Predicted global warming scenarios impact on the mother plant to alter seed dormancy and germination behavior in Arabidopsis.

Running Head: Global warming and seed production

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Seed characteristics are key components of plant fitness that are influenced by temperature in their maternal environment, and temperature will change with global warming. To study the effect of such temperature changes, *Arabidopsis thaliana* plants were grown to produce seeds along a uniquely designed polyethylene tunnel having a thermal gradient reflecting local global warming predictions. Plants therefore experienced the same variations in temperature and light conditions, but different mean temperatures. A range of seed related plant fitness estimates were measured. There were dramatic non-linear temperature effects on the germination behaviour in two contrasting ecotypes. Maternal temperatures lower than 15-16 $^{\circ}$C resulted in significantly greater primary dormancy. In addition, the impact of nitrate in the growing media on dormancy was shown only by seeds produced below 15-16 $^{\circ}$C. However, there were no consistent effects on seed yield, number or size. Effects on germination behaviour were shown to be a species characteristic responding to temperature and not time of year. Elevating temperature above this critical value during seed development has the potential to dramatically alter the timing of subsequent seed germination and the proportion entering the soil seed bank. This has potential consequences for the whole plant life cycle and species fitness.

Key Words: Seed germination, seed dormancy, germination timing, life cycle, seed yield, global warming, temperature, *Arabidopsis*

Introduction

Evidence for warming of the climate system resulting from anthropomorphic greenhouse gas emissions is now unequivocal (IPCC, 2014). Such global warming has not only increased mean temperatures, but reduced the diurnal temperature range as minimum temperature has increased at twice the rate of maximum temperature (Walther et al. 2002), and impacted on a

seasonal scale as biological spring is now earlier and biological winter is later (Penuelas et al. 2009). Temperature is the primary signal determining the timing of the two major transitions in the plant life cycle; germination (seed to plant transition) and reproduction (plant to seed transition) that are key components of plant fitness. Temperature also affects depth of dormancy during seed maturation (Donohue, 2009; Chiang et al. 2011; Kendall et al. 2011; Kendall and Penfield, 2012; Huang et al. 2014) and during dormancy cycling in the soil following shedding (Probert, 2000; Finch-Savage and Leubner-Metzger, 2006; Footitt et al. 2011, 2013, 2014). Thermal control both before and after shedding therefore determines when seeds germinate and the timing of seedling emergence in seasonal climates (Donohue et al. 2010; Donohue et al. 2015; Burghardt et al. 2016). This control is likely to be disrupted in the event of future climate change to impact upon plant regeneration from seeds (Walck, 2011). Such potential for compromised seedling emergence and vigour, and shifts in germination phenology are likely to influence population dynamics, and therefore, species composition and diversity of communities (Walck et al. 2011). Nevertheless, seedling emergence timing has until now been largely neglected in global warming studies (Hedhly et al. 2009).

Fitness can be considered as the ability of species to survive and reproduce in the environment in which they find themselves (Orr, 2009) and therefore the probability of surviving to the next generation. The ability of species to adapt to future climates depends on the existence of phenotypic plasticity in the life history traits that impact on fitness under increasing temperatures (Nicotra et al. 2010). In addition to phenotypic plasticity, genetic variation within populations is also a primary mechanism for adaptation (Jump et al. 2009) that may ‘preadapt’ them to future climates. There is considerable genetic evidence of adaptation, for example, Postma and Ågren (2016) in a reciprocal life cycle experiment using

a Recombinant Inbred Line population produced from Swedish and Italian *Arabidopsis* ecotypes found that fitness selection during seedling establishment was favoured by local alleles in the establishment Quantitative Trait Loci. Although they have limitations, the commonly used measures of fitness include seed related variables such as seed number, size and yield (Primack, 1989). However, the probability for survival to the next generation, particularly of ruderal annual monocarp species such as *Arabidopsis,* also involves seed behavioral characteristics such as seed dormancy, germination phenology, longevity and persistence in the soil seed bank, which can also be influenced indirectly by seed mass (Fenner and Thompson, 2005; Poschlod et al. 2005; Springthorpe and Penfield, 2015). Temperature, water stress and nitrate in the maternal environment influence the phenotypic expression of all these seed characteristics (Fenner, 1991, Case et al. 1996; Meyer & Allen, 1999; Lacey & Herr, 2000; Alboresi et al. 2005; Kochanek et al. 2010; Chiang et al. 2009; Kendall et al. 2011; He et al. 2014; He et al. 2016). Walck et al. (2011) point out that parental environments can therefore facilitate the evolutionary divergence of life history patterns among plant populations. Furthermore, they suggest that as there is substantial variation in both genetic and phenotypic plasticity for seed dormancy and germination within most species over elevational and latitudinal gradients (Meyer et al. 1995; Baskin & Baskin, 1998; Cavieres & Arroyo, 2000; Daws et al. 2006; Vidigal et al. 2016) populations may therefore be buffered from some of the effects of projected climate change. A degree of environmental buffering may also occur in the soil seed bank. Fenner and Thompson (2005) concluded that most evidence suggests that direct effects of global warming on the soil seed bank will be limited, but there may be large indirect effects of climate change on seed banks. Such indirect effects may result from changes in the dormancy level and seed mass of newly dispersed seeds; this may alter the balance between the short-term and persistent seed banks.

A recent study of phenotypic plasticity in seed dormancy highlights the importance of considering varying weather conditions rather than just constant average temperatures when assessing responses to global warming (Fernández-Pascual and Jiménez-Alfaro 2014). In the present work, we investigate the extent of this phenotypic plasticity in *Arabidopsis* using a unique thermogradient polyethylene tunnel. The tunnel provides realistic seasonal and diurnal temperature fluctuations, but with a gradient of simulated global warming depending on the position that the plant is grown in the tunnel (Wurr et al. 1996). A projected median emissions scenario for the local experimental area used in this work (West Midlands, UK) indicates an increase in the summer mean temperature of 3.7 ˚C by 2080 (UK Climate Change Projections, 2014; http://ukclimateprojections.metoffice.gov.uk/). We therefore adjusted the tunnel to a gradient from ambient to *c.* + 4 ˚C. To avoid the confounding effects of temperature on the timing of flowering and on seed maturation we established the temperature gradient at the start of seed development in the first three sowings. To compare with this, at the fourth sowing the gradient was applied throughout plant growth to seed harvest.

Other environmental variables not linked to climate change can also impact plant growth, and seed characteristics (yield, size, dormancy) and may interact with the effect of increases in mean temperature; a principal one of these is nitrate availability. For example, the nitrate content in both soil and seed has an impact on dormancy in *Arabidopsis* (Alboresi et al. 2005; Matakiadis et al. 2009). Furthermore, maternal temperature and nitrate availability both alter dormancy and expression of *CYP707A2* (ABA catabolism) and genes involved in nitrate metabolism (Matakiadis et al. 2009; Kendall et al. 2011; He et al. 2016). Thus temperature could potentially regulate dormancy by influencing nitrate metabolism during seed development. We therefore include nitrate availability as a further variable in this study.

Here, a comparison is made between two *Arabidopsis* ecotypes (Cvi and Bur) that have adapted to unique environments (Fig. S1), which has resulted in contrasting obligate winter (Cvi) and summer (Bur) annual behaviours when grown in the local environment used for this study (Footitt et al. 2013). These two ecotypes were therefore employed to provide contrasts in key life cycle variables that determine fitness i.e. their flowering time (*c.* 35 and 46 days at 20^{o}C for Cvi and Bur respectively), seed yield, seed size, and in their dormancy response to temperature that influences germination time (Huang et al. 2014, 2015). Previous work has also shown that their ecotypic differences in seed responses to germination conditions are greatly influenced by seed maturation in different laboratory environments (Huang et al. 2014, 2015). Comparison of these very different phenotypes within a species helps to shed light on the potential within-species life cycle plasticity in the face of global warming; and to determine whether effects are a species characteristic or ecotype specific. We investigate whether the limited temperature increases in realistic simulated global warming scenarios could impact significantly upon seed characteristics that can influence species fitness. We found that these scenarios gave no consistent effects on seed yield, number or size. However, there were dramatic non-linear temperature effects on the germination behaviour of the seeds produced in both the contrasting ecotypes. We discuss how these effects may have long-term consequences for the stability of soil seed banks as native flora comes under increasing pressure from climate change.

Materials and methods

Experiments were carried out with *Arabidopsis* over two years (2011, 2012) in a field-based thermogradient tunnel (Wurr et al. 1996) to investigate the impact of global warming scenarios on plant growth and development, and seed parameters considered as measures of

147 fitness. The experiments compared two ecotypes, Cape Verde Islands (Cvi) and Burren (Bur)
148 because they exhibit obligate winter and summer annual behaviour respectively at the
149 experimental site used. Seeds of both ecotypes were sown to coincide with the time of seed
150 maturity and shedding of each ecotype grown in the UK (Footitt *et al.* 2013). Key plant
151 development stages were monitored and seeds were harvested at maturity and their dormancy
152 characteristics were determined in the laboratory. Furthermore, seed yield (total seed weight)
153 and seed size (1000-seed weight) were also determined.
154
155 ***Thermogradient tunnel:*** The polyethylene tunnel (32 m long x 9 m wide) structure enabled
156 plants to be grown at natural day lengths with a high percentage (76%) of natural levels of
157 irradiance. The ambient air temperature was constantly monitored outside of the tunnel.
158 Reacting to this an electronic climate control system operated fans that generated opposing
159 warmed and ambient air flows to establish and maintain a temperature gradient from ambient
160 at one end of the tunnel to *c.* ambient + 4 °C at the other end (Wurr *et al*. 1996; Fig. 1). Air
161 and soil temperatures were monitored continuously along the tunnel. Realistic seasonal and
162 diurnal temperature fluctuations were therefore maintained within the tunnel, but with
163 varying degrees of simulated climate warming depending on the position that plants are
164 grown along the tunnel. Four positions along the tunnel were selected to provide *c.* T1,
165 ambient; T4, ambient + 4 ºC and at two equally spaced temperatures (T2 &T3) in between
166 (Fig. 1).
167
168 ***Seed material for tunnel experiments:*** Bulk seed stocks of *Arabidopsis* ecotypes Bur and
169 Cvi, were initially produced in a temperature-controlled glasshouse (23/17˚C, 16/8 h, light
170 /dark) and harvested as described below. The glasshouse was vented and heated to control
171 temperature and had supplementary lighting to maintain light levels and photoperiod. At

harvest seeds were dried to an equilibrium relative humidity of 55% above a saturated calcium nitrate solution at 20 °C for six days (seed moisture content 9.9% on a dry weight basis). A proportion of the freshly harvested seeds were placed in separate sealed moisture-proof containers and after-ripened (AR) at 20˚C/dark for eight months. This ensured all seeds were non-dormant before subsequent use to avoid delay in seedling emergence.

Sowing occasions: Experiments were set up on 4 occasions (early and late sowings in each of two years; Table S1): 11 February and 27 July, 2011 at a single nitrogen concentration; 9 February and 1 May 2012 at three nitrate concentrations detailed below. On the first three occasions seeds were sown in a temperature-controlled glasshouse (23/17 ˚C, 16/8 h, light /dark) and then transferred to the tunnel at the initiation of bolting and therefore before opening of the first flower. Plants were then grown to maturity for seed harvest. On the final occasion (late sowing 2012) seeds were sown directly into the tunnel to record the full life cycle: seedling emergence, bolting, flowering, seed maturation and yield components. Across the four occasions sowing was timed to compare the performance of seeds produced under a wide range of temperatures experienced during natural winter and summer annual production times at the experimental site. For example, early sowings in both years were timed for seed maturation and shedding consistent with a winter annual; the late sowing in 2011 was timed for seed maturation consistent with a summer annual; the final late sowing in 2012 coincided with shedding of the winter annual Cvi. At this final sowing, because seeds had been after-ripened to relieve dormancy and allow rapid germination, seed maturation occurred on these plants during higher temperatures in summer, and therefore intermediate between summer and winter annual phenotypes.

196 Depending on the experiment, growth media contained three different levels of nitrate:
197 standard N (SN; Levingtons F1 compost: sand: vermiculite 6:1:1); Low N (LN; 4:1:1); and
198 very low N (VLN; 4:2:2). They contained 304.3, 263.5 and 127.8 NO_3-N mg/kg dry weight
199 respectively. Each experiment had a randomized split-plot design with three replicates at each
200 tunnel position. Plots were P24 cellular trays (24 cells, each 5 x 5 x 5 cm) containing either
201 SN, LN or VLN media. Each tray was placed in a second undivided tray lined with capillary
202 matting to ensure all the plants had an adequate uniform water supply from below. Within
203 each tray there were two separate subplots of 8 cells sown with seed of Cvi or Bur. Plants
204 were watered regularly throughout from below to ensure they did not experience differential
205 water stress along the tunnel. No further nutrient was applied to the trays during the
206 experiments.
207
208 ***Seedling emergence:*** For recording seedling emergence twenty-five seeds were sown onto
209 the surface of pre-watered compost in each of four replicate cells per treatment. The trays
210 were placed into the four locations along the tunnel. After 24 h exposure to light to remove
211 the final layer of dormancy the seeds were covered with a uniform layer of clean horticultural
212 sand (0.5 cm). Seedling emergence through the sand was recorded daily.
213
214 ***Bolting time and plant growth to maturity*:** For plants grown to maturity, five seeds were
215 sown into each of the eight cells per treatment and maintained as above; except the trays were
216 initially covered with transparent propagator lids for at least four days, by which time all the
217 seedlings had established. One week after sowing, the seedlings were thinned to one per cell.
218 Plants were visually scored daily for bolting (inflorescence extended 1 cm). At that stage the
219 rosette diameter and leaf number were also recorded. Aracon bases (Arasystem, Belgium)
220 were then placed on plants. When the plant had grown through the bases, Aracon tubes

(Arasystem, Belgium) were added to the bases to isolate each plant during pollination and to facilitate collection of all the seeds produced.

Seed harvesting and yield measurement: In all cases watering stopped as seeds became fully mature (i.e. when all the siliques had turned yellow and dry on the plant). Seven days later the plants were cut just above the rosettes, the height of the inflorescence was measured and following seed extraction it (minus seeds and siliques) was placed in a paper bag. The bags were then left to dry at room temperature for 7 days, placed in an oven at 80 ˚C for 24 h and the inflorescence dry weight was recorded. Following extraction, seeds were sieved (500 μm) and then placed at 55% relative humidity/20 °C for six days to equilibrate as above. This resulted in a seed-moisture content of 8-10% on a dry weight basis. At this point seed yield (total seed weight) and seed size (1000-seed weight) were determined. Seeds were then sealed in aluminium foil bags (11×24 cm) (Moore and Buckle, St. Helens, UK) and stored at -80 ˚C for germination experiments.

Seed germination: Germination tests used seeds directly from -80 ˚C or seeds AR at 20 ˚C/dark for 30 days as stated for each experiment. Seeds were surface-sterilized in a 0.125 % sodium hypochlorite solution (household bleach: 5% sodium hypochlorite, diluted to 2.5% v/v) for five minutes and then washed three times with distilled water. Germination experiments were conducted in temperature controlled incubators. Seeds were placed on two layers of 3MM chromatography paper in clear plastic boxes (8×12×2 cm) (Stewart Plastics Ltd, Croydon, UK) containing 8ml of distilled water or 1 mM or 10 mM KNO_3. For each treatment, there were three replicates of 40 seeds of each ecotype. Germination (radical emerged through endosperm and testa) was recorded either in the light or dark, for 28 days. In experiments with dark treatments (germination boxes wrapped in a double layer of

246 aluminum foil) seeds were surface sterilized, sown and germination was recorded in the dark
247 under a green safe light (Kodak 7B safelight filter/Green**,** Kodak Limited, London).
248
249 ***Seed nitrate content measurement:*** Triplicate 150 mg samples of fresh dry seeds were
250 ground using a pestle and mortar, and transferred to a 20 ml scintillation vial that was
251 weighed before and after drying at 80˚C for 16 h. Deionised water (10 ml) was added and the
252 samples were shaken for one hour and then centrifuged for five minutes at 5000 rpm. The
253 supernatant was filtered using nitrogen free filter paper, and analysed for NO_3-N by a steam
254 distillation method using a FOSS FIAstar 5000 Flow Injection Analyser (Gerber Instruments,
255 Effretikon, CH) for end point determination (Bremner and Keneney, 1965).
256
257 ***Data analysis:*** Analysis of variance was used to detect the differences and interactions
258 between variates. Statistical analysis was carried out using the software package GenStat
259 (VSN International, 2012 or Payne et al. 2003). All percentage germination data were first
260 angular transformed. The regression analysis function of Sigmaplot (Systat Software Inc,
261 UK) was used to obtain curves with the best fit in Figs. 4 and 5. Details of fitted curves are
262 given in Table S2. Mean maturation temperature was calculated for increasing periods of
263 time prior to harvest to determine best fit to the data (30 days for all occasions and ecotype
264 except 18 days for Cvi at the early sowing of 2011).
265
266 **Results**
267 ***Global warming scenarios:*** A thermal gradient was established along the experimental
268 polyethylene tunnel and four positions were selected with different air and soil temperature
269 scenarios (Fig. 1a). The first position (T1) remained at ambient and the fourth (T4) remained
270 *c.* 4 ˚C higher, with two intermediate positions (T2 and 3). Fig. 1b,c shows this gradient was

maintained throughout the year as ambient temperature rose and fell. There was a linear relationship ($R^2 = 0.936$, $P<0.001$) between air temperature (1m above soil level) and soil temperature (5 cm below soil surface) measured across all four sites. Therefore, a similar gradient of soil temperature was also established and maintained in the plant growing containers along the tunnel.

Effect of temperature and nitrate on the life cycle: A full life cycle was recorded at the four selected positions along the tunnel for both Cvi and Bur following sowing on 1 May 2012 (Fig. 2). In general, at progressively warmer positions along the tunnel the duration of the plant life cycle decreased in both ecotypes. This reduction resulted largely from a reduced post-flowering period, which included seed development and subsequent drying to harvest maturity (siliques sufficiently dry to extract seeds). There was little effect of nitrate content in the growth media on the length of the life cycle in Bur. The relative delay in seedling emergence in the high nitrate regime was offset by a reduced period of vegetative growth (rosette formation) prior to extension of the inflorescence (bolting) (Fig. 2). Cvi seedlings grown in the low and very low nitrate regimes failed to reach maturity and produce seeds and therefore post-seeding emergence data is only presented for the standard nitrate regime for this ecotype.

Effect of temperature on seedling emergence, plant growth and seed yield components: Final percentage seedling emergence was significantly ($P<0.001$) higher in Bur than Cvi, but there was no overall significant effect of tunnel position (temperature regime) or nitrate regime in either ecotype (Fig. S2) and no interaction between the variates. However, in both ecotypes there was a significant effect ($P<0.001$) of nitrate regime on seedling emergence rate (1/T50, time to 50% seedling emergence from viable seeds). In both ecotypes the

296 standard nitrate (SN) regime delayed seedling emergence compared to the lower nitrate
297 regimes (LN, VLN) and seedling emergence rate was fastest in the LN regime.
298
299 There were significant (P<0.001) effects of both temperature regime and ecotype on the time
300 to bolting time (extension of the inflorescence). This decreased with increasing temperature
301 along the tunnel in both ecotypes in the SN regime (Table 1). However, there was a
302 significant (P<0.001) interaction with the effect more marked in Bur (35.6- 30.8 days; T1 to
303 T4 respectively) than Cvi (34.2-31.3 days; T1 to T4 respectively). In Bur, bolting was
304 recorded in three nitrate regimes, but as reported above development in Cvi was limited in
305 the LN and VLN regimes. In Bur, the effect was not significantly different in the three nitrate
306 regimes, and there was no significant interaction with temperature. In general, Bur tended to
307 have faster bolting times in the SN regime (Table 1).
308 Table 1 near here
309 There were significant (P<001) effects of ecotype on both rosette diameter (measured at
310 bolting) and leaf number with Bur plants being larger and having much greater vegetative
311 growth than those of Cvi in all temperature regimes. Where they were compared in the SN
312 regime, the mean rosette diameters were 5.58 ± 0.09 cm and 3.67 ± 0.14 cm for Bur and Cvi
313 respectively. Mean leaf number was 15.1 ± 0.2 and 8.7 ± 0.3 for Bur and Cvi respectively. In
314 Bur, there was a significant (P<0.001) increase in rosette diameter and leaf number with
315 increasing nitrate and no interaction with temperature (Table S2). In general, Bur rosette
316 diameter tended to increase with temperature along the tunnel except in the SN regimes,
317 however, Bur leaf number at bolting was significantly (P<0.001) reduced as temperature
318 increased (Table S2). However, there was no trend with temperature in these two measures in
319 Cvi.
320

There was no consistent effect of temperature on seed yield, seed size (1000-seed weight) or seed nitrate content across sowings in either ecotype in the SN regime. However, there was a significant effect ($P<0.001$) of nitrate regime in Bur. For example, the VLN regime significantly ($P<0.001$) reduced seed yield, and in the LN regime both highest and lowest temperature regimes reduced seed yield (Fig. 3a) resulting in a significant ($P<0.001$) temperature x nitrate interaction. In Bur, there was no consistent effect of nitrate regime on seed size (Fig. 3b), but seed nitrate content was significantly ($P<0.001$) higher in the SN regime at the highest temperature compared to any other combination of treatments (Fig. 3c). There were clear highly significant ($P<0.001$) positive linear relationships in Bur between inflorescence dry weight and height, seed yield and seed number (Fig. S3). Data from all tunnel positions and nitrate regimes could be fitted to the same relationship with a clear demarcation between low and high values from VLN and SN regimes respectively. In contrast, these same relationships were not significant in Cvi in the SN regime (Fig. S4).

Effect of seed production environment (temperature, nitrate) on dormancy and germination: Germination experiments were carried out at 10 and 25 $^{\circ}$C in Bur and at 10 and 20 ^{o}C in Cvi as their seeds exhibit contrasting germination at lower compared to higher temperatures. In general, Cvi is more dormant at high temperatures and Bur more dormant at lower temperatures consistent with their winter and summer annual behaviour respectively (Huang *et al.*, 2014). In the experiments there were significant ($P<0.001$) effects of temperature regime, nitrate regime and ecotype and significant ($P<0.001$) interactions between these variables on the percentage germination of seeds produced, which are detailed below.

In Bur: Mean temperatures during maturation along the tunnel overlapped between occasions in 2011 but not in 2012 (Fig. 4). The relationship between mean temperature and percentage seed germination in the light was continuous across both sowings in each year showing the extent of germination to be a function of maturation temperature not time of year (Fig. 4a-d). In all combinations of temperature and nitrate regimes germination was lower when seeds were matured at lower temperatures and higher at higher temperatures; importantly there was a sharp transition at *c.* 16 $^{\circ}$C. These data show dormancy was greater when seeds were matured at mean temperatures lower than 16 $^{\circ}$C. In general, the level of dormancy displayed was greater when seeds were germinated at 25 compared to that at 10 $^{\circ}$C. In 2012, seeds were produced in three nitrate regimes and this had a highly significant ($P<0.001$) effect on depth of dormancy when seeds were produced below 16 $^{\circ}$C, but not at higher temperatures (Fig. 4c, d). This germination response occurred despite there being no consistent effect on seed nitrate concentration (Fig. 3e). Surprisingly, the relationship between nitrate level in the growing media and dormancy of seeds produced at lower temperature was positive; seeds produced in the VLN regime having least dormancy (highest germination).

When Bur germination was tested in the dark at 10 or 25 °C, dark-germination was less than 5% in seed produced under the LN and SN regimes. In seeds produced in the VLN regime dark germination peaked at 30% at 10 °C and 11% at 25 °C, but only below a maturation temperature of 16 °C (Fig. S6). Nitrate could substitute for the light requirement at 10 °C, but only in seeds matured at below 16 °C (Fig. 4e) indicating maturation temperature is a determinant of nitrate sensitivity in Bur. When tested at the higher temperature of 25 °C with nitrate in the dark the response approached that seen in the light (Fig. 4f).

In Cvi: Seeds were dormant in all production environments in both years and consequently there was no germination in the light of freshly harvested seeds at temperatures from 5 to 25 °C. However, depth of dormancy did differ and this was illustrated by germination of freshly harvested seeds on Gibberrellin solution at 20 °C (Fig. 5a,c). Gibberrellin, depending on concentration, can reduce the depth of dormancy allowing germination in the light. Seeds produced in 2011 under winter annual conditions (flowered in spring; early sowing) were less dormant at a given production temperature than those grown as a summer annual (flowered in autumn; late sowing) (Fig. 5a). In 2012, production temperatures on the two occasions did not overlap and those produced at lower temperature (early sowing) had greater dormancy than those produced at higher temperature (late sowing). Interestingly a transition occurred at *c.* 16 °C as it did for Bur. Germination was also recorded on water at a range of temperatures following dry storage (after-ripening; AR) for 30 days (Fig. 5b,d; Fig. S5). Such storage, depending on temperature and seed moisture, progressively relieves dormancy. When these AR seeds were placed to germinate at 15 °C there was a clear relationship between seed maturation temperature and depth of dormancy (Fig. 5b,d). In general, depth of dormancy was greater when seeds were matured at lower temperatures than when matured at higher temperatures; again, in both years there was a clear transition at *c.* 16 °C. However, this relationship differed dependent on the germination conditions. For example, freshly harvested 2011 seeds on Gibberrellin at 20 °C showed seeds from early sown plants were less dormant than those from late sown plants (Fig. 5a), interestingly, the reverse is shown when 30 day AR seeds were germinated at 10 °C on water (Fig. S5a). At 25 °C dormancy persisted and there was no germination after 30 days AR. These relationships may change with further AR.

Discussion

Arabidopsis plants and seeds were produced under realistic global warming scenarios (mean temperature increase to 2080; UK Climate Change Projections 2014) and under this limited range of temperature elevation there was no consistent effect on seed yield and size. In contrast, there were dramatic non-linear temperature effects on the germination behaviour of the seeds produced in both the contrasting ecotypes studied. We show that maternal temperatures lower than 15-16 ˚C resulted in significantly greater primary dormancy than higher temperatures. In addition, the impact of nitrate availability in the growing media was shown only by seeds produced below 15-16 ˚C. A similar dramatic difference in seed dormancy over a small range of constant temperatures (either side of 14-15 ˚C) in the laboratory also occurs in the Col ecotype of *Arabidopsis* (Springthorpe and Penfield, 2015). Importantly, we show in 2011 these effects occurred along the tunnel in a single experiment showing they are driven by temperature and not related to the production time of year. These results therefore illustrate the potentially large impact of small mean temperature increases in this critical temperature range, and that the impact of global warming in the maternal environment can dramatically alter subsequent seed performance. This effect is particularly relevant to temperate regions where seeds are produced in this temperature range. In these regions, there may be long-term consequences for the stability of soil seed banks as native flora comes under increasing pressure from climate change. Such differences could greatly influence phenology expression and future evolution (Burghardt et al. 2016).

Despite expressing contrasting obligate winter and summer annual behaviours, and representing the more extreme ends of the dormancy spectrum in Arabidopsis, the general effect of the local UK global warming scenarios used was similar in both ecotypes. A species characteristic relationship was therefore revealed between maternal temperature (during maturation) and level of dormancy having a sharp cut off at 15-16 ˚C. The impact of this

species characteristic appears tempered by the ecotypes characteristic higher or lower reference dormancy levels (Cvi or Bur respectively). For example, the impact of the maternal temperature was shown to be dependent on conditions in the germination environment that alter the expression of thermodormancy, and this is greatest in the more dormant Cvi.

The maternal temperature effect on dormancy is tempered by the availability of nitrate to the mother plant in Bur, but not Cvi, showing the nitrate effect to be ecotype specific. Bur has a greater nitrate use efficiency (Chardon et al. 2010) that enabled growth to maturity in the VLN and LN regimes while Cvi seedling mortality was 100% in the same conditions. In Bur, there was an interaction between the maternal temperature and nitrate regimes that manifested itself post maturation exclusively in seeds produced below 16 °C. This resulted in altered germination in the light and dark at 10 °C (Fig. 4) at the early sowings. Seeds produced above 16 °C, lost sensitivity to the maternal nitrate regime and light was required for germination at 10 °C. As maturation temperature determined nitrate sensitivity in Bur this may negatively impact low temperature spring germination.

The enhanced nitrate sensitivity of Bur may serve to exploit the impact of temperature and nitrate availability on dormancy level. Seed maturation under low temperature and low nitrate conditions both result in increased dormancy and down regulation of genes involved in nitrogen metabolism (He et al., 2016) while warm temperatures result in reduced dormancy and increased expression of genes involved in nitrogen metabolism (Kendal et al., 2011). Expression of the ABA catabolism gene *CYP707A2* is regulated by nitrate signaling via NITRATE TRANSPORTER1.1 (See discussion in Finch-Savage and Footitt, 2017). As such in the Bur ecotype increased dormancy induced by seed maturation at low temperature is

444 tempered by increased nitrate sensitivity an adaptation that promotes seedling emergence in
445 cool spring conditions.
446
447 On the first three occasions the temperature gradient was applied at the start of seed
448 development so that the effects of temperature during seed development would not be
449 confounded with the effects of temperature on the timing of flowering and start of seed
450 development. For comparison, at the fourth sowing the gradient was applied throughout plant
451 growth to seed harvest. In both ecotypes, the relationship between seed maturation
452 temperature and the depth of seed dormancy (Figs. 4 and 5) fitted to data from all four
453 occasions. This occurred even though on the fourth occasion seed development was occurring
454 earlier at the warm end of the tunnel than at the cooler end. Therefore, temperature during
455 seed maturation is an important environmental factor influencing depth of dormancy.
456 However, the temperature history experienced by mother plants during their life cycle before
457 seed development can also impact on seed characteristics and seed performance in the next
458 generation (Chen et al. 2014; Auge et al. 2017). It is also important to point out that increased
459 global warming is likley to be accompanied by other changes to the environment such as
460 rainfall and the likelihood of drought that may impact on seed dormancy. As winter and
461 summer annuals the annual life cycle timings of these two ecotypes differ and thus the impact
462 of these changes may also differ.
463
464 Correlations and trade-offs between traits such as germination and flowering time may limit
465 the ability of species to adapt to climate change (Etterson & Shaw 2001); this is pertinent in
466 *Arabidopsis* since flowering time affects seed dormancy under field conditions in this species
467 (Chiang et al. 2013). Furthermore, Springthorpe and Penfield (2015) suggest that the
468 temperature control of flowering time may have evolved to constrain when seeds are set (i.e.

around 15 ºC) to ensure that plants produce seeds with different levels of dormancy. They predict, low dormant progeny will enter a rapid cycle if the climate permits, to flower and set seeds later the same summer. This switch therefore represents part of a bet-hedging strategy where the proportion of the seed population with low dormancy emerge immediately while the more dormant portion may avoid reproductive failure in variable environments by entering the persistent seed bank. As the environment varies with global warming so will the proportion of seeds entering these two strategies. This represents an indirect effect of predicted warming on the size of the seed bank (Fenner and Thompson, 2005) and will likley also alter its genetic composition. Seed banks tend to average out the effects of environmental heterogeneity (Venable and Brown 1998) and therefore the greater the extent of disturbance and environmental heterogeneity in a habitat the greater the need for seed banks (Long et al. 2014). Thus, any reduction in seed bank size may reduce resilience to the other aspects of climate change such as the increased likelihood for extreme environmental conditions, which increases the risk of reproductive failure.

Genetically identical cohorts of seeds can adapt to contrasting life cycles (Montesinos-Navarro et al. 2012) and both spring and autumn germination windows have been described in coastal, but not montane Spanish populations (Montesinos-Navarro et al. 2009). In cold years, the impact of low temperature will result in increased dormancy (Fernández-Pascual and Jiménez-Alfaro, 2014) as shown in lab experiments (Chiang et al. 2011; Kendall et al. 2011; Kendall and Penfield, 2012; Huang et al. 2014). This behaviour supports the predictions of Springthorpe and Penfield (2015) in the Col ecotype at different locations. However, the strongly contrasting ecotypes used here germinate only in Autumn (Cvi, obligate winter annual; Footitt et al. 2011) and spring (Bur, obligate summer annual; Footitt et al. 2013) in the UK so that flowering and seed set occur at different times and therefore

494 temperatures. The species characteristic of a sharp temperature transition in its effect on
495 depth of dormancy is therefore likely to impact differently in such ecotypes. In contrast those
496 ecotypes with facultative annual life cycles (e.g. Col-0) are likely to exhibit greater
497 adaptability.
498
499 A further complicating effect, in addition to the effect of warming on depth of dormancy at
500 shedding, is that warming during dormancy relief in the soil seed bank could also differ
501 between these contrasting ecotypes. For example, a greater effect could be expected when
502 dormancy relief is by low temperature in winter (Bur) rather than by warm temperature in
503 summer (Cvi; Footitt et al. 2013). Fenner and Thompson (2005) suggest that such potential
504 side effects of warmer temperatures in winter not relieving dormancy is unlikely since
505 dormancy relief may occur up to 15 ˚C. However, this does not take into account that the rate
506 at which dormancy relief occurs alters with temperature (more rapid at low temperatures).
507 Furthermore, low and high temperatures in the seed bank can have opposite effects on
508 dormancy induction and relief in the winter and summer annual ecotypes used here (Huang et
509 al. 2015; Finch-Savage and Footitt, 2017). Therefore, the consequences of global warming
510 for seed bank stability (both seed entry and persistence) are currently unclear.
511
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517
518 **Author contributions**

W.E.F-S and S.F. conceived the experiments; S.F., ZH and AT performed the experiments; SF, ZH analysed data; W.E.F-S, S.F. and Z.H. wrote the manuscript.

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733 **Table 1. Bolting time responses of Bur and Cvi sown in May 2012 to different**
734 **nitrate compost levels along the thermal gradient tunnel.** Bolting time (days) was
735 recorded in response to growth on very low (VLN), low (LN) and standard nitrate
736 (SN) compost for Bur and SN for Cvi. Data are mean values of three replicates of
737 eight plants ± standard error. Differences between the means are compared by the
738 L.S.D. at the P<0.05 level for Bur only (0.604) and Bur and Cvi under SN conditions
739 (1.341).
740

Temperature location	Bolting time (days)			
	Bur			Cvi
	VLN	LN	SN	SN
T1	36.79 ± 0.15	36.56 ± 0.15	35.63 ± 0.19	34.15 ±0.52
T2	32.72 ± 0.36	32.67 ± 0.67	31.29 ± 0.56	33.68 ± 0.54
T3	30.29 ± 0.36	29.71 ± 0.21	30.58 ± 0.55	34.36 ± 0.45
T4	31.08 ± 0.29	31.3 ± 0.32	30.83 ± 0.17	31.29 ± 0.29

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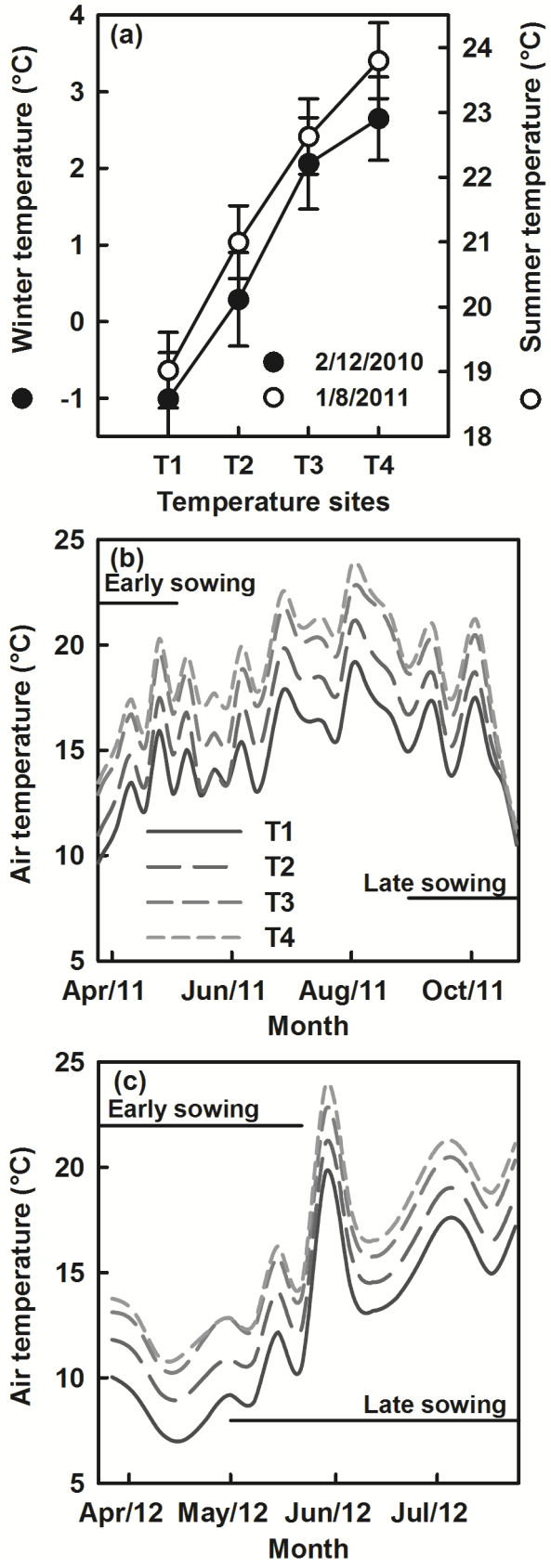
744

745 **Figure legends:**

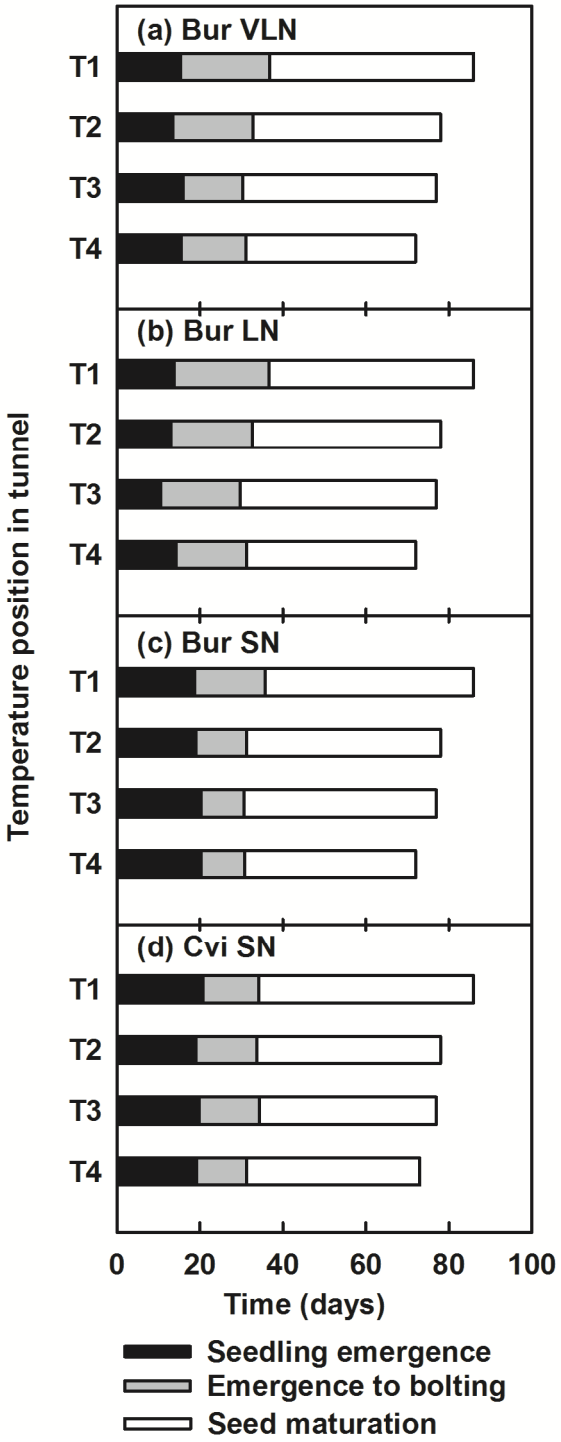
746

747 **Figure 1.** Warming scenarios established along the thermogradient tunnel. (a) Examples of
748 the linear temperature gradients in the winter (2/12/2010) and summer (1/08/2011). T1 is the
749 ambient and T4 the warm end of the tunnel. (b) Mean weekly air temperature recorded at
750 these four positions along the thermogradient tunnel in 2011 and (c) in 2012.
751 The horizontal lines marked early and late sowing denote the time plants spent in the tunnel
752 from transfer at bolting to seed harvest or in the case of the late sowing in 2012 from sowing
753 of seeds to seed harvest. Exact dates are to be found in Table S1.


(a)
Winter temperature (°C)
Summer temperature (°C)
2/12/2010
1/8/2011
T1
T2
T3
T4
Temperature sites
(b)
Early sowing
Late sowing
Air temperature (°C)
Apr/11
Jun/11
Aug/11
Oct/11
Month
(c)
Early sowing
Late sowing
Air temperature (°C)
Apr/12
May/12
Jun/12
Jul/12
Month

754

Figure 2. The impact of temperature (tunnel position) on the life cycle time course of Bur and Cvi. Seeds were sown on 1 May 2012 and progress was recorded through to seed maturity and harvest. The seeds were sown at four positions along the thermogradient tunnel (T1 ambient – T4 warm end). The Bur accession was exposed to three levels of nitrate in the growth media (SN =standard N, LN = low N, VLN = very low N). Cvi failed to complete its' life cycle at the two lower levels of nitrate.



761

762 **Figure 3.** The impact of temperature (tunnel position) during seed maturation on seed
763 components in Bur sown May 2012. Seed yield (a), seed size (b; 1000 seed wt) and nitrate
764 content (c) in the seed were recorded following harvest at four positions along the
765 thermogradient tunnel (T1 ambient –T4 warm end). Plants were exposed to three levels of

nitrate in the growth media (SN =standard N, LN = low N, VLN = very low N). Data are the mean ± standard error. No error bar indicates symbol is larger than the error.

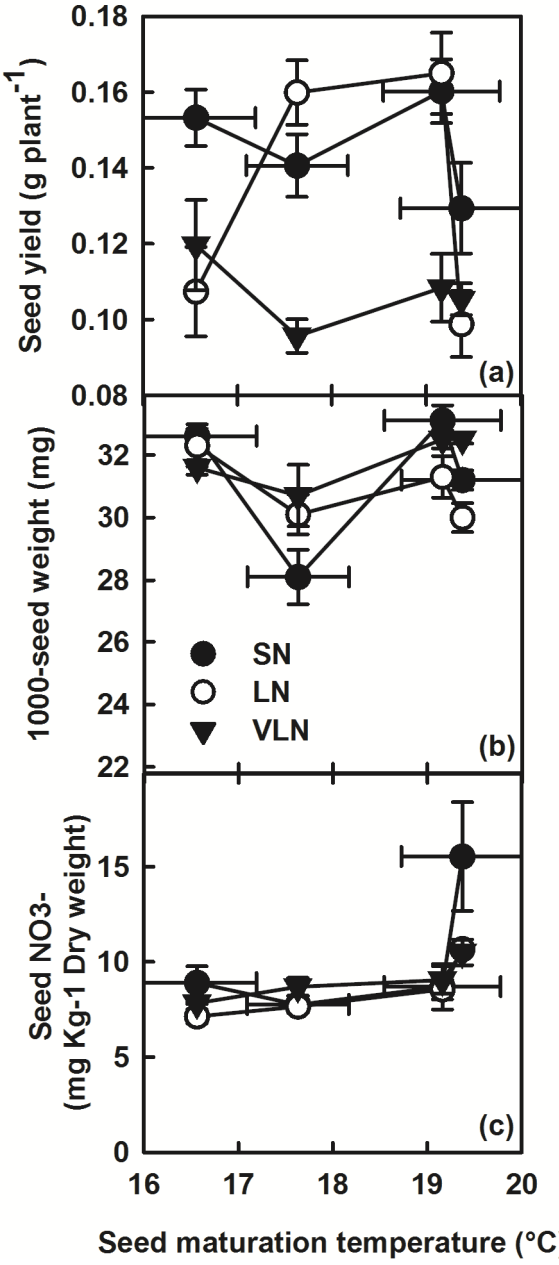


Figure 4. The impact of temperature (tunnel position) during seed maturation on germination performance of Bur. Seeds were collected at harvest maturity following early sowing (closed symbols) and late sowing (open symbols) in both 2011 ((a), (b)) and 2012 ((c) – (f)) at four positions along the thermogradient tunnel (T1 ambient –T4 warm end). The Bur accession was exposed to three levels of nitrate in the growth media (SN =standard N, LN = low N, VLN =

774 very low N). Germination was recorded at ((a),(c),(e)) 10 and ((b),(d),(f)) 25 ˚C, both on ((a)–
775 (d)) water in the light and ((e),(f)) on a nitrate solution in the dark. Data are the mean ± standard
776 error. No error bar indicates symbol is larger than the error. For details of fitted curves see
777 Table S3.

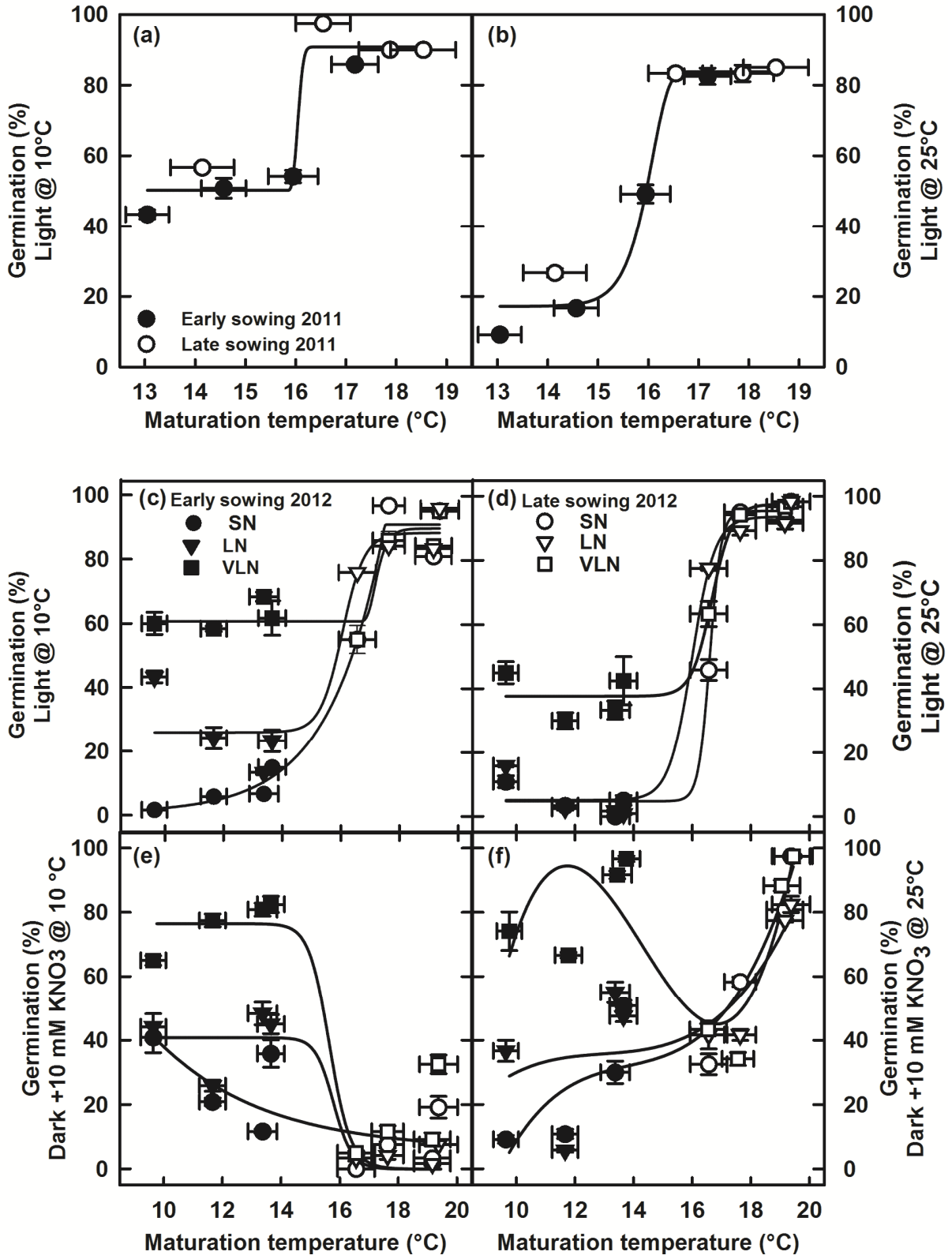
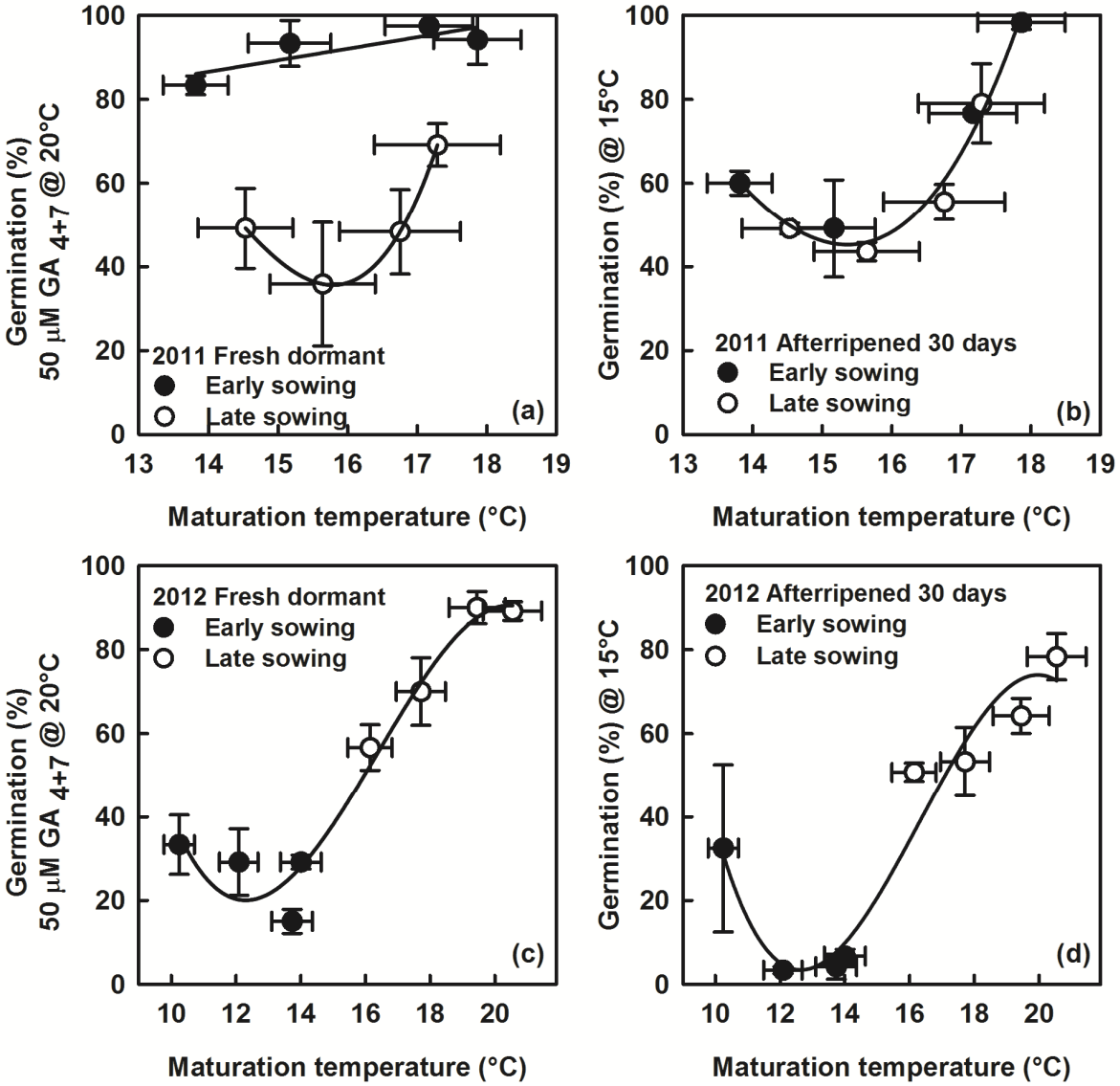


Figure 5. The impact of temperature (tunnel position) during seed maturation on germination performance of Cvi. Seeds were collected at harvest maturity following early sowing (closed symbols) and late sowing (open symbols) in both 2011 ((a),(b)) and 2012 ((c),(d)) at four

positions along the thermogradient tunnel (T1 ambient –T4 warm end). Germination in the light was recorded at 20 ˚C on 50 µM GA_{4+7} (a,c) and at 15 ˚C following 30 days AR ((b),(d)). Data are the mean ± standard error. No error bar indicates symbol is larger than the error. For details of fitted curves see Table S3.



Supporting information

Fig. S1 Emergence from seeds at four positions in the thermal gradient tunnel.

Fig. S2 The relationship between plant size and seed number, seed yield and plant height in Bur.

Fig. S3 The relationship between plant size and seed number, and between seed yield and plant height in Cvi.

Fig. S4 The impact of temperature (tunnel position) during seed maturation on germination performance of *Cvi*.

Fig. S5 The impact of temperature (tunnel position) on dark germination of Bur seeds produced in 2012 under VLN conditions.

Fig. S6 Mean environmental data in the environment of origin for Bur and Cvi

Table S1 Dates of seed production in the thermal gradient tunnel

Table S2 Types of curve used to fit data sets for germination of Burren (Fig. 4) and Cape Verde Islands (Fig. 5) ecotypes. Nitrate level is standard nitrate (SN), low nitrate (LN) and very low nitrate (VLN). The correlation values for each fitted curve are also given (R and R^2).

Table S3 Time to bolting, rosette diameter and leaf number at bolting of Bur sown in May 2012 in different nitrate compost levels along the thermal gradient tunnel in Bur