Hülber K, Klonner G, et al (2018) Range dynamics of mountain plants decrease with elevation. Proc Natl Acad Sci 115:1848–1853. doi.org/10.1073/pnas.1713936115

Sadok W, Lopez JR, Smith KP (2021) Transpiration increases under high-temperature stress: potential mechanisms, trade-offs and prospects for crop resilience in a warming world. Plant Cell Environ 44:2102–2116. doi.org/10.1111/pce.13970

Saini K, Dwivedi A, Ranjan A (2022) High temperature restricts cell division and leaf size by coordination of PIF4 and TCP4 transcription factors. 190:2380-2397. doi.org/ 10.1093/plphys/kiac345

Sánchez-Castro D, Perrier A, Willi Y (2022) Reduced climate adaptation at range edges in North American *Arabidopsis lyrata*. Glob Ecol Biogeogr 31:1066–1077. doi.org/10.1111/geb.13483

Sánchez-Salguero R, Camarero JJ, Gutiérrez E, et al (2017) Assessing forest vulnerability to climate warming using a process-based model of tree growth: bad prospects for rear-edges. Glob Chang Biol 23:2705–2719. doi.org/10.1111/gcb.13541

Santos del Blanco L, Bonser S, Valladares F, et al (2013) Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress. J Evol Biol 26:1912-1924. doi.org/10.1111/jeb.12187

Savin R, Nicolas ME (1996) Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. Funct Plant Biol 23:201–210

Scheiner SM, Callahan HS (1999) Measuring natural selection on phenotypic plasticity. Evol 53:1704–1713. doi.org/10.1111/j.1558-5646.1999.tb04555.x

Schmickl R, Jørgensen MH, Brysting AK, Koch MA (2010) The evolutionary history of the *Arabidopsis lyrata* complex: a hybrid in the amphi-Beringian area closes a large distribution gap and builds up a genetic barrier. BMC Evol Biol 10:98. doi.org/10.1186/1471-2148-10-98

Seleiman MF, Al-Suhaibani N, Ali N, et al (2021) Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10:259. doi.org/10.3390/plants10020259.

Sher A, Asghar Y, Qayum A, et al (2022) A review on strong impacts of thermal stress on plants physiology, agricultural yield; and timely adaptation in plants to heat stress. J Bioresour Manag 9:15

Sicher RC, Timlin D, Bailey B (2012) Responses of growth and primary metabolism of water-stressed barley roots to rehydration. J Plant Physiol 169:686–695. doi.org/10.1016/j.jplph.2012.01.002

Stewart JJ, Demmig-Adams B, Cohu CM, et al (2016) Growth temperature impact on leaf form and function in *Arabidopsis thaliana* ecotypes from northern and southern Europe. Plant Cell Environ 39:1549–1558. doi.org/10.1111/pce.12720

Suzuki N, Rivero RM, Shulaev V, et al (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43. doi.org/10.1111/nph.12797

Tardieu F (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Front Physiol 4:17. doi.org/10.3389/fphys.2013.00017

Tardieu F (2012) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J Exp Bot 63:25–31. doi.org/10.1093/jxb/err269

Taseski GM, Keith DA, Dalrymple RL, Cornwell WK (2021) Shifts in fine root traits within and among species along a fine-scale hydrological gradient. Ann Bot 127:473–481. doi.org/10.1093/aob/mcaa175

Taylor MA, Cooper MD, Schmitt J (2019) Phenological and fitness responses to climate warming depend upon genotype and competitive neighbourhood in *Arabidopsis thaliana*. Funct Ecol 33:308–322. doi.org/10.1111/1365-2435.13262

Verslues PE, Juenger TE (2011) Drought, metabolites, and *Arabidopsis* natural variation: a promising combination for understanding adaptation to water-limited environments. Curr Opin Plant Biol 14:240–245. doi.org/10.1016/j.pbi.2011.04.006

Vile D, Pervent M, Belluau M, et al (2012) *Arabidopsis* growth under prolonged high temperature and water deficit: Independent or interactive effects? Plant Cell Environ 35:702–718. doi.org/10.1111/j.1365-3040.2011.02445.x

Wickham H, François R, Lionel K, Müller H (2022) dplyr: a grammar of data manipulation. R package version 1.1.2, dplyr.tidyverse.org

Willi Y, Van Buskirk J (2022) A review on trade-offs at the warm and cold ends of geographical distributions. Philos Trans R Soc B Biol Sci 377:20210022. doi.org/10.1098/rstb.2021.0022

Wos G, Willi Y (2018). Genetic differentiation in life history traits and thermal stress performance across a heterogeneous dune landscape in *Arabidopsis lyrata*. Ann Bot, 122:473—484. doi.org/10.1093/aob/mcy090

Wright IJ, Reich PB, Cornelissen JHC, et al (2005) Modulation of leaf economic traits and trait relationships by climate. Glob Ecol Biogeogr 14:411–421. doi.org/10.1111/j.1466-822x.2005.00172.x

Yadav MR, Choudhary M, Singh J, et al (2022) Impacts, tolerance, adaptation, and mitigation of heat stress on wheat under changing climates. Int J Mol Sci 23:5. doi.org/10.3390/ijms23052838

Zandalinas SI, Mittler R (2022) Plant responses to multifactorial stress combination. New Phytol 234:1161–1167. doi.org/10.1111/nph.18087

Zeileis A, Hothorn T (2002) Diagnostic checking in regression relationships. R News 2:7–10

Zhang H, Sonnewald U (2017) Differences and commonalities of plant responses to single and combined stresses. Plant J 90:839–855. doi.org/10.1111/tpj.13557

Zhao J, Lu Z, Wang L, Jin B (2021) Plant responses to heat stress: physiology, transcription, noncoding RNAs, and epigenetics. Int J Mol Sci 22:117. doi.org/10.3390/ijms22010117

Zhou H, Zhou G, He Q, et al (2020) Environmental explanation of maize specific leaf area under varying water stress regimes. Environ Exp Bot 171:103932. doi.org/10.1016/j.envexpbot.2019.103932

Zhou M, Wang J, Bai W, et al (2019) The response of root traits to precipitation change of herbaceous species in temperate steppes. Funct Ecol 33:2030–2041. doi.org/10.1111/1365-2435.13420

Table 1: Effect of heat and drought on performance and leaf and root functional traits of *Arabidopsis lyrata*

	Estimates of fixed effects				Difference in log-likelihood		
Variable	Intercept	Heat	Drought	Heat+Drought	Pop.	Pop.*Heat	Pop.*Drought
Survival	**3.51** ***	**-1.62** ***	**-1.84** **	**-2.71** ***	0.81		2.96
Flowering	-0.33		**-1.76** ***		0.89		**113.32** ***
Size	**1464.85** ***	-209.25	**-374.06** *	**-62.1** **	<0.01	1.85	2.51
x_{mid}	**27.12** ***	**-1.48** ***	**-1.38** ***	**-2.30** ***	<0.01	<0.01	<0.01
Growth rate	**0.08** ***	**0.01** **	**<-0.01** ***	**0.03** ***	<0.01		**109.62** ***
SLA	**1.04** ***	0.07	-0.07	**0.53** ***	<0.01	1.87	1.90
LDMC	**2.46** ***	**-0.06** ***	**-0.04** ***	0.04	<0.01	<0.01	<0.01
Root:shoot	**0.13** **	**0.06** *	**0.05** *	0.01	**29.92** **		54.64

Estimates of fixed effects and the difference in log-likelihood for random effects are reported. Significance is indicated in bold (* P<0.05, ** P<0.01, *** P<0.001). Models for size, x_{mid} (time to fastest growth), SLA (specific leaf area) and LDMC (leaf dry matter content) assessed variances of intercepts, slopes on temperature and watering, and all covariances, for population and family (testing of an aspect included its variance and the two covariances). Models for survival, flowering, growth rate and root:shoot ratio assessed variances of intercepts only, for population (Pop.) and family (Fam.). The random-effects structure of models was determined based on model selection (Online Resource 2 Table S4).

Tables

Table 2: Effect of heat and drought on performance and leaf and root functional traits differing between southern and northern/central populations

	P-values			
Variable	Intercept	Heat	Drought	Heat+Drought
Survival	0.394	0.138	0.200	**0.004**
Flowering	0.721		0.964	
Size	0.252	0.268	0.483	0.661
x_{mid}	0.781	0.417	0.806	0.621
Growth rate	0.515	0.76	0.081	0.495
SLA	**0.003**	0.064	0.133	**0.018**
LDMC	0.431	0.989	0.384	0.880
Root:shoot	**0.037**	**<0.001**	**<0.001**	0.312

x_{mid} is the time to fastest growth, SLA the specific leaf area, and LDMC the leaf dry matter content. P-values based on pairwise Wilcox tests are shown. Significant differences are indicated in bold ($P<0.05$).

Table 3: Selection analysis of plant growth, leaf and root functional traits under the four treatments, based on the performance measures [W] of flowering, survival or longevity

	Univariate selection				Multivariate selection	
Variable	AIC $_{lin.}$	AIC $_{quad.}$	Coef.$_x$		Coef.$_{x^2}$	Coef.$_x$	
Control; W = *flowering [0/1]*							
Size	755	758	**0.06**	**		-0.02	
x_{mid}	761	761	0.02			-0.02	
Growth rate	761	761	-0.01			-0.03	
SLA	290	309	**0.33**	***		**0.29**	***
LDMC	499	466	**0.22**	***		**0.10**	***
Root:shoot	624	567	**-0.19**	***		**-0.04**	*
Heat; W = *survival [0/1]*							
Size	553	552	<0.01			<0.01	
x_{mid}	546	547	0.03			<0.01	
Growth rate	549	549	-0.03			<0.01	
SLA	-27626	-27621	<0.01			<0.01	
LDMC	-27366	-27372	<0.01		<0.01	<0.01	
Root:shoot	-27385	-27394	<0.01		<0.01	<0.01	
Drought; W = *survival [0/1]*							
Size	623	623	0.02			<0.01	
x_{mid}	613	621	**0.06**	**		<0.01	
Growth rate	608	619	**-0.07**	***		<0.01	
SLA	-24473	-24476	<0.01			<0.01	
LDMC	-24472	-24474	<0.01			<0.01	
Root:shoot	-24805	-24806	<0.01			<0.01	
Heat+Drought; W = *longevity*							
Size	1497	1512	**0.18**	***		**0.14**	**
x_{mid}	1494	1502	**0.16**	***		-0.12	
Growth rate	1448	1466	**-0.32**	***		**-3.60**	***
$SLA_{Heat\&Drought}$	1429	1429	-0.07			**-0.11**	*
$LDMC_{Heat\&Drought}$	1432	1434	0.06			0.06	
$Root:shoot_{Heat\&Drought}$	1449	1447	-0.01			0.02	

x_{mid} is the time to fastest growth, SLA the specific leaf area, and LDMC the leaf dry matter content. In the univariate selection models, each trait was explored for the importance of the linear and quadratic term by AIC, and for the model with the lower AIC, estimated coefficients are reported. The last column shows the estimated coefficients of a model of multivariate selection, with all six traits as linear effects. Significant coefficients (coef.) are indicated in bold (* P<0.05, ** P<0.01, *** P<0.001)

Figures

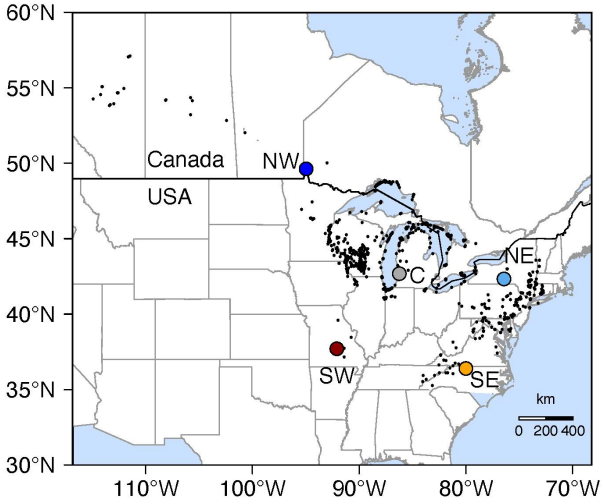

Fig. 1: Range of *Arabidopsis lyrata* in North America. The black dots indicate species occurrences reported since 1960 of a thinned dataset. Coloured dots show the locations of the populations used in this study: one from the centre of the range (C), and the others from the range edges, from the north-east (NE), north-west (NW) south-east (SE) and south-west (SW).

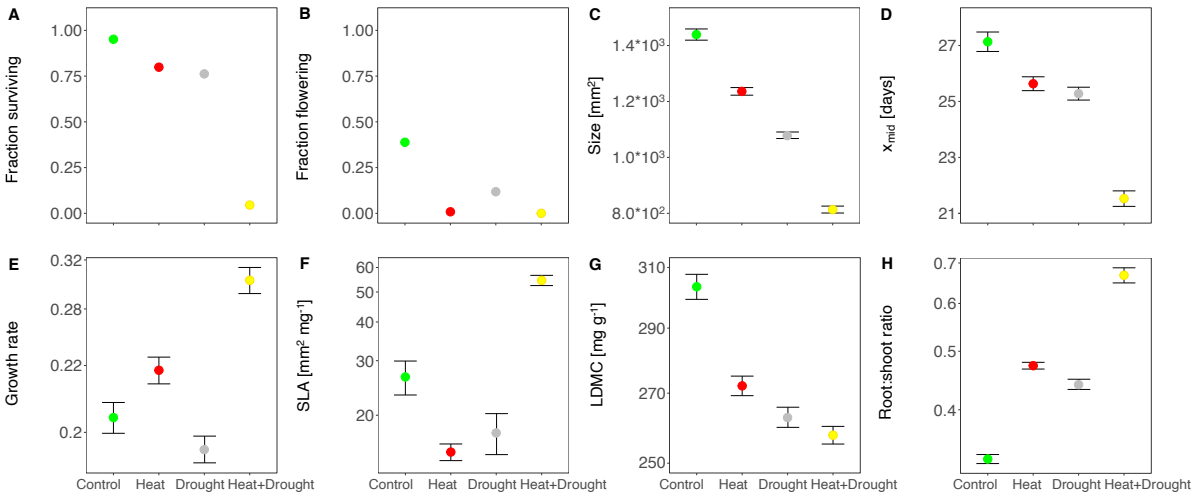

Fig. 2: Effect of heat, drought and combined stress on performance and leaf and root functional traits of *Arabidopsis lyrata*. For each of the four treatment combinations of Control, Heat, Drought, and Heat+Drought, the overall corrected means with standard error (for non-binary traits) are shown. Please note the $\log_{10}$ scale for growth rate, SLA, LDMC, and root:shoot ratio.

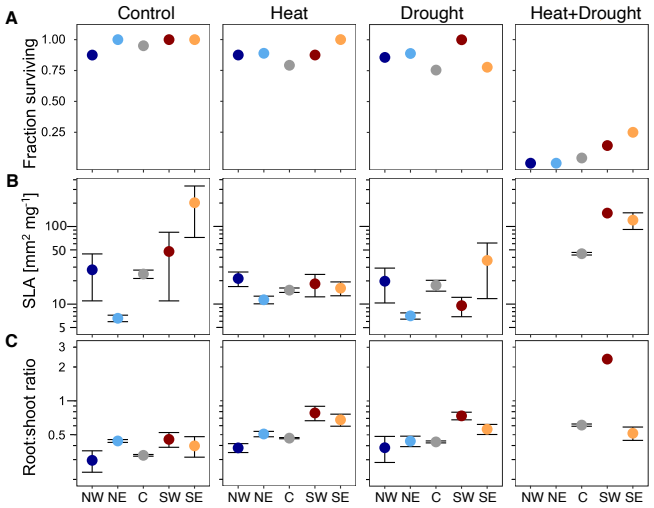

Fig. 3: Effect of heat, drought and combined stress on performance and leaf and root functional traits of *Arabidopsis lyrata*. For each of the four treatment combinations, population corrected means with standard error are shown. The five populations are sorted on the x-axis from left/north to right/south. Please note the $\log_{10}$ scale for SLA and root:shoot ratio. SLA had a wider than usual range of values because leaf area was approximated by rosette surface area, resulting in particularly low values in the case of overlapping leaves and particularly high values in the case leaves had long petioles.

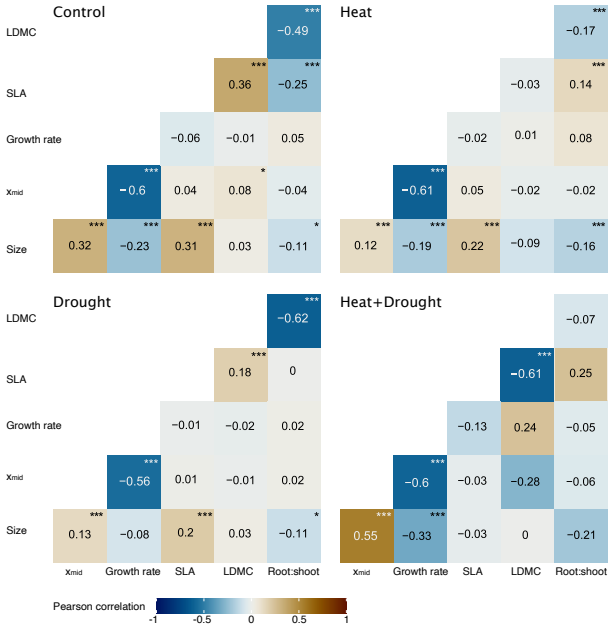

Fig. 4: Phenotypic correlations between all trait pairs of the central population in the four treatments. Negative correlations are indicated in shades of blue, positive ones in brown. Colour intensity indicates the strength of the correlation. Significance is indicated (* P<0.05, ** P<0.01, *** P<0.001).

Chapter 3

Increased connectedness among plant traits, root exudates and the rhizosphere microbiome under drought

Judith R. Schepers[1*], Jan Waelchli[2], Axel Birkholz[3], Jessica Heblack[1], Klaus Schlaeppi[2], Yvonne Willi[1]

* Correspondence

Affiliations:

[1] Plant Ecology and Evolution, Department of Environmental Sciences, University of Basel, Schoenbeistrasse 6, 4056 Basel, Switzerland
[2] Plant Microbe Interactions, Department of Environmental Sciences, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland
[3] Environmental Geosciences, Department of Environmental Sciences, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland

E-mail: judith.schepers@unibas.ch, jan.waelchli@unibas.ch, axel.birkholz@unibas.ch, jessica.heblack@unibas.ch, klaus.schlaeppi@unibas.ch, yvonne.willi@unibas.ch

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Abstract

Plants interact with soil microbes by secreting root exudates that nourish them. In turn, soil microbes can improve access to nutrients that plants take up with soil water. The importance of both, exudates and microbes, may increase for plant performance under drought stress, as they increase soil water availability and thus support the plants' continued access to water and nutrients. Consequently, tighter interactions between plant allocation and performance, exudates and microbes are expected under drought. We tested this expectation in a drought experiment with *Arabidopsis lyrata*. Exudate compounds were quantified using gas chromatography, and the rhizosphere bacteria and fungi were identified by sequencing. We found that drought stress changed the composition of root exudates and rhizosphere bacteria significantly, but not of fungi. Correlation network analysis revealed higher connectedness under drought, indicating the presence of more interactions among plant performance, exudates and the rhizosphere microbiota. Positive correlations between performance traits and known plant growth-promoting bacteria confirmed an increase in beneficial interactions for the stressed plants. The study reveals interconnected dynamic responses of plants, root exudates and soil microorganisms to drought, highlighting the importance of these interactions for plant survival and growth under challenging conditions.

Keywords: Correlation network, drought, interaction, plant performance, rhizosphere microorganisms, root exudates

Introduction

Climate change has altered precipitation patterns, with longer and more extreme droughts being more frequent (IPCC 2023). The resulting fluctuations in soil water content impact the growing conditions and performance of many plants (Benard et al. 2019; Cahill et al. 2014; Paquette and Hargreaves 2021). Water is crucial for the survival and performance of plants, as it is needed for transpiration, photosynthesis, nutrient transport and maintaining cell turgidity for structure and growth (Beauzamy et al. 2014; Crawford et al. 2012; Hillel et al. 1998; Kramer and Boyer 1995; McElrone et al. 2013). Recent research has indicated complex interactions between plant traits, such as aspects of the root architecture, root exudates and microbes in the rhizosphere, which together seem to determine water and nutrient uptake (Cheng et al. 2019; Fitzpatrick et al. 2019; Oppenheimer-Shaanan et al. 2022; Williams and de Vries, 2020). In a drought experiment, we assessed all these components, analysed changes in the correlation network and singled out key components that were linked to performance under drought.

Many studies have shown that the interaction between plants and soil microorganisms can buffer the impact of harsh weather conditions, such as extreme drought, on plant performance (Classen et al. 2015; Fitzpatrick et al. 2019; Karlowsky et al. 2018a; Redman et al. 2011; Rodriguez et al. 2008; Rolli et al. 2015). The mechanisms by which bacteria and fungi in the soil and rhizosphere can help plants better tolerate and adjust to abiotic stress are manifold. First, by increasing the space of exploration in the soil, root-associated fungi, such as arbuscular mycorrhizal fungi, can contribute to a higher uptake of water and nutrients (Meddich et al. 2015; Tang et al. 2022). Even in plants without mycorrhiza, such as those of the family Brassicaceae (Cosme et al. 2018), fungi can fulfil this role. For example, it was shown that root fungi can promote their growth in phosphorous-limited conditions by providing nutrients (Almario et al. 2017; Hiruma et al. 2018). Second, soil microorganisms produce extra polymeric substances (EPS) that can increase the amount of root-adhering soil and form filamental bridges among the soil particles. As a consequence, the water holding capacity of

soils increases, evaporation is lowered and water loss during drying is reduced (Alami et al. 2000; Chenu 1993; Dudman, 1977; Roberson and Firestone 1992; Rosenzweig et al. 2012; Sutherland 2001). Third, microbes can increase the availability of nutrients and essential minerals to plants, which is important as plant nutrient uptake is lower under drought (Bista et al. 2018). For example, some microorganisms increase the bioavailability of iron through the excretion of siderophores, small and high-affinity iron-chelating compounds (Singh et al. 2022). Others produce organic acids that convert inorganic phosphate to plant-available phosphate (Vassilev et al. 2012), and some root bacteria can fix nitrogen (Franche et al. 2009). Finally, root-colonizing fungi can increase stress tolerance under drought conditions through increased antioxidant activity, thereby reducing oxidative damage in the plants (Huang et al. 2017; Sun et al. 2010).

In turn, plants generate and release a variety of micro- and macromolecules at their roots. These substances attract beneficial bacteria and fungi from the bulk soil because they forage on this carbon source (Buée et al. 2009; Curl and Truelove 1986; Sasse et al. 2018). Root exudates mainly consist of sugars and sugar compounds, organic acids, fatty acids and amino acids (Naveed et al. 2017; Sasse et al. 2018), which have specific functions. For example, organic acids are essential for phosphorous uptake by plants, as they mobilize compounds that are otherwise attached to soil particles and are not available to plants (Wu et al. 2018). Together with amino acids, they are also often involved in nutrient uptake regulation and signaling pathways that are activated under nutrient deficiency (Canarini et al. 2019). The detailed composition of exudates can vary among plant genotypes, the stage of plant development and with environmental stress (Enagbonma et al. 2023; Micallef et al. 2009; Sasse et al. 2018). Under environmental stress, exudates may help plants not only indirectly by promoting beneficial microbes but also by direct effects (Williams and de Vries, 2020). Under drought, root hairs and exudated mucilage reduce the increasing gaps among soil particles and roots. Thus, similar to EPS, exudates improve hydraulic conductivity and water flow in drying soils,

and they can slow drying by reducing soil-water evaporation (Ahmed et al. 2014; Benard et al. 2019; Carminati et al. 2009, 2016; Karlowsky et al. 2018b; McCully 1999; Passioura 1988). A recent study highlighted the importance of root exudates under drying conditions by documenting enzymatic hotspots where the plants keep conditions habitable for microbes (Zhang et al. 2023).

The obvious link between soil microbes, exudates and plants are roots. Together with many other plant traits, roots typically change in response to drought. In an immediate response to drought, plants close their stomata, ensuring that less water is lost and the leaf water potential remains above critical levels for metabolic processes to continue (Tardieu 2013; Verslues and Juenger 2011). In combination with increased water uptake from the soil, the plant can thereby maintain a stable water balance (Rodrigues et al. 2019). A response that requires longer adjustment is a more extended root system to assure water uptake (Dinneny 2019). Another common adjustment to drought are small leaves and a change in biomass partitioning, such as an increased root:shoot ratio and reduced leaf surface area per dry weight (specific leaf area, *SLA*) (Dovrat et al. 2019; Matsui and Singh 2003; Poorter and Nagel 2000; Wright and McConnaughay 2002). Other responses can include drought escape by phenological adjustments, such as accelerated reproductive development, a shorter growth period, earlier germination or prolonged dormancy (Balachowski et al. 2016; Basu et al. 2016; Franks 2011; Franks et al. 2007; Tardieu 2013; Verslues and Juenger 2011). Responses are likely to co-occur and/or depend on each other.

In summary, the above-described effects of drought on the expression of plant traits and the direct and indirect ways exudates and microbes can help plants suggest tight interdependencies (Fig. 1). Many of these interactions may affect plant performance under benign conditions but could become critical under drought (Carbone et al. 2021; Lau et al. 2012; Rolli et al. 2015; Santos-Medellín et al. 2017; Ulrich et al. 2022; Windisch et al. 2021a). Under drought stress, plant size is likely to decrease, but a relatively higher investment in the root

system could cause the root:shoot ratio to increase. Exudation in the roots may increase locally, but possibly not at the level of the entire root system. More likely are changes in the composition, e.g., the favouring of specific sugars to attract beneficial soil organisms. Apart from the direct interactions between the three players of plants, root exudates and microbiota, indirect mechanisms of root exudates and soil microorganisms via soil properties should also increase plant performance (circles in Fig. 1). However, exudates may come at some allocation cost, which may weaken positive links.

By choosing a holistic approach, we studied the interplay between plants, exudates and soil microbes depending on soil water conditions. We set up a drought experiment with *Arabidopsis lyrata*, imitating dry versus benign conditions. During the experiment, we tracked plant performance and growth. At the end of the experiment, we assessed allocation traits and collected root exudates of whole roots in a soil-hydroponic system. Using sequencing, we determined the rhizosphere microbiome of the exact same plant. This allowed us to directly relate plant performance, allocation, root exudates and microbiome composition. We further investigated whether there was genotypic variation in all these traits. Specifically, we addressed the following questions: What is the influence of drought on plant traits, root exudates and the microbiome? And what are the interactions between plant traits, exudates and the soil microbiome, and are they stronger under drought as compared to benign conditions?

Methods

We used *Arabidopsis lyrata* ssp. *lyrata* from a genetically highly diverse population in the centre of the species' range, occurring in a dune landscape in the Saugatuck Dunes State Park, Michigan, USA (Wos and Willi 2018). The species is in the Brassicaceae family that does not associate with arbuscular mycorrhiza fungi but still shares mutualistic interactions with other microorganisms present at the roots, which makes it a good model organism (Cosme et al. 2018; Hassani et al. 2018). Plants were propagated from field-collected seeds for one generation to

reduce carryover effects of the environment of origin. They were crossed in pairs, of which 10 were selected for this experiment that had different environmental backgrounds to consider high within population diversities.

Plant raising and measuring of phenotypic traits

Plants (10 families x 5 replicates x 2 treatments = 100) were raised in pots (dimensions: 4 cm diameter, 5 cm depth) filled to the rim with a mixture of washed river sand (0-4 mm) and peat (2:1; peat: Jiffy Products International BV, Netherlands). Pots were split into 5 spatial blocks, with one replicate per family of the two treatments in each block. For germination, pots with seeds were well watered, covered with a mesh net and stratified for twelve days at 4 °C in climate chambers (ClimeCab 1400, KÄLTE 3000 AG, Landquart, Switzerland). Day length increased from 8 h after stratification to 16 h during the experiment, by 1 h every 3-4 days, with a constant light intensity of 200 µM m^{-2} s^{-1}. During the germination period, the temperature was set to 20 °C during the day and 18 °C during the night. One week after stratification, when 75% of the plants had germinated, the mesh nets were removed and after two additional weeks, when about 80% of the germinated plants had four leaves, treatments started. Blocks were randomized twice per week within and/or between two greenhouse chambers. We fertilized every four weeks (0.2% Wuxal universal fertilizer, Westland Schweiz GmbH, Dielsdorf, Switzerland) and applied an insecticide after 14 weeks once a week to protect the plants from insect infestations (1.5% Kendo gold, Westland Schweiz GmbH). Plants were harvested five weeks after the start of flowering or 190 days after the end of stratification if no flowering occurred before.

Treatment. Control plants were watered 8.4 ml every second day, which – in amount – is about average precipitation during growing season at the low-latitudinal range edge of *A. lyrata*. In the drought treatment, watering of 5 ml every second day was close to precipitation of the driest month at the range edge (Heblack et al. 2024). Watering was increased in both

treatments after 2 weeks by 20% due to early wilting in the drought treatment. Soil moisture was measured in 40 pot per treatment four times on consecutive days, one day after watering and right before watering (this was coupled with a larger experiment, Schepers et al., 2024; ECH_2O Check, DECAN DEVICES, Pullman, WA, USA). The results demonstrated significant differences in soil moisture between treatments (Fig. S1). Daytime temperature was 22 °C, with an one-hour heat peak of 25 °C at noon. Night time temperature was 18 °C (8 hours) and air humidity was set to 70%. Daytime temperature and heat peak were selected to also imitate conditions in late spring/early summer at the low-latitude range edge.

Plant traits. Starting with treatment, images of pots were taken twice a week to model rosette growth (see details in Heblack et al. 2024). The three-parameter logistic model was considered the best performing across multiple environments, with the parameters being the maximum asymptotic rosette size (in mm^2), the time to the inflection point thus to fastest growth (in days), and the growth rate. At the end of the experiment, allocation traits were assessed. These were the root dry weight (in g), rosette dry weight (in g), total dry biomass (in g), the specific leaf area (SLA, rosette size [mm^2] per dry weight of rosette [mg], excluding dead leaves), the leaf dry matter content (LDMC, dry weight rosette [mg] per wet weight rosette [g], excluding dead leaves) and the root:shoot ratio (dry weight roots [g] per dry weight of all leaves and inflorescences [g]). Drying was done separately for roots, alive leaves, dead leaves, and inflorescences at 60 °C for 48 h. Additionally, we calculated the propensity to survive. For plants that survived until the first plant flowered, we assessed the propensity that they flowered.

Root exudates

Root exudates were collected based on an adapted protocol from Herz et al. (2018). Plants with roots were taken out of pots, and loose bulk soil was carefully removed by hand and set aside. After the collection of the root rhizosphere as described below, the roots were carefully cleaned with ultrapure water and then placed for 2 hours in a nutrient solution to allow the plants to

recover from any stress. Even though some damage to roots might have occurred during this process (Oburger and Jones 2018), these had been shown to be insignificant if plants were handled with care (Herz et al. 2018). Exudates were collected for 2 hours in ultrapure water, filtered, freeze dried and stored at -80 °C until further processing. Collection in a low-ionic-strength solution, such as distilled water, was compatible for any downstream analysis (Oburger and Jones 2018; Valentinuzzi et al. 2015). In a final step, roots were separated from the rosette, and the fresh and dry weights of both were taken to assess biomass allocation.

For analysis, defrosted exudate samples were cleaned, derivatized and sylilized. For compound identification, six random samples, three from drought, three from control, were analysed on a gas chromatograph (Trace GC Ultra) coupled with a mass spectrometer (GC-MS; DSQ II, both Thermo Scientific, Waltham, MA, USA). Compounds measured with the GC-MS were identified based on the digital database of the National Institute of Standards and Technology (NIST MS Search 2.0, MD, USA) and the Excalibur GC software (v2.0.7, Thermo Fisher Scientific Inc., 1998–2007). We generated a list of reference compounds that were found at least in two of the six samples and by comparison with measurements of single compounds (Table S2). For each identified compound the linear retention index (RI) in the GC-FID chromatogram was determined by using the RI of the alkane standards (C12, C15, C19, C22, C28, C32; van Den Dool and Dec. Kratz 1963). The RI of each peak in each sample was calculated. If a peak had the same RI as an identified compound (RI ±15, range verified with the six GC-MS samples), we assigned it as such. If several peaks were in this RI range, the major one was used. The area under each peak was used to calculate the relative abundance of each compound. Known concentrations of added alkane standards were used to correct for any changes in signal intensity. Output was a library of retention times and indices of compounds of interest. Finally, all samples were analysed on the same gas-chromatograph coupled with a flame ionization detector (GC-FID; Waltham, MA, Thermo Scientific, USA) for compound

detection and quantification, based on GC-MS results. A detailed protocol is in the appendix "methods S1".

Rhizosphere microbiota

Rhizosphere bacteria and fungi were collected according to the description in Schlaeppi et al. (2014). The roots plus the attached rhizosphere were placed in a 50 ml falcon tube filled with 40 ml of a phosphate-buffered saline solution (PBS-T; 130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, 0.02% Silwet77 and 0.05% Tween). The rhizosphere was removed from the roots by carefully shaking the tubes for 20 minutes. To collect as much rhizosphere soil as possible, this step was repeated in a second falcon tube. Samples were centrifuged at 4000 g, the supernatant was removed, and the remaining soil was flash-frozen with liquid nitrogen and stored at -80°C until further processing.

DNA was extracted from grinded rhizosphere samples with the DNeasy Power Soil Pro Kit (Qiagen Inc., Germantown, MD, USA). Amplicon libraries were prepared using ITS1F and ITS2R PCR primers (EUROFINS, NGSgrade) for fungi and 515F and 806R PCR primers for bacteria (Apprill et al. 2015; Parada et al. 2016). Barcodes from the Access array barcode system of Fluidigm were attached with CS1/CS2 linkers (Fluidigm, San Francisco, CA, USA). The pooled library was sequenced at the NGS platform of the University of Bern on an Illumina Miseq v2 standard flow cell with 500 cycles. More details about DNA extraction, PCR cycles, cleanup and library preparation are in appendix "Methods S2".

To prepare the data for analysis, we first controlled the raw sequencing quality using FastQC v0.11.8 (Andrews 2015) and removed primers with Cutadapt v3.4 (Martin 2011). Barcodes had been removed previously by the NGS platform and were written in the sequence headers. We used this sequence header information to demultiplex data. Following the methods in Gfeller et al. (2023), we used dada2 v1.26.0 (Callahan et al. 2016) in R v4.2.0 (R Core Team 2022) to infer exact amplicon sequence variants (ASV). The taxonomic group was assigned

with a naive Bayesian classifier to bacteria with the SILVA v.132 database (Quast et al. 2013) and to fungi with the UNITE v8.3 database (Abarenkov et al. 2010). In a second round of assignment, naive Bayesian classifier assignments were replaced if the IDTAXA classifier could assign more taxonomic levels with the SILVA r138 and UNITE 8.2 databases from DECIPHER (Wright 2016).

For downstream analysis, a core microbiome was defined by removing taxa of groups other than those of interest, such as cyanobacteria and mitochondria for bacteria, and Protista, Plantae, Protozoa and Animalia for fungi. Further, ASVs were discarded if they had less than 0.01 percent abundance in the whole dataset, if they were detected in only one sample, or if the outlier ratio was below 0.1 (ratio from the second most abundant to the most abundant sample for each ASV). Then the sequence data was rarefied. Based on rarefaction curves, the rarefaction threshold for bacteria was set to 20 000 sequences, leading to the removal of 21 samples. For fungi, the threshold was set to 5000 sequences, leading to the removal of 12 samples (vegan package of R, v2.6-4; Oksanen et al. 2022). We calculated the proportion of reads of each genera and phylum within the bacteria and fungi. The largest group was further split on the level of classes to reduce their dominance, which were *Proteobacteria* and *Ascomycota* (Fig. S2).

Statistical analysis

If not stated differently, all data preparation and analysis was done using R v4.2.1 (R-Core-Team,2022) and the packages broom v1.0.3 (Robinson et al. 2023), dplyr v1.1.2 (Wickham et al. 2023a), ggplot2 v3.4.2 (Wickham 2016), ggpubr v0.6.0 (Kassambara 2023), reshape2 v1.4.4 (Wickham 2007) and tidyr v1.0.3 (Wickham et al. 2023b).

Effects of treatment. For plant traits, root exudates and diversity estimates of bacteria and fungi, analysis of variance or logistic regression for binary traits were performed to test for the effects of treatment and treatment varying among families (for plants and exudates with the

car package v3.1-1 (Fox and Weisberg 2019); for bacteria and fungi on ASV level permutational multivariate analysis of variance (PERMANOVA) with adonis2 and vegdist of the vegan package). Beforehand, continuous plant traits were tested and corrected for an effect of block, tray within block, position in tray (edge/centre) and days to germination for the two treatments separately (note that the 100 plants were part of a much larger experiment, described in Schepers et al. 2024 and Heblack et al. 2024). To approach normality of data, SLA, growth rate and root biomass were $\log_{10}$-transformed. Root exudates were grouped into amino acids, sugars and organic acids (fatty acids and benzoic acid). For both, bacteria and fungi, the alpha-diversity was estimated based on the richness and Shannon index of ASVs (parallel and vegan packages). The two microbe data sets were further explored by principal coordinate analysis (PCoA) with the phyloseq package (McMurdie and Holmes, 2013).

Correlation network analysis. We tested our main hypothesis of stronger interactions under drought as compared to benign conditions by correlation network analysis. Pairwise Spearman correlation coefficients were calculated for each treatment separately including plant traits and relative abundance of root exudates, bacteria and fungi per sample. Correlations were plotted as network diagrams with igraph excluding interactions below ±0.7 (Csárdi et al. 2023; Gao et al. 2021; Hartman et al. 2018). The modularity of networks was calculated with the igraph package including the degree, strength, betweenness and closeness and tested for significant differences with a Kruskal-Wallice test (Table S7, Fig. 3D, ggpubr v0.6.0, Kassambara, 2023). The strength of a network member (vertex) is the sum of all correlations (edges) above 0.7, while the degree indicates the number of interactions of a vertex above absolute 0.7. Further, the closeness centrality (harmonic centrality, mode “all”) measures how many steps are needed to connect a vertex with every other vertices and the betweenness combines the number and strength of interactions of each vertex to other vertices (Csárdi et al. 2023). The differences in number of positive and negative correlations above ±0.7 was tested with a chi-square test (Fig. 3C). To investigate, if very highly abundant network members also

have the most high correlations, we plotted the degree against the abundance or concentration (abundance estimate) of all network members (Fig. 3B).

Results

Drought reduces plant performance and changes the composition of exudates and microbes

Plant traits. The following plant traits were significantly different between treatments: maximum rosette size, total biomass, growth rate, survival and flowering propensity (Table 1, Fig. 2A). That means in detail that under drought, plants were smaller, had a reduced total biomass, including a diminished rosette biomass and smaller root biomass. They also had a slower growth rate and a greater SLA. In general, we observed a lower survival and less flowering plants under drought conditions. However, there were no significant changes between the two treatments regarding the time to fastest growth, LDMC and root:shoot ratio. We observed significant variations among plant seed families regarding their specific growth trajectories, their rosette weights and their propensities to flower.

Exudates. Next, we analysed the compounds in the semi-hydroponic collected root exudate samples. The total amount of exudates differed between treatments, with lower exudation under drought and a significant difference between compounds as well as compound groups (Table 2). The main groups of exudates that decreased significantly in relative abundance were the fatty acids within the organic acids and the amino acids. In control conditions, the largest fractions consisted of organic acids, sugars and low proportions of amino acids. In contrast to these observations, in drought conditions, the largest fraction of exudates consisted of sugars, of which glucose was the most abundant, followed by sucrose and, in lower amounts, glucopyranose, myoinositol and ribose. Organic acids, mainly fatty acids, were the second-most abundant; we identified benzoic acid and several fatty acids, with dodecanoic acid being the most common. Most rare was the group of amino acids, with valine, proline and

alanine. On the level of single compounds, only dodecanoic acid had a significant different concentration between both treatments (Table S3). Plant seed families varied regarding the amount of amino acids they exudated (Table 2).

Bacteria. The largest fraction of rhizosphere bacteria were Proteobacteria (62-59%, in both treatments mainly Alpha-Proteobacteria), followed by Actinobacteria (22-27%, Fig. S2A). Under control conditions, the proportionally most common bacteria on the genus level were *Nocardioides* (4.4%) and *Conexibacter* (4.0%), both Actinobacteria, and the Proteobacteria *Bauldia* (4.2%). However, in drought, the most abundant genera were the Proteobacteria *Sphingomonas* (5.4%), followed by *Nocardioides* (5.1%) and *Conexibacter* (4.2%; Table S5). The bacteria whose relative abundance increased most with drought were the Actinoabacteria *Lechevalieria* and the Proteobacteria *Sphingomonas* and *Azospirillum*. Analysis of alpha-diversity estimates showed no significant effect of treatment (Table 3, Fig. 2C). However, PERMANOVA revealed a significant difference between control and drought in bacterial beta-diversity (Table 3, Fig. 2C). The PCoA separates the two treatments along the first axis, which explained 25.0% of variance in the data. Also, treatment and seed family interacted in their effect on beta diversity.

Fungi. In both, control and drought conditions, the most abundant group was Ascomycota, with over 80% in each watering treatment, and the second-commonest phylum was Basidiomycota, with 5-9% in each watering treatment (Fig. S2B). The most common genus was the Ascomycota *Cladosporium* (control 18.1%, drought 32.3%), which also showed the strongest change in relative abundance between treatments (Table S5). *Alternaria* (17.2%, 14.2%) and *Stachybotrys* (17.1%, 14.4%) turned out to be the second and third most abundant genus in both treatments. Analysis of variance (ANOVA) on alpha-diversity estimates revealed no significant effect of treatment, but an effect of the plant seed family (Table 3, Figs. 2C, S2B). Beta diversity of rhizosphere fungi was not affected by treatment, which was also visually confirmed by PCoA, as there was a big overlap of fungal composition between the two

treatments (Fig. 2C). In summary, we found a significant effect of drought on plant performance and the microorganisms in the rhizosphere, particularly in bacteria.

Drought strengthens correlation network

The main correlation analysis was performed for exudates on the level of compounds and for rhizosphere bacteria and fungi on the level of the genus, based on correlations with $r \geq 0.7$ (Fig. 3A). We included all measurements as individual replicates. Within the different groups of plant traits, exudate compounds, bacteria and fungi, the metric betweenness was not significantly different between treatments. However, under drought conditions, the degree and closeness were significantly higher, overall and also within the groups of plant traits, exudate compounds, bacteria and fungi. Additionally, the strength was lower across the four groups (Fig. 3D). Based on a chi-square test, significantly more correlations above 0.7 and below -0.7 were found in drought conditions, with more positive than negative correlations (Fig. 3C). The network of the drought treatment was highly interconnected, with many high correlations between the groups of plant traits, exudates, bacteria and fungi (Fig. 3A). In control and drought conditions, the top ten vertices, based on the highest degree and greatest closeness were always bacteria (Table S7, Fig. 3B). The abundance estimate (abundance of microorganisms, concentration of exudates and zero-to-one scaled plant traits) was plotted against the degree to visualize the key vertices (yellow part in Fig. 3B). In control conditions, the ten most abundant bacteria had a slight increase in number of high correlations, while in drought, the most abundant genera had fewer correlations >0.7. In the control network, *Sphingomonas* had the highest degree, while in the drought network this role had *Thauera*.

We found significant correlations that involved plant traits such as maximum rosette size and total biomass as estimates of performance under drought (Table 4). Rosette size was negatively correlated with *Gymnostellatospora*. In contrast, total biomass, was positively related to several plant traits as well as *Brevundimonas*. In drought, but not control root weight

was highly positively correlated with *Vivinamibacter* and the root exudate alanin and negatively with *Dongia, Hyphomicrobium* and *Nitratreductor.* Additionally, the root:shoot ratio was positively correlated to *Sphingomonas* and *Qipenqyuania*. Flowering, as proxy for reproduction, was positively correlated under drought with the fungi *Leptodontidium*. Taken together this indicates a change in interactions of plants and their close environment between control and drought conditions.

Discussion

Our experimental study supported the expectation that, under drought, interactions among plant traits, root exudates and soil microorganisms become tighter. Under drought stress, plants suffered, growing slightly slower and to a smaller rosette size, and they had a slightly higher mortality rate and a lower fraction of plants flowering (Table 1, Fig. 2A). Their exudation was reduced, and the community of bacteria but not the fungi in the rhizosphere changed significantly (Tables 2, 3, Figs. 2B, C). The analysis of stronger correlations between the four aspects of plant traits, root exudates, bacteria and fungi revealed that there were more high correlations under drought, with the highest centrality of bacteria (Fig. 3D). In the following, we discuss the effect of treatment on each of these aspects and then in the context of a correlation network.

Drought-induced changes in plants and soil microorganisms

Plants did not respond to drought stress by increased resistance such as the expression of a higher leaf dry matter content or a higher root:shoot ratio. Changes in rosette size, biomass, SLA, survival, and the propensity to flower however, clearly indicated that they were stressed.

Exudate composition. Our analysis allowed the detection of various sugars, organic acids and amino acids. In drought, sugars made up the largest fraction of identified root exudates (Fig. 2B). They are important for plant nutrient uptake and a crucial carbon source for

microorganisms (Hammond and White 2011; Kamilova et al. 2006; Sasse et al. 2018; Ulrich et al. 2022). Unlike sugars, the amount of excreted organic acids, particularly the fatty acids, was significantly influenced by watering treatment. Fatty acids are important chemo-attractants that can act as pathogen defense agents and alter plant growth, which may explain the relative increase of organic acids under drought as compared to the other compounds, even though their total amount decreased (Table S4; Li et al. 2017; Ma et al. 2021; Vlot et al. 2009). Phenolic acids, such as benzoic acid, were previously linked negatively to rhizosphere pathogen abundance and positively to beneficial fungi (Clocchiatti et al. 2021; Liu et al. 2017; Windisch et al. 2017). In times of drought, the relationship between soil bacteria and amino acids released by roots – which, for instance, stimulate bacterial growth by nutrient provision – becomes even more crucial (Katznelson et al. 1954; Lochhead and Thexton 1947).

Bacteria. Under control conditions, we found *Nocardioides*, common plant growth promoting bacteria (Hou et al. 2015; Liu et al. 2022) , and *Bauldia,* nitrogen-fixing bacteria, in relatively high amounts (Table S5; Yee et al. 2010). However, under dry conditions, the Proteobacteria *Sphingomonas* was most abundant. It has been recorded in arid habitats and is known to enhance plant growth under drought (Fan et al. 2023; Luo et al. 2019; Refai et al. 2023; Wang et al. 2022). In general, the phylum *Actinobacteria* increased under drought (blue in Fig. S2A; Fan et al. 2023; Zhang et al. 2021). In contrast, the overall level of Proteobacteria was reduced under dry conditions (different shades of red in Fig. S2A). Gram-negative bacteria (such as Proteobacteria) prefer labile carbon compounds, which are abundant under well-watered conditions. But gram-positive bacteria (such as Actinobacteria) can also use recalcitrant carbon sources during water-limited conditions (Naylor and Coleman-Derr 2018). In line, our analysis showed that beta-, but not alpha-diversity, was influenced by treatment, and plant seed families combined with treatment varied significantly in their response to beta-diversity (Fan et al. 2023; Zhang et al. 2017). Thus, we could find a relatively higher abundance

of some bacteria under drought compared to control conditions, with most of them already well-known for their positive impact on plant growth under drought.

Fungi. Rhizosphere fungi have been shown to be important in drought avoidance and drought adaptation (Almario et al. 2017; Hiruma et al. 2018; Huang et al. 2017; Sun et al. 2010). The Ascomycota *Cladosporium* was the most prevalent genus in both treatments, with a higher abundance under dry conditions (Table S5). It is a known root fungus that improves plant growth and especially enhances drought tolerance, for example, through the production of the hormone gibberellin (Dastogeer et al. 2018; Hamayun et al. 2009; Hereira-Pacheco et al. 2023; Răut et al. 2021). The second and third most common fungi were the pathogens *Alternaria* and *Stachybotrys*, which are biocontrol agents against plant pathogens (Al-Lami et al. 2019; Christ 1990; Morissette et al. 2003; Taylor et al. 2003). However, these reported changes in rhizosphere fungi composition were not very strong, resulting in non-significant differences in alpha- and beta-diversity between treatments (Table 3). The lack of difference in fungi as compared to the beta-diversity of bacteria might indicate that fungi have higher drought resistance than bacteria (Guhr et al. 2015; Veach et al. 2020). Furthermore, we saw that plant seed families significantly varied in the root-associated fungi, which is in line with what other studies had shown (Chen et al. 2018; Gaete et al. 2021). In summary, we found some fungal phyla in the rhizosphere that are known to promote plant growth regardless of treatment, as well as variations in the fungi that plant seed families are typically associated with.

Drought increases centrality of correlation network

To investigate the effect of drought on plants, it is important to not only consider the direct effect but also the indirect effect it has on root exudates and plant-associated microorganisms, as they can buffer the negative impacts (Bastías et al. 2022; Chen et al. 2022). As illustrated in Fig. 1, there are several direct and indirect connections between plants, root exudates and rhizosphere microorganisms that overall are assumed to help plants under drought. Therefore,

we expected interactions and correlations to become stronger under drought. And indeed, the correlation network between the four groupings differed between the control and the drought treatment (Fig. 3A). While under control conditions the plant traits and root exudates were mostly separated, they were often attached to the central cluster in drought conditions. The degree and closeness of the interaction network was significantly higher under drought conditions, overall and within the different groups, which point to more high correlations under drought (Fig. 3C, D). Interestingly, we identified *Sphingomonas* in control to be best connected with other players, despite being more abundant under drought. However, it was the denitrifying *Thauera* that had the most correlations above 0.7 under drought conditions, emphasizing its role in nutrient provision to other players in dry soils (Mao et al. 2014).

Also, correlations involving plant traits were substantially more common under drought conditions, compared to control conditions (Table 4). A high aboveground growth, including the traits of high weight, low SLA and flowering rate, correlated positively with several plant growth promoting bacteria such as *Brevundimonas* or *Leptodontidium*, which have for example, been reported to reduce oxidative damage (Fernando and Currah 1996; Hou and Guo 2009; Naqqash et al. 2020; Tran et al. 2023). Negative correlations between maximum rosette size and the pathogenetic Ascomycota *Gymnostellatospora*, which was previously found on decaying wood, might be explained with a reduction of beneficial microorganisms in the plants vicinity (Sigler et al. 2000). Root:shoot ratio correlated positively with the plant growth promoting and very abundant bacteria *Sphingomonas* (Wang et al. 2022). At first glance, the negative correlations of the plant growth promoting microorganisms *Hyphomicrobium, Dongia* and *Nitratreductor* with root weight under drought conditions were surprising. However, this might be a sign that they are enriched in the rhizosphere of plants that adjusted to drought stress by overall smaller growth, above and below ground. And to get enough nutrients, despite a smaller root system, the plants have more interactions with plant growth promoting organisms, that for example can counteract the lower nutrient levels in drought conditions via

denitrification (Alahmad et al. 2023; Fesefeldt et al. 1998; Marasco et al. 2023; Palla et al. 2022; Sperl and Hoare 1971). This highlights how important some microorganisms are in their interplay between plants and their below-ground surroundings. Both, sugar and amino acids, correlated positively with below-ground allocation, showing the importance of below-ground investment of plants under dry conditions (Boukhatem et al. 2022; Buée et al. 2009; Franco-Correa and Chavarro-Anzola 2016; Lundberg et al. 2012; Silambarasan et al. 2022; Tecon and Or 2017). To a lower degree, several sugars and amino acids were also correlated with above-ground growth such as total biomass, time to fastest growth, lower SLA and rosette biomass (Table S6). Sugars like glucose are well known for their importance as metabolic substrates and signal molecules but also as crucial parts of fatty acid biosynthesis (Zhai et al. 2021). Additionally, the amino acids directly protect against oxidation and increase nitrogen uptake (Fig. 1; Canarini et al. 2019; Ulrich et al. 2022). This demonstrates the importance of root exudates in overall plant growth, beginning with the roots, where they are exuded and nutrients and water are absorbed. In conclusion, we were able to demonstrate that particularly stressful conditions such as drought cause plants to rely more on beneficial interactions with their environment.

Conclusion

Here we performed a drought experiment with *A. lyrata* and assessed plant traits, root exudates, the bacteria and fungi in the rhizosphere to assess their interactions. Under drought, we found a much tighter connection between the four aspects. The study revealed complex interactions, with a particular emphasis on exudation and the beneficial role of rhizosphere organisms in assisting plants during water scarcity. Plants showed signs of stress, but microbial communities demonstrated resilience, especially with an increase in drought-tolerant bacteria. These findings emphasize the crucial role of plant-microbe interactions in enhancing plant survival and growth under challenging environmental conditions.

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Author contributions

Yvonne Willi, Klaus Schlaeppi and Judith R. Schepers conceived the ideas and designed methodology; Jessica Heblack, Axel Birkholz and Judith R. Schepers collected the data; Jan Waelchli and Judith R. Schepers analysed the data; Judith R. Schepers led the writing of the manuscript. Jan Waelchli and Axel Birkholz contributed to sections of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Availability of data and material

The raw sequencing data are available from the European Nucleotide Archive (http://www.ebi.ac.uk/ena) with the study accession PRJEB75649 (sample ID ERS19767548). The plant growth and biomass data and root exudate chromatography data will be available on figshare (https://doi.org/10.6084/m9.figshare.26086168).

References

Abarenkov K, Henrik Nilsson R, Larsson K-H, et al (2010) The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytol 186:281–285. doi.org/10.1111/j.1469-8137.2009.03160.x

Ahmed MA, Kroener E, Holz M, et al (2014) Mucilage exudation facilitates root water uptake in dry soils. Funct Plant Biol 41:1129–1137. doi.org/10.1071/FP13330

Al-Lami HFD, You MP, Barbetti MJ (2019) Incidence, pathogenicity and diversity of *Alternaria* spp. associated with alternaria leaf spot of canola (*Brassica napus*) in Australia. Plant Pathol 68:492–503. doi.org/10.1111/ppa.12955

Alahmad A, Edelman L, Castel L, et al (2023) Prebiotics: A solution for improving plant growth, soil health, and carbon sequestration? J Soil Sci Plant Nutr 23:6647–6669. doi.org/10.1007/s42729-023-01517-8

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398. doi.org/10.1128/AEM.66.8.3393-3398.2000

Almario J, Jeena G, Wunder J, et al (2017) Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. Proc Natl Acad Sci U S A 114:E9403–E9412. doi.org/10.1073/pnas.1710455114

Andrews S (2015) FastQC: A quality control tool for high throughput sequence data. www.bioinformatics.babraham.ac.uk/projects/fastqc/

Apprill A, McNally SP, Parsons RJ, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137. doi.org/10.3354/ame01753

Balachowski JA, Bristiel PM, Volaire FA (2016) Summer dormancy, drought survival and functional resource acquisition strategies in California perennial grasses. Ann Bot 118:357–368. doi.org/10.1093/aob/mcw109

Bastías DA, Balestrini R, Pollmann S, Gundel PE (2022) Environmental interference of plant−microbe interactions. Plant Cell Environ 45:3387–3398. doi.org/10.1111/pce.14455

Basu S, V R, Kumar A, Pereira A (2016) Plant adaptation to drought stress. F1000Research 5:1554. doi.org/10.12688/f1000research.7678.1

Beauzamy L, Nakayama N, Boudaoud A (2014) Flowers under pressure: Ins and outs of turgor regulation in development. Ann Bot 114:1517–1533. doi.org/10.1093/aob/mcu187

Benard P, Zarebanadkouki M, Brax M, et al (2019) Microhydrological niches in soils: How mucilage and EPS alter the biophysical properties of the rhizosphere and other biological hotspots. Vadose Zo J 18:180211. doi.org/10.2136/vzj2018.12.0211

Bista DR, Heckathorn SA, Jayawardena DM, et al (2018) Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and -tolerant grasses. Plants 7. doi.org/10.3390/plants7020028

Boukhatem ZF, Merabet C, Tsaki H (2022) Plant growth promoting Actinobacteria, the most promising candidates as bioinoculants? Front. Agron. 4. doi.org/10.3389/fagro.2022.849911

Buée M, de Boer W, Martin F, et al (2009) The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant Soil 321:189–212. doi.org/10.1007/s11104-009-9991-3

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, et al (2014) Causes of warm-edge range limits: Systematic review, proximate factors and implications for climate change. J Biogeogr 41:429–442. doi.org/10.1111/jbi.12231

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. doi.org/10.1038/nmeth.3869

Canarini A, Kaiser C, Merchant A, et al (2019) Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front. Plant Sci. 10. doi.org/10.3389/fpls.2019.00157

Carbone MJ, Alaniz S, Mondino P, et al (2021) Drought influences fungal community dynamics in the grapevine rhizosphere and root microbiome. J. Fungi 7. doi.org/10.3390/jof7090686

Carminati A, Vetterlein D, Weller U, et al (2009) When roots lose contact. Vadose Zo J 8:805. doi.org/10.2136/vzj2008.0147

Carminati A, Zarebanadkouki M, Kroener E, et al (2016) Biophysical rhizosphere processes affecting root water uptake. Ann Bot 118. doi.org/10.1093/aob/mcw113

Chen M, Arato M, Borghi L, et al (2018) Beneficial services of arbuscular mycorrhizal fungi–from ecology to application. Front Plant Sci 9:1270. doi.org/10.3389/fpls.2018.01270

Chen Y, Yao Z, Sun Y, et al (2022) Current studies of the effects of drought stress on root exudates and rhizosphere microbiomes of crop plant species. Int. J. Mol. Sci. 23. doi.org/10.3390/ijms23042374

Cheng YT, Zhang L, He SY (2019) Plant-microbe interactions facing environmental challenge. Cell Host Microbe 26:183–192. doi.org/10.1016/j.chom.2019.07.009

Chenu C (1993) Clay polysaccharide or sand polysaccharide associations as models for the interface between microorganisms and soil - water related properties and microstructure. Geoderma 56:143–156. doi.org/10.1016/0016-7061(93)90106-U

Christ BJ (1990) Influence of potato cultivars on the effectiveness of fungicide control of early blight. Am Potato J 67:419–425. doi.org/10.1007/BF03044510

Classen AT, Sundqvist MK, Henning JA, et al (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6:art130. doi.org/10.1890/ES15-00217.1

Clocchiatti A, Hannula SE, van den Berg M, et al (2021) Evaluation of phenolic root exudates as stimulants of saptrophic fungi in the rhizosphere. Front Microbiol 12. doi.org/10.3389/fmicb.2021.644046

Cosme M, Fernández I, Van Der Heijden MGA, et al (2018) Non-mycorrhizal plants: The exceptions that prove the rule. Trends Plant Sci 23:577–587. doi.org/10.1016/j.tplants.2018.04.004

Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA (2012) High temperature exposure increases plant cooling capacity. Curr Biol 22:R396–R397. doi.org/10.1016/j.cub.2012.03.044

Csárdi G, Nepusz T, Traag V, et al (2023) igraph: Network analysis and visualization in R. doi.org/10.5281/zenodo.7682609

Curl EA, Truelove B (1986) The rhizosphere. Advanced series in agricultural sciences. Springer-Verlag, Berlin

Dastogeer KMG, Li H, Sivasithamparam K, et al (2018) Fungal endophytes and a virus confer drought tolerance to *Nicotiana benthamiana* plants through modulating osmolytes, antioxidant enzymes and expression of host drought responsive genes. Environ Exp Bot 149:95–108. doi.org/10.1016/j.envexpbot.2018.02.009

Dinneny JR (2019) Developmental responses to water and salinity in root systems. Annu Rev Cell Dev Biol 35:239–257. doi.org/10.1146/annurev-cellbio-100617-062949

Dovrat G, Meron E, Shachak M, et al (2019) Plant size is related to biomass partitioning and stress resistance in water-limited annual plant communities. J Arid Environ 165:1–9. doi.org/10.1016/j.jaridenv.2019.04.006

Dudman WF (1977) Role of surface polysaccharides in natural environments. In: Sutherland I (ed) Surface Carbohydrates of the Procaryotic Cell. Academic Press, London, pp 357–3414

Enagbonma BJ, Fadiji AE, Ayangbenro AS, Babalola OO (2023) Communication between plants and rhizosphere microbiome: Exploring the root microbiome for sustainable agriculture. Microorganisms 11. doi.org/10.3390/microorganisms11082003

Fan W, Tang F, Wang J, et al (2023) Drought-induced recruitment of specific root-associated bacteria enhances adaptation of alfalfa to drought stress. Front. Microbiol. 14. doi.org/10.3389/fmicb.2023.1114400

Fernando AA, Currah RS (1996) A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (Fungi Imperfecti) on the growth of some subalpine plants in culture. Can J Bot 74:1071–1078. doi.org/10.1139/b96-131

Fesefeldt A, Kloos K, Bothe H, et al (1998) Distribution of denitrification and nitrogen fixation genes in *Hyphomicrobium* spp. and other budding bacteria. Can J Microbiol 44:181–186. doi.org/10.1139/w97-139

Fitzpatrick CR, Mustafa Z, Viliunas J (2019) Soil microbes alter plant fitness under competition and drought. J Evol Biol 32:438–450. doi.org/10.1111/jeb.13426

Fox J, Weisberg S (2019) An R companion to applied regression. Third edition. Sage, Thousand Oaks CA. socialsciences.mcmaster.ca/jfox/Books/Companion/

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi.org/10.1007/s11104-008-9833-8

Franco-Correa M, Chavarro-Anzola V (2016) Actinobacteria as plant growth-promoting rhizobacteria. In: Dhanasekaran D, Jiang Y (eds). IntechOpen, Rijeka, p Ch. 10. doi.org/10.5772/61291

Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. New Phytol 190:249–257. doi.org/10.1111/j.1469-8137.2010.03603.x

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proc Natl Acad Sci 104:1278–1282. doi.org/10.1073/pnas.0608379104

Gaete A, Pulgar R, Hodar C, et al (2021) Tomato cultivars with variable tolerances to water deficit differentially modulate the composition and interaction patterns of their rhizosphere microbial communities. Front. Plant Sci. 12. doi.org/10.3389/fpls.2021.688533

Gao M, Xiong C, Gao C, et al (2021) Disease-induced changes in plant microbiome assembly and functional adaptation. Microbiome 9:187. doi.org/10.1186/s40168-021-01138-2

Gfeller V, Waelchli J, Pfister S, et al (2023) Plant secondary metabolite-dependent plant-soil feedbacks can improve crop yield in the field. Elife 12:e84988. doi.org/10.7554/eLife.84988

Guhr A, Borken W, Spohn M, Matzner E (2015) Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization. Proc Natl Acad Sci 112:14647–14651. doi.org/10.1073/pnas.1514435112

Hamayun M, Afzal Khan S, Ahmad N, et al (2009) *Cladosporium sphaerospermum* as a new plant growth-promoting endophyte from the roots of *Glycine max* (L.) Merr. World J Microbiol Biotechnol 25:627–632. doi.org/10.1007/s11274-009-9982-9

Hammond JP, White PJ (2011) Sugar signaling in root responses to low phosphorus availability. Plant Physiol 156:1033–1040. doi.org/10.1104/pp.111.175380

Hartman K, van der Heijden MGA, Wittwer RA, et al (2018) Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6:14. doi.org/10.1186/s40168-017-0389-9

Hassani MA, Durán P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6:58. doi.org/10.1186/s40168-018-0445-0

Heblack J, Schepers JR, Willi Y (2024) Reduced evolutionary potential under heat and drought stress at the southern range edge of North American *Arabidopsis lyrata*. J Evol Biol voae045. doi.org/10.1093/jeb/voae045

Hereira-Pacheco SE, Estrada-Torres A, Dendooven L, Navarro-Noya YE (2023) Shifts in root-associated fungal communities under drought conditions in *Ricinus communis*. Fungal Ecol 63:101225. doi.org/10.1016/j.funeco.2023.101225

Herz K, Dietz S, Gorzolka K, et al (2018) Linking root exudates to functional plant traits. PLoS One 13:e0204128. doi.org/10.1371/journal.pone.0204128

Hillel D, Warrick AW, Baker RS, Rosenzweig C (1998) Environmental soil physics. Academic Press, San Diego, CA

Hiruma K, Kobae Y, Toju H (2018) Beneficial associations between Brassicaceae plants and fungal endophytes under nutrient-limiting conditions: evolutionary origins and host–symbiont molecular mechanisms. Curr Opin Plant Biol 44:145–154. doi.org/10.1016/j.pbi.2018.04.009

Hou J, Liu W, Wang B, et al (2015) PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. Chemosphere 138:592–598. doi.org/10.1016/j.chemosphere.2015.07.025

Hou X-Q, Guo S-X (2009) Interaction between a dark septate endophytic isolate from *Dendrobium* sp. and roots of *D. nobile* seedlings. J Integr Plant Biol 51:374–381. doi.org/10.1111/j.1744-7909.2008.00777.x

Huang Y-M, Zou Y-N, Wu Q-S (2017) Alleviation of drought stress by mycorrhizas is related to increased root H(2)O(2) efflux in trifoliate orange. Sci Rep 7:42335. doi.org/10.1038/srep42335

Intergovernmental Panel on Climate Change (IPCC) (2023): Climate Change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge Univ Press. doi.org/10.1017/9781009157896

Kamilova F, Kravchenko L V, Shaposhnikov AI, et al (2006) Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. Mol Plant-Microbe Interact 19:250–256

Karlowsky S, Angela A, Ingrisch J, et al (2018a) Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. J Ecol 106:1230—1243. doi.org/10.1111/1365-2745.12910

Karlowsky S, Augusti A, Ingrisch J, et al (2018b) Drought-induced accumulation of root exudates supports post-drought recovery of microbes in mountain grassland. Front Plant Sci 9:1593. doi.org/10.3389/fpls.2018.01593

Kassambara, A (2023) ggpubr: 'ggplot2' based publication ready plots. rpkgs.datanovia.com/ggpubr/.

Katznelson H, Rouatt JW, Payne TMB (1954) Liberation of amino-acids by plant roots in relation to desiccation. Nature 174:1110–1111. doi.org/10.1038/1741110a0

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic press

Lau JA, Lennon JT (2012) Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc Natl Acad Sci U S A 109:14058–14062. doi.org/10.1073/pnas.1202319109

Liu J, Li X, Jia Z, et al (2017) Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases. Appl Soil Ecol 110:34–42. doi.org/10.1016/j.apsoil.2016.11.001

Liu S-Y, Wei C-Y, Tong Y, et al (2022) *Cyperus rotundus* L. drives arable soil infertile by changing the structure of soil bacteria in the rhizosphere, using a maize field as an example. Environ Sci Pollut Res 29:79579–79593. doi.org/10.1007/s11356-022-21480-8

Lochhead AG, Thexton RH (1947) Qualitative studies of soil micor-organisms: VII. The "rhiszosphere effect" in relation to the amino acid nutrition of bacteria. Can J Res 25c:20–26. doi.org/10.1139/cjr47c-003

Lundberg DS, Lebeis SL, Paredes SH, et al (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86–90. doi.org/10.1038/nature11237

Luo Y, Wang F, Huang Y, et al (2019) *Sphingomonas* sp. Cra20 increases plant growth rate and alters rhizosphere microbial community structure of *Arabidopsis thaliana* under drought stress. Front. Microbiol. 10. doi.org/10.3389/fmicb.2019.01221

Mao Y, Xia Y, Wang Z, Zhang T (2014) Reconstructing a *Thauera* genome from a hydrogenotrophic-denitrifying consortium using metagenomic sequence data. Appl Microbiol Biotechnol 98:6885–6895. doi.org/10.1007/s00253-014-5756-x

Marasco R, Michoud G, Sefrji FO, et al (2023) The identification of the new species *Nitratireductor thuwali* sp. nov. reveals the untapped diversity of hydrocarbon-degrading culturable bacteria from the arid mangrove sediments of the Red Sea. Front Microbiol 14. doi.org/10.3389/fmicb.2023.1155381

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10–12. doi.org/ISSN 2226-6089

Matsui T, Singh BB (2003) Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp Agric 39:29–38. doi.org/10.1017/S0014479703001108

McCully ME (1999) Roots in soil: Unearthing the complexities of roots and their rhizospheres. Annu Rev Plant Physiol Plant Mol Biol 50:695–718. doi.org/10.1146/annurev.arplant.50.1.695

McElrone A, Choat B, Gambetta GA, Brodersen CR (2013) Water uptake and transport in vascular plants. Nat Educ Knowl 4:6

McMurdie PJ, Holmes S (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217. doi.org/10.1371/journal.pone.0061217

Meddich A, Jaiti F, Bourzik W, et al (2015) Use of mycorrhizal fungi as a strategy for improving the drought tolerance in date palm (*Phoenix dactylifera*). Sci Hortic (Amsterdam) 192:468–474. doi.org/10.1016/j.scienta.2015.06.024

Micallef SA, Shiaris MP, Colón-Carmona A (2009) Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. J Exp Bot 60:1729–1742. doi.org/10.1093/jxb/erp053

Morissette DC, Driscoll BT, Jabaji-Hare S (2003) Molecular cloning, characterization, and expression of a cDNA encoding an endochitinase gene from the mycoparasite *Stachybotrys elegans*. Fungal Genet Biol 39:276–285. doi.org/10.1016/S1087-1845(03)00050-1

Naqqash T, Imran A, Hameed S, et al (2020) First report of diazotrophic *Brevundimonas* spp. as growth enhancer and root colonizer of potato. Sci Rep 10:12893. doi.org/10.1038/s41598-020-69782-6

Naveed M, Brown LK, Raffan AC, et al (2017) Plant exudates may stabilize or weaken soil depending on species, origin and time. Eur J Soil Sci 68:806–816. doi.org/10.1111/ejss.12487

Naylor D, Coleman-Derr D (2018) Drought stress and root-associated bacterial communities. Front. Plant Sci. 8. doi.org/10.3389/fpls.2017.02223

Oburger E, Jones DL (2018) Sampling root exudates – Mission impossible? Rhizosphere 6. doi.org/10.1016/j.rhisph.2018.06.004

Oksanen J, Simpson GL, Blanchet FG, et al (2022) vegan: Community Ecology Package. github.com/vegandevs/vegan

Oppenheimer-Shaanan Y, Jakoby G, Starr ML, et al (2022) A dynamic rhizosphere interplay between tree roots and soil bacteria under drought stress. Elife 11:e79679. doi.org/10.7554/eLife.79679

Palla M, Turrini A, Cristani C, et al (2022) Impact of sheep wool residues as soil amendments on olive beneficial symbionts and bacterial diversity. Bioresour Bioprocess 9:45. doi.org/10.1186/s40643-022-00534-2

Paquette A, Hargreaves AL (2021) Biotic interactions are more often important at species' warm versus cool range edges. Ecol Lett 24:2427–2438. doi.org/10.1111/ele.13864

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18:1403–1414. doi.org/10.1111/1462-2920.13023

Passioura JB (1988) Water transport in and to roots. Annu Rev Plant Physiol Plant Mol Biol 39:245–265. doi.org/10.1146/annurev.pp.39.060188.001333

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Funct Plant Biol 27:1191. doi.org/10.1071/PP99173_CO

Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. doi.org/10.1093/nar/gks1219

R-Core-Team (2021) R: A language and environment for statistical computing. www.r-project.org

Răut I, Călin M, Capră L, et al (2021) *Cladosporium* sp. isolate as fungal plant growth promoting agent. Agronomy 11. doi.org/10.3390/agronomy11020392

Redman RS, Kim YO, Woodward CJDA, et al (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6:e14823. doi.org/10.1371/journal.pone.0014823

Refai MY, Abulfaraj AA, Hakeem IJ, et al (2023) Rhizobiome signature and Its alteration due to watering in the wild plant *Moringa oleifera*. Sustainability 15. doi.org/10.3390/su15032745

Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Appl Environ Microbiol 58:1284–1291. doi.org/10.1007/BF00172568

Robinson D, Hayes A, Couch S (2023) broom: Convert statistical objects into tidy tibbles. broom.tidymodels.org/

Rodrigues J, Inzé D, Nelissen H, Saibo NJM (2019) Source–sink regulation in crops under water deficit. Trends Plant Sci 24:652–663. doi.org/10.1016/j.tplants.2019.04.005

Rodriguez RJ, Henson J, Van Volkenburgh E, et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416. doi.org/10.1038/ismej.2007.106

Rolli E, Marasco R, Vigani G, et al (2015) Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environ Microbiol 17:316–331. doi.org/10.1111/1462-2920.12439

Rosenzweig R, Shavit U, Furman A (2012) Water retention curves of biofilm-affected soils using xanthan as an analogue. Soil Sci Soc Am J 76:61. doi.org/10.2136/sssaj2011.0155

Santos-Medellín C, Edwards J, Liechty Z, et al (2017) Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes. MBio 8:10.1128/mbio.00764-17. doi.org/10.1128/mbio.00764-17

Sasse J, Martinoia E, Northen T (2018) Feed your friends: Do plant exudates shape the root microbiome? Trends Plant Sci 23:25–41. doi.org/10.1016/j.tplants.2017.09.003

Schepers, JR, Heblack, J, Willi, Y, (2024). Negative interaction effect of heat and drought stress at the warm end of species distribution. Oecologia. 204:173–185. doi.org/10.1007/s00442-023-05497-5

Sigler L, Lumley TC, Currah RS (2000) New species and records of saprophytic ascomycetes (Myxotrichaceae) from decaying logs in the boreal forest. Mycoscience 41:495–502. doi.org/10.1007/BF02461670

Silambarasan S, Logeswari P, Vangnai AS, et al (2022) Plant growth-promoting actinobacterial inoculant assisted phytoremediation increases cadmium uptake in *Sorghum bicolor* under drought and heat stresses. Environ Pollut 307:119489. doi.org/10.1016/j.envpol.2022.119489

Singh P, Chauhan PK, Upadhyay SK, et al (2022) Mechanistic insights and potential use of siderophores producing microbes in rhizosphere for mitigation of stress in plants grown in degraded land. Front. Microbiol. 13. doi.org/10.3389/fmicb.2022.898979

Sperl GT, Hoare DS (1971) Denitrification with methanol: A selective enrichment for *Hyphomicrobium* Species. J Bacteriol 108:733–736. doi.org/10.1128/jb.108.2.733-736.1971

Sun C, Johnson JM, Cai D, et al (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017. doi.org/10.1016/j.jplph.2010.02.013

Sutherland IW (2001) Biofilm exopolysaccharides: A strong and sticky framework. Microbiology 147:3–9. doi.org/10.1099/00221287-147-1-3

Tang H, Hassan MU, Feng L, et al (2022) The critical role of arbuscular mycorrhizal fungi to improve drought tolerance and nitrogen use efficiency in crops. Front. Plant Sci. 13

Tardieu F (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Front Physiol 4:. doi.org/10.3389/fphys.2013.00017

Taylor G, Wang X, Jabaji-Hare SH (2003) Detection of the mycoparasite *Stachybotrys elegans*, using primers with sequence- characterized amplification regions in conventional and real-time PCR. Can J Plant Pathol 25:49–61. doi.org/10.1080/07060660309507049

Tecon R, Or D (2017) Biophysical processes supporting the diversity of microbial life in soil. FEMS Microbiol Rev 41:599–623. doi.org/10.1093/femsre/fux039

Tran CT, Al Azzawi TN, Khan M, et al (2023) *Brevundimonas vesicularis* (S1T13) mitigates drought-stress-associated damage in *Arabidopsis thaliana*. Int. J. Mol. Sci. 24. doi.org/10.3390/ijms242316590

Ulrich DEM, Clendinen CS, Alongi F, et al (2022) Root exudate composition reflects drought severity gradient in blue grama (*Bouteloua gracilis*). Sci Rep 12:12581. doi.org/10.1038/s41598-022-16408-8

Valentinuzzi F, Cesco S, Tomasi N, Mimmo T (2015) Influence of different trap solutions on the determination of root exudates in *Lupinus albus* L. Biol Fertil Soils 51:757–765. doi.org/10.1007/s00374-015-1015-2

van Den Dool H, Dec. Kratz P (1963) A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J Chromatogr A 11:463–471. doi.org/10.1016/S0021-9673(01)80947-X

Vassilev N, Eichler-Löbermann B, Vassileva M (2012) Stress-tolerant P-solubilizing microorganisms. Appl Microbiol Biotechnol 95:851–859. doi.org/10.1007/s00253-012-4224-8

Veach AM, Chen H, Yang ZK, et al (2020) Plant hosts modify belowground microbial community response to extreme drought. mSystems 5:10.1128/msystems.00092-20. doi.org/10.1128/msystems.00092-20

Verslues PE, Juenger TE (2011) Drought, metabolites, and *Arabidopsis* natural variation: A promising combination for understanding adaptation to water-limited environments. Curr Opin Plant Biol 14:240–245. doi.org/10.1016/j.pbi.2011.04.006

Wang F, Wei Y, Yan T, et al (2022) *Sphingomonas* sp. Hbc-6 alters physiological metabolism and recruits beneficial rhizosphere bacteria to improve plant growth and drought tolerance. Front. Plant Sci. 13. doi.org/10.3389/fpls.2022.1002772

Wickham H (2016) ggplot2: Elegant graphics for data analysis. ggplot2.tidyverse.org

Wickham H (2007) Reshaping data with the {reshape} package. J Stat Softw 21:1–20. jstatsoft.org/v21/i12/

Wickham H, François R, Henry L, et al (2023a) dplyr: A grammar of data manipulation. dplyr.tidyverse.org

Wickham H, Vaughan D, Girlich M (2023b) tidyr: Tidy messy data. tidyr.tidyverse.org

Williams A, de Vries FT (2020) Plant root exudation under drought: Implications for ecosystem functioning. New Phytol 225:1899–1905. doi.org/10.1111/nph.16223

Windisch S, Bott S, Ohler M-A, et al (2017) *Rhizoctonia solani* and bacterial inoculants stimulate root exudation of antifungal compounds in lettuce in a soil-type specific manner. Agronomy 7. doi.org/10.3390/agronomy7020044

Windisch S, Sommermann L, Babin D, et al (2021) Impact of long-term organic and mineral fertilization on rhizosphere metabolites, foot–microbial interactions and plant health of lettuce. Front. Microbiol. 11. doi.org/10.3389/fmicb.2020.597745

Wos G, Willi Y (2018) Genetic differentiation in life history traits and thermal stress performance across a heterogeneous dune landscape in *Arabidopsis lyrata*. Ann Bot 122:473—484. doi.org/10.1093/aob/mcy090

Wright E (2016) Using DECIPHER v2.0 to analyze big biological sequence data in R. R J 8:352–359. doi.org/10.18129/B9.bioc.DECIPHER

Wright SD, McConnaughay KDM (2002) Interpreting phenotypic plasticity: The importance of ontogeny. Plant species Biol. 17:119–131. doi.org/10.1046/j.1442-1984.2002.00082.x

Wu L, Kobayashi Y, Wasaki J, Koyama H (2018) Organic acid excretion from roots: A plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil Sci Plant Nutr 64:697–704. doi.org/10.1080/00380768.2018.1537093

Yee B, Oertli GE, Fuerst JA, Staley JT (2010) Reclassification of the polyphyletic genus Prosthecomicrobium to form two novel genera, *Vasilyevaea* gen. nov. and *Bauldia* gen. nov. with four new combinations: *Vasilyevaea enhydra* comb. nov., *Vasilyevaea mishustinii* comb.nov., *Bauldia consociata* comb. nov. and *Bauldia litoralis* comb.nov.. Int J Syst Evol Microbiol 60:2960. doi.org/10.1099/ijs.0.018234-0

Zhai Z, Keereetaweep J, Liu H, et al (2021) The role of sugar signaling in regulating plant fatty acid synthesis. Front. Plant Sci. 12. doi.org/10.3389/fpls.2021.643843

Zhang J, Nasir F, Kong Y, et al (2017) Drought stress shapes the root-associated bacterial and fungal community structure in soybean genotypes. Pak J Bot 49:1933–1942

Zhang X, Bilyera N, Fan L, et al (2023) The spatial distribution of rhizosphere microbial activities under drought: Water availability is more important than root-hair-controlled exudation. New Phytol 237:780–792. doi.org/10.1111/nph.18409

Zhang X, Myrold DD, Shi L, et al (2021) Resistance of microbial community and its functional sensitivity in the rhizosphere hotspots to drought. Soil Biol Biochem 161:108360. doi.org/10.1016/j.soilbio.2021.108360

Tables

Table 1: Effect of drought, plant family and their interaction on performance, as well as leaf and root functional traits of *A. lyrata*. F-ratios and estimated coefficants of drought treaments of continiuous traits and the difference in percentrage of binary traits are reported. P-values are included in parentheses, with significant P-values (P<0.05) indicated in bold. Sample sizes (N) are given for both treatments together. Sample sizes per treatment are given in table S1.

Trait	N	Treatment	Seed Family	Treatment:Family
Maximum Rosette Size	100	**44.5, -184 (<0.01)**	**4.83 (<0.01)**	1.52 (0.16)
Biomass	81	**42.3, -0.02 (<0.01)**	0.66 (0.74)	1.42 (0.20)
Time to Fastest Growth	100	0.02, -1.8 (0.88)	**2.58 (0.01)**	0.84 (0.58)
Growth Rate	100	**5.16, -0.01 (0.03)**	**4.35 (<0.01)**	1.11 (0.36)
SLA	78	**7.4, 0.19 (0.01)**	**2.7 (0.01)**	1.53 (0.16)
LDMC	78	0.23, -53 (0.63)	**3.17 (<0.01)**	1.43 (0.20)
Root:Shoot	78	1.91, 0.42 (0.17)	1.20 (0.31)	1.40 (0.21)
Survival	100	**6.92, -18% (0.01)**	0.47 (0.89)	**2.01 (0.05)**
Flowering	97	**7.93, -17% (0.01)**	**4.28 (<0.01)**	**2.22 (0.03)**

Table 2: Effect of drought, plant family and their interaction on relative root exudate abundance collected from *A. lyrata* by compound group. F-value, estimated coefficients and P-value are reported in parentheses. Significant P-values (P<0.05) are indicated in bold. Sample sizes (N) are given for both treatments.

Group	**Treatment**	**Seed Family**	**Treatment:Family**
Overall (N_C=41, N_D=32)	**6.86, -0.20 (0.01)**	**2.21 (0.02)**	1.26 (0.257)
Sugars	2.03, -1.6 (0.155)	1.42 (0.177)	0.9 (0.522)
Benzoic Acid	0.27, 0.20 (0.609)	0.96 (0.479)	0.38 (0.939)
Fatty Acids	**7.21, -0.19 (0.008)**	0.91 (0.514)	0.77 (0.641)
Amino Acids	**5.98, -0.20 (0.016)**	1.19 (0.302)	0.71 (0.696)

Table 3: Effect of drought, plant family and their interaction on alpha diversity (ANOVA on richness, Shannon index) and beta diversity (PERMANOVA) of bacteria and fungi. F-values and P-values in parentheses are reported. Significant P-values (P<0.05) are indicated in bold. Sample sizes (N) are given for both treatments and the number of taxonomic groups (ASVs, amplicon sequence variants).

Index	Treatment	Seed Family	Treatment:Family
Bacteria (N_C=33, N_D=23, N_{ASV}=726)			
Richness	0.09 (0.88)	0.92 (0.21)	0.96 (0.32)
Shannon	0.08 (0.51)	0.38 (0.80)	0.90 (0.41)
Beta Diversity	**3.00 (<0.01)**	1.16 (0.12)	**1.54 (<0.01)**
fungi (N_C=39, N_D=28, N_{ASV}=122)			
Richness	0.25 (0.50)	**3.18 (<0.01)**	1.21 (0.94)
Shannon	0.18 (0.31)	**1.87 (0.02)**	0.61 (0.90)
Beta Diversity	1.03 (0.38)	0.96 (0.56)	0.87 (0.73)

Table 4: Correlation of plant traits with other plant traits, root exudates, and rhizosphere bacteria and fungi on genus level above 0.7 under control and dry conditions. Positive correlations are indicated in red and negative correlations in blue. A list with correlations of plant traits above 0.5 can be found in the appendix (Table S6).

Trait	Treatment	Plants	Exudates	Bacteria	Fungi
Maximum Rosette Size	Control	-	-	-	-
	Dry	-	-	-	*Gymnostellatospora*
Total Biomass	Control	Root biomass	-	-	-
	Dry	SLA, Rosette biomass, Root biomass	-	*Dongia*, *Brevunimonas*	-
Time to Fastest Growth	Control	Growth Rate	-	-	-
	Dry	Growth Rate	-	-	-
Growth Rate	Control	Time to Fastest Growth	-	-	-
	Dry	Time to Fastest Growth	-	-	-
SLA	Control	Rosette Biomass	-	*Brevunimonas*	-
	Dry	Rosette Biomass, Biomass, Root Biomass	-	-	-
LDMC	Control	-	-	-	-
	Dry	-	-	*OLB13*, *Lacibacter*	-
Root:Shoot	Control	Root Biomass	-	-	-
	Dry	Root Biomass	-	*Sphingomonas*, *Quipengyuania*, *Stella*	-
Root Biomass	Control	Root:Shoot, Biomass	-	*Defluviicoccus*	-
	Dry	Root:Shoot, Biomass, SLA	Alanin	*Vicinamibacter*, *Nitratireductor*, *Hyphomicrobium*, *Dongia*	-
Rosette Biomass	Control	SLA	-	-	-
	Dry	Biomass, SLA	-	*Legionella*	-
Flowering	Control	-	-	-	-
	Dry	-	-	-	*Leptodontidium*

Figures

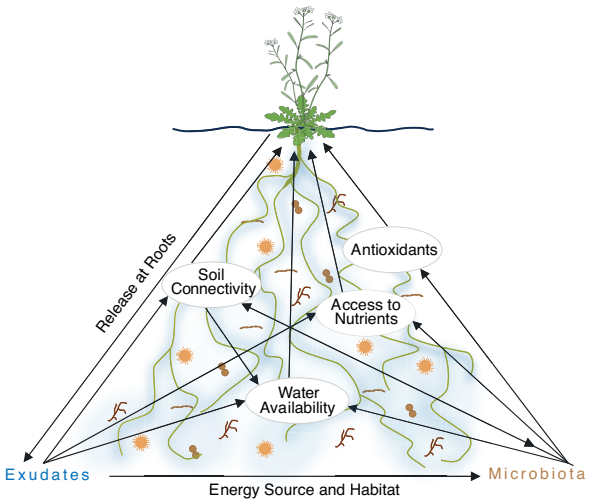

Fig. 1: Direct and indirect interactions among plants, their root exudates and the microbiome in the rhizosphere.

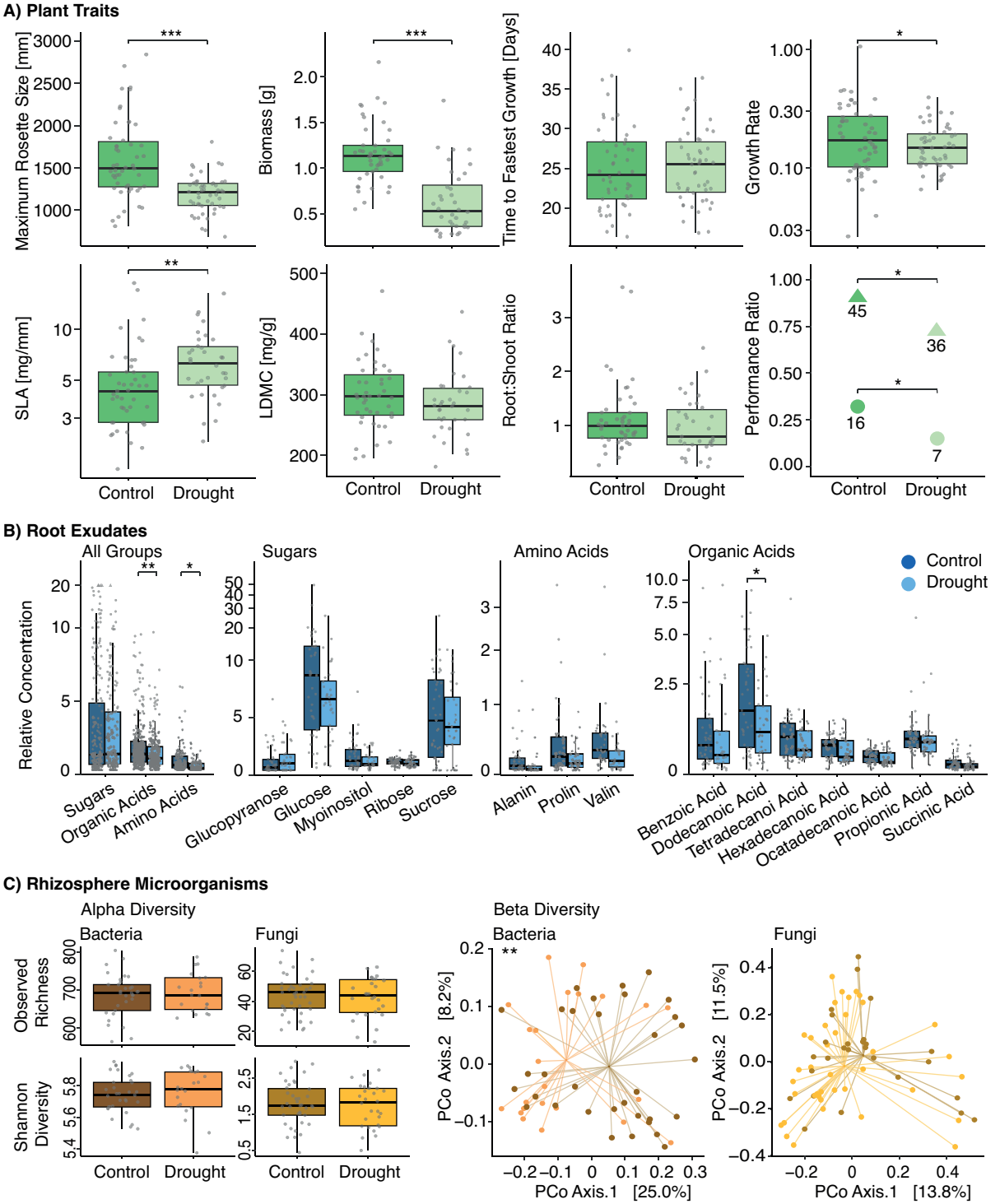

Fig. 2: Treatment effect on plant traits as well as root exudates, bacteria and fungi.
A) Effect of drought on performance and allocation in *A. lyrata*. For treatments, the overall medians (thick horizontal lines) with 1.5 inter-quartile range of the upper and lower quartile, (whiskers; for non-binary traits) are shown. Grey dots show all data points. The performance ratio gives the fraction and absolute number of surviving (triangle) and flowering (circle) plants in both treatments. Note the log scale on the y-axis of growth rate and SLA. Significant differences between treatments are indicated in all plots with stars (P<0.05*, P<0.01**, P<0.001***).
B) Relative concentration of different compound groups and compounds identified in root exudates in the control (dark blue) and drought (light blue) treatments. Grey dots indicate, as

above, single measurements, while grey triangles measurements above the given scale. Note the log scale for all plots.
C) Diversity measures of alpha and beta diversity of bacteria and fungi. Boxplots show the observed richness and Shannon diversity of bacteria and fungi in both treatments. Ordination plots show the first two principal coordinates of rhizosphere bacteria and fungi in the control (darker brown) and drought treatment (lighter brown).

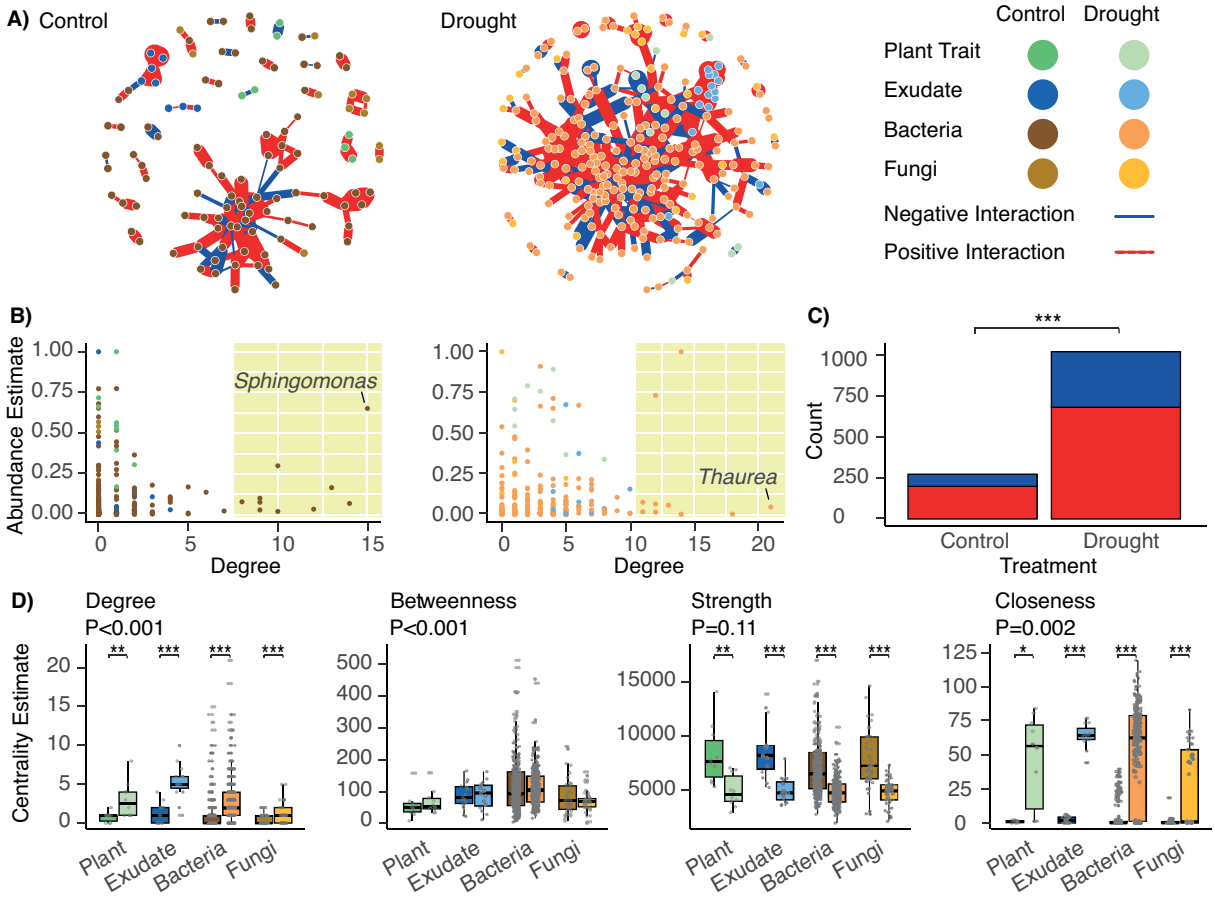

Fig. 3: Correlation network of plant traits, root exudates, bacteria and fungi in control and drought conditions.
A) Correlation networks of plant traits (green), root exudates (blue), bacteria (light brown) and fungi (dark brown) of correlations above 0.7. Positive interactions are indicated in red, negative in blue. The line thickness reflects the strength of correlation.
B) Degree of correlation networks and relative concentration of exudates, abundance of microorganisms and zero-to-one scaled plant traits. Colours refer to plant trait, exudates, bacteria or fungi. Top 10 of degree of correlation have a yellow background
C) Cumulative positive and negative correlations above 0.7 in the control and drought treatment. Significant differences between treatments were calculated with a chi-square test and are indicated with stars (P<0.05*, P<0.01**, P<0.001***).
D) Centrality estimates (degree, betweenness strength and closeness) of correlation networks in control and drought conditions. Significant differences between treatments were calculated with a Kruskal-Wallice test and are indicated in all plots with stars (P<0.05*, P<0.01**, P<0.001***).

Concluding discussion

Species are restricted in their distribution, and the already challenging growth conditions at the rear edge are often worsening with climate change (Hampe and Petit 2005). However, the adaptability of plants to changing climatic conditions that affect several stressors simultaneously has rarely been studied systematically. In my thesis, I assessed the effects of the most limiting environmental conditions, temperature and precipitation, on the performance of the North American model plant *Arabidopsis lyrata* spp. *lyrata*. I disentangled the effects of heat and drought on plant growth and persistence, and analysed the different adaptive strategies *A. lyrata* has evolved to cope with those harsh conditions. This was achieved by performing a transplant experiment across the rear range edges of its distribution and a greenhouse experiment including heat and drought conditions. My thesis comprises three projects, each of which I summarise here briefly.

Summary of findings

In **Chapter 1,** I investigated how temperature and precipitation influence plant performance along a transect across the warm end of *A. lyrata* species distribution limits. I monitored local weather conditions in this 1.5-year transplant experiment. With this data, I analysed the impact of weather on the transition of several key life stages, including germination, flowering and mortality. When linking the measured temperatures and precipitation levels during life state transitions, I observed a positive effect of warm temperatures on immediate germination and flowering success, but also a positive effect of colder temperatures on state transition in the weeks prior to germination and flowering. Mortality was increased by heavy rainfall events and long-term drought, as well as too warm temperatures or extreme cold. As a consequence of climate change, temperatures have generally increased during the last decades, and precipitation has become more variable and extreme (IPCC 2023). Therefore, the conditions at the low latitudinal range edge will continue to become less suitable, plants are less likely to persist at

the rear edge, and the geographical location of the species‘ optimum within the current range will change to higher latitudes (Sánchez-Castro et al. 2024).

In Chapter 2, I focused on the individual and combined effects of the two stressors, heat and drought, on plant performance. In a greenhouse experiment, I exposed plants to heat and drought, simulating the environmental conditions *A. lyrata* has experienced at the warm range edges over the last few decades. An important observation was that plant survival was only slightly reduced under single-stress conditions. Though a single stressor like heat or drought had a negative impact on plant performance, a synergistic effect was observed when both stressors were combined. Plants adapted to drought or heat with an increased root-to-shoot ratio, meaning a higher allocation to root systems. However, plants exposed to both stressors had a significant lower survival rate and showed a significant higher specific leaf area (SLA, rosette area per rosette dry mass). Analysis revealed that under both stress conditions, drought as well as drought combined with heat, plants did not have a higher survival or longevity if they had a higher SLA, but a better performance was rather connected to a delayed and slower growth. Plants of populations originating from the southern end of the distribution displayed a higher root-to-shoot ratio and thinner leaves and coped slightly better with the intensive stress caused by the combination of heat and drought than plants originating from other areas. These populations seemed to have an adaptive advantage as they already grew under comparatively challenging conditions in nature (Hampe and Petit 2005; Estarague et al. 2022). Overall, this experiment suggests that plants develop strategies to escape single plant stresses in time through changes in phenology, even though this strategy is not favourable for higher longevity under stress conditions (Levitt 1980; Ludlow and Muchow 1990). However, the ability to adapt to single stresses does not imply that plants are resilient to combined stresses.

In **Chapter 3,** I aimed to study the interaction of *A. lyrata* with its rhizosphere under stress conditions. In a greenhouse experiment, I collected phenotypic and phenological plant data, root exudates, and rhizosphere microorganisms from plants grown for several months

under dry and well-watered conditions. Using next-generation sequencing, I identified the bacteria and fungi present under each condition. I employed gas chromatography coupled with a flame-ionisation detector to quantify the most common primary root exudate compounds. In line with the above findings, plants grown under drought had a slightly reduced survival and flowering rate and responded to drought stress with a smaller rosette size and biomass and a higher SLA. The composition of the bacterial genera identified in the rhizosphere varied significantly between treatments. Under drought, as compared to control treatment, I discovered a higher abundance of gram-positive bacteria, which access carbon sources that are unavailable for gram negative bacteria (Naylor and Coleman-Derr 2018). The most abundant bacteria under drought stress was *Sphingomonas*, which is known to improve plant growth under drought conditions (Luo et al. 2019; Wang et al. 2022; Fan et al. 2023; Refai et al. 2023). Rhizosphere fungi are important partners in maintaining plant performance under drought (Almario et al. 2017; Hiruma et al. 2018; Huang et al. 2017; Sun et al. 2010). However, contrary to bacteria, fungi composition was not significantly different between treatments, suggesting a higher drought tolerance of fungi (Guhr et al. 2015; Veach et al. 2020). Further, I observed under drought conditions a higher relative concentration of organic acids in the root exudate, especially of fatty acids. Fatty acids are important not only for the nutrient supply of plants but also for communication with microorganisms in the soil, including both pathogens as well as beneficial microbes (Ma et al. 2021; Li et al. 2017; Vlot et al. 2009). A comparison of the correlation matrices demonstrated that the intensity of interactions increased under dry conditions compared to control conditions. Under dry conditions, especially positive interactions were prominent, highlighting the importance of plant-microbe interactions under stress (Berendsen et al. 2012). Under drought conditions, a higher plant performance was, for example, linked with *Brevundimonas* or *Leptodontidium*, both plant-growth-promoting bacteria (Fernando and Currah 1996; Hou and Guo 2009; Naqqash et al. 2020; Tran et al. 2023).

Characterising the range edge at the warm end of the distribution

Plants have their growth optima at locations with the highest environmental suitability (Hargreaves et al. 2014; Lee-Yaw et al. 2016). If the environmental conditions deviate too much from the optimum, plants perform worse, thus defining the abiotic limitations of a species range. In the three projects, I demonstrated the negative effect of inadequate water availability and temperature conditions on plant performance by studying the limits of plant growth based on temperature and precipitation, both in field and controlled experimental set-ups. The results demonstrate that precipitation and especially temperature have a high impact on the suitability of a location regarding the growth of a species (Moles et al. 2014; Tao et al. 2017; Lee-Yaw et al. 2018). My research contributes to the understanding of abiotic limitations on plant growth, particularly focusing on the effects of temperature, precipitation, their combination, and geographical location on plant performance. It also sheds light on species range limits and why they are not expanding beyond a certain range. The studies suggest that deviations from the optimal growth conditions, be they too hot, too cold, too dry, or too wet, negatively influence plant growth and performance. My work focuses in particular on the negative effects of long-term heat and drought on plant performance and the mitigation of stress through interactions with the soil microbiome. This leads to the hypothesis that, at the warm range edge, population persistence might be under threat and, if not limited by other factors, perform better at the colder locations.

What are the consequences of climate change?

With progressive climate change, it is very likely that conditions at the warm range edge of *A. lyrata* get more severe over time, as it is the rear edge (Cahill et al. 2014; Lenoir et al. 2024). Even if the climatic conditions are not so extreme in the centre of distribution, plants have a similar level of vulnerability, as populations are less adapted to warm and extreme events (Bennet et al. 2015). As already indicated by its common name, lyre-leaved rock-cress, my

model species, *A. lyrata*, grows naturally on substrates that tend to be poor in water retention, such as sand and rock cracks. This suggests that the drought effect might have an even bigger impact on populations in their natural habitats, as water is not accessible for extended periods after rainfall. This can also explain the detrimental effect of heavy rainfall in our transplant experiment on common soil (Chapter 1). In addition to an altered precipitation pattern, temperatures have increased in the home range of my model species but also of many other species (USGCRP 2023; IPCC 2023). This motivated me to investigate the effects of both environmental factors, single and combined, in more detail. With more frequent extreme weather conditions, it is likely that these stressors will appear together during the growing season of *A. lyrata*, when plants are most vulnerable. However, it should not be neglected that plants have the ability to adapt to new conditions over a couple of generations (Franks 2011; Kristensen et al. 2020). Without this adaptation process, plants only have a minor chance to survive in the context of global warming (Chapter 2). Nonetheless, under drought stress, the interactions with the soil microbiome increased, which suggests that some of the drought effects may be mitigated (Chapter 3). Several reviews reported benefits of plant-microbe interactions on plant performance under environmental stress, which is confirmed by the increased positive interaction under drought stress in my last chapter (Pineda et al. 2013; Vimal et al. 2017; Bastías et al. 2022). However, to what extent the plant-microbe interactions might compensate the negative impact of climate change on plant survival is to be determined. As the type and intensity of interactions vary under different conditions, a subsequent experiment could investigate the change of correlations along environmental gradients, not only under controlled settings in the greenhouse but also in natural conditions. Additional studies on the interactions including more plant species and different kinds of root exudate compounds, a gradient of environmental stress levels, or a combination of stressors, would help to understand how these relationships might be helpful during stress and if they can be employed purposefully to support plant growth under harsh conditions.

References

Almario J, Jeena G, Wunder J, et al (2017) Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. Proc Natl Acad Sci U S A 114:E9403–E9412. doi.org/10.1073/pnas.1710455114

Bastías DA, Balestrini R, Pollmann S, Gundel PE (2022) Environmental interference of plant−microbe interactions. Plant Cell Environ 45:3387–3398. doi.org/10.1111/pce.14455

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, et al (2014) Causes of warm-edge range limits: Systematic review, proximate factors and implications for climate change. J Biogeogr 41:429–442. doi.org/10.1111/jbi.12231

Estarague A, Vasseur F, Sartori K, et al (2022) Into the range: a latitudinal gradient or a center-margins differentiation of ecological strategies in *Arabidopsis thaliana*? Ann Bot 129:343–356. doi.org/10.1093/aob/mcab149

Fan W, Tang F, Wang J, et al (2023) Drought-induced recruitment of specific root-associated bacteria enhances adaptation of alfalfa to drought stress. Front. Microbiol. 14. doi.org/10.3389/fmicb.2023.1114400

Fernando AA, Currah RS (1996) A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (Fungi Imperfecti) on the growth of some subalpine plants in culture. Can J Bot 74:1071–1078. doi.org/10.1139/b96-131

Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. New Phytol 190:249–257. doi.org/10.1111/j.1469-8137.2010.03603.x

Guhr A, Borken W, Spohn M, Matzner E (2015) Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization. Proc Natl Acad Sci 112:14647–14651. doi.org/10.1073/pnas.1514435112

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: The rear edge matters. Ecol Lett 8:461–467. doi.org/10.1111/j.1461-0248.2005.00739.x

Hargreaves AL, Samis KE, Eckert CG (2014) Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. Am Nat 183:157–173. doi.org/10.1086/674525

Hiruma K, Kobae Y, Toju H (2018) Beneficial associations between Brassicaceae plants and fungal endophytes under nutrient-limiting conditions: evolutionary origins and host–symbiont molecular mechanisms. Curr Opin Plant Biol 44:145–154. doi.org/10.1016/j.pbi.2018.04.009

Hou X-Q, Guo S-X (2009) Interaction between a dark septate endophytic isolate from *Dendrobium* sp. and roots of *D. nobile* seedlings. J Integr Plant Biol 51:374–381. doi.org/10.1111/j.1744-7909.2008.00777.x

Huang Y-M, Zou Y-N, Wu Q-S (2017) Alleviation of drought stress by mycorrhizas is related to increased root H(2)O(2) efflux in trifoliate orange. Sci Rep 7:42335. doi.org/10.1038/srep42335

Intergovernmental Panel on Climate Change (IPCC) (2023): Climate Change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge Univ Press. doi.org/10.1017/9781009157896

Kristensen TN, Ketola T, Kronholm I (2020) Adaptation to environmental stress at different timescales. Ann N Y Acad Sci 1476:5–22. doi.org/10.1111/nyas.13974

Lee-Yaw JA, Fracassetti M, Willi Y (2018) Environmental marginality and geographic range limits: a case study with *Arabidopsis lyrata* ssp. *lyrata*. Ecography (Cop) 41:622–634. doi.org/10.1111/ecog.02869

Lee-Yaw JA, Kharouba HM, Bontrager M, et al (2016) A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. Ecol Lett 19:710–722. doi.org/10.1111/ele.12604

Lenoir J, Svenning J-C, Sheffer MM (2024) Latitudinal and elevational range shifts under contemporary climate change. In: Scheiner SMBT-E of B (Third E (ed). Academic Press, Oxford, pp 690–709

Levitt J (1980) Volume 1: Chilling, freezing, and high temperature stresses. In: Responses of plants to environmental stress, 2nd edn. Academic Press, New York

Li S, Xu C, Wang J, et al (2017) Cinnamic, myristic and fumaric acids in tobacco root exudates induce the infection of plants by *Ralstonia solanacearum*. Plant Soil 412:381–395. doi.org/10.1007/s11104-016-3060-5

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron 43:107–153. doi.org/10.1016/S0065-2113(08)60477-0

Luo Y, Wang F, Huang Y, et al (2019) *Sphingomonas* sp. Cra20 increases plant growth rate and alters rhizosphere microbial community structure of *Arabidopsis thaliana* under drought stress. Front. Microbiol. 10. doi.org/10.3389/fmicb.2019.01221

Ma K, Kou J, Khashi U Rahman M, et al (2021) Palmitic acid mediated change of rhizosphere and alleviation of Fusarium wilt disease in watermelon. Saudi J Biol Sci 28:3616–3623. doi.org/10.1016/j.sjbs.2021.03.040

Moles AT, Perkins SE, Laffan SW, et al (2014) Which is a better predictor of plant traits: Temperature or precipitation? J Veg Sci 25:1167–1180. doi.org/10.1111/jvs.12190

Naqqash T, Imran A, Hameed S, et al (2020) First report of diazotrophic *Brevundimonas* spp. as growth enhancer and root colonizer of potato. Sci Rep 10:12893. doi.org/10.1038/s41598-020-69782-6

Naylor D, Coleman-Derr D (2018) Drought stress and root-associated bacterial communities. Front. Plant Sci. 8. doi.org/10.3389/fpls.2017.02223

Pineda A, Dicke M, Pieterse CMJ, Pozo MJ (2013) Beneficial microbes in a changing environment: are they always helping plants to deal with insects? Funct Ecol 27:574–586. doi.org/10.1111/1365-2435.12050

Refai MY, Abulfaraj AA, Hakeem IJ, et al (2023) Rhizobiome signature and Its alteration due to watering in the wild plant *Moringa oleifera*. Sustainability 15. doi.org/10.3390/su15032745

Sánchez-Castro D, Patsiou T-S, Perrier A, et al (2024) Uncovering the cause of breakup between species' range limits and niche limits under climate warming. J Biogeogr 00:1-14. doi.org/10.1111/jbi.14796

Sun C, Johnson JM, Cai D, et al (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017. doi.org/10.1016/j.jplph.2010.02.013

Tao Z, Wang H, Liu Y, et al (2017) Phenological response of different vegetation types to temperature and precipitation variations in northern China during 1982–2012. Int J Remote Sens 38:3236–3252. doi.org/10.1080/01431161.2017.1292070

Tran CT, Al Azzawi TN, Khan M, et al (2023) *Brevundimonas vesicularis* (S1T13) mitigates drought-stress-associated damage in Arabidopsis thaliana. Int. J. Mol. Sci. 24. doi.org/10.3390/ijms242316590

USGCRP (2023) Fifth National Climate Assessment. In: Crimmins AR, Avery CW, Easterling DR, et al. (eds) Global Climate Change Impacts in the United States. Cambridge University Press, Washington, DC, USA. doi.org/10.7930/NCA5.2023

Veach AM, Chen H, Yang ZK, et al (2020) Plant hosts modify belowground microbial community response to extreme drought. mSystems 5:10.1128/msystems.00092-20. doi.org/10.1128/msystems.00092-20

Vimal SR, Singh JS, Arora NK, Singh S (2017) Soil-plant-microbe interactions in stressed agriculture management: A review. Pedosphere 27:177–192. doi.org/10.1016/S1002-0160(17)60309-6

Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47:177–206. doi.org/10.1146/annurev.phyto.050908.135202

Wang F, Wei Y, Yan T, et al (2022) *Sphingomonas* sp. Hbc-6 alters physiological metabolism and recruits beneficial rhizosphere bacteria to improve plant growth and drought tolerance. Front. Plant Sci. 13. doi.org/10.3389/fpls.2022.1002772

Supplementary Information

Chapter 1

Table S1: Population mean and standard deviation of selected performance traits.

Population	Germination Time [days]	Germination Success [%]	Flowering Time [days]	Times Flowering	Flowering Success [%]	Longevity [days]	Winter Survival [%]
NW	22 ± 7.5	31	163 ± 20	1 ± 0	11	193 ± 23	41
NE	16 ± 7.6	46	221 ± 112	1.25 ± 0.5	12	512 ± 5.9	49
C	20 ± 13	39	166 ± 41	1.1 ± 0.45	10	315 ± 130	40
SW	39 ± 7.8	19	151 ± 16	1 ± 0	4.4	158 ± 4.9	34
SE	21 ± 5.2	45	154 ± 17	1.05 ± 0.22	19	234 ± 97	39

Table S2: Overview of characteristics of experimental sites during the experiment. Average mean, minimum and maximum precipitation (mm) and temperature (Celsius) of weeks, where plants changed their stage from seed to germinated, alive to death or growing to flowering.

	Germination						Flowering						Death					
Site	T_{mean}	T_{min}	T_{max}	P_{mean}	P_{min}	P_{max}	T_{mean}	T_{min}	T_{max}	P_{mean}	P_{min}	P_{max}	T_{mean}	T_{min}	T_{max}	P_{mean}	P_{min}	P_{max}
N_{in}	6.8	-8.3	26.1	0.3	0.0	1.2	15.0	2.2	28.9	0.7	0.5	1.4	7.69	-12.5	32.5	14	0.0	39.8
N_{edge}	11.0	-3.4	26.9	0.4	0.0	11.3	13.1	-3.3	28.6	4.2	0.0	10.3	11.5	-13.3	34.4	12.5	0.0	107
N_{out}	14.1	1.1	31.0	0.5	0.0	4.3	18.4	1.1	38.1	0.9	0.0	2.1	11.6	-6.39	35.0	26.6	0.0	86.9
S_{in}	6.6	-8.5	32.5	1.3	0.0	8.4	14.0	-1.5	34.1	1.3	0.1	2.0	8.46	-13.3	28.3	6.32	0.0	38.4
S_{edge}	13.0	-6.7	31.7	0.2	0.0	3.8	15.0	-3.3	35.0	0.6	0.0	1.7	19.2	-8.5	39.4	22.2	0.0	75.6
S_{out}	12.3	-3.3	33.0	0.3	0.0	2.7	18.9	-6.4	38.3	1.3	0.0	5.0	10.5	-13.1	34.7	11.1	0.0	77.8

Table S3: P-values of type three ANOVA including models analysing the influence of weather on stage transitions from seed to germinating, plant to flowering and plant to death, belonging to Fig. 4. A glmmTMB model was used including precipitation (P), mean temperature (T_{mean}), difference to minimum temperature (ΔT_{min}) and maximum temperature (ΔT_{max}) with site as random effect. Week indicates the week before stage transitions. Flowering and survival additionally included the interaction with age as week after germination (age interaction). Death included the interaction of precipitation with all temperature variables and P^2 with ΔT_{max} (P interaction). Also, mortality was separated in the warmer and colder half of the year based on moving four-week T_{mean} averages.

Effect	Week	P	P^2	T_{mean}	ΔT_{min}	ΔT_{max}
Germination Intercept: -5.29 (<0.01)						
Single	0	-0.12 (<0.01)			0.06 (<0.01)	0.28 (<0.01)
Single	1	0.02 (<0.01)		-0.12 (<0.01)	0.09 (<0.01)	-0.17 (<0.01)
Flowering Intercept: -51.60 (<0.01), log(Age): 9.37(<0.01)						
Single	1 and 2	-0.22 (<0.01)		2.0 (<0.01)	-0.19 (0.41)	-0.65 (0.05)
Single	3 and 4	-0.20 (<0.01)			-1.40 (<0.01)	-0.62 (0.06)
Single	5 and 6	0.13 (0.04)			-1.09 (<0.01)	0.91 (<0.01)
Age Interaction	1 and 2	0.06 (<0.01)		-0.52 (<0.01)	0.06 (0.34)	0.25 (<0.01)
Age Interaction	3 and 4	0.05 (<0.01)			0.37 (<0.01)	0.25 (<0.01)
Age Interaction	5 and 6	-0.04 (0.02)			0.26 (<0.01)	-0.25 (<0.01)
Mortality Winter Intercept: -1.03 (0.41), log(Age): -1.99(<0.01)						
Single	1	1.62 (<0.01)	0.72 (<0.01)	0.23 (<0.01)	-0.18 (<0.01)	0.12 (0.13)
Single	2	-1.95 (<0.01)	0.86 (<0.01)		-0.11 (0.14)	0.12 (0.19)
Age Interaction	1	-0.35 (<0.01)		-0.02 (0.11)	0.00 (0.92)	0.00 (0.90)
Age Interaction	2	-0.14 (<0.01)			0.06 (0.01)	0.02 (0.50)
P Interaction	1			0.1 (<0.01)	-0.1 (<0.01)	0.10 (<0.01)
P Interaction	2				0.02 (<0.01)	0.16 (<0.01)
P^2 Interaction	1					-0.07 (<0.01)
P^2 Interaction	2					-0.06 (<0.01)
Mortality Summer Intercept: -27.49(<0.01), log(Age): 5.09(<0.01)						
Single	1	12.84 (<0.01)	-0.11 (0.02)	3.02 (<0.01)	5.25 (<0.01)	-1.01 (<0.01)
Single	2	0.50 (0.29)	0.23 (<0.01)		-1.12 (<0.01)	0.15 (0.41)
Age Interaction	1	-4.01 (<0.01)		-0.73 (<0.01)	-1.61 (<0.01)	0.33 (<0.01)
Age Interaction	2	-0.11 (0.33)			0.33 (<0.01)	0.04 (0.45)
P Interaction	1			0.25 (<0.01)	-0.12 (<0.01)	0.11 (<0.01)
P Interaction	2				0.14 (<0.01)	-0.14 (<0.01)
P^2 Interaction	1					-0.01 (0.11)
P^2 Interaction	2					-0.01 (<0.01)

Table S4: Selection of non-correlated parameters for mixed effect models.
As T_{mean} of the different weeks was often correlated, only one T_{mean} was included in the model. The lower triangle reports the correlation coefficient and the upper triangle the p-value with *** indicating p<0.005, ** p<0.001 and * p<0.05.

Germination

	P W_0	T_{mean} W_0	ΔT_{min} W_0	ΔT_{max} W_0	P W_1	T_{mean} W_1	ΔT_{min} W_1	ΔT_{max} W_1
P W_0		***	***	***	***	***	*	***
T_{mean} W_0	0.14		***	***		***	***	***
ΔT_{min} W_0	0.19	0.23		***	***	***	***	***
ΔT_{max} W_0	-0.09	-0.48	-0.57		***	***	***	***
P W_1	-0.18	0.01	0.23	-0.17		***	***	***
T_{mean} W_1	0.21	0.83	0.4	-0.46	0.1		***	***
ΔT_{min} W_1	0.01	0.19	0.5	-0.44	0.21	0.19		***
ΔT_{max} W_1	-0.12	-0.33	-0.48	0.48	-0.08	-0.45	-0.58	

Flowering

	P W_{12}	T_{mean} W_{12}	ΔT_{min} W_{12}	ΔT_{max} W_{12}	P W_{34}	T_{mean} W_{34}	ΔT_{min} W_{34}	ΔT_{max} W_{34}	P W_{56}	T_{mean} W_{56}	ΔT_{min} W_{56}	ΔT_{max} W_{56}
P W_{12}		***	***	***	***	***	***	***	***	***	***	0.5603
T_{mean} W_{12}	0.06		***	***	***	***	***	***	***	***	***	***
ΔT_{min} W_{12}	0.09	0.49		***	***	***	***	***	***	***	***	***
ΔT_{max} W_{12}	-0.02	-0.63	-0.64		***	***	***	***	***	***	***	***
P W_{34}	-0.09	0.08	0.13	-0.2		***	***	***	***	***	***	***
T_{mean} W_{34}	0.13	0.89	0.59	-0.68	0.07		***	***	***	***	***	***
ΔT_{min} W_{34}	0.04	0.44	0.49	-0.44	0.08	0.51		***	***	***	***	***
ΔT_{max} W_{34}	-0.05	-0.51	-0.37	0.53	-0.03	-0.63	-0.64		***	***	***	***
P W_{56}	-0.11	0.11	0.13	-0.08	-0.08	0.09	0.13	-0.19		***	***	***
T_{mean} W_{56}	0.12	0.82	0.57	-0.68	0.14	0.9	0.61	-0.67	0.06		***	***
ΔT_{min} W_{56}	-0.01	0.41	0.43	-0.33	0.05	0.45	0.54	-0.47	0.1	0.54		***
ΔT_{max} W_{56}	0	-0.43	-0.42	0.47	-0.08	-0.52	-0.38	0.55	-0.03	-0.64	-0.63	

Death

	P W_1	T_{mean} W_1	ΔT_{min} W_1	ΔT_{max} W_1	P W_2	T_{mean} W_2	ΔT_{min} W_2	ΔT_{max} W_2
P W_1		***	***	***	***	***	***	***
T_{mean} W_1	0.07		***	***	***	***	***	***
ΔT_{min} W_1	-0.15	-0.19		***	***	***	***	***
ΔT_{max} W_1	-0.03	-0.44	0.45		***	***	***	***
P W_2	-0.04	0.07	-0.07	-0.21		***	***	***
T_{mean} W_2	0.22	0.85	-0.34	-0.4	0.07		***	***
ΔT_{min} W_2	-0.05	-0.19	0.36	0.3	-0.09	-0.18		***
ΔT_{max} W_2	-0.06	-0.26	0.3	0.33	-0.01	-0.43	0.47	

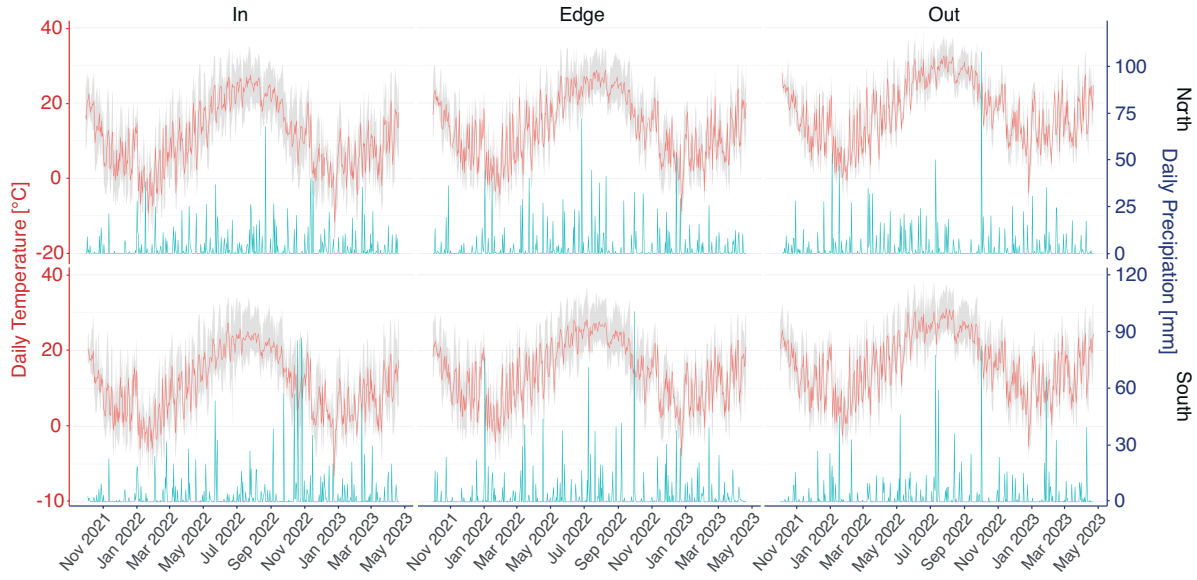

Fig. S1: Weather at sites during the experiment with mean daily records of temperature (red, with minimum and maximum indicated by grey shaded areas) and cumulative precipitation in blue (mm).

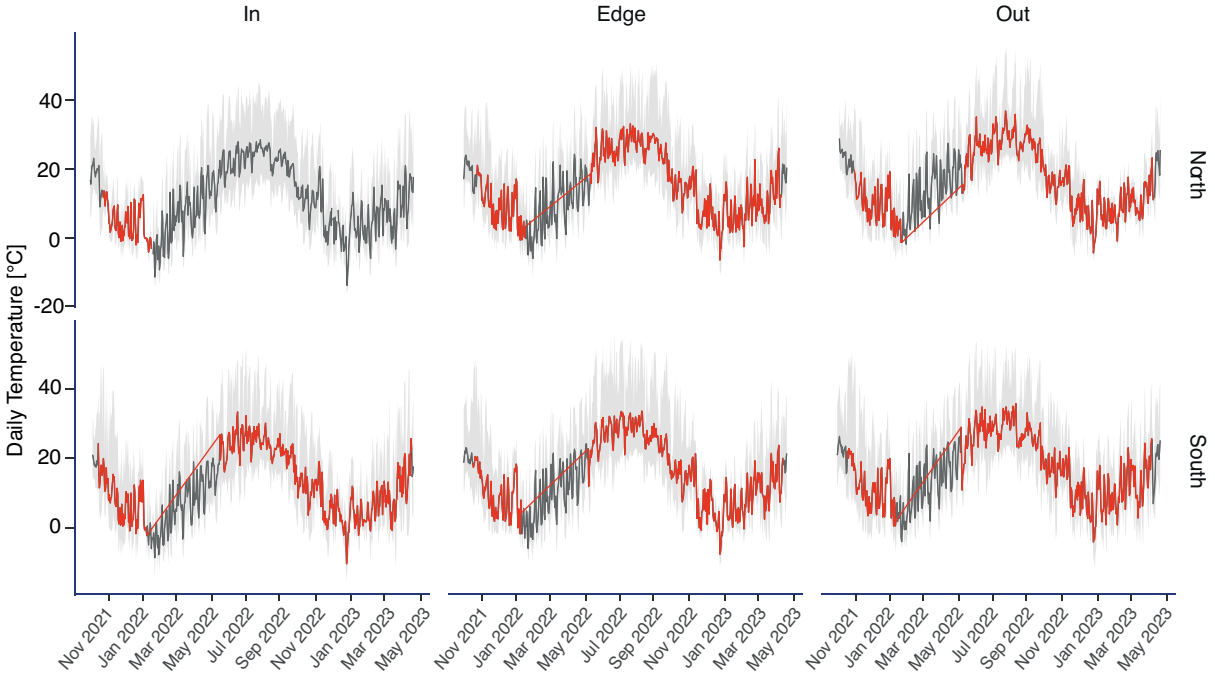

Fig. S2: Prediction of temperature at plant level. Measured temperatures at each common garden site (red line) and predicted temperatures based on data of the nearest weather station (dark grey line) with maximum and minimum temperatures (light grey shaded area) at daily resolution.

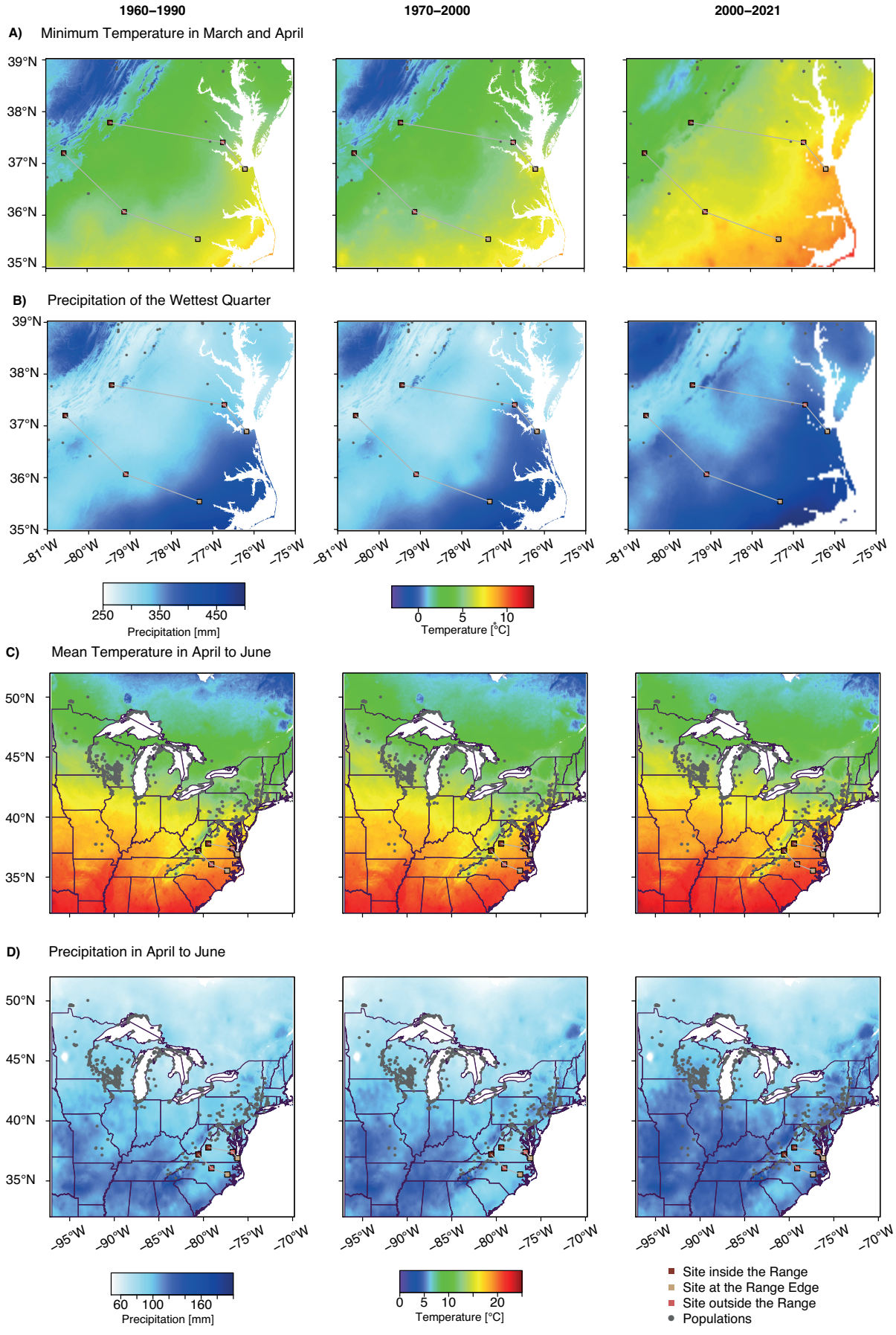

Fig. S3: Climate across the species distribution of *Arabidopsis lyrata* across different decades

In all temperature plots darker reds indicate warmer temperatures and darker blues indicate colder temperatures, while in precipitation plots lighter blues indicate less precipitation and darker blues more. A and B are zoomed into the area of the experiment, while C and D include the whole range of *A. lyrata*. Coloured squares indicate experimental sites and grey circles known populations. A: Minimum temperature in early spring (March & April); B: Precipitation of the wettest quarter; C: Precipitation in March-May and April-June; D: Average temperature in March-May and April-June

Chapter 2

Table S1: Climate averages in the last 60 years at the locations of the five populations of *Arabidopsis lyrata* studied.

	Average April-June			Average June-August			Yearly
Population and period	Tmean [°C]	Tmax [°C]	P [mm]	Tmean [°C]	Tmax [°C]	P [mm]	Pmin [mm]
NW							
1960-1990	9.9	15.8	62	17.8	23.6	85	18
1970-2000	9.7	15.9	65	17.5	23.3	92	16
2000-2018	9.5	15.5	78	17.9	23.6	96	13
NE							
1960-1990	12.5	19	87	19.2	25.7	90	52
1970-2000	12.6	18.7	88	19.2	25.3	92	55
2000-2018	12.9	18.9	99	20	25.4	109	43
C							
1960-1990	13.4	19.2	82	20.3	25.9	85	38
1970-2000	13.1	18.8	80	20.1	25.8	84	34
2000-2018	13.7	19.2	91	21.2	26	84	30
SW							
1960-1990	18.1	25.3	106	24.1	31.1	94	49
1970-2000	18	24.8	108	24.3	30.7	95	50
2000-2018	18.9	25.3	116	25.2	30.8	100	26
SE							
1960-1990	18.6	25.5	96	24	30.4	104	77
1970-2000	18.5	25.4	98	24.1	30.4	107	80
2000-2018	19.1	25.8	105	24.6	30.4	120	35
Mean SW and SE							
1960-1990	18.4	25.4	106	24.1	30.7	99	63
1970-2000	**18.3**	**25.1**	108	24.2	**30.5**	**101**	**65**
2000-2018	18.9	25.6	116	24.9	30.6	110	30
Overall mean							
1960-1990	15.1	21.7	87	17.6	22.8	76.3	39
1970-2000	15	21.5	88	17.5	22.6	78.3	39.2
2000-2018	15.5	21.7	98	18.2	22.7	84.9	24.5
Difference 1960-1990 to 2000-2018	0.4	0	11	0.6	-0.1	8.6	-14.5

Climate data were downloaded from WorldClim 1.4 and 2.1.

Table S2: Origin of the *A. lyrata* populations included in this study

Population	State of origin	Latitude, N [°]	Longitude, W [°]	Year of seed collection
NW	Ontario, Canada	49.65	94.92	2011
NE	New York, USA	42.35	76.39	2011
C	Michigan, USA	42.7	89.19	2007-2014
SW	Missouri, USA	37.72	92.05	2011
SE	North Carolina, USA	36.41	79.96	2011

Table S3: Overview of means, standard deviations, and number of observations per population, treatment, and trait

	N (germination)	N (survival)	Survival	Longevity [days]	N (flowering)	Flowering time [days]	Flowering	N (x_{mid})	Xmid [days]	N (size)	Size [mm^2]	N (growth rate)	Growth rate	N (SLA)	SLA [mm^2 mg^{-1}]	N (LDMC)	LDMC [mg g^{-1}]	N (root:shoot)	Root:shoot ratio
Total	2310	1479	0.64	149+-48	297	132+-30	0.37	2287	25+-429	2297	1142+-0	2287	0.23+-0.19	1403	21+-61	1405	280+-89	1456	0.42+-7.09
Control																			
NW	8	7	0.88	175+-19	6	129+-18	0.75	8	32+-5.1	8	1390+-372	8	0.19+-0.05	5	25+-51	5	323+-59	6	0.27+-0.1
NE	9	9	1	189+-1	0		0	9	25+-0.57	9	1797+-108	9	0.15+-0.01	9	7+-1	9	246+-4	9	0.46+-0.03
C	546	519	0.95	186+-18	208	134+-13	0.38	541	27+-4	543	1436+-165	541	0.21+-0.14	467	25+-62	468	308+-65	506	0.33+-0.08
SW	7	7	1	186+-0.7	4	109	0.57	7	24+-4.2	7	841+-48	7	0.28+-0.12	6	87+-3	6	272+-11	7	0.44+-0.04
SE	7	7	1	189+-0.5	6	129+-25	0.86	7	28+-1.3	7	1767+-140	7	0.34+-0.54	6	345+-105	6	329+-52	7	0.41+-0.07
Heat																			
NW	8	7	0.88	152+-3.6	0		0	8	29+-1.5	8	1171+-60	8	0.2+-0.06	7	26+-9	7	286+-143	7	0.38+-0.05
NE	9	8	0.89	157+-0.24	0		0	9	25+-1.5	9	1610+-98	9	0.19+-0.04	8	14+-1	8	261+-30	8	0.51+-0.05
C	548	434	0.79	152+-17	5	123+-14	0.01	539	26+-2.4	542	1234+-114	539	0.24+-0.13	431	15+-18	432	274+-62	434	0.47+-0.06
SW	8	7	0.88	153+-0.9	0		0	8	23+-3.7	8	853+-129	8	0.3+-0.05	7	21+-8	7	258+-23	7	0.79+-0.31
SE	9	9	1	157+-0.5	0		0	9	26+-0.86	9	1336+-161	9	0.34+-0.42	9	17+-6	9	243+-37	9	0.68+-0.09
Drought																			
NW	7	6	0.86	186+-0.8	3	136	0.43	7	24+-0.22	7	1008+-15	7	0.2+-0.01	6	16+-22	6	248+-46	6	0.48+-0.13
NE	9	8	0.89	178+-30	0		0	9	25+-0.32	9	1351+-46	9	0.14+-0.01	8	7+-1	8	227+-13	8	0.43+-0.11
C	542	409	0.75	167+-24	62	149+-13	0.11	540	25+-2.2	540	1075+-73	540	0.19+-0.11	392	18+-63	392	268+-45	400	0.43+-0.13
SW	9	9	1	185+-0.6	0		0	9	24+-1	9	817+-136	9	0.34+-0.27	9	10+-5	9	238+-32	9	0.74+-0.08
SE	9	7	0.78	177+-29	3	146	0.33	9	28+-4.7	9	1177+-176	9	0.21+-0.11	7	30+-79	7	242+-7	7	0.53+-0.02
Heat+Drought																			
NW	6	0	0	93+-19	0		0	6	23+-3.5	6	829+-95	6	0.31+-0.18	0		0		0	
NE	9	0	0	65+-2.9	0		0	8	19+-2.3	9	881+-162	8	0.51+-0.02	0		0		0	
C	545	23	0.04	89+-10	0		0	539	22+-2.8	543	811+-134	539	0.3+-0.13	23	46+-2	23	251+-5	23	0.59+-0.5
SW	7	1	0.14	132+-4.5	0		0	7	21+-1.9	7	600+-49	7	0.29+-0.23	1	149	1	363	1	2.35
SE	8	2	0.25	97+-19	0		0	8	22+-2.4	8	1020+-152	8	0.31+-0.26	2	121	2	216	2	0.51

Sample sizes are written to the left of the traits.

Table S4: Model selection for testing the effect of heat and drought on performance and leaf/ root functional traits of Arabidopsis lyrata, with models differing in the random-effects part.

Model	a	b	c
Variable	AIC	AIC	AIC
Survival	1677	1682	1659
Longevity	-1670	-1658	-1621
Flowering	1026	1030	1019
Size	32830	32852	32883
Xmid	15097	15110	15119
Growth rate	-6231	-6224	-6243
SLA	1582	1601	1608
LDMC	-2115	-2120	-2127
Root:shoot	-4907	-4920	-4929

Model a. y~temperature*watering + (1 + temperature + watering|population) + (1 + temperature + watering|population:family).
Model b. y~temperature*watering + (1|population) + (0 + temperature|population) + (0 + watering |population) + (1|population:family) + (0 + temperature|population:family) + (0 + watering|population:family).
Model c. y~temperature*watering + (1|population) + (1|population:family).

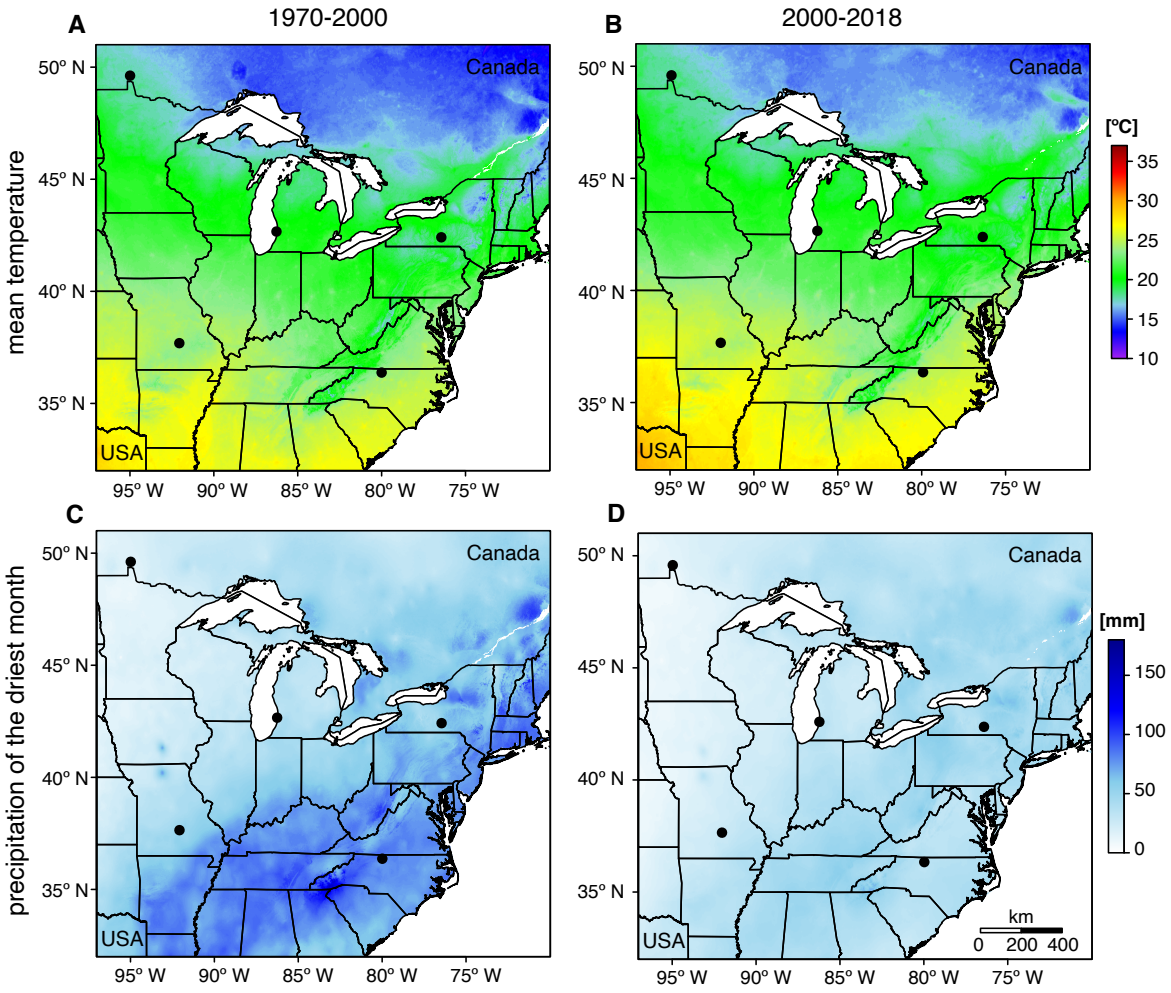

Fig. S1: Maps representing climate of different periods of the recent past in the area of distribution of North American *Arabidopsis lyrata*. A) Mean temperature averaged for April, May, and June in the time period of 1970-2000 and B) mean temperature averaged for June, July, and August in the time period of 2000-2018 in ºC (scale of colours on the right). C) Precipitation during the driest month in the time period of 1970-2000 and D) of 2000-2018 in mm. Black dots indicate the location of populations used in this experiment. The border between the US and Canada, as well as US state borders are indicated by black lines.

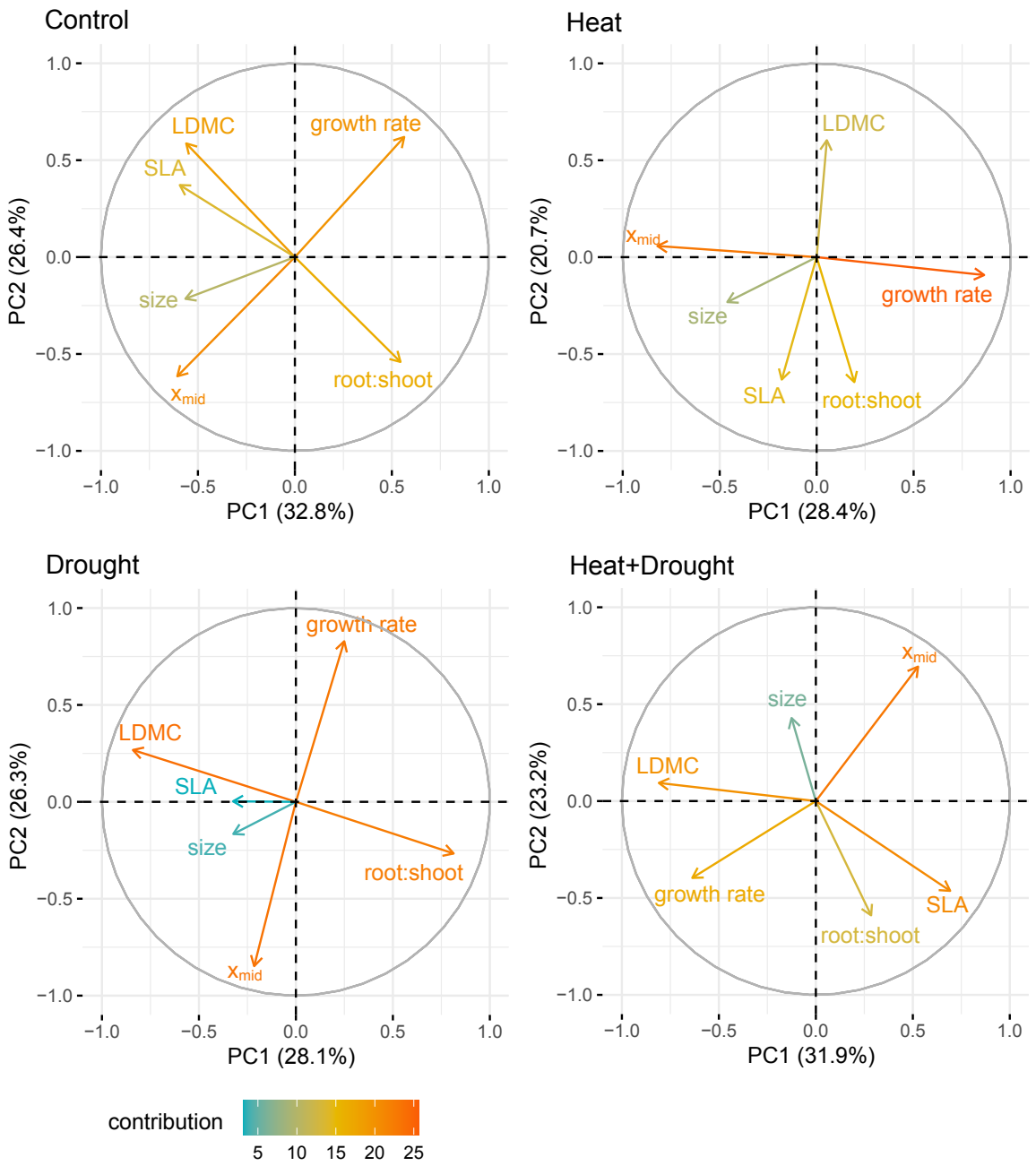

Fig. S2: Principial component analysis on plant growth, leaf and root traits of a diverse *Arabidopsis lyrata* central population for four temperature and watering treatments - Control, Heat, Drought, and Heat+Drought. Arrow length and color represent the contribution of each trait to the first two principal component axes (with the amount of phenotypic variation they depict written in parenthesis).

Table S1: Seed family means of measured plant traits under control (C) and drought (D) treatments: rosette size [mm^2], total biomass [g], time of fastest growth [day], growth rate, SLA (specific leaf area) [mg/ mm^2], LDMC (leaf dry matter content) [mg/ g], root:shoot, fraction of plants surviving and fraction of plants flowering.

Seed Family	Rosette Size		Biomass		Time To Fastest Growth		Growth Rate		SLA		LDMC		Root:Shoot		Root Biomass		Rosette Biomass		Fraction Survival		Fraction Flowering	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
20	1567	1025	1.13	0.53	21	22	0.17	0.17	4.5	8.2	326	256	1.24	0.71	0.63	0.18	0.35	0.17	1	0.8	1	0.25
85	1175	907	1.28	0.59	30	25	0.09	0.12	2.8	3.7	270	341	1.12	0.83	0.66	0.28	0.46	0.25	1	0.6	0.6	0
120	1501	1318	0.86	0.86	30	28	0.12	0.10	13.4	6.5	339	315	1.02	1.41	0.44	0.51	0.19	0.18	0.8	0.6	0.5	0.33
179	1570	1321	1.14	0.40	20	23	0.38	0.21	12.5	8.5	254	241	1.89	0.92	0.75	0.18	0.21	0.16	0.8	1	0.25	0
206	2100	1338	1.56	0.59	24	25	0.19	0.14	7.0	7.2	323	362	1.78	1.04	0.99	0.31	0.32	0.19	1	0.6	0.2	0
212	2199	1334	1.21	0.68	31	26	0.10	0.17	3.8	5.9	267	268	0.68	0.87	0.5	0.3	0.56	0.21	0.8	0.8	0.25	0.25
260	1414	1099	1.12	0.76	26	28	0.18	0.16	2.8	5.0	297	227	0.89	1.03	0.53	0.39	0.48	0.26	0.8	0.8	0	0
325	1554	1160	1.16	0.93	25	28	0.15	0.13	2.9	5.7	346	385	0.81	1.36	0.51	0.54	0.53	0.24	1	0.4	0	0.5
335	1362	1207	1.09	0.54	21	25	0.39	0.19	3.7	10.0	284	264	1.11	0.94	0.57	0.27	0.42	0.18	0.8	0.6	0	0
384	1580	1197	0.94	0.63	23	24	0.21	0.13	5.6	5.8	275	346	0.93	0.79	0.45	0.3	0.36	0.23	1	0.6	0.6	0.66
Sample Size	50	50	45	36	50	50	50	50	43	35	43	35	43	35	45	36	45	36	50	50	50	47

Table S2: Retention index of root exudate compounds the study focused on, including their compound group that was used for analysis.

Retention Index	Compound	Group
1074	Alanin	Amino Acid
1187	Valin	Amino Acid
1538	Prolin	Amino Acid
1225	Benzoic Acid	Organic Acid
1020	Propionic Acid	Fatty Acid/ Organic Acid
1287	Succinic Acid	Fatty Acid/ Organic Acid
1659	Dodecanoic Acid	Fatty Acid/ Organic Acid
1885	Tetradecanoic Acid	Fatty Acid/ Organic Acid
2100	Hexadecanoic Acid	Fatty Acid/ Organic Acid
2301	Octadecanoic Acid	Fatty Acid/ Organic Acid
1788	Ribose	Sugar
1983	Glucose	Sugar
2191	Myoinositol	Sugar
2705	Sucrose	Sugar
3624	Glucopyranose	Sugar

Table S3: Effect of drought and plant seed family and their interaction on exudate compounds. F-ratios, estimates as well as P-values and coefficients for treatment in parenthesis, are reported. Significant P-values (P<0.05) are indicated in bold.

Group	Treatment	Family	Treatment:Family
Sugars			
Glucopyranose	1.47, -3.82 (0.236)	1.33 (0.266)	0.65 (0.748)
Glucose	2.77, -0.09 (0.102)	1.02 (0.435)	0.81 (0.607)
Myoinositol	0.99, -0.27 (0.325)	1.24 (0.291)	0.98 (0.468)
Ribose	0.26, 0.12 (0.613)	0.62 (0.777)	1.42 (0.204)
Sucrose	0.23, -2.71 (0.633)	1.07 (0.406)	0.64 (0.738)
Organic Acids			
Benzoic Acid	0.27, 0.20 (0.609)	0.96 (0.479)	0.38 (0.939)
Dodecanoic Acid	**5.01, -0.72 (0.03)**	0.87 (0.555)	0.64 (0.76)
Tetradecanoic Acid	2.47, -0.09 (0.122)	0.79 (0.624)	0.98 (0.47)
Hexadecanoic Acid	0.93, -0.02 (0.34)	0.96 (0.481)	1.01 (0.445)
Octadecanoic Acid	0.74, 0.01 (0.393)	1.05 (0.415)	1.09 (0.387)
Propionic Acid	0.85, -0.27 (0.361)	0.47 (0.887)	0.99 (0.461)
Succinic Acid	1.65,0.04 (0.205)	0.86 (0.563)	0.69 (0.71)
Amino Acids			
Alanin	0.85, -0.06 (0.361)	0.84 (0.582)	0.55 (0.826)
Valin	1.63, -0.25 (0.208)	0.61 (0.782)	0.35 (0.952)
Prolin	4.41, -0.43 (0.041)	0.78 (0.636)	0.5 (0.865)

Table S4: Relative concentration of root exudate compound groups measured under control and drought conditions.

Group	**Sum**		**Mean ± Standard Deviation**		**Percentage**	
	Control	Drought	Control	Drought	Control	Drought
Sugars	610	318	3.32 ± 6.18	2.24 ± 3.57	66.4	66.4
Organic Acids	49	32	1.20 ± 1.65	1.03 ± 1.79	5.3	6.7
Fatty Acids	217	115	0.89 ± 1.26	0.62 ± 0.68	23.6	24.0
Amino Acids	43	14	0.38 ± 0.57	0.19 ± 0.20	4.7	2.9

Table S5: ASV taxonomies for bacteria and fungi and their abundances under control and drought conditions. The mean with standard deviation as well as the percentages are given.

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	% Under Drought
Bacteria								
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter	0.09 ± 0.08	0.05 ± 0.06	0.001	0.001
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	PAUC26f	0.02 ± 0.02	0.01 ± 0.02	0.000	0.000
Acidobacteria	Blastocatellia_(Subgroup_4)	Blastocatellales	Blastocatellaceae	Aridibacter	0.07 ± 0.17	0.03 ± 0.04	0.001	0.000
Acidobacteria	Blastocatellia_(Subgroup_4)	Blastocatellales	Blastocatellaceae	Blastocatella	0.02 ± 0.04	0.02 ± 0.07	0.000	0.000
Acidobacteria	Blastocatellia_(Subgroup_4)	Blastocatellales	Blastocatellaceae	JGI_0001001-H03	0.03 ± 0.03	0.02 ± 0.02	0.000	0.000
Acidobacteria	Subgroup_6	Unknown_Order	Unknown_Family	Luteitalea	0.01 ± 0.01	0.02 ± 0.03	0.000	0.000
Acidobacteria	Subgroup_6	Unknown_Order	Unknown_Family	Vicinamibacter	0.11 ± 0.1	0.14 ± 0.1	0.001	0.002
Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup_10	0.05 ± 0.04	0.07 ± 0.05	0.001	0.001
Acidobacteriota	Blastocatellia	Blastocatellales	Blastocatellaceae	JGI 0001001-H03	0.29 ± 0.25	0.31 ± 0.17	0.004	0.004
Acidobacteriota	Blastocatellia	Blastocatellales	Blastocatellaceae	Stenotrophobacter	0.73 ± 0.52	0.86 ± 0.42	0.010	0.012
Acidobacteriota	Vicinamibacteria	Vicinamibacterales	Vicinamibacteraceae	Luteitalea	0.23 ± 0.1	0.3 ± 0.11	0.003	0.004
Actinobacteria	Acidimicrobiia	Microtrichales	Iamiaceae	Iamia	0.43 ± 0.25	0.71 ± 0.32	0.006	0.010
Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29_marine_group	0.02 ± 0.03	0.01 ± 0.02	0.000	0.000
Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	Ilumatobacter	0.23 ± 0.1	0.24 ± 0.1	0.003	0.003
Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	Sva0996_marine_group	0.02 ± 0.03	0.02 ± 0.05	0.000	0.000
Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	0.53 ± 0.37	0.89 ± 0.48	0.007	0.012
Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia	0.05 ± 0.07	0.05 ± 0.03	0.001	0.001
Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	0.02 ± 0.02	0.02 ± 0.03	0.000	0.000
Actinobacteria	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	0.08 ± 0.09	0.1 ± 0.05	0.001	0.001
Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Blastococcus	0.52 ± 0.26	0.61 ± 0.26	0.007	0.008
Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Geodermatophilus	0.06 ± 0.11	0.09 ± 0.1	0.001	0.001
Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Modestobacter	0.72 ± 0.61	0.9 ± 0.45	0.010	0.012
Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	Nakamurella	0.17 ± 0.32	0.14 ± 0.47	0.002	0.002
Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Sporichthya	0.02 ± 0.02	0.03 ± 0.02	0.000	0.000

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	% Under Drought
Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineosporia	0.01 ± 0.02	0.02 ± 0.04	0.000	0.000
Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Quadrisphaera	0.05 ± 0.05	0.07 ± 0.05	0.001	0.001
Actinobacteria	Actinobacteria	Micrococcales	Bogoriellaceae	Georgenia	0.02 ± 0.04	0.04 ± 0.05	0.000	0.001
Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Actinotalea	0.15 ± 0.22	0.13 ± 0.12	0.002	0.002
Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	0.11 ± 0.06	0.09 ± 0.04	0.002	0.001
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Janibacter	0.24 ± 0.29	0.19 ± 0.17	0.003	0.003
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Knoellia	0.04 ± 0.07	0.04 ± 0.07	0.001	0.000
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Lapillicoccus	0.04 ± 0.04	0.06 ± 0.05	0.001	0.001
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Ornithinimicrobium	0.01 ± 0.02	0.02 ± 0.03	0.000	0.000
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus	0.1 ± 0.08	0.12 ± 0.13	0.001	0.002
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	0.02 ± 0.04	0.02 ± 0.03	0.000	0.000
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Lysinimonas	0.02 ± 0.03	0.03 ± 0.04	0.000	0.000
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Yonghaparkia	0.03 ± 0.03	0.04 ± 0.03	0.000	0.000
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	0.53 ± 0.6	0.47 ± 0.29	0.007	0.006
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Kocuria	0.38 ± 0.25	0.37 ± 0.2	0.005	0.005
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	1.45 ± 1.34	1.6 ± 1.23	0.020	0.021
Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	Promicromonospora	0.02 ± 0.07	0.04 ± 0.08	0.000	0.001
Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Actinoplanes	0.03 ± 0.12	0.02 ± 0.08	0.000	0.000
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium	0.06 ± 0.08	0.16 ± 0.13	0.001	0.002
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Kribbella	0.19 ± 0.13	0.27 ± 0.14	0.003	0.004
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola	0.73 ± 0.27	0.62 ± 0.2	0.010	0.008
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	3.23 ± 1.01	3.77 ± 0.94	0.044	0.051
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinokineospora	0.05 ± 0.09	0.17 ± 0.2	0.001	0.002
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinophytocola	0.02 ± 0.04	0.04 ± 0.04	0.000	0.001
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Alloactinosynnema	0.06 ± 0.04	0.08 ± 0.05	0.001	0.001
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Amycolatopsis	0.01 ± 0.02	0.02 ± 0.06	0.000	0.000
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Lechevalieria	1.65 ± 2.14	2.97 ± 2.35	0.022	0.040
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	0.35 ± 0.18	0.46 ± 0.34	0.005	0.006
Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	0.09 ± 0.08	0.16 ± 0.1	0.001	0.002

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	% Under Drought
Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Nocardiopsis	0 ± 0.01	0.03 ± 0.11	0.000	0.000
Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Actinomadura	0.01 ± 0.02	0.02 ± 0.02	0.000	0.000
Actinobacteria	Nitriliruptoria	Euzebyales	Euzebyaceae	Euzebya	0.04 ± 0.05	0.05 ± 0.06	0.001	0.001
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella	0.58 ± 0.22	0.77 ± 0.22	0.008	0.010
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter	2.94 ± 1.08	3.12 ± 1.14	0.040	0.042
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Parviterribacter	0.09 ± 0.09	0.08 ± 0.08	0.001	0.001
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	0.12 ± 0.06	0.17 ± 0.2	0.002	0.002
Actinobacteriota	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella	0.07 ± 0.04	0.1 ± 0.04	0.001	0.001
Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter	0.01 ± 0.03	0.01 ± 0.02	0.000	0.000
Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonas	0.06 ± 0.04	0.05 ± 0.04	0.001	0.001
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter	0.02 ± 0.03	0.02 ± 0.02	0.000	0.000
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavihumibacter	0.03 ± 0.03	0.04 ± 0.03	0.000	0.001
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavisolibacter	0.01 ± 0.02	0.02 ± 0.02	0.000	0.000
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavitalea	0.33 ± 0.27	0.45 ± 0.21	0.005	0.006
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Lacibacter	0.01 ± 0.02	0.01 ± 0.03	0.000	0.000
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Niastella	0.07 ± 0.16	0.03 ± 0.04	0.001	0.000
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas	0.1 ± 0.09	0.12 ± 0.07	0.001	0.002
Bacteroidetes	Bacteroidia	Cytophagales	Cytophagaceae	Rhodocytophaga	0.04 ± 0.06	0.03 ± 0.05	0.001	0.000
Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	Chryseolinea	0.01 ± 0.02	0.01 ± 0.01	0.000	0.000
Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	Ohtaekwangia	0.05 ± 0.05	0.09 ± 0.09	0.001	0.001
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Dyadobacter	0.02 ± 0.03	0.04 ± 0.04	0.000	0.001
Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	Chryseolinea	0.01 ± 0.02	0.02 ± 0.03	0.000	0.000
Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	Ohtaekwangia	0.03 ± 0.03	0.03 ± 0.04	0.000	0.000
Chloroflexi	Anaerolineae	SBR1031	A4b	OLB13	0.03 ± 0.04	0.03 ± 0.03	0.000	0.000
Chloroflexi	Chloroflexia	Chloroflexales	Chloroflexaceae	Chloronema	0.03 ± 0.08	0.11 ± 0.48	0.000	0.001
Chloroflexi	Chloroflexia	Chloroflexales	Herpetosiphonaceae	Herpetosiphon	0.24 ± 0.38	0.33 ± 0.33	0.003	0.004
Deinococcus-Thermus	Deinococci	Deinococcales	Trueperaceae	Truepera	0.36 ± 0.27	0.49 ± 0.31	0.005	0.007
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.06 ± 0.15	0.07 ± 0.18	0.001	0.001

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	% Under Drought
Firmicutes	Bacilli	Bacillales	Family_XII	Exiguobacterium	0.16 ± 0.39	0.07 ± 0.1	0.002	0.001
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0.01 ± 0.01	0.01 ± 0.01	0.000	0.000
Firmicutes	Bacilli	Bacillales	Planococcaceae	Domibacillus	0.02 ± 0.07	0.01 ± 0.04	0.000	0.000
Firmicutes	Bacilli	Bacillales	Planococcaceae	Paenisporosarcina	0.02 ± 0.06	0.02 ± 0.03	0.000	0.000
Firmicutes	Bacilli	Bacillales	Planococcaceae	Planomicrobium	0.06 ± 0.08	0.07 ± 0.06	0.001	0.001
Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	Baia	0.01 ± 0.02	0.02 ± 0.03	0.000	0.000
Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	Geothermomicrobium	0 ± 0.02	0.02 ± 0.06	0.000	0.000
Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.28 ± 1.18	0.02 ± 0.07	0.004	0.000
Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_13	0.03 ± 0.07	0.01 ± 0.01	0.000	0.000
Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_3	0.04 ± 0.13	0.02 ± 0.03	0.001	0.000
Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_8	0.02 ± 0.06	0 ± 0.01	0.000	0.000
Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_9	0.02 ± 0.06	0.02 ± 0.08	0.000	0.000
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Desulfosporosinus	0.02 ± 0.08	0.01 ± 0.02	0.000	0.000
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Pelosinus	0.49 ± 0.92	0.1 ± 0.19	0.007	0.001
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Sporomusa	0.02 ± 0.07	0 ± 0	0.000	0.000
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	0.01 ± 0.02	0.02 ± 0.03	0.000	0.000
Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	Longimicrobium	0.03 ± 0.12	0.04 ± 0.1	0.000	0.000
Gemmatimonadota	Longimicrobia	Longimicrobiales	Longimicrobiaceae	Longimicrobium	0.01 ± 0.05	0.02 ± 0.07	0.000	0.000
Gemmatimonadota	Longimicrobia	Longimicrobiales	Longimicrobiaceae	YC-ZSS-LKJ147	0.07 ± 0.14	0.07 ± 0.09	0.001	0.001
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Fimbriiglobus	0.97 ± 0.37	0.66 ± 0.28	0.013	0.009
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Gemmata	0.66 ± 0.32	0.71 ± 0.33	0.009	0.009
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Telmatocola	0.02 ± 0.02	0.03 ± 0.03	0.000	0.000
Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	Paludisphaera	0.02 ± 0.02	0.01 ± 0.01	0.000	0.000
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Blastopirellula	0.12 ± 0.09	0.09 ± 0.07	0.002	0.001
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pir4_lineage	1.17 ± 0.39	1.2 ± 0.47	0.016	0.016
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula	1.76 ± 0.58	1.54 ± 0.68	0.024	0.021

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Rhodopirellula	0.04 ± 0.04	0.09 ± 0.08	0.000	0.001
Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	Planctomicrobium	0.16 ± 0.1	0.15 ± 0.13	0.002	0.002
Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	SH-PL14	0.68 ± 0.23	0.73 ± 0.28	0.009	0.010
Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae	Fimbriiglobus	0.01 ± 0.04	0.01 ± 0.03	0.000	0.000
Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae	Zavarzinella	0.01 ± 0.02	0.01 ± 0.01	0.000	0.000
Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Pir4 lineage	0.07 ± 0.05	0.07 ± 0.04	0.001	0.001
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas	0.07 ± 0.06	0.05 ± 0.05	0.001	0.001
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacterales_Incertae_Sedis	Zavarzinia	0.04 ± 0.06	0.01 ± 0.01	0.001	0.000
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Azospirillum	0.57 ± 1.46	1.56 ± 4.24	0.008	0.021
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella	0.14 ± 0.15	0.06 ± 0.09	0.002	0.001
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillales_Incertae_Sedis	Stella	0.04 ± 0.04	0.02 ± 0.03	0.001	0.000
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	0.22 ± 0.13	0.3 ± 0.21	0.003	0.004
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	0.23 ± 0.12	0.28 ± 0.17	0.003	0.004
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0.17 ± 0.1	0.15 ± 0.07	0.002	0.002
Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Hirschia	0.07 ± 0.05	0.06 ± 0.06	0.001	0.001
Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	SWB02	0.06 ± 0.05	0.07 ± 0.09	0.001	0.001
Proteobacteria	Alphaproteobacteria	Dongiales	Dongiaceae	Dongia	0.4 ± 0.27	0.27 ± 0.21	0.006	0.004
Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	Micropepsis	0.32 ± 0.11	0.25 ± 0.06	0.004	0.003
Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	0.25 ± 0.12	0.28 ± 0.31	0.003	0.004
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	alphaI_cluster	0.03 ± 0.03	0.05 ± 0.03	0.000	0.001
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	0.35 ± 0.16	0.4 ± 0.19	0.005	0.005
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Chelatococcus	0.01 ± 0.01	0.02 ± 0.03	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	0.02 ± 0.03	0.02 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	0.05 ± 0.06	0.06 ± 0.05	0.001	0.001
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Neo-b11	0.01 ± 0.02	0.02 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Psychroglaciecola	0.01 ± 0.02	0.01 ± 0.03	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Arsenicitalea	0.03 ± 0.04	0.02 ± 0.03	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia	1.95 ± 0.54	1.86 ± 0.48	0.027	0.025
Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Maritalea	0.12 ± 0.17	0.04 ± 0.07	0.002	0.001

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	% Under Drought
Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Pelagibacterium	0.11 ± 0.12	0.16 ± 0.17	0.001	0.002
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	1.79 ± 0.4	1.58 ± 0.41	0.024	0.021
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium	1.27 ± 0.47	1 ± 0.37	0.017	0.013
Proteobacteria	Alphaproteobacteria	Rhizobiales	Pleomorphomonadaceae	Chthonobacter	0.18 ± 0.16	0.12 ± 0.16	0.002	0.002
Proteobacteria	Alphaproteobacteria	Rhizobiales	Pleomorphomonadaceae	Prosthecomicrobium	0.28 ± 0.2	0.14 ± 0.08	0.004	0.002
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aliihoeflea	0.4 ± 0.17	0.5 ± 0.31	0.005	0.007
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.78 ± 0.4	0.86 ± 0.59	0.011	0.011
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aminobacter	0.63 ± 0.23	0.42 ± 0.18	0.009	0.006
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aurantimonas	0.03 ± 0.05	0.03 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aureimonas	0.05 ± 0.07	0.06 ± 0.05	0.001	0.001
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Brucella	0.04 ± 0.1	0.06 ± 0.11	0.000	0.001
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ensifer	0.3 ± 0.59	0.15 ± 0.2	0.004	0.002
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	1.67 ± 0.56	1.71 ± 0.48	0.023	0.023
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Neorhizobium	0.37 ± 0.47	0.56 ± 0.53	0.005	0.007
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Nitratireductor	0.07 ± 0.1	0.02 ± 0.03	0.001	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Pseudaminobacter	0.19 ± 0.12	0.15 ± 0.13	0.003	0.002
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Shinella	0.05 ± 0.11	0.03 ± 0.06	0.001	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Alsobacter	0.01 ± 0.02	0.02 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Bauldia	3.07 ± 0.93	2.67 ± 0.69	0.042	0.036
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Nordella	0.58 ± 0.18	0.48 ± 0.22	0.008	0.006
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Phreatobacter	0.04 ± 0.02	0.04 ± 0.04	0.001	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Afipia	0.02 ± 0.02	0.02 ± 0.01	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	0.45 ± 1.26	0.21 ± 0.16	0.006	0.003
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys	2.78 ± 0.82	2.96 ± 0.8	0.038	0.040
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudorhodoplanes	1.27 ± 0.37	1.2 ± 0.43	0.017	0.016
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudoxanthobacter	0.02 ± 0.02	0.02 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Rhodoplanes	1.09 ± 0.32	1 ± 0.25	0.015	0.013
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Rhodopseudomonas	0.07 ± 0.05	0.06 ± 0.04	0.001	0.001

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	Phylum
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amaricoccus	0.34 ± 0.38	0.18 ± 0.33	0.005	0.002
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Defluviimonas	0.01 ± 0.04	0.02 ± 0.06	0.000	0.000
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Gemmobacter	0.12 ± 0.43	0.07 ± 0.18	0.002	0.001
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	0.91 ± 0.64	0.97 ± 0.81	0.012	0.013
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudorhodobacter	0.13 ± 0.09	0.08 ± 0.07	0.002	0.001
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	1.07 ± 0.69	0.54 ± 0.5	0.015	0.007
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rubellimicrobium	0.44 ± 0.34	0.52 ± 0.37	0.006	0.007
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Tabrizicola	0.65 ± 0.46	0.3 ± 0.36	0.009	0.004
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodopirillaceae	Defluviicoccus	0.03 ± 0.03	0.03 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Sneathiellales	Sneathiellaceae	Ferrovibrio	0.02 ± 0.02	0.01 ± 0.01	0.000	0.000
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Altererythrobacter	2.44 ± 0.77	2.71 ± 0.94	0.033	0.036
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Ellin6055	0.46 ± 0.26	0.67 ± 0.38	0.006	0.009
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Erythrobacter	0.01 ± 0.01	0.02 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	0.25 ± 0.13	0.24 ± 0.11	0.003	0.003
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Plot4-2H12	0.02 ± 0.03	0.01 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Porphyrobacter	0.01 ± 0.02	0.02 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Qipengyuania	0.17 ± 0.08	0.26 ± 0.31	0.002	0.004
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	0.08 ± 0.11	0.03 ± 0.05	0.001	0.000
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	2.87 ± 2.04	4.06 ± 2	0.039	0.054
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	0.24 ± 0.21	0.12 ± 0.1	0.003	0.002
Proteobacteria	Alphaproteobacteria	Thalassobaculales	Thalassobaculaceae	Thalassobaculum	0.01 ± 0.02	0.01 ± 0.02	0.000	0.000
Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Candidatus_Alysiosphaera	0.12 ± 0.15	0.1 ± 0.14	0.002	0.001
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	Peredibacter	0.08 ± 0.05	0.09 ± 0.06	0.001	0.001
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	0.14 ± 0.08	0.17 ± 0.09	0.002	0.002
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	OM27_clade	0.1 ± 0.1	0.11 ± 0.08	0.001	0.001
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	0.03 ± 0.1	0 ± 0.01	0.000	0.000
Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	Anaeromyxobacter	0.03 ± 0.04	0.04 ± 0.03	0.000	0.001
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium	0.39 ± 0.29	0.61 ± 0.64	0.005	0.008
Proteobacteria	Deltaproteobacteria	Myxococcales	Phaselicystidaceae	Phaselicystis	0.03 ± 0.04	0.07 ± 0.11	0.000	0.001

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	Phylum
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Jahnella	0.05 ± 0.05	0.1 ± 0.06	0.001	0.001
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Pajaroellobacter	0.01 ± 0.01	0.02 ± 0.02	0.000	0.000
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Sorangium	0.03 ± 0.02	0.03 ± 0.03	0.000	0.000
Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	Sandaracinus	0.09 ± 0.09	0.15 ± 0.1	0.001	0.002
Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	Oligoflexus	0.08 ± 0.08	0.09 ± 0.1	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Aquabacterium	0.04 ± 0.04	0.06 ± 0.06	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Azohydromonas	1.16 ± 0.9	0.86 ± 0.9	0.016	0.011
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Caenimonas	0.18 ± 0.12	0.13 ± 0.09	0.002	0.002
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Hydrogenophaga	0.46 ± 0.3	0.37 ± 0.2	0.006	0.005
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Lautropia	0.14 ± 0.11	0.1 ± 0.08	0.002	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnobacter	0.01 ± 0.03	0 ± 0.01	0.000	0.000
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	0.23 ± 0.29	0.12 ± 0.16	0.003	0.002
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Methylibium	0.45 ± 0.3	0.26 ± 0.23	0.006	0.004
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Noviherbaspirillum	0.05 ± 0.06	0.04 ± 0.05	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Oxalicibacterium	0.1 ± 0.1	0.1 ± 0.09	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Piscinibacter	0.36 ± 0.32	0.2 ± 0.32	0.005	0.003
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Pseudorhodoferax	2.16 ± 1.05	2.08 ± 0.82	0.029	0.028
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Ramlibacter	0.64 ± 0.31	0.68 ± 0.3	0.009	0.009
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter	0.03 ± 0.04	0.01 ± 0.01	0.000	0.000
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhodoferax	0.05 ± 0.06	0.03 ± 0.06	0.001	0.000
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rubrivivax	0.13 ± 0.14	0.19 ± 0.35	0.002	0.002
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Xylophilus	0.11 ± 0.09	0.1 ± 0.11	0.002	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chromobacteriaceae	Vogesella	0.02 ± 0.04	0 ± 0.01	0.000	0.000
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Methylobacillus	0.05 ± 0.05	0.04 ± 0.03	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	UBA6140	0.06 ± 0.07	0.04 ± 0.06	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	DSSD61	0.11 ± 0.15	0.06 ± 0.05	0.001	0.001
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067	0.28 ± 0.17	0.4 ± 0.19	0.004	0.005
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	IS-44	0.4 ± 0.46	0.29 ± 0.48	0.006	0.004
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	mle1-7	0.06 ± 0.04	0.07 ± 0.06	0.001	0.001

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	Phylum
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1	0.46 ± 0.57	0.24 ± 0.26	0.006	0.003
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Dechloromonas	0.01 ± 0.05	0 ± 0	0.000	0.000
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Ferribacterium	0.02 ± 0.05	0 ± 0.01	0.000	0.000
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Methyloversatilis	0.21 ± 0.22	0.18 ± 0.26	0.003	0.002
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Thauera	0.91 ± 1.67	0.17 ± 0.51	0.012	0.002
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Zoogloea	0.05 ± 0.16	0.01 ± 0.03	0.001	0.000
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	Cellvibrio	0.06 ± 0.09	0.06 ± 0.07	0.001	0.001
Proteobacteria	Gammaproteobacteria	Coxiellales	Coxiellaceae	Coxiella	0.04 ± 0.08	0.02 ± 0.03	0.000	0.000
Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	Aquicella	0.12 ± 0.17	0.07 ± 0.12	0.002	0.001
Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter	0.32 ± 0.38	0.29 ± 0.21	0.004	0.004
Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Candidatus_Berkiella	0.01 ± 0.01	0.02 ± 0.02	0.000	0.000
Proteobacteria	Gammaproteobacteria	Immundisolibacterales	Immundisolibacteraceae	Immundisolibacter	0.02 ± 0.03	0.01 ± 0.02	0.000	0.000
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	0.17 ± 0.25	0.13 ± 0.17	0.002	0.002
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.05 ± 0.11	0.03 ± 0.13	0.001	0.000
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.62 ± 1.27	0.23 ± 0.28	0.009	0.003
Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Solimonas	0.01 ± 0.02	0.01 ± 0.02	0.000	0.000
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	Steroidobacter	0.02 ± 0.04	0.01 ± 0.01	0.000	0.000
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Ahniella	0.01 ± 0.02	0.04 ± 0.04	0.000	0.000
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dokdonella	0.03 ± 0.02	0.02 ± 0.02	0.000	0.000
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	0.02 ± 0.01	0.01 ± 0.01	0.000	0.000
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas	0.15 ± 0.13	0.18 ± 0.13	0.002	0.002
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	0.07 ± 0.07	0.06 ± 0.05	0.001	0.001
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	0.08 ± 0.08	0.11 ± 0.07	0.001	0.002
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	0.7 ± 0.78	0.91 ± 0.97	0.010	0.012
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Thermomonas	0.03 ± 0.08	0.04 ± 0.02	0.000	0.000
Sumerlaeota	Sumerlaeia	Sumerlaeales	Sumerlaeaceae	Sumerlaea	0.14 ± 0.11	0.21 ± 0.13	0.002	0.003
Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus_Nitrocosmicus	0.04 ± 0.03	0.06 ± 0.04	0.001	0.001
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	0.13 ± 0.16	0.14 ± 0.12	0.002	0.002

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	Phylum
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	IMCC26134	0.03 ± 0.07	0.02 ± 0.02	0.000	0.000
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Lacunisphaera	0.02 ± 0.02	0.01 ± 0.03	0.000	0.000
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Opitutus	0.05 ± 0.06	0.09 ± 0.1	0.001	0.001
Verrucomicrobia	Verrucomicrobiae	Opitutales	Puniceicoccaceae	Verruc-01	0 ± 0.01	0.03 ± 0.06	0.000	0.000
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter	0.34 ± 0.21	0.44 ± 0.36	0.005	0.006
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Roseimicrobium	0.08 ± 0.07	0.11 ± 0.11	0.001	0.002
Verrucomicrobiota	Chlamydiae	Chlamydiales	Parachlamydiaceae	Neochlamydia	0.04 ± 0.05	0.03 ± 0.06	0.001	0.000
Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Ellin517	0.01 ± 0.03	0.02 ± 0.02	0.000	0.000
Fungi								
Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	7.45 ± 11.1	13.7 ± 13.1	0.181	0.323
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Acrodontium	0.02 ± 0.06	0 ± 0.01	0.000	0.000
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	0.03 ± 0.12	0.48 ± 1.44	0.001	0.011
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Pseudopithomyces	0.52 ± 2.43	0 ± 0	0.010	0.002
Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	5.44 ± 7.00	5.36 ± 10.7	0.173	0.142
Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Stemphylium	0.01 ± 0.03	0.05 ± 0.23	0.000	0.001
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	0.04 ± 0.13	0 ± 0	0.001	0.001
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	0.77 ± 0.79	1.29 ± 1.45	0.020	0.034
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	0.46 ± 0.37	0.46 ± 0.36	0.013	0.012
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	0.07 ± 0.09	0.09 ± 0.28	0.002	0.002
Ascomycota	Eurotiomycetes	Onygenales	Onygenales_fam_Incertae_sedis	Chrysosporium	2.22 ± 1.9	2.37 ± 3.24	0.059	0.056
Ascomycota	Leotiomycetes	Erysiphales	Erysiphaceae	Erysiphe	0.01 ± 0.05	0.06 ± 0.14	0.002	0.004
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Meliniomyces	0.07 ± 0.15	0.05 ± 0.11	0.003	0.001
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Scytalidium	0.04 ± 0.06	0.03 ± 0.07	0.001	0.001
Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_Incertae_sedis	Leptodontidium	0.02 ± 0.06	0.01 ± 0.03	0.001	0.000
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	Oidiodendron	1.57 ± 1.65	1.14 ± 1.56	0.034	0.036
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Geomyces	0.11 ± 0.12	0.08 ± 0.12	0.003	0.002
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Gymnostellatospora	0.43 ± 0.47	0.43 ± 0.87	0.011	0.011
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Pseudeurotium	0.02 ± 0.05	0.11 ± 0.22	0.001	0.002
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Pseudogymnoascus	0.09 ± 0.1	0.14 ± 0.31	0.002	0.003

Phylum	Class	Order	Family	Genus	Absolut Control	Absolut Drought	% Under Control	Phylum
Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetales_fam_Incertae_sedis	Candida	0.33 ± 0.35	0.86 ± 1.45	0.008	0.020
Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetales_fam_Incertae_sedis	Nadsonia	0.03 ± 0.05	0.02 ± 0.04	0.001	0.000
Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	0.02 ± 0.05	0.01 ± 0.02	0.001	0.000
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Beauveria	0 ± 0	0.5 ± 1.64	0.000	0.009
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Lecanicillium	0 ± 0	0.37 ± 1.17	0.000	0.007
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	0.26 ± 0.32	0.2 ± 0.28	0.006	0.004
Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertae_sedis	Acremonium	2.09 ± 7.06	1.83 ± 3.3	0.061	0.049
Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertae_sedis	Emericellopsis	0.62 ± 1.43	1.03 ± 2.97	0.030	0.019
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	1.02 ± 4.36	0.34 ± 0.87	0.029	0.007
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Ilyonectria	0.03 ± 0.14	0 ± 0	0.001	0.000
Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Stachybotrys	3.87 ± 12.8	5.33 ± 10.8	0.171	0.144
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Podospora	0.04 ± 0.06	0.04 ± 0.06	0.001	0.001
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	Lepiota	0.03 ± 0.08	0.03 ± 0.06	0.001	0.001
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Gymnopilus	0.05 ± 0.12	0.01 ± 0.04	0.001	0.000
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Clitopilus	0.1 ± 0.25	0.07 ± 0.11	0.003	0.002
Basidiomycota	Agaricomycetes	Agaricomycetes_ord_Incertae_sedis	Agaricomycetes_fam_Incertae_sedis	Xenasmatella	0.02 ± 0.06	0.02 ± 0.07	0.001	0.000
Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	Ganoderma	0.01 ± 0.03	0.03 ± 0.04	0.001	0.001
Basidiomycota	Agaricostilbomycetes	Agaricostilbales	Chionosphaeraceae	Ballistosporomyces	0.04 ± 0.07	0.05 ± 0.1	0.001	0.001
Basidiomycota	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	Bannoa	0.15 ± 0.49	0.04 ± 0.07	0.004	0.001
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma	1.55 ± 2.04	1.01 ± 0.76	0.044	0.025
Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	Saitozyma	1.1 ± 1.42	0.61 ± 1.23	0.033	0.018
Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	Apiotrichum	0.1 ± 0.14	0.07 ± 0.12	0.002	0.002
Blastocladiomycota	Blastocladiomycetes	Blastocladiales	Catenariaceae	Catenaria	1.89 ± 5.07	1.58 ± 2.73	0.069	0.035
Chytridiomycota	Spizellomycetes	Spizellomycetales	Spizellomycetaceae	Gaertneriomyces	0.11 ± 0.36	0 ± 0	0.003	0.000
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	0.31 ± 0.32	0.16 ± 0.23	0.009	0.005
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	Umbelopsis	0.05 ± 0.07	0.09 ± 0.22	0.002	0.002

Table S6: Correlation of plant traits with other plant traits, root exudate groups, and rhizosphere bacteria and fungi above 0.5 under control and dry conditions. Positive interactions are red, negative blue. Bold indicates interaction above 0.7.

Trait	Treatment	Plant Traits	Exudates	Bacteria	Fungi
Size	Control	SLA		*Actinomadura*	*Coniochaeta*, *Lapillicoccus*
	Dry	-	Benzoic Acid	*Zavarzinia*, *Zoogloea*, *Longimicrobium*, *Leifsonia*, *Pseudomonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Novosphingobium*, *Aquabacterium*, *Caulobacter*, *Herpetosiphon*, *Modestobacter*, *Chelatococcus*	***Gymnostellatospora***, *Stachybotrys*, *Apiotrichum*, *Pseudeurotium*, *Talaromyces*, *Mortierella*, *Oidiodendron*
Biomass	Control	**Root Biomass**, **Rosette Biomass**	Glucose	*Defluviicoccus*, *Bosea*	-
	Dry	**SLA**, **Rosette Biomass**, **Root Biomass**	Alanin, Valin	***Brevunimonas***, ***Dongia***, *Legionella*, *Vicinamibacter*, *Methylibium*, *Hyphomicrobium*, *Nitratireductor*	-
Time to Fastest Growth	Control	**Growth Rate**	-	*Kribbella*, *Phreatobacter*, *Parviterribacter*	-
	Dry	**Growth Rate**, LDMC	-	*Aeromicrobium*, *Baia*	*Coniochaeta*
Growth Rate	Control	**Time to Fastest Growth**	-	***Brevundimonas***, *SWB02*, *Mesorhizobium*, *Rhodanobacter*, *Reyranella*, *OLB13*	*Cladosporium*
	Dry	**Time to Fastest Growth**	Succinic Acid	*Lysobacter*, *Paenisporosarcina*, *Longimicrobium*	*Xenasmatella*, *Coniochaeta*
SLA	Control	**Rosette Biomass**, Size		*Rhodobacter*, *Pseudaminobacter*, *Ramlibacter*, *Paludisphaera*, *alphaI_cluster*, *Dyadobacter*	*Stachybotrys*, *Penicillium*, *Geomyces*
	Dry	**Rosette Biomass**, **Biomass**, **Root Biomass**, LDMC	Glucopyranose, Alalin, Valin	*Nitratireductor*, *Zavarzinia*, *Dongia*, *Hyphomicrobium*, *Planctomicrobium*, *Methylibium*, *Reyranella*, *Blastopirellula*, *Rhodococcus*, *Vicinamibacter*, *Thermomonas*	*Clitopilus*, *Aspergillus*
LDMC	Control	-	-	-	-
	Dry	Time to Fastest Growth, SLA	-	***OLB13***, ***Lacibacter***, *Legionella*, *Gemmatimonas*, *Hyphomicrobium*, *Gemmata*, *Ensifer*, *Fimbriiglobus*, *Planctomicrobium*, *Chthoniobacter*, *Sandaracinus*	*Clitopilus*, *Scytalidium*
Root:Shoot	Control	**Root Biomass**, **Rosette Biomass**	-	-	-
	Dry	-	Alanin	***Sphingomonas***, ***Quipengyuania***, ***Stella***, *Phaselicystis*, *Ellin6055*, *Aeromicrobium*, *Terrimonas*, *Actinophytocola*, *Georgenia*, *Chelatococcus*, *Altererythrobacter*, *Lechevalieria*, *Arthrobacter*, *Flavisolibacter*, *Rubellimicrobium*, *Blastococcus*, *Vicinamibacter*, *Nitratireductor*, *Pedomicrobium*, *Rhodobacter*, *Lacunisphaera*, *Dongia*, *Phreatobacter*, *Hyphomicrobium*, *Methylibium*, *Maritalea*, *Psychroglaciecola*	-

Trait	Treatment	Plant Traits	Exudates	Bacteria	Fungi
Root Biomass	Control	**Root:Shoot**, **Biomass**	-	***Defluviicoccus***	-
	Dry	-	**Alanin**, Valin, Benzoic Acid	***Vicinamibacter***, ***Nitratireductor***, ***Hyphomicrobium***, ***Dongia***, *Phaselicystis*, *Devosia*, *Altererythrobacter*, *Pajaroellobacter*, *Qipengyuania*, *Aeromicrobium*, *Terrimonas*, *Ahniella*, *Sphingomonas*, *Ellin6055*, *Thermomonas*, *Brevundimonas*, *Stella*, *OM27_clade*, *Rhodobacter*, *Zoogloea*, *Maritalea*, *Sphingopyxis*, *Methylibium*, *Prosthecomicrobium*, *Amaricoccus*, *Psychroglaciecola*	-
Rosette Biomass	Control	**SLA**, Root:Shoot, Flowering	-	*Geomyces*, *IMCC26134*, *Paludisphaera*	*Chrysosporium*
	Dry	Root Biomass	Alanin, Valin, Glucopyranose	***Lengionalla***, *Thermomonas*, *Rhodococcus*, *Chthoniobacter*, *Sorangium*, *Reyranella*, *Lysinimonas*, *Hyphomicrobium*, *Dongia*, *Afipia*	*Aspergillus*
Flowering	Control	-	Benzoic Acid, Myoinositol	*Terrimonas*	-
	Dry	-	-	*Chthonobacter*, *Xylophilus*, *Desulfosporosinus*, *Gaiella*, *Coxiella*, *Subgroup_10*, *alphaI_cluster*, *Pelagibacterium*, *Acidibacter*, *Rhodanobacter*, *Chryseolinea*, *Planctomicrobium*	*Catenaria*

Table S7: Centrality measures of correlation networks in both treatments. Networks are based on correlations <-0.7 and >0.7. Strength is the sum of weight of edges to the vertex, degree is the number of edges to a vertex, betweenness is including the shortest path between two vertices and closeness is the mean inverse distance to all other vertices.

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Plant Traits								
Size	28	159	5699	4127	0	1	0	55
Biomass	33	52	10919	4306	1	5	2	73
Time to Fastest Growth	52	35	10110	6912	1	1	1	1
Growth Rate	70	33	7241	7067	1	1	1	1
SLA	158	57	8052	4945	1	4	1	67
LDMC	8	71	14101	5042	0	2	0	38
Root:Shoot	65	48	5898	3495	1	4	2	84
Root Biomass	45	100	7801	3953	2	8	3	81
Rosette Biomass	57	45	7545	6759	1	3	1	58
Flowering	48	81	5350	2978	0	1	0	1
Exudates								
Glucopyranose	22	87	12209	3605	0	1	0	66
Glucose	137	129	7175	4765	0	5	0	65
Myoinositol	107	110	8041	3690	0	8	0	73
Ribose	60	103	9225	4941	4	4	6	53
Sucrose	114	123	6680	3842	0	6	0	64
Alanin	51	95	9000	3940	0	5	0	77
Prolin	26	60	9002	7914	0	2	0	44
Valin	82	163	6160	5565	1	7	2	74
Benzoic Acid	117	140	13881	4657	2	4	3	65
Dodecanoic Acid	90	118	8177	6029	0	10	0	77
Tetradecanoic Acid	130	49	7942	6105	2	6	5	62
Hexadecanoic Acid	75	28	12616	6116	2	6	4	61
Octadecanoic Acid	65	11	5279	4920	2	5	4	61
Propionic Acid	72	38	8810	4670	3	5	6	60
Succinic Acid	165	70	5657	4316	1	5	2	64
Microorganisms								
Acidobacteria;Acidobacteriia;Solibacterales;Solibacteraceae_(Subgroup_3);Candidatus_Solibacter	215	116	5663	4674	7	3	30	52
Acidobacteria;Acidobacteriia;Solibacterales;Solibacteraceae_(Subgroup_3);PAUC26f	143	94	6261	3659	0	5	0	73
Acidobacteria;Blastocatellia_(Subgroup_4);Blastocatellales;Blastocatellaceae;Aridibacter	89	131	4923	4830	1	2	1	65
Acidobacteria;Blastocatellia_(Subgroup_4);Blastocatellales;Blastocatellaceae;Blastocatella	124	84	4772	4648	1	7	2	82

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Acidobacteria;Blastocatellia_(Subgroup_4);Blastocatellales;Blastocatellaceae;JGI_0001001-H03	18	52	6640	6039	0	1	0	1
Acidobacteria;Subgroup_6;Unknown_Order;Unknown_Family;Luteitalea	4	150	5585	6636	0	0	0	0
Acidobacteria;Subgroup_6;Unknown_Order;Unknown_Family;Vicinamibacter	293	182	7825	3931	0	8	0	81
Acidobacteria;Thermoanaerobaculia;Thermoanaerobaculales;Thermoanaerobaculaceae;Subgroup_10	123	207	4519	4257	3	8	28	94
Acidobacteriota;Blastocatellia;Blastocatellales;Blastocatellaceae;JGI 0001001-H03	351	144	3352	4647	14	11	38	98
Acidobacteriota;Blastocatellia;Blastocatellales;Blastocatellaceae;Stenotrophobacter	220	166	4468	3321	13	7	37	94
Acidobacteriota;Vicinamibacteria;Vicinamibacterales;Vicinamibacteraceae;Luteitalea	122	82	6878	4286	0	5	0	77
Actinobacteria;Acidimicrobiia;Microtrichales;Iamiaceae;Iamia	51	221	5188	3774	0	6	0	85
Actinobacteria;Acidimicrobiia;Microtrichales;Ilumatobacteraceae;CL500-29_marine_group	28	93	10741	4595	0	3	0	80
Actinobacteria;Acidimicrobiia;Microtrichales;Ilumatobacteraceae;Ilumatobacter	87	59	7533	4595	0	1	0	61
Actinobacteria;Acidimicrobiia;Microtrichales;Microtrichaceae;Sva0996_marine_group	20	16	6158	6173	0	0	0	0
Actinobacteria;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium	279	131	3734	5374	2	7	26	94
Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae;Nocardia	80	40	8626	6497	0	0	0	0
Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae;Rhodococcus	9	53	10736	5204	0	5	0	73
Actinobacteria;Actinobacteria;Frankiales;Acidothermaceae;Acidothermus	86	60	4789	10831	0	0	0	0
Actinobacteria;Actinobacteria;Frankiales;Geodermatophilaceae;Blastococcus	110	100	7094	4064	2	1	3	47
Actinobacteria;Actinobacteria;Frankiales;Geodermatophilaceae;Geodermatophilus	103	43	8536	5222	1	4	2	67
Actinobacteria;Actinobacteria;Frankiales;Geodermatophilaceae;Modestobacter	239	149	5626	3588	3	4	26	73
Actinobacteria;Actinobacteria;Frankiales;Nakamurellaceae;Nakamurella	81	77	6162	5001	0	1	0	67
Actinobacteria;Actinobacteria;Frankiales;Sporichthyaceae;Sporichthya	47	60	7520	5274	0	0	0	0
Actinobacteria;Actinobacteria;Kineosporiales;Kineosporiaceae;Kineosporia	53	67	10569	4053	0	1	0	1
Actinobacteria;Actinobacteria;Kineosporiales;Kineosporiaceae;Quadrisphaera	126	104	9646	5014	1	7	18	89
Actinobacteria;Actinobacteria;Micrococcales;Bogoriellaceae;Georgenia	26	105	6496	5002	0	2	0	54
Actinobacteria;Actinobacteria;Micrococcales;Cellulomonadaceae;Actinotalea	66	26	4540	5295	2	2	13	59
Actinobacteria;Actinobacteria;Micrococcales;Cellulomonadaceae;Cellulomonas	168	79	7215	3805	0	0	0	0
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Janibacter	173	155	10579	5763	0	5	0	79
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Knoellia	92	105	4987	4350	0	4	0	68
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Lapillicoccus	119	139	6894	4509	1	3	17	68
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Ornithinimicrobium	117	170	5596	4110	2	1	16	42
Actinobacteria;Actinobacteria;Micrococcales;Intrasporangiaceae;Phycicoccus	252	238	7396	3565	2	7	24	84
Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Leifsonia	110	208	10938	4923	1	1	13	65
Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Lysinimonas	61	128	7698	3731	0	1	0	60
Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Yonghaparkia	51	78	10821	3860	0	0	0	0
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;Arthrobacter	150	120	3631	4636	6	2	30	66
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;Kocuria	29	39	8738	4309	0	2	0	75
Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;Pseudarthrobacter	129	148	7117	5062	2	1	25	1

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Actinobacteria;Actinobacteria;Micrococcales;Promicromonosporaceae;Promicromonospora	34	123	6924	3891	0	3	0	76
Actinobacteria;Actinobacteria;Micromonosporales;Micromonosporaceae;Actinoplanes	101	57	3528	5911	2	1	3	1
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae;Aeromicrobium	110	121	5042	3469	1	3	23	66
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae;Kribbella	56	229	6667	4406	0	3	0	72
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae;Marmoricola	53	46	7106	4537	0	3	0	79
Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioidaceae;Nocardioides	57	78	6694	5852	1	3	19	62
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Actinokineospora	283	317	7200	4095	0	8	0	95
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Actinophytocola	103	147	5767	3361	0	10	0	104
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Alloactinosynnema	78	101	9408	4397	1	0	1	0
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Amycolatopsis	72	50	7004	4184	0	1	0	53
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Lechevalieria	513	228	5174	5016	10	12	34	108
Actinobacteria;Actinobacteria;Pseudonocardiales;Pseudonocardiaceae;Pseudonocardia	48	144	7151	6651	0	0	0	0
Actinobacteria;Actinobacteria;Streptomycetales;Streptomycetaceae;Streptomyces	67	49	4407	4985	0	1	0	60
Actinobacteria;Actinobacteria;Streptosporangiales;Nocardiopsaceae;Nocardiopsis	182.5	66	6522	3755	0	0	0	0
Actinobacteria;Actinobacteria;Streptosporangiales;Thermomonosporaceae;Actinomadura	50	67	15050	4648	0	1	0	42
Actinobacteria;Nitriliruptoria;Euzebyales;Euzebyaceae;Euzebya	60	32	12280	4404	0	1	0	54
Actinobacteria;Thermoleophilia;Gaiellales;Gaiellaceae;Gaiella	67	173	9742	3165	0	5	0	77
Actinobacteria;Thermoleophilia;Solirubrobacterales;Solirubrobacteraceae;Conexibacter	143	66	9437	4016	0	4	0	69
Actinobacteria;Thermoleophilia;Solirubrobacterales;Solirubrobacteraceae;Parviterribacter	152	192	10617	3403	0	4	0	84
Actinobacteria;Thermoleophilia;Solirubrobacterales;Solirubrobacteraceae;Solirubrobacter	80	31	7800	7297	0	0	0	0
Actinobacteriota;Thermoleophilia;Gaiellales;Gaiellaceae;Gaiella	59	42	11267	8307	0	0	0	0
Actinobacteriota;Thermoleophilia;Solirubrobacterales;Solirubrobacteraceae;Conexibacter	76	81	5156	3431	0	3	0	62
Armatimonadetes;Armatimonadia;Armatimonadales;Armatimonadaceae;Armatimonas	99	37	5683	4053	0	0	0	0
Ascomycota;Dothideomycetes;Capnodiales;Cladosporiaceae;Cladosporium	57	67	6067	4955	0	0	0	0
Ascomycota;Dothideomycetes;Capnodiales;Mycosphaerellaceae;Acrodontium	29	5	8188	2638	0	0	0	0
Ascomycota;Dothideomycetes;Capnodiales;Mycosphaerellaceae;Mycosphaerella	56	96	2843	6436	1	1	2	58
Ascomycota;Dothideomycetes;Pleosporales;Didymosphaeriaceae;Pseudopithomyces	7	64	3077	2147	0	1	0	63
Ascomycota;Dothideomycetes;Pleosporales;Pleosporaceae;Alternaria	60	66	9942	4955	0	1	0	45
Ascomycota;Dothideomycetes;Pleosporales;Pleosporaceae;Stemphylium	21	1	2804	2969	0	0	0	0
Ascomycota;Eurotiomycetes;Eurotiales;Aspergillaceae;Aspergillus	242	75	11977	5295	0	0	0	0
Ascomycota;Eurotiomycetes;Eurotiales;Aspergillaceae;Penicillium	126	130	6619	5307	2	2	3	47
Ascomycota;Eurotiomycetes;Eurotiales;Trichocomaceae;Talaromyces	54	84	6734	6157	0	0	0	0
Ascomycota;Eurotiomycetes;Onygenales;Onygenales_fam_Incertae_sedis;Chrysosporium	81	76	5810	4887	2	5	3	50
Ascomycota;Leotiomycetes;Erysiphales;Erysiphaceae;Erysiphe	44	24	8879	6432	0	0	0	0
Ascomycota;Leotiomycetes;Helotiales;Helotiaceae;Meliniomyces	72	69	7715	5621	0	2	0	65
Ascomycota;Leotiomycetes;Helotiales;Helotiaceae;Scytalidium	144	136	8908	6002	0	1	0	45

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Ascomycota;Leotiomycetes;Helotiales;Helotiales_fam_Incertae_sedis;Leptodontidium	71.5	48	10758	5117	1	1	2	1
Ascomycota;Leotiomycetes;Helotiales;Myxotrichaceae;Oidiodendron	47	52	7296	5362	0	2	0	60
Ascomycota;Leotiomycetes;Thelebolales;Pseudeurotiaceae;Geomyces	118	87	4717	5363	2	2	3	48
Ascomycota;Leotiomycetes;Thelebolales;Pseudeurotiaceae;Gymnostellatospora	234	150	6158	7319	2	3	3	67
Ascomycota;Leotiomycetes;Thelebolales;Pseudeurotiaceae;Pseudeurotium	27	162	6356	4115	0	3	0	83
Ascomycota;Leotiomycetes;Thelebolales;Pseudeurotiaceae;Pseudogymnoascus	73	59	5717	4701	0	0	0	0
Ascomycota;Saccharomycetes;Saccharomycetales;Saccharomycetales_fam_Incertae_sedis;Candida	103	76	6853	4967	0	5	0	58
Ascomycota;Saccharomycetes;Saccharomycetales;Saccharomycetales_fam_Incertae_sedis;Nadsonia	127	34	9387	4631	0	0	0	0
Ascomycota;Sordariomycetes;Coniochaetales;Coniochaetaceae;Coniochaeta	122	68	5161	6810	0	0	0	0
Ascomycota;Sordariomycetes;Hypocreales;Hypocreaceae;Trichoderma	30	77	9602	4113	0	1	0	1
Ascomycota;Sordariomycetes;Hypocreales;Hypocreales_fam_Incertae_sedis;Acremonium	121	65	11447	5216	1	1	18	1
Ascomycota;Sordariomycetes;Hypocreales;Hypocreales_fam_Incertae_sedis;Emericellopsis	39	42	11857	2484	0	1	0	1
Ascomycota;Sordariomycetes;Hypocreales;Nectriaceae;Fusarium	24	69	7249	3801	0	1	0	62
Ascomycota;Sordariomycetes;Hypocreales;Stachybotryaceae;Stachybotrys	26	53	8034	5545	0	1	0	49
Ascomycota;Sordariomycetes;Sordariales;Lasiosphaeriaceae;Podospora	188	69	5982	5428	0	1	0	1
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Dinghuibacter	187	34	3594	4517	0	0	0	0
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Flavihumibacter	105	47	5639	4438	0	0	0	0
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Flavisolibacter	226	103	6292	4866	1	5	23	90
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Flavitalea	292	186	4298	3061	8	9	34	87
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Lacibacter	28	107	6179	5598	1	2	1	34
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Niastella	128	64	4244	5548	0	2	0	63
Bacteroidetes;Bacteroidia;Chitinophagales;Chitinophagaceae;Terrimonas	210	110	5379	3395	2	3	25	78
Bacteroidetes;Bacteroidia;Cytophagales;Cytophagaceae;Rhodocytophaga	39	56	5030	5636	0	1	0	1
Bacteroidetes;Bacteroidia;Cytophagales;Microscillaceae;Chryseolinea	36	198	5393	5101	0	5	0	77
Bacteroidetes;Bacteroidia;Cytophagales;Microscillaceae;Ohtaekwangia	53	38	9833	4453	0	3	0	53
Bacteroidetes;Bacteroidia;Cytophagales;Spirosomaceae;Dyadobacter	213	80	3388	6359	0	2	0	64
Bacteroidota;Bacteroidia;Cytophagales;Microscillaceae;Chryseolinea	69	124	9609	3795	0	4	0	76
Bacteroidota;Bacteroidia;Cytophagales;Microscillaceae;Ohtaekwangia	24	36	13933	5760	0	1	0	1
Basidiomycota;Agaricomycetes;Agaricales;Agaricaceae;Lepiota	62	50	5118	5113	0	3	0	68
Basidiomycota;Agaricomycetes;Agaricales;Cortinariaceae;Gymnopilus	61	23	10001	5838	0	1	0	1
Basidiomycota;Agaricomycetes;Agaricales;Entolomataceae;Clitopilus	85	104	5819	4304	0	1	0	67
Basidiomycota;Agaricomycetes;Agaricomycetes_ord_Incertae_sedis;Agaricomycetes_fam_Incertae_sedis;Xenasmatella	100	59	6730	3896	0	0	0	0
Basidiomycota;Agaricomycetes;Polyporales;Ganodermataceae;Ganoderma	85	45	8772	3127	0	0	0	0
Basidiomycota;Agaricostilbomycetes;Agaricostilbales;Chionosphaeraceae;Ballistosporomyces	14	78	10819	6402	0	1	0	44
Basidiomycota;Cystobasidiomycetes;Erythrobasidiales;Erythrobasidiaceae;Bannoa	62	49	7069	5515	1	0	1	0

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Basidiomycota;Tremellomycetes;Filobasidiales;Piskurozymaceae;Solicoccozyma	183	88	6155	5116	0	3	0	50
Basidiomycota;Tremellomycetes;Tremellales;Trimorphomycetaceae;Saitozyma	36	95	13558	3011	0	0	0	0
Basidiomycota;Tremellomycetes;Trichosporonales;Trichosporonaceae;Apiotrichum	46	71	6895	4542	1	0	1	0
Blastocladiomycota;Blastocladiomycetes;Blastocladiales;Catenariaceae;Catenaria	91	52	14637	4878	0	1	0	36
Chloroflexi;Anaerolineae;SBR1031;A4b;OLB13	117	96	6601	5013	0	3	0	42
Chloroflexi;Chloroflexia;Chloroflexales;Chloroflexaceae;Chloronema	25	109	11887	3781	0	5	0	88
Chloroflexi;Chloroflexia;Chloroflexales;Herpetosiphonaceae;Herpetosiphon	38	66	6516	5867	0	2	0	3
Deinococcus-Thermus;Deinococci;Deinococcales;Trueperaceae;Truepera	81	55	5695	6774	0	1	0	1
Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	52	114	13038	6828	0	0	0	0
Firmicutes;Bacilli;Bacillales;Family_XII;Exiguobacterium	70	210	4747	4563	0	0	0	0
Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus	46	51	11209	9287	0	0	0	0
Firmicutes;Bacilli;Bacillales;Planococcaceae;Paenisporosarcina	48	114	9620	6382	0	0	0	0
Firmicutes;Bacilli;Bacillales;Planococcaceae;Planomicrobium	85	100	6662	5462	0	1	0	62
Firmicutes;Bacilli;Bacillales;Thermoactinomycetaceae;Baia	18	73	8511	5161	0	1	0	60
Firmicutes;Bacilli;Bacillales;Thermoactinomycetaceae;Geothermomicrobium	41	99	4564	3682	0	3	0	50
Firmicutes;Clostridia;Clostridiales;Clostridiaceae_1;Clostridium_sensu_stricto_1	183	86	5634	4283	4	2	19	61
Firmicutes;Clostridia;Clostridiales;Clostridiaceae_1;Clostridium_sensu_stricto_13	120	95	6049	6004	2	1	21	2
Firmicutes;Clostridia;Clostridiales;Clostridiaceae_1;Clostridium_sensu_stricto_3	136	142	13172	7619	0	4	0	67
Firmicutes;Clostridia;Clostridiales;Clostridiaceae_1;Clostridium_sensu_stricto_8	182	137.5	5215	4384	3	4	16	60
Firmicutes;Clostridia;Clostridiales;Clostridiaceae_1;Clostridium_sensu_stricto_9	49	137.5	6097	4384	0	4	0	60
Firmicutes;Clostridia;Clostridiales;Peptococcaceae;Desulfosporosinus	53	111	6670	2688	0	4	0	67
Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Pelosinus	238	230	8736	5434	2	7	26	91
Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Gemmatimonas	67	213	4938	3578	0	7	0	90
Gemmatimonadetes;Longimicrobia;Longimicrobiales;Longimicrobiaceae;Longimicrobium	73	140	5295	3867	1	1	1	44
Gemmatimonadota;Longimicrobia;Longimicrobiales;Longimicrobiaceae;Longimicrobium	26	86	6401	5933	0	2	0	39
Gemmatimonadota;Longimicrobia;Longimicrobiales;Longimicrobiaceae;YC-ZSS-LKJ147	107	100	7103	4420	1	2	1	64
Mortierellomycota;Mortierellomycetes;Mortierellales;Mortierellaceae;Mortierella	97	67	9931	4135	1	0	1	0
Mucoromycota;Umbelopsidomycetes;Umbelopsidales;Umbelopsidaceae;Umbelopsis	159	75	9970	3361	1	3	1	54
Planctomycetes;Planctomycetacia;Gemmatales;Gemmataceae;Fimbriiglobus	163	184	7519	3745	1	2	1	52
Planctomycetes;Planctomycetacia;Gemmatales;Gemmataceae;Gemmata	78	38	11325	6851	0	0	0	0
Planctomycetes;Planctomycetacia;Gemmatales;Gemmataceae;Telmatocola	126	61	6284	4522	1	4	24	68
Planctomycetes;Planctomycetacia;Isosphaerales;Isosphaeraceae;Paludisphaera	74	114	7235	4894	0	2	0	70
Planctomycetes;Planctomycetacia;Pirellulales;Pirellulaceae;Blastopirellula	149	132	6807	4924	0	1	0	66
Planctomycetes;Planctomycetacia;Pirellulales;Pirellulaceae;Pir4_lineage	169	78	4169	4772	0	1	0	30
Planctomycetes;Planctomycetacia;Pirellulales;Pirellulaceae;Pirellula	170	180	7106	5356	0	2	0	72
Planctomycetes;Planctomycetacia;Planctomycetales;Rubinisphaeraceae;Planctomicrobium	146	174	4235	4538	0	2	0	46

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Planctomycetes;Planctomycetacia;Planctomycetales;Rubinisphaeraceae;SH-PL14	58	133	6056	3708	0	7	0	84
Planctomycetota;Planctomycetes;Gemmatales;Gemmataceae;Fimbriiglobus	123	81	5067	4555	0	5	0	83
Planctomycetota;Planctomycetes;Gemmatales;Gemmataceae;Zavarzinella	52	140	9795	5429	0	1	0	50
Planctomycetota;Planctomycetes;Pirellulales;Pirellulaceae;Pir4 lineage	58	67	7684	6481	0	1	0	1
Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacteraceae;Roseomonas	252	111	7437	3227	3	3	27	71
Proteobacteria;Alphaproteobacteria;Acetobacterales;Acetobacterales_Incertae_Sedis;Zavarzinia	123	173	4290	3289	0	0	0	0
Proteobacteria;Alphaproteobacteria;Azospirillales;Azospirillaceae;Azospirillum	59	93	6769	3861	0	1	0	59
Proteobacteria;Alphaproteobacteria;Azospirillales;Azospirillaceae;Skermanella	125	77	9248	3311	0	0	0	0
Proteobacteria;Alphaproteobacteria;Azospirillales;Azospirillales_Incertae_Sedis;Stella	413	130	3727	3777	5	5	28	84
Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Brevundimonas	109	187	3812	5635	0	5	0	67
Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Caulobacter	163	234	4903	4170	0	3	0	80
Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Phenylobacterium	58	126	11256	3667	0	0	0	0
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Hirschia	61	148	6629	3863	1	5	1	76
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;SWB02	151	116	5199	4246	0	5	0	77
Proteobacteria;Alphaproteobacteria;Dongiales;Dongiaceae;Dongia	301	140	3639	4421	9	7	35	91
Proteobacteria;Alphaproteobacteria;Micropepsales;Micropepsaceae;Micropepsis	179	236	6935	6701	4	5	27	78
Proteobacteria;Alphaproteobacteria;Reyranellales;Reyranellaceae;Reyranella	61	170	6253	4465	0	5	0	88
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;alphaI_cluster	93	89	4627	4727	1	2	1	68
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;Bosea	165	62	7718	4663	0	4	0	74
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;Chelatococcus	65	54	5354	4764	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;Methylobacterium	53	63	5047	6007	0	1	0	1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;Microvirga	490	73	4350	3413	0	4	0	84
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;Neo-b11	143	208	6978	3404	0	8	0	94
Proteobacteria;Alphaproteobacteria;Rhizobiales;Beijerinckiaceae;Psychroglaciecola	66	454	4433	4033	1	14	1	110
Proteobacteria;Alphaproteobacteria;Rhizobiales;Devosiaceae;Arsenicitalea	86	132	5816	3548	0	3	0	78
Proteobacteria;Alphaproteobacteria;Rhizobiales;Devosiaceae;Devosia	95	110	7798	3862	0	1	0	2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Devosiaceae;Maritalea	181	127	6970	4132	2	10	25	94
Proteobacteria;Alphaproteobacteria;Rhizobiales;Devosiaceae;Pelagibacterium	106	77	5162	6018	0	4	0	71
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Hyphomicrobium	139	134	8409	4997	1	2	1	69
Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Pedomicrobium	216	91	7793	4779	1	0	1	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Pleomorphomonadaceae;Chthonobacter	137	136	6325	5359	1	0	4	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Pleomorphomonadaceae;Prosthecomicrobium	117	55	13829	5631	2	1	5	1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Aliihoeflea	16	86	9527	5594	1	1	1	1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	95	68	6843	5037	0	0	0	0

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Aminobacter	188	65	7664	5078	1	5	17	90
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Aurantimonas	26	92	5860	7027	0	3	0	61
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Aureimonas	45	94	9128	5631	0	2	0	52
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Brucella	180	78	4851	5507	0	4	0	75
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Ensifer	103	80	5628	5659	0	1	0	51
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Mesorhizobium	127	88	5160	5330	1	0	1	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Neorhizobium	79	120	8421	5388	0	3	0	56
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Nitratireductor	331	230	2797	3524	10	7	34	87
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Pseudaminobacter	414	81.5	5375	5131	0	3	0	62
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Shinella	58	76	10083	5139	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiales_Incertae_Sedis;Alsobacter	339	77	4229	6016	3	3	23	72
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiales_Incertae_Sedis;Bauldia	83	160	11827	5295	0	3	0	62
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiales_Incertae_Sedis;Nordella	70	155	5720	3883	0	2	0	71
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiales_Incertae_Sedis;Phreatobacter	175	166	6280	3921	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Afipia	56	76	4384	4322	0	2	0	73
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Bradyrhizobium	37	38	8559	7762	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Pseudolabrys	197	162	5444	4004	0	6	0	76
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Pseudorhodoplanes	30	130	4519	4870	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Pseudoxanthobacter	17	96	9948	4221	1	3	1	81
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Rhodoplanes	169	41	3786	6343	0	3	0	53
Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Rhodopseudomonas	100	97	7384	6844	0	3	0	44
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Amaricoccus	197	117	5556	5055	0	6	0	86
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Defluviimonas	84	53	9379	4473	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Gemmobacter	61	89	7499	7895	0	1	0	63
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Paracoccus	171	106	4645	4962	0	0	0	0
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudorhodobacter	76	98	8321	7556	0	1	0	57
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Rhodobacter	303	212	5730	4302	1	7	1	94
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Rubellimicrobium	166	110	4168	5612	1	1	2	46
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tabrizicola	305	132	9276	7158	2	7	22	93
Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodopirillaceae;Defluviicoccus	93	43	7073	5383	0	1	0	53
Proteobacteria;Alphaproteobacteria;Sneathiellales;Sneathiellaceae;Ferrovibrio	143	53	4309	4931	1	1	1	1
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Altererythrobacter	343	112	4601	5377	0	4	0	66
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Ellin6055	85	171	6011	4691	0	4	0	62
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Erythrobacter	57	155	5661	5686	0	4	0	76
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium	195	157	6371	4232	0	0	0	0

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Plot4-2H12	60	63	9405	5221	0	1	0	62
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Porphyrobacter	116	89	5586	7099	1	2	20	38
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Qipengyuania	82	46	9759	5024	0	4	0	73
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingobium	48	95	8149	4328	0	0	0	0
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas	411	257	3505	3244	15	14	40	106
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingopyxis	159	64	7567	4861	3	1	22	1
Proteobacteria;Alphaproteobacteria;Thalassobaculales;Thalassobaculaceae;Thalassobaculum	25	52	8750	3542	0	4	0	80
Proteobacteria;Alphaproteobacteria;Tistrellales;Geminicoccaceae;Candidatus_Alysiosphaera	203	61	4841	3571	2	0	21	0
Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;Peredibacter	63	130	6117	5093	0	5	0	77
Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio	49	51	8052	5112	0	0	0	0
Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;OM27_clade	188	92	16993	5369	0	1	0	62
Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter	30	39	4574	4598	0	2	0	51
Proteobacteria;Deltaproteobacteria;Myxococcales;Archangiaceae;Anaeromyxobacter	59	47	4593	6497	0	0	0	0
Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae;Haliangium	139	164	5029	4421	0	2	0	63
Proteobacteria;Deltaproteobacteria;Myxococcales;Phaselicystidaceae;Phaselicystis	34	129	5124	4890	0	1	0	70
Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;Jahnella	133	114	5167	6224	1	3	19	74
Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;Pajaroellobacter	83	173	9420	3883	0	7	0	90
Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;Sorangium	90	96	7784	5813	0	1	0	73
Proteobacteria;Deltaproteobacteria;Myxococcales;Sandaracinaceae;Sandaracinus	63	123	7106	7394	0	3	0	53
Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexus	44	60	6216	6704	0	0	0	0
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Aquabacterium	114	133	5149	4631	0	3	0	80
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Azohydromonas	41	109	5522	3352	0	6	0	92
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Caenimonas	40	147	10397	4792	1	5	1	80
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Hydrogenophaga	197	127	5360	5804	4	1	28	57
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Lautropia	225	81	4757	4766	1	1	2	1
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Limnohabitans	264	325	6459	3152	2	8	21	84
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Methylibium	204	239	5501	4790	0	5	0	89
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Noviherbaspirillum	132	55	6261	6140	2	1	3	59
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Oxalicibacterium	23	78	9789	9427	0	1	0	58
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Piscinibacter	321	232	3880	3146	9	13	35	100
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Pseudorhodoferax	126	62	6440	6028	1	0	1	0
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Ramlibacter	156	42	9150	5532	0	0	0	0
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Rhizobacter	44	96	14804	6187	0	1	0	70
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Rhodoferax	20	159	8137	3305	1	4	1	79
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Rubrivivax	70	119	12102	5651	0	4	0	71

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Burkholderiaceae;Xylophilus	8	39	11280	6225	0	0	0	0
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Chromobacteriaceae;Vogesella	160	181	5741	5197	0	4	0	81
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Methylophilaceae;Methylobacillus	79	47	8252	5832	1	1	1	1
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Methylophilaceae;UBA6140	114	142	9283	2974	0	6	0	92
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Nitrosomonadaceae;DSSD61	119	165	13700	3492	2	12	22	105
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Nitrosomonadaceae;Ellin6067	37	182	6840	3602	0	0	0	0
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Nitrosomonadaceae;IS-44	211	150	7414	3043	0	2	0	74
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Nitrosomonadaceae;mle1-7	69	109	7554	4317	0	1	0	43
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Nitrosomonadaceae;MND1	289	172.5	5225	4624	4	12	26	100
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Rhodocyclaceae;Ferribacterium	76	87	4655	1991	0	1	0	44
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Rhodocyclaceae;Methyloversatilis	137	116	9335	5229	0	1	0	1
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Rhodocyclaceae;Thauera	47	441	9069	3469	0	21	0	119
Proteobacteria;Gammaproteobacteria;Betaproteobacteriales;Rhodocyclaceae;Zoogloea	71	164	5512	3849	0	2	0	83
Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Cellvibrio	50	179	5646	2807	1	7	1	90
Proteobacteria;Gammaproteobacteria;Coxiellales;Coxiellaceae;Coxiella	43	200	4787	3887	1	1	1	78
Proteobacteria;Gammaproteobacteria;Diplorickettsiales;Diplorickettsiaceae;Aquicella	63	236	3884	3558	9	11	34	100
Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Acidibacter	194	145	6212	3819	0	3	0	74
Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Candidatus_Berkiella	51	71	7395	5704	0	0	0	0
Proteobacteria;Gammaproteobacteria;Immundisolibacterales;Immundisolibacteraceae;Immundisolibacter	242	233	5055	3473	5	18	30	111
Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;Legionella	13	54	10530	3924	0	2	0	55
Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter	129	159	5881	5267	3	5	16	73
Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	219	132	10509	3347	5	2	23	52
Proteobacteria;Gammaproteobacteria;Salinisphaerales;Solimonadaceae;Solimonas	34	177	4862	4249	1	3	1	79
Proteobacteria;Gammaproteobacteria;Steroidobacterales;Steroidobacteraceae;Steroidobacter	87	155	13669	5382	0	4	0	79
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Rhodanobacteraceae;Ahniella	153	72	11258	2984	0	3	0	57
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Rhodanobacteraceae;Dokdonella	111	76	4775	3824	0	0	0	0
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Rhodanobacteraceae;Rhodanobacter	104	38	6190	6276	0	1	0	1
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Arenimonas	297	105	5468	4366	1	4	21	87
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Luteimonas	47	88	10311	6637	0	0	0	0
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Lysobacter	207	155	5096	4516	0	6	0	88
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Pseudoxanthomonas	138	70	8218	6468	2	3	20	58
Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Thermomonas	29	122	8217	6034	0	3	0	80

Variable	Betweenness		Strength		Degree		Closeness	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Sumerlaeota;Sumerlaeia;Sumerlaeales;Sumerlaeaceae;Sumerlaea	259	130	11161	4785	12	6	38	93
Thaumarchaeota;Nitrososphaeria;Nitrososphaerales;Nitrososphaeraceae;Candidatus_Nitrocosmicus	84	51	9615	7690	0	0	0	0
Verrucomicrobia;Verrucomicrobiae;Chthoniobacterales;Chthoniobacteraceae;Chthoniobacter	194	67	4804	4864	0	1	0	71
Verrucomicrobia;Verrucomicrobiae;Opitutales;Opitutaceae;IMCC26134	138	57	9422	5727	0	0	0	0
Verrucomicrobia;Verrucomicrobiae;Opitutales;Opitutaceae;Lacunisphaera	70	103	4898	3631	2	2	23	63
Verrucomicrobia;Verrucomicrobiae;Opitutales;Opitutaceae;Opitutus	52	150	5329	4080	0	9	0	83
Verrucomicrobia;Verrucomicrobiae;Opitutales;Puniceicoccaceae;Verruc-01	36	94	7983	4498	0	5	0	64
Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Luteolibacter	42	59	4719	5304	0	1	0	50
Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseimicrobium	81	174	4094	3818	0	7	0	85
Verrucomicrobiota;Chlamydiae;Chlamydiales;Parachlamydiaceae;Neochlamydia	140	105	4692	4866	0	3	0	52
Verrucomicrobiota;Verrucomicrobiae;Pedosphaerales;Pedosphaeraceae;Ellin517	54	154	6684	5279	1	3	1	60

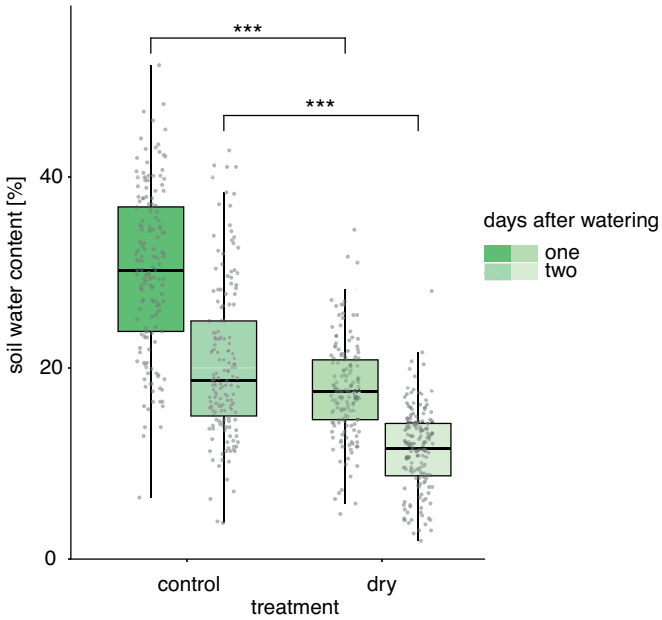

Fig. S1: Soil moisture in the control and drought treatment.
Soil moisture was measured one or two days after watering. The x-axis indicates the treatment and the day after watering, while the y-axis gives the soil water content in %. Differences between treatments were determined using a t-test with bonferroni-correction and resulting P-values are indicated with stars (P<0.05*, P<0.01**, P<0.001***).

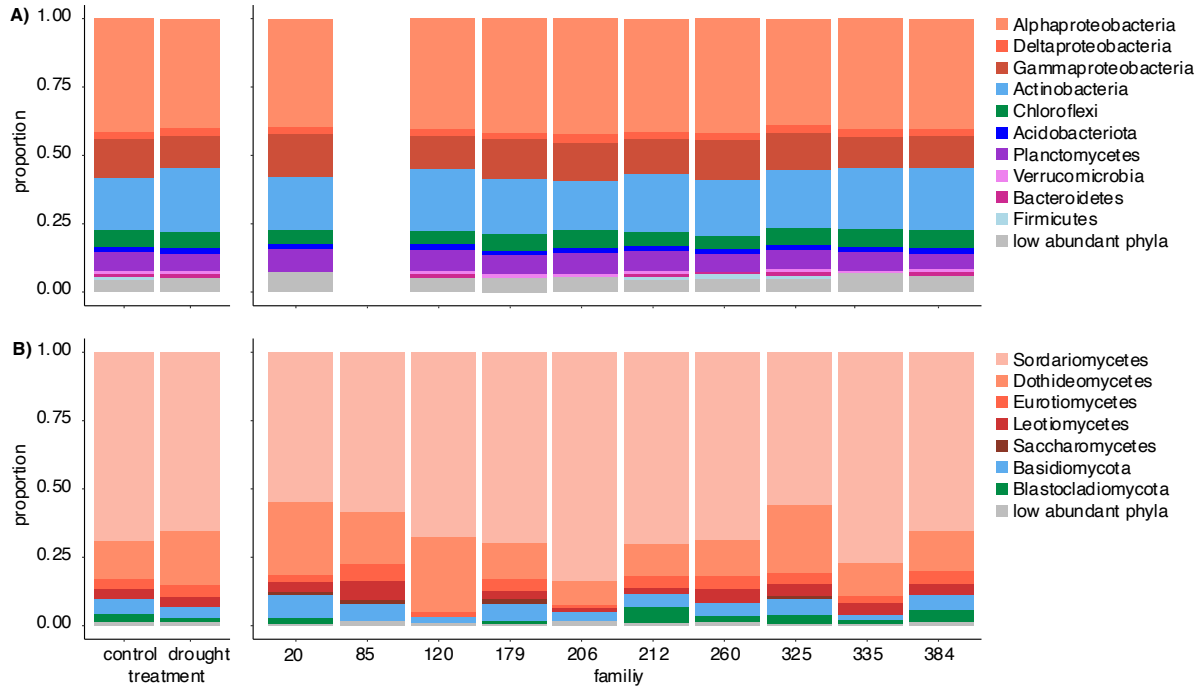

Fig. S2: Distribution of bacteria (A) and fungi (B) phyla in each of the two treatments (left) and plant families (right). For the most common phyla (Proteobacteria/ Ascomycota), the classes are shown in different shades of red.

Method S1: Preparation and analysis of root exudate samples

Sample preparation

After collecting the root rhizosphere as described under 2.3, the roots were carefully cleaned with ultrapure water (type 1 water, PURELAB flex 2, ELGA LabWater, Veolia, Celle, Germany). To allow the plants to recover from any stress, they were placed for 2 hours in a nutrient solution consisting of 18 g of soil mixed in 40 ml of ultrapure water. After cleaning plant roots a second time, they were placed for another 2 hours in a dark falcon tube containing 25 ml of distilled water to collect root exudates (Thermo Scientific Nalgene, Rochester, NY, USA). The collected solution was filtered (Whatman nr. 42, 90 mm, Global Life Sciences Solutions USA LLC, MA, USA), freeze dried for 48 hours (Scanvac CoolSave, Labogene, Allerød, Denmark), weighed, and stored at -80 °C until further processing.

Following Herz et al. (2018), the thawed root exudate samples and controls containing deionized water were dissolved twice in 0.75 ml of methanol and sonicated each time for 10 minutes at 20 °C in 2 ml tubes (SafeSeal, Sarstedt, Nuermbrecht, Germany). The solutions were evaporated to dryness at 40 °C with a vacuum centrifuge (CentriVap, Labcono, Kansas City, MO, USA) attached to a vacuum pump (PC3001 VARIO^{pro} EK Peltronic, VACCUBRAND, Wertheim, Germany) and dissolved in 0.275 ml of 80% methanol containing 20 µg/ mL 2-(2,4-dichlorophenoxy) acetic acid and 10 µM ribitol as internal standards. Next, each sample was centrifuged for 5 minutes at 8000 rpm to precipitate the remaining particles (Centrifuge 5430 Eppendorf, Hamburg, Germany). The supernatant was transferred, dried in the vacuum centrifuge, and stored at -20 °C until further analysis. Samples were derivatized performing a methoxylamination with 50 µl of methoxylamin-hydrochloride (20 mg/ ml in pyridine) for 90 minutes at 37 °C on a heating block. Then, they were silylated with 50 µl *N,O*-Bis-(trimethylsilyl)-trifluoracetamid (BSTFA) with added alkane retention time indices (C12, C15, C19, C22, C28 (each 0.1 mg/ ml final concentration) and C32 (0.4 mg/ ml final concentration))

for 30 minutes at 37 °C on a heating block (ThermoMixer C, Eppendorf, Hamburg, Germany; Herz et al., 2018).

Chromatography

Samples, 1 µl injection volume, out of 100 µl, were analysed on a gas chromatograph, (Trace GC Ultra; Thermo Fisher Scientific, Waltham, MA, USA), equipped with a flame-ionization detector and a Rxi-5ms GC column (60 m, iD 0.25 mm, df 0.25 µm; Restek, Bellefonte, PA, USA). The carrier gas flow was at 1.2 ml/min. Injector temperature was set to 300 °C, the transfer line temperature was set to 260 °C and the FID base temperature to 320 °C. Further, six random samples were measured on the same gas chromatograph coupled to a mass spectrometer to verify the identification of the target compounds (GC-MS, DSQ II, Thermo Scientific, Waltham, MA, USA).

Single compound measurements

For compound verification, some expected compounds were measured on the GC-FID in single compound measurements. Lactose, Sucrose, benzoic acid, glucose (each 0.1 mg/ 0.1ml), succinic acid and oxalic acid (both 1 mg/ 0.1ml) were prepared with the same derivatization and silylation protocol as root exudate samples.

Method S2: Preparation and analysis of microorganisms

DNA extraction

Samples were freeze-dried for 72 hours (Scanvac CoolSave, Labogene, Allerød, Denmark) and later ground with a ball mill for one minute at 30 Hz (Retsch MM 400, Haan, Germany; 25 ml chambers with a 1 cm ball; Hartman et al. 2017). DNA was extracted with the DNeasy PowerSoil Pro kit (Quiagen, Hilden, Germany) with the following adaptations: Homogenization of samples was done with a ball mill (Retsch MM400) for 5 minutes at 25 Hz twice (step 2c in the detailed manufactures protocol), and to increase yield, 50 µl of solution C6 was added twice to the filter membrane (steps 16 and 17 of the detailed manufactures protocol were repeated). DNA concentration was quantified with a Qbit 3 fluorometer with the dsDNA HS Assay kit (Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania) and set to 1 ng/ µl. Finally, extracted DNA was stored at -80°C.

Target amplification and barcodes

We used the Platinum Hot Start PCR kit (Invitrogen) for primer and barcode binding. The total PCR reaction volume of 20 ml contained 0.8-fold the Platinum Hot Start PCR mastermix, 200 nM of each primer, 0.3% of bovine serine albumin for fungi, and 3 µl of template DNA for bacteria or 5 µl of template DNA for fungi. PCRs were run on the Sensoquest Labcycler (Sensoquest, Göttingen, Germany) with a setup of first 3 minutes initial denaturation at 94 °C, then cycles of 30 seconds denaturation at 94 °C, 1 minute primer annealing at 50 °C, 90 seconds extension at 72 °C, and final extensions for 10 minutes at 72 °C with 25 cycles for bacteria and 30 cycles for fungi (EMP, McGuire et al. 2013). Fungi samples after PCR were used for bacteria analysis. We validated results, including positive and negative controls on agarose gels (electrophoresis system: Mupid-One by Advance, Tokyo, Japan; gel scanning: Ebox by Vilber, Collegien, France). PCR products were cleaned with the ChargeSwitch PCR Clean-Up kit

(Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania) on the cleaning robot Kingfisher Flex (Thermo Scientific, Thermo Fisher Scientific, Waltham, MA, USA).

In a second PCR, barcodes were attached to each of the samples. We used Access Array barcodes (Fluidigm Corporation, South San Francisco, USA) with the primers PE1-CS1-F and PE2-Barcode-CS2-R. The 25 ml of reaction volume included 5 µl of water, 0.8-fold Platinum Hot Start PCR Mastermix, 400 nM primers, and 5 µl of template DNA. PCR settings were 2 minutes initial denaturation at 94 °C, then cycles of 30 seconds denaturation at 94 °C, 30 seconds annealing at 60 °C, one minute extension at 72 °C, and 10 minutes final extension at 72 °C with 10 cycles (step 2 until 4; Bai et al. 2015).

Concentration, pooling and sequencing

PCR products were cleaned with the Charge Switch PCR Clean-Up kit. Concentration was determined with a fluorescence microplate reader (BioTek Synergy H1, Santa Clara, CA, USA) using the BioTak Gen5 software (Agilent Technologies, Santa Clara, CA, USA). Samples for that were prepared with the Myra robot (Labgene Instruments, Chatel-St-Denis, Switzerland) using standards from the AccuClear Kit (Biotium, Fremont, CA, USA). Samples were diluted (1:1) with elution buffer (Charge Switch PCR Clean-Up kit). If concentrations were too low, we reran PCRs but replaced water with template DNA and increased concentrations during cleanup using the CleanNGS kit (CleanNA, Waddinxvveen, Netherlands). This was done by reducing the sample volume of PCR1 products from 35 µl to 15 µl and of PCR2 products from 25 µl to 20 µl. Finally, samples were pooled, with 40 ng of PCR products per sample. Sequencing was done on the Illumina MiSeq Personal Sequencer (Illumina, San Diego, CA, USA) with a 500-cycle v2 flowcell using Fludigm custom spike-in primers.

Additional co-authored papers

Heblack J, Schepers JR, Willi Y (2024) Reduced evolutionary potential under heat and drought stress at the southern range edge of North American *Arabidopsis lyrata*. J Evol Biol 204:173-185. voae045. doi.org/10.1093/jeb/voae045

Sánchez-Castro D, Patsiou T-S, Perrier A, Schepers JR, Willi Y (2024) Uncovering the cause of breakup between species' range limits and niche limits under climate warming. J Biogeogr 00:1-14. doi.org/10.1111/jbi.14796

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Evolutionary potential under heat and drought stress at the southern range edge of North American *Arabidopsis lyrata*

Jessica Heblack, Judith R. Schepers, Yvonne Willi

Department of Environmental Sciences, University of Basel, Basel, Switzerland

Corresponding author: Jessica Heblack, Department of Environmental Sciences, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland. Email: jessica.heblack@unibas.ch

Abstract

The warm edges of species' distributions are vulnerable to global warming. Evidence is the recent range retraction from there found in many species. It is unclear why populations cannot easily adapt to warmer, drier, or combined hot and dry conditions and locally persist. Here, we assessed the ability to adapt to these stressors in the temperate species *Arabidopsis lyrata*. We grew plants from replicate seed families of a central population with high genetic diversity under a temperature and precipitation regime typical of the low-latitude margin or under hotter and/or drier conditions within naturally occurring amplitudes. We then estimated genetic variance–covariance (G-) matrices of traits depicting growth and allocation as well as selection vectors to compare the predicted adaptation potential under the different climate-stress regimes. We found that the sum of genetic variances and genetic correlations were not significantly different under stress as compared to benign conditions. However, under drought and heat drought, the predicted ability to adapt was severely constrained due to strong selection and selection pointing in a direction with less multivariate genetic variation. The much-reduced ability to adapt to dry and hot-dry conditions is likely to reduce the persistence of populations at the low-latitude margin of the species' distribution and contribute to the local extinction of the species under further warming.

Keywords: adaptation, climatic gradient, evolutionary potential, genetic variation, G-matrix, range edge, trade-offs

Introduction

Species' distribution limits often reflect endpoints of the ecological niche of a species, with the latter defined as the ranges of abiotic factors, availability of resources, and the abundance of interacting species that enable long-term persistence (Hargreaves et al., 2014; Paquette & Hargreaves, 2021). However, for many species, climate alone is a good predictor of where a species reaches its geographical or elevational limit (Lee-Yaw et al., 2016; Patsiou et al., 2021), suggesting that failing climate adaptation at range limits plays a major role in determining distributions. Constrained climate adaptation at range limits is also indicated by the many examples of species that have shifted their distributions under recent climate warming, with expansions at the cold margins and retractions from the warm margins (Chen et al., 2011; Lenoir et al., 2020; Rumpf et al., 2018). In parallel, macroevolutionary studies have revealed that adaptation to climate is evolutionary constrained, particularly adaptation to heat (Bennett et al., 2021; Liu et al., 2020). Still, the causes of constraint are unknown. Here, we focus on the genetic architecture of growth traits under selection and its role in constraining climate adaptation at warm range limits, as species seem mostly unable to adapt there (Parmesan, 2006).

Evolutionary theory has come up with several hypotheses as to why adaptation to changing conditions can fail at range limits (Sexton et al., 2009). These include steepening environmental gradients, too little or too much dispersal, small population size, and, linked with low dispersal and small population size, low genetic variation (Holt, 2003; Kirkpatrick & Barton, 1997; Polechová, 2018; Polechová & Barton, 2015). An aspect that has received relatively less attention is the nature of genetic variation. There may be ample genetic variation for traits under selection when evaluated individually, though genetic variation may still be constraining if selection acts on several traits and these are tied in genetic correlations antagonistic to the direction of selection (Blows & Hoffmann, 2005; Hansen et al., 2019; Lande, 1979). Within a population, genetic correlations antagonistic to the direction of selection, or genetic trade-offs, may be the result either of physical linkage or antagonistic pleiotropy (Falconer & Mackay, 1996, p. 312). Evolutionary trade-offs can be detected within populations if genotypes differ enough in regard to the expression of traits involved in the trade-off, often under stressful conditions (Stearns, 1992) or across habitat types (Falconer & Mackay, 1996, p. 321–324). For the latter scenario, genotypes that are favoured in one habitat are less favoured in another habitat (Fry, 2003), thus preventing the niche expansion of specialized organisms (Holt & Gaines, 1992) and the evolution of favourable traits at distribution margins (Hoffmann & Blows, 1994; Roff et al., 2002).

Genetic variance–covariance (G-) matrices are useful for disentangling correlations among multiple traits, estimating genetic integration, and assessing constraints on recent or future multivariate evolution (Arnold, 1992; Lande, 1979).

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Genetic variances of specific traits are the elements on the main diagonal axis, whereas genetic covariances are the off-diagonal elements of G. G-matrices of different populations or revealed under different environmental conditions can be compared with each other and in regard to how easily they can contribute to a selection response (Roff & Fairbairn, 2012). An important estimate of G capturing genetic correlations in one value is the effective number of dimensions (Kirkpatrick, 2009). If genetic correlations are absent, this number equals the number of traits included in the matrix. The other extreme is when all genetic variation aligns along one axis, with the effective number of dimensions being 1. Angles between G or its components/eigenvectors and other vectors can predict the constraining nature of genetic correlations more specifically. A first such angle involves the vector of population divergence to assess the adaptability in a likely direction of selection (Schluter, 1996). A second involves a selection vector to predict the immediate response to selection (Blows & Hoffmann, 2005).

So far, few studies have assessed the role that genetic trade-offs may play in constraining adaptive evolution at range margins and/or under climate change on a microevolutionary scale (Willi & Van Buskirk, 2022). Paccard et al. (2016) compared the G-matrices of populations of *Arabidopsis lyrata* of a latitudinal gradient and found that populations at range limits had reduced genetic variances, but genetic covariances were such that they constrained evolution less than those of more centrally located populations. Sheth and Angert (2016) imposed artificial selection on scarlet monkeyflowers (*Mimulus cardinalis*) from replicate populations of the latitudinal range, either for early or late flowering. They detected correlated responses in early flowering lines, namely higher specific leaf area (SLA) and leaf nitrogen content. However, population divergence across latitudes did not follow the pattern of correlations, suggesting that past evolution had gone in the direction of less multivariate genetic variation. Etterson and Shaw (2001) performed a quantitative genetics crossing experiment with three populations of *Chamaecrista fasciculata* from a latitudinal gradient, estimated G-matrices at the three sites of origin, and predicted responses to selection based on single traits or G. The predicted multivariate responses were mostly reduced compared to predicted univariate responses due to genetic correlations antagonistic to the direction of selection.

The traits included in the estimation of G need special consideration. Sessile organisms, such as herbaceous plants, seem to respond to environmental stress either by a strategy of escape or tolerance (e.g., Kooyers, 2015; Puijalon et al., 2011; Upadhyay, 2019). Under stress, growth and development may be accelerated to finish an important life-history phase before the effect of stress becomes too severe, a strategy of escape. Alternatively, growth and development may be slowed down in favour of expressing protective traits. Sartori et al. (2019) showed in *A. thaliana* that an acceleration of phenology is related to lower precipitation and higher temperature along the species' range from high to low latitudes, indicating escape from stress under low-latitude conditions. For our study organism, *Arabidopsis lyrata* ssp. *lyrata*, of the many traits that were previously tested for latitudinal clinal variation, only plant size, reproductive development, and thermal resistance were found to vary. Plants of low-latitude areas grew to smaller sizes under benign temperatures and had a slower transition to flowering, higher thermal tolerance, and higher heat resistance, indicating a strategy of slow development and tolerance/protection at low latitudes (Paccard et al., 2014; Wos & Willi, 2015). Hence, adjustments on the continuum of fast versus slow growth or development may be key for coping with stress (Sartori et al., 2019), and aspects of growth and development are, therefore, good candidate traits in investigations on G in the context of low-latitude/warm range limits.

In this study, we compared G-matrices of one large outcrossing population of North American *Arabidopsis lyrata* ssp. *lyrata* (*A. lyrata* in short) grown under experimental temperature and precipitation similar to those at the low-latitude range margin. In climatized glasshouse chambers, we simulated average temperature and precipitation, or extreme conditions, i.e., increased temperature or decreased precipitation, or both types of stressors combined, as they can occur in spring to summer at the southern range edge. Environmental niche modelling revealed that the distribution of the species in the south and north is restricted by climate, and the major climatic factor associated with range limits was the mean minimum temperature in early spring (Lee-Yaw et al., 2018; Sánchez-Castro et al., 2024). Apart from warmer temperatures, we chose drier conditions, as low precipitation during the growing season may reduce the transpiration capacity of plants, which is their typical way of coping with heat (Irvine et al., 1998). We focussed on traits of growth and allocation based on previous findings that indicated the importance of growth progression and allocation in coping with stress. To achieve solid estimates on genetic correlations, we worked with one population only, but we included many replicate families. For the same reason, we chose a population of the southerly centre of distribution with high genetic variation, including in expressed traits. Populations of the southern range limit generally harbour low genomic variation and genetic variation for expressed traits (Paccard et al., 2016; Willi et al., 2018), making the detection of trade-offs difficult. We addressed the following questions: (a) Do genetic variances of traits differ under benign and climate-stress conditions? (b) Are there multivariate genetic constraints? (c) How well can *A. lyrata* respond to selection and adapt under heat, drought, or combined heat drought?

Materials and methods

Seed material and propagation

Arabidopsis lyrata subsp. *lyrata* occurs in temperate eastern and mid-western North America on sand dunes or rocky outcrops with some natural disturbance. It is a short-lived perennial that produces basal rosettes, out of which inflorescences grow in late spring/early summer. We selected a population from the south centre of the *A. lyrata* distribution at Saugatuck Dunes State Park, Michigan, United States (42.70° N, 86.20° W), with high genomic variation and a history of little genetic drift despite some postglacial range expansion (Willi et al., 2018). Furthermore, the population was found to harbour genetic variation in plant size and reproductive development under control conditions and in frost resistance, with traits being associated with environmental gradients of the dune landscape: position on the dune, distance from the canopy, vegetation cover, and intraspecific density (Paccard et al., 2013; Wos & Willi, 2018). The same three traits were confirmed as being variable among populations across the

latitudinal distribution of the species (Paccard et al., 2014; Wos & Willi, 2015).

Seeds of >600 maternal plants were collected between 2007 and 2014 in the field. We assumed that over the 7 years, there had been little change in allele frequencies as the species is common over a large surface area, with a large census size. Seeds of maternal plants were grown in separate pots in a glasshouse and thinned to one plant per pot (conditions in Supplementary Table S1). Plants were cross-pollinated in pairs, with a preference for pairing within one of several habitat aspects, e.g., both plants from dune tops (Supplementary Methods S1). The intention was to keep some of the potentially existent adaptive variants linked to a habitat aspect at a higher frequency in some offspring genotypes. The design resulted in 271 successful cross pairs or "families," of which 120 were randomly selected for offspring raising. Crosses were performed reciprocally, but cross direction was not included in the statistical models. Additionally, the crossing design included three families, each from two northern and two southern populations. These were used later to compare the within-population variation of the Saugatuck population with the within-species and latitudinal trait variation (Supplementary Table S2; Supplementary Figure S1). One pair of northern/southern populations came from the eastern ancestral cluster of *A. lyrata*, and one, together with the Saugatuck population, from the western ancestral cluster (Willi et al., 2018). The obtained seeds were stored in paper bags at 4 °C with silica beads to reduce moisture.

Experimental setup

We designed a 2 × 2 factorial stress experiment with average or extreme temperatures and average or low precipitation occurring in the two populations at the southern range limit (Supplementary Table S1). Low-temperature conditions (*Control* and *Dry*) were close to the average temperature in late spring/early summer, with the corresponding experimental conditions of 18 °C at night, 22 °C during the day, and 25 °C for the daily 1-hr heat peak (Figure 1A; climate data at the two southern edge sites in Schepers et al., 2024). High-temperature conditions (*Hot* and *Hot&Dry*) resembled the summer climate, with 23 °C at night, 27 °C during the day, and 30 °C for the daily 1-hr heat peak. Experimental temperatures during night-time were not as low as those at the two southern sites. The baseline for watering (*Control* and *Hot*) was about average precipitation in late spring/early summer, 100 mm per month. Low watering (*Dry* and *Hot&Dry*) was chosen close to precipitation during the driest month, 60 mm per month. Precipitation amounts were broken down to watering the pots every second day, which was set to either 8.4 or 5 ml per pot. Because some mortality was observed early on, we increased watering after two weeks by ~20% to 10 or 6 ml.

Five replicate plants per family were grown in each of the four treatment combinations (in short, treatments), split over 5 blocks. Seeds were sown in pots (1 per pot, pot diameter/depth: 4/5 cm) of 54-multi-pot-trays filled with a mixture of 1:2 of peat and sand (120 families × 4 environments × 5 replicate blocks = 2,400 pots, plus 3 families × 4 marginal populations × 4 environments × 3 replicates = 144 pots). Pots were watered to saturation and covered with mesh nets, and seeds were stratified at 4 °C in dark climate cabinets for 12 days (ClimeCab 1400, KÄLTE 3000 AG, Landquart, Switzerland). Trays were then moved to the glasshouse for germination and kept moist by spraying from above and keeping the mesh nets until ~75% of seeds had germinated (for 7 days). After 3 weeks, when ~80% of the plants had reached the 4-leaf stage, the stress experiment started. The experiment involved four glasshouse chambers, two with the low-temperature regime and two with the high-temperature regime. Within each of these, five blocks of multiport trays were maintained, with multiport trays allocated to either baseline- or low-watering. To reduce the effects of the glasshouse chamber and position within the block, blocks and trays within blocks were randomly repositioned across the two glasshouse chambers of the same temperature regime twice a week. Plants received fertilizer every fourth week and some insecticide to combat thrips infestation. The stress experiment was terminated after 5 months for plants under the high-temperature regime and after 6 months for plants under the low-temperature regime.

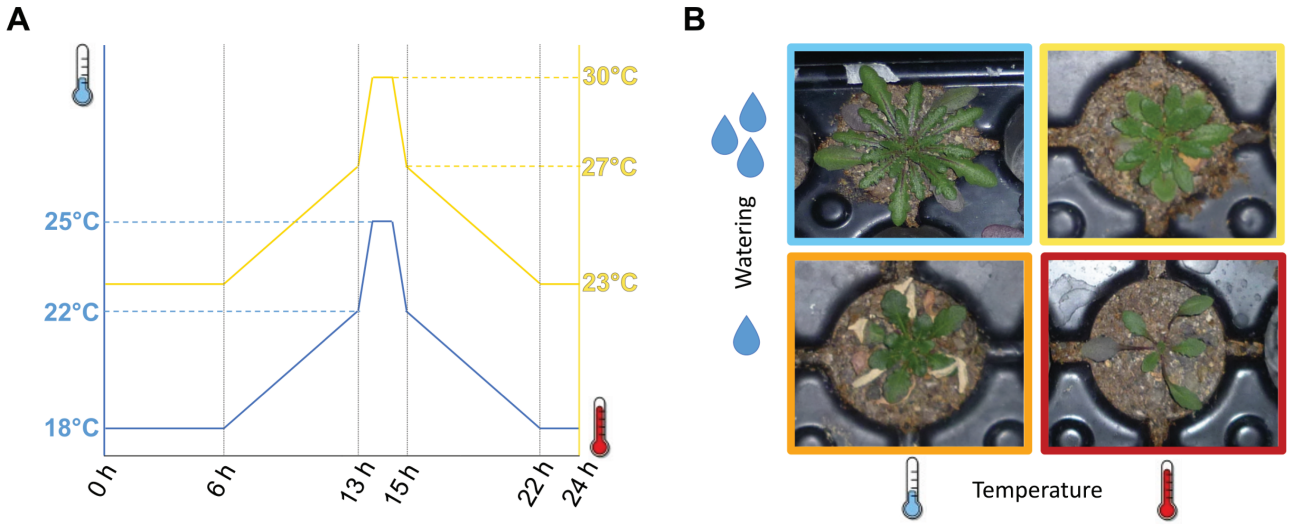

Figure 1. Climate stress experiment with *Arabidopsis lyrata* in the glasshouse. (A) The two temperature treatments were benign (left axis) and high temperature (right axis). Daily temperature profiles included an amplitude of 7 K per day. (B) Differences in performance among plants of the same seed family in the respective treatment combinations (from top left to bottom right)—*Control* (benign temperature and watering), *Hot* (high temperature), *Dry* (low watering), and *Hot&Dry* (high temperature and low watering). Colours indicate the respective treatments.

Trait assessment

Growth

Seed germination was checked every day for the first 2 weeks. The starting size for the day of germination was set to 2 mm^2, representing about four times the mean seed size of *A. lyrata* (Willi, 2013). Growth was tracked by taking pictures of each tray twice a week (every 3–4 days) until at least bolting (Figure 1B). At the same time, mortality was recorded. Camera setup, photo box, and image analysis were based on descriptions by Exposito-Alonso et al. (2018) and were adapted to fit this study design. A detailed description and access to the image analysis script can be found in the Supplementary Methods S2. Overrepresentation of late time points with size data occurred, and therefore, size values that were recorded after the four largest sizes of a plant were removed from the growth curve calculation. All remaining size measures of individual plants were used to fit seven growth models: linear, exponential, power, two- and three-parametric logistic, Gompertz, and von Bertalanffy, using the R package *minpack.lm* (Elzhov et al., 2022). Based on weighted AIC (AIC for each model and plant, and weighted relatively for each plant), the Gompertz model was overall the best but was only in third position for the *Hot&Dry* treatment (Supplementary Table S3). For this reason, the next best model, the three-parameter logistic, was chosen for trait extraction. For 11 plants (0.4%), this model could not be fitted, and the asymptotic size was set to the mean of the four highest size values (no data for growth rate and time to half the asymptotic size). Model output for plant growth included the following three parameters: asymptotic size (s_{asym}, in mm^2), maximal growth rate (r_{max}), and time until half the asymptotic size and fastest growth were achieved (x_{mid}, in days).

Allocation

At the end of the experiment, all available plant material per pot was split into the following categories and weighted separately: green rosette tissue, dead rosette tissue, roots, and inflorescences. Soil particles were washed away, and saturated weight was measured. After 48 hr of drying the material in an oven at 60 °C, the dry weight was measured. We then calculated *SLA* (s_{asym} [mm^2] per green rosette dry matter [mg]), leaf dry matter content (green rosette dry matter [mg] per green rosette saturated weight [g]), and root-shoot ratio (RS_{ratio}; the dry weight of roots to dry weight of all aboveground biomass). Final sample sizes for all populations, growth traits, and allocation traits are listed in Supplementary Table S4.

G-matrices and their analysis

G-matrix

G-matrices were calculated with a focus on growth traits. The first reason for focusing on this set of traits was the modularity among growth and allocation traits (see *Results* section), with considerable correlations within the two sets of traits, but not between them. A second reason was that allocation estimates for the *Hot&Dry* treatment were few (n = 21–23), as many plants died after accelerated growth in this treatment, which precluded the comparison of G for these traits and treatment. For allocation traits and all traits combined, we ran the same set of analyses on G-matrices as for the growth traits but by excluding the *Hot&Dry* treatment (results in the Supplementary Material).

Around 1,800 growth data points per treatment were available: 120 families × 5 replicates × 3 growth traits. Trait estimates were first corrected for the effects of block, tray within block, and position in the multi-pot tray for each treatment separately. The data points were then centred and rescaled across treatments, with a mean of 0 and a variance of 1. We calculated G-matrices for each treatment combination using a Bayesian approach with *MCMCglmm* (Hadfield, 2010). The mixed-effects model was:

$$Y_{ijk} \sim \mu + F_{jk} + \varepsilon_{ijk},$$

where Y_{ijk} is an observation for plant i of family j on trait k, the intercept (μ) is a fixed effect, F_{jk} is the random effect of the family, and ε_{ijk} is the random residuals. Iterations were set to 200,000, with a burn-in of 5,000 and thinning of 50. Priors for G came from a restricted maximum likelihood model (*lme4*; Bates et al. 2015). The significance of family-level covariance and variance estimates was evaluated by comparing deviance information criterion values (DIC; generalization of the Akaike information criterion) of (a) a model with a full G-matrix to (b) one with a matrix with family-level variances only, and (b) to (c) one without variances or covariances on the family level (Paccard et al., 2016; Puentes et al., 2016). For further analyses and presentation, all obtained G-matrices were multiplied by 2 to approximate genetic variances and covariances given the full-sib design.

Comparison of Gs

G-matrices of the four treatment combinations were compared by estimates of G-matrix geometry (Hansen & Houle, 2008; Kirkpatrick, 2009; Milocco & Salazar-Ciudad, 2022; Paccard et al., 2016). The first was the sum of the genetic variances across the traits, the trace of G (Kirkpatrick, 2009). The second was the effective number of dimensions (n_D), calculated as the sum of all eigenvalues of G divided by the first eigenvalue (eq. [2] in Kirkpatrick, 2009). The third measure was the angle between the dominant eigenvector of G, g_{max}, and the dominant eigenvector of the matrix of latitudinal trait divergence (D) among northern and southern populations, d_{max}. D matrices were established for the four environments in the same way as the G matrices, but with the input data of plant traits of the above-mentioned edge populations and including the random effect of southern position (north/south as 0/1). The fourth was the deviation of the predicted selection response from the end point of the selection vector, a measure of adaptive potential. We produced selection vectors using longevity (days of survival) as a fitness proxy. As with the three growth traits, longevity was first corrected for the effects of the block, tray within the block, and position in the multi-pot tray within treatment, followed by dividing by the mean in that treatment. We used *blme* (Chung et al., 2013) to overcome singularity and the model (in *blme* format):

$$\frac{\text{Longevity}}{\text{Mean longevity}} \sim s_{\text{asym}} + r_{\text{max}} + x_{\text{mid}} + (1|j),$$

with family, j, being the random factor. The obtained coefficients of the fixed effects of traits are the selection coefficients, which, taken together, build the selection vector (β) of the specific treatment (Hansen & Houle, 2008). The response to selection (Δz) can now be calculated by multiplying G with the selection vector (β) using the multivariate breeder's equation ($\Delta z = G^*\beta$; Lande, 1979). Selection deviation is the distance of the end points between the selection vector and the predicted response to selection after one generation. As a fifth measure, we calculated evolvability (evo_{HH}) by the method

of Hansen and Houle (2008, eq. [1]), which incorporates the strength of selection (the length of the selection vector) and its orientation. More precisely, evo_{HH} is the projection of the predicted response to selection on the selection vector. All comparisons involving aspects of G were made based on the posterior distribution of 3,900 G-matrices per treatment, following the approach described in Aguirre et al. (2014). Testing was done based on 95% highest posterior density (HPD) intervals, and when HPD intervals were overlapping, a comparison of the region of practical equivalence (Kruschke, 2018) followed. For this, the posterior distributions of the two treatments were divided. If the 95% HPD interval of the distribution of differences did not overlap with ROPE, i.e., a range between 0.9 and 1.1 (±10%; Henry and Stinchcombe, 2023; Kruschke, 2018), then a difference between treatments was assumed to exist.

Heritability

We estimated broad-sense heritability (H^2) by analysis of variance on mean-centred data across treatments. H^2 was calculated as twice the variance explained by family (V_f) over the phenotypic variance ($V_z = V_f + V_{error}$). In a full sib design, $2V_f$ represents an upper-bound estimate of additive genetic variance (V_g), likely inflated by a fraction of dominance variance and variance due to common-environment/maternal effects that also contribute to V_f (Walsh & Chenoweth, 2017). However, maternal effects were shown to be insignificant beyond very early life stages in *A. lyrata* (Paccard et al., 2013), and empirical (Wolak & Keller, 2014) and theoretical results (Clo & Opedal, 2021) show that dominance variance is generally much lower than additive variance. To compare variance estimates among traits and treatments, we standardized them by the square of the trait mean of the specific environment as proposed by Houle (1992)—now I_g and I_e. Standardized genetic variance, I_g, is another measure of evolvability that, compared to heritability, estimates the response relative to the trait mean before selection (Houle, 1992). The standard error of H^2 was approximated based on sample sizes (Walsh & Chenoweth, 2017). All analyses were done in R v. 4.0.5 (R Core Team, 2021).

Results

The four treatment combinations varied in stress, indicated by the varying mean sizes the plants achieved. Plants had declining asymptotic sizes from *Control* (14.5 ± 0.3 cm^2) to *Hot* (12.4 ± 0.2 cm^2), *Dry* (10.8 ± 0.1 cm^2), and *Hot&Dry* (8.2 ± 0.2 cm^2; Supplementary Table S5; Supplementary Figure S1). Correlation analysis among growth and allocation traits within treatments revealed a modular pattern (Supplementary Table S6). Growth traits (s_{asym}, r_{max}, x_{mid}) were often highly correlated with each other, and allocation traits (*SLA*, leaf dry matter content, RS_{ratio}) were often highly correlated, but correlations between the two sets of traits were weak. This, along with the low sample sizes for allocation traits in the *Hot&Dry* treatment (Supplementary Table S4), motivated the focus on growth traits in further analyses.

We found that genetic co-/variances for growth traits were overall significant in all treatments. Models with covariances, as compared to those without covariances, always had significantly lower DIC values, and models with variances only as compared to models without had lower DICs (Table 1). The comparison of the trace and dimensionality of treatment-specific G-matrices revealed little variation among the four environments. Neither the trace of Gs nor their dimensionality significantly differed between any of the four treatments, as 95% of HPD intervals were highly overlapping (Figure 2A and B). Dimensionality varied between averages of 1.3 and 1.6 for the three aspects of the logistic growth trajectory, indicating the presence of considerable correlations. The strongest correlations across treatments were revealed between maximal growth rate and time to the mid-point of growth (Supplementary Tables S6 and S7; Supplementary Figure S2). Plants either grew early (low x_{mid}) and had a high growth rate (r_{max}), or they grew late with a slow growth rate. In the *Hot&Dry* treatment, the two traits were associated with trade-offs with maximum size. Early and fast-growing plants reached small final size, while late and slow-growing plants reached large asymptotic size.

The next five estimates related the direction of G with vectors of population divergence and selection. Two were angles, with higher angles (up to 180°) indicating stronger constraints. The angle between g_{max} and d_{max} (dominant eigenvectors of G and the matrix of latitudinal trait divergence, D) was highest in the *Hot* and *Dry* treatments and lowest in the *Hot&Dry* treatment, with differences being significant (Figure 2C; G-matrices in Supplementary Table S7; D-matrices in Supplementary Table S8). The result indicates a good alignment between G and latitudinal trait divergence under combined stress. The angle between the selection vector and the predicted response to selection based on G required the assessment of selection in each of the experimental environments. We found selection (vector length; $|\beta|$) to be strongest under *Hot&Dry* ($|\beta| = 0.136$), considerably lower under *Dry* ($|\beta| = 0.058$) and lowest under *Control* ($|\beta| = 0.011$) and *Hot* ($|\beta| = 0.007$; Figure 3). The angle between the selection vector and the predicted response to selection revealed for the four treatment combinations decreased in the following order: *Dry* (close to 60°), *Hot&Dry*, *Control*, *Hot* (close to 20°) (Figures 2D and 3). Similarly, the deviation between the endpoints of the selection vector and the predicted response significantly differed between treatments, with the distance decreasing from *Hot&Dry* and *Dry* to *Control* and *Hot* (Figures 2E and 3). Somewhat in line, the projection of the selection response onto the selection vector (evo_{HH}) was lowest in the *Dry* treatment and significantly higher in the other three treatments (Figures 2F; Supplementary Figure S3). This latter estimate indicated the strongest constraints under *Dry*, followed by *Hot&Dry*.

Average broad-sense heritability deviated from the trace of G in predicting genetic variation across the four

Table 1. DIC values for G-matrices that include both variances and covariances on the family level ($DIC_{(co)variances}$), variances only ($DIC_{variances}$), or only family effects (DIC_{null}) for each treatment.

	$DIC_{(co)variances}$	$DIC_{variances}$	DIC_{null}
Control	4,391.379	4,440.294	4,556.135
Hot	3,722.478	3,785.262	4,039.161
Dry	2,951.876	2,982.765	3,410.213
Hot&Dry	3,846.137	3,875.810	4,001.464

Note. Models with smaller DIC are better supported—those with variances and covariances on the family level as compared to variances only and those with variances as compared to none.

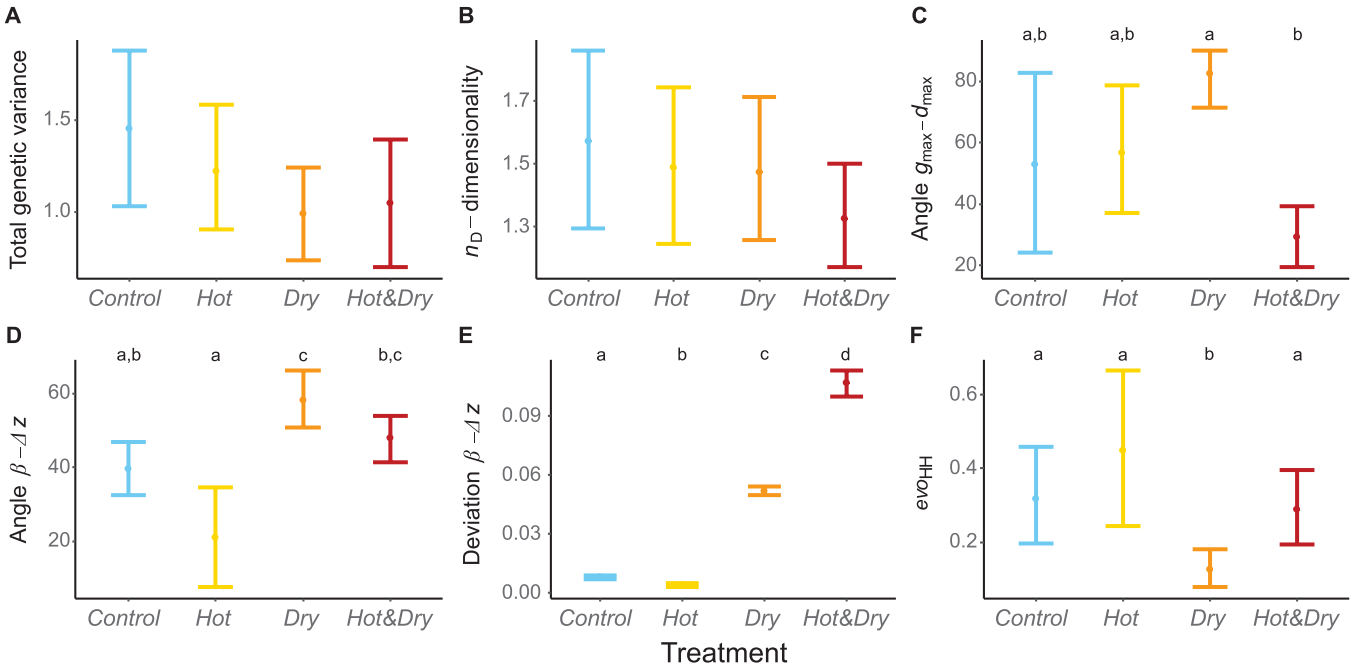

Figure 2. Comparison of geometric aspects of genetic variance–covariance (G-) matrices estimated under benign and stress conditions. (A) Total genetic variance, the trace of G. (B) Effective number of dimensions, n_D. (C) Change in the angle between g_{max}, the dominant eigenvector of G, and d_{max}, the dominant eigenvector of the matrix of latitudinal trait divergence. (D) Angle and (E) deviation distance between selection vector (β) and predicted selection response (Δ*z*). (F) Hansen and Houle's measure of evolvability (evo_{HH}). The colours represent the respective treatments: *Control*, *Hot*, *Dry*, *Hot&Dry*. Dots indicate the predicted model estimates and bars the 95% highest posterior density intervals. The letters above the bars indicate differences between treatments based on the region of practical equivalence.

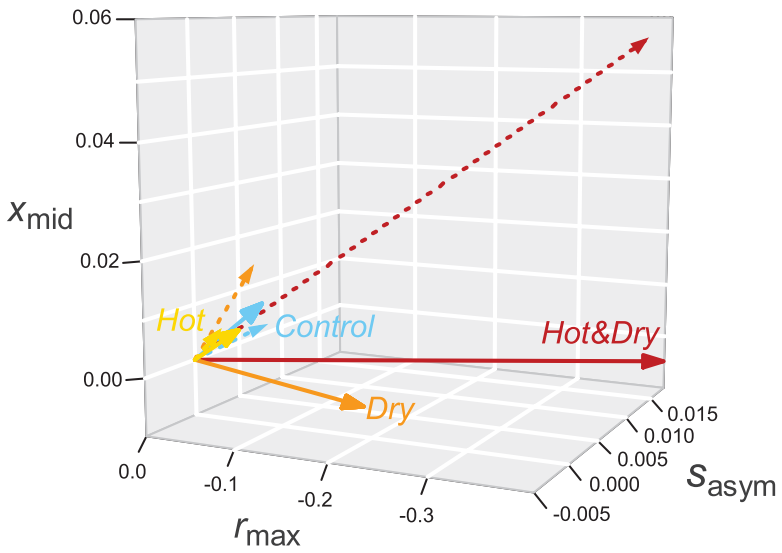

Figure 3. Direction and strength of viability selection (β, solid lines) and predicted selection response after two generations (Δ*z*, dotted lines) for each treatment along the three aspects of logistic growth (asymptotic size—s_{asym}, maximal growth rate—r_{max}, time to fastest growth—x_{mid}).

treatments. Heritability tended to be lower—across traits—in the *Control* (mean: 0.359, range: 0.278—0.440), the *Hot&Dry* treatment (0.370, 0.289—0.451), and the *Hot* treatment (0.477, 0.392—0.561), and higher in the *Dry* treatment (0.567, 0.481—0.653; Supplementary Figure S4). The maximal growth rate and the time to the mid-point of growth had heritabilities that were generally low across the four treatments (mean: 0.431 and 0.292, respectively; mean for asymptotic size: 0.606). Estimates of genetic and environmental variances as well as Houle's *I* varied across traits, with no consistent patterns across the four treatments (no systematic increase or decline with increasing stressfulness; Supplementary Figure S4).

G-matrices for allocation traits, as well as growth and allocation traits combined, revealed similar patterns as for growth traits. The three allocation traits had lower trace values, and differences among treatments were not significant (Supplementary Figure S5A). Furthermore, the dimensionality of G did not differ among treatments (Supplementary Figure S5B). The higher discrepancy between the selection vector and selection response was pronounced under *Dry* (Supplementary Figure S5C). However, the mean was about

four times smaller than for G-matrices with growth traits only. G-matrices, including allocation and growth traits, did not differ in trace or dimensionality among treatments, but the distance between the selection vector and selection response was again pronounced under *Dry* (Supplementary Figure S5D–F). Despite the seemingly low correlation between growth and allocation traits, the dimensionality of G when all six traits were included was considerably lower than the sum of n_D of the two separate matrices with three traits.

Discussion

There is no consensus on the causes of species' distribution limits when species have range limits that equal niche limits, as evolution should progress towards expanding the niche and the range if habitat is generally available (Sexton et al., 2009; Willi & Van Buskirk, 2019). Our study focussed on the potential contribution of genetic correlations constraining adaptive responses to cope with extreme conditions at range limits. We picked conditions typical for the low-latitude range limit of *A. lyrata*, as numerous studies have shown that warm margins of species' distributions are places where constrained evolution becomes most evident under climate change (Clark et al., 2020; Parmesan, 2006). The population studied was from the southerly centre of distribution with high genetic variation, which was assumed to make the detection of genetic correlations more likely. Furthermore, the population was reported to harbour genetic variation for traits that also vary along the latitudinal cline both in the eastern and western ancestral cluster of *A. lyrata* (see *Materials and methods* section, first paragraph). We found support that heat stress imposes multivariate selection to which the specific population can respond by adaptation. However, drought stress or the combination of heat and drought led to strong selection and in a direction away from high multivariate genetic variation, resulting in a high predicted lag of adaptation. We will discuss the results in light of aspects of G, the role of stress in affecting them, and what the results imply for low-latitude populations under climate warming. The focus is on traits of the growth trajectory.

Genetic variation and covariation in growth traits under climate stress

Across treatments, we found significant genetic variation in growth traits (Figure 2A). Similarly, broad-sense heritabilities were considerably too high (range of means across environments: 0.260—0.799), with the lowest for the trait of time to fastest growth. However, the trace of G and average heritabilities did not vary concordantly. While the sum of genetic variances did not differ significantly across treatments (Figure 2A), average heritability tended to be higher in the dry treatment (Supplementary Figure S4). Deviations were the result of environmental variances being relatively reduced under dry conditions. Furthermore, we found genetic covariances to be significant and important. They reduced the number of dimensions or sphericity of G by one-half relative to no correlations, and there was little variation in this among treatments (Figure 2B).

Environmental stress was hypothesized to either increase genetic variances or decrease them (Hoffmann & Merilä, 1999). Our results do not support a systematic increase or decrease of genetic variances or heritabilities under stress. The trace of G for growth and/or allocation traits did not significantly differ between treatments. Heritabilities across traits tended to be lowest in the benign and the most stressful environment. Furthermore, genetic and environmental variances did not reveal a linear-like pattern with stressfulness (Supplementary Figure S4). Another way of depicting genetic variation for individual traits was suggested for fitness-relevant traits, the variance standardized by the square of the trait mean (Houle, 1992). In previous research, those estimates were shown to increase consistently with the level of stress, including thermal stress (Willi et al., 2010, 2011). Here, the mean-standardized variances also did not reveal a linear-like pattern with stressfulness (Supplementary Figure S4), supporting inconsistent responses of genetic variation to stress.

Environmental stress has also been discussed to affect genetic correlations systematically. Empirical studies covering a wide range of taxa have documented that genetic correlations are ubiquitous, with the effective number of dimensions of G often being considerably lower than the number of traits studied (e.g., Chenoweth & Blows, 2008; Eroukhmanoff & Svensson, 2011; Kirkpatrick & Lofsvold, 1992; McGuigan & Blows, 2007; Mezey & Houle, 2005). In a previous study on *A. lyrata* populations of a latitudinal gradient, the dimensionality of Gs was relatively more reduced than shown here (Paccard et al., 2016), possibly because more traits were studied. Stearns (1992) argued that negative correlations between traits in regard to their fitness implications, or trade-offs, might be expressed more likely under considerable stress. Our results and those of Paccard et al. (2016), who applied a dry treatment, suggest that genetic correlations are not necessarily altered by stress. We found no significant changes in the dimensionality of G despite dry and hot-dry conditions being the most stressful (e.g., based on the effect on plant size).

Instead, our results and those of Paccard et al. (2016) point to the increased divergence between the direction of G and the direction of selection under water stress (Figures 2D and 3). Similar results were revealed in a meta-analysis by Wood and Brodie (2015). Despite only small differences in genetic correlations among environments, variation in the discrepancy (angle) between the direction of genetic correlations and the direction of selection was found to be considerable. The direction of multivariate genetic variation relative to the direction of selection plays a major role, as genetic constraints may seriously limit adaptive evolution only if they are directed against selection (Agrawal & Stinchcombe, 2009; Conner, 2002). Therefore, despite very similar G-matrices, the orientation of genetic constraints compared to selection as well as the strength of selection might be the most important factors for a species' adaptive potential under differing selection regimes (Arnold et al., 2008; Phillips & Arnold, 1999).

Lastly, a reason for some consistency in the magnitude of genetic covariances in growth and allocation traits may be their generally high integration. There was one consistent and considerable genetic correlation among growth traits, namely between the time to the mid-point of growth and the maximal growth rate of the logistic growth model (Supplementary Table S6; Supplementary Figure S2). Plants either grew early and fast, or they grew late and slowly. Furthermore, under combined stress, the two traits of time to the mid-point of growth and the maximal growth rate were tied in trade-offs with asymptotic size. Early-growing plants and plants that grew fast had a smaller final size, while late- and slow-growing plants achieved larger sizes. These results are in line with the slow-fast continuum suggested by Grime and

Hunt (1975) and later extended by Stearns (1992, 1983), that organisms either grow fast, have a short lifecycle, and are small, or the opposite. Support for the hypothesis is numerous (e.g., Oliveira et al., 2021; Salguero-Gómez, 2017; Sartori et al., 2019, 2022). Interestingly, a similar trade-off complex among the three growth traits was found in the latitudinal divergence matrices. To variable extents across treatments, time to the mid-point of growth and growth rate were negatively correlated, and, with the exception of one of these traits under heat, early and fast growth implied a smaller final size (Supplementary Table S8). Southern populations had generally smaller sizes within each of the two ancestral clusters, though the association with earlier and faster growth was not consistent (Supplementary Table S5).

Predicted selection response under climate stress at the low-latitude range edge

Unlike genetic variances and genetic correlations, the predicted ability to adapt varied significantly among treatments for growth traits, allocation traits, and all traits combined. On the one hand, selection was stronger both under drought and heat drought as compared to benign or hot conditions. This strongly affected the deviation between ideal and predicted selection response (Figure 2E; for allocation and all traits, see Supplementary Figure S5C and F). Under both drought and heat with drought, the deviation was high. This pattern was also depicted by the estimate of evolvability (evo_{HH}), though only the estimate under drought was significantly lower (Figure 2F; Supplementary Figure S3). On the other hand, the genetic correlations were involved in lowering the ability to adapt. However, and as discussed further up, what changed was that under drought and heat with drought, selection took a direction more antagonistic to the direction of the highest multivariate genetic variation; the genetic correlations changed little (Figures 2D and 3). Results confirm previous results by Lau et al. (2014) on *A. thaliana* that certain stressors, particularly combined stressors, impose strong selection, and combined stress reduces the evolutionary potential along a phenotypic selection gradient. Furthermore, they are in line with the constraining aspect of genetic correlations as found, e.g., in the transplant experiment by Etterson and Shaw (2001). Covering gradients of temperature and water availability, they showed that genetic correlations antagonistic to the direction of selection decreased the evolutionary potential in a plant despite considerable genetic variances and heritabilities in the traits under selection.

If drought and combined heat with drought become more frequent at the low-latitude range limit of *Arabidopsis lyrata*, this will seriously impede population persistence. Niche modelling indicated that temperature was the main driver of distribution limits in the south and north (Lee-Yaw et al., 2018; Sánchez-Castro et al., 2024). This suggests that the species occurs in areas with marginal temperature conditions at the range limit, which was confirmed in a transplant experiment with sites within and beyond the southern and northern range limits (Sánchez-Castro et al., 2024). With climate warming, drought will become an additional stressor. For the southern and eastern United States, climate change has been associated not only with increasing temperature but also with longer periods of drought (Easterling et al., 2017; Schepers et al., 2024; Vose et al., 2017). For several accessions of the closely related *A. thaliana*, Vile et al. (2012) found that the fitness proxy of biomass production was mostly higher under heat than drought conditions, suggesting that drought is more of a stressor than heat. A meta-study on a variety of organisms revealed a more even picture, whereby at low latitudes, water availability is of similar importance for survival than temperature (Pearce-Higgins et al., 2015). Given that temperatures are marginal at the southern range limits for *A. lyrata* and drought phases are increasing, our results of low adaptation potential under these conditions suggest that populations at the low-latitude range limit are at risk of extinction.

Conclusions

Our study shows that drought and combined heat and drought—at magnitudes that may occur in nature at the low-latitude range limit of *Arabidopsis lyrata*—impose strong selection on traits related to the growth trajectory. At the same time, multivariate genetic variation for these traits is reduced due to some consistent genetic correlations. Correlations generally follow the continuum between slow and fast growth and become more constraining under drought and combined heat and drought because selection takes a direction more antagonistic to the direction of high multivariate genetic variation. When occurring together, strong selection and such constrained genetic variation led to a relatively low predicted selection response. If the future climate exposes low-latitude populations of *A. lyrata* to drought or heat with drought more frequently, populations may therefore fail to persist due to excessive deaths linked with selection.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

Data will be available on DRYAD: https://doi.org/10.5061/dryad.2rbnzs7sw. The image analysis script (Supplementary Methods S2) is available at https://github.com/HeblackJ/automated_image_analysis.git or https://doi.org/10.5281/zenodo.10897248.

Author contributions

Jessica Heblack (Conceptualization [Equal], Data curation [Lead], Formal analysis [Lead], Methodology [Equal], Software [Lead], Visualization [Lead], Writing—original draft [Equal], Writing —review & editing [Equal]), Judith Schepers (Conceptualization [Equal], Data curation [Supporting], Formal analysis [Supporting], Methodology [Equal], Writing—original draft [Supporting], Writing—review & editing [Supporting]), and Yvonne Willi (Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Lead], Methodology [Equal], Writing—original draft [Equal], Writing—review & editing [Equal])

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Conflicts of interest

None declared.

References

Agrawal, A. F., & Stinchcombe, J. R. (2009). How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *276*(1659), 1183–1191. https://doi.org/10.1098/rspb.2008.1671

Aguirre, J. D., Hine, E., McGuigan, K., & Blows, M. W. (2014). Comparing G: Multivariate analysis of genetic variation in multiple populations. *Heredity*, *112*(1), 21–29. https://doi.org/10.1038/hdy.2013.12

Arnold, S. J. (1992). Constraints on phenotypic evolution. *The American Naturalist*, *140*(Suppl 1), S85–107. https://doi.org/10.1086/285398

Arnold, S. J., Bürger, R., Hohenlohe, P. A., … Jones, A. G. (2008). Understanding the evolution and stability of the G-matrix. *Evolution*, *62*(10), 2451–2461. https://doi.org/10.1111/j.1558-5646.2008.00472.x

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 48. https://doi.org/10.18637/jss.v067.i01

Bennett, J. M., Sunday, J., Calosi, P., … Olalla-Tárraga, M. (2021). The evolution of critical thermal limits of life on Earth. *Nature Communications*, *12*(1), 1198. https://doi.org/10.1038/s41467-021-21263-8

Blows, M. W., & Hoffmann, A. A. (2005). A reassessment of genetic limits to evolutionary change. *Ecology*, *86*(6), 1371–1384. https://doi.org/10.1890/04-1209

Chen, I. -C., Hill, J. K., Ohlemüller, R., … Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026. https://doi.org/10.1126/science.1206432

Chenoweth, S. F., & Blows, M. W. (2008). Q_{ST} meets the G matrix: The dimensionality of adaptive divergence in multiple correlated quantitative traits. *Evolution*, *62*(6), 1437–1449. https://doi.org/10.1111/j.1558-5646.2008.00374.x

Chung, Y., Rabe-Hesketh, S., Dorie, V., … Liu, J. (2013). A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. *Psychometrika*, *78*(4), 685–709. https://doi.org/10.1007/s11336-013-9328-2

Clark, J. S., Poore, A. G. B., Coleman, M. A., & Doblin, M. A. (2020). Local scale thermal environment and limited gene flow indicates vulnerability of warm edge populations in a habitat forming macroalga. *Frontiers in Marine Science*, *7*, 711. https://doi.org/10.3389/fmars.2020.00711

Clo, J., & Opedal, H. (2021). Genetics of quantitative traits with dominance under stabilizing and directional selection in partially selfing species. *Evolution*, *75*(8), 1920–1935. https://doi.org/10.1111/evo.14304

Conner, J. K. (2002). Genetic mechanisms of floral trait correlations in a natural population. *Nature*, *420*(6914), 407–410. https://doi.org/10.1038/nature01105

Easterling, D. R., Kunkel, K. E., Arnold, J. R., … Wehner, M. F. (2017). Chapter 7: Precipitation change in the United States. In D. J. Wuebbles, D. W. Fahey, K. A. Hibbard, D. J. Dokken, B. C. Stewart, & T. K. Maycock (Eds.), *Climate science special report: Fourth national climate assessment* (Vol. *I*, pp. 207–230). U.S. Global Change Research Program. https://doi.org/10.7930/J0H993CC

Elzhov, T. V., Mullen, K. M., Spiess, A. -N., & Bolker, B. (2022). *minpack.lm: R Interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, Plus support for bounds* (R package version 1.2-2) [Computer software]. https://CRAN.R-project.org/package=minpack.lm

Eroukhmanoff, F., & Svensson, E. I. (2011). Evolution and stability of the G-matrix during the colonization of a novel environment: G-matrix evolution. *Journal of Evolutionary Biology*, *24*(6), 1363–1373. https://doi.org/10.1111/j.1420-9101.2011.02270.x

Etterson, J. R., & Shaw, R. G. (2001). Constraint to adaptive evolution in response to global warming. *Science*, *294*(5540), 151–154. https://doi.org/10.1126/science.1063656

Exposito-Alonso, M., Vasseur, F., Ding, W., … Weigel, D. (2018). Genomic basis and evolutionary potential for extreme drought adaptation in *Arabidopsis thaliana*. *Nature Ecology & Evolution*, *2*(2), 352–358. https://doi.org/10.1038/s41559-017-0423-0

Falconer, D. S., & Mackay, T. (1996). *Introduction to quantitative genetics* (4th ed.). Pearson, Prentice Hall.

Fry, J. D. (2003). Detecting ecological trade-offs using selection experiments. *Ecology*, *84*(7), 1672–1678. https://doi.org/10.1890/0012-9658(2003)084[1672:detuse]2.0.co;2

Grime, J. P., & Hunt, R. (1975). Relative growth-rate: Its range and adaptive significance in a local flora. *The Journal of Ecology*, *63*(2), 393. https://doi.org/10.2307/2258728

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, *33*(2), 22. https://doi.org/10.18637/jss.v033.i02

Hansen, T. F., & Houle, D. (2008). Measuring and comparing evolvability and constraint in multivariate characters. *Journal of Evolutionary Biology*, *21*(5), 1201–1219. https://doi.org/10.1111/j.1420-9101.2008.01573.x

Hansen, T. F., Solvin, T. M., & Pavlicev, M. (2019). Predicting evolutionary potential: A numerical test of evolvability measures. *Evolution*, *73*(4), 689–703. https://doi.org/10.1111/evo.13705

Hargreaves, A. L., Samis, K. E., & Eckert, C. G. (2014). Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. *The American Naturalist*, *183*(2), 157–173. https://doi.org/10.1086/674525

Henry, G. A., & Stinchcombe, J. R. (2023). G-matrix stability in clinally diverging populations of an annual weed. *Evolution*, *77*(1), 49–62. https://doi.org/10.1093/evolut/qpac005

Hoffmann, A. A., & Blows, M. W. (1994). Species borders: Ecological and evolutionary perspectives. *Trends in Ecology & Evolution*, *9*(6), 223–227. https://doi.org/10.1016/0169-5347(94)90248-8

Hoffmann, A. A., & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, *14*(3), 96–101. https://doi.org/10.1016/s0169-5347(99)01595-5

Holt, R. D. (2003). On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research*, *5*, 159–178.

Holt, R. D., & Gaines, M. S. (1992). Analysis of adaptation in heterogeneous landscapes: Implications for the evolution of fundamental niches. *Evolutionary Ecology*, *6*(5), 433–447. https://doi.org/10.1007/bf02270702

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics*, *130*(1), 195–204. https://doi.org/10.1093/genetics/130.1.195

Irvine, J., Perks, M. P., Magnani, F., & Grace, J. (1998). The response of *Pinus sylvestris* to drought: Stomatal control of transpiration and hydraulic conductance. *Tree Physiology*, *18*(6), 393–402. https://doi.org/10.1093/treephys/18.6.393

Kirkpatrick, M. (2009). Patterns of quantitative genetic variation in multiple dimensions. *Genetica*, *136*(2), 271–284. https://doi.org/10.1007/s10709-008-9302-6

Kirkpatrick, M., & Barton, N. H. (1997). Evolution of a species' range. *The American Naturalist*, *150*(1), 1–23. https://doi.org/10.1086/286054

Kirkpatrick, M., & Lofsvold, D. (1992). Measuring selection and constraint in the evolution of growth. *Evolution*, *46*(4), 954–971. https://doi.org/10.1111/j.1558-5646.1992.tb00612.x

Kooyers, N. J. (2015). The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Science*, *234*, 155–162. https://doi.org/10.1016/j.plantsci.2015.02.012

Kruschke, J. K. (2018). Rejecting or accepting parameter values in Bayesian estimation. *Advances in Methods and*

Practices in Psychological Science, *1*(2), 270–280. https://doi.org/10.1177/2515245918771304

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain: Body size allometry. *Evolution*, *33*(1, Part2), 402–416. https://doi.org/10.1111/j.1558-5646.1979.tb04694.x

Lau, J. A., Shaw, R. G., Reich, P. B., & Tiffin, P. (2014). Indirect effects drive evolutionary responses to global change. *The New Phytologist*, *201*(1), 335–343. https://doi.org/10.1111/nph.12490

Lee-Yaw, J. A., Fracassetti, M., & Willi, Y. (2018). Environmental marginality and geographic range limits: A case study with *Arabidopsis lyrata* ssp. *lyrata*. *Ecography*, *41*(4), 622–634. https://doi.org/10.1111/ecog.02869

Lee-Yaw, J. A., Kharouba, H. M., Bontrager, M., … Angert, A. L. (2016). A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. *Ecology Letters*, *19*(6), 710–722. https://doi.org/10.1111/ele.12604

Lenoir, J., Bertrand, R., Comte, L., … Grenouillet, G. (2020). Species better track climate warming in the oceans than on land. *Nature Ecology & Evolution*, *4*(8), 1044–1059. https://doi.org/10.1038/s41559-020-1198-2

Liu, H., Ye, Q., & Wiens, J. J. (2020). Climatic-niche evolution follows similar rules in plants and animals. *Nature Ecology & Evolution*, *4*(5), 753–763. https://doi.org/10.1038/s41559-020-1158-x

McGuigan, K., & Blows, M. W. (2007). The phenotypic and genetic covariance structure of drosophilid wings. *Evolution*, *61*(4), 902–911. https://doi.org/10.1111/j.1558-5646.2007.00078.x

Mezey, J. G., & Houle, D. (2005). The dimensionality of genetic variation for wing shape in *Drosophila melanogaster*. *Evolution*, *59*(5), 1027–1038. https://doi.org/10.1111/j.0014-3820.2005.tb01041.x

Milocco, L., & Salazar-Ciudad, I. (2022). Evolution of the G matrix under nonlinear genotype-phenotype maps. *The American Naturalist*, *199*(3), 420–435. https://doi.org/10.1086/717814

Oliveira, R. S., Eller, C. B., Barros, F. D. V., … Bittencourt, P. (2021). Linking plant hydraulics and the fast–slow continuum to understand resilience to drought in tropical ecosystems. *New Phytologist*, *230*(3), 904–923. https://doi.org/10.1111/nph.17266

Paccard, A., Fruleux, A., & Willi, Y. (2014). Latitudinal trait variation and responses to drought in *Arabidopsis lyrata*. *Oecologia*, *175*(2), 577–587. https://doi.org/10.1007/s00442-014-2932-8

Paccard, A., Van Buskirk, J., & Willi, Y. (2016). Quantitative genetic architecture at latitudinal range boundaries: Reduced variation but higher trait independence. *The American Naturalist*, *187*(5), 667–677. https://doi.org/10.1086/685643

Paccard, A., Vance, M., & Willi, Y. (2013). Weak impact of finescale landscape heterogeneity on evolutionary potential in *Arabidopsis lyrata*. *Journal of Evolutionary Biology*, *26*(11), 2331–2340. https://doi.org/10.1111/jeb.12220

Paquette, A., & Hargreaves, A. L. (2021). Biotic interactions are more often important at species' warm versus cool range edges. *Ecology Letters*, *24*(11), 2427–2438. https://doi.org/10.1111/ele.13864

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Patsiou, T., Walden, N., & Willi, Y. (2021). What drives species' distributions along elevational gradients? Macroecological and -evolutionary insights from Brassicaceae of the central Alps. *Global Ecology and Biogeography*, *30*(5), 1030–1042. https://doi.org/10.1111/geb.13280

Pearce-Higgins, J. W., Ockendon, N., Baker, D. J., … Tanner, E. V. J. (2015). Geographical variation in species' population responses to changes in temperature and precipitation. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *282*(1818), 20151561. https://doi.org/10.1098/rspb.2015.1561

Phillips, P. C., & Arnold, S. J. (1999). Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution*, *53*(5), 1506–1515. https://doi.org/10.1111/j.1558-5646.1999.tb05414.x

Polechová, J. (2018). Is the sky the limit? On the expansion threshold of a species' range. *PLoS Biology*, *16*(6), e2005372. https://doi.org/10.1371/journal.pbio.2005372

Polechová, J., & Barton, N. H. (2015). Limits to adaptation along environmental gradients. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(20), 6401–6406. https://doi.org/10.1073/pnas.1421515112

Puentes, A., Granath, G., & Ågren, J. (2016). Similarity in G matrix structure among natural populations of *Arabidopsis lyrata*: G matrix structure in populations of *A. lyrata*. *Evolution*, *70*(10), 2370–2386. https://doi.org/10.1111/evo.13034

Puijalon, S., Bouma, T. J., Douady, C. J., … Bornette, G. (2011). Plant resistance to mechanical stress: Evidence of an avoidance–tolerance trade-off. *The New Phytologist*, *191*(4), 1141–1149. https://doi.org/10.1111/j.1469-8137.2011.03763.x

R Core Team. (2021). *R: A language and environment for statistical computing* [Computer software]. R Foundation for Statistical Computing. https://www.R-project.org/

Roff, D. A., & Fairbairn, D. J. (2012). The evolution of trade-offs under directional and correlational selection: Selection on a trade-off. *Evolution*, *66*(8), 2461–2474. https://doi.org/10.1111/j.1558-5646.2012.01634.x

Roff, D. A., Mostowy, S., & Fairbairn, D. J. (2002). The evolution of trade-offs: Testing predictions on response to selection and environmental variation. *Evolution*, *56*(1), 84–95. https://doi.org/10.1111/j.0014-3820.2002.tb00851.x

Rumpf, S. B., Hülber, K., Klonner, G., … Dullinger, S. (2018). Range dynamics of mountain plants decrease with elevation. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(8), 1848–1853. https://doi.org/10.1073/pnas.1713936115

Salguero-Gómez, R. (2017). Applications of the fast–slow continuum and reproductive strategy framework of plant life histories. *New Phytologist*, *213*(4), 1618–1624. https://doi.org/10.1111/nph.14289

Sánchez-Castro, D., Patsiou, T., Perrier, A., … Willi, Y. (2024). Uncovering the cause of breakup between species' range limits and niche limits under climate warming. *Journal of Biogeography*, 1–14. https://doi.org/10.1111/jbi.14796

Sartori, K., Vasseur, F., Violle, C., … Vile, D. (2019). Leaf economics and slow-fast adaptation across the geographic range of *Arabidopsis thaliana*. *Scientific Reports*, *9*(1), 10758. https://doi.org/10.1038/s41598-019-46878-2

Sartori, K., Violle, C., Vile, D., … Kazakou, E. (2022). Do leaf nitrogen resorption dynamics align with the slow-fast continuum? A test at the intraspecific level. *Functional Ecology*, *36*(5), 1315–1328. https://doi.org/10.1111/1365-2435.14029

Schepers, J. R., Heblack, J., & Willi, Y. (2024). Negative interaction effect of heat and drought stress at the warm end of species distribution. *Oecologia*, *204*(1), 173–185. https://doi.org/10.1007/s00442-023-05497-5

Schluter, D. (1996). Adaptive radiation along genetic lines of least resistance. *Evolution*, *50*(5), 1766–1774. https://doi.org/10.1111/j.1558-5646.1996.tb03563.x

Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317

Sheth, S. N., & Angert, A. L. (2016). Artificial selection reveals high genetic variation in phenology at the trailing edge of a species range. *The American Naturalist*, *187*(2), 182–193. https://doi.org/10.1086/684440

Stearns, S. C. (1983). The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. *Oikos*, *41*(2), 173. https://doi.org/10.2307/3544261

Stearns, S. C. (1992). *The evolution of life histories* (1st ed.). Oxford University Press.

Upadhyay, P. (2019). Climate change and adaptation strategies: A study of agriculture and livelihood adaptation by farmers in Bardiya District, Nepal. *Advances in Agriculture and Environmental Science:*

Open Access (AAEOA), *2*(1), 47–52. https://doi.org/10.30881/aaeoa.00022

Vile, D., Pervent, M., Belluau, M., … Simonneau, T. (2012). Arabidopsis growth under prolonged high temperature and water deficit: Independent or interactive effects?: Plant responses to high temperature and water deficit. *Plant, Cell & Environment*, *35*(4), 702–718. https://doi.org/10.1111/j.1365-3040.2011.02445.x

Vose, R. S., Easterling, D. R., Kunkel, K. E., … Wehner, M. F. (2017). Chapter 6: Temperature changes in the United States. In D. J. Wuebbles, D. W. Fahey, K. A. Hibbard, D. J. Dokken, B. C. Stewart, & T. K. Maycock (Eds.), *Climate science special report: Fourth national climate assessment*. (Vol. *I*, pp. 185–206). U.S. Global Change Research Program. https://doi.org/10.7930/J0N29V45

Walsh, B., & Chenoweth, S. F. (2017). *Summer institute in statistical genetics—Module1: Principles of quantitative genetics*. https://cnsgenomics.com/data/teaching/SISG/module_1/Module01-Lecture-Notes.pdf

Willi, Y. (2013). The battle of the sexes over seed size: Support for both kinship genomic imprinting and interlocus contest evolution. *The American Naturalist*, *181*(6), 787–798. https://doi.org/10.1086/670196

Willi, Y., Follador, R., Keller, N., … McDonald, B. A. (2010). Heritability under benign and stressful conditions in the plant pathogenic fungus *Mycosphaerella graminicola*. *Evolutionary Ecology Research*, *12*, 761–768.

Willi, Y., Fracassetti, M., Zoller, S., & Van Buskirk, J. (2018). Accumulation of mutational load at the edges of a species range. *Molecular Biology and Evolution*, *35*(4), 781–791. https://doi.org/10.1093/molbev/msy003

Willi, Y., Frank, A., Heinzelmann, R., … Ceresini, P. C. (2011). The adaptive potential of a plant pathogenic fungus, *Rhizoctonia solani* AG-3, under heat and fungicide stress. *Genetica*, *139*(7), 903–908. https://doi.org/10.1007/s10709-011-9594-9

Willi, Y., & Van Buskirk, J. (2019). A practical guide to the study of distribution limits. *The American Naturalist*, *193*(6), 773–785. https://doi.org/10.1086/703172

Willi, Y., & Van Buskirk, J. (2022). A review on trade-offs at the warm and cold ends of geographical distributions. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *377*(1848), 20210022. https://doi.org/10.1098/rstb.2021.0022

Wolak, M. E., & Keller, L. F. (2014). Dominance genetic variance and inbreeding in natural populations. In A. Charmentier, D. Garant, & L. E. B. Kruuk (Eds.), *Quantitative genetics in the wild*. Oxford University Press.

Wood, C. W., & Brodie, E. D. (2015). Environmental effects on the structure of the G-matrix: Environmental effects on genetic correlations. *Evolution*, *69*(11), 2927–2940. https://doi.org/10.1111/evo.12795

Wos, G., & Willi, Y. (2015). Temperature-stress resistance and tolerance along a latitudinal cline in North American *Arabidopsis lyrata*. *PLoS One*, *10*(6), e0131808. https://doi.org/10.1371/journal.pone.0131808

Wos, G., & Willi, Y. (2018). Genetic differentiation in life history traits and thermal stress performance across a heterogeneous dune landscape in *Arabidopsis lyrata*. *Annals of Botany*, *122*(3), 473–484. https://doi.org/10.1093/aob/mcy090

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RESEARCH ARTICLE

Uncovering the cause of breakup between species' range limits and niche limits under climate warming

Darío Sánchez-Castro[1] | Theofania-Sotiria Patsiou[2] | Antoine Perrier[1] | Judith Schepers[1] | Yvonne Willi[1]

[1]Department of Environmental Sciences, University of Basel, Basel, Switzerland

[2]Institute of Plant Sciences, Department of Biology, University of Bern, Bern, Switzerland

Correspondence
Yvonne Willi, Department of Environmental Sciences, University of Basel, Basel 4056, Switzerland.
Email: yvonne.willi@unibas.ch

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Abstract

Aim: Global climate change has been linked to shifts in species' geographic and elevational distributions, with taxa varying in responsiveness. This variation may be due to a time lag in response or climate alone not being a simple determinant of distribution limits. To tease apart the role of climate in distribution, we compared the temperature response of predicted occurrence revealed by ecological niche modelling (ENM) on historical climate with that of performance in a multi-population transplant experiment. Congruence would support that climate is a main driver of distribution limits of a species.

Location: North America.

Taxon: *Arabidopsis lyrata* subsp. *lyrata*.

Methods: Seeds of 20 populations of North American *Arabidopsis lyrata* from across the entire range were collected, propagated and then sown along a latitudinal transect across and beyond the species' range. Lifetime performance was related to the main niche- and range-determining climatic variable revealed by ENM.

Results: Lifetime performance did not consistently decline towards the high-latitude range limit, but it did so towards the low-latitude range limit. This decline was slightly weaker for low-latitude populations, indicating divergent climate adaptation. The overall performance curve on the field-measured minimum temperature in early spring was fairly congruent with the curve of ENM-predicted suitability on this important niche-determining variable. ENM-based projections revealed that the southernmost populations were vulnerable under climate warming.

Main Conclusions: Results verified that ENM based on species occurrences can well-predict plant performance under field conditions. Congruence teaches us that with the climate change so far, the species exhibits a colonisation deficit in the north. Furthermore, the southernmost populations are vulnerable to extinction. A likely outcome is the shrinking of the species' range.

KEYWORDS

Arabidopsis lyrata, climate warming, ecological niche, local adaptation, niche limit, range boundary, species distribution modelling, transplant experiment

Darío Sánchez-Castro and Theofania-Sotiria Patsiou contributed to this paper equally.

1 | INTRODUCTION

Current climate warming has been linked to range shifts of species to higher latitudes and elevations (Chen et al., 2011). Shifts have been observed across many taxa, including plants, insects, birds and mammals (e.g. Fazlioglu et al., 2020; Geppert et al., 2020; Gillings et al., 2015; McCain et al., 2021; McCain & Garfinkel, 2021; Rumpf et al., 2018). However, research in the field has also documented great variability in the extent of range shifts among species in response to increasing temperatures (e.g. Lenoir et al., 2008; Parmesan & Yohe, 2003). Deviations from precise tracking were hypothesised to be due to colonisation lags at the cold ends and extinction lags at the warm ends of ranges, to ecological factors other than temperature, or some interactions affecting distributions (Chen et al., 2011). At the same time as reports on range shifts started increasing, predictive modelling was used more widely to project the future distribution of species under climate warming scenarios, working with the assumption of temperature and, more generally, climate tracking (reviewed in Pearson & Dawson, 2003). Both observations on incomplete range shifts and predictive modelling can be interpreted meaningfully only if we know the link between temperature and species' distribution. So far, efforts to verify this link in field experiments have been rare.

The extent to which climatic factors determine species' geographic distributions has—in recent years—been mainly based on relating species' occurrence data with climatic variables. Correlative approaches were developed, termed ecological niche modelling (ENM), species distribution modelling (SDMs) or habitat suitability modelling (HSM; Guisan et al., 2017, p. 8). One premise of modelling is that geographic distributions generally reflect niche limits, and that the environment and species' ranges are in some equilibrium that established over time (Guisan & Zimmermann, 2000). Even though modelling is not restricted to climate variables, they are typically included, which is appropriate given that large-scale distribution limits have long been shown to be explained well by one or few climate variables (e.g. Boucher-Lalonde et al., 2012; Chown & Gaston, 1999; Normand et al., 2009; Patsiou et al., 2021; Root, 1988; Woodward, 1987). Nevertheless, modelling is more likely to characterise the realised niche instead of the fundamental niche (sensu Hutchinson, 1957), as the input variables are based on where the species is found, and not where it could be found due to abiotic conditions (and resource availabilities) alone. The alternative has been mechanistic niche modelling (Kearney & Porter, 2009). The approach often integrates immediate physiological and behavioural responses of organisms to temperature (or other environmental factors) to predict spatial distributions. Particularly the former approach has been used intensively for projecting distributions under climate warming (e.g. Di Febbraro et al., 2018; Normand et al., 2013; Thomas et al., 2004; Thuiller et al., 2005).

An alternative approach to study whether specific factors, such as certain temperature aspects, determine distribution limits is transplant experiments over environmental gradients. Transplant experiments have been considered the gold standard of testing if they include sites within and beyond a species' range and if lifetime performance of organisms is assessed (Hargreaves et al., 2014). Such set-ups allow testing whether populations are generally viable within the range and some part of the environmental gradient but not outside of the range, at the environmental extremes, therefore unequivocally documenting whether range limits reflect niche limits. Added insight comes from including populations from range edges and the core of distribution to detect intraspecific variation in performance under different conditions, thus, divergent adaptation (Kawecki & Ebert, 2004). Hargreaves et al. (2014) performed a meta-level analysis on transplant experiments across geographic (latitudinal and longitudinal) and elevational range limits, and they found that lifetime performance declined from within to beyond the range edge in 75% and 88% of the experiments, respectively, and that populations grown beyond the range were not long-term viable in all cases. Even though distances of beyond-range sites from the range edge were variable, it can be assumed that transplant sites across latitude and elevation covered temperature gradients, and therefore, results reflect that range limits—at least in part—are due to temperature niche limits.

Better insight into the role of temperature in distribution may come from the combined modelling and field experimentation (Lee-Yaw et al., 2016; Willi & Van Buskirk, 2019). The combined results can tell about the mechanisms involved in the discordance between climate niche limits and range limits under current and upcoming climate change. Specifically the response curves of the predicted distribution derived from ENM (e.g. HOF curves; Huisman et al., 1993; Oksanen & Minchin, 2002; Jansen & Oksanen, 2013) can point to ecologically significant variables driving the distribution of species. A transplant experiment can provide similar data, namely lifetime performance on the same niche- and range-determining environmental variables. If the two types of curves for an important climate variable align, we have good evidence that climate has been important in shaping the niche and a species' range, and that ENM is reliable in predicting distributions. At this point, other variables, including biotic factors, can be considered as less important players, which means that current distribution can be interpreted in terms of a lag or its absence of tracking climate change. Lee-Yaw et al. (2016) used ENM based on climatic variables to verify whether range limits equal niche for the transplants considered in Hargreaves et al. (2014) and found congruence between methods in 31 of 40 cases, supporting that range limits often reflect climate niche limits.

Here, we tested for accordance between plant responses to climate once revealed by environmental niche modelling and once by a transplant experiment to conclude about the species' responsiveness to climate change. Testing included populations from across a species' range, with differences in history and climatic backgrounds varying from temperate to boreal, to check for within-species consistency in accordance. Our study organism was the short-lived perennial plant *Arabidopsis lyrata* subsp. *lyrata* of North America. A previous study on range limits by environmental niche modelling revealed that suitability was predicted well by minimum temperature

in early spring (Lee-Yaw et al., 2018). Furthermore, specific testing indicated that low- and high-latitude range limits coincided well with a decline in predicted suitability. We ran an updated version of ENM with more species' occurrences, recent climate data and four algorithms. We found that minimum temperature in early spring remained one of the most important predicting variables. The transplant experiment covered a wide latitudinal gradient and included sites across the species' distribution and beyond (data analysed in the context of drift effects at range limits in Perrier et al., 2020, 2022; Sánchez-Castro, Perrier et al., 2022). We assessed whether the performance curve on minimum temperature in early spring across the 3 years of the experiment had the same shape as the density curve of ENM-predicted suitability on the same variable (I), whether populations differed in the performance curve depending on their origin (II), and when in life patterns established (III). Results were used to interpret current distribution in the light of the species' ability to respond to climate change by shifting its range.

2 | MATERIALS AND METHODS

North American *A. lyrata* subsp. *lyrata* (from now on abbreviated as *A. lyrata*) occurs in the eastern United States, from North Carolina to Upstate New York, and in the Midwest, from Missouri to southwestern Ontario (few populations occur westward up to southern Alberta; Willi et al., 2022). Populations are mostly self-incompatible and mainly pollinated by wild bees and syrphids (Sánchez-Castro, Armbruster et al., 2022). Some populations had been described as self-compatible and selfing, with a bias in distribution at the edges of the species' eastern and western ranges (Griffin & Willi, 2014). The habitat of the species includes dynamic sand deposits or rocky areas on the shores of larger lakes and rivers, eroded sandstone or rocky outcrops, such as those found on the knobs of the Appalachians.

2.1 | ENM modelling

2.1.1 | Occurrences

The *Arabidopsis lyrata* occurrence data set was compiled with data from Lee-Yaw et al., (2018), the GBIF database with occurrence data entries after 1960 (GBIF.org, September 14, 2022, https://doi.org/10.15468/dl.vv75kt) and various herbaria collections. We excluded occurrence points of northern Alberta and Saskatchewan as they were found to consist of hybrids with *Arabidopsis arenicola* in a secondary contact zone formed after the divergence of the two species (Willi et al., 2022). We first thinned the occurrences to one per square kilometre to match the resolution of the climate data. We found no spatial autocorrelation of occurrence data based on a Mantel correlogram. However, to reduce sampling bias, we thinned to the median Euclidean distance among occurrences (i.e. c.a. 10–11 km). The final data set used for the ENM analysis for *A. lyrata* contained 433 occurrences (of the initial 1656).

2.1.2 | Climate variables and variable selection

We used the CHELSA v2 climate data set 1980–2010 (Brun et al., 2022; Karger et al., 2017). Topographic, soil and vegetation variables were not included as they received no support in earlier modelling (Lee-Yaw et al., 2018). We removed highly correlated variables (correlation coefficient threshold between −0.6 and 0.6) and further tested for collinear structure in the data using the variance inflation factor (VIF; using the *vif_func* function, https://gist.github.com/fawda123/4717702#file-vif_fun-r). The final data set included six ecologically meaningful variables for running ENMs: minimum temperature in early spring (TMinESpr), mean temperature of the growing season (TMeanGrS), frost change frequency (FrostChFreq), snow water equivalent (SnowWEq), precipitation of the coldest quarter (PrecColdQ) and precipitation of the warmest quarter (PrecWarmQ).

2.1.3 | Niche modelling algorithms and settings

All analyses were run with R v4.2 (R Core Team, 2022). We used the biomod2 package (Thuiller et al., 2023) and four models/algorithms: generalised lineal model (GLM; McCullagh & Nelder, 1989), maximum entropy model (MaxEnt; Phillips et al., 2006), Random Forest (RF; Breiman, 2001) and boosted regression trees (GBM; Friedman, 2001) (see supplementary information for model/algorithm settings). We assessed algorithm performance (Table S1) by Kappa statistic, maximum true skill statistic (TSS) and by receiver operating characteristic (ROC) curve (explained in Allouche et al., 2006). Variable permutation importance (Table S2) was assessed by permuting each variable 100 times. We produced model ensembles by committee averaging of binary projections based on maximum TSS and equal weight for using the threshold value that maximised specificity (true-negative rate) and sensitivity (true-positive rate), with consensus (4/4 algorithms agree), majority (3/4), mean (2/4) and minority agreement (1/4). We summarised the niche space in two axes of variation using a density grid (ecospat package; Di Cola et al., 2017).

We then projected the predicted distribution for the period 2011–2040 to compare it with the potential suitable distribution of the recent-historical reference period. We used five CMIP6 climate models of the CHELSA v2.1 data set with the ssp585 (i.e. rcp 8.5 carbon emission scenario) as a more likely scenario (Karger et al., 2017). The five models used were gfdl-esm4, ukesm1-0-ll, mpi-esm1-2-hr, ipsl-cm6a-lr and mri-esm2-0 (documentation: https://chelsa-climate.org/wp-admin/download-page/CHELSA_tech_specification_V2.pdf). We summed the binary projection across the four algorithms per climate model and divided values by four. We then summed across the five climate models, considered as presence values ≥2 and rescaled values to a range of 0–4 (to match the range of near-historical projection values). To refine predictions for different parts of the range, we performed cluster analysis on reported occurrences based on the climate attributes of the variables used in ENM. We used the K-means algorithm of the eclust function (100 times bootstrapping; factoextra package; Kassambara & Mundt, 2020).

2.2 | Transplant experiment

We selected 20 populations of *A. lyrata*, covering the total distribution of the species in North America (Figure 1, Table S3). Mature fruits of 30–50 plants were collected over a surface area of about 450 m^2 at each site in 2007, 2011 or 2014. We grew a first generation of plants indoors and performed within-population crosses to reduce carry-over effects of the sites of origin and time since collection. Twenty-six plants of different seed families per population were grown in pots in a greenhouse and randomly designated to be pollen-receiving mother plants (12), pollen-donating father plants (12) or backup plants (2). Plants of the same population were assigned to pairs, and hand pollinations were performed on emasculated flowers at the bud stage to avoid cross contamination. We repeated all cross combinations until six to seven fruits or about 60 viable seeds were obtained, having a total of 224 seed/cross combinations for the transplant experiment.

Five transplant sites were set up along a latitudinal gradient in the eastern United States based on the position relative to the species' range and suitability: beyond the northern range edge in the Adirondacks, NY (CG1; ENM suitability score of 0); near the northern range edge in Williamstown, MA (CG2; 4); in the centre of distribution in Harrisonburg, VA (CG3; 2); at the southern range edge in Winston-Salem, NC (CG4; 3); and beyond the southern range edge in Athens, GA (CG5; 0) (Figure 1, Table S4). At each site, the date of sowing was selected as 6 weeks before the long-term daily mean temperature fell to 10°C. Sowing started in August 2017 at the site beyond the northern edge (CG1) and ended 2 months later at the southernmost site (CG5). Sowing had to be repeated at the southern range edge (CG4) in December of the same year.

At each transplant site, two seeds from the same cross combination were sown in each of three replicate pots. The three pots were then haphazardly split into three spatial blocks, and within blocks, they were randomly assigned to 13 multi-pot trays with 38 pots each (not all pots filled with seeds were analysed here; others contained between-population crosses; see Perrier et al., 2020, 2022). Pots were perforated at the bottom to allow root growth into the local soil. They had a diameter of 7 cm and a depth of 6 cm, filled with a 1.5:1 mix of unfertilised peat moss and washed sand. A total of 7098 seeds were sown across transplant sites (5 transplant sites x 20 populations ×12 crosses ×3 blocks ×2 seeds per pot, minus 102 missing seeds because of low seed numbers for some lines, Table S5). Pots were placed outdoors in the field. Initially, a temporary greenhouse and a white mesh cloth (for up to 19 days) protected the pots and seedlings from being washed away by heavy rain, high sun exposure and rapid drying. Tubs were watered regularly until the first snow or night frost, because the species germinates only under prolonged phases of high soil moisture, which can occur naturally at the sites at any time during most of the year but with high variability among sites. An exception was sowing in

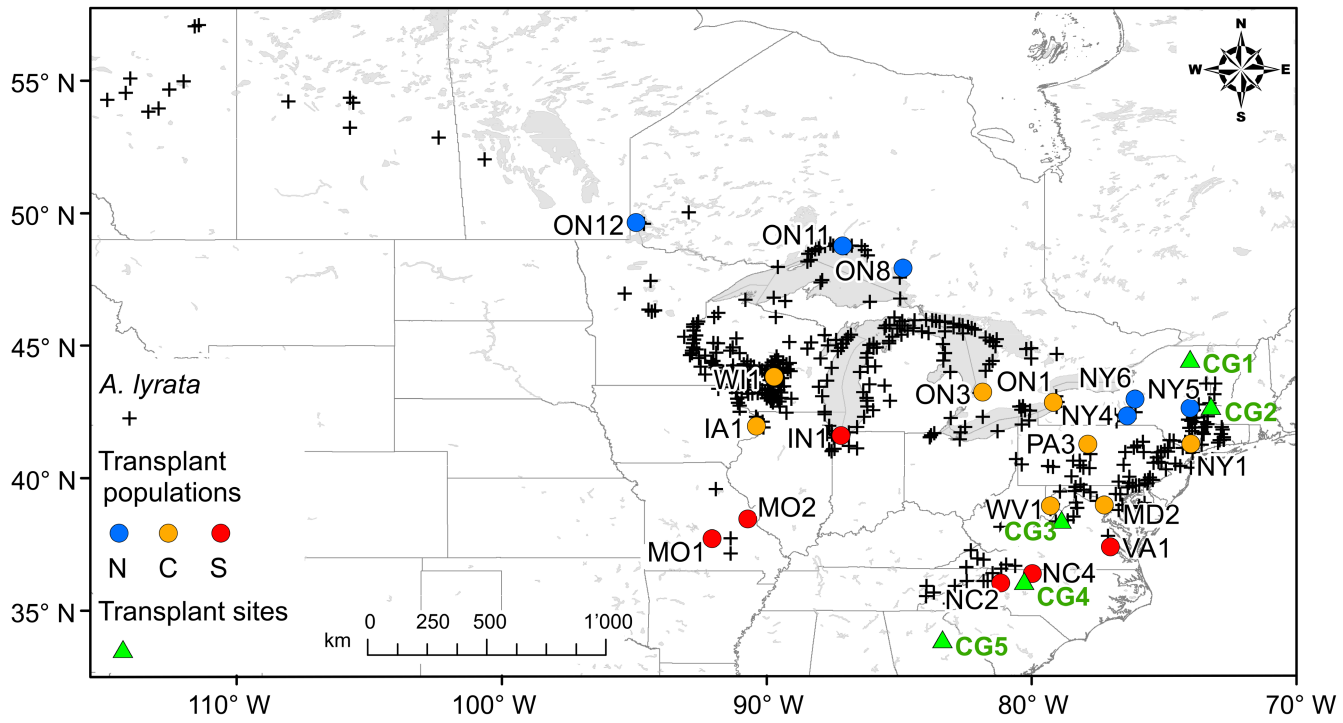

FIGURE 1 Map of the distribution of *Arabidopsis lyrata*, with information on the populations studied and the transplant sites. Black crosses indicate occurrences of *A. lyrata* (without the region of hybrids with *A. arenicola* at Lake Athabasca). Coloured circles accompanied by a three-digit abbreviation represent the sites of origin of *A. lyrata* populations included in the transplant experiment (Table S3). The populations were categorised relative to their latitudinal position within the western (with Lake Erie population ON1) and eastern ancestral clusters (Willi et al., 2018) in southern-edge (red), centre (orange) and northern-edge (blue). Green triangles represent the locations of transplant sites, abbreviated with CG (common garden) and a number indicating the sequence of initial sowing.

CG4; it had to be redone; and to speed up germination and initial growth before winter, plants were kept in a ventilated greenhouse before being placed in the field. Thereafter, transplants were exposed to the natural local conditions at each site. Plant competition was avoided by surrounding the multi-pot trays with a black foil, and herbivory by larger mammals was prevented by a fence around the experiment. With this procedure, we aimed at climate being the main factor causing differences in performance between sites.

Plant performance was recorded weekly, starting with germination in autumn 2017 until the end of the reproductive season in June 2019 (see Table S6 for the description of traits recorded). If two seedlings germinated in the same pot, one was removed haphazardly, keeping one plant per pot. *Reproductive output* was measured in 2018 and 2019, 9 weeks after the first few plants flowered at each site in year 2, and 5 weeks after flowering began in year 3. Reproductive output was calculated as the total number of fruits, pedicels (flowers that did not produce a fruit but possibly contributed with pollen), flowers and buds. We calculated lifetime *multiplicative performance* (*MP*) as *germination rate* observed in a pot multiplied by the reproductive output. The transplant site in the centre (CG3) needed to be removed in fall 2018, and therefore, analysis on *MP* up to year 2 was also performed. The following additional variables related to performance were recorded: survival, time to flowering, damage and severity of damage on rosettes and inflorescences, and root length at the end of the experiment (description in Table S6, further details under Statistical Analysis).

At each transplant site, temperature loggers recorded data hourly. The data loggers were installed 1.5 m above the ground, in the shade and close to the transplant site. The mean minimum temperature in early spring, during March and April, was calculated for each site as the average of daily minimum temperatures of March and April, averaged across months and then averaged across the two spring periods the experiment was running. Precipitation data for the transplant sites were obtained from PRISM (http://prism.oregonstate.edu) for the duration of the experiment. For comparison, minimum temperature in early spring and precipitation during the wettest quarter were extracted from the WorldClim database v2.1 (Fick & Hijmans, 2017) for the transplant sites and the sites of origin of populations. The climate was generally warmer during the experiment compared to averages between 1970 and 2000 (Table S4).

2.3 | Statistical analysis

2.3.1 | Performance curve on minimum temperature in early spring, and differences among populations

All main analyses were performed on multiplicative performance as the dependent variable, on pot-level data and population means (means of transplant sites in Table S7). Analysis on the level of the pot was performed within a Bayesian framework (MCMCglmm in R; Hadfield, 2010; R Core Team, 2019) because *MP* was 0-inflated and required the analysis of both the logistic part (0s vs. >0, with the latter coded as 1) and the Gaussian part of the distribution (values $\log_{10}$-transformed if >0). MCMCglmm was run on 10 parallel chains, with a burn-in of 5000, thinning every 100 iterations, with 200,000 iterations in total. Analysis on the level of the population used linear mixed-effects models (LM; package lme4; Bates et al., 2015). Fixed effects were mean minimum temperature in early spring, its square term (both centred to a mean of 0), origin of populations (southern edge, centre and northern edge, with the reference being the centre populations) and the two-way interactions with temperature. Random effects were common garden, block nested within common garden, population and family nested within population in the analysis on the level of the pot, and common garden and population in the analysis on the level of the population.

2.3.2 | When in life patterns established across transplant sites and populations

Life stages were tested for their contribution to performance in the common gardens at the level of the pot using generalised linear mixed-effects model (GLMM), and at the population level. In the former case, *germination* and *survival* were estimated as binary variables (0, 1). $Survival_{year\ 1}$ took into account the germination state (i.e. NA if the seed did not germinate; 0 if the plant died before the end of winter in 2017/18; 1 if it survived); and $survival_{year\ 2}$ was based on *survival* in year 1 (i.e. NA if the plant had died before). *Damage* on rosettes or inflorescences was also treated as binary. *Time to flowering*, *damage severity* (fraction in four categories; 0.25: >0%–25%, 0.5: 26%–50%, 0.75: 51%–75%, 1: 76%–100% of leaf or inflorescence tissue affected), *reproductive output* and *root length* were continuous variables. All these variables were analysed individually with restricted maximum likelihood, with the R package lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017). Fixed and random effects were the same as specified for performance.

3 | RESULTS

ENM confirmed that temperature, especially minimum temperature in early spring, was a significant predictor of *A. lyrata* distribution (Figure 2; density of occurrence aligns with the vector of TMinESpr). Furthermore, the 20 populations used in the transplant experiment varied considerably along this particular temperature axis. This justified the comparison of curves on the minimum temperature in early spring and considering the origin of populations in further analyses.

3.1 | Performance curve on minimum temperature in early spring, and differences among populations

We first analysed performance estimated as population means on temperature (Table 1). The linear and the quadratic terms of

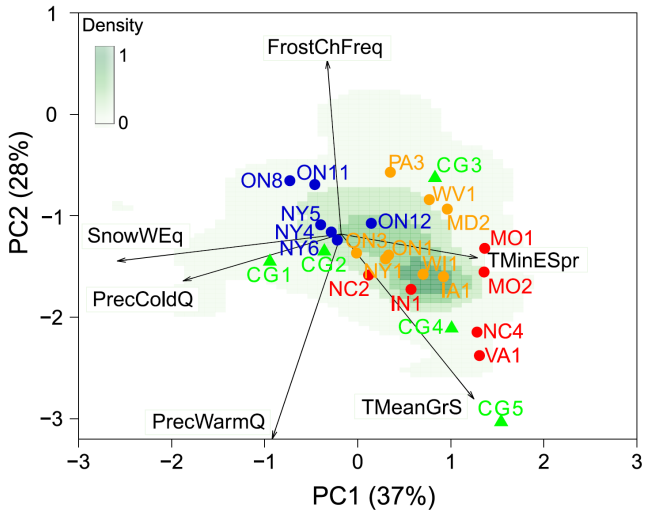

FIGURE 2 Position of the 20 transplant populations in multivariate niche space as predicted by ecological niche modelling. Ordination diagram based on the first two axes of a principle component analysis on climate in suitable areas predicted by the consensus ensemble ENM. Eigenvectors of the climate variables in two major axes indicate niche variation. The position of the abbreviations for populations (of southern edge in red, centre in orange and northern edge in blue) and transplant sites (CG, written in green) reflects their place in climate niche space. Shades of green represent the predicted suitability density in niche space. The graph illustrates that suitability aligns well with minimum temperature in early spring, with southern-edge populations tending to be at the higher end of that axis and northern-edge populations at the lower end.

minimum temperature in early spring were significant and negative, indicating that multiplicative performance was highest in the mid-range and declined less towards cooler temperatures than warmer temperatures (Figure 3a,b). We found neither a significant effect of the origin of populations nor interactions between the origin of populations and temperature. Pot-level analysis deviated in that they revealed that southern-edge populations, compared to centre populations, showed less of a decline in performance towards increasing temperatures, and northern populations showed a less negative curvilinear relationship between temperature and performance (Table S8). These deviations were observed only for the logistic aspect of performance. For the log-normal aspect, slightly significant positive effects were revealed for temperature and its square term independent of origin, suggesting compensatory effects.

The curve of the population mean performance on the minimum temperature in early spring predicted by data of the transplant experiment was in good agreement with the curve of ENM-predicted suitability on the same temperature variable (Figure 3c,d). For multiplicative performance, the optimum temperature was −1.0°C, and 0.2*optimum was at −8.5 and 6.6°C. The curve of predicted occurrence in early spring measured between 1980 and 2010 had the optimum very close, at −1.7°C, and 0.2*optimal suitability was at −7.9 and 6.9°C.

3.2 | When in life patterns established across transplant sites and populations

Further analyses focused on life stage components, on the level of population means (Table 1) and the level of the pot (Table S9). For germination, a significant positive linear effect and a negative quadratic effect of temperature were found in both analyses, with the pattern of increasing germination with increasing temperature up to a peak at the southern range edge (Figure S1a). Survival in the first year and additionally in the second year in pot-level analysis, however, declined significantly with increasing temperature, with a pattern of strong decline in survival between the northern beyond-range site to the centre in the first year, and between the northern beyond-range site to the northern edge in the second year (Figure S1c,d). Time to flowering revealed significant positive coefficients for the linear and the quadratic terms of temperature; plants flowered early in the north and centre and about one month later at the two southern sites (Figure S1b). Reproductive output was related neither linearly nor quadratically to temperature in analysis on population means but revealed evidence of a decline with increasing temperature in pot-level analysis. For damage assessed binarily and the severity of rosette damage in pot-level analysis, significant negative linear and quadratic effects of temperature were found; damage was lowest at the two southern sites and intermediate at the northern beyond-range site (Figure S1e,f). Furthermore, results indicated no strong difference between the origin of populations or that they responded differently to temperature. An exception was the responses of southern-edge populations to temperature—as compared to the response of centre populations—revealed in pot-level analysis. Under increasing temperature, southern-edge populations had increased germination and survival in year 1, time to flowering was not lengthened as much and reproductive output to year 3 was higher (Table S9).

3.3 | Projection of the distribution of *A. lyrata* given climate warming

Based on ENM, we compared the recent historical distribution with the near-future projected distribution of *A. lyrata*. ENM revealed that all known occurrence points were within the predicted suitable area under recent historical conditions (Figure 4a,c). ENM projections for the period of 2011–2040 showed a general shift of suitable conditions towards the north, with southern parts of the range becoming unsuitable (Figure 4b). Support for occurrence points falling into four clusters along the climate variables considered in ENM motivated summarising results also for each of them (silhouette width statistics with average silhouette width = 0.43). The four clusters were northern, centre–west, centre–east and south. Figure 4c shows the predicted suitability in the recent past (solid line) and the near future (dashed line). Sites of the northern and central-western environmental clusters were predicted to experience little change in suitability. In contrast, those of the centre-east and south were

TABLE 1 The effect of minimum temperature in early spring, its square term, the origin of populations (north/N or south/S compared to centre) and interactions on population mean multiplicative performance (*MP*) and plant traits of different life stages in a transplant experiment of *Arabidopsis lyrata*.

		Performance		Germination & survival			Reproduction & roots				Damage in year 2			
		$MP_{\text{to year 2}}$	$MP_{\text{to year 3}}$	Germ	$\text{Survival}_{\text{year 1}}$	$\text{Survival}_{\text{year 2}}$	Time to flower	$\text{Reprod. output}_{\text{year 2}}$	$\text{Reprod. output}_{\text{to year 3}}$	Root length	Rosettes	Inflorescences	$\text{Severity}_{\text{rosettes}}$	$\text{Severity}_{\text{inflorescences}}$
Fixed effects	*N*	100	100	100	100	100	92	99	99	57	99	92	89	82
$T_{\text{min spring}}$	Estimate	**−0.06*****	**−0.06*****	**0.02*****	**−0.02*****	−0.01	**3.26*****	−0.04	−0.04	**10.77*****	−0.04(*)	0.01	**−0.04*****	−0.01
	SE	0.01	0.01	0.01	0.004	0.01	0.57	0.04	0.04	3.36	0.02	0.03	0.01	0.01
$T_{\text{min spring}}^2$	Estimate	**−0.01*****	**−0.01*****	**−0.01***	0.002	0.001	**0.46*****	−0.01	0.0005	**3.02*****	−0.004	0.002	**−0.01*****	−0.001
	SE	0.003	0.003	0.002	0.001	0.002	0.16	0.01	0.01	1.09	0.01	0.01	0.003	0.003
Origin, N	Estimate	−0.11	−0.09	0.02	−0.01	0.02	−1.41	−0.07	0.002	17.69	−0.03	0.01	−0.07	0.05
	SE	0.14	0.14	0.07	0.04	0.03	3.93	0.24	0.25	34.26	0.11	0.11	0.08	0.07
Origin, S	Estimate	0.14	0.15	0.03	−0.02	−0.03	1.51	0.16	0.18	3.77	0.08	−0.1	−0.03	0.06
	SE	0.14	0.14	0.07	0.04	0.03	3.91	0.24	0.25	34.01	0.11	0.11	0.08	0.08
$T_{\text{min spring}} \times N$	Estimate	−0.01	−0.004	−0.0001	−0.001	−0.001	0.12	**−0.05***	−0.03	**7.51***	0.01	−0.004	0.01	0.001
	SE	0.01	0.01	0.01	0.004	0.003	0.53	0.02	0.02	3.56	0.01	0.02	0.01	0.01
$T_{\text{,min spring}} \times S$	Estimate	−0.005	0.003	0.01(*)	0.001	−0.002	−0.80(*)	0.01	0.03	6.00(*)	0.01	0.0001	0.001	0.003
	SE	0.01	0.01	0.01	0.004	0.003	0.45	0.02	0.02	3.50	0.01	0.01	0.01	0.01
$T_{\text{min spring}}^2 \times N$	Estimate	0.004	0.004	0.002	0.001	−0.0003	0.02	−0.01	−0.01	1.08	0.002	−0.001	0.001	−0.002
	SE	0.004	0.004	0.002	0.001	0.001	0.13	0.01	0.01	1.08	0.004	0.004	0.002	0.002
$T_{\text{min spring}}^2 \times S$	Estimate	−0.001	−0.0003	0.001	0.001	0.002(*)	−0.06	0.001	0.003	0.41	0.002	0.002	0.003	−0.001
	SE	0.004	0.004	0.002	0.001	0.001	0.13	0.01	0.01	1.09	0.004	0.004	0.002	0.002

Note: Traits are described in more detail in Table S6. Test statistics include regression coefficients of each fixed effect (*estimate*) and standard error (*SE*). Estimates with *p*-values <0.05 are written in bold; significance is indicated: (*) $p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Results for random effects are not shown. The *bobyqa* optimizer was used to improve model convergence.

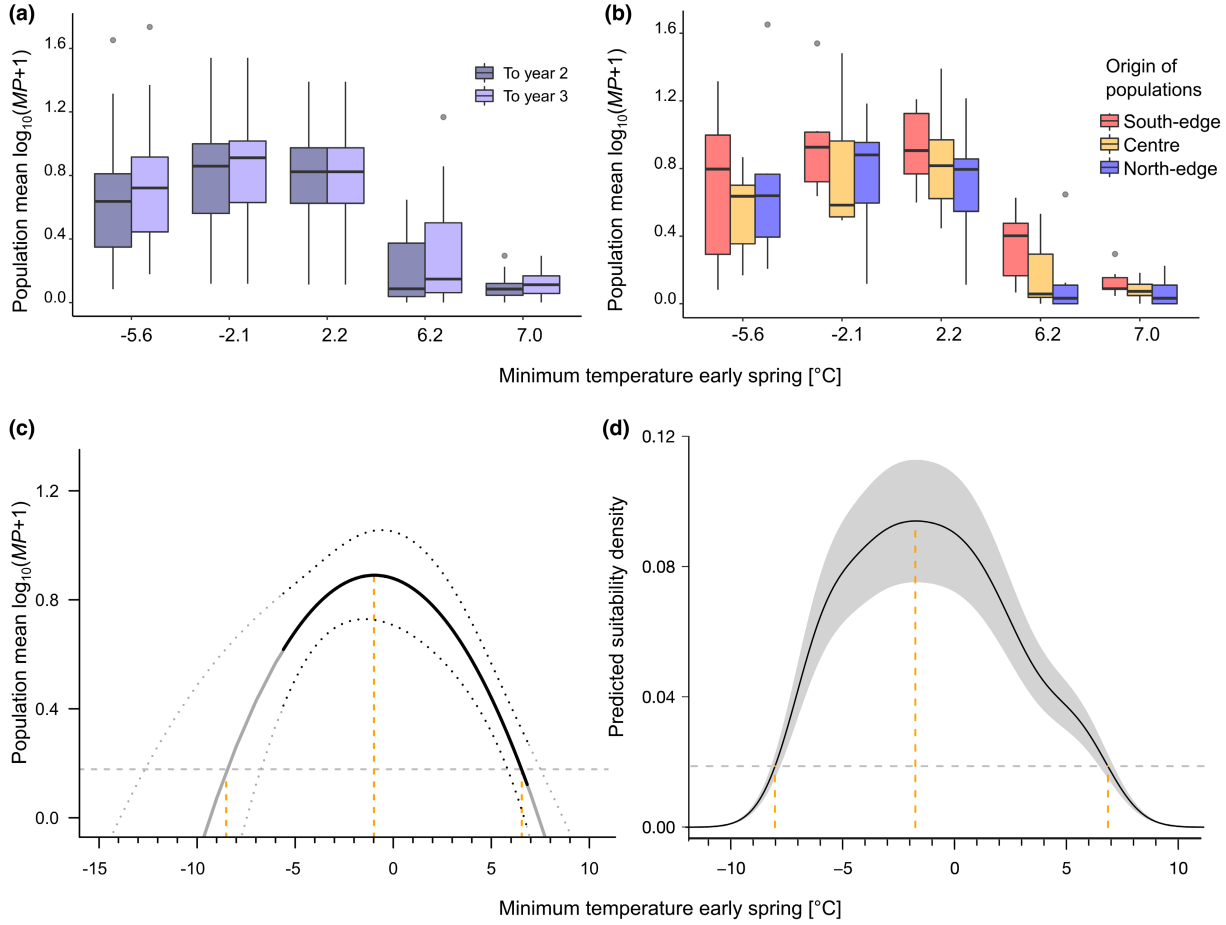

FIGURE 3 Relationship between performance of *Arabidopsis lyrata* in a latitudinal transplant experiment or suitability revealed by ecological niche modelling and minimum temperature in early spring. (a) Box plots based on population mean multiplicative performance (*MP*) up to year 2 or year 3, independent of the population origin at the five transplant sites. (b) Box plots of multiplicative performance up to year 3 for southern-edge (red), centre (orange) and northern-edge populations (blue) at the five transplant sites. Population means were based on family means of pot-level performance that were first log-transformed. Box plot elements: The centre line is the median, box limits are the upper and lower quartiles, whiskers are the 1.5× interquartile ranges and the points are the outliers. Box plots are sorted from north (left) to south (right), with their respective minimum temperature in early spring (not to scale). (c) Model-predicted quadratic relationship between population-level *MP* and temperature with 95% confidence intervals (dashed lines; in grey the range outside of observations). (d) Density curve of ENM-predicted suitability (consensus projections) on temperature. The grey band indicates the 95% credible interval. The dashed orange lines in (c) and (d) indicate the temperatures at which 0.2 of the optimum or the optimum is reached.

predicted to experience a decline in suitability in about half of the more suitable locations. Declines in climate suitability appeared systematic in some areas of the south, in the Ozarks of Missouri and the foothills of the Appalachians in North Carolina and Virginia.

4 | DISCUSSION

A main goal of this study was to compare the response of a species to temperature as revealed by niche modelling with that of performance assessed in a field experiment over a large latitudinal gradient. Our results are supportive that the two curves are fairly congruent on the one most important niche- and range-determining variable of North American *Arabidopsis lyrata*, minimum temperature in early spring (Figure 3c,d). Furthermore, the transplant experiment revealed some evidence of divergent climate adaptation of low-latitude populations to low-latitude conditions but with little effect on population means (Figure 3b, Table 1). Based on the good overlap of results between ENM analysis and the transplantation experiment, we can conclude that currently, the distribution of *Arabidopsis lyrata* lags behind global warming as it has not—to the extent that is projected—expanded the range to higher latitudes. On the warm end of distribution, the species has some capacity to cope with higher temperatures, but probably not under predicted warming in the near future (Figure 4c). We discuss these results in more detail below and relate them to the mechanisms (not) at play.

Our study is one of few that validated response curves of ENM on specific niche variables with those revealed experimentally. Validation of niche modelling has typically been based on comparing outcomes of different modelling approaches and techniques,

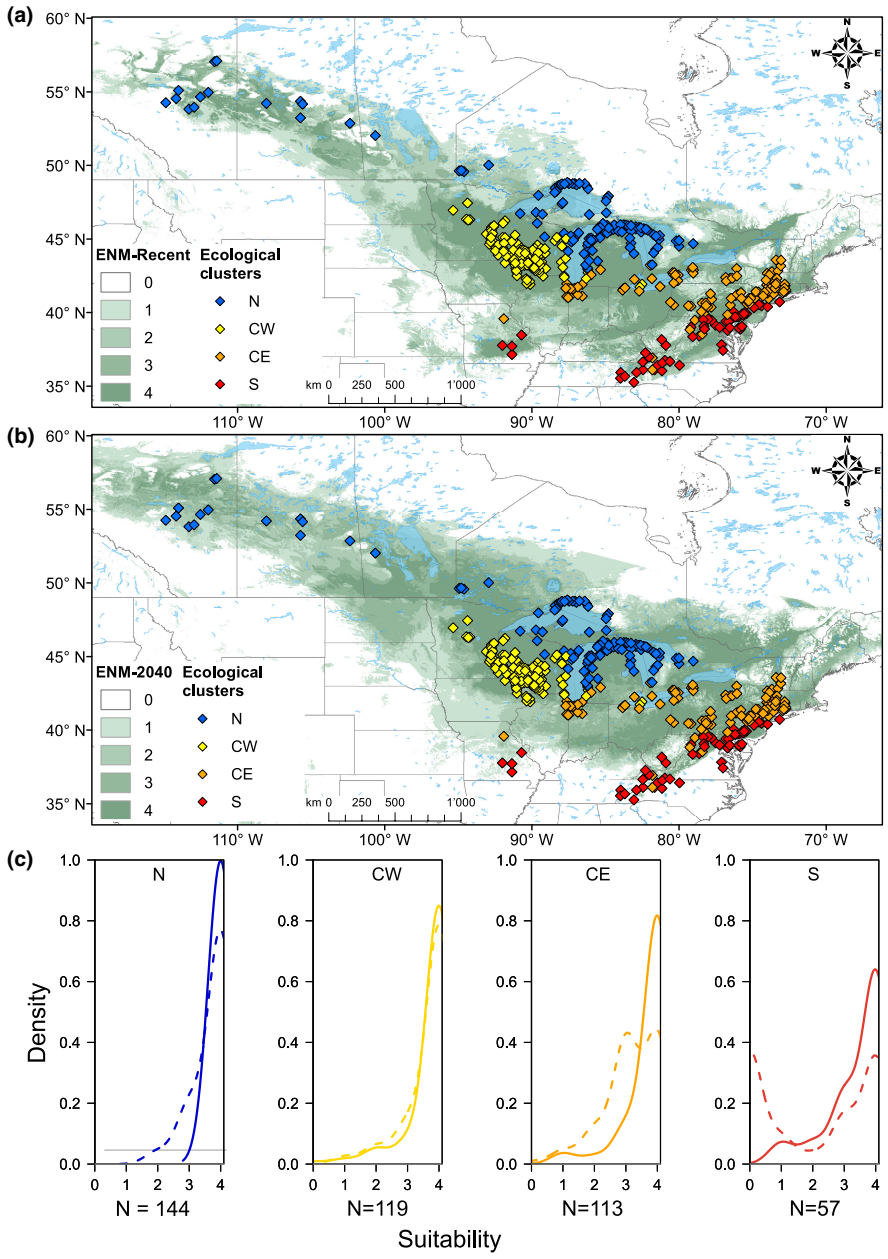

FIGURE 4 Predicted distribution under recent historical and near-future conditions. (a) Recent-historical potential distribution is expressed as the number of ENM algorithms indicating presence (in shades of green). (b) Projection of the potential distribution of the species in 2011–2040 averaged across algorithms per climate model and consequently summed across five climate models. The diamonds on the maps represent the known occurrence points of the species, and their colours represent the four clusters in niche space they fall into. (c) Suitability density of occurrences within the ecological clusters (N: north; CW: centre–west; CE: centre–east; S: south) under recent (continuous lines) and near-future conditions (dashed lines). The higher the number on the *x*-axis, the more algorithms and climate models predict presence under recent and future climate conditions.

such as between correlative and mechanistic modelling (e.g. reviewed in Tourinho & Vale, 2023) or different algorithms within the field of ecological niche modelling (e.g. Ashraf et al., 2017; Shabani et al., 2016). Several studies also validated niche modelling by relating predicted suitability to local abundance, with generally good support, especially for vertebrates (meta-analysis in Weber et al., 2017). However, few have verified predictions with experimental data of transplants and releases, though studies seem to be increasing. A literature search, summarised in Table 2, revealed at least four studies that tested whether the most important variable(s) revealed by niche modelling similarly explained performance under experimental conditions such as by transplants to sites differing in those variables. They all found accordance, with three of them providing strong support (Goff et al., 2021; Guerra-Coss et al., 2021; Swab et al., 2015). More studies (bottom part of Table 2) looked into the relationship between general suitability and performance in experimental transplants directly. Most of these reported positive relationships, with four studies revealing consistent links (Draper et al., 2019; Hemrová et al., 2019; Monnet et al., 2015; Wright et al., 2006), six studies partially or untested positive links (Hemrová & Münzbergová, 2012; Merlin et al., 2018; Sanczuk et al., 2022; Sangüesa-Barreda et al., 2018; Sheppard et al., 2014; Tojibaev et al., 2019) and three studies showing no such links (Bayly & Angert, 2019; Greiser et al., 2020; Swab et al., 2015). Overall, suitability revealed by modelling seems to translate often into performance, and maybe even more so if the focus is on important niche axes instead of general suitability. However, more studies on particular niche axes are needed to come to conclusive answers.

Our study revealed little deviation between the curves of ENM-predicted occurrence on temperature and performance on

TABLE 2 Published studies on the accordance in predicting environmental suitability between environmental niche modelling (ENM) and transplant/translocation experiments under field and/or manipulated conditions (M).

Organism	Species	Location	Phenotypic data	Venue (M*)	Accordance: +, −, or .		Source
Studies comparing ENM-predicted occurrence and performance on most important environmental variable(s)							
Plant	2 *Clarkia* sp.	USA	Seed production	Greenhouse*	+		Goff et al. (2021)
Plant	*Schinus molle*	Mexico	Recruitment	Field*	+		Guerra-Coss et al. (2021)
Plant	*Carlina vulgaris*	Europe	Fecundity potential	Field	+	In 1 out of 2 tests	Swab et al. (2015)
Plant	A grass species	USA	Biomass	Greenhouse*	+	No explicit testing	Zhang et al. (2021)
Studies comparing ENM-predicted habitat suitability with performance							
Plant	*Collinsia sparsiflora*	USA	Performance	Field*	+		Wright et al. (2006)
Bird	Houbara bustard	Marocco	Survival	Field	+		Monnet et al. (2015)
Plant	*Narcissus cavanillesii*	Portugal	Germination	Field	+		Draper et al. (2019)
Plant	4 orchid species	Czech Republic	Germination	Field	+	In 7 out of 8 tests	Hemrová et al. (2019)
Plant	*Jasione montana*	Czech Republic	Germination, survival	Field*	+	In 6 out of 32 tests	Hemrová and Münzbergová (2012)
Plant	3 woody plants	New Zealand	Biomass, survival	Field	+	In 4 out of 9 tests	Sheppard et al. (2014)
Plant	10 tree species	France	Seedling growth, survival	Field	+	In 11 out of 28 tests	Merlin et al. (2018)
Plant	Mistletoe	Spain	Recruitment	Field	+	No explicit testing	Sangüesa-Barreda et al. (2018)
Plant	*Otostegia bucharica*	Uzbekistan	Survival	Field	+	No explicit testing	Tojibaev et al. (2019)
Plant	*Hyacinthoides* sp.	Belgium	Pop. and ind. performance	Field	+	No explicit testing	Sanczuk et al. (2022)
Plant	*Erythranthe cardinalis*	USA	Demographic growth rate	Field	., −		Bayly and Angert (2019)
Plant	*Carlina vulgaris*	Europe	Fecundity potential	Field	.		Swab et al. (2015)
Plant	*Lathyrus vernus*	Sweden	Demographic growth rate	Field*	.		Greiser et al. (2020)

Note: The literature search was performed on 26 January 2023 with the string (TS=("species distribution model*" OR "environmental niche model*" OR "ecological niche model*" OR "habitat suitability model*")) AND (TS=(experiment* OR transplant* OR translocat*)) NOT (TS=(physiolog*)), Web of Science. The search produced 538 hits, which were filtered first based on title and the first three lines of the abstract, second based on reading the entire abstract (32 hits remaining), third based on reading the article (16 studies remaining, shown in the table). Studies were split based on whether accordance was found between ENM-predicted occurrence and performance on one or several important environmental variable(s) (top) or whether accordance was found by relating ENM-predicted habitat suitability with performance directly (bottom).

Abbreviation: M*, experimentation involved treatments.

temperature (Figure 3c,d). Some divergent climate adaptation of low-latitude populations did not lead to considerable deviation (Figure 3b). Evidence that populations were divergently adapted was gathered in pot-level analysis of plant performance and previously on the same data set in formal testing for climate adaptation (Sánchez-Castro, Perrier et al., 2022). Pot-level analysis revealed that the decline in performance with increasing temperature, towards the south, was less negative in southern-edge populations (Table S8); they had relatively higher germination and survival in the first year as compared to central populations (Table S9). While southern-edge populations were significantly the fittest under southern conditions, they were not fitter than central populations across transplant sites (boxplots suggest this visually, but variances are high). It appears that ecological niche modelling can account for such regional adaptation at range limits and does not predict narrower niche breaths across populations due to regional adaptation (but see Wright et al., 2006).

Our result of general congruence between the response of predicted occurrence to temperature and of performance to temperature allows clear conclusions for the species' range under climate warming. Given the current warmer climate at the northern range edge and beyond compared to the recent past, there is much more suitable habitat in the north where the species could live, based on climate alone. The species thrives in relatively open habitats exposed to wind erosion, erosion by water or the regular occurrence of wildfires. Even if dispersal was not limited and the species would have the capacity to spread, boreal forests to the north limit habitat availability. At the other end of distribution, at the southern edge and beyond, both the transplant experiment and the ENM predictions for minimum temperature in early spring indicated that parts of the southern edge had become very marginal. Likely regions where we can expect local extinctions under warming are the Ozarks and the piedmont of the Appalachians of North Carolina and Virginia (Figure 4b). Beyond, the projection of species distribution in the close future revealed that many sites in the eastern part of species' distribution will see a decline in suitability (Figure 4c, third panel from the left). In contrast, occurrences in the north and centre-west will on average experience little change in suitability (Figure 4c, first two panels from the left).

Despite the good overlap between the two approaches in predicting suitability depending on temperature, minimum temperature in early spring likely does not affect performance directly. Secondary analyses of life stage variables revealed that the linear aspect of decline with increasing temperature was supported by survival in the first year, with lower survival under warmer fall/winter conditions (Table 1, Figure S1c). However, what seemed to cause high mortality at those (low-latitude) sites in the first year were not warm temperatures but frost nights in the absence of a snow cover. A negative hump shape was supported by germination data, with lower germination especially in the north (Figure S1a). Time to flowering showed the inverse relationship, with a positive slope and a positive hump shape on temperature. Flowering tended to be delayed towards the north, though only slightly, and strongly towards the south (Figure S1b). Pot-level analyses revealed the additional contribution of later life stages, of reproductive output and survival in the second year (Table S9). These analyses uncover that lower performance at the warmer and cooler ends of the temperature gradient manifested across life stages. This cautions against concluding about direct causal effects of minimum temperature in early spring in shaping performance. Instead, the variable seems to depict best critical temperatures generally associated with a warmer or cooler climate. Similarly, Woodward (1987) described that temperature minima were often best at predicting performance on the cold end of distribution, even in areas with snow cover, though just reflecting general low-temperature climate differences and not direct effects. Also, we found that at none of the sites, catastrophic events happened throughout the nearly 3 years of experimentation; patterns fell into place gradually. This speaks against the commonheld belief that extreme events alone produce range limits, as they are confounded with more extreme climate in general.

Our study also provides insights into the role biotic interactions play in determining warm range limits. Ever since Darwin, there has been the assumption that towards higher latitudes, cold range limits are determined by climate and warm range limits by biotic interactions (Louthan et al., 2015). A recent meta-level analysis supported that cold range limits seem often affected by temperature and climate, while warm range limits seem often affected by species interactions, namely predation or herbivory, followed by habitat and climate (Paquette & Hargreaves, 2021). In *A. lyrata*, we did not find any evidence that herbivory is more important at the warm end of species' distribution. On the contrary, we found that the severity of damage was lowest towards the southern edge and beyond, and intermediate in the north (Table 1, Figure S1f). Pot-level analysis confirmed the pattern and further suggested the same for the chance of rosette attack (Table S9, Figure S1e). While herbivory seems not contributing to southern range limits, pollinators may play a role in the system. In a parallel study on natural populations of *A. lyrata* along a latitudinal gradient, southern edge populations were shown to be visited less often by pollinators as compared to more northern populations, indicating that pollination could be constrained at the southern range edge (Sánchez-Castro, Armbruster et al., 2022).

5 | CONCLUSION

Ecological niche modelling or species' distribution modelling has been widely applied to predict range shifts of species under climate warming. However, there have been few attempts to validate results on model-predicted important niche axes. Here, we used the framework of relating model-predicted occurrence (habitat suitability) on a temperature variable found to be the most niche- and range-determining. We compared the curve with that of performance on the same variable in a field experiment and found high congruence. On the one hand, this shows that niche modelling makes meaningful predictions applicable to field-climate conditions. On the other

hand, the overlap between findings together with projections for the close future provide very strong support that the particular species, *Arabidopsis lyrata*, is unable to track climate change. Range shift at the cold end of distribution does not seem to occur, and the species is likely to go extinct under further warming at the warm end of distribution, leading to the shrinking of its range.

AUTHOR CONTRIBUTIONS

Darío Sánchez-Castro and Yvonne Willi designed the study. Yvonne Willi collected seeds in the field. Darío Sánchez-Castro and Antoine Perrier performed the crossing and transplant experiments. Theofania-Sotiria Patsiou performed ENM and related analyses. Darío Sánchez-Castro analysed the transplant data. Darío Sánchez-Castro, Theofania-Sotiria Patsiou and Yvonne Willi wrote the manuscript, and Judith Schepers contributed to sections.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Occurrence data and performance data are stored on Dryad (https://doi.org/10.5061/dryad.bcc2fqzkp, https://doi.org/10.5061/dryad.n8pk0p2x7) .

ORCID

Yvonne Willi https://orcid.org/0000-0001-7654-3261

REFERENCES

Allouche, O., Tsoar, A., & Kadmon, R. (2006). Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, *43*, 1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x

Ashraf, U., Peterson, A. T., Chaudhry, M. N., Ashraf, I., Saqib, Z., Ahmand, S. R., & Ali, H. (2017). Ecological niche model comparison under different climate scenarios: A case study of *Olea* spp. in Asia. *Ecosphere*, *8*, e01825. https://doi.org/10.1002/ecs2.1825

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bayly, M. J., & Angert, A. L. (2019). Niche models do not predict experimental demography but both suggest dispersal limitation across the northern range limit of the scarlet monkeyflower (*Erythranthe cardinalis*). *Journal of Biogeography*, *46*, 1316–1328. https://doi.org/10.1111/jbi.13609

Boucher-Lalonde, V., Morin, A., & Currie, D. J. (2012). How are tree species distributed in climatic space? A simple and general pattern. *Global Ecology and Biogeography*, *21*, 1157–1166. https://doi.org/10.1111/j.1466-8238.2012.00764.x

Breiman, L. (2001). Random forests. *Machine Learning*, *45*, 5–32. https://doi.org/10.1023/A:1010933404324

Brun, P., Zimmermann, N. E., Hari, C., Pellissier, L., & Karger, D. (2022). Data from: CHELSA-BIOCLIM+ A novel set of global climate-related predictors at kilometre-resolution. *EnviDat*. https://doi.org/10.16904/envidat.332

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*, 1024–1026. https://doi.org/10.1126/science.1206432

Chown, S. L., & Gaston, K. L. (1999). Exploring links between physiology and ecology at macro-scales: The role of respiratory metabolism in insects. *Biological Reviews*, *74*, 87–120. https://doi.org/10.1111/j.1469-185X.1999.tb00182.x

Di Cola, V., Broennimann, O., Petitpierre, B., Breiner, F. T., D'Amen, M., Randin, C., Engler, R., Pottier, J., Pio, D., Dubuis, A., Pellissier, L., Mateo, R. G., Hordijk, W., Salamin, N., & Guisan, A. (2017). Ecospat: An R package to support spatial analyses and modeling of species niches and distributions. *Ecography*, *40*, 774–787. https://doi.org/10.1111/ecog.02671

Di Febbraro, M., D'Amen, M., Raia, P., De Rosa, D., Loy, A., & Guisan, A. (2018). Using macroecological constraints on spatial biodiversity predictions under climate change: The modelling method matters. *Ecological Modelling*, *390*, 79–87. https://doi.org/10.1016/j.ecolmodel.2018.10.023

Draper, D., Marques, I., & Iriondo, J. M. (2019). Species distribution models with field validation, a key approach for successful selection of receptor sites in conservation translocations. *Global Ecology & Conservation*, *19*, e00653. https://doi.org/10.1016/j.gecco.2019.e00653

Fazlioglu, F., Wan, J. S. H., & Chen, L. (2020). Latitudinal shifts in mangrove species worldwide: Evidence from historical occurrence records. *Hydrobiologia*, *847*, 4111–4123. https://doi.org/10.1007/s10750-020-04403-x

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*, 4302–4315. https://doi.org/10.1002/joc.5086

Friedman, J. H. (2001). Greedy function approximation: A gradient boosting machine. *Annals of Statistics*, *29*, 1189–1232. https://doi.org/10.1214/aos/1013203451

Geppert, C., Perazza, G., Wilson, R. J., Bertolli, A., Prosser, F., Melchiori, G., & Marini, L. (2020). Consistent population declines but idiosyncratic range shifts in Alpine orchids under global change. *Nature*

Communications, *11*, 5835. https://doi.org/10.1038/s41467-020-19680-2

Gillings, S., Balmer, D. E., & Fuller, R. J. (2015). Directionality of recent bird distribution shifts and climate change in Great Britain. *Global Change Ecology*, *21*, 2155–2168. https://doi.org/10.1111/gcb.12823

Goff, K. A., Martinez Del Rio, C., & Kay, K. M. (2021). A greenhouse experiment partially supports inferences of ecogeographic isolation from niche models of *clarkia* sister species. *American Journal of Botany*, *108*, 2002–2014. https://doi.org/10.1002/ajb2.1756

Greiser, C., Hylander, K., Meineri, E., Luoto, M., & Ehrlén, J. (2020). Climate limitation at the cold edge: Contrasting perspectives from species distribution modelling and a transplant experiment. *Ecography*, *43*, 637–647. https://doi.org/10.1111/ecog.04490

Griffin, P. C., & Willi, Y. (2014). Evolutionary shifts to self-fertilisation restricted to geographic range margins in north American *Arabidopsis lyrata*. *Ecology Letters*, *17*, 484–490.

Guerra-Coss, F. A., Badano, E. I., Cedillo-Rodríguez, I. E., Ramírez-Albores, J. E., Flores, J., Barragán-Torres, F., & Flores-Cano, J. A. (2021). Modelling and validation of the spatial distribution of suitable habitats for the recruitment of invasive plants on climate change scenarios: An approach from the regeneration niche. *Science of the Total Environment*, *777*, 146007. https://doi.org/10.1016/j.scitotenv.2021.146007

Guisan, A., Thuiller, W., & Zimmermann, N. E. (2017). *Habitat suitability and distribution models, with applications in R*. Cambridge University Press.

Guisan, A., & Zimmermann, N. E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling*, *135*, 147–186.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, *33*(2), 1–22. https://doi.org/10.18637/jss.v033.i02

Hargreaves, A. L., Samis, K. E., & Eckert, C. G. (2014). Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. *American Naturalist*, *183*, 157–173. https://doi.org/10.1086/674525

Hemrová, L., Kotilínek, M., Konečná, M., Paulič, R., Jersáková, J., Těšitelová, T., Knappová, J., & Münzbergová, Z. (2019). Identification of drivers of landscape distribution of forest orchids using germination experiment and species distribution models. *Oecologia*, *190*, 411–423. https://doi.org/10.1007/s00442-019-04427-8

Hemrová, L., & Münzbergová, Z. (2012). Identification of suitable unoccupied habitats: Direct versus an indirect approach. *Preslia*, *84*, 925–937.

Huisman, J., Olff, H., & Fresco, L. F. M. (1993). A hierarchical set of models for species response analysis. *Journal of Vegetation Science*, *4*, 37–46.

Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–427.

Jansen, F., & Oksanen, J. (2013). How to model species responses along ecological gradients—Huisman–Olff–Fresco models revisited. *Journal of Vegetation Science*, *24*, 1108–1117. https://doi.org/10.1111/jvs.12050

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., Zimmermann, N. E., Linder, H. P., & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, *4*, 170122. https://doi.org/10.1038/sdata.2017.122

Kassambara, A., & Mundt, F. (2020). Factoextra: Extract and visualize the results of multivariate data analyses. (R Package Version 1.0.7). https://CRAN.R-project.org/package=factoextra

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*, 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Kearney, M., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, *12*, 334–350. https://doi.org/10.1111/j.1461-0248.2008.01277.x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Lee-Yaw, J. A., Fracassetti, M., & Willi, Y. (2018). Environmental marginality and geographic range limits: A case study with *Arabidopsis lyrata* ssp. *lyrata*. *Ecography*, *41*, 622–634. https://doi.org/10.1111/ecog.02869

Lee-Yaw, J. A., Kharouba, H. M., Bontrager, M., Mahony, C., Csergő, A. M., Noreen, A. M. E., Li, Q., Schuster, R., & Angert, A. L. (2016). A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. *Ecology Letters*, *19*, 710–722. https://doi.org/10.1111/ele.12604

Lenoir, J., Gégout, J. C., Marquet, P. A., de Ruffray, P., & Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. *Science*, *320*, 1768–1771. https://doi.org/10.1126/science.1156831

Louthan, A. M., Doak, D. F., & Angert, A. L. (2015). Where and when do species interactions set range limits? *Trends in Ecology & Evolution*, *30*, 780–792. https://doi.org/10.1016/j.tree.2015.09.011

McCain, C. M., & Garfinkel, C. F. (2021). Climate change and elevational range shifts in insects. *Current Opinion in Insect Science*, *47*, 111–118. https://doi.org/10.1016/j.cois.2021.06.003

McCain, C. M., King, S. R. B., & Szewczyk, T. M. (2021). Unusually large upward shifts in cold-adapted, montane mammals as temperature warms. *Ecology*, *102*, e03300. https://doi.org/10.1002/ecy.3300

McCullagh, P., & Nelder, J. A. (1989). *Generalized linear models, second edition. Monographs on statistics and applied probability 37*. Chapman & Hall.

Merlin, M., Duputié, A., & Chuine, I. (2018). Limited validation of forecasted northward range shift in ten European tree species from a common garden experiment. *Forest Ecology & Management*, *410*, 144–156. https://doi.org/10.1016/j.foreco.2018.01.001

Monnet, A.-C., Hardouin, L. A., Robert, A., Hingrat, Y., & Jiguet, F. (2015). Evidence of a link between demographic rates and species habitat suitability from post release movements in a reinforced bird population. *Oikos*, *124*, 1089–1097. https://doi.org/10.1111/oik.01834

Normand, S., Randin, C., Ohlemueller, R., Bay, C., Hoye, T. T., Kjaer, E. D., Körner, C., Lischke, H., Maiorano, L., Paulsen, J., Pearman, P. B., Psomas, A., Treier, U. A., Zimmermann, N. E., & Svenning, J.-C. (2013). A greener Greenland? Climatic potential and long-term constraints on future expansions of trees and shrubs. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *368*, 20120479. https://doi.org/10.1098/rstb.2012.0479

Normand, S., Treier, U. A., Randin, C., Vittoz, P., Guisan, A., Svenning, J.-C., & Field, R. (2009). Importance of abiotic stress as a range-limit determinant for European plants: Insights from species responses to climatic gradients. *Global Ecology and Biogeography*, *18*, 437–449. https://doi.org/10.1111/j.1466-8238.2009.00451.x

Oksanen, J., & Minchin, P. R. (2002). Continuum theory revisited: What shape are species responses along ecological gradients? *Ecological Modelling*, *157*, 119–129.

Paquette, A., & Hargreaves, A. L. (2021). Biotic interactions are more often important at species' warm versus cool range edges. *Ecology Letters*, *24*, 2427–2438. https://doi.org/10.1111/ele.13864

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*, 37–42.

Patsiou, T.-S., Walden, N., & Willi, Y. (2021). What drives species' distributions along elevational gradients? Macroecological and -evolutionary insights from Brassicaceae of the Central Alps. *Global Ecology & Biogeography*, *30*, 1030–1042. https://doi.org/10.1111/geb.13280

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology & Biogeography*, *12*, 361–371.

Perrier, A., Sánchez-Castro, D., & Willi, Y. (2020). Expressed mutational load increases towards the edge of a species' geographic range. *Evolution*, *74*, 1711–1723. https://doi.org/10.1111/evo.14042

Perrier, A., Sánchez-Castro, D., & Willi, Y. (2022). Environment dependence of the expression of mutational load and species' range limits. *Journal of Evolutionary Biology*, *35*, 731–741. https://doi.org/10.1111/jeb.13997

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*, 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Root, T. (1988). Environmental factors associated with avian distributional boundaries. *Journal of Biogeography*, *15*, 489–505.

Rumpf, S. B., Hülber, K., Klonner, G., Moser, D., Schütz, M., Wessely, J., Willner, W., Zimmermann, N. E., & Dullinger, S. (2018). Range dynamics of mountain plants decrease with elevation. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 1848–1853. https://doi.org/10.1073/pnas.1713936115

Sánchez-Castro, D., Armbruster, G., & Willi, Y. (2022). Reduced pollinator service in small populations of *Arabidopsis lyrata* at its southern range limit. *Oecologia*, *200*, 107–117. https://doi.org/10.1007/s00442-022-05237-1

Sánchez-Castro, D., Perrier, A., & Willi, Y. (2022). Reduced climate adaptation at range edges in north American *Arabidopsis lyrata*. *Global Ecology and Biogeography*, *31*, 1066–1077. https://doi.org/10.1111/geb.13483

Sanczuk, P., De Lombaerde, E., Haesen, S., Van Meerbeek, K., Van der Veken, B., Hermy, M., Verheyen, K., Vangansbeke, P., & De Frenne, P. (2022). Species distribution models and a 60-year-old transplant experiment reveal inhibited forest plant range shifts under climate change. *Journal of Biogeography*, *48*, 537–550. https://doi.org/10.1111/jbi.14325

Sangüesa-Barreda, G., Camarero, J. J., Pironon, S., Gazol, A., Peguero-Pina, J. J., & Gil-Pelegrín, E. (2018). Delineating limits: Confronting predicted climatic suitability to field performance in mistletoe populations. *Journal of Ecology*, *106*, 2218–2229. https://doi.org/10.1111/1365-2745.12968

Shabani, F., Kumar, L., & Ahmadi, M. (2016). A comparison of absolute performance of different correlative and mechanistic species distribution models in an independent area. *Ecology and Evolution*, *6*, 5973–5986. https://doi.org/10.1002/ece3.2332

Sheppard, C. S., Burns, B. R., & Stanley, M. C. (2014). Predicting plant invasions under climate change: Are species distribution models validated by field trials? *Global Change Biology*, *20*, 2800–2814. https://doi.org/10.1111/gcb.12531

Swab, R. M., Regan, H. M., Matthies, D., Becker, U., & Bruun, H. H. (2015). The role of demography, intra-species variation, and species distribution models in species' projections under climate change. *Ecography*, *38*, 221–230. https://doi.org/10.1111/ecog.00585

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., Ferreira de Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L., & Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*, 145–148. https://doi.org/10.1038/nature02121

Thuiller, W., Georges, D., Gueguen, M., Engler, R., Breiner, F., Lafourcade, B., & Patin, R. (2023). *Package 'biomod2': Ensemble platform for species distribution modeling*. (4.2.4). https://cran.r-project.org/web/packages/biomod2/index.html

Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T., & Prentice, I. C. (2005). Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 8245–8250. https://doi.org/10.1073/pnas.0409902102

Tojibaev, K., Beshko, N., & Volis, S. (2019). Translocation of *Otostegia bucharica*, a highly threatened narrowly distributed relict shrub. *Plant Diversity*, *41*, 105–108. https://doi.org/10.1016/j.pld.2019.01.005

Tourinho, L., & Vale, M. M. (2023). Choosing among correlative, mechanistic, and hybrid models of species' niche and distribution. *Integrative Zoology*, *18*, 93–109. https://doi.org/10.1111/1749-4877.12618

Weber, M. M., Stevens, R. D., Diniz-Filho, J. A. F., & Grelle, C. E. V. (2017). Is there a correlation between abundance and environmental suitability derived from ecological niche modelling? A meta-analysis. *Ecography*, *40*, 817–828. https://doi.org/10.1111/ecog.02125

Willi, Y., Fracassetti, M., Zoller, S., & Van Buskirk, J. (2018). Accumulation of mutational load at the edges of a species range. *Molecular Biology and Evolution*, *35*, 781–791. https://doi.org/10.1093/molbev/msy003

Willi, Y., Lucek, K., Bachmann, O., & Walden, N. (2022). Recent speciation associated with range expansion and a shift to self-fertilization in north American *Arabidopsis*. *Nature Communications*, *13*, 7564. https://doi.org/10.1038/s41467-022-35368-1

Willi, Y., & Van Buskirk, J. (2019). A practical guide to the study of distribution limits. *American Naturalist*, *193*, 773–785. https://doi.org/10.1086/703172

Woodward, F. I. (1987). *Climate and Plant Distribution*. Cambridge University Press.

Wright, J. W., Davies, K. F., Lau, J. A., McCall, A. C., & McKay, J. K. (2006). Experimental verification of ecological niche modeling in a heterogeneous environment. *Ecology*, *87*, 2433–2439.

Zhang, B., Yuan, Y., Shu, L., Grosholz, E., Guo, Y., Hastings, A., Cuda, J. P., Zhang, J., Zhai, L., & Qiu, J. (2021). Scaling up experimental stress responses of grass invasion to predictions of continental-level range suitability. *Ecology*, *102*, e03417. https://doi.org/10.1002/ecy.3417

BIOSKETCH

Research in the team of **Yvonne Willi** centers around the causes and impacts of evolutionary constraints. Specifically, constraints in the evolution of the climate niche are studied using species' geographic and elevational range limits as model systems (see https://duw.unibas.ch/en/ecoevo/).

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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