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ARTICLE
Vegetation Ecology

Population persistence, phenotypic divergence, and metabolic adaptation in yarrow (*Achillea millefolium* L.)

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Abstract

Climate change threatens biodiversity as populations can persist if they migrate or adapt to the rapidly changing conditions of the Anthropocene. However, the metabolic mechanisms underlying plant population persistence under the long-term trends of increasing temperature and drought remain unclear. Here, we investigate the persistence and adaptation of yarrow (*Achillea millefolium* L.) populations over 100 years of climate change. We resurveyed historical sites spanning a broad climatic gradient (from 1 m to 3200 m above sea level) and analyzed metabolic diversity in a common-garden experiment. We report that nine out of ten populations persisted locally, showing phenotypic and metabolic differentiation. The only population potentially extirpated is that of the hottest and driest site. A complex interaction between increasing temperatures and changing precipitation patterns shaped plant growth across populations. Populations from warmer sites in coastal and mountain regions grew taller than 100 years ago, whereas populations from drier sites in lowlands and foothills became shorter. Furthermore, we document differentiation in metabolic diversity involving plant defenses and stress response. These findings suggest that ongoing adaptation is constrained by long-term changes in temperature and precipitation as well as by local biotic interactions. Preserving locally adapted populations and their metabolic diversity is key for conservation efforts in the face of accelerating climate change.

KEYWORDS
biodiversity change, climate change, growth response, local adaptation, metabolomics, phytochemical diversity, species persistence

INTRODUCTION

Climate change is a complex alteration of global and local climatic patterns compared with a last century-based line which includes warming and precipitation regime shifts (IPCC, 2022). Considerable effort has been invested in understanding the effects of rising temperatures on organisms and populations (Manrique-Ascencio et al., 2024; Parmesan & Hanley, 2015; Steinbauer et al., 2018; Zandalinas et al., 2021). Yet, besides temperature, the

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long-term effects of increasing drought remain unclear (Exposito-Alonso et al., 2018; Losapio & Schöb, 2017). As the acceleration of these changes in climate poses new challenges to species' survival and conservation (Parmesan & Hanley, 2015), understanding species' response to rapid environmental change is urgent and critical to guide policy for halting the biodiversity loss crisis (Dirzo et al., 2022).

The seminal, pioneering work of Hall, Clausen, Keck, and Hiesey (Clausen et al., 1948) documented for the first time the adaptive responses of species to changing environments. Through field observations and common-garden experiments, Clausen et al. (1948) demonstrated that geographically distinct populations of yarrow (*Achillea millefolium* L.) have adapted to specific local climate conditions. They documented how certain traits, particularly plant height and phenology, vary across geographical gradients in response to changes in temperature and precipitation (Baldwin, 2006; Clausen et al., 1948). They proposed that yarrow ecotypes possess varying degrees of genetic diversity, which contributes to their local adaptation and broad distribution across different environments. Today, after one hundred years of increasing temperature and drought, it remains unknown if those locally adapted populations persisted or went extinct, and whether the growth signature (plant height) has changed in response to locally changing climatic conditions.

As climate change accelerates, it poses new challenges to species' survival and conservation (Parmesan & Hanley, 2015). Genetic diversity is key to the ability of populations to survive, reproduce, and adapt under changing climatic conditions (Bastias et al., 2024; Kawecki & Ebert, 2004; Liu et al., 2019). Associated with genetic diversity, chemical diversity plays a central role in species' potential to adapt to changing climatic conditions and biotic environments (Agrawal et al., 2009; Walker et al., 2023; Wetzel & Whitehead, 2020). Also, plant secondary metabolism may be affected by changes in climatic factors (Fernandez-Conradi et al., 2022). Specialized plant metabolites have key functions in mediating species interactions and driving the response of plants to environmental stress (Agrawal et al., 2009; Fernandez-Conradi et al., 2022; Volf et al., 2024). However, how the potential contribution of chemical diversity to local adaptation changes over space–time remains poorly documented. Understanding the metabolic mechanisms underlying local adaptation is essential for anticipating species responses to climate change (Agrawal et al., 2009; Walker et al., 2023). This knowledge is crucial for devising effective conservation and restoration strategies that safeguard biodiversity and ecosystem integrity.

Here we explore whether historical populations of yarrow persist or not after one hundred years of climate change, and if/how they have phenotypically changed. We resurveyed 10 locally adapted yarrow populations that were originally surveyed in the 1920s (Clausen et al., 1948) to compare them with current populations from the same locations (Figure 1). We then established a common-garden experiment to assess the metabolic diversity of different populations under the same controlled climatic conditions. By means of metabolomics, we examined if populations from different regions exhibit inherited metabolic adaptations that may have allowed them to persist over one hundred years of increasing temperature and drought. We address the following research questions: (1) Do yarrow populations persist or are they locally extirpated? (2) How did yarrow height change across populations over the last 100 years? (3) Which metabolic components predict differences among populations?

MATERIALS AND METHODS

Resurvey data

A. millefolium L. (known as Biranjasif, Colchón de pobre, Flor de pluma, Huzambil, Manzanilla de los montes, Mil de rama, Millefoglio, Milenrama, Shavella, Yarrow, Yerba de Carpintero) is a perennial herbaceous plant belonging to the family Asteraceae (POWO, 2024). Native to temperate regions of the Northern Hemisphere, it has become naturalized in many parts of the world, including North and Central America, due to its adaptability to a wide range of environments, where it grows from lowland to the alpine zone. The species is characterized by its finely divided, feathery leaves and clusters of small, white to pink flowers, which bloom from late spring to early autumn. The average lifespan of an individual plant is several years, although populations may persist much longer due to clonal reproduction.

A. millefolium is one of the most diverse polyploid complexes of the Northern Hemisphere in terms of morphological, genetic, and ecological features (Ehrendorfer & Guo, 2006). It includes different autopolyploids and allopolyploids members (2×, 4×, 6×, and 8×) that in the past were considered as separate species, including *A. borealis*, *A. californica*, and *A. lanulosa*. It is commonly used in the traditional medicine of several Eurasian cultures for the treatment of spasmodic gastrointestinal disorders, hepatobiliary, gynecological disorders, against inflammation, and for wound healing (Ali et al., 2017). The phytochemistry of the species includes a broad, diversified range of phenols,

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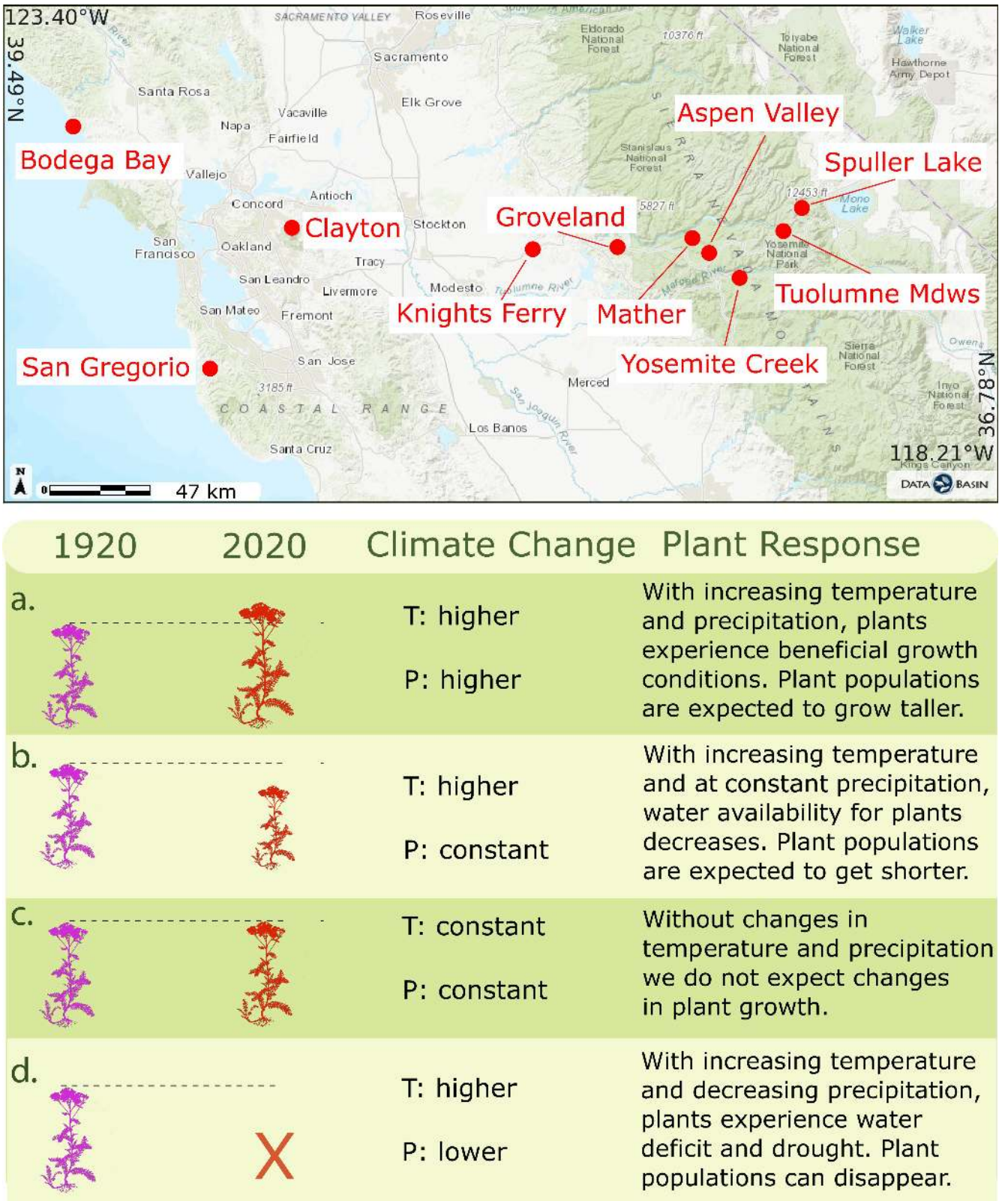


FIGURE 1 Location of study sites along the elevational gradient in California, USA. Sites were resurveyed after 100 years of changing temperature and precipitation. The height of plant populations is expected to change according to climate change.

flavonoids, monoterpenes, sesquiterpene, and sesquiterpenoids with antioxidant and antimicrobial properties (Candan et al., 2003).

To address the first question on population persistence, we revisited historical study sites of yarrow reported by Clausen et al. (1948) during the spring and

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summer of 2020 in California, USA (Figure 1). We selected the 10 sites where the evidence of local adaptation was documented (Appendix S1: Table S1). These 10 sites span a broad climatic gradient, ranging from the Pacific coast at 1 m above sea level (asl) to the alpine zone at 3200 m asl. In each site, we (1) recorded the occurrence of the yarrow population, (2) measured plant height (1-cm accuracy from bottom to top of inflorescence) on 10 randomly chosen plants in reproductive phase, and (3) collected seeds (Appendix S1: Table S2).

To assess whether observed changes in plant height could be attributed to factors other than climate, we evaluated the historical and current land-use context of each site. Based on historical context (Clausen et al., 1948) and field observations during surveys, we found no evidence of land-use changes or other significant disturbances that might have influenced plant growth over the past century. All sites remain within natural habitats with no visible signs of habitat conversion, intensive management, or anthropogenic infrastructures that could confound the observed effects of climate change. However, we acknowledge that it is not possible to completely exclude or control for all potential non-climatic changes that may have occurred over the last century.

Data analysis

To address the second question on population changes over space–time, we extracted the original plant height data for each site as reported from the 1920s studies (Clausen et al., 1948; Appendix S1: Table S3). We resampled plant height data from two uniform distributions. Twenty plants were randomly sampled from the min–max interval of each site for the two years (i.e., the 1920s and 2020). We gathered climate data for both 1920 and 2020 for mean annual temperature and annual precipitation from https://calclim.dri.edu/.

We used a linear regression model to test for differences in plant height (response variable) between years (categorical predictor), temperature, precipitation, and their statistical interactions. To this end, we used the *lm* function of *base* R software package (R Core team, 2023). The model was evaluated in terms of residuals dispersion, variance explained, and Cohen's effect size was calculated (R Core team, 2023).

We first tested for differences in plant height among sites between years using a linear regression model. We estimated marginal (least-squares) means for each combination of site and year ($n = 18$) and compared plant height within each site between years. We used the *emmeans* function of the R software package of the same name (Lenth, 2016).

Then, we analyzed the possible change of plant height over time using a stochastic ecological model of plant growth in response to climate change (Soetaert & Herman, 2009), which was parametrized using field survey data. Changes in plant height H of each population i over time t were modeled as

$$\frac{d\mathrm{H}_i}{dt} = \mathrm{H}_i \times \mu_i \times (\rho_i - \varepsilon),$$

where μ represents plant growth rate and ρ the plant response to climate between 1920 and 2020. The parameters for plant growth rate μ and climate sensitivity ρ were estimated using our field data and historical records of Clausen et al. (1948). Specifically, plant growth rate was derived from the mean plant height changes over time using marginal means contrasts between years at each site i. The population-specific climate sensitivity parameter ρ was calculated combining the differences in temperature and precipitation between 1920 and 2020 at each site into a single metric as

$$\rho_i = \frac{\eta_i(\eta_{2020} - \eta_{1920})}{100} + \frac{\theta_i(\theta_{2020} - \theta_{1920})}{100},$$

where sensitivity to precipitation η and sensitivity to temperature θ were derived from principal components analysis (PCA) and weighted for differences in precipitation and temperature between two sampling years (i.e., 2020 and 1920) at each site i. PCA was performed on standardized variables of temperature η and θ precipitation across sites and years. The first PC represents the dominant gradient of climatic change across sites, integrating temperature and precipitation changes, and explains the majority of variance (~72%). The metrics η and θ were then extracted as the loading (i.e., coordinates on the PCA plan) of each site along PC1 (Appendix S1: Table S3).

Stochasticity ε was introduced from a normal distribution with mean $= 2.5 \times 10^{-3}$ and SD $= 1 \times 10^{-2}$ to account for random mortality. Initial conditions were plant average height as reported in Clausen et al. (1948). The model was run for 100 years from 1920 to 2020 with one-year interval using automatic switching solver for Ordinary Differential Equations (ODE) (Soetaert et al., 2010). While this model simplifies complex interactions influencing plant growth (height) over a century—such as interannual climate variability and additional abiotic and biotic factors—it focuses on the primary drivers of observed changes (temperature and precipitation), providing a first approximation that allows us to capture a range of adaptive responses while relying on a limited number of parameter assumptions under data-limited conditions.

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Metabolomic analysis

To address the third question on plant metabolic diversity among populations, we sowed 1 g of seeds per ecotype in germination trays with well-watered potting soil in an open-chamber greenhouse. Germination rates were recorded every day for the first two weeks and then once per week. After 60 days, we transplanted one plant per pot (common potting soil in 8-cm pots) and let the yarrow grow for another 60 days before metabolomic analysis. We analyzed the ecotypes of Bodega Bay, Groveland, Mather, and Yosemite Creek, representing the Pacific coast, low, medium, and high-elevation adaptation, respectively.

Plant secondary metabolites, particularly volatile organic compounds (VOCs), were analyzed via headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME-GC-MS). We used an Agilent gas chromatograph (GC 7890A) coupled with a single quadrupole mass spectrometer (MS 5975C INERT XL MSD) and a CTC ANALYTICS PAL autosampler. To perform sample chromatography, a 5MS column (30 m × 0.25 mm × 0.25 μm + 10 m of pre-column) was employed (see Appendix S1 for details). For the experiments, 1 g of fresh plant material was used. Before injection, the samples were equilibrated for 20 min, at room temperature, in a 20-mL sealed glass vial.

Metabolomic analysis was performed using MetaboAnalyst 5.0 (Pang et al., 2022). Metabolomic data were first normalized using the MS-DIAL mTIC (total ion current). We used partial least-squares discriminant analysis (PLS-DA) to distinguish populations and identify compounds most contributing to those differences. Then, we used a machine learning algorithm (i.e., random forest) to select compounds with the highest discriminatory power; a threshold >1 was set for variable importance in the projection (VIP) score (Pang et al., 2022). The PLS-DA model was validated using Q2 as a performance measure through 10-fold cross-validation. Chemical information was retrieved from the open chemistry database at the National Institutes of Health (Kim et al., 2023).

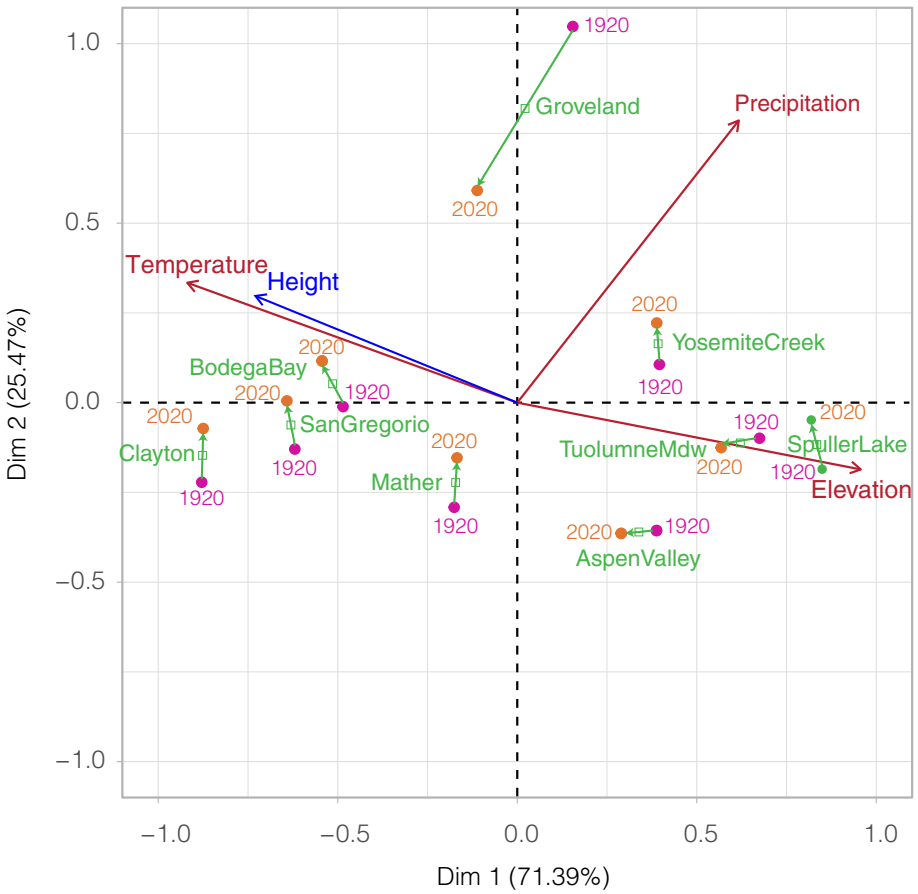


FIGURE 2 Factor map of the principal components analysis of the relationships between temperature, precipitation, and elevation across sites and between years (1920 and 2020). Plant height (blue arrow) was fitted passively, while the other variables (brown arrows) actively contribute to the components.

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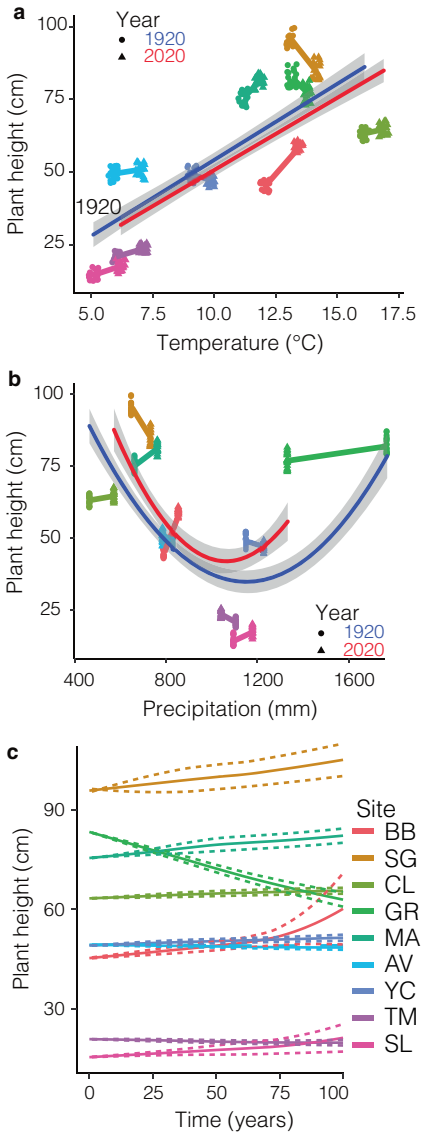


FIGURE 3 Changes in yarrow plant height in response to changes in (a) temperature and (b) precipitation between the 1920s (blue line and circles) and 2020 (red line and triangles). The line connecting the two average data points indicates the strength and direction of the change between years. (c) An ecological model of plant growth for changes in height over 100 years of changing temperature and precipitation across populations. AV, Aspen Valley; BB, Bodega Bay; CL, Clayton; GR, Groveland; MA, Mather; SG, San Gregorio; SL, Spuller Lake; TM, Tuolumne Meadows; YC, Yosemite Creek.

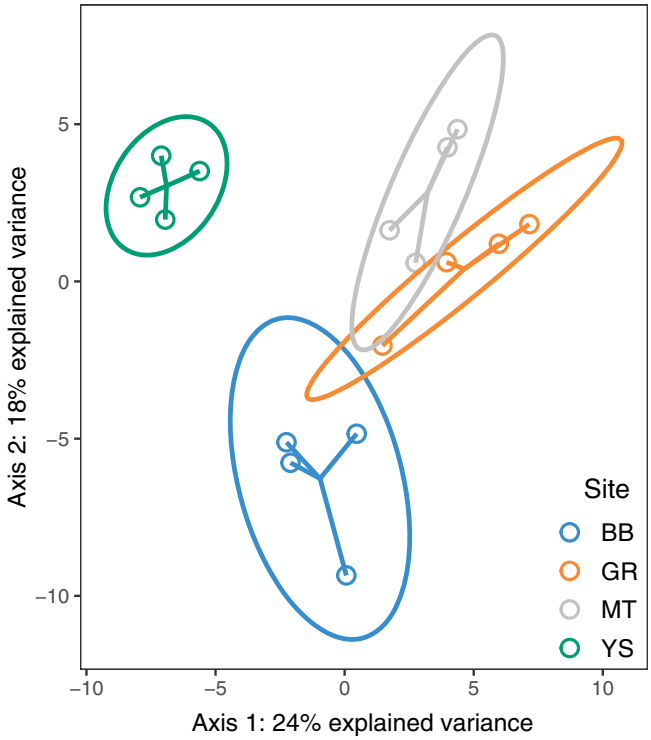


FIGURE 4 Biplot of partial least square-discriminant analysis of phytochemicals of different populations (BB, Bodega Bay; GR, Groveland; MT, Mather; YS, Yosemite Creek) in the common-garden experiment.

RESULTS

Climatic conditions have changed over the last one hundred years in all sites (Figure 2). The temperature increased monotonically across sites, with changes between +4.63% and +23.82%; changes were more pronounced at high-elevation sites than at lower elevation. On the contrary, changes in precipitation were idiosyncratic as precipitation increased in coast sites while decreased in lowlands and western slopes of the Sierra Nevada (Appendix S1: Table S1).

Yarrow populations occurred at nine out of 10 historical sites (Appendix S1: Table S4). The only population not recorded, potentially extirpated, was that of the hottest and driest site in the lowland (Knights Ferry). Inspection of this site did not reveal habitat conversion due to land-use change or any other visible disturbance (personal observation).

Overall, yarrow plants grew taller over one hundred years on average across sites (coef: 1.04 ± 0.25 SE, $p < 0.001$). Changes in yarrow plant height were significantly correlated with changes in temperature (coef: 0.09 ± 0.02, $p < 0.001$; Figure 3a), precipitation ($\text{coef}_{\text{linear}}$: -22.89 ± 4.29, $p < 0.001$; $\text{coef}_{\text{quadratic}}$: 9.57 ± 5.35, $p = 0.074$; Figure 3b), and by the interaction temperature–precipitation–years ($\text{coef}_{\text{linear}}$: -1.32 ± 0.42, $p = 0.002$; $\text{coef}_{\text{quadratic}}$: -2.40 ± 0.50, $p < 0.001$) (Appendix S1: Table S5).

A global regression analysis was performed to assess population-level trends. The degree of change in yarrow height between years varies among populations ($F_{\text{site:years}} = 70.0$, $p < 0.001$; Appendix S1: Table S6). Between 1920s and 2020, yarrow plants grew significantly

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taller at Bodega Bay (contrast: 0.261 ± 0.015, $p < 0.001$), Mather (contrast: 0.076 ± 0.015, $p < 0.001$), Aspen Valley (contrast: 0.029 ± 0.015, $p = 0.047$), Tuolumne Meadow (contrast: 0.125 ± 0.015, $p < 0.001$), and Spuller Lake (contrast: 0.193 ± 0.015, $p < 0.001$). On the contrary, yarrow plants were significantly shorter at San Gregorio (contrast: −0.121 ± 0.015, $p < 0.001$), Groveland (contrast: −0.063 ± 0.015, $p < 0.001$), and Yosemite Creek (contrast: 0.039 ± 0.015, $p = 0.009$). Yarrow plants were not statistically different between years at Clayton (contrast: 0.023 ± 0.015, $p = 0.121$) (Appendix S1: Table S7).

Results of a theoretical model parametrized with field data indicate, in seven out of nine cases, that changes in yarrow height are driven by local adaptation to changes in both temperature and precipitation ($F_{\text{years:site}} = 0.49$, $p < 0.001$; Figure 3c; Appendix S1: Tables S8 and S9).

The metabolomic analysis identified 102 plant compounds (Appendix S1: Table S10), 42 of which were found in significantly different concentrations among populations ($p < 0.001$). Overall, yarrow populations showed distinct metabolic profiles (Figure 4). In particular, Yosemite Creek and Bodega Bay are distinct from Groveland and Mather. Random forest results indicate 14 metabolites predict differences in metabolic profile (all VIP scores >1.6). In particular, the concentrations of 4-terpinyl acetate, isobornyl formate, p-menth-3-en-1, dehydrosabinenol, cis-3-hexenyl, and α-terpinen were the highest in high-elevation populations (Figure 5). In contrast, the concentrations of santolina triene, artemisia ketone, 3-methyl-4-pentenal, and myrtenyl acetate were the highest in low-elevation populations. The concentration of Camphor, p-mentha-1,4-diene, 2,3-dehydro-1,8-cineole, and eucalyptol was the highest in populations from mid-elevation. The clustered image map (Figure 6) indicates that those compounds are clustered among populations. Bodega Bay and Yosemite Creek are clearly differentiated on the basis of their metabolic profiles, while Groveland and Mather are more closely related and have more intermingled profiles.

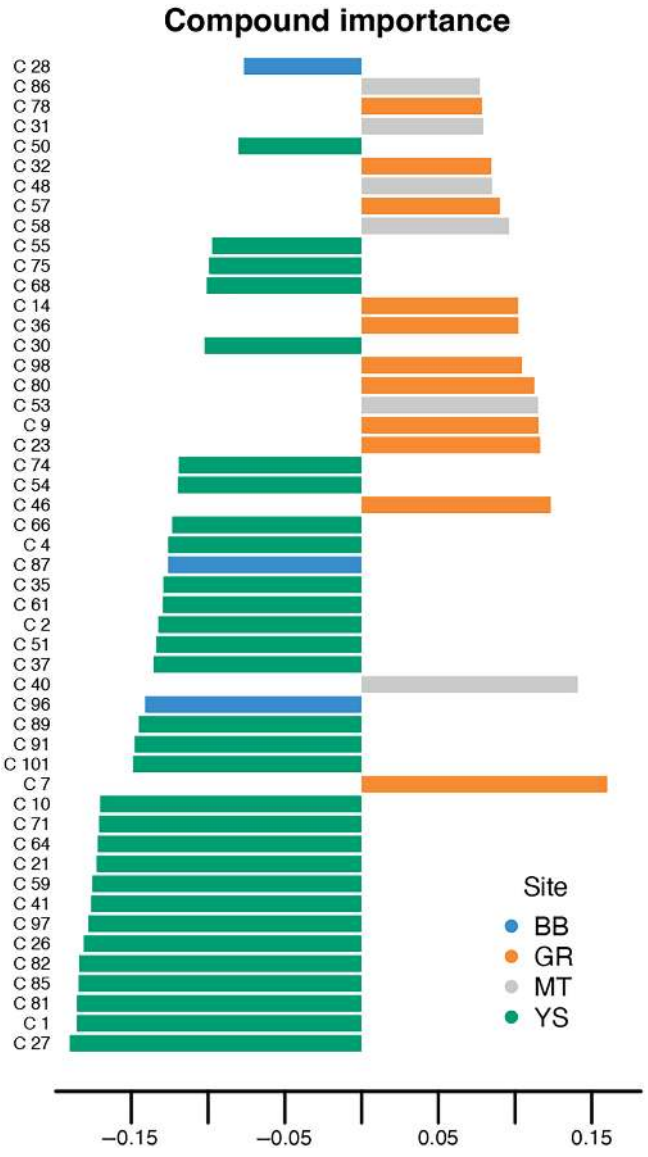


FIGURE 5 The most important metabolites responsible for differentiating populations (BB, Bodega Bay; GR, Groveland; MT, Mather; YS, Yosemite Creek) in the common-garden experiment. For compound names, see Appendix S1: Table S10.

DISCUSSION

Our results provide novel insights into the responses of yarrow populations (*A. millefolium* L.) to one hundred years of climate change. We report that nine out of ten historical populations were still viable, indicating the ongoing local adaptation of this species. However, the population of Knights Ferry, the hottest and driest site, may have been potentially extirpated, highlighting the vulnerability of populations to increasing both temperature and aridity at the range edge (Bastias et al., 2024). There, extirpation might have been driven by additional factors such as land-use change other than climate.

We document an increase in plant height over one hundred years in most populations parallel to a warming climate. Our results suggest that yarrow plants tend to grow taller as temperature has increased during the last century. This finding is consistent with global trends in the growth–temperature relationship, whereby plants become taller with increasing temperature or, vice versa, become shorter with increasing altitude or latitude (Buckley & Puy, 2022). Furthermore, increased nitrogen deposition, although not tested here, may also have increased plant growth (Losapio & Schöb, 2017).

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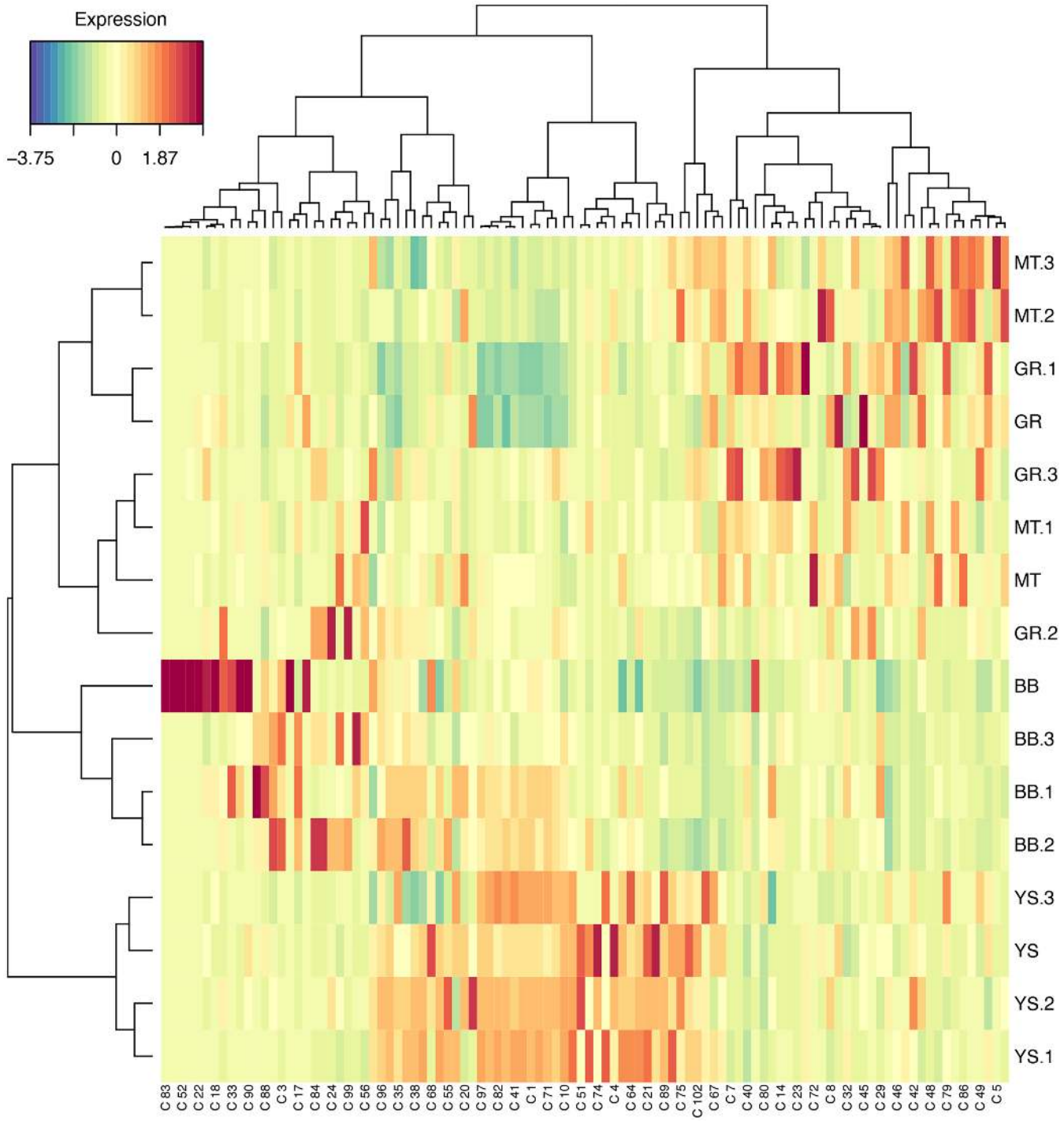


FIGURE 6 Clustered image map representing expression values of phytochemical compounds across populations (BB, Bodega Bay; GR, Groveland; MT, Mather; YS, Yosemite Creek) in the common-garden experiment. For compound names, see Appendix S1: Table S10.

Besides rising temperatures, many populations also experienced a dramatic change in precipitation regimes. Consistently, we found a complex, nonlinear relationship between yarrow growth and precipitation patterns. Significant interactions between temperature and precipitation were found as the effects of increasing temperature depend on how precipitation changes: As temperature and drought increased, yarrow plants became shorter. An ecological model simulation indicates that yarrow plants get shorter if precipitation decreases while temperature increases. These results are consistent with physiological constraints, as increasing temperature at constant precipitation leads to higher evapotranspiration and hence water deficit (Parmesan & Hanley, 2015).

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In addition to the observed effects of temperature and precipitation on plant height, it is important to consider the potential role of increased atmospheric CO_2 as a contributing factor. Elevated CO_2 levels are well known to enhance photosynthesis and net primary production, particularly in woody plants, by increasing carbon assimilation efficiency and reducing photorespiration (Graumlich et al., 1989; LaMarche et al., 1984). Such effects have been documented across diverse ecosystems, including tundra environments, where plant height has increased significantly in response to rising CO_2 levels (Bjorkman et al., 2018). In our study, the observed increase in height among Californian populations of *A. millefolium* in warmer and non-arid regions may partly reflect the physiological benefits of increasing CO_2 levels during the last century, in addition to the direct effects of warming temperatures and altered precipitation regimes. While our data do not explicitly parse out CO_2 effects, their potential contribution highlights the need for future studies to quantify the interactive effects of CO_2, temperature, and precipitation on plant growth and metabolic diversity over long time scales.

Identifying distinct phytochemical profiles among populations provides further evidence supporting ongoing local adaptation and differentiation. We identified diverse sets of plant secondary metabolites that play crucial roles in ecological interactions and responses to environmental stress (Agrawal et al., 2009; Fang et al., 2019; Walker et al., 2023). The phytochemical 4-Terpinyl acetate may contribute to defense against herbivores and fungal pathogens (Kim et al., 2023). Isobornyl formate may be involved in the yarrow's response to abiotic stressors by acting as a signaling molecule (Kim et al., 2023). p-Menth-3-en-1 contributes to the yarrow aromatic scent (Kim et al., 2023). Dehydrosabinenol can have allelopathic effects impacting the growth and survival of neighboring plants (Kim et al., 2023). cis-3-Hexenyl is a volatile terpenoid that might be involved in response to herbivory, attracting natural enemies of herbivores and communicating with neighboring plants (Kim et al., 2023). α-Terpinen has microbial properties. Santolina triene contributes to drought and high-temperature resistance (Kim et al., 2023). Artemisia ketone and myrtenyl are volatile biomarkers acting as signaling molecules; methyl-4-pentenal may have allelopathic effects (Kim et al., 2023). Camphor has insecticidal properties, and Eucalyptol is an antimicrobial (Kim et al., 2023); p-mentha-1,4-diene and 2,3-dehydro-1,8-cineole might contribute to plant signaling and communication (Kim et al., 2023).

These compounds contribute to plant defenses against herbivores and pathogens. They may also play a role in competitive interactions with other plant species and mutualistic interactions with pollinators, microbes and beneficial insects. As these compounds are often specific, our results suggest that yarrows may adapt and differentiate to changes in local biotic communities too, in addition to climate. Future studies shall address how species interactions vary across yarrow populations. Environmental conditions could also strongly impact the production of specialized metabolites involved in plant protection. Among them, plant VOCs play crucial roles in plant adaptation to the environment and act as infochemicals in interactions involving multiple trophic levels (Agrawal et al., 2009; Walker et al., 2023). The production of these compounds, which is pivotal for plant adaptation, may be influenced by global climate change factors like elevated atmospheric carbon dioxide, ozone, and temperature. This critical aspect of plant responses to climate change warrants further research.

The documented differences in plant height and metabolic profile among yarrow populations highlight how these populations may have evolved environmental-specific responses leading to their persistence over one hundred years of increasing temperature and drought. This adaptive variation in growth and metabolism may drive population differentiation and diversification (Kawecki & Ebert, 2004; Liu et al., 2019), a process essential for the distribution and persistence of populations across diverse changing climatic conditions (Parmesan & Hanley, 2015). Understanding these drivers and the consequences of local adaptation has broader implications for conservation biology. Recognizing plant populations' metabolic diversity traits is key to making informed decisions regarding ecological restoration projects. Utilizing locally adapted populations can increase restoration efforts' success and enhance restored ecosystems' resilience in the face of climate change.

AUTHOR CONTRIBUTIONS

Gianalberto Losapio, Roger Guevara, and Rodolfo Dirzo conceived the study. Gianalberto Losapio conducted field work. Baptiste Doussot conducted lab work with help from Fabrizio Araniti and Leonardo Bruno. Gianalberto Losapio analyzed the data with input from Baptiste Doussot. Gianalberto Losapio drafted the manuscript. All authors commented and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Losapio, 2024) are available from the Environmental Data Initiative (EDI) Data Portal: https://doi.org/10.6073/pasta/88aad2d5c2677ed759d836f3f58564b4. All specimens are stored in the institutional collections of Città Studi Botanic Garden of the University of Milan.

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