PHENOTYPIC RESPONSES OF SAGEBRUSH TO THE SOUTHWESTERN NORTH AMERICA MEGADROUGHT: A GENOTYPE-BY-ENVIRONMENT (GxE) APPROACH

by

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The following individuals read and discussed the thesis submitted by Kara A. Navock, and they evaluated their presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

For my family and friends who provided support and encouragement throughout this wild adventure.

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ABSTRACT

The Southwestern North America megadrought is an extreme climate event. *Artemisia tridentata* (big sagebrush) is the dominant, keystone species of sagebrush-steppe ecosystems in arid and semi-arid habitats of western North America. I conducted a genotype-by-environment (GxE) experiment on two putative genotypes (drought-tolerant, G1 and drought-sensitive, G2) and two cytotypes, diploid (2x) and tetraploid (4x), to determine the phenotypic responses of big sagebrush seedlings to drought. For three chlorophyll fluorescence parameters, my results indicate a complex set of factors influence sagebrush responses to drought, including canalization, adaptive phenotypic plasticity, cryptic genetic diversity, and GxE interactions. Variation in leaf temperature profiles of sagebrush seedlings is exclusively driven by treatment effects, suggesting that variation for this trait is determined by non-adaptive phenotypic plasticity. I did not detect significant treatment effects for root to shoot (R:S) length ratios for 2x and 4x families exposed to drought, although I did detect significant differences among G1 and G2 genotypes of both cytotypes. Tetraploid seedlings significantly outperformed 2x seedlings for R:S length ratios across all three watering treatments. My results indicate that sagebrush populations differ in their capacity to respond to megadrought; thus, proper sourcing of seeds for restoration efforts should account for the genotypes and cytotypes of populations.

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LIST OF ABBREVIATIONS

BSU	Boise State University
G1	Drought tolerant genotype
G2	Drought sensitive genotype
GC	Graduate College
GxE	Genotype-by-Environment
ST1	Sample time one
ST2	Sample time two
SWNA	Southwestern North America
T1	Treatment one (well-watered)
T2	Treatment two (imposed-drought)
T3	Treatment three (drought-recovery)
TDC	Thesis and Dissertation Coordinator

INTRODUCTION

Extreme climate events such as excessive heat, precipitation extremes, flooding, frosts, and droughts are important components of human-caused climate change (Gutschick and BassiriRad, 2003; Niu et al., 2014). The severe and persistent 21st-century megadrought in southwestern North America (SWNA) has been identified recently (Williams et al., 2020) based on hydrological modeling coupled with 1200-year tree-ring reconstruction of summer soil moisture. Williams et al. (2020) demonstrated that the 2000–2018 SWNA megadrought was the second driest 19-year period since 800 CE, exceeded only by a megadrought in the late-1500s. This recent extreme climate event was reported to be driven by natural variability superimposed on drying due to anthropogenic warming (i.e., human activities account for 47% of the response) (Williams et al., 2020). Although significant progress has been made in understanding the origin of recent megadroughts, we still lack fundamental knowledge to predict how organisms will respond to these extreme climate events, which are likely to intensify in the future (Stott, 2016).

Drought occurs due to a lack of precipitation and concomitant reduction in soil moisture, which has large-scale impacts on plant species, communities, and ecosystems (Ault, 2020). Drought is a major cause of seedling mortality because seedling are the most vulnerable stage of the plant life cycle, and the persistence and sustainability of plant communities is dependent on the survival and reproduction of species that form

these communities (Leck et al., 2008). Thus, drought, and other conditions of the abiotic and biotic environment, exert variable selection on populations of species that leads to the evolution of local adaptation (i.e., ecotypic differentiation among populations) (Via and Lande, 1985). Consequently, plants that are locally adapted to specific environmental conditions will have high fitness while those conditions are maintained, but will however be more vulnerable to recruitment and reproductive failure with changing conditions, such as those associated with megadrought. Deciphering the mechanisms that produce the phenotypes (adaptive traits) contributing to seedling survival and recruitment and successful reproduction is paramount to predict the impact of climate change on species, and ultimately communities and ecosystems.

Another mechanism contributing to variation in phenotypic expression is the plastic response of individuals with different genotypes to variable environmental conditions (i.e., phenotypic plasticity) (Nicotra et al., 2010). Two types of phenotypic plasticity have been described, non-adaptive and adaptive phenotypic plasticity; although ascertaining the relative role of each of these forms of plasticity across a species range has proven to be difficult to demonstrate and thus remains an open question (Van Kleunen and Fischer, 2005; Ghalambor et al., 2007; Nicotra et al., 2010; Murren et al., 2015). Non-adaptive plasticity is believed to occur in response to novel stressful environments, as a result of reductions in performance due to limited resources (Van Kleunen and Fischer, 2005), and is therefore associated with reduced fitness relative to the ancestral phenotype (Ghalambor et al., 2007). Adaptive plasticity is plasticity that is maintained by natural selection in different, or new, environments, and increases a genotype's (population's) long-term fitness (Miner et al., 2005; Nicotra et al., 2010).

Ghalambor et al. (2007) define adaptive plasticity as a reaction norm that produces a phenotype "…that is in the same direction as the optimal value favored by selection in the new environment." Adaptive plasticity reduces the cost of directional selection; thereby providing time for a population to become established with sufficient standing genetic variation to generate a range of heritable phenotypes which can respond to local selection pressures (see Ghalambor et al., 2007, and references therein). Thus, adaptive plasticity reduces the probability of population extirpation under changing environmental conditions, as is occurring with the SWNA megadrought.

Currently, there is a lack of knowledge concerning the role of polyploidy in enabling the rapid response (i.e., tolerance) of plants to megadrought. In the context of drought-tolerance, polyploids might not necessarily outperform their diploid progenitors (Hao et al., 2013). Polyploids usually have larger xylem vessels than diploids due to chromosome doubling, which leads to larger cell size (e.g. Hao et al., 2013). Larger xylem vessels confer higher water-transport efficiency (Maherali et al., 2009), but may also be more vulnerable to cavitation under drought stress due to the inverse relationship between hydraulic conductance and prevention of embolisms (Piñol and Sala, 2000; Martínez-Vilalta et al., 2002). Conversely, narrow xylem vessels tend to have fewer and smaller pit membrane pores to reduce the occurrence of air seeding (air entry) under high xylem tension (Wheeler et al., 2005). Narrow xylem vessels may offer a selective advantage to diploids under drought stress by minimizing cavitation and therefore maintaining hydraulic conductivity for longer amounts of time, compared to polyploids. In this context, one prediction is that polyploids would be maladapted to the climate conditions associated with the SWNA megadrought (see below).

Research exploring the phenotypic responses of plants to extreme climate events is especially salient in ecosystems dominated by one or a few foundational, keystone plant species. The sagebrush steppe ecosystem of western North America is characterized by the dominant, keystone species *Artemisia tridentata* (Asteraceae, common name, big sagebrush; but hereafter referred to as sagebrush), and this ecosystem largely occurs within the region experiencing the SWNA megadrought. In addition to being ecologically dominant, sagebrush provides shelter and food for many herbivores, including the endemic pygmy rabbit (*Brachylagus idahoensis*) and two species of sage-grouse (*Centrocercus* spp.; Welch, 2005; Prevéy et al., 2010). The sagebrush steppe was once distributed over roughly 1 million km^2 in western North America (Requena-Mullor et al., 2019), but has since been destroyed and fragmented due to threats from invasive species (Prevéy et al., 2010), increased fire frequency and intensity (Shriver et al., 2019), habitat destruction (Thompson, 2007), and climate change (Richardson et al., 2017; Still and Richardson, 2015). Because of these threats, land managers have prioritized restoration efforts of sagebrush in these ecosystems (Chaney et al., 2017), yet these efforts have not investigated how adaptive capacity may influence the successful restoration of this keystone species.

To assess the phenotypic responses of sagebrush to megadrought, we focused on populations in two locations, one in Idaho (ID) and one in Utah (UT), which exhibit contrasting precipitation regimes and where two different cytotypes of sagebrush co-occur (see below). Historically, the ID location received one-tenth the annual precipitation of the UT location. In addition, sagebrush populations at the ID location typically experienced a four-month period of summer drought (accompanied by heat

stress), whereas UT populations of sagebrush experienced lower water deficits during the summer. Consequently, sagebrush seedlings of UT populations would be expected to be less adapted to drought (due to the onset of the North American Monsoon in August) than populations in more xeric locations such as ID (Supp Mat Figs. S1, S2). Williams et al. (2020) indicates that the ID and UT locations have undergone significant declines in soil moisture in the last two decades, although these declines are especially acute for the UT location. Common garden experiments demonstrated that sagebrush populations from UT experienced high mortality when translocated to ID (Chaney et al., 2017; Supp Mat Fig. S3). This result suggests that sagebrush populations in UT (and from similar climates) might be maladapted to summer drought, and especially to megadrought. Because of the lower precipitation levels at the ID location and the higher survival of populations from ID in the ID common garden, we propose that individuals from ID populations would possess a drought-tolerant genotype (G1) and individuals from UT populations would exhibit a drought-sensitive genotype (G2).

The objectives of this study are to evaluate the role of local adaptation versus phenotypic plasticity in influencing the phenotypic responses of these two genotypes to experimentally-imposed megadrought, and to assess the influence of ploidy on phenotypic expression by including sagebrush populations with the diploid (2n=2x=18) and tetraploid (2n=4x=36) cytotypes. Both cytotypes occur in sympatry in the ID and UT locations (McArthur, 1994). I simulated megadrought conditions by growing seedlings in a greenhouse, in small containers. Soils in these containers lost most of their water content within 48 hours, following the cessation of watering. In order to assess seedling performance under megadrought conditions, I conducted a genotype-by-environment

(GxE) experiment. Specifically, my study aims to address the following questions: (i) can sagebrush seedlings rapidly respond to megadrought?, (ii) does ploidy influence the expression of drought tolerance?, and (iii) when exposed to megadrought, do populations that have historically experienced more intense summer drought (plants with the G1 genotype) outperform populations that have historically experienced less intense summer drought (plants with the G2 genotype)? If the answer to question (iii) is yes, this would suggest that the G2 genotype is maladapted to megadrought, compared to the G1 genotype. Based on the results of this study, I provide recommendations to land managers for developing strategies to mitigate the effect of megadrought on this critically important keystone species.

METHODS

(A) Plant Material

This experiment was conducted using sagebrush seedlings grown from sympatric populations of 2x and 4x cytotypes at the ID location (Lat. 43.336, Long. -116.964; Mountain Home, Idaho; the G1 genotype) and the UT location (Lat. 43.336, Long. -116.964; La Sal, Utah; the G2 genotype). I sampled two populations at each location, representing each genotype and each cytotype: the diploid (2x) cytotype, G1 = IDT3 (IDT3 is a population identification code) and G2 = UTT2, and the tetraploid (4x) cytotype, G1 = IDW3 and G2 = UTW2. Seeds from ten maternal plants per population were provided by the US Forest Service, Rocky Mountain Research Station, Moscow, ID. Seeds from each maternal plant (hereafter referred to as a family) were sown on 10 July 2019 in the research greenhouse facility at Boise State University (Boise, Idaho, USA). Due to the highly outcrossing, wind-pollinated mating system of sagebrush, I consider the seeds from each maternal plant to be half siblings (half-sibs). Three seeds from a maternal plant were sown directly into 983cm^3 Deepot™ (Stuewe & Sons, Inc.) containers with a soil mix consisting of one-half soil conditioner (one part volcanic cinder: two parts vermiculite: one part peat moss) and one-half greenhouse potting soil. The soil conditioner in the soil mix allowed for sufficient drainage for the seedlings growing in the containers. I verified drainage of the soil mix by observing that soil at the top of the containers dried within 48 hours following watering; drying also indicated that re-watering was required to maintain my well-watered experimental treatment (see

below). Greenhouse temperature was maintained at 20°C (+/- 2°) and a 16/8 h day/night photoperiod was maintained throughout the experiment. Seeds and seedlings were watered on alternating days for optimum growth. Once seeds were sown, containers were randomly placed into racks that could accommodate 20 containers, and the positions of the racks in the greenhouse were randomized every two weeks to minimize any greenhouse microclimate effects.

Random thinning of seedlings occurred when more than one seed germinated, this ensured that there was only one seedling per container. Mortality data was collected every two weeks, for approximately seven months. Once mortality stabilized (Supp Mat Fig. S4), plants were grown under optimum conditions for two more weeks before starting the GxE megadrought experiment. This 7½ month growth period allowed seedlings to acclimate to the greenhouse environment and was meant to eliminate/reduce maternal environmental effects. Although seedlings were grown in the greenhouse for a longer period of time than typically occurs under natural conditions, before the onset of summer drought, this approach was necessary to ensure that individual leaves and entire plants were large enough for fluorescence and stomatal conductance measurements.

(B) Conducting the Gxe Experiment

Norms of reaction analyses were conducted to determine treatment effects, which estimates the phenotypic effects of environment (E) and provides an estimate of phenotypic plasticity; the effects of genotypes and cytotypes (G), which estimates the genetic component; and their interactions (GxE), in relation to the responses of seedlings to drought (Hendry, 2020). In my study, the tolerance of sagebrush seedlings to

megadrought was analyzed by measuring their phenotypic responses to well-watered conditions (T1, simulating the summer soil moisture conditions of the UT location) and comparing them to their responses to drought conditions (T2, simulating the summer soil conditions of the ID location; Supp Mat Fig. S1). Additionally, the ability of seedlings to recover from megadrought was quantified by conducting a drought-recovery experiment (T3).

My goal for the GxE megadrought experiment was to include six randomly selected seedlings per family, and 10 families per population, resulting in 60 individuals per population (i.e., genotype/cytotype). Therefore, I intended to include a total of 240 individuals in this experiment. However, due to what appeared to be random mortality, some families had less than six individuals surviving; thus, the total sample size at the start of the experiment was 229 seedlings (Table 1). Seedlings were randomly allocated to treatments and trays within treatments. Trays were randomized each week to avoid any greenhouse microclimate effects.

The GxE megadrought experiment was subdivided into two phases starting on 24 February 2020. Phase 1 lasted 16 days and seedlings were divided into two treatments: T1 and T2. For T1, I watered seedlings every two days so that the soil mix was at field capacity; for T2, seedlings were watered on day one, and then watering was withheld for 15 days. Withholding water caused the soil mix to dry from the top-down, which mimics the typical soil drying pattern of sagebrush habitats during the late spring and summer, and simulates megadrought conditions (Hacke, Sperry, & Pittermenn, 2000). Phase 2 lasted six days and was split into two treatments: well-watered seedlings (T1), maintained as in part one, and drought-recovery seedlings (T3) for which half of the T2 seedlings

were randomly selected for re-watering. Re-watering was conducted following the same procedure as T1 except for day one, when seedlings received 15 ml of water. Sagebrush seedlings were harvested at the end of each phase of the experiment, imaged (Nikon, model d5600) and stored at -80°C freezer for subsequent analyses. Hereafter, I refer to these sampling times as ST1 (harvesting at the end of Phase 1) and ST2 (harvesting at the end of Phase 2).

(C) Validating Megadrought Treatment Effectiveness

The effectiveness of my imposed-drought treatments was assessed by weighing all T2 and T3 containers, and a random subset of T1 containers, daily using an Ohaus Scout SPX8200 portable balance (Supp Mat Table S1). These data were used as a proxy of water content of the soil mix, which determines the water availability for photosynthesis and plant growth. An R script was used to infer daily container weight differences ($weight_{t1}$ - $weight_{t0}$) and those were plotted through time (x=time, and y=Daily container weight loss or gain, g).

(D) Stomatal Conductance Measurements: Initiating the Re-Watering Treatment (T3)

In this study, stomatal conductance measurements were used in combination with thermal imagery to ascertain the timing of stomatal closure (Supp Mat Figure S4). Stomatal closure is considered a sign that leaves have reached negative carbon balances because of severe soil water deficits, and this was used as a cue to initiate Phase 2 of the experiment by beginning the re-watering treatment (T3). Stomatal conductance was measured using a model SC-1 leaf porometer (Decagon Devices, Inc., Pullman, WA,

USA). Instrument calibration was conducted before each set of measurements (at ST1 and ST2) according to the manufacturer's guidelines. Leaves of sagebrush seedlings are relatively small and have stomata on both sides (Downs and Black, 1999). For each measurement, three perennial leaves were inserted in the porometer to fully cover the sensor aperture.

(E) Phenotypic Measurements

I assessed phenotypic response to imposed drought by measuring photosynthetic performance (photosynthetic electron transport), leaf temperature profiles, and root to shoot (R:S) length ratios. I also conducted phenotypic measurements on three groups of sagebrush seedlings: seedlings receiving the imposed-drought treatment (T2), seedlings under drought-recovery condition (T3), and those maintained under well-watered conditions (T1). I randomly sampled half of the seedlings (n=120) to conduct daily measurements of chlorophyll fluorescence and temperature profiles. Daily phenotypic measurements started at 9:30 am (MST) and lasted for a four-hour period. Destructive sampling occurred at ST1 and ST2 to measure R:S length ratio and stomatal conductance. Due to their small size, some seedlings were not suitable for chlorophyll fluorescence and stomatal conductance measurements (see below) and were therefore not included in my analysis (Supp Mat Table S2).

(I) Chlorophyll Fluorescence Measurements

Photosynthetic electron transport relies on water to produce chemical energy (ATP and NADPH), which is then used for carbon fixation and the production of sugar molecules that sustain plant growth (Murchie and Lawson, 2013). Photosynthesis

performance was quantified by measuring chlorophyll fluorescence. The effect of drought on the photosynthetic performance of sagebrush seedlings was assessed in light-adapted leaves using a MultiSpeq v2 a PAM fluorometer (PhotosynQ Inc., East Lansing, MI, USA) following the Photosynthesis RIDES protocol (Kuhlgert et al., 2016; Supp Mat Table S3). I calculated PHI2 ($F_{q'}/F_{m'}$; the operating efficiency of PSII photochemistry), $F_{v'}/F_{m'}$ (maximum quantum yield of PSII), and $F_{o'}/F_{m'}$ (minimal chlorophyll fluorescence over maximal chlorophyll fluorescence, which is a measure of the structure and function of PSII). $F_{v'}/F_{m'}$ has been utilized as an indicator of drought stress in previous studies (e.g. Li et al., 2006), likely signaling photo-inhibitory damage under water-stressed conditions, and $F_{o'}/F_{m'}$ has been proposed as an alternate metric (e.g. Banks, 2018). This study did not use dark-adapted leaves due to spatial and time limitations. However, relative comparisons of chlorophyll fluorescence measures were performed. For each measure, a single mature leaf was inserted into the device cuvette so that the entire leaf was within the light guide and this procedure was repeated twice. The order that the plants were measured was randomized daily.

(II) Leaf Temperature Profiles

Thermal imaging of leaves can provide a measure of a plant's response to drought (Liu et al., 2011): as soil water becomes limiting, stomal closure takes place and evapotranspiration rates decrease; as evapotranspiration decreases, leaf temperatures increase. The FLIR (C3) thermal camera (FLIR systems, Inc., Wilsonville, OR, USA) and its associated software were used to image seedlings and infer leaf temperature profiles (Supp Mat Table S4). To obtain accurate thermal images, I determined the reflected apparent temperature each day using the reflector method as described in FLIR

C3 user manual (section 15.2.1.1.2). Individual seedlings were imaged daily using non-reflective black felt as a background. An R function was developed to automatically recognize leaf tissues using RBG images obtained from the thermal camera and to extract temperature data from associated csv files. The R function began by converting RBG images into rasters (using *magick* and *raster* packages) (Hijmans et al., 2016), with the same resolution as the csv files outputted by the FLIR software. It next inferred the color of each cell using *colourvalues* and *plotrix* (Lemon et al., 2020) packages, and finally it excluded non-plant cells by searching for gray, ivory, brown, wood, white, puff, snow, wheat and yellow cells. Because this process was time consuming, a first filtering step was applied by excluding cells with RBG green channel values < 150 (= removing the black background). Finally, unique cell numbers reflecting plant tissues were used to infer average and 5-95% quantile temperatures by cross-referencing this information with the csv files.

(III) Root to Shoot (R:S) Length Ratio

In this study, I assessed the response of seedlings to my imposed-drought treatments by determining their R:S length ratios by (i.e., I measured the length of the root and shoot of each seedling, Supp Mat Table S5). I chose this approach because rapid preservation of root and shoot tissue samples was required for future transcriptomic analyses (thus, I did not determine R:S ratio by measuring the dry-weight biomass of root and shoot tissues). At both sampling times (ST1 and ST2), seedlings were gently removed from their containers and soil was removed from roots carefully to maintain root structure. Once a seedling was removed from its container, the entire seedling (root and shoot) was placed onto white paper next to a ruler, and imaged using a digital camera

(Nikon, model d5600). The Fiji software package (Schindelin et al., 2012) was used to conduct measurements using the digital images. R:S length ratios were calculated for each seedling, and then averaged for each family.

(F) Determining Drought Treatment Effects

(I) Statistical Analysis

To compare the performance of genotypes and cytotypes to imposed-drought treatments, a two-fold approach was utilized. Approach 1 examined the effects of drought/watering treatments (T1 vs T2 and T2 vs T3) within cytotypes (2x, 4x). Three-way ANOVAs were used to investigate treatment, genotype, family (to investigate if genetic response differs among families within populations), and interaction effects (treatment x genotype, treatment x family) using chlorophyll fluorescence (PHI2, F_o'/F_m', F_v'/F_m'), temperature profiles, and R:S length ratio as response variables. Approach 2 compared the performance of cytotypes (2x,4x) within treatments (T1, T2, T3), using two-way ANOVAs of response variables (chlorophyll fluorescence, temperature profiles, and R:S length ratio). All ANOVAs were performed in base R (R Core Team, 2020) with the *aov* function and a significance level defined as p-value ≤ 0.05. Variables deemed significant were subsequently analyzed using a post-hoc Tukey's test with the *TukeyHSD* function in base R (R Core Team, 2020) to identify significant pairwise comparisons using adjusted p-values (*<0.05, **<0.01, ***<0.001).

(II) Norms of Reaction

For each cytotype (2x, 4x), norms of reaction comparing the phenotypic responses of genotypes (displayed at the family level) across treatments were inferred based on

chlorophyll fluorescence (PHI2, F_o'/F_m', F_v'/F_m'), temperature profiles, and R:S length ratios. An R script (R Core Team, 2020) was written using basic functions to infer mean and 5-95% quantiles phenotypic responses for each measurement at the family level, sorted by genotype. The phenotypic responses of families with significant treatment x family adjusted p-values (from the Tukey's tests; see above) are interpreted to be due to phenotypic plasticity, whereas the phenotypic responses of families with non-significant p-values are interpreted as being under "hard" genetic control.

RESULTS

(A) Predicted Results

Unless these traits exhibit a canalized response (i.e., seedlings express the same phenotype regardless of environmental variability or genotype; *sensu* Waddington, 1942), I predicted that both chlorophyll fluorescence and leaf temperature profiles would increase as water availability decreases and the negative physiological effects of drought increase. Additionally, I predicted that an increase of those metrics should be more severe for G2 and 4x seedlings compared to G1 and 2x seedlings. In addition, less drought-tolerant seedlings should close their stomata faster in response to drought, to avoid cavitation, and would therefore more quickly transition to the starvation phase. Finally, I evaluated resource allocation in seedlings by quantifying root to shoot (R:S) length ratios. Mašková and Herben (2018) reported that increased allocation to resource-acquiring organs was enormously important for seedling survival and the future success of the plant. In this context, I predicted that drought-tolerant seedlings will allocate more resources to their roots (more precisely to taproots) to track water availability in the soil compared to drought-sensitive seedlings (since these individuals receive frequent precipitation throughout summer and would have reduced selection favoring individuals with long taproots).

Overall, I predicted that G1 genotype will outperform G2 genotype under megadrought conditions by exhibiting higher root to shoot length ratios, and less damage

to their photosynthetic apparatus. As noted earlier, the effects of ploidy on drought tolerance are unclear. I predicted that diploid (2x) sagebrush seedlings will outperform their tetraploid (4x) counterparts under drought conditions due to their hypothesized ability to minimize cavitation and therefore maintain water and nutrient uptake for longer periods (Piñol and Sala, 2000; Martínez-Vilalta, et al., 2002, Wheeler et al., 2005).

(B) The GxE Experiment

Although seedlings responded to the imposed-drought treatment (T2; Table 2), no mortality was recorded during the GxE megadrought experiment. For each treatment, seedlings of the two genotypes generally exhibited similar response patterns. With the imposition of T2 for 16 days, I found that decreases in the weight of containers were minimal (Supp Mat Fig. S5). This result suggested that major declines in leaf transpiration was likely occurring, this was my signal to start measuring stomatal conductance and determine when to terminate T2 and begin re-watering (T3; Supp Mat Fig. S6).

(C) Determining Drought Treatment Effects

Traits such as chlorophyll fluorescence, leaf temperature profiles, and R:S length ratio were used to evaluate the phenotypic responses of sagebrush seedlings to imposed-drought (Supp Mat Tables S2-S6).

(I) Approach 1: Comparisons of T1 with T2 and T2 with T3

Comparisons of T1 with T2 revealed significant treatment effects for all phenotypic traits, with the exception of R:S length ratio, which is non-significant for both cytotypes (Table 2; Supp Mat Table S7). Significant genotype effects were detected for

all phenotypic traits, for 2x seedlings, with the exception of leaf temperature profiles; whereas, only two phenotypic traits (Phi2 and R:S length ratio) showed significant genotype effects for the 4x seedlings (Table 2). Significant family effects were detected for both cytotypes, but only for measurements associated with chlorophyll fluorescence (Table 2). The following cytotypes and families exhibiting significant treatment effects (based on the Tukey's test): the 2x cytotype, IDT3c, IDT3d, UTT2d, UTT2j for F_v'/F_m' and F_o'/F_m' and IDT3c, IDT3d, and UTT2d for PHI2, and 4x cytotype, IDW1b, IDW1e, IDW1g, UTW2f, UTW2i for F_v'/F_m' and F_o'/F_m' and IDW1b, IDW1e, UTW2f, UTW2h, UTW2i for PHI2 (more details on the performance of these families, based on norms of reaction, is provided below). Significant treatment x genotype interactions were detected for chlorophyll fluorescence, but only for 4x seedlings; whereas significant treatment x family interactions were revealed for chlorophyll fluorescence for seedlings of both cytotypes (Table 2).

Comparisons of T2 with T3 revealed significant treatment effects for both cytotypes for all phenotypic traits, with the exception of F_o'/F_m' and F_v'/F_m' in 2x seedlings (Table 2). Genotype and family effects for the comparison of T2 vs T3 for 2x seedlings were very similar to the patterns observed for the comparison of T1 with T2, whereas significant GxE interactions were only detected for chlorophyll fluorescence (Table 2). Only two 2x families, UTT2e (for F_o'/F_m' and PHI2) and UTT2j (for PHI2), exhibited significant treatment effects, whereas IDW1e (for F_o'/F_m' and PHI2) was the only 4x family with significant treatment effects (see below for more details on performance of families based on norms of reactions).

Norms of reaction comparing the performance of families (sorted by genotypes, for both cytotypes) for T1 and T2 revealed complex patterns of phenotypic response to imposed drought. For 2x seedlings, most families did not exhibit significant differences for PHI2 between the well-watered and drought treatments (Fig. 1). However, several families did exhibit variation in their phenotypic responses. Two families (UTT2d, IDT3d) exhibited low values for PHI2 under T1 and then showed a significant increase in PHI2 under T2, and one family, IDT3c, had a high value of PHI2 under T1, but exhibited a low value of PHI2 under T2 (Fig. 1A). A similar pattern was observed for 2x families for other measures of chlorophyll fluorescence. For F_o'/F_m' and F_v'/F_m', two families (UTT2d and IDT3d) had higher PHI2 values under T2, compared to T1, and two families, IDT3c and UTT2j, had higher PHI2 values under T1 than T2 (Supp Mat Figs. S7 and S8). For 4x seedlings, four families (UTW2i, UTW2h, IDW1b, and IDW1e) exhibited lower PHI2 values with drought (T2), compared to well-watered conditions (T1); whereas one family (UTW2f) had higher PHI2 values under T2 (Fig. 1B). Similar results occurred for the other measures of chlorophyll fluorescence, for 4x seedlings (Fig. 1B; Supp Mat Figs. S7 and S8).

Norms of reaction comparing the performance of families (sorted by genotypes, for both cytotypes) for T2 and T3 also revealed complex patterns of phenotypic response to imposed drought. Seedlings from most 2x families did not exhibit significant differences for PHI2 between the drought and re-watering treatments (Fig. 2), and only the UTT2e family showed significant treatment effects, it exhibited lower values of PHI2 and F_o'/F_m' under imposed-drought conditions (T2), compared to its values with re-watering (T3) (Fig. 2A; Supp Mat Fig. S9). I observed similar values of F_v'/F_m' for

seedlings from 2x families under drought (T2) and re-watering (T3) treatments (Supp Mat Fig. S10). For 4x seedlings, a pattern similar to 2x seedlings was observed: most families exhibit similar values of PHI2 for T2 and T3, and two families (IDW1e and UTT2j) exhibit significantly higher PHI2 value for T3, compared to T2 (Fig. 2B). The F_o'/F_m' norms of reaction analysis revealed that IDW1e was the only 4x family with a decreased value with re-watering (T3, Supp Mat Fig. S9). As noted above for 2x seedlings, I observed similar values of F_v'/F_m' for 4x seedlings under T2 and T3 (Supp Mat Fig. S10).

Norms of reaction analyses based on R:S length ratios are consistent with statistical analyses, and show clear differences between G1 and G2 families of 2x seedlings, with G1 families generally having higher values for this phenotypic trait; whereas 4x families with these two genotypes were intermixed (Fig. 3). Norms of reactions analyses of temperature profiles are also consistent with statistical analyses and show that responses were predominantly driven by the three different treatments (Supp Mat Figs. S11 and S12).

(II) Approach 2: Comparisons of T1, T2, And T3

A comparison of the performance of the two cytotypes for each treatment (T1, T2, T3) does not reveal consistent patterns (Table 3). Under well-watered conditions (T1), phenotypic differences were only observed for R:S length ratio, with the 4x seedlings slightly outperformed the 2x seedlings (adjusted p-value of 0.039; Supp Mat Table S8). With imposed drought (T2), the cytotypes exhibited statistically significant differences for all phenotypic traits except temperature profile (Table 3). Diploid seedlings

outperformed 4x seedlings for PHI2 and F_v'/F_m'; conversely, 4x seedlings outperformed 2x seedlings for F_o'/F_m' and R:S length ratio (Supp Mat Table S8). After the re-watering treatment (T3), I observed a pattern similar to that of T1, 4x seedlings outperforming 2x seedlings for R:S length ratio (Table 3; Supp Mat Table S8).

DISCUSSION

Artemisia tridentata (sagebrush) is the dominant, keystone species of sagebrush steppe ecosystems that occupy arid and semi-arid habitats of western North America. This species and these ecosystems are currently under threat from multiple human-induced stresses and disturbances (Chambers et al., 2014; 2017); including the SWNA megadrought, which has been ongoing for the last 19 years (Williams et al., 2020). Consequently, there is an urgent need to better understand the characteristics of sagebrush that determine its capacity to cope with these rapidly changing conditions, especially this severe climate event. Sagebrush consists of three major subspecies and two cytotypes, *A. t. wyomingensis* is a tetraploid that occupies lower elevation, drier habitats where annual precipitation can be less than 160 mm per year, *A. t. tridentata* is a diploid or tetraploid that occupies deep, well-drained soils at lower elevation, and *A. t. vaseyana* is a diploid or tetraploid that occupies higher elevations that generally have cooler and moister conditions (precipitation ranges between 500-750 mm per year) (Kolb and Sperry 1999; Mahalovich & McArthur, 2004; Brabec et al., 2017). These observations suggest niche differentiation among sagebrush subspecies and cytotypes, although their distributions do overlap in certain areas across the landscape.

Because sagebrush lives within arid and semiarid habitats of western North America, its adaptations to drought have been well-documented and include various physiological, growth, resource allocation, and phenological traits (Evans et al., 1991;

1992; Evans and Black, 1993; Kolb and Sperry, 1999). However, while sagebrush subspecies and cytotypes may exhibit similar responses to drought (reduction in transpiration, reduced stomatal conductance, foliage abscission), variation in drought tolerance among these subspecies and cytotypes has been documented. For instance, Kolb and Sperry (1999) showed that cavitation resistance, which maintains xylem water conductance, was highest for ssp. *wyomingensis*, intermediate for ssp. *tridentata*, and lowest for ssp. *vaseyana*, with these differences apparently under genetic control. Because of this earlier research, I anticipate that I will detect differences in the drought responses of the two genotypes (G1 and G2) and the two cytotypes (2x and 4x) included in my experimental design (see the Introduction). Moreover, it is likely that the phenotypic traits I measure in this study will reveal different response patterns following the imposition of drought.

To my knowledge, this study is the first GxE common-garden experiment aimed at evaluating the phenotypic responses of sagebrush to imposed drought, and recovery from drought after re-watering. With these experiments I assessed three phenotypic traits that are related to sagebrush's response to drought: chlorophyll fluorescence measurements, leaf temperature profiles, and root to shoot (R:S) length ratios. In addition, this design allowed me to partition phenotypic variation into various components: treatment effects (a measure of phenotypic plasticity), two genetic effects (at the genotype and family levels), GxE interaction effects, and the effect of cytotype. My results demonstrate that a complex combination of factors including phenotypic canalization, phenotypic plasticity, genetics (both at the genotype and family levels), GxE interactions, and cytotype influence the rapid response of sagebrush seedlings to drought

(Table 2, Figs. 1-3). These findings suggest that certain populations may be better able to survive and persist as the SWNA megadrought continues and other populations may be in jeopardy; they also provide guidelines for translocating plants so that populations of sagebrush can be maintained or restored (see below).

As suggested by Menezes-Silva et al. (2017) and Banks (2018), I used multiple measures of chlorophyll fluorescence to assess the photosynthetic response (i.e., photochemical efficiency) of sagebrush seedlings to imposed drought: PHI2, $F_{v'}/F_{m'}$, and $F_{o'}/F_{m'}$. Because the photosynthetic apparatus of drought-sensitive genotypes are likely to be damaged during drought, the value of these parameters will be lower for drought-sensitive genotypes; whereas, the value of these parameters will be relatively higher for drought-tolerant genotypes because their photosynthetic apparatus is less likely to be damaged (Percival and Sheriffs, 2002; Li et al., 2006). While Table 2 shows statistically significant results for these three chlorophyll fluorescence parameters at the treatment, genetic, and interaction levels; the norms of reaction diagrams provides insights into factors influencing the expression of these phenotypic traits. While G1 and G2 2x families exhibit statistically significant treatment, genotype, and family effects for PHI2 with imposed drought (T1 vs T2, Table 2), I also note that many of these families exhibit only minor variation in their PHI2 values (Fig. 1). This observation indicates that families of both genotypes have similar phenotypic responses, regardless of treatment (i.e., they have a canalized response). However, variation in the phenotypic response of these families may signal the influence of adaptive phenotypic plasticity and/or cryptic genetic diversity (*sensu* Schlichting and Wund, 2014); this diversity is most likely due to the wind-pollinated, highly outcrossing mating system of sagebrush.

Two families show increased PHI2 values under drought conditions; suggesting that these two families have the capacity to rapidly adapt to imposed drought. One family exhibited a reduced PHI2 value, indicating damage to its photosynthetic apparatus and a maladaptive phenotypic response to drought. Most G1 and G2 4x families (Fig. 1) had decreased PHI2 values under drought conditions (indicating drought-sensitivity of these families), with only a few families showing increased PHI2 values. Most G1 and G2 families of both cytotypes have increased PHI2 values with re-watering (Fig. 2), indicating that most of these families recovered from drought imposed for 16 days.

Results obtained for $F_{v'}/F_{m'}$ and $F_{o'}/F_{m'}$ with drought (T1 vs T2) and re-watering (T2 vs T3) (Supp Mat Figs. S7, S8, S9, and S10) were generally similar to those just described for PHI2, with the exception that most G1 and G2 4x families exhibited either increased or decreased values with drought, indicating GxE interactions for these families. Taken together, my results indicate a complex set of factors influence the response for these three chlorophyll fluorescence parameters to drought: 1) the canalized response of most G1 and G2 2x families indicate that they do not drastically alter their phenotypes; thus they appear to be genetically capable of withstanding drought, 2) while my data indicates canalization occurs, some of the variation in the expression of these three chlorophyll fluorescence traits appears to be influenced by adaptive phenotypic plasticity and cryptic genetic diversity, 3) while G1 and G2 2x families do exhibit some differences, most of these families do not appear to exhibit strong ecotypic differentiation for these chlorophyll fluorescence parameters, 4) a few 2x families appear to have the ability to adapt to drought; whereas a few families have maladaptive responses, 5) many G1 and G2 4x families exhibit strong GxE interactions, especially with the comparison of

T1 vs T2, and 6) I did not detect clear-cut differences between the two cytotypes for these three chlorophyll fluorescence parameters with imposed drought: 2x seedlings outperformed 4x seedlings for PHI2 and F_v'/F_m', but 4x seedlings outperformed 2x seedlings for F_o'/F_m'.

Leaf temperature profiles increase in response to drought as plants close their stomates due to soil-water deficits (Liu et al., 2011). Leaf temperature profiles of sagebrush seedlings reflect this pattern; they increased with drought (T1 vs T2) and decreased after re-watering (T2 vs T3), indicating recovery from drought (Supp Mat Figs. S11 and S12). Variation in leaf temperature profile data for sagebrush seedlings is exclusively driven by treatment effects (Table 2), and there is no effect of genotypes, families, interactions, or cytotypes (Table 3). These data suggest that phenotypic variation for leaf temperature profiles in the sagebrush population included in this study is determined by non-adaptive phenotypic plasticity.

Functional root traits, including root to shoot (R:S) ratios, have been used to assess the response of woody plants to drought conditions (Paganova et al., 2019). An increase in the R:S ratio of plants is considered an adaptive response to drought because it indicates that root extension is taking place to increase water uptake. I did not detect significant treatment effects for R:S length ratios for 2x and 4x families exposed to imposed drought (T1 vs T2), although I did detect significant differences among G1 and G2 genotypes for both the 2x and 4x cytotypes (Table 2). This was especially true for families of the diploid cytotypes, G1 families had significantly higher R:S length ratios compared to G2 families (Fig. 3). With re-watering (T2 vs T3), I detected significant treatment effects for R:S length ratios for 2x and 4x families and I also detected

significant differences among G1 and G2 genotypes for both cytotypes (Table 2, Fig. 3). Tetraploid seedlings significantly outperformed 2x seedlings for R:S length ratio across all three watering treatments, T1, T2, and T3 (Table 3). These results indicate strong genetic differentiation between G1 and G2 families and between the two cytotypes.

The expression of this phenotypic trait by G1 families of both cytotypes provides evidence for local adaptation: G1 families allocate more resources into the production of root tissue compared to G2 families, regardless of watering treatment. This suggest that the larger R:S length ratio phenotype of G1 families of both cytotypes contributes to their survival and persistence within the more xeric conditions found in ID. Differences in R:S length ratios among G1 and G2 families may partially explain the survival and mortality patterns of sagebrush populations in the ID common garden reported by Chaney et al. (2017). Larger R:S length ratio values of 4x seedlings, compared to 2x seedlings, also provides evidence for the influence of polyploidy in the expression of this phenotypic trait.

My original hypothesis stated that 2x sagebrush seedlings would outperform 4x seedling due to their ability to minimize cavitation, which leads to embolism and eventually to plant death, because they possess smaller xylem vessels. I did not explicitly test the role of ploidy on cavitation; rather, I assessed cavitation indirectly by measuring the ability of seedlings to maintain photosynthetic performance in response to imposed drought (T2). My data do not provide unequivocal support for this hypothesis: 2x seedlings outperformed 4x seedlings for two chlorophyll fluorescence parameters (PHI2 and F_v'/F_m'), but 4x seedlings outperformed 2x seedlings for the third parameter (F_o'/F_m'). Both cytotypes experienced a significant increase in leaf temperature profiles

in response to drought, (Supp Mat Figs. S11, S12), most likely due to reduced water availability and closure of stomata (Supp Mat Fig. S6) to avoid cavitation. Additionally, both cytotypes experienced a significant decrease in leaf temperature profiles following re-watering (T3), indicating the seedlings were capable of re-opening their stomata once a sufficient amount of soil-water was available. In contradiction of my hypothesis, under imposed drought (T2), 4x seedlings exhibited higher R:S length ratio values compared to 2x seedlings (Table 2; Fig. 3). Because this rapid response was detected in my GxE common-garden experiment, it is clearly under genetic control.

The two distinct R:S length ratio phenotypes detected for 2x and 4x seedlings of sagebrush suggests that their root architecture may be determined by different genes and/or by contrasting gene expression mechanisms, which are controlled by hormonal signaling (Chen et al., 2012). Under well-watered conditions, auxin promotes lateral root growth in plants to ensure the uptake of water and nutrients (Leyser, 2018). However, under drought stress, resource allocation to lateral roots is reduced, presumably facilitating primary (tap) root elongation for water and nutrient uptake (see Chen et al., 2012 and references therein). In addition, the stress hormone ABA inhibits lateral root growth during periods of drought. In the auxin signaling pathway, the binding of auxin to the F-box TIR1 family of auxin receptors promotes the interaction of receptors and Aux/IAA proteins. Chen et al. (2012) discovered a miRNA (*miR393*) in *Arabidopsis*, which is induced by ABA and targets TIR1 family mRNAs for degradation. The cleavage of TIR1 mRNAs prevents auxin from initiating lateral root growth and therefore redirects root growth into primary root elongation. Future transcriptomic and hormonal analyses in sagebrush will reveal the potential roles played by auxin and ABA in controlling root

growth and architecture during drought, which could be mediated by miRNAs. I believe that these mechanisms are operating because R:S length ratios under well-water conditions were similar across genotypes and cytotypes, thereby suggesting that this phenotypic response is mediated by miRNAs.

Given the many human-induced stresses and disturbances negatively impacting sagebrush steppe habitats across western North America, including the SWNA megadrought, application of a triage-based approach (*sensu* Nicotra et al., 2015) means that there is an urgent need for management and conservation strategies that maintain populations of sagebrush and improve restoration efforts focused on this keystone species (Davies et al., 2011). A critical component of successful management is the proper selection of seed sources used in plant translocations and restoration efforts (Godefroid et al., 2016). Thus, the selection of seed sources should consider variation in phenotypic trait expression of genotypes and cytotypes across an environmental gradient. My results indicate that certain sagebrush populations (e.g., G1 and 4x populations) possess the capability to withstand and adapt to drought; this information needs to be incorporated into efforts to manage and restore sagebrush; and is timely, considering the ongoing SWNA megadrought. My results can also be used to identify populations that might be in jeopardy due to the SWNA megadrought. For example, the diploid G2 population from UT I analyzed (and populations with similar genotypes) appears to be drought-sensitive and possesses less capability of surviving future climate change.

Because a complex concatenation of factors is threatening sagebrush steppe ecosystems across western North America; a complex set of approaches must be employed to better understand these processes. Thus, results of my GxE common-garden

experiment should be merged with climate models (Williams et al., 2020), sagebrush demographic models (Requena-Mullor et al., 2019), and other approaches, to improve the management and conservation of these ecosystems and enhance restoration of sagebrush, the dominant, keystone species of these ecosystems.

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TABLES

Table 1. Sagebrush seedling sample size for each genotype and cytotype (n=229 seedlings) utilized in GxE experiment. T1: well-watered treatment, T2: imposed drought treatment, T3: re-watering treatment, ST1 and ST2: different times when tissues were harvested (see text for more details).

Genotype	Cytotype	T1	T2	T3	ST1	ST2
G1	2x	19	20	20	20 (T2)	19 (T1) 20 (T3)
G2	2x	18	20	20	20 (T2)	18 (T1) 20 (T3)
G1	4x	20	20	14	20 (T2)	20 (T1) 14 (T3)
G2	4x	20	20	20	20 (T2)	18 (T1) 20 (T3)

Table 2. Three-way ANOVA analyses (confirmed by Tukey tests) assessingtreatment effects for Approach 1(T1 vs T2 and T2 vs T3), for each cytotype (2x and 4x). Asterisks indicate level of statistical significance, * P < 0.05, ** P < 0.001, and *** P < 0.001.

			Phenotypic plasticity	Genetics		GxE Interactions	
Comparison	Cytotype	Dependent Variable	Treatment	Genotype	Family	Treatment x Genotype	Treatment x Family
T1 vs. T2	2x	Phi2	***	***	***	.	***
		F_o'/F_m'	**	***	***	.	***
		F_v'/F_m'	**	***	***	.	***
		Temperature profiles	***	.	.	.	.
		R:S ratio	.	***	.	.	.
	4x	Phi2	***	*	***	**	***
		F_o'/F_m'	***	.	***	**	***
		F_v'/F_m'	***	.	***	**	***
		Temperature profiles	***	.	.	.	.
		R:S ratio	.	*	.	.	.

T2 vs. T3	2x	Phi2	***	*	***	***	***
		F_o'/F_m'	.	***	***	***	***
		F_v'/F_m'	.	***	***	***	***
		Temperature profiles	***	.	.	.	.
		R:S ratio	**	***	.	.	.
	4x	Phi2	***	.	***	.	.
		Fo'/Fm'	*	.	***	.	.
		Fv'/Fm'	*	.	***	.	.
		Temperature profiles	***	.	.	.	.
		R:S ratio	**	**	*	.	.

Table 3. Two-way ANOVA analyses for assessing the influence of cytotype and treatment (T1, T2, T3) on three chlorophyll fluorescence parameters, leaf temperature profile, and R:S length ratio. Asterisks indicate level of statistical significance, * P < 0.05, ** P < 0.001, and *** P < 0.001.

Treatment	Dependent Variable	Cytotype
T1	Phi2	.
	Fo'/Fm'	.
	Fv'/Fm'	.
	Temperature profiles	.
	R:S ratio	*
T2	Phi2	***
	Fo'/Fm'	***
	Fv'/Fm'	***
	Temperature profiles	.
	R:S ratio	***
T3	Phi2	.
	Fo'/Fm'	.
	Fv'/Fm'	.
	Temperature profiles	.
	R:S ratio	*

FIGURES

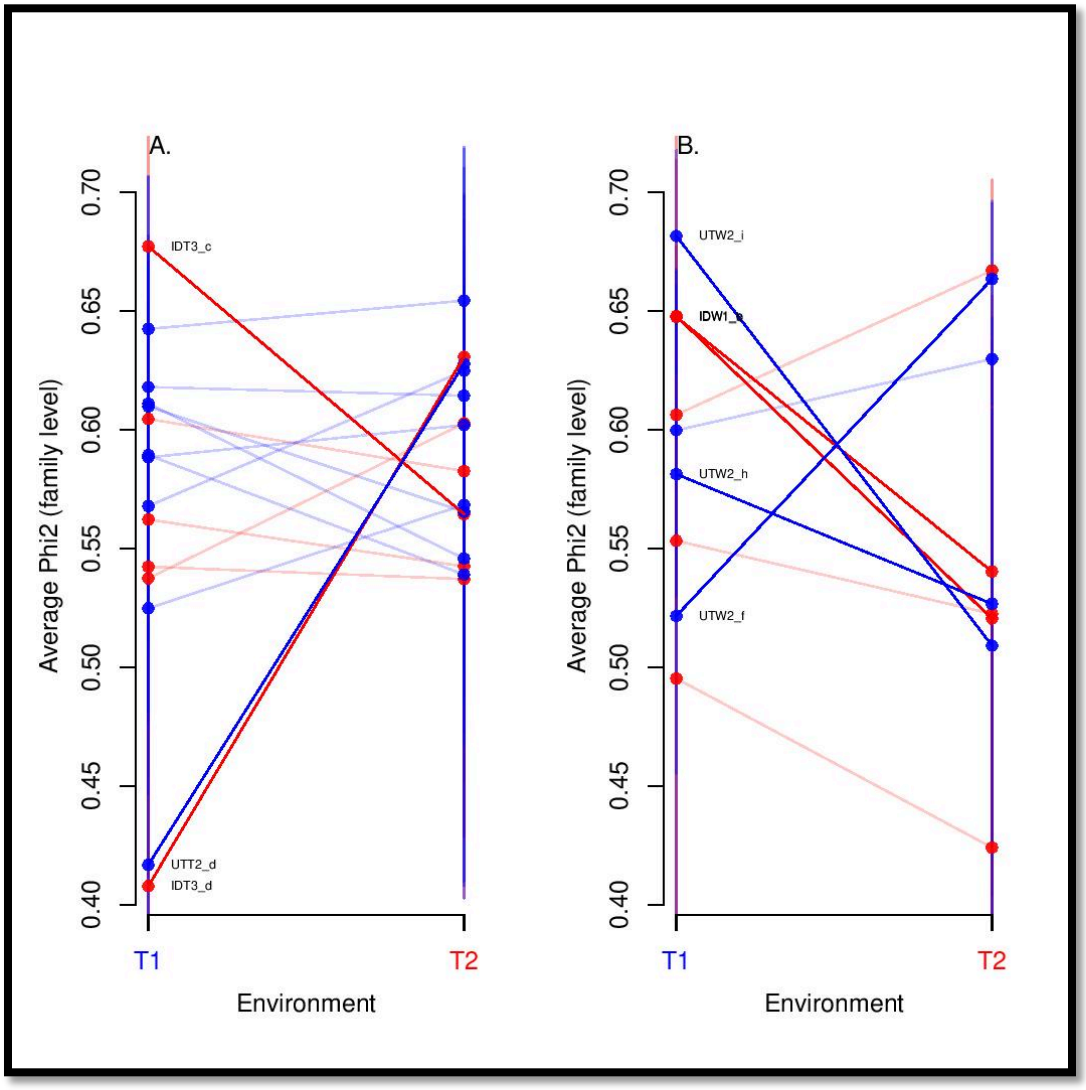

Figure 1. Norms of reaction for PHI2 values of sagebrush seedlings under well-watered (T1) and imposed drought (T2) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Lines in bold indicate families with either a significant increase or decrease in PHI2 values between treatments, and lines not bolded indicate families that do not have significantly different PHI2 values between treatments. Seedlings within each family were assigned to treatments randomly.

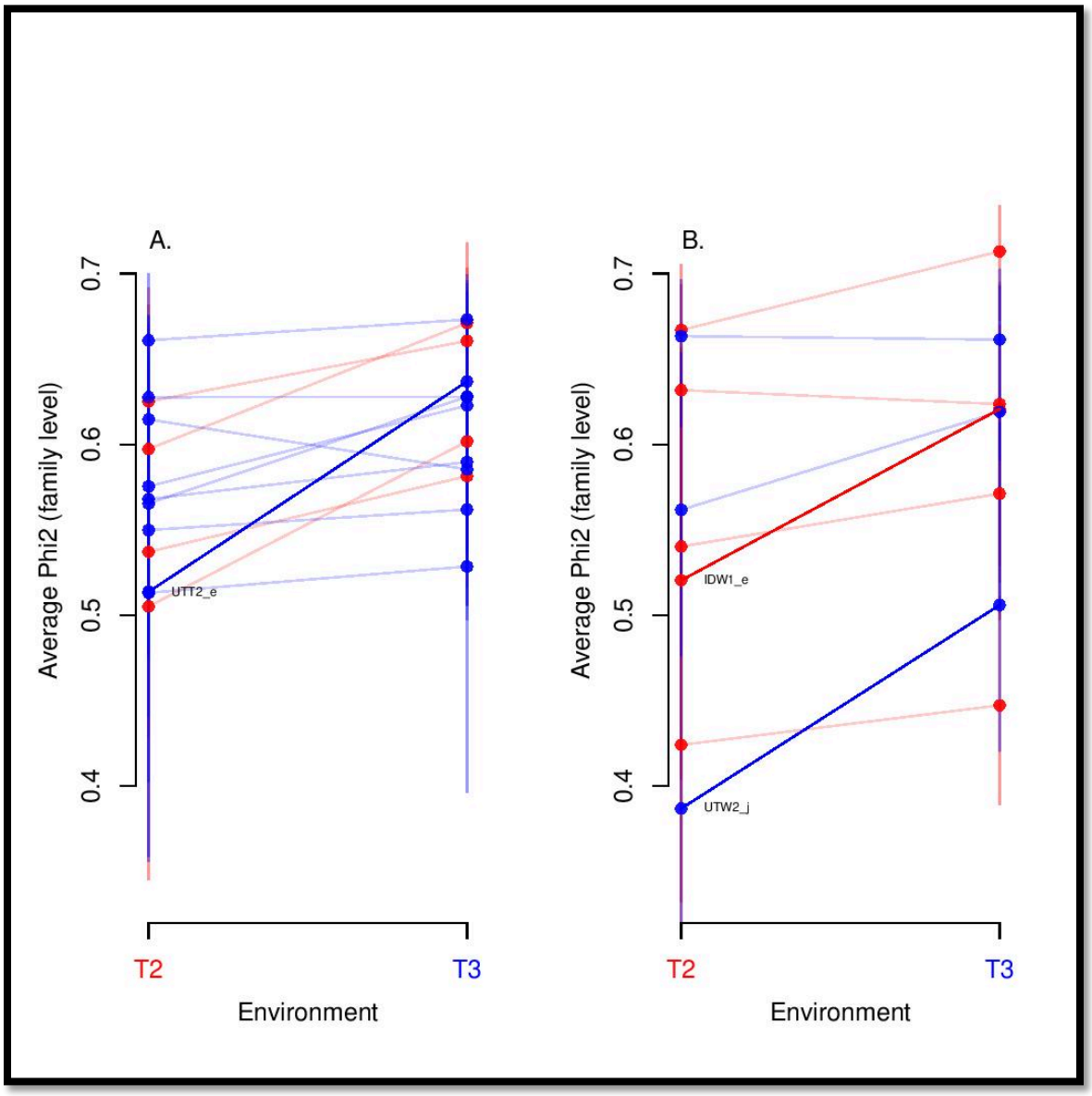

Figure 2. Norms of reaction for PHI2 values of sagebrush seedlings under imposed-drought (T2) and re-watering (T3) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Lines in bold indicate families with either a statistically significant increase or decrease in PHI2 values between treatments, and lines not bolded indicate families that do not have significantly different PHI2 values between treatments. Seedlings within each family were assigned to treatments randomly.

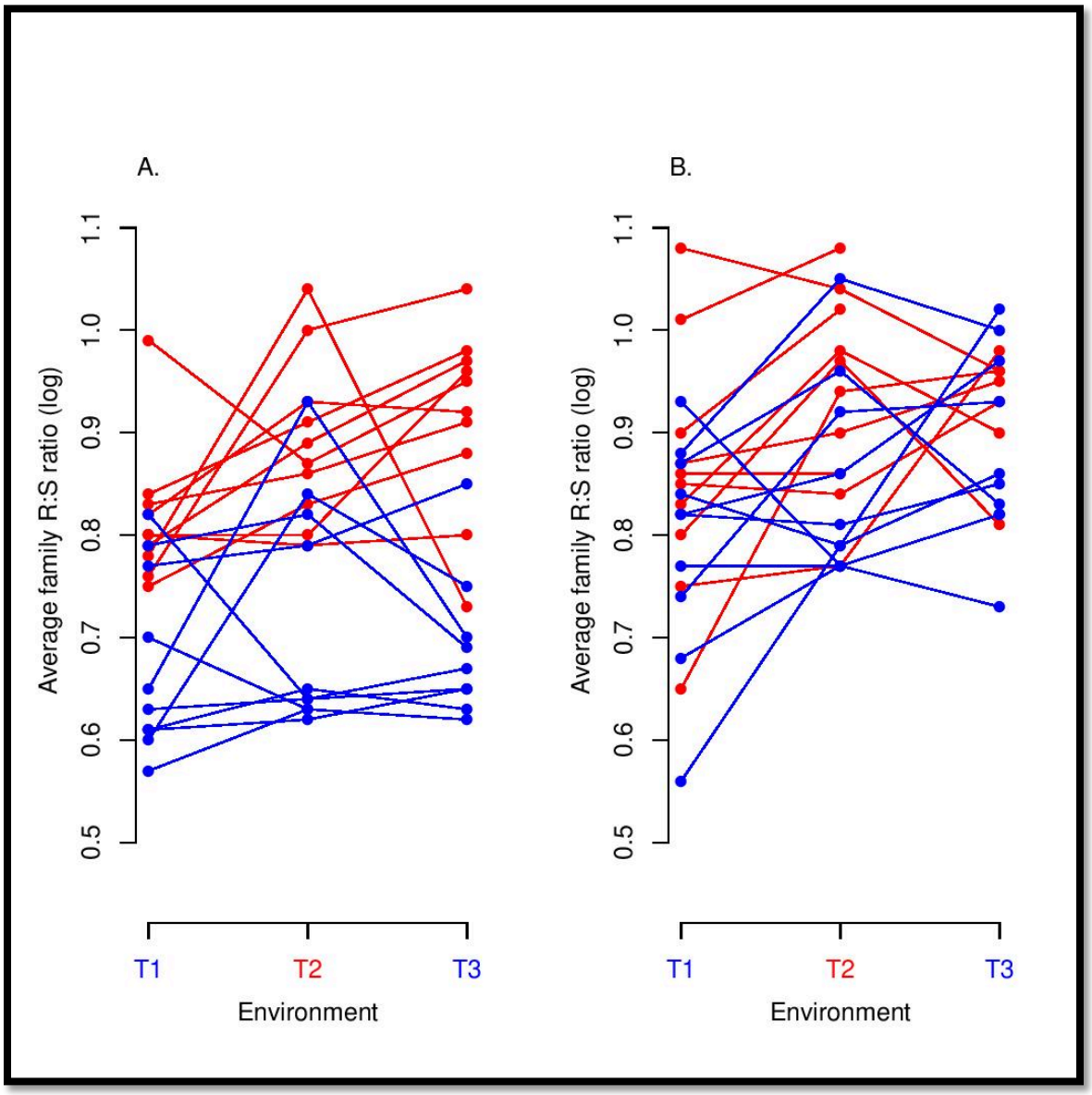

Figure 3. Norms of reaction for the log of average R:S length ratio values of sagebrush seedlings under well-watered (T1), imposed drought (T2), re-watering (T3) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Seedlings within each family were assigned to treatments randomly.

SUPPLEMENTARY MATERIAL

Supplementary Tables

Table S1. Daily container weight (g) measurements for all sagebrush seedlings in the GxE experiment (n=229). Each seedling is identified by a corresponding SeedlingID, and the weight of all containers was recorded every day, for the duration of the GxE experiment. Data are available upon request.

Table S2. The number of sagebrush seedlings that was included in each treatment, well-watered (T1), imposed drought (T2), and re-watering (T3). Each seedling is identified by the corresponding individual SeedlingID. Data are available upon request.

Table S3. Sagebrush seedling chlorophyll fluorescence measurements. Data are available upon request.

Table S4. Sagebrush seedling index for images taken with the FLIR C3 thermal camera used to infer leaf temperature profile values, for the duration of the GxE experiment. Data are available upon request.

Table S5. Root to shoot (R:S) length ratio measurements for all sagebrush seedlings in the GxE experiment. Each seedling corresponds to a unique SeedlingID. Data are available upon request.

Table S6. Stomatal conductance values for a subset of sagebrush seedlings after 16 days of imposed drought treatment (T2). Data are available upon request.

Table S7. Results of the post-hoc Tukey's statistical test following ANOVA of the results from Approach 1 (see the Methods for a description of Approach 1). Data are available upon request.

Table S8. Summary of Tukey analyses following ANOVA results for Approach 2 (see Methods for description of Approach 2). Data are available upon request.

Supplementary Figures

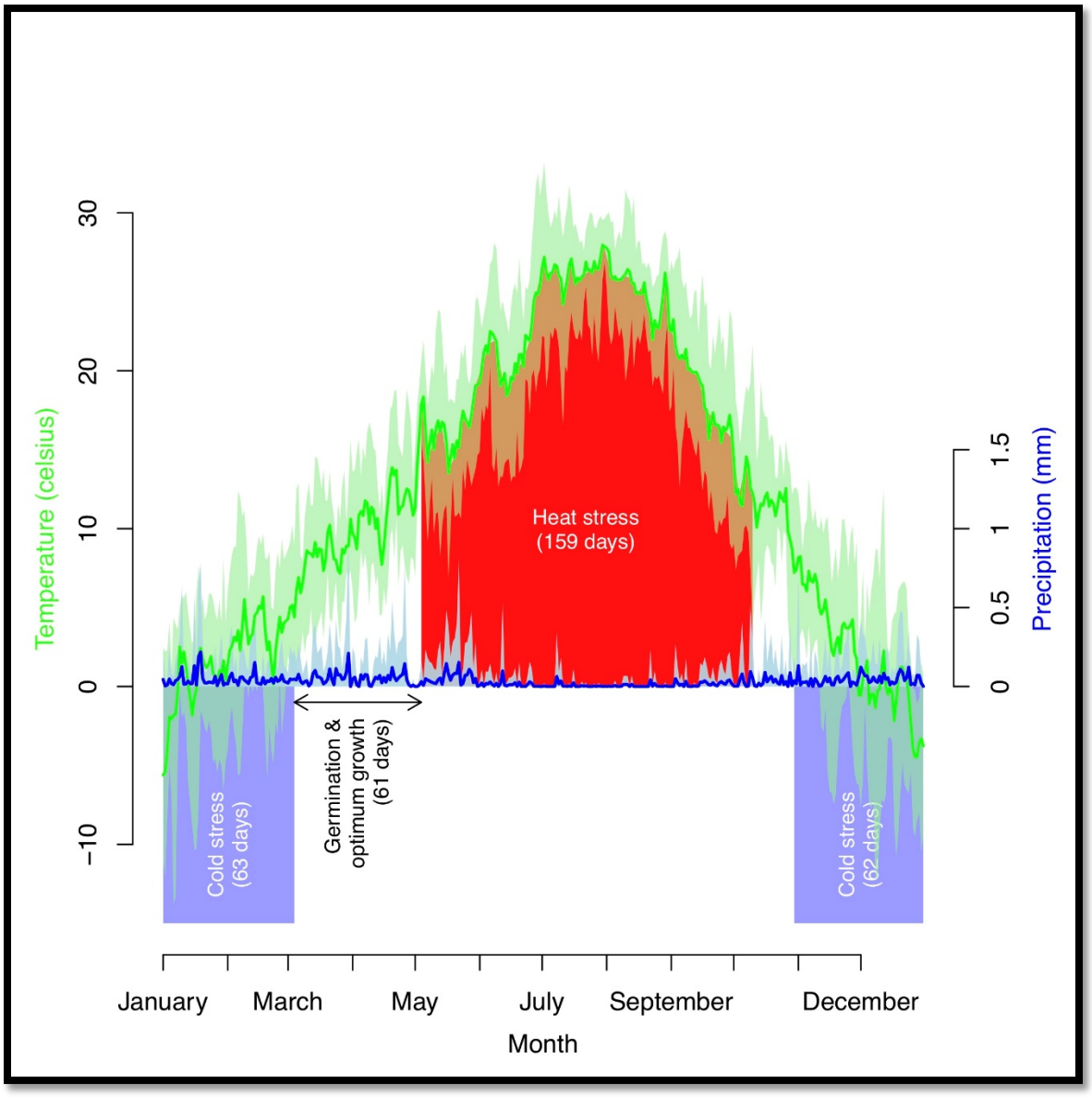

Figure S1A. Average daily climatic conditions inferred from 30 years of precipitation and temperature data for locations where sagebrush seeds were collected in Idaho. See text for more details on methodology.

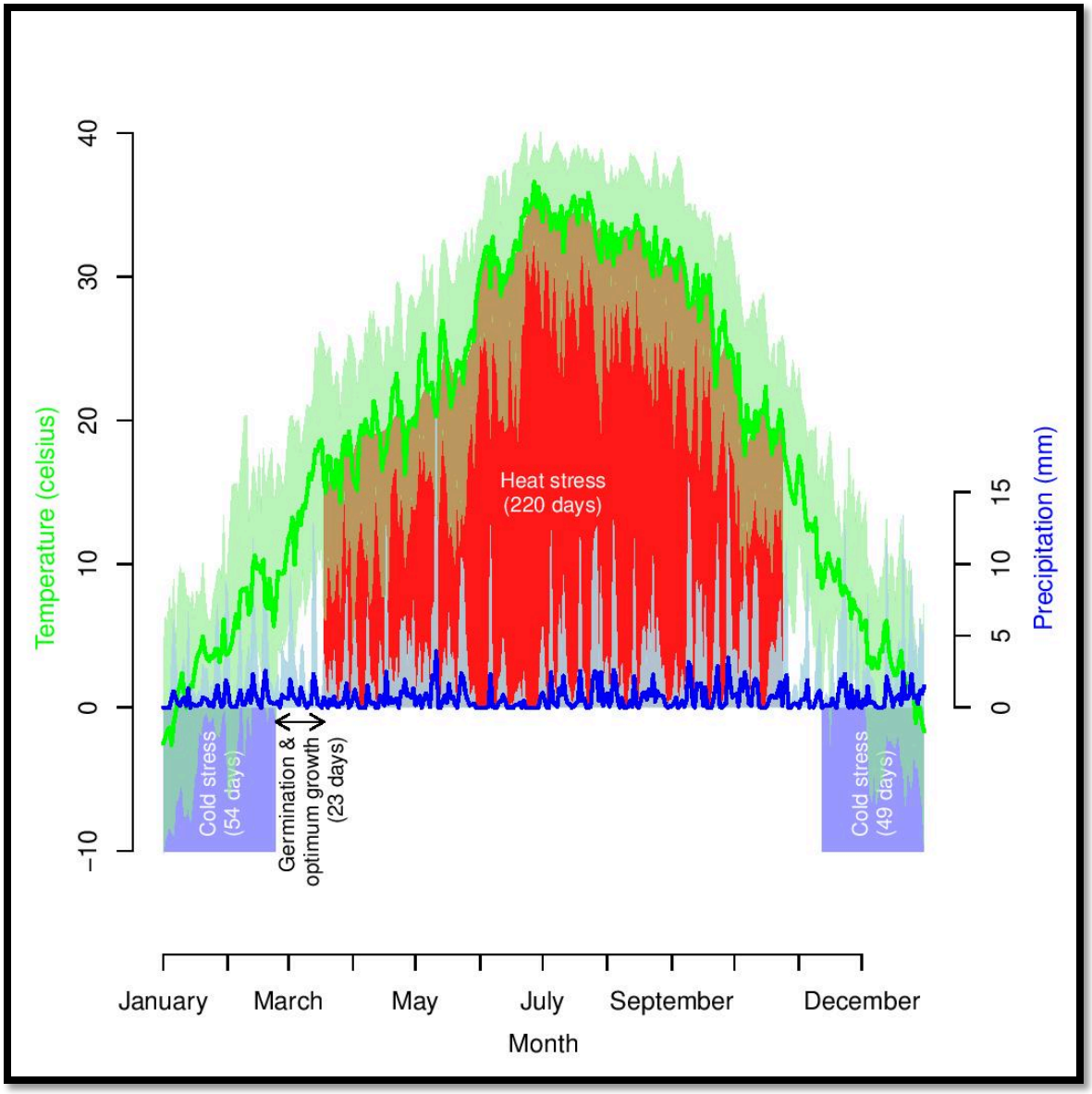

Figure S1B. Average daily climatic conditions inferred from 30 years of precipitation and temperature data for locations where sagebrush seeds were collected in Utah. See text for more details on methodology.

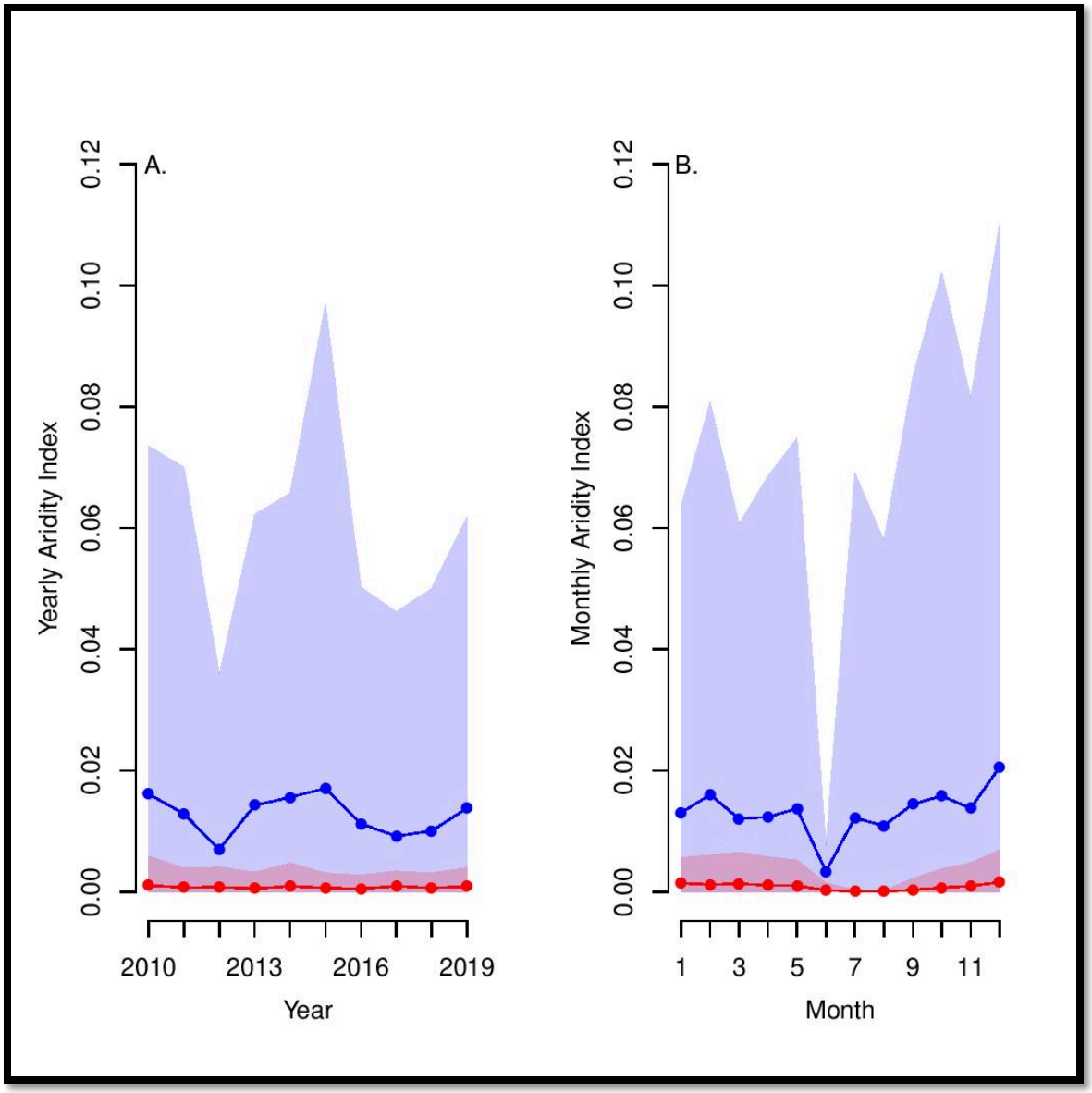

Figure S2. Aridity index values for Idaho and Utah populations. (A) yearly aridity index from 2010 to 2019. (B) monthly aridity index from January (1) to December (12). The blue color indicates the Utah location, and the red color indicates the Idaho location.

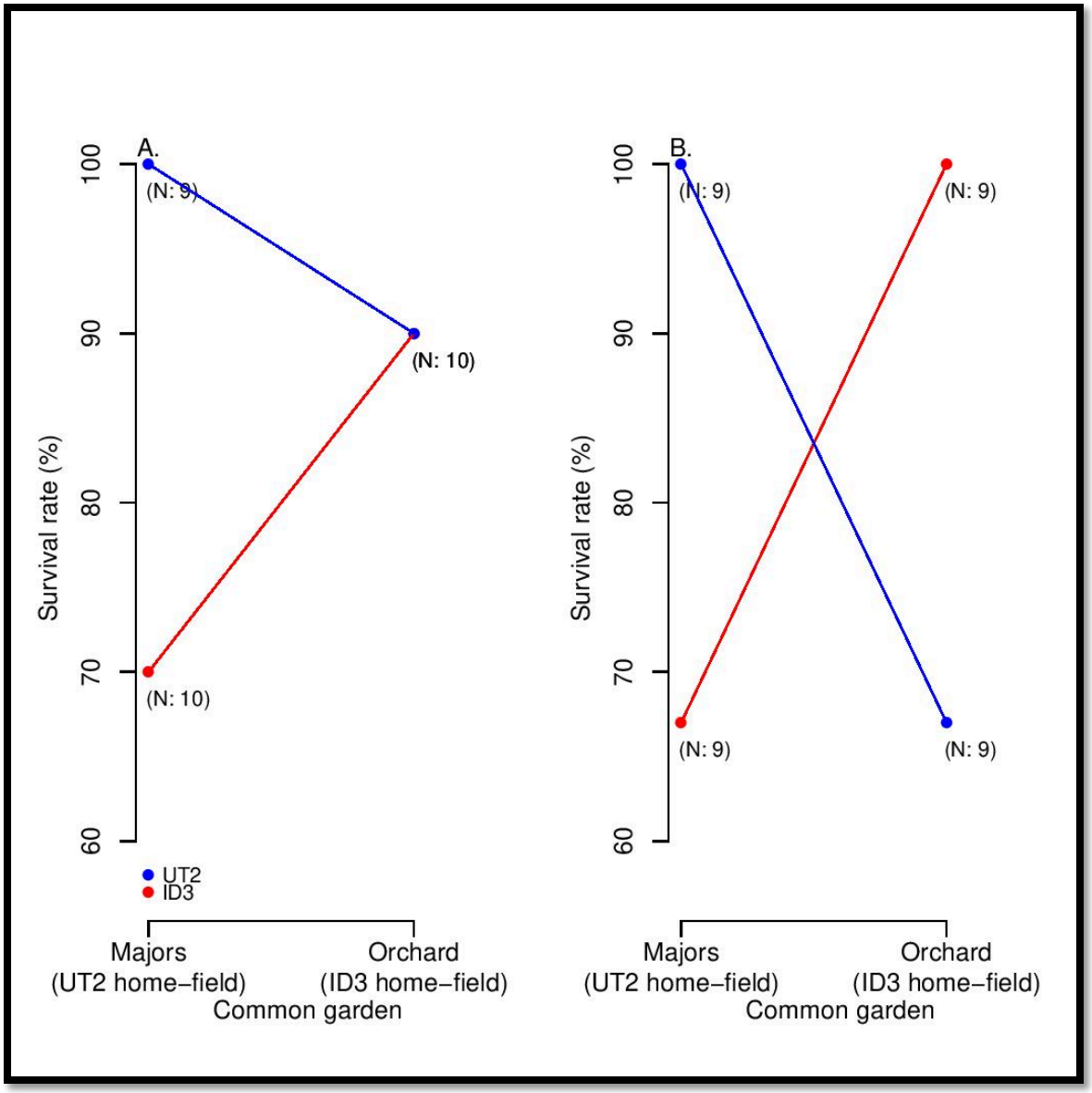

Figure S3. Survival rates of the parental populations of sagebrush included in this GxE experiment in two common gardens. The Majors, Utah common garden experiences the UT2 precipitation and temperature regimes associated with drought-sensitive sagebrush genotypes, and the Orchard, ID common garden experiences the ID3 precipitation and temperature regimes associated with drought-tolerant sagebrush genotypes. (A) survival rate for plants of the 2x Idaho (N= 10) parent population and the Utah (N=9) parent populations, and (B) survival rate for plants with 4x Idaho (N=9) and Utah (N=9) parent populations. The ID3 (Idaho) populations are indicated in red and the UT2 (Utah) populations are indicated in blue. Data are from Chaney et al. (2010).

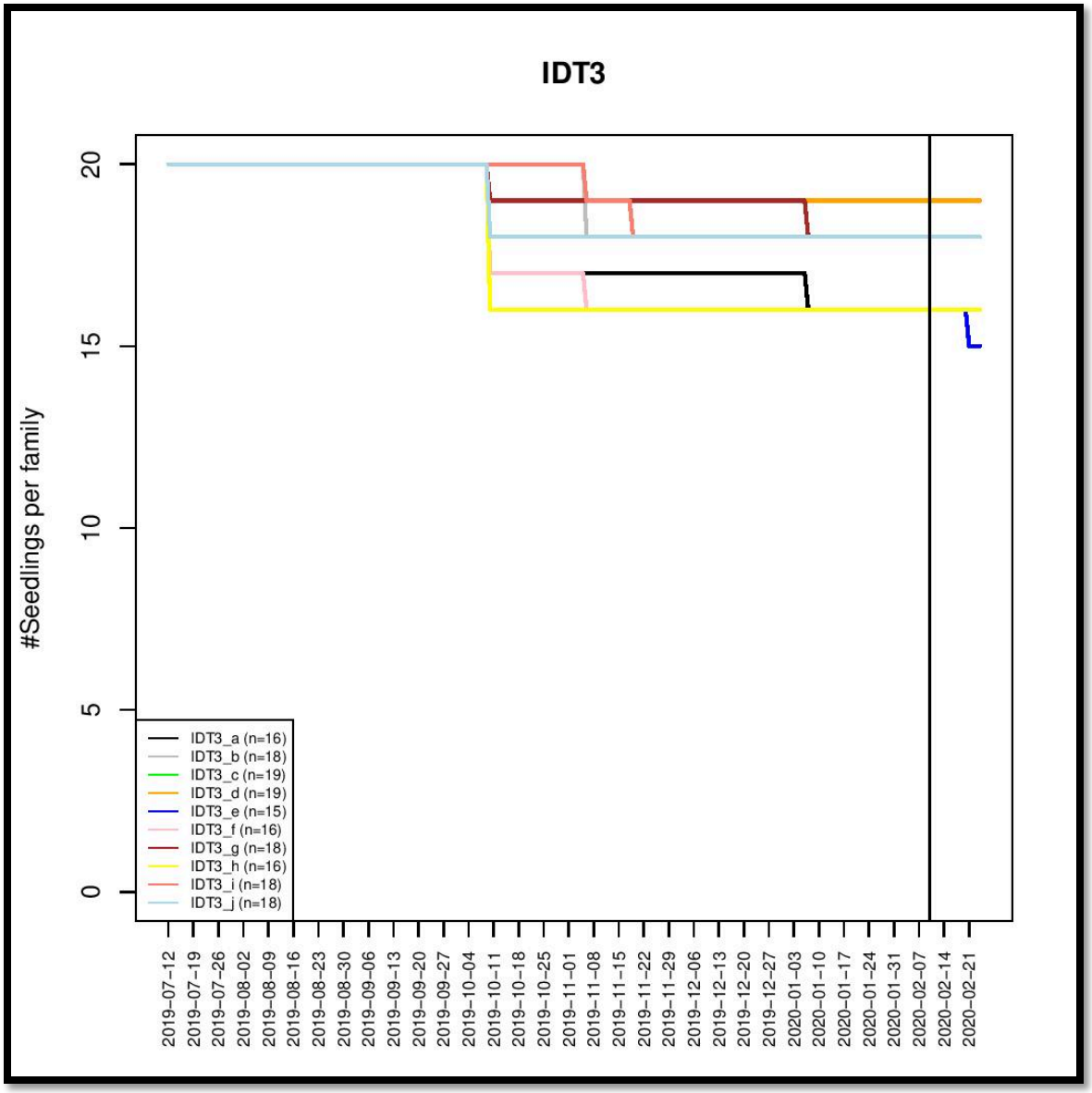

Figure S4A. Survivorship curves for sagebrush seedling families included in GxE common garden experiment, during acclimation phase. The identity of the population is indicated at the top of each figure (A, IDT3; B, IDW1; C, UTT2; and D, UTW2). All families consisted of 20 seedlings per parent. The number of seedlings per family is on the y-axis, and date is on the x-axis. Each line represents a different family within the population, and the total number of seedlings that survived the acclimation phase in each per family is indicated in the figure legend.

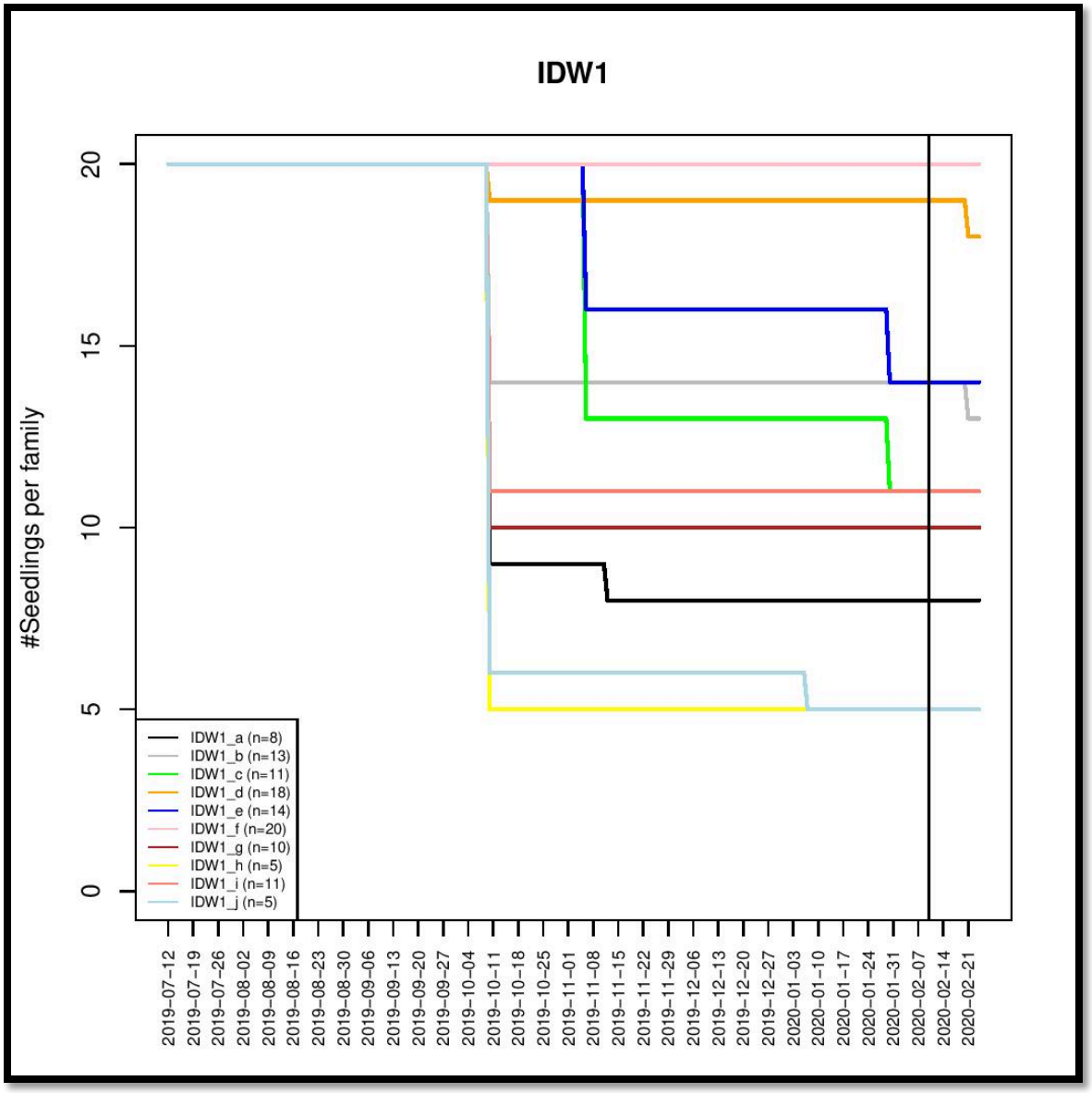

Figure S4B. Survivorship curves for sagebrush seedling families included in GxE common garden experiment, during acclimation phase. The identity of the population is indicated at the top of each figure (A, IDT3; B, IDW1; C, UTT2; and D, UTW2). All families consisted of 20 seedlings per parent. The number of seedlings per family is on the y-axis, and date is on the x-axis. Each line represents a different family within the population, and the total number of seedlings that survived the acclimation phase in each per family is indicated in the figure legend.

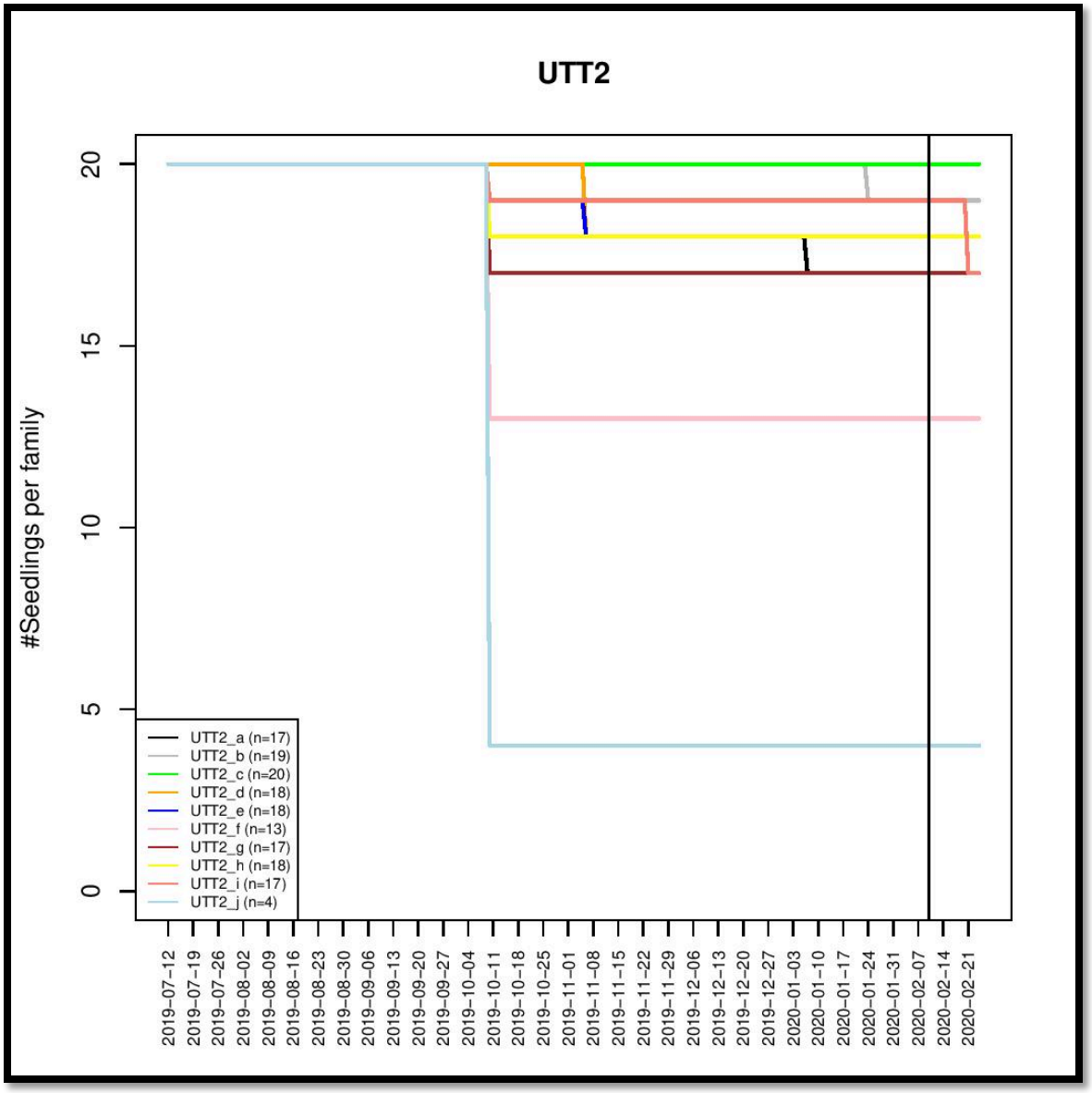

Figure S4C. Survivorship curves for sagebrush seedling families included in GxE common garden experiment, during acclimation phase. The identity of the population is indicated at the top of each figure (A, IDT3; B, IDW1; C, UTT2; and D, UTW2). All families consisted of 20 seedlings per parent. The number of seedlings per family is on the y-axis, and date is on the x-axis. Each line represents a different family within the population, and the total number of seedlings that survived the acclimation phase in each per family is indicated in the figure legend.

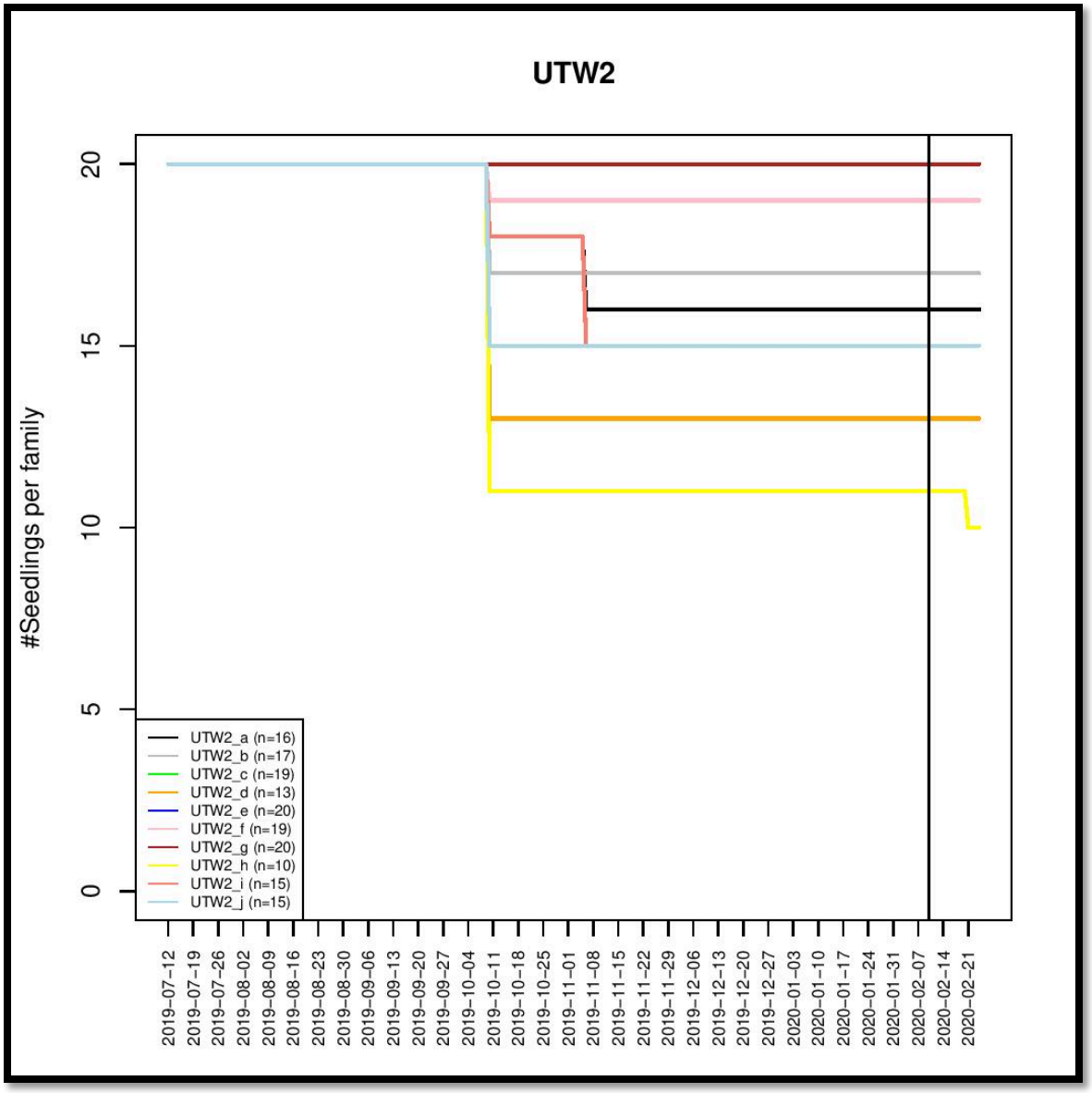

Figure S4D. Survivorship curves for sagebrush seedling families included in GxE common garden experiment, during acclimation phase. The identity of the population is indicated at the top of each figure (A, IDT3; B, IDW1; C, UTT2; and D, UTW2). All families consisted of 20 seedlings per parent. The number of seedlings per family is on the y-axis, and date is on the x-axis. Each line represents a different family within the population, and the total number of seedlings that survived the acclimation phase in each per family is indicated in the figure legend.

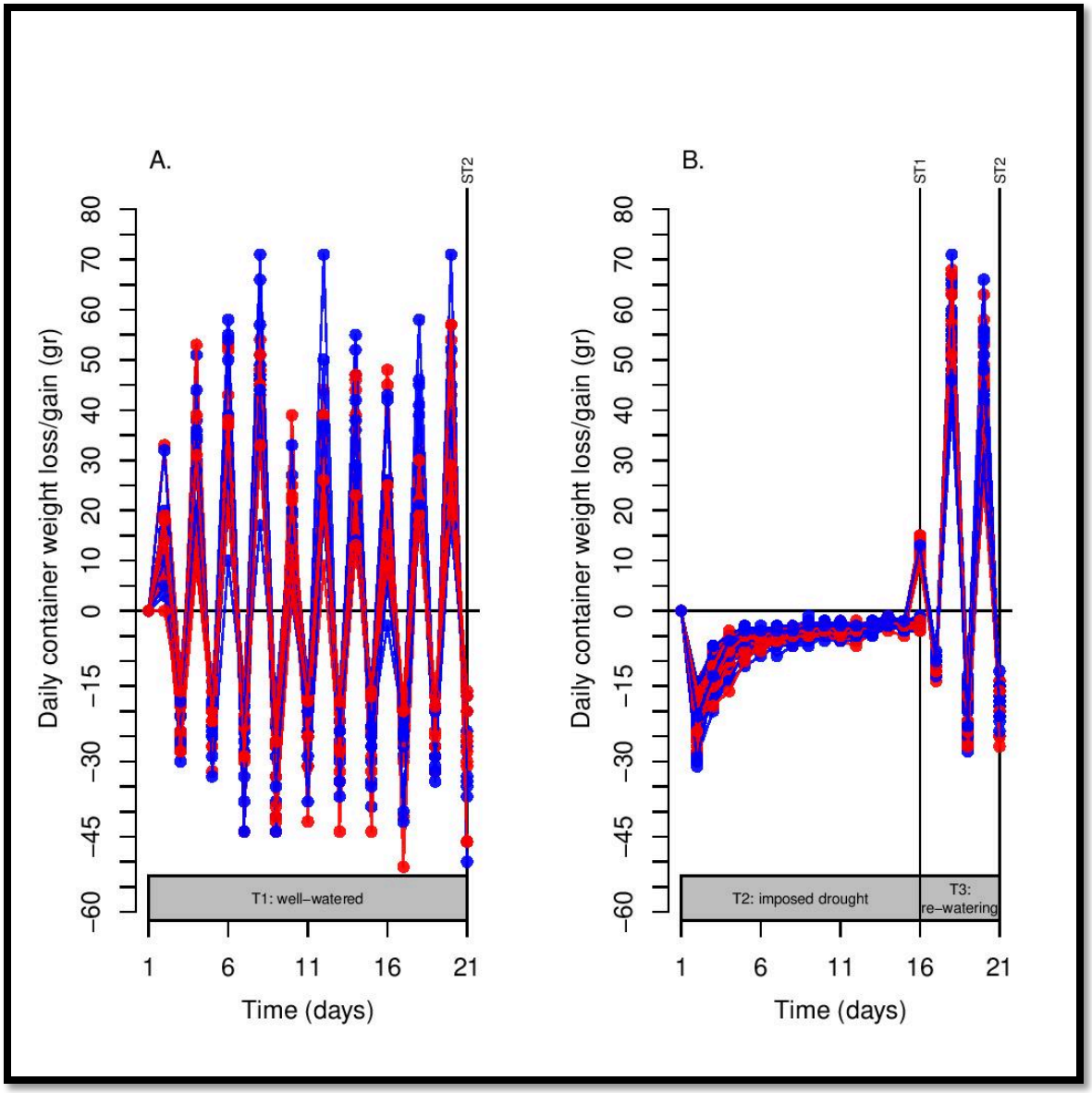

Figure S5A. Container weight loss and/or gain (g) measured and plotted daily throughout the GxE experiment. (A) is the container weights for 2x sagebrush seedlings grown under well-watered treatment (T1) plotted over 21 days (i); and for 2x seedlings grown under imposed-drought treatment (T2) for 16 days, followed by five days of the re-watering treatment (T3) and (B) is the container weights for 4x sagebrush seedlings grown under well-watered treatment (T1) plotted over 21 days (i); and for 4x seedlings grown under imposed-drought treatment (T2) for 16 days, followed by five days of the re-watering treatment (T3). (ST2). Red symbols and lines indicate drought-tolerant genotypes (G1) and blue symbols and lines indicate drought-sensitive genotypes (G2)4x.

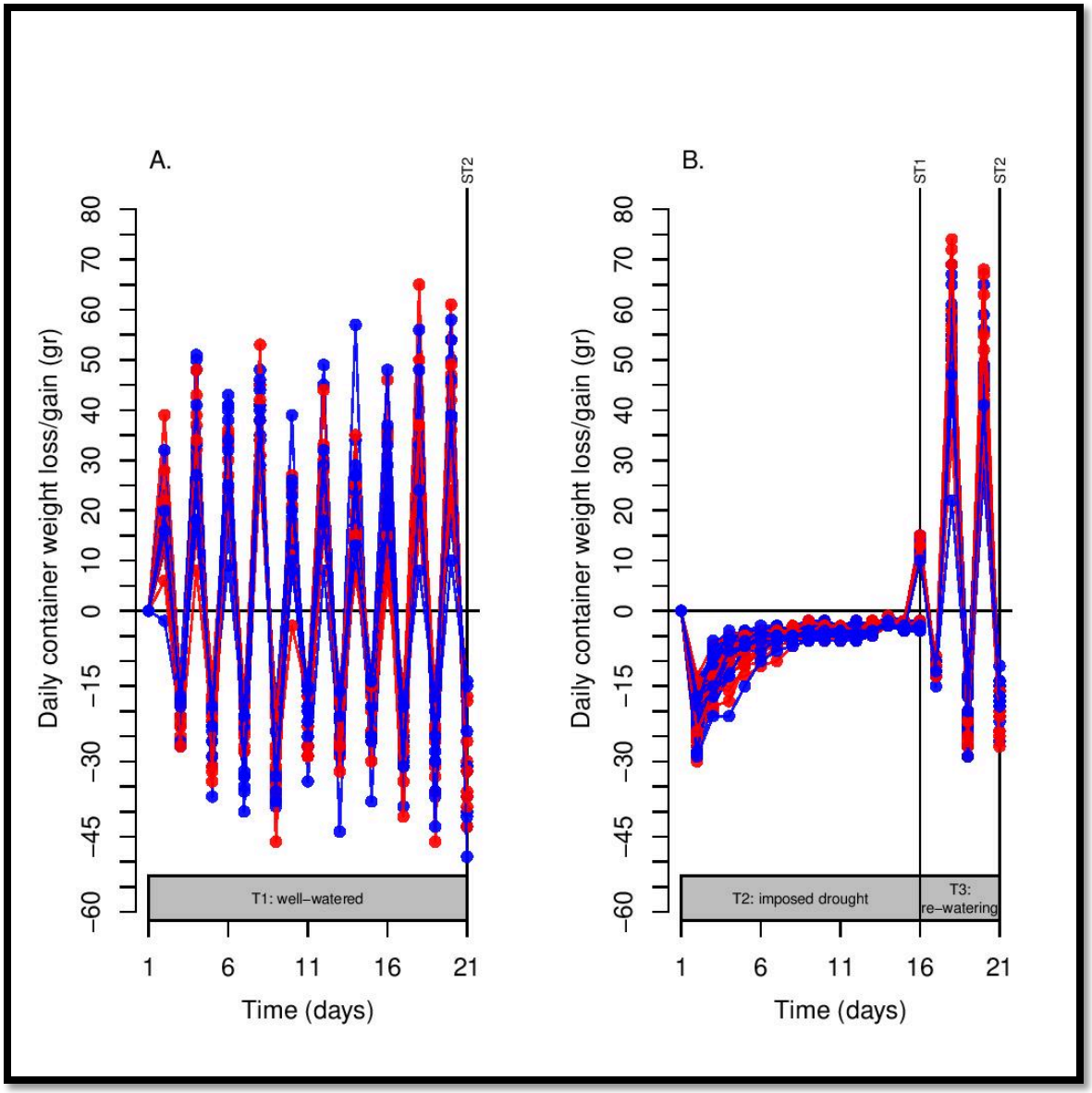

Figure S5B. Container weight loss and/or gain (g) measured and plotted daily throughout the GxE experiment. (A) is the container weights for 2x sagebrush seedlings grown under well-watered treatment (T1) plotted over 21 days (i); and for 2x seedlings grown under imposed-drought treatment (T2) for 16 days, followed by five days of the re-watering treatment (T3) and (B) is the container weights for 4x sagebrush seedlings grown under well-watered treatment (T1) plotted over 21 days (i); and for 4x seedlings grown under imposed-drought treatment (T2) for 16 days, followed by five days of the re-watering treatment (T3). (ST2). Red symbols and lines indicate drought-tolerant genotypes (G1) and blue symbols and lines indicate drought-sensitive genotypes (G2)4x.

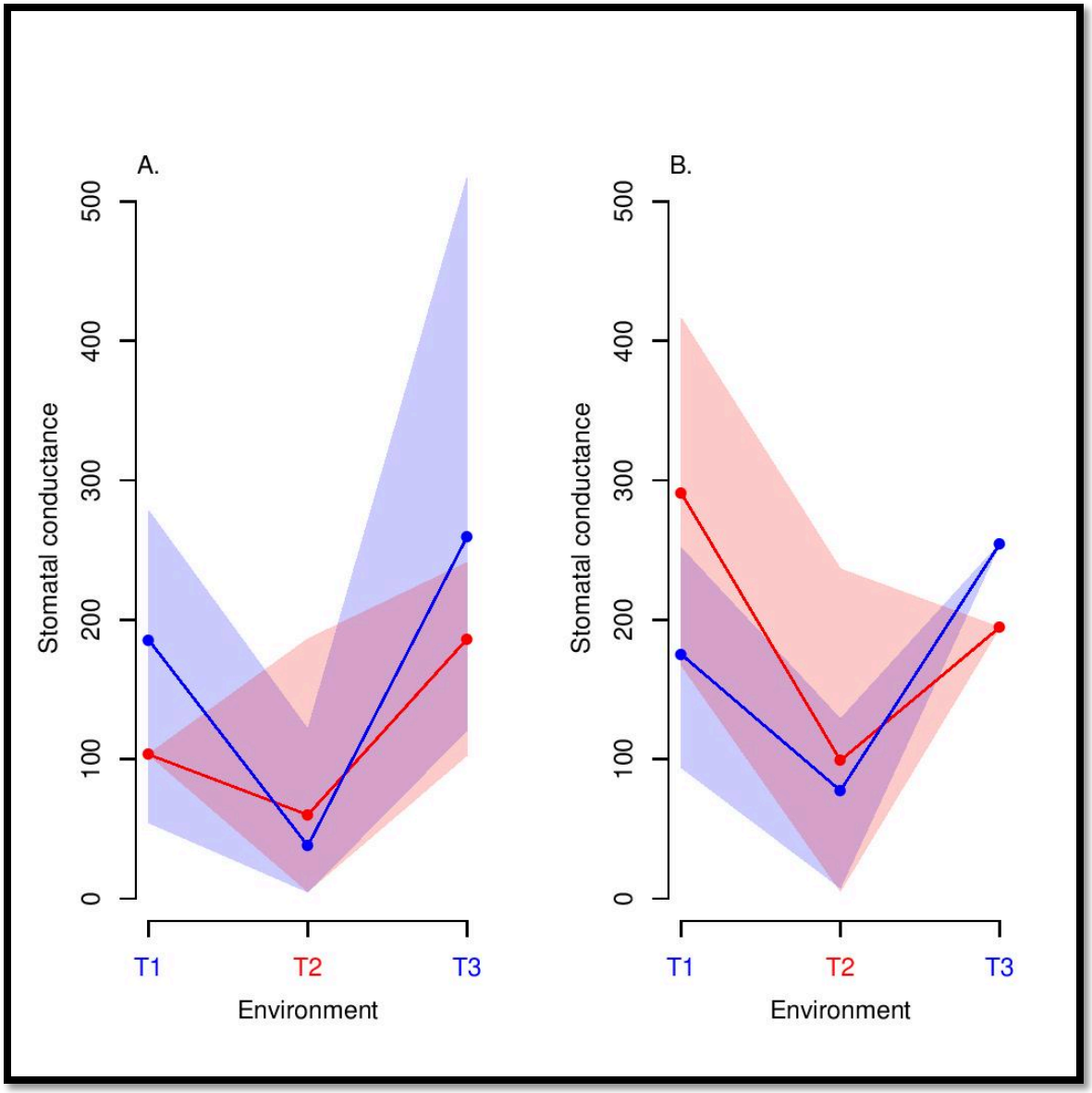

Figure S6. Stomatal conductance values of sagebrush seedlings grown under each treatment (T1, T2, T3). (A) presents the stomatal conductance values of 2x seedlings and (B) presents the stomatal conductance values of 4x seedlings. Values are summarized for each genotype, with the red color showing data for seedlings with the putative drought-tolerant genotype (G1) and the blue color showing data for seedlings with the putative drought-sensitive genotype (G2).

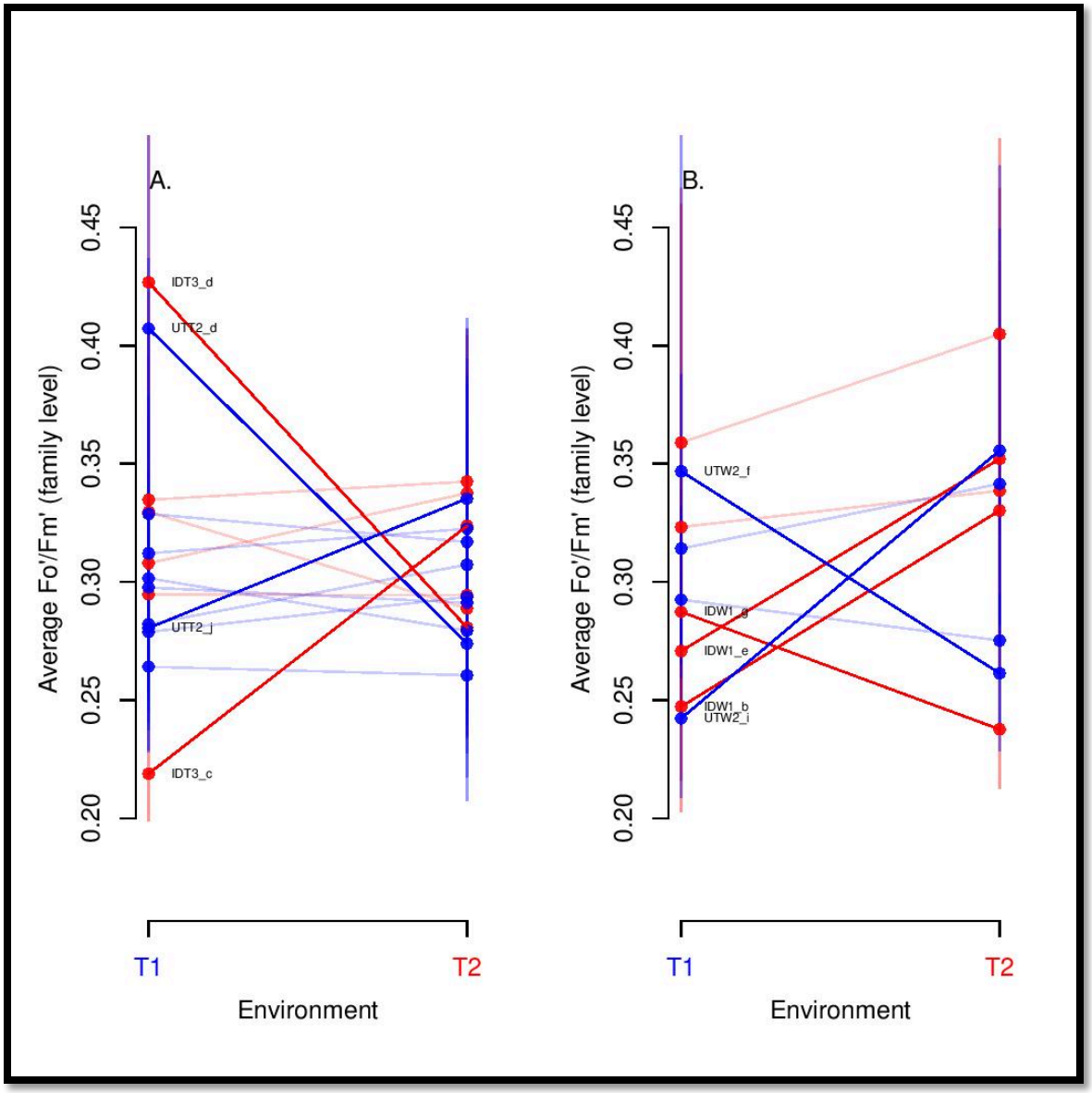

Figure S7. Norms of reaction for Fo'/Fm' values of sagebrush seedlings under well-watered (T1) and imposed drought (T2) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Lines in bold indicate families with either a significant increase or decrease in Fo'/Fm' values between treatments, and lines not bolded indicate families that do not have significantly different Fo'/Fm' values between treatments. Seedlings within each family were assigned to treatments randomly.

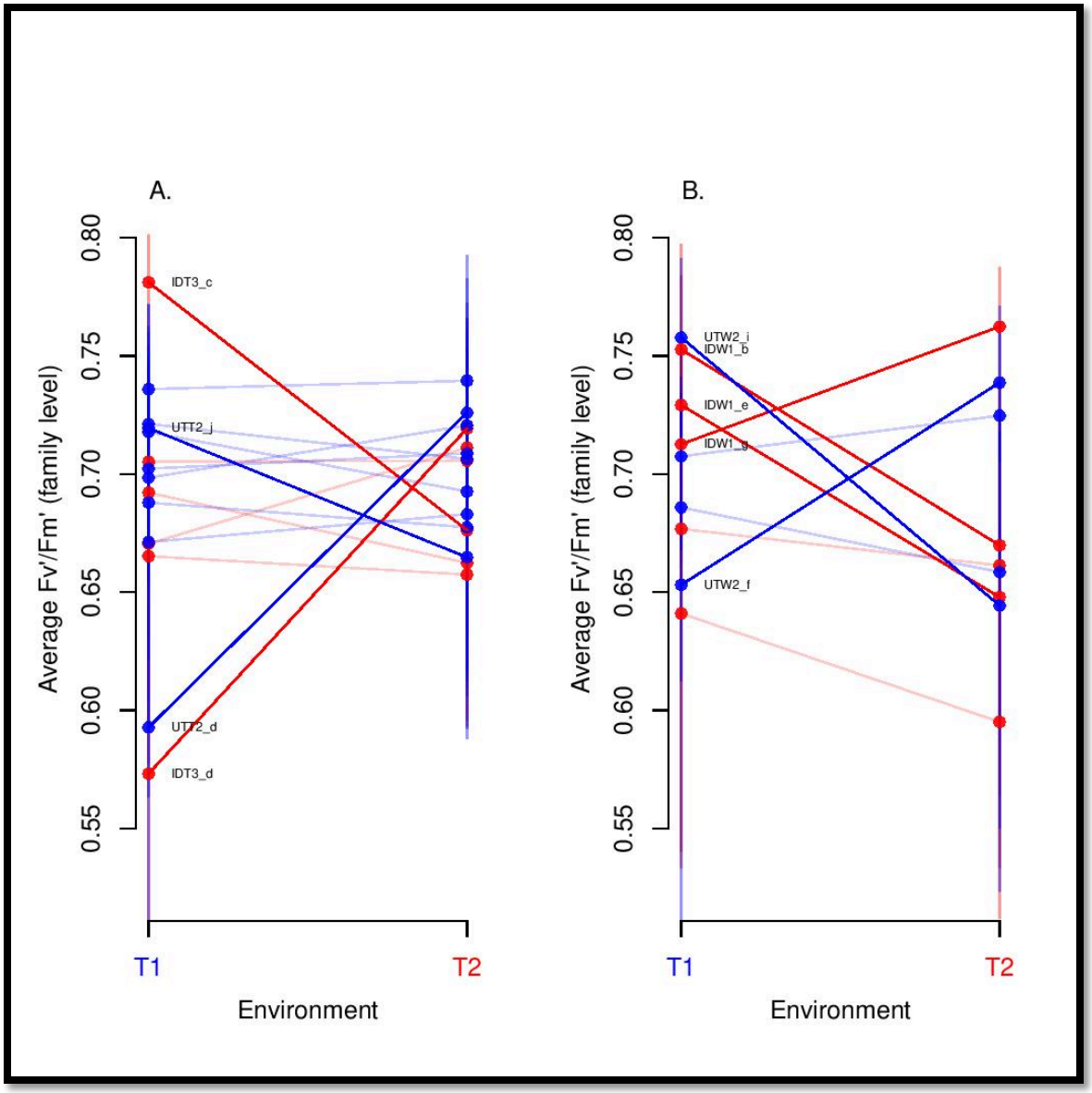

Figure S8. Norms of reaction for Fv'/Fm' values of sagebrush seedlings under well-watered (T1) and imposed drought (T2) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Lines in bold indicate families with either a significant increase or decrease in Fv'/Fm' values between treatments, and lines not bolded indicate families that do not have significantly different Fv'/Fm' values between treatments. Seedlings within each family were assigned to treatments randomly.

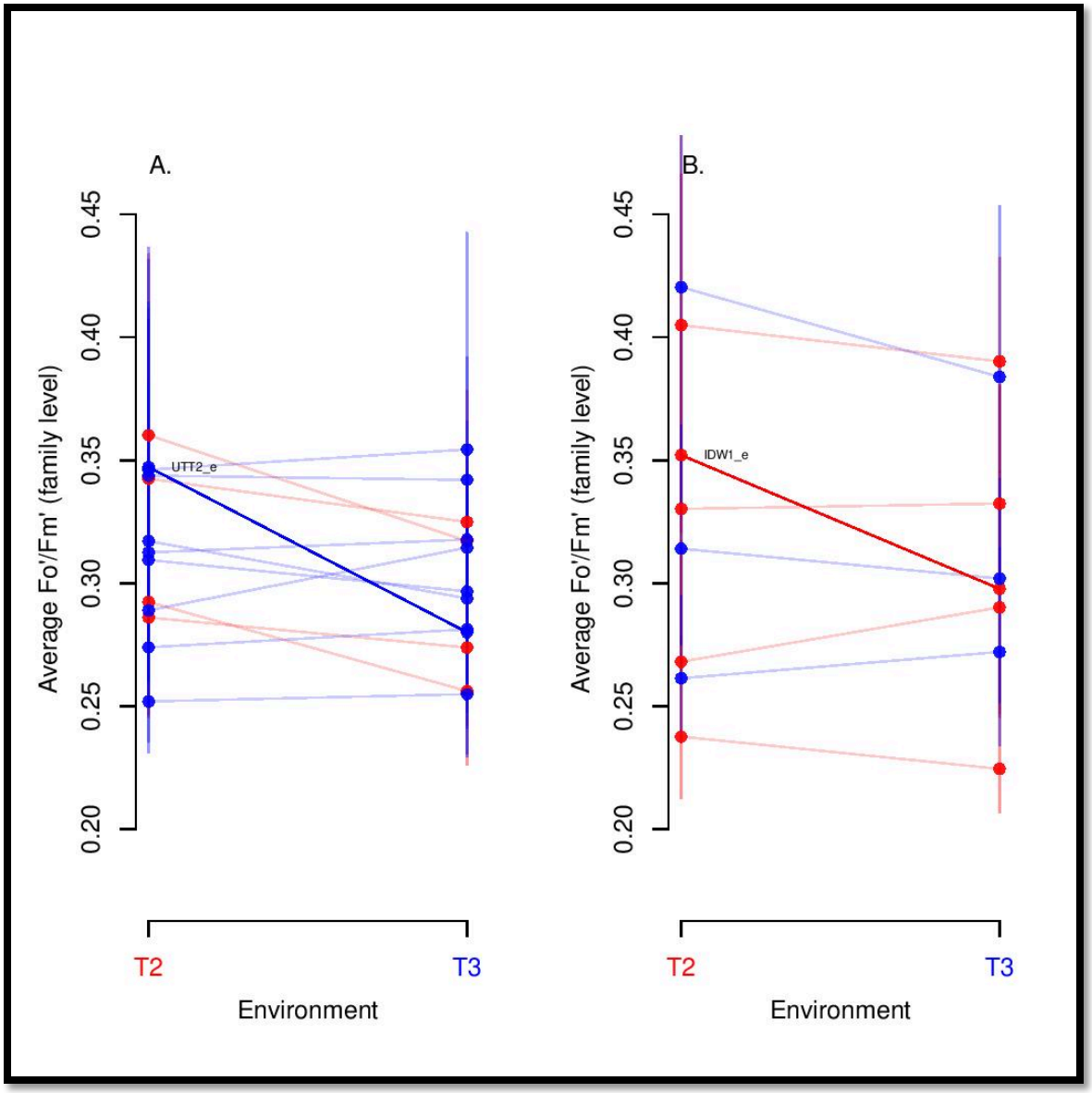

Figure S9. Norms of reaction for Fo'/Fm' values of sagebrush seedlings under imposed-drought (T2) and re-watering (T3) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Lines in bold indicate families with either a significant increase or decrease in Fo'/Fm' values between treatments, and lines not bolded indicate families that do not have significantly different Fo'/Fm' values between treatments. Seedlings within each family were assigned to treatments randomly.

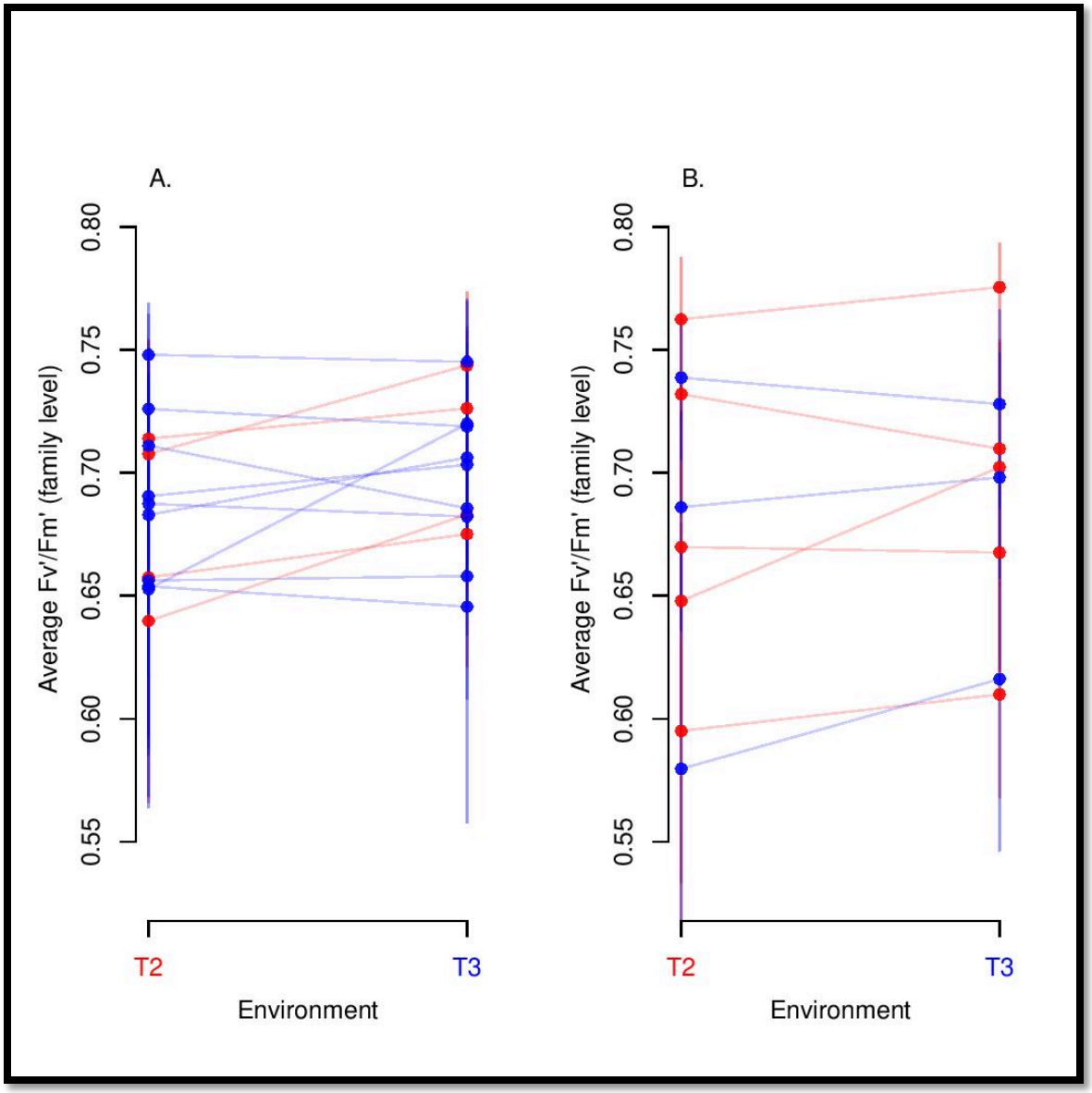

Figure S10. Norms of reaction for Fv'/Fm' values of sagebrush seedlings under imposed-drought (T2) and re-watering (T3) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. Lines in bold indicate families with either a significant increase or decrease in Fv'/Fm' values between treatments, and lines not bolded indicate families that do not have significantly different Fv'/Fm' values between treatments. Seedlings within each family were assigned to treatments randomly.

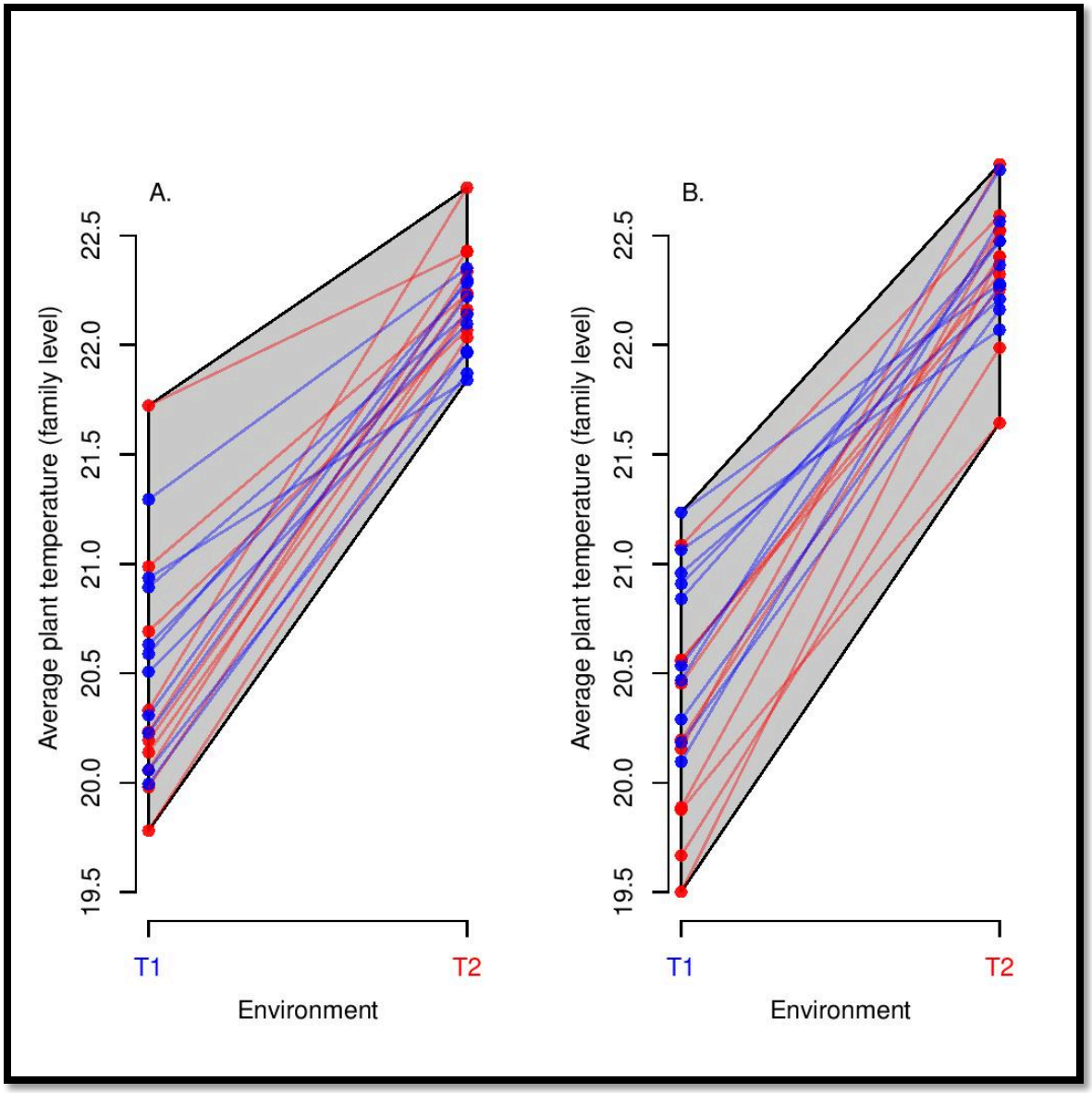

Figure S11. Norms of reaction for leaf temperature profile values of sagebrush seedlings under well-watered (T1) and imposed drought (T2) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. All G1 and G2 families for both cytotypes exhibit significant increase in leaf temperature profile values between treatments. The gray shading indicates the range of responses of these families to these treatments. Seedlings within each family were assigned to treatments randomly.

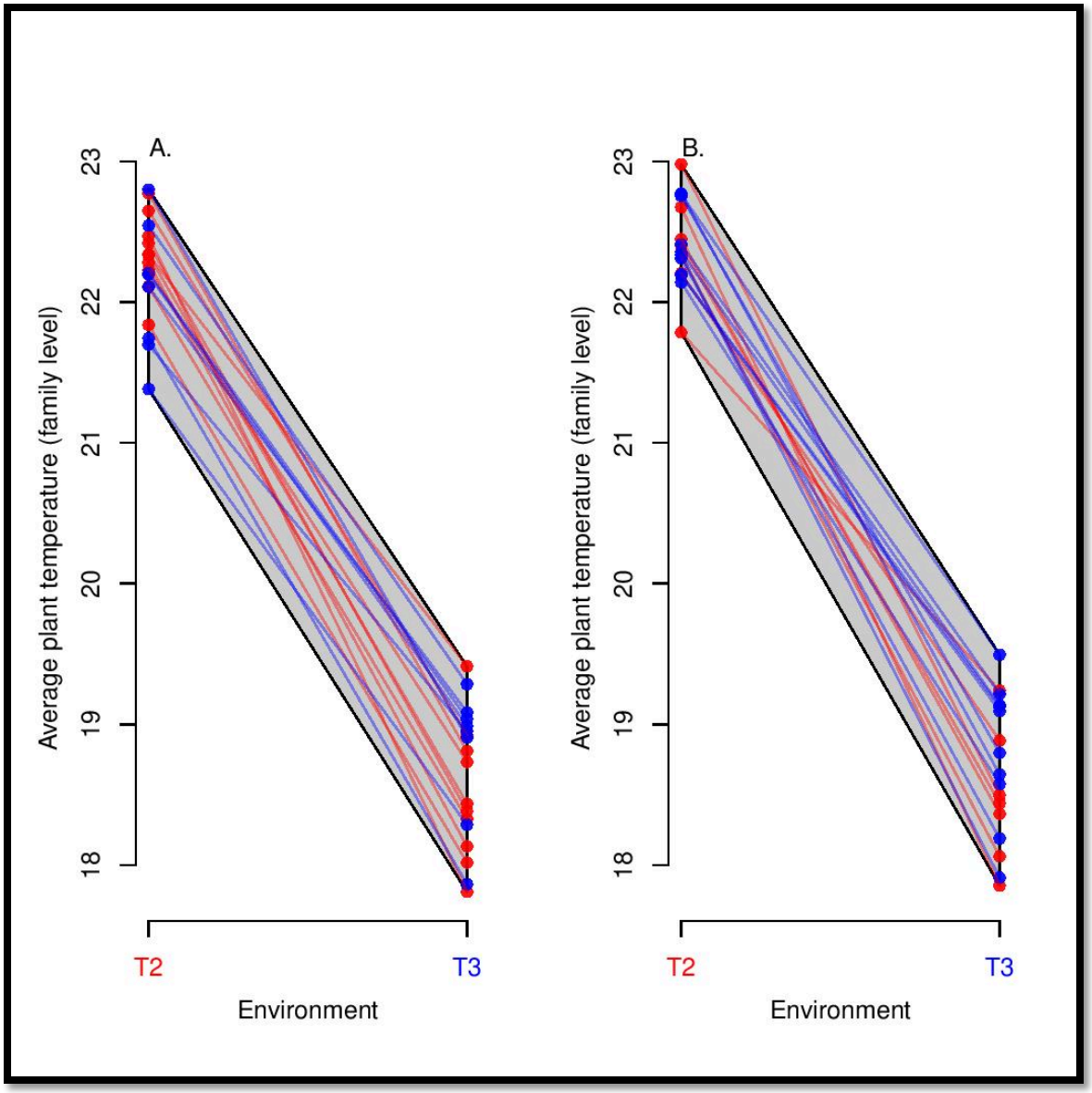

Figure S12. Norms of reaction for leaf temperature profile values of sagebrush seedlings under imposed-drought (T2) and re-watering (T3) treatments, for (A) 2x families and (B) 4x families. Putative drought-tolerant families (G1) are indicated by red lines and drought-sensitive families (G2) are indicated by blue lines. All G1 and G2 families for both cytotypes exhibit significant decrease in leaf temperature profile values between treatments. The gray shading indicates the range of responses of these families to these treatments. Seedlings within each family were assigned to treatments randomly