

TEMPERATURE SENSITIVITY, PHYSIOLOGICAL MECHANISM, AND
IMPLICATIONS OF DROUGHT-INDUCED TREE MORTALITY

by

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ABSTRACT

Drought-induced tree mortality is an emerging global phenomenon that appears related to climate change and rising temperatures in particular, and may be an early indication of vegetation change. However, vegetation response to climate change is uncertain, particularly for future novel climates. Notably, no current models of vegetation change attempt to mechanistically predict plant mortality, and in particular, mortality of trees, which exerts strong influences on ecological function. Resolving uncertainties surrounding the physiological mechanism and temperatures sensitivity of tree mortality is a current challenge in global change ecology.

The objectives of this dissertation were to 1) consider tree mortality consequences for earth system processes related to carbon, water, and energy exchange that include climate regulation; 2) explore tree mortality effects on the water cycle by developing hypotheses and research needs; 3) quantify the temperature sensitivity of drought-induced tree mortality and gain insight into the physiological mechanism of mortality; 4) quantify the relationships among temperature, stored carbohydrate resources, and gas exchange to further elucidate physiological tree mortality mechanisms; and 5) quantify the sensitivity of two species of pine seedlings to progressively elevated temperatures and relate mortality to the effect of temperature on carbon metabolism.

Major findings of this dissertation relate to the temperature sensitivity, physiological mechanism, and implications of tree mortality. Assessment of the potential consequences of tree mortality for earth system processes documented the contrasting influences of tree mortality on the terrestrial C cycle and land-surface energy exchange,

the balance of which will determine the net effects on climate regulation (Appendix A). Following a survey of the ecohydrology literature, thresholds for tree mortality to cause watershed changes were hypothesized at ~20% loss of canopy cover, ~500 mm of annual precipitation, and whether flows are snowmelt dominated (Appendix B). Elevated temperature (~+4°C) accelerated tree mortality by 28% during experimental drought, a difference related to cumulative respiration dynamics in piñon pine (Appendix C). Stored carbohydrate resources were declined during lethal drought but were not entirely depleted prior to mortality (Appendix D). Seedlings exhibited progressive declines in time-to mortality with increased temperatures, a response related to C metabolism (Appendix E).

INTRODUCTION

Anthropogenic activities have increased the atmospheric concentrations of heat-trapping, greenhouse gases since the industrial revolution, such that global average temperatures have risen by 0.76°C since 1850 (IPCC 2007). Under varying scenarios, modeling assessments project further temperature increase of 2-4°C by 2100. Set against this trend of rising temperatures is the expectation of increased extreme heat events and an intensification of the hydrological cycle expected to cause more heavy precipitation events and result in increased drought, which could have large effects on vegetation (IPCC 2007, Huxman et al. 2004, Jentsch et al. 2007, Smith 2011a, 2011b). Rising temperatures are projected to be accompanied by a cooling of the stratosphere, causing poleward expansion of Hadley circulation, reducing precipitation at subtropical latitudes while increasing precipitation at higher latitudes (IPCC 2007, Seidel et al. 2008, Johanson and Fu 2009). While overall precipitation is expected to increase in some regions with global change, a warmer atmosphere has a greater capacity for water vapor, so precipitation increase will be accompanied by an increase in potential evapotranspiration, with uncertain effects on soil moisture (IPCC 2007, Kingston et al. 2009).

Many models of vegetation response to global change assume that as climate conditions shift, plant communities will simply shift their boundaries to follow. Such envelope models of vegetation response rely on determining current climate at species' range limits, predicting where the same conditions will exist in the future, and moving species distributions to match (Iverson and Prasad 1998, Malcolm et al. 2002, Sato et al.

2007). These models are unrealistic since they do not include factors such as natural migration and dispersal rates, as well as the movement of plants across complex terrain (Loehle and LeBlanc 1996, Loehle 2000, Urban et al. 2000, Neilson et al. 2005). Few current vegetation change models mechanistically represent the basic processes of plant establishment and mortality (Moorcroft et al. 2001, Scheiter and Higgins 2009), and none account for rapid, widespread die-off that has been observed in response to recent extreme events (McDowell et al. 2011). Additionally, global change may bring novel climates, temperature and precipitation regimes for which there is no modern analog, to many regions of the world (Williams and Jackson 2007, Williams et al. 2007). These novel climates may encourage the assembly of no-analog plant communities and result in ecological surprises (Williams et al. 2007, Kimball et al. 2010).

The effects of recent temperature increases on vegetation distributed along elevation gradients related to climate provide natural experiments in vegetation change. Observations from long-term elevation gradient datasets show a variety of responses to the changing climate of the last few decades. These responses include shifts in plant species distribution that can be characterized as a “march”, “crash”, or “lean”, outcomes which may depend on whether climate drivers are extreme events or slow changes in climatic means (Breshears et al. 2008). Across elevation gradients in the Green Mountains of Vermont, researchers observed a ~100 m upward shift of the northern hardwood – boreal forest ecotone over forty years, during which temperature increased by ~1°C and precipitation by 34% (Beckage et al. 2008). In northern New Mexico, the lower forest boundary between piñon-juniper woodland and ponderosa pine forest rapidly

shifted uphill during a severe drought (Allen and Breshears 1998). These two studies found responses that exhibit an uphill “march” of populations in response to climate change. Over the course of 30 years in the Santa Rosa Mountains of southern California, the centroid of woody plant species canopy cover distributions shifted uphill along a ~2300 m elevation gradient, while species overall range limits were unchanged, indicating a “lean” response (Kelly and Goulden 2008, Breshears et al. 2008). However, some of the observed changes in these vegetation distributions reported in this study may have been complicated by recovery from disturbance by fire (Schwilk & Keeley 2012). Tree die-off observed in piñon pine across the southwest US following a severe and unusually warm drought resulted in fairly even mortality rates across the species’ entire elevation range, a “crash” response (Breshears et al. 2005, Shaw et al. 2005, Koepke et al. 2010).

Tree mortality related to climate, particularly drought and elevated temperatures, and tree pest and/or pathogen outbreak, is of particular interest as a vegetation response to climate change because it can occur rapidly (Allen and Breshears 1998, Breshears and Allen 2002). Recent research has documented that tree mortality in the last few decades is widespread and found on all forested continents (Allen et al., 2010). Tree mortality can be subtle, such as observations of increased background population mortality rates seen in forests of the western US (van Mantgem et al. 2009) and boreal regions of Canada (Peng et al. 2011). Mortality can also be selective, affecting only a single tree species in a diverse forest, such as mortality of eastern hemlock in the Appalachians of the US from hemlock woolly adelgid, a phloem-consuming insect (Ford and Vose 2007).

Tree mortality can also manifest as a widespread die-off, such as the 600,000+ km² of pine forests in western North America from Mexico to Alaska affected by drought, higher temperatures, and bark beetle outbreak (Raffa et al. 2008, Hicke et al., 2012) or a 50,000 km² die-off of Eucalypt species across Australia related to drought (Fensham et al. 2009, Allen et al. 2010). Causes of tree mortality can be complex, as they often involve interrelationships between direct climate stress on trees, stress-related reductions in tree defense, and positive climate effects on multiple insect pests and fungal or other pathogens (Raffa et al. 2008). Mortality in ponderosa pine in the southwest US has been related to tree drought stress, increasing vulnerability to bark beetle attack by multiple species of two beetle genera, *Dendroctonus* and *Ips*, which reproduce in the phloem, effectively girdling the tree (Gaylord et al. 2006, 2007). These beetles typically carry blue-stain fungus (*Grosmannia clavigera*), which may further exacerbate tree stress by cavitating xylem water transport and inhibiting resin production for beetle defense (Gaylord et al. 2011). Mortality causes can also be simple, no biotic agent was found to be associated with mortality of *Juniperus* species in the southwest US, indicating tree drought stress as the sole likely cause (Floyd et al, 2009). Elevated temperatures appear to be a common causal factor in tree mortality, as they increase drought stress and often promote biotic agent populations and spread (Breshears et al, 2005, Raffa et al. 2008).

The ecological consequences of tree mortality include effects on populations that translate to impacts to community composition and structure. When die-off affects the dominant plant species in the community, interactions with a large number of other species can be affected. Piñon pine, a co-dominant species in Southwest affected by

widespread die-off, is associated with approximately 1000 other species, and piñon loss means a restructuring of these relationships, which raises biodiversity conservation concerns (Mueller et al., 2005a, 2005b, Gitlin et al. 2006, Sthultz et al. 2007). Drought mortality in piñon pine differentially affected trees of differing genotypes, resulting in a loss of heritable insect resistance (Sthultz et al. 2009). Conservation implications of die-off are not always negative, mortality of ponderosa pine in British Columbia is expected to create improved wildlife habitat with an increase in open areas and standing snags (Klenner and Arsenault 2009). Transition toward an herbaceous dominated community with tree mortality increases light inputs to the understory (Royer et al. 2011), alters patterns of phenology and primary production, and can promote invasion by undesirable exotic species (Rich et al. 2008, Kane et al. 2011). Tree mortality also can accelerate biogeochemical cycles; increased N and P inputs to soils and stream water were observed following pine die-off in Colorado (Clow et al. 2011).

Forests exert a strong effect on earth system processes relative to their spatial extent, which is in part due to the 3-dimensional nature of vegetation structure in forests (Bonan 2008). This influence occurs through the effect of forests on the terrestrial carbon sink, land-surface energy balance, and hydrological cycling (Bonan 2008, Chapin et al., 2008). Forests sequester more C than other vegetation types and contain 55% of stored terrestrial C. Forest canopy is often darker than non-forest, especially so at high latitudes where snow covers the ground for much of the year, such that forest cover reduces albedo, the reflectance of energy by the land surface (Bonan 2008). Forests are also very influential over the water cycle through canopy effects on interception and

evapotranspiration (Chapin et al. 2008). Therefore, large-scale tree mortality can rapidly affect exchanges of carbon, energy, and water between the biosphere and atmosphere due to a sudden loss of canopy cover (Breshears and Allen 2002, McDowell et al. 2008). Altered carbon sequestration following widespread die-off has affected North American forest carbon budgets with a net warming forcing on climate, influencing policy in Canada (Kurz et al., 2008a, 2008b, Hicke et al., 2012). Die-off threats to carbon sequestration in the Amazon are of particular concern in these C-rich and productive forests (Metcalf et al. 2010). However, altered energy balance from tree mortality could result in a localized cooling effect in drylands where canopy cover has an insulative effect on surface temperatures (Rotenberg and Yatir, 2010) and at high latitude forests where mortality exposes a snow-covered landscape (Betts, 2000, Lee et al., 2011). Potential hydrological effects of tree mortality range from flooding risk to decreased water resources (Carver et al. 2009, Guardiola-Claramonte et al., 2011). Through its effects on energy and carbon balance, widespread tree mortality caused by increased temperatures is a potential and uncertain feedback on global climate change. The balance of die-off effects on forest carbon sink strength, a potential net warming forcing, and albedo and energy exchange, a likely net cooling forcing, could disrupt the climate regulation provided by the biosphere.

The balance and degree of these effects will ultimately be determined by ecological responses to tree die-off, which include successional return to the previous forest, shifts in species composition, conversion to non-forest, and other outcomes along this spectrum of responses. For forests where tree mortality is driven primarily by insect

outbreak in the absence of drought, indications of forest recovery are reported (Veblen et al., 1991, Axelson et al., 2009, 2010, Brown et al., 2010, Collins et al., 2011). This response has been particularly noted in western North American forests affected by mountain pine beetle outbreak where beetles preferentially attacked larger trees, often resulting in >95% overstory mortality, but entirely avoided smaller trees in the understory (Axelson et al., 2009, Klenner and Arsenault, 2009, Axelson et al., 2010, Brown et al., 2010). Natural regeneration in some of these stands meets timber production guidelines for restocking without re-planting (3000 seedlings/ha, Axelson et al., 2010). Although such observations suggest quick recovery, many assessments of these forests fail to consider the effect of a changing climate on tree recruitment and future beetle outbreaks, particularly at high latitudes where temperature increases are expected to be greatest (IPCC, 2007).

However, in forests and woodlands where drought was an important contributing factor to widespread tree mortality, understory saplings and seedlings were affected by the same drought that killed overstory trees. Following the 2002/2003 drought and bark beetle outbreak in the southwest US, surveys showed mortality in ~25-80% of adult and ~50-80% of ponderosa pine seedlings (Gitlin et al., 2006), and where ~80% of overstory piñon trees died, in ~25-45% of piñon seedlings (Mueller et al., 2005a). These observations are not limited to the southwest US, low post-drought recruitment was also correlated with overstory tree mortality for *Nothofagus dombeyi* in Patagonia, Argentina (Suarez and Kitzberger, 2008) and *Pinus sylvestris* in the Pyrenees of Spain (Galiano et al., 2010). In these cases, relatively more recruitment of less-affected, drought-resistant

tree species were observed, indicating that a shift in overstory composition and stand structure will follow mortality. These responses indicate that the consequences of tree mortality for carbon, water, and energy budgets will persist in such forests and woodlands.

In order to more realistically predict vegetation response to global change and associated ecological and earth system consequences, alternatives have been suggested to replace envelope models. Mechanistic models which scale plant responses to large areas offer a potential solution and may prove more useful for making predictions under novel climate regimes (Moorcroft et al. 2001, McDowell et al. 2008, 2011). However, key data on the physiological sensitivities and mechanisms of plant mortality and recruitment required to build such models are lacking. Correlative relationships determined from observations are not always available and may not be sufficient to determine responses to future novel climates. Experimental manipulations that simulate extreme events, including drought, are necessary to reveal causal relationships between climate factors and plant physiology (Jentsch et al. 2007).

Two hypothesized mechanisms of drought-induced tree mortality linked to species differences in drought tolerance strategies as well as the intensity and duration of drought have been proposed, hydraulic failure and carbon starvation (McDowell et al. 2008). Hydraulic failure from drought could occur if high xylem tension causes catastrophic cavitation by air embolism, impeding the flow of water and leading to a loss of cellular turgor pressure that results in death. Carbon starvation could occur if trees reduce stomatal conductance during drought in an effort to prevent xylem failure,

limiting access to atmospheric CO₂. When photosynthesis could no longer meet respiratory demands, death would occur when trees had exhausted stored carbohydrates. Mortality by both mechanisms could be exacerbated by tree pests and pathogens.

Further conceptual assessments have led to an expansion of initially proposed hypotheses, postulating that tree drought mortality is a complex process which likely occurs by multiple interrelated mechanisms (Sala 2009, Sala et al. 2010, McDowell and Sevanto 2010, McDowell 2011, McDowell et al. 2011). Carbon starvation could occur by a combination of two pathways, exhaustion of carbohydrate reserves and mobilization/transportation failure (Sala 2009, Sala et al. 2010, McDowell and Sevanto 2010). The reserve exhaustion pathway is carbon starvation as initially envisioned a depletion of stored carbohydrates during drought that leads to mortality. However, this assumes that the physiological processes of mobilizing non-structural carbohydrates as sugars and transporting them in phloem to carbon sinks will remain unaffected by drought (Sala et al. 2010). Yet, both the metabolism of stored starches into sugar and the generation of positive hydrostatic pressure flow in the phloem require water, making mobilization/transport failure probable under severe water stress (Münch 1930, Taiz and Zeiger 2002, Cernusak et al. 2003, Höllta et al. 2006, Sala et al. 2010, Sevanto et al. 2011, McDowell et al. 2011).

A common drought response in trees is the reduction of growth, which reduces respiration during drought (Ayub et al. 2011), and subsequently causes non-structural carbohydrate reserves (NSC) to be increased and may reduce photosynthesis through feedback inhibition (Körner 2003, Würth et al. 2005, Sala and Hoch 2009, Galvez et al.

2010, Muller et al. 2011). This response has been termed sink limitation and can seem incompatible with tree drought mortality by carbon starvation (Leuzinger et al. 2009, Sala 2009), which essentially implies a source limitation related to reduced photosynthesis with stomatal closure during drought (McDowell et al. 2008, 2011). Although reduced NSC in trees during drought has also been observed (Körner 2003, Sayer and Haywood 2006, Eilmann et al. 2010), mortality mechanisms should not be inferred from responses to non-lethal drought (McDowell and Sevanto 2010). Drought mortality by carbon starvation, as currently hypothesized, includes an initial period of increased NSC sink limitation that would be followed by NSC depletion (reserve exhaustion), or a loss of tree's ability to transfer carbohydrates among tissues (transportation/ mobilization failure) prior to death (Sala et al. 2010, McDowell et al., 2011). Moreover, both sink and source dynamics are well-integrated such that tree metabolism under stress is likely co-limited by both (Bansal and Germino 2008). Carbohydrate pools are also actively maintained and accumulated in plant tissue for use in signaling, cryoprotection, and desiccation protection are not simply passive pools resulting from photosynthetic supply and respiration demand (Obendorf 1997, Ogren et al. 1997, Li et al. 2003, Ryan 2011, Sala et al. 2012). Additionally, NSC may be important in reversing cavitation in the xylem under negative pressure, implying a further level of interdependence between carbon starvation and hydraulic failure (McDowell et al. 2011, Sala et al. 2012). Stored NSC is also likely important for the production of resins used in tree defense against bark beetle attack. Recent studies reported that NSC resources were lower for drought-killed *Pinus sylvestris* in Spain (Galiano et al. 2011) and near-dead seedlings of *Nothofagus*

nitida, but not *Nothofagus dombeyi*, from Chile (Piper 2011), or aspen ramets in Colorado, USA (Anderegg et al. 2012) relative to surviving individuals. These results suggest that carbohydrates may be diminished during lethal drought for some species, but not others, and highlight that the physiological mechanisms of tree mortality remain unresolved.

In summary, despite its emergence as global phenomenon related to rising temperatures, tree mortality is a relatively understudied aspect of vegetation change in response to a changing climate. Tree mortality has important consequences for ecosystems and earth system consequences, including the potential to disrupt terrestrial climate regulation. Key data relating to the temperature sensitivity and physiological mechanism of tree mortality, which could be used to project responses to climate change, are lacking.

PRESENT STUDY

The methods, results and conclusions of this research program are presented fully in manuscripts appended to this dissertation. The following is a description and summary of the most important findings in these documents.

Climate induced tree mortality: earth system consequences.

(Author's note: The following describes the results of a multi-authored manuscript in which I was the primary contributor. Coauthors of this research contributed editorial support. This manuscript is an invited, peer-reviewed, feature article published in *Eos, Transactions, American Geophysical Union*, which is the member newspaper of the American Geophysical Union).

Feedbacks between the biosphere and the earth system are an important component of models that predict future climate change. However, there is much uncertainty regarding the stability of these feedbacks with pressures on the biosphere from ongoing climate changes. Terrestrial ecosystems, and in particular forests, have strong effects on the global carbon cycle, water cycle and surface energy budgets (Bonan 2008; Chapin et al. 2008). Therefore, recent and widespread tree mortality, now documented on 6 continents, related to increased temperatures, drought, and insect infestation (Allen et al., 2010) is a threat to ecosystem function, including the earth's climate regulation.

Recent examples of tree mortality include extensive "die-offs" in which high proportions of trees die at regional scales, which has affected 12,000 km² of piñon pine woodlands in the southwestern US (Breshears et al. 2005), 130,000 km² of pine species in western Canada (Kurz et al., 2008a), and extensive areas on all other forest continents (Allen et al. 2010). Rates of background mortality are also on the rise (van Mantgem et

al. 2009), and mortality is altering tree species distributions at the vulnerable edges of their ranges (Allen and Breshears 1998; Kelly and Goulden 2008). Early warning signs indicate potential vulnerability in tropical and boreal forests, which are particularly important for climate regulation due to the high productivity and C stocks of tropical forests and the influence of boreal forest cover on surface albedo (Bonan 2008).

Reducing the uncertainty surrounding forest-atmosphere feedbacks, will require development of a predictive capability for tree mortality, but there are large gaps regarding the specific vulnerabilities of different tree species and forest types. There is a particular interest in the physiological mechanism mortality as a means to predict die-off under future climate; hypotheses that relate mortality to tree carbon balance and xylem water tension have been proposed (McDowell et al. 2008) and some initial studies conducted (Raffa et al. 2008; Adams et al. 2009).

Forests contain ~ 55% of the carbon in terrestrial ecosystems, absorbed 33% of anthropogenic carbon emissions during the 1990s, and alter the energy exchange with the atmosphere by affecting land surface albedo (Bonan 2008). The stability of these effects is threatened by tree mortality. In British Columbia, forest die-off is projected to reduce carbon sequestration by 270 megatons over 20 years, undoing the sequestration gains of the last 20 years across a the region (Kurz et al. 2008a) Hydrological changes are also expected as tree mortality alters landscape-level evaporation: transpiration ratios (Chapin et al., 2008). These threats to climate regulation are an urgent call for research on die-off observation, tree mortality physiology, and post-mortality ecosystem effects.

Quantifying and predicting tree mortality effects on biosphere-atmosphere feedbacks are essential for improved global change models.

Ecohydrological consequences of drought- and infestation-triggered tree die-off: insights and hypotheses.

(Author's note: The following describes the results of a multi-authored manuscript in which I was the primary contributor. Coauthors of this research contributed editorial support. This manuscript was submitted for a special issue and is in press with *Ecohydrology*, a peer-reviewed Wiley journal.)

Global climate change is projected to affect the hydrological cycle through altered precipitation patterns and increased temperatures, but may also lead to ecological change that could influence hydrology. Tree mortality caused by a combination of drought, increased temperatures, and tree pest infestation appears to be on the rise globally (Allen et al. 2010). Tree canopy cover exerts important influences on forest water balance from the stand to watershed level. Since tree mortality patterns are often linked to precipitation variability that affects hydrological response, assessing feedbacks between vegetation and the water cycle is a pressing challenge in ecohydrology. However, there has been scant research on the ecohydrological consequences of forest die-off and these remain highly uncertain.

To explore this knowledge gap, we summarized the limited existing die-off ecohydrology literature, and also relied on the extensive forest harvest hydrology literature. Among forest disturbances that cause canopy cover loss, die-off and harvest have more in common than do die-off and fire, which can also affect the understory and soils more substantially. The primary effects of die-off and harvest are a loss of canopy cover that reduces overstory transpiration and interception. Therefore we relied on both

die-off and harvest research to develop testable hypotheses on the ecohydrological consequences of tree mortality.

Canopy cover loss from tree mortality directly alters evaporation, transpiration, and canopy interception, and the sum of these competing processes will determine if overall evapotranspiration and interception (ETI) are affected. Any overall effect on ETI by mortality will indirectly alter the processes of infiltration, runoff, groundwater recharge, and streamflow. Harvest research suggests that for most forests, streamflow can be expected to increase following > 20% loss of tree cover, likely due to a decrease in ETI (Stednick 1996; Brown et al. 2005). In die-off-affected watersheds, canopy cover reductions of ~15 - ~80% led to increased (~10 - ~26%), unaffected, and even decreased (~30 - ~50%) streamflow. Two out of seven reviewed studies found decreased flows following die-off, an unusual prevalence, given that such a result is very rare in the harvest literature (Brown et al. 2005). Both of these studies accounted for changes in precipitation before and after die-off, and researchers could only speculate that unmeasured ETI increased following mortality (Somor 2010; Guardiola-Claramonte et al., 2011). The response of snow hydrology to die-off is of particular interest due to extent of mortality (130,000 km²) in pine forests at higher elevations and latitudes in western North America (Kurz et al. 2008a). In typical years, increased snowpack accumulation and faster melt rates in die-off-affected forests resembled those clear-cut or treeless areas (Boon 2007; 2012; Pugh and Small 2012). This is consistent with earlier, faster, and more synchronous snowmelt that occurs with harvest in forests (Jones 2000; Tonina et al. 2008).

Following this review, we hypothesized conditions necessary for the detection of increased watershed flows as a result of ETI reduction. We expect increases if annual precipitation exceeds ~500 mm or water yield is dominated by snowmelt and if absolute canopy cover loss from die-off exceeds 20%. If these conditions are not met, we hypothesized that flows will generally be unaffected, but could decrease in drier forests. We also hypothesized that the causes of tree mortality can influence potential ecohydrological outcomes. For example, if mortality is primarily drought related, reduced precipitation is likely to mute ecohydrological responses.

To reliably predict die-off responses, more research is needed to test these hypotheses, including observations of multiple water budget components and the persistence of ecohydrological effects with the post-die-off successional dynamics of tree recruitment and understory growth. Management options to prevent die-off and restore affected forests will be limited and costly. Therefore, to improve predictions of the threat to water resources, die-off ecohydrology should be a high priority for future research.

Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global change-type drought

(Author's note: The following describes the results of a multi-authored in which I was the primary contributor. Coauthors of this research contributed editorial, logistical, and material support. This manuscript was published in the *Proceedings of The National Academy of Sciences of the United States of America*, a peer-reviewed journal published by the National Academy of Sciences.)

Large-scale vegetation shifts are predicted in response to the increased temperature and altered precipitation regimes associated with global climate change.

Vegetation shifts have profound ecological impacts and are an important climate-

biosphere feedback through their effects on carbon, water, and energy exchange between the land-surface and atmosphere (Breshears and Allen 2002, Bonan 2008, Chapin et al. 2008). Of particular concern is the potential for warmer temperatures to exacerbate plant stress in response to increasingly severe droughts, triggering vegetation shifts via woody plant mortality (Allen and Breshears 1998, Breshears et al., 2005). Around the world, many tree species are experiencing widespread, historically unprecedented mortality in response to climate and tree pest outbreaks (Allen et al. 2010). The sensitivity of tree mortality to temperature is dependent on which of two non-mutually exclusive mechanisms predominates—carbon starvation in response to a period of protracted water stress or sudden cavitation-induced hydraulic failure under extreme water stress (McDowell et al., 2008).

To test the hypothesis that temperature exacerbates tree stress and accelerate mortality, we transplanted small, reproductively mature into the Biosphere 2 glasshouse. Trees were randomly allocated to two temperature treatments, ambient and elevated (+4.3°C), and then two moisture treatments, well-watered and drought. Drought trees received no water through mortality, defined as the point of foliar browning. We measured net photosynthesis, pre-dawn respiration and water potential, among other variables

We found that experimentally-induced warmer temperatures (~4°C) accelerated the time to drought-induced mortality in *Pinus edulis* (piñon pine) trees by nearly a third. Drought trees under both temperatures experienced the same decline in pre-dawn water potential, indicating that hydraulic failure occurred but was not the primary driver of

differences in time-to-mortality between treatments. Cumulative, time-integrative respiration was initially higher in warmer drought trees than ambient drought trees, but just prior to mortality for both there was no difference in cumulative respiration costs. This indicates that trees in both treatments respired the same mean cumulative CO₂, but warmer trees did so faster and died earlier. This result implicated temperature-sensitive carbon starvation as the primary mechanism of mortality. Extrapolating the temperature difference of survival time to the historic frequency of water deficit in the southwestern U.S. predicts a five-fold increase in the frequency of regional-scale tree die-off events for this species due to temperature alone. Although a simplistic estimate, this is a conservative one as projected increases in drought frequency due to changes in precipitation and increases in stress from biotic agents (e.g. bark beetles) would further exacerbate mortality. Our results demonstrate that warmer temperatures have exacerbated recent regional die-off events and background mortality rates and were consistent with the mortality mechanism of carbon starvation. Because of pervasive projected increases in temperature, our results portend widespread increases in extent and frequency of vegetation die-off.

Non-structural leaf carbohydrate dynamics of Pinus edulis during drought-induced tree mortality support role for carbon metabolism in mortality mechanism.

(Author's note: The following describes the results of a multi-authored manuscript in which I am the primary contributor. Coauthors of this research contributed editorial, logistical, and material support. This manuscript will be resubmitted to *New Phytologist*, a peer-reviewed journal published by Wiley-Blackwell.)

Widespread tree mortality associated with drought, increased temperatures, and tree pest/pathogen infestation appears to be a common ecological response to global

change recently observed across all six forested continents (Allen et al. 2010). Die-off from tree mortality has affected over 605,000 km² of pine forests in North America alone (Hicke et al., 2012). Widespread tree die-off can alter ecosystem function, hydrology, and land-surface energy exchange, and potentially producing strong feedbacks on climate through the loss of forest carbon sinks (Breshears and Allen 2002, Bonan 2008, Chapin et al. 2008, Kurz et al., 2008a, Rotenberg and Yakir 2010, Hicke et al. 2012). Despite these effects, no models of vegetation response to climate change mechanistically predict tree mortality (McDowell et al., 2011). The physiological mechanisms underlying tree mortality are of great interest but are unresolved, particularly the roles of complete xylem cavitation and/or carbon starvation.

Here we present analysis of foliar non-structural carbohydrates (NSC) and associated physiology collected during the Biosphere 2 piñon experiment where drought-induced mortality of *Pinus edulis* was highly temperature-sensitive (Adams et al. 2009). In this experiment, trees under drought at higher temperatures had higher time-integrated cumulative respiration than ambient-temperature trees, but died earlier. As such, trees under both temperatures had similar mean total cumulative respiration just prior to death, consistent with temperature-sensitive carbon starvation. We predicted that in this experiment we would measure a faster decline in NSC for warmed trees than for ambient-temperature trees, consistent with cumulative respiration dynamics and carbon starvation.

Total foliar NSC declined by ~30% during drought, through mortality. Moreover, droughted trees had substantially lower leaf NSC than controls as control tree NSC increased during the experiment. NSC decline in drought trees was primarily due to

decreased sugar concentrations, while starch initially decreased, but then increased above pre-drought concentrations prior to mortality. Unexpectedly, temperature had no detectable effect on total leaf NSC or sugar concentrations for droughted trees or well-watered controls. However, significant differences in the decline and then increase of starch between temperature treatments suggest differential effects of temperature on photosynthetic feedback inhibition and possible mobilization/ transportation failure (Sala et al. 2010). Structural equation modeling revealed a strong stomatal limitation of photosynthesis and a positive effect of photosynthesis on sugar content during drought. Our results support an important role for carbon metabolism in the mortality mechanism of this species during drought. However, changes in NSC during drought did not deplete stored leaf carbon resources entirely, and mortality was preceded by hydraulic failure in the xylem. Our results demonstrate that for some tree species mortality is a complex process of interrelated mechanisms (McDowell et al., 2011). Improved determination of the contribution of carbon starvation to mortality will require accounting for whole-plant carbon dynamics, maintenance of NSC for non-metabolic functions, and internal NSC conversions.

Drought-induced Pinus seedling mortality hastens progressively with temperature as reflected in respiration during stomatal closure.

(Author's note: The following describes the results of a multi-authored manuscript in which I am the primary contributor. Coauthors of this research contributed editorial, logistical, and material support. This manuscript is intended for submission to *Global Change Biology*, or a similar peer-reviewed journal.)

Extensive tree die-off in forested ecosystems caused by drought and warmer temperatures has been recently observed from the tropics to high latitudes (Allen et al. 2010). In the next century, predicted increases in temperature and drought frequency could cause more die-off, potentially altering ecosystem function, hydrology, and land-surface energy exchange (Adams et al. 2010). The degree and persistence of these consequences of die-off depend directly on the demographic response of tree species following overstory mortality (Suarez and Kitzberger 2008). In some die-off-affected communities, large recruitment pulses following overstory die-off suggest successional recovery (Klenner and Arsenault 2009, Brown et al. 2010), while for other communities, the same drought that caused overstory tree mortality also killed a high proportion of understory seedlings (Mueller et al. 2005a, Gitlin et al. 2006, Galiano et al. 2010). However the sensitivity of drought-induced mortality in tree seedlings has not been evaluated across range of higher temperatures projected to accompany future drought.

In this work, we investigated sensitivity of drought-induced mortality to progressively increased temperatures in seedlings of two pine species, the more drought-tolerant *Pinus edulis*, and the less drought-tolerant *Pinus ponderosa*, both of which were affected by large-scale die-off. We withheld water from seedlings at ambient summer temperatures of the lower forest boundary where these two pines co-occur, and at three

elevated temperatures, +3.6, +5.9, and +7.7°C until all seedlings died. Gas exchange of CO₂ and H₂O was measured during the drought to relate time-to- mortality to physiological drought tolerance and carbon metabolism.

Drought-induced mortality in both pine species was highly temperature sensitive, exhibiting earlier mortality progressively with each temperature increase. While the more drought-tolerant *P. edulis* showed greater drought resistance, outlasting *P. ponderosa* at all temperatures, time-to-mortality in *P. edulis* declined more with increasing temperature than *P. ponderosa*, indicating greater within-species sensitivity to temperature in *P. edulis*. Earlier mortality during drought was associated with a greater effect of temperature on the decline of stomatal conductance, net photosynthesis, and respiration rates, indicating a similar drought tolerance strategy in both species. The pre-mortality period of stomatal closure lasted longer in *P. edulis* than in *P. ponderosa* and was shorter with increasing temperature in both species. Lower mean respiration rate during this period was associated with a longer time-to-mortality in both species.

Under increased temperatures more frequent shorter droughts become sufficient to cause tree mortality so reduced time-to-mortality leads to progressive increases in future episodes of seedling mortality from future climate warming alone, even without projected increases in drought frequency. Our results indicate that a successional return to previous tree community conditions should not be expected in communities where drought mortality affects both overstory and understory components. This vulnerability of seedlings to drought at higher temperatures suggests that recruitment may not offset

adult mortality and that woodland and forest communities may undergo substantial future changes that affect ecosystem and earth system function.

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APPENDIX A: CLIMATE-INDUCED TREE MORTALITY: EARTH SYSTEM
CONSEQUENCES

Adams HD, Macalady AK, Breshears DD, Allen CD, Stephenson NL, Saleska SR,
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Climate-Induced Tree Mortality: Earth System Consequences

PAGE 153–154

One of the greatest uncertainties in global environmental change is predicting changes in feedbacks between the biosphere and the Earth system. Terrestrial ecosystems and, in particular, forests exert strong controls on the global carbon cycle and influence regional hydrology and climatology directly through water and surface energy budgets [Bonan, 2008; Chapin *et al.*, 2008].

According to new research, tree mortality associated with elevated temperatures and drought has the potential to rapidly alter forest ecosystems, potentially affecting feedbacks to the Earth system [Allen *et al.*, 2010]. Several lines of recent research demonstrate how tree mortality rates in forests may be sensitive to climate change—particularly warming and drying. This emerging consequence of global change has important effects on Earth system processes (Figure 1).

Observations and Patterns of Tree Mortality

Reports of tree mortality associated with heat and drought from around the world have increased in the past decade, and although each cannot be conclusively linked to climate change, they collectively illustrate the vulnerability of many forested ecosystems to rapid increases in tree mortality due to warmer temperatures and more severe drought [Allen *et al.*, 2010]. Recent examples include extensive “die-offs” in which high

proportions of trees die at regional scales [Breshears *et al.*, 2005].

In the southwestern United States, widespread drought and insect-driven mortality of piñon pine in the early 2000s affected more than 12,000 square kilometers in less than 3 years, killing 40–97% of those trees at some sites [Breshears *et al.*, 2005; McDowell *et al.*,

2008]. Although episodic tree mortality is an intrinsic process in many forests, the recent mortality in the southwestern United States occurred during an unusually warm drought and appears to have been more severe than mortality associated with a cooler yet drier drought in the 1950s.

In western Canada, drought and unusually warm temperatures weakened trees and accelerated mountain pine beetle population growth and range expansion, causing a massive outbreak that killed millions of trees across 130,000 square kilometers of pine forest in 6 years [Kurz *et al.*, 2008a]. Other

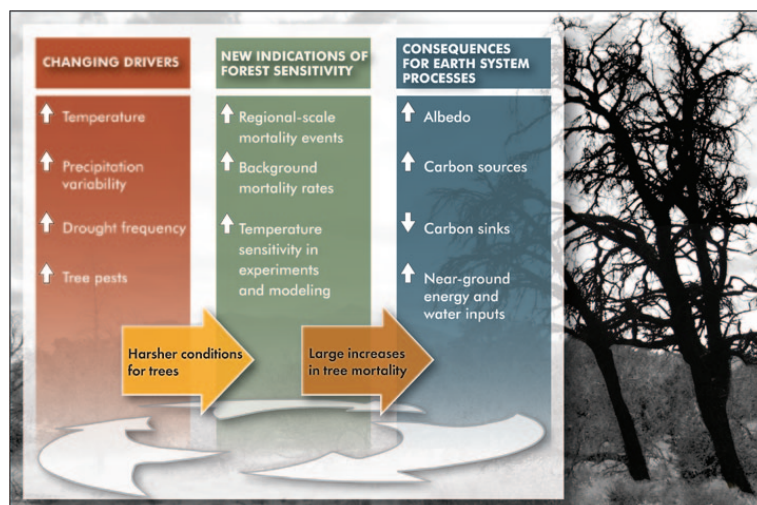


Fig. 1. Climate change can affect tree mortality both directly (such as through drought) and indirectly (such as by favoring tree pests). Recent observations have revealed apparent warming-induced increases in both background tree mortality [van Mantgem *et al.*, 2009] and regional-scale forest die-off [Allen *et al.*, 2010]. Observations, theory, and experiments have begun to unravel sensitivities and mechanisms driving these events [McDowell *et al.*, 2008; Adams *et al.*, 2009a]. Accelerating tree mortality resulting from ongoing climate changes could have potentially profound effects on Earth system processes, providing positive feedbacks that further enhance climate change.

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extensive insect outbreaks triggered at least in part by climate have been documented in North America from Alaska to Mexico, with drought and warming appearing as common drivers [Raffa *et al.*, 2008]. Instances of extensive tree mortality also have recently been reported from Africa, Asia, Australia, Europe, and South America [Allen *et al.*, 2010].

In addition to extensive, insect-mediated tree mortality, slower, less obvious changes in tree mortality are equally concerning. Over the past few decades in old forests of the western United States, background (non-catastrophic) tree mortality rates have more than doubled, an apparent consequence of rising temperatures [van Mantgem *et al.*, 2009]. Changes in mortality rates associated with rising temperatures and drought also may be driving elevation shifts in tree species, especially through mortality at lower forest boundaries, effectively pushing tree species uphill and into smaller geographic ranges [Allen and Breshears, 1998; Kelly and Goulden, 2008].

The possibility of rising tree mortality rates in tropical and boreal forests is of particular interest because tropical forests contain more than half of the total stored carbon in global forests, and boreal forests play a critical role in Earth's surface albedo, which is the ratio of reflected to total incoming solar radiation [Bonan, 2008]. Observations in boreal ecosystems suggest that forests may become increasingly vulnerable to insect outbreaks because of warmer temperatures and elevated drought stress in host trees [Berg *et al.*, 2006]. In the Amazon, modeling studies have raised concerns that forests may approach a tipping point in the coming century where climatic thresholds are exceeded, triggering widespread tree mortality [Phillips *et al.*, 2008; Malhi *et al.*, 2009]. Long-term data from pan-Amazonian forest surveys recently documented effects from a severe drought in 2005, with reduced growth and increased tree mortality driving a marked shift in forest carbon balance [Phillips *et al.*, 2009]. Uncertainty surrounding the responses of forests that greatly influence global climate points to a need for a better understanding of tree mortality.

Mechanisms of Mortality

Scientists are far from understanding the specific vulnerabilities of different tree species or forest types that are needed to predict climatically induced changes in tree mortality. Current studies lack a fundamental mechanistic understanding of mortality at all spatial scales, from the level of individual trees, through forest stands, to regional landscapes. Accurate model forecasts of the effects of changing tree mortality on the Earth system require a more robust understanding of the causal links between climate and tree death.

Recent research targeting gaps in this mechanistic understanding has provided insight into the role of drought in tree mortality. Two nonexclusive mechanisms—carbon starvation and

hydraulic failure—have been proposed to explain drought-induced tree mortality, based on differing tree strategies [McDowell *et al.*, 2008]. Carbon starvation occurs when isohydric species, which strongly regulate transpiration through stomatal closure to avoid excessive water loss when water-stressed, forgo access to the atmospheric carbon dioxide (CO₂) necessary for photosynthesis. Isohydric plants must then outlast the drought while relying primarily on stored carbon for the respiratory demands of tissue maintenance. If this respiratory consumption exceeds stored resources, death results from carbon starvation. In contrast, anisohydric species only weakly regulate transpiration to continue photosynthesizing, yet this strategy risks mortality via hydraulic failure if sufficient xylem cavitation occurs, rupturing water transport structures under tension and preventing needed water flow.

Warmer temperatures during drought can exacerbate hydraulic failure via higher evaporative demand or especially carbon starvation via elevated respiration. A recent experimental assessment of drought-induced mortality in piñon pine, an isohydric species, found that elevated temperatures increased respiratory load and reduced survival time during drought by 28%, consistent with carbon starvation [Adams *et al.* [2009a, 2009b, 2009c]; but see Leuzinger *et al.* [2009] and Sala [2009]]. However, mortality also could be caused by a lack of access to stored carbon resources within the plant [Sala *et al.*, 2010]. Thus research into metabolic and carbon transport limitations is needed to determine if starvation occurs from reduced photosynthesis or a water-stress-induced inability to use stored carbon. Increased temperatures also can enhance the success of tree pests (e.g., bark beetles or fungi) directly, by encouraging pest reproduction, growth, survival, and dispersal, and indirectly, by reducing tree defensive capabilities during drought [Raffa *et al.*, 2008].

Effects on Earth System Processes

The observations and experimental results summarized above highlight the vulnerability of global forests to widespread mortality, which in turn could affect carbon, energy, and water cycles (Figure 1). Forests are important sinks for anthropogenic CO₂ emissions and exert disproportionately strong controls on Earth system processes relative to their geographic extent [Bonan, 2008]. Forests contain close to 55% of the carbon in terrestrial ecosystems and contribute substantially to the terrestrial sink, absorbing 33% of anthropogenic carbon emissions during the 1990s [Bonan, 2008].

Determining the future of this sink is vital to projecting future climate change, as accelerating climate-induced tree mortality and subsequent decomposition could switch forests from carbon sinks to sources for several decades following extensive tree mortality. This has occurred in British

Columbia, where mortality associated with recent beetle outbreaks reduced carbon sinks by 270 megatons over 20 years. This event reversed the carbon sequestration gains of the previous 20 years across millions of hectares of forest [Kurz *et al.*, 2008a] and influenced Canadian climate change policy [Kurz *et al.*, 2008b]. Further, CO₂ released following tree mortality could easily exceed carbon sequestration enhancements from elevated CO₂ promoting forest growth [Chapin *et al.*, 2008].

Tree mortality also is expected to have strong feedbacks on local and regional climate by altering surface albedo and energy exchange between the land surface and atmosphere. Albedo increases, which help mitigate warming, occur when tree loss exposes a lighter land surface, an effect that may be particularly important for boreal and semiarid ecosystems. In boreal forests, large increases in albedo due to tree loss and exposure of snow-covered ground could partially offset climate forcing due to carbon releases [Bonan, 2008; Chapin *et al.*, 2008]. In coniferous semiarid forests, even small increases in albedo due to tree loss could also result in significant negative feedbacks to global warming because the total incoming energy available in these systems is so high [Roitenberg and Yakir, 2010]. Changes in hydrology also are expected, as a loss of tree cover can decrease transpiration while increasing surface evaporation through near-ground inputs of energy and water [Chapin *et al.*, 2008].

Future Research, Assessment, and Modeling Needs

The links between global carbon, energy, and water cycles and forest dynamics reveal the critical need for forecasting the extent and patterns of changing forest properties as affected by tree mortality, disturbances, and regeneration under climate change (Figure 1). An improved network of observations, both ground-based and remotely sensed, is needed to document tree mortality annually [Allen *et al.*, 2010]. Improved experiments assessing mechanisms of tree mortality in relation to climate drivers are needed for more biomes. Both observations and experiments must be linked to modeling efforts to improve forecasts. Future needs also include assessment of management actions, such as forest thinning, that might increase the resistance of forested ecosystems to climatic changes.

Last, extensive observations of the effect of increasing tree mortality on fluxes of carbon, energy, and water are needed. Such observations need to quantify not only the magnitude and direction of these response but also the effects of subsequent forest regeneration and recovery, which ultimately will influence the persistence of impacts. Addressing these information gaps will improve our understanding of climate-induced tree mortality and associated

shifts in Earth system feedbacks, helping researchers to project global changes and anticipate their effects on society.

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APPENDIX B: ECOHYDROLOGICAL CONSEQUENCES OF DROUGHT- AND
INFESTATION-TRIGGERED TREE DIE-OFF: INSIGHTS AND HYPOTHESES

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Ecohydrological consequences of drought- and infestation-triggered tree die-off: insights and hypotheses

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ABSTRACT

Widespread, rapid, drought-, and infestation-triggered tree mortality is emerging as a phenomenon affecting forests globally and may be linked to increasing temperatures and drought frequency and severity. The ecohydrological consequences of forest die-off have been little studied and remain highly uncertain. To explore this knowledge gap, we apply the extensive literature on the ecohydrological effects of tree harvest in combination with the limited existing die-off ecohydrology research to develop new, relevant hypotheses. Tree mortality results in loss of canopy cover, which directly alters evaporation, transpiration, and canopy interception and indirectly alters other watershed hydrologic processes, including infiltration, runoff, groundwater recharge, and streamflow. Both die-off and harvest research suggest that for most forests, water yield can be expected to increase following substantial loss of tree cover by die-off. We hypothesize that where annual precipitation exceeds ~500 mm or water yield is dominated by snowmelt, watersheds will experience significantly decreased evapotranspiration and increased flows if absolute canopy cover loss from die-off exceeds 20%. However, recent observations suggest that water yield following die-off can potentially decrease rather than increase in drier forests. To reliably predict die-off responses, more research is needed to test these hypotheses, including observations of multiple water budget components and the persistence of ecohydrological effects with the post-die-off successional dynamics of tree recruitment, understorey growth, and interactions with additional disturbances. With die-off, mitigation and restoration options are limited and costly, necessitating societal adaptation; therefore, die-off ecohydrology should be a high priority for future research. Published in 2011. This article is a US Government work and is in the public domain in the USA.

KEY WORDS ecohydrology; die-off; tree mortality; forest mortality; evapotranspiration; recharge; water yield; bark beetle; tree pests

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INTRODUCTION

Global climate change is projected to directly alter the hydrological cycle through changes in precipitation and temperature (IPCC, 2007). In addition, if such climate changes are sufficient to trigger changes in vegetation, as expected, additional potentially important hydrological changes to water budgets will occur (Troch *et al.*, 2009). Ecohydrology research assessing such feedbacks between hydrological and ecological aspects is a current pressing challenge, particularly if rapid and large-scale changes in vegetation are triggered (Jackson *et al.*, 2009; Wilcox 2010). An emerging picture of global change ecology suggests that the nature and pace of climate-driven vegetation adjustments can be rapid and abrupt (Allen and Breshears, 1998; Overpeck and Cole, 2006; Backlund *et al.*, 2008).

Of particular concern is the potential for changes in climate to trigger rapid and widespread vegetation die-off through tree mortality due to a combination of drought, warmer temperatures, and/or infestation by pathogen and/or pests (Allen *et al.*, 2010). A recent global assessment of tree die-off over the past 30 years on all six forested continents suggests that climate-driven forest die-off may be emerging as a global phenomenon (Allen *et al.*, 2010). Several recently documented die-offs were extensive in scale, for example, affecting 130 000 km² of pine (*Pinus* spp.) forests in western Canada by the end of 2006, 55 000 km² of Australian eucalypts (*Eucalyptus* spp.) by the late 1990s, and over 600 000 km² in total of non-contiguous coniferous forests in western North America in the last decade (Fensham and Holman, 1999; Kurz *et al.*, 2008; Bentz *et al.*, 2009; Fensham *et al.*, 2009; Allen *et al.*, 2010).

Patterns of mortality are often linked to the interaction between climate conditions and pest dynamics. Precipitation variability can have both direct effects on vegetation

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growth and survival as well as predisposing forests to pest outbreaks (McDowell *et al.*, 2008; Raffa *et al.*, 2008; Allen *et al.*, 2010). Projected trends in precipitation portend substantial poleward shifts in storm tracks that will decrease mean precipitation at mid-latitudes as well as increased temporal precipitation variability (Easterling *et al.*, 2000; IPCC, 2007; Milly *et al.*, 2008). Significant increases in drought severity and frequency have been observed in association with even minor trends in mean precipitation (Luce and Holden, 2009), and these can have important consequences for die-off vulnerability. Warmer temperatures amplify drought effects, weaken trees, and reduce their defenses, while simultaneously facilitating increased populations of tree pests (Breshears *et al.*, 2005; Stahl *et al.*, 2006; McDowell *et al.*, 2008; Raffa *et al.*, 2008; Worrall *et al.*, 2008; Allen *et al.*, 2010), and likely increase mortality even without accounting for associated changes in precipitation variability (Adams *et al.*, 2009). Tree mortality can occur along a progression of severity, from subtle increases in background mortality (e.g., van Mantgem *et al.*, 2009), to affecting only a single species in a diverse forest (e.g., through a host-specific tree pest; MacDonald and Hoff, 2001; Ellison *et al.*, 2005; Ford and Vose, 2007), and, of particular concern ecohydrologically, die-off of a high percentage of individuals in forests dominated by a few tree species (e.g., Fensham *et al.*, 2003; Breshears *et al.*, 2005; Kurz *et al.*, 2008).

The ecohydrological consequences of forest die-off have not been widely studied and remain highly uncertain. Despite recent projections of the importance of die-off (Allen *et al.*, 2010) and its potential to impact ecohydrological processes, assessments of ecohydrological consequences of drought- and infestation-triggered tree die-off and associated research priorities are lacking. However, a few studies do exist, and relevant insights can be obtained from studies of ecohydrological responses to other types of forest disturbance. To explore this knowledge gap and develop relevant hypotheses about the potential ecohydrological consequences of die-off, we consider the extensive literature on the ecohydrological effects of disturbances that reduced tree cover in combination with the limited existing die-off ecohydrology research. More specifically, we (1) review the limited literature on tree die-off hydrological effects and link it to the much larger body of work on responses to harvest

(after determining that responses following fire are much less relevant); (2) consider the direct and indirect ecohydrological effects of tree die-off; (3) compare short- and long-term potential ecohydrological responses to die-off; (4) examine applications and implications for forest management; and (5) identify related research hypotheses and priorities. Regarding our overall approach, an important aspect of ecohydrology is the partitioning of the water budget among relevant components (Newman *et al.*, 2006), which are often lumped together, particularly with respect to components of evapotranspiration (Savenije, 2004). To be explicit about the components—soil evaporation (E), plant transpiration (T), and evaporation of water intercepted by the plant canopy (I)—we refer them either individually, collectively as ETI, or as ET when we are referring to the total of only E and T without accounting for I.

COMPARING CHARACTERISTICS OF TREE MORTALITY BY DIE-OFF TO HARVEST AND FIRE

We first begin by comparing the general characteristics of changes from die-off to the changes associated with tree removal by harvest and fire. Here, we refer to harvest as externally applied tree removal that includes multiple felling methods, cabling, scraping, and/or herbicide application; and fire as burning substantial enough to remove overstorey canopy cover. The primary effect of die-off is a loss of tree canopy cover that reduces T and I by the overstorey. Tree canopy cover loss is also a dominant effect of forest harvest and fire, so hydrological responses to these forest manipulations could provide useful insights into the hydrological consequences of die-off. Moreover, extensive literature exists on the ecohydrological consequences of tree harvest and fire (e.g. Stednick, 1996; Andréassian, 2004; Brown *et al.*, 2005; Shakesby and Doerr, 2006; Montes-Helu *et al.*, 2009).

Although die-off, harvest, and fire all result in overstorey canopy cover reduction, important distinctions exist among their effects on other ecohydrological characteristics (Table I). For example, in contrast to some types of forest harvesting practices (e.g., complete clear cuts with no residual seed trees) and to much high-severity, stand-replacing wildfire, in most documented tree die-off events the mortality is not continuous across the landscape and rarely results in complete conversion to

Table I. A comparison of effects relevant to hydrological processes for three disturbance types: die-off, harvest, and fire.

Associated changes	Presence of change with disturbance type		
	Die-off	Harvest	Fire
Canopy cover loss	X	X	X
Soil compaction (from roads and skid trails)		X	
Standing snags remain	X		X
Heated-soil water repellency			X
Litter layer/understorey burning			X

Canopy cover loss is common to all three, but fire causes more changes relevant to hydrological processes, suggesting that the potential hydrological consequences of die-off have more in common with those of harvest than for fire.

non-forest (Allen *et al.*, 2010). An important distinction between harvest and die-off is that harvesting has effects associated with the building and use of roads and skid trails required to remove timber. Decreased infiltration on compacted surfaces can increase overland flow affecting both water yield and peak flows at the watershed level (Jones and Grant, 1996; Luce, 2002; Wemple and Jones, 2003; Hubbart *et al.*, 2007).

Although fire can certainly reduce overstorey tree canopy cover, its reported ecohydrological effects make fire less similar to die-off than harvest. Unlike harvest and die-off, fire abruptly alters ground surface cover, by consuming the litter layer and understorey vegetation. Fire can also create soil water repellency when soil heating vaporizes organic material, which condenses and bonds with cooler, underlying mineral soils (Table I, DeBano, 2000; Lewis *et al.*, 2006; Shakesby and Doerr, 2006; Lane *et al.*, 2010). Immediately after fires, T and I diminish due to the lack of overstorey and understorey canopy, but increased exposure of bare soil and dark charred surfaces likely results in increased E. Increases in overland flow due to combinations of water repellency and loss of soil organic horizons can drive substantial rill formation and erosion, including debris flows that transport sediment and alter stream channel morphology while reducing soil infiltration (Istanbulluoglu *et al.*, 2002, 2003; Cannon *et al.*, 2009; Moody and Martin, 2009). Low severity fires rarely yield such dramatic outcomes, but also commonly result only in understorey biomass consumption and some mortality of smaller trees, differing from die-off for which mostly larger canopy elements are killed and understorey plants are potentially released for increased growth (Klenner and Arsenault, 2009).

Although both die-off and fire leave dead standing snags while harvest does not, harvest has more effects in common with die-off relevant to hydrological process than does fire (Table I). Die-off effects on forest structure often resemble a 'thinning-from-above' harvesting treatment, where larger trees are selectively removed from the stand, since larger trees are often more likely to be killed during die-off (Mueller *et al.*, 2005; Floyd *et al.*, 2009; Klenner and Arsenault, 2009) if the drought effects are not completely overwhelming with respect to tree size/age. Ideally, a consideration of the ecohydrological consequences of tree die-off should be entirely based on die-off results. However, the limited literature on die-off ecohydrology and the very extensive literature on forest harvesting hydrology together necessitate considering both to hypothesize consequences of tree die-off broadly across different forest types. Therefore, we will draw on both harvest and die-off literature to explore the ecohydrological consequences of die-off in the following sections, while keeping in mind that die-off is distinct from harvest in some effects (we will not consider fire effects further). Key lessons from studies of the ecohydrological effects of harvest are that both the intensity of the initial disturbance and the successional pathways

after disturbance affect the magnitude of changes, and their persistence (Stednick, 1996; Brown *et al.*, 2005).

THE EFFECTS OF TREE MORTALITY

We next review the limited available literature on ecohydrological responses to drought- and infestation-induced die-off and evaluate it in the context of direct effects, indirect effects, and persistence of effects. We relate each section to relevant literature on harvest (but not fire, as addressed in the previous section), as well as to more general water budget research. The published research on ecohydrological responses to drought- and infestation-triggered tree die-off is relatively limited and is currently restricted to mortality of conifer species, mostly in western North American watersheds (Table II). Much of the recent research has been conducted in response to two regionally extensive forest mortality events: piñon pine (*Pinus edulis*) die-off in the southwestern US (Breshears *et al.*, 2005) and lodgepole pine (*Pinus contorta*) die-off in western Canada (e.g. Kurz *et al.*, 2008). Studies include empirical and modelling assessment of the effects of tree mortality on ETI, snow ablation and accumulation, peak flow, and water yield (Table II). Among this small group of studies, mostly with similar forest cover types, responses vary considerably, preventing the crafting of well-supported, broadly applicable conclusions on the hydrological effects of die-off. Nonetheless, the studies do provide important insights that help frame relevant hypotheses and future research priorities. To consider the ecohydrological effects of tree mortality, we begin with direct effects, specifically the ETI consequences of tree cover loss and then consider indirect effects associated with the results of ETI change on other water balance components.

Direct effects

Die-off direct effects. The direct ecohydrological effects of tree die-off relate to changes in canopy and ground cover, which exert important influences on many ecosystem processes (Breshears, 2006). Tree canopy cover plays an important role in regulating ETI through opposing influences from its E, T, and I components. Decreased canopy cover reduces overstorey T and I, a negative feedback on overall ETI (Figure 1). Yet at the same time, canopy cover loss increases the wind and solar energy reaching the land surface, which drives bare soil E and understorey T, a positive feedback on ETI. The balance of these competing E, T, and I responses to canopy cover reduction ultimately determines the extent of direct tree die-off effects on overall ETI. Studies that partition ETI into these components, at both canopy and near-surface levels, are needed to better understand these opposing effects on die-off hydrology (Savenije *et al.*, 2004; Newman *et al.*, 2006; Spittlehouse 2007; Wang *et al.*, 2010).

Table II. A summary of research on the hydrological effects of tree die-off.

Location	Ecosystem type	Species affected	Component studied	Effect of mortality	References
Arizona, USA	Conifer forest and woodland	<i>Pinus edulis</i> , <i>Pinus ponderosa</i>	Near-ground energy	Increased near-ground energy and potential surface ET	Royer <i>et al.</i> (2010, 2011)
British Columbia, Canada	Conifer forest	<i>Pinus contorta</i>	ETI, drainage	Decreased ETI, increased drainage	Spittlehouse (2007), Redding <i>et al.</i> (2008)
British Columbia, Canada	Conifer forest	<i>P. contorta</i>	Snow accumulation and ablation	Increased snow accumulation and ablation	Boon (2007, 2009)
British Columbia, Canada	Conifer forest	<i>P. contorta</i>	Snow surface albedo	Decreased albedo, increased snow loss	Winkler <i>et al.</i> (2010)
British Columbia, Canada	Conifer forest	<i>P. contorta</i>	ETI, water yield, snowmelt peak flow	Increased streamflow	Carver <i>et al.</i> (2009a, 2009b), Weiler <i>et al.</i> (2009)
Colorado, USA	Conifer forest	<i>Picea engelmannii</i>	Water yield	Increased streamflow	Bethlahmy (1974, 1975)
Colorado, USA	Conifer forest	<i>P. contorta</i> , <i>Abies lasiocarpa</i>	Water yield	Unchanged and decreased streamflow	Somor (2010)
Colorado, USA	Conifer forest	<i>P. contorta</i>	Soil and stream chemistry	Increased soil N, NO ₃ , NH ₄ , unchanged stream NO ₃	Clow <i>et al.</i> , (2011)
Germany	Mixed hardwood/conifer forest	<i>Picea abies</i>	Water yield, groundwater, runoff, NO ₃ concentration	Increased streamflow, groundwater recharge, runoff, and NO ₃ concentration	Beudert <i>et al.</i> (2007)
Montana, USA	Conifer forest	<i>P. contorta</i>	Water yield	Increased streamflow	Potts (1984)
North Carolina, USA	Mixed hardwood/conifer forest	<i>Tsuga canadensis</i>	Transpiration	Reduced spring and winter stand transpiration	Ford and Vose (2007)
Southwest (AZ, CO, NM, UT) USA	Conifer woodland/savanna	<i>P. edulis</i>	Water yield	Decreased streamflow	Guardiola-Claramonte (2009), Guardiola-Claramonte <i>et al.</i> (In press)

Studies include modelling and empirical assessments of energy budgets, overstorey transpiration and interception, snow dynamics, water yield, groundwater recharge, runoff and NO₃ concentration responses. Nearly all studies are for North American ecosystems and watersheds (mostly western North America) where evergreen conifer species experienced substantial climate-related mortality, often associated with insect pests.

Few published studies have directly considered ETI changes following tree mortality (Table II). In the southern Appalachian Mountains of the United States, recent eastern hemlock (*Tsuga canadensis*) mortality was caused by an infestation of the host-specific hemlock woody adelgid (*Adelges tsugae*), a phloem-consuming insect (Ford and Vose, 2007). This affected hemlock species, an evergreen conifer, transpires year round, even while

the dominant hardwood species are inactive in the winter, giving it a unique role in that forest ecosystem. Ford and Vose (2007) estimated that the loss of this species from the forest would initially reduce annual overstorey T by 10% and spring T by 30%. In British Columbia, an assessment of water balance for a forested lodgepole pine watershed suggested that infestation-induced tree mortality reduced overall ETI, by

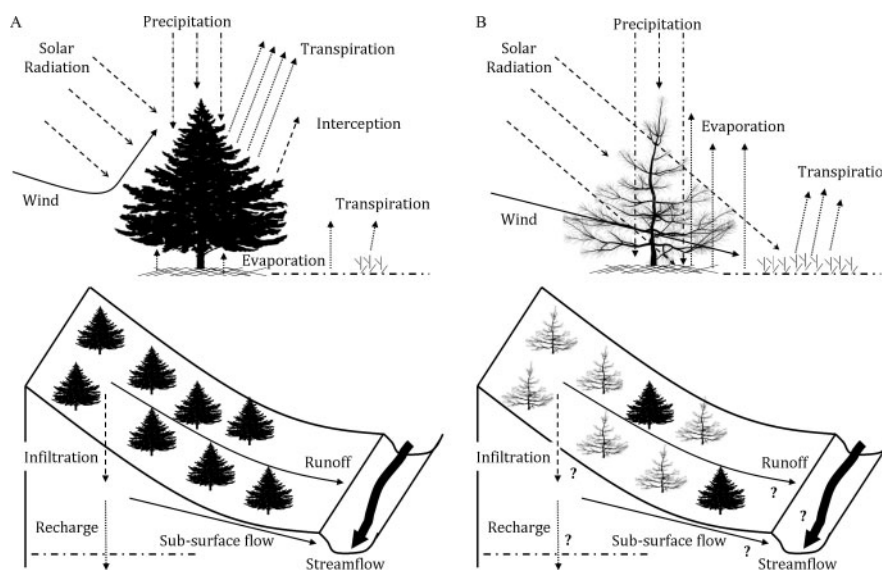


Figure 1. The relative effects of canopy cover on water budget components at the patch and hillslope scale under normal conditions (A) and after die-off (B). Live trees transpire soil water and block solar radiation, wind, and precipitation from reaching the soil surface. After tree mortality, more solar radiation, wind, and precipitation reaches the surface as interception is greatly reduced; overstorey transpiration decreases greatly; understorey transpiration increases; and evaporation at the surface is increased. The interactions among all of these tree-cover-related evapotranspiration factors determine the net direct effects of canopy cover reductions on infiltration, runoff, groundwater recharge, subsurface flows, and streamflow.

approximately 50% (Spittlehouse, 2007; Redding *et al.*, 2008). In two northern Arizona ecosystems, tree die-off increased near-ground energy inputs that drive near-ground surface ET (understorey T and bare soil E only, not overstorey T or I) by 12% for piñon pine and 19% for ponderosa pine (Royer *et al.*, 2010, 2011). These increases in near-ground energy depended on pre-mortality canopy cover and were greatest at intermediate canopy cover.

Snow I and ground snowpack accumulation are highly dependent on canopy cover, so tree mortality can exert a strong influence on the hydrology of snowmelt-dominated watersheds (Boon, 2007, 2009, 2011; Redding *et al.*, 2008). Storage of intercepted snowfall by tree canopies varies with branch and snow characteristics, but can be an order of magnitude greater (in water equivalent) than canopy storage of rainfall (for non-die-off effects, see Lundberg and Halldin, 2001). Because sublimation of intercepted snowfall is also greater than that of snowpack on the ground, tree canopies can block a significant portion of precipitation from reaching the land surface when it falls as snow (Boon, 2007, 2009, 2011; Redding *et al.*, 2008). Canopy cover declines associated with tree mortality result in greater snow throughfall to the ground, while also allowing increased solar energy inputs to melt snow compared to areas with undiminished canopy cover (Boon, 2007, 2009, 2011).

The broad regional extent of the ongoing conifer die-off associated with a massive mountain pine beetle (*Dendroctonus ponderosae*) outbreak in British Columbia, a region with snowmelt-dominated hydrology, has spurred

a number of studies into the effects of die-off on snow processes and hydrology, many of which are still ongoing (Winkler and Boon, 2010). Results from recent studies showed that ablation rate (the sum of E and sublimation) of ground snowpack was only slightly elevated for beetle-killed stands relative to live stands, and the snow accumulation in beetle-killed stands resembled that in clear-cut and treeless areas, except in years when high snowfall overwhelmed the canopy interception of live stands (Boon, 2007, 2009, 2011). Snowpack persisted longest in living stands, indicating that tree mortality can trigger earlier, intensified snow losses as continued needle loss causes dead stands to resemble cleared areas with time. At several sites, litterfall from dead conifers landing directly onto snowpack reduced surface albedo and increased ablation and melt (Winkler *et al.*, 2010). A small amount of litter on the snow surface had a large, nonlinear effect, causing snowpack loss that was similar to that for an open area. Results of research on the response of snow dynamics to pine beetle mortality in Colorado, USA, are consistent with work in British Columbia, showing increased accumulation and faster snowpack loss in affected stands (Pugh and Small 2011).

Other relevant research on direct effects. Regarding other relevant research on snow, the reported changes in snow dynamics following die-off are consistent with snow interception and snowpack responses to harvest, including snowmelt timing (Wilm, 1944; Troendle and King, 1985; Berris and Harr, 1987; Jost *et al.*, 2007), and their associated responses to amount of canopy cover (Molotch

et al., 2009; Veatch *et al.*, 2009). Canopy cover reduction by harvest can lead to earlier, faster, and more synchronous snowmelt (Jones, 2000; Tonina *et al.*, 2008). Lacking from die-off studies, however, is research on the partitioning of ETI, which has been studied in the context of other changes or variation in canopy cover. Studies that have addressed canopy cover reductions due to factors other than die-off on ET partitioning and overall ETI demonstrate the potentially opposing influences of E, T, and I components. In thinned and unthinned semi-arid ponderosa pine (*Pinus ponderosa*) stands in northern Arizona, assessment of ET partitioning indicated that an 82% reduction in basal area (45% reduction in leaf area index) increased E and ET overall during a dry summer, when the T component was small due to low tree stomatal conductance (Simonin *et al.*, 2007). However, during the following wet spring when tree stomatal conductance was high, overall ET was higher for the unthinned treatment when the T component dominated ET. A study of semi-arid mesquite (*Prosopis chilensis*) trees, in an experimental manipulation that varied canopy cover to multiple levels in a large controlled-environment glasshouse, showed that overall ET rose with increased canopy cover, as a result of increasing T exceeding the corresponding reduction in E (Wang *et al.*, 2010). Although I is highly dependent on rainfall event size, canopy storage capacity is thought to increase linearly with canopy cover (Gash 1979; Gash *et al.*, 1995; Valente *et al.*, 1997). Thinning of a Mediterranean oak (*Quercus ilex*) stand with a reduction of tree basal area by 33% caused a decrease of 34% in I at an experimental forest in southern France (Limousin *et al.*, 2008). However, increased I does not always equate with increased moisture loss to the atmosphere. Particularly for forests where cloud and fog interception are important moisture inputs, throughfall can exceed precipitation, and canopy loss could decrease moisture reaching the ground (Harr, 1982; Brauman *et al.*, 2010).

Indirect effects

Die-off indirect effects. Direct hydrological consequences of tree mortality include changes in E, T, I, and snow accumulation and melt dynamics. Considering these changes to the water balance, tree mortality may indirectly affect other aspects of hydrological functioning, such as infiltration and flow path partitioning, which would subsequently result in changes in soil moisture status, groundwater recharge dynamics, and streamflow volume and timing. For example, if tree mortality decreases overall ETI, as discussed above, and precipitation remains the same, then more water is available for these other components as an indirect effect of tree die-off. Whether this water enters the soil or becomes overland flow will depend on the infiltration capacity of the soil, which could be increased through inputs of dead material like needle litter from dying trees and creation of macropores when dead trees fall, or decreased if soil organic material is washed away following tree mortality.

Subsurface connections such as fractured bedrock that create flow paths to groundwater are not directly affected by tree mortality (although there could be potential effects from roots in rock; Schwinning, 2010), yet affect whether water that enters the soil following tree mortality will contribute to groundwater recharge (for non-die-off effects, see Wilcox, 2002; Seyfried and Wilcox, 2006; Wilcox *et al.*, 2006; Wilcox and Huang, 2010). Published studies on the indirect effects of tree mortality on flow path partitioning are rare, and assessments of groundwater recharge from die-off are almost non-existent. At one intensively studied experimental watershed in southern Germany, Grosse Ohe, an isotopic tracer model was used to partition discharge response for a catchment that underwent a ~53% forest cover loss through Norway spruce (*Picea abies*) mortality (Beudert *et al.*, 2007). Tree mortality caused a 39% decrease in ET associated with a 135% increase in runoff and a 125% increase in groundwater flow. Additionally, NO₃ concentrations of soil water increased from 10 to 200 mg/l at 40 cm in depth and to 130 mg/l at 100 cm, demonstrating that tree die-off can affect water quality. In a watershed in Colorado experiencing extensive mortality of lodgepole pine NO₃, NH₄, and total N increased in soils under stands of dead trees, but this did not translate to elevated NO₃ in stream water in the near-term following die-off (Clow *et al.*, 2011).

Increased groundwater recharge can translate to increased streamflow volumes and higher flows during low flow periods, and in areas where the water table is already close to the ground surface, it may increase the potential for saturation excess overland flow or unfavourable conditions for seedling establishment and tree growth. This combination of higher antecedent wetness conditions, elevated groundwater levels, and more area available for overland flow generation could lead to changes in the timing and magnitude of responses to rainfall and snowmelt. Streamflow, which integrates overland, subsurface, and groundwater flows differently depending on watershed properties, may ultimately be indirectly affected by tree mortality. Quantified as water yield, streamflow will increase if tree mortality decreases watershed ETI, and conversely decrease if ETI is increased.

The variation in water yield responses reported for die-off ecohydrology studies, all in watersheds with conifer mortality involving bark beetle outbreaks, demonstrates a wide range of possible hydrological responses to tree die-off (Table II). In two northern Colorado river drainages in the United States annual water yield increased by 10% following a bark beetle outbreak that killed up to 80% of trees in the late 1930s and early 1940s, mostly from nonproportional streamflow increases during wet years (Bethlahmy, 1974, 1975). At Jack Creek in southern Montana, United States, a mountain pine beetle outbreak killed 35% of trees across the watershed from 1975 to 1977 (Potts, 1984). This event caused a 15% increase in annual water yield, a 2–3 week advance in the onset of

snowmelt-driven flows, and a 10% increase in low flows over a period of 5 years post-mortality. The research in southern Germany at Gross Ohe showed that peak flows in a catchment with ~53% tree mortality increased by a factor of 2.2 relative to an adjacent catchment mostly unaffected by die-off (Beudert *et al.*, 2007).

In contrast, research examining the streamflow response to mortality of lodgepole pine and subalpine fir (*Abies lasiocarpa*) in eight Colorado, US catchments found that streamflow relative to precipitation was unchanged in seven catchments where canopy cover loss averaged 43%, and even decreased by 31% in one catchment that had 50% tree mortality (Somor, 2010; unpublished manuscript). In addition, an assessment of water yield changes in the southwestern United States after extensive piñon pine die-off found that five semi-arid basins, ranging in size from ~1000 to ~5000 km² and which lost 11–21% of tree cover, had on average ~50% less water yield post-mortality after correcting for precipitation changes (Guardiola-Claramonte, 2009; Guardiola-Claramonte *et al.*, in press). In addition, these basins had significantly delayed streamflow generation compared to similar unaffected basins. The responses of these watersheds were attributed to rapid post-die-off understorey growth detected in a remote sensing analysis for the same basins (Rich *et al.*, 2008). This increase in understorey cover was speculated to have reduced overland flow (consistent with Zou *et al.*, 2010) and increased infiltration, T, and I, increasing overall ETI in these dry, low-elevation watersheds (Guardiola-Claramonte, 2009; Guardiola-Claramonte *et al.*, in press).

The extensive mountain pine beetle outbreak across British Columbia, Canada in the last decade has driven development of a process-based hydrological model to estimate peak- and low-flow responses across the mostly ungauged Fraser River Basin (Carver *et al.*, 2009a,b; Weiler *et al.*, 2009). Incorporating changes in ETI and snow dynamics with flow components and discharge responses, the model suggests that with complete mortality of all trees in the watershed, snowmelt-induced peak flows increase with area of forest affected, up to a maximum of 140%, with a 26% increase predicted at the Fraser River outlet. High variability in these projections across the basin revealed potential nonlinear thresholds in the hydrological response and specific effects of differences in runoff generation processes (i.e., Hortonian overland flow, saturation excess overland flow, subsurface flow) among the watersheds with increasing mortality.

Other relevant research on indirect effects. As noted previously, the wide range of responses documented in the small set of die-off hydrology literature calls for drawing on the forest harvest literature to further consider likely indirect effects of forest die-off. Early reviews of paired catchment studies in US watersheds found harvest responses to be highly variable (Bosch and Hewlett, 1982). However, more recent reviews have

focused on organizing responses by climate and treatment characteristics (e.g. Brown *et al.*, 2005). Harvesting intensity is a primary determinant of water yield response, with canopy cover removal thresholds of 20–25% suggested to enable statistical detection of a response across several environments (Bosch and Hewlett, 1982; Stednick, 1996; Brown *et al.*, 2005; Troendle *et al.*, 2010). However, for cases with a reduction of less than 20% forest cover, lack of significant hydrological responses could be due to low statistical power from short post-treatment records or the effects of different harvesting techniques (McMinn and Hewlett, 1975).

Locations with greater annual precipitation tend to show greater hydrological sensitivity to forest treatments compared to drier locales (Bosch and Hewlett, 1982; Stednick, 1996; Troendle *et al.*, 2010). Similarly, results from high elevation lodgepole pine forest also show that at a given site, water yield in wetter years is more sensitive to harvest (Troendle and King, 1987). A global analysis of catchment studies comparing the ET of forested versus non-forested watersheds with similar climates demonstrated that potential water yield changes could be predicted from annual precipitation (Zhang *et al.*, 2001). This assessment suggested that little change should be expected with a shift from forest to grassland for sites below 500 mm of annual rainfall because potential ET at these sites is a large proportion of precipitation. The largest increases in water yield

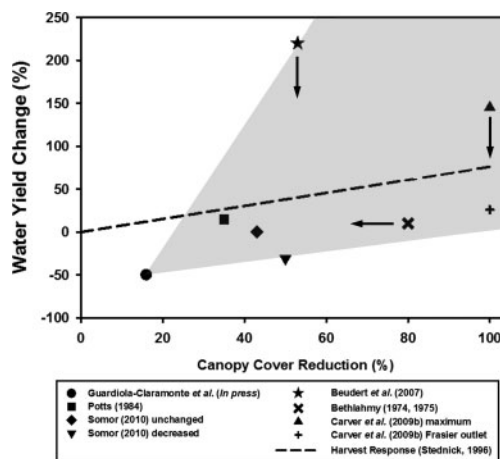


Figure 2. The relationship between canopy cover reduction and annual water yield change for die-off hydrology studies that measured or estimated water yield. Points represent individual studies from Table II. For Somor (2010) the response of seven catchments where water yield was unchanged (diamond) and the single catchment with decreased flows (triangle pointing down) are shown separately. Values shown for Carver *et al.* (2009b) are modelled peak flow maximum (triangle) and Fraser River outlet peak flow changes (plus sign). The grey area represents the range of hypothesized water yield responses to die-off. Arrows indicate that for Beudert *et al.* (2007) values reported are for peak flows and annual water yield change is likely lower, and that for Bethlahmy (1974, 1975) canopy cover reduction was up to 80% and average canopy cover reduction is likely much lower. Also shown is the relationship between canopy cover reduction by harvest and water yield increase (dashed line), calculated from the dataset of Stednick (1996).

have occurred when coniferous forest cover was removed in mesic environments, while removal of 'scrub' cover elicited the smallest response across a variety of climates (Brown *et al.*, 2005; see also hypotheses in Huxman *et al.*, 2005).

The seasonality of precipitation and streamflow in a watershed determined the season that experienced the greatest shifts in water yield after treatment (Brown *et al.*, 2005). For example, in watersheds where most precipitation occurred in summer, forest removal increases in annual water yield were driven by proportionally higher increases in summer water yield. Likewise, for watersheds dominated by snowmelt peak flows, tree harvest increased annual water yield through higher and earlier snowmelt peak flow (Brown *et al.*, 2005; Zou *et al.*, 2010).

Ecohydrological responses to die-off vary substantially and are much more inconsistent than well-reviewed responses to forest harvest (Figure 2). In particular, initial responses of decreased streamflow following canopy loss from die-off in drier forests are not seen in response to forest harvest, suggesting caution should be used in overgeneralizing from these results. Specifically, the finding of decreased streamflow following mortality in the piñon-juniper ecosystem (Guardiola-Claramonte, 2009; Guardiola-Claramonte *et al.*, in press) contrasts with previous tree removal research in watersheds of the same vegetation type. At the Beaver Creek experimental watershed in northern Arizona, two watershed treatments where 100% of tree cover was removed by harvest resulted in unchanged flows (Clary *et al.*, 1974; Baker, 1984). A third watershed was treated with an herbicide targeted at junipers that removed 83% of tree cover. This increased annual streamflow initially by 65% in 4 years post-treatment (Clary *et al.*, 1974) and by 157% over 8 years post-treatment (Baker, 1984). The larger watersheds considered in the recent die-off study also include some higher-elevation, mesic forests (Guardiola-Claramonte, 2009; Guardiola-Claramonte *et al.*, in press). Changes in precipitation dynamics at high elevations could have exerted a disproportionate influence on whole basin

water yield numbers following piñon die-off (Guardiola-Claramonte, 2009; Guardiola-Claramonte *et al.*, in press). On the other hand, at Beaver Creek, in addition to killing junipers, the herbicide treatment also initially damaged piñon pines and led to a shift in the understory from perennial to annual grasses (possibly further depressing T); harvested watersheds also were subjected to some burning of slash (Clary *et al.*, 1974; Baker, 1984). Other assessments of water yield response to tree removal in piñon-juniper watersheds found that ETI still accounted for almost all precipitation following treatment, and that flows did not increase unless slash was burned (Gifford, 1975; Wright *et al.*, 1976). Assessment of other shrub-dominated watersheds suggests that subsurface characteristics which permit deep drainage of soil water are key to determining if shrub removal leads to increased streamflow (Wilcox, 2002; Seyfried and Wilcox, 2006; Wilcox *et al.*, 2006).

In summary, studies of die-off effects on indirectly affected hydrological processes are limited in scope and not always consistent with relevant harvest study results, perhaps due to a variety of factors. Inconsistencies in measuring and reporting canopy cover loss among die-off studies may also be contributing to the variability in responses: it is easier to measure the impact of an externally applied forest harvest than to quantify a tree mortality event that varies greatly with space and time. We hypothesize that the interaction of three influences—annual precipitation, level of canopy loss, and belowground characteristics—determines many of the ecohydrological differences among studies of responses to tree cover loss both within and between die-off and harvest responses, all of which will vary with successional dynamics.

THE PERSISTENCE OF ECOHYDROLOGICAL EFFECTS FROM DIE-OFF

Persistence of die-off effects

The persistence of hydrological responses to tree mortality through time will depend on the trajectory of each

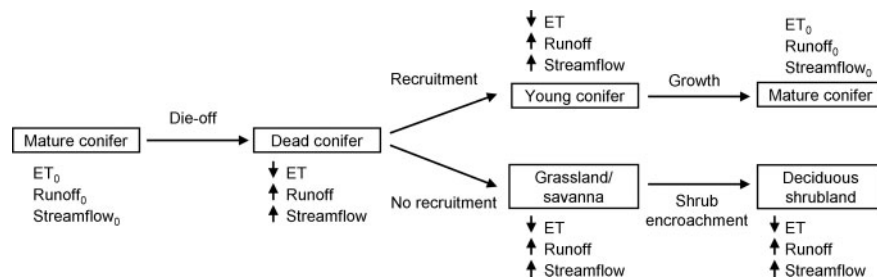


Figure 3. An example scenario of how ecosystem trajectories will ultimately influence post-mortality hydrological effects over time. Hydrological outcomes over longer time-scales depend on whether disturbance from die-off is sufficient to cross a threshold that triggers a shift to an alternate state of ecosystem structure and function (Ryan *et al.*, 2008). Arrows next to water budget components indicate relative change from the initial forest community. After mature conifer forest die-off episodes, the relative success of conifer recruitment can determine whether the forest returns to its previous composition, structure, and hydrological function or transforms into an alternate state such as an open savanna or a deciduous shrubland with lower ETI and higher runoff and streamflow than the initial forest state. Adapted for die-off and hydrological function from Goetz *et al.* (2007).

ecological response to die-off. Ecological responses following mortality of a high percentage of overstorey trees could range from a successional return to previous community structure and composition to an emergence of a new community with an altered ecosystem function (Figure 3). Predicting ecological responses to die-off in a changing climate is inherently difficult as multiple life-stage, survival thresholds are affected (Jackson *et al.*, 2009), as are cross-scale interactions with other ecological disturbance processes (Allen, 2007). One of the few, if not the only, study to date measuring the long-term effect of die-off on hydrological processes showed the persistence of a 10% increase in water yield in two Colorado watersheds for 25 years following a bark beetle outbreak that caused up to 80% tree mortality (Bethlahmy, 1974). Long-term ecological responses to tree die-off are poorly understood (Allen *et al.*, 2010), but will ultimately determine the persistence of any mortality-induced hydrological changes.

Observed vegetation responses following tree mortality vary and are mostly short-term. Sites in the southwest United States affected by piñon pine die-off underwent a resetting of a successional trajectory (Clifford *et al.*, 2011), show little immediate recruitment of overstorey trees (Barger *et al.*, 2009), and were chronically affected by episodic mortality (Mueller *et al.*, 2005) but exhibited a rapid understorey growth response (Rich *et al.*, 2008). At many sites affected by die-off in British Columbia, recruitment of ponderosa and lodgepole pine seedlings and saplings, many of which survived the die-off event, are expected to restore forest cover (Klenner and Arsenault, 2009; Axelson *et al.*, 2009, 2010; Brown *et al.*, 2010), although changing climate could preclude this (Loarie *et al.*, 2009). Shifts in dominance patterns of tree species are also possible if recruitment of a drought-tolerant species follows mortality that primarily affected a codominant, less drought-tolerant species (Suarez and Kitzberger, 2008). For such watersheds, the pace of tree recruitment will determine persistence of the initial hydrological effects of die-off.

Die-off may increase the vulnerability of an ecosystem to additional disturbances such as biological invasion or fire that would have further ecohydrological consequences (Mack *et al.*, 2000; Allen, 2007). Tree mortality could create the conditions for invasion of novel and alien species into watersheds though dynamic changes in the diversity–invasibility relationship (Clark and Johnston, 2011). Invasive species, whether woody or herbaceous, could further affect post-mortality watersheds by altering hydrological processes in unexpected ways (Wilcox and Thurrow, 2006; Boxell and Drohan, 2009; Wilcox, 2010). Tree die-off also affects fire dynamics in multiple ways—e.g., initially decreasing crown fire risk as dead canopies drop flammable fine fuels, but conversely increasing surface fire risk as this organic litter reaches the forest floor and understorey vegetation increases (Allen, 2007; Bentz 2009). High-density tree recruitment following overstorey mortality could create

conditions that increase crown fire risk. Fire following mortality would further alter other water budget component responses (Shakesby and Doerr, 2006), potentially with greater consequences than die-off (See Table I, and its earlier discussion.)

Other relevant research on persistence of effects. In paired catchment studies that include forest manipulation, the persistence of increases in water yield attributed to the loss of overstorey T depended on both management after treatment (Brown *et al.*, 2005) and climate (Troendle *et al.*, 2010). In colder climates, where regeneration is slower, streamflow effects may last many decades (Troendle and King, 1987). When regeneration of forest cover occurs rapidly, initial increases in streamflow typically persist for less than a decade, and in some cases water yield may eventually decrease below pre-treatment levels, particularly following species conversion treatments (Hornbeck *et al.*, 1993; Jones and Post, 2004; Brown *et al.*, 2005). Conversely, for some watersheds where forest clearing resulted in negligible or small initial changes in water yield, water yield increased for 10–20 years following regrowth or afforestation, possibly due to reduced T, before appearing to reach a new equilibrium (Brown *et al.*, 2005).

MANAGEMENT IMPLICATIONS AND OPTIONS

Options for land managers dealing with hydrological changes following die-off will likely differ with climate and forest type, and will need to be considered in the context of site-specific objectives. For some regions, increased water yield caused by tree die-off could be desirable, as long as water quality is unaffected and peak flows do not cause excessive flooding. In other regions, preventing die-off from causing decreased water yield, water quality reduction, or flooding damage may be the goal. However, the current state of knowledge on the hydrological impacts of die-off precludes precise prediction of the effects on a specific watershed.

Preventing broad-scale forest die-off through forest management may not be feasible due to the great extent of potentially affected areas, which makes management actions cost-prohibitive and logistically difficult. For example, spraying trees with pesticides to reduce bark beetle success can be very effective, yet is probably limited only to high value areas due to expense and risks of negative environmental side-effects (Fettig *et al.*, 2006). Forest thinning has long been suggested as a management strategy for reducing vulnerability of trees to drought and pests (see Fettig *et al.*, 2007; Millar *et al.*, 2007). Thinning can result in increased soil moisture per tree (or per leaf area or unit biomass), as seen in ponderosa pine forests (Feeney *et al.*, 1998; Simonin *et al.*, 2007; Zou *et al.*, 2008). However, research findings on the effect of stand density on mortality rates are mixed. A number of studies have shown that mortality increased with tree density (Fettig *et al.*, 2007; Greenwood and

Weisberg, 2008; Horner *et al.*, 2009; Klos *et al.*, 2009; Negron *et al.*, 2009), yet others have found no relationship between density and mortality (Mueller *et al.*, 2005; Floyd *et al.*, 2009). Therefore, the effectiveness of thinning for preventing tree die-off may depend on the density-dependence of mortality drivers, species traits, and the type of thinning used (Waring and Pitman 1985; Fettig *et al.*, 2007; Allen *et al.*, 2010). For example, a thinning from below to remove small trees can reduce competition for soil water and drought stress on large trees (McDowell *et al.*, 2006). Such a treatment would be most effective in forests where drought is the dominant cause of tree mortality and less effective where pests and/or pathogens cause mortality independent of tree drought stress. Thinning to prevent undesirable die-off-induced hydrological changes could be self-defeating, if effective treatment requires high levels of tree removal that would cause hydrological changes on their own. Many areas vulnerable to die-off may also be at risk of high severity fire from a legacy of fire suppression, and thinning can be an effective method to reduce the risk of fire impacts (Covington *et al.*, 1997), which include

hydrological changes that exceed those of die-off (See earlier discussion).

Post-mortality options for mitigating undesirable hydrological effects will likely consist mostly of trying to guide the ecological trajectories of watersheds following die-off (Millar *et al.*, 2007). Restoring a watershed's water balance to pre-die-off conditions potentially could be achieved by encouraging recruitment and regrowth that leads to recovery of forest cover that existed prior to mortality. However, ongoing climate changes may mean that a different mix of future tree species should be considered to enhance probabilities of future forest sustainability. For large undesirable hydrological impacts such as flooding, planting of non-native fast-growing tree species may present a short-term local solution at local spatial scales, but the expense of treating large areas and the time lag between tree planting and mitigating eco-hydrological effect both limit the potential of such an approach. Additionally, the risks of undesired long-term hydrological and ecological effects from such an action, which could include altered flows and habitat loss, can be high. In regions with extensive die-off, lack of resources

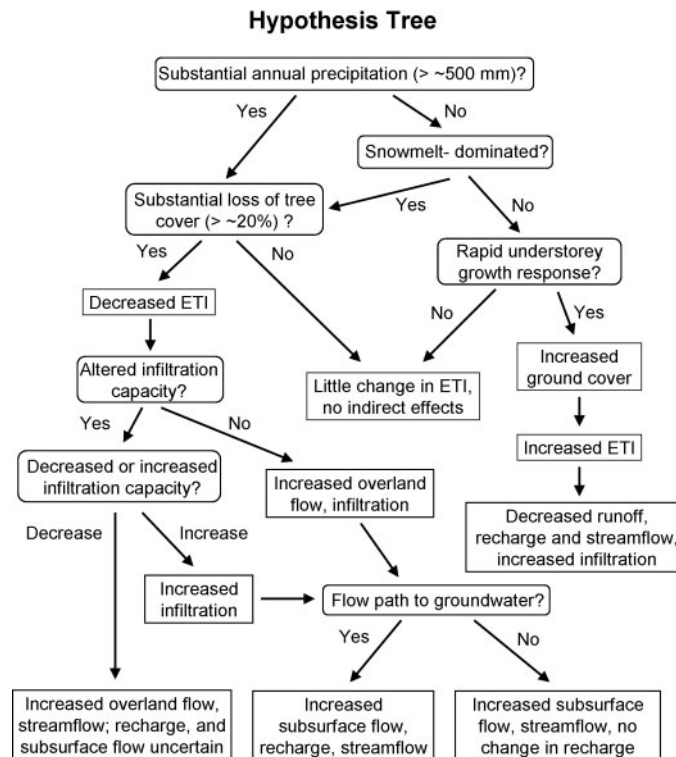


Figure 4. A hypothesis tree of potential effects of tree die-off on key water budget components. Possible effects include increases and decreases in ETI, runoff, infiltration, groundwater recharge, subsurface flow, and streamflow following tree mortality. Important factors for determining these outcomes include an annual precipitation threshold at 500 mm, the dominance of snowmelt on watershed flows, percent tree cover lost from mortality, understorey growth and transpiration response, the effect of tree mortality on infiltration capacity, and the existence of subsurface groundwater flow paths. Testing these hypotheses will require modelling assessments and empirical studies in watersheds affected by die-off that vary widely in climate, forest type, and soil properties.

will limit mitigation of these risks, necessitating adaptation to post-mortality hydrological conditions.

When die-off occurs in forests of commercially valuable timber, salvage logging is often considered a means of recovering financial losses. In the extensive post-mortality forests of British Columbia, salvage harvesting of lodgepole pine is now proceeding at a massive scale (Carver *et al.*, 2009a,b). This harvesting includes removal of standing dead trees as well as the cutting of live, higher value, unaffected tree species. Salvage logging could amplify hydrological changes brought on by tree mortality, initially through road-building and associated soil compaction and disturbance, as well as through additional live tree removal, and over the long term by affecting forest ecological trajectories that otherwise would lead to restoration of hydrological function. Modelling assessment of the Fraser River Basin in British Columbia suggests that salvage harvesting could double post-mortality increases in peak flows (Carver *et al.*, 2009a; Weiler *et al.*, 2009). Therefore, forest managers should consider the potential for undesirable hydrological impacts when planning post-mortality salvage harvest. More generally, the limited management options for preventing tree die-off across extensive areas highlight the importance of future research to improve our ability to predict the ecohydrological consequences of die-off.

EMERGENT HYPOTHESES AND RESEARCH NEEDS

Our evaluation of the small group of published studies on hydrological responses to die-off, considered in concert with the larger body of research on forest harvest effects, reveals a wide range of possible outcomes. With the aim of guiding and prioritizing future research, we present a set of simplified hypotheses that are generally consistent with the existing die-off studies, applying insights from the other relevant research reviewed. We present these as a hypotheses tree (Figure 4). Note that these are hypotheses for future testing rather than validated conclusions. The research summarized above suggests that the most likely direct effect of tree mortality is a reduction in ETI and an associated indirect increase in flows and groundwater recharge (Stednick, 1996; Zhang *et al.*, 2001; Brown *et al.*, 2005; see also Table II). Notably, however, in drier regions, which includes many areas where mortality is occurring (Allen *et al.*, 2010), die-off may not cause significant increases in water yield (Guardiola-Claramonte, 2009; Somor, 2010; Guardiola-Claramonte *et al.*, in press; consistent with Zhang *et al.*, 2001; Wilcox, 2002; Wilcox *et al.*, 2006). Therefore, we hypothesize that watersheds receiving more than ~500 mm of annual precipitation will experience decreases in ETI from a loss of overstorey T and I that lead to increased annual flows and potential groundwater recharge, while watersheds with less than ~500 mm of annual precipitation will not (Figure 4, consistent with Zhang *et al.*, 2001). However, for watersheds

with less than ~500 mm of precipitation that also have water yields dominated by snowmelt, we hypothesize that die-off will produce increased flows (Figure 4, Carver *et al.*, 2009a; Weiler *et al.*, 2009).

The hydrological consequences of tree mortality also will likely depend on the level of canopy cover reduction. Harvest-related literature suggests a threshold of ~20% loss of forest cover is required to statistically detect a change in water budget components (Stednick, 1996; Brown *et al.*, 2005). We hypothesize that this threshold of ~20% loss of forest cover for producing a detectable change in water budget components also applies to die-off (Figure 2). Mortality in many die-off affected areas falls below this threshold (Allen *et al.*, 2010), and we hypothesize such watersheds will not experience much hydrological change. We also hypothesize that seasonal effects on watershed responses following die-off will be similar to those reported in response to harvest (Brown *et al.*, 2005), with annual changes in water yield depending on peak flows (Figure 5), particularly where snowmelt dominates watershed dynamics. This hypothesis is consistent with a modelling assessment of bark beetle effects in the Fraser Basin, British Columbia, Canada (Carver *et al.*, 2009a,b). Exceptions to this seasonal effect might exist when mortality selectively kills species with a unique hydrological function, as highlighted in the case of die-off of eastern hemlock in the southeast United States (Ford and Vose, 2007). Because eastern hemlock is the only evergreen species in an otherwise deciduous forest, its loss is hypothesized to reduce early spring T, causing increased spring flows that could raise annual water yield (Figure 5).

In addition, we hypothesize that the causes of tree mortality can influence potential ecohydrological outcomes. When drought is an important cause of tree mortality, we expect reduced precipitation from drought to mute ecohydrological responses (Guardiola-Claramonte, 2009; Somor, 2010; Guardiola-Claramonte *et al.*, in press;). In

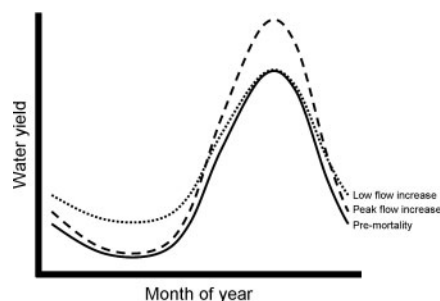


Figure 5. A depiction of two possible seasonal affects on annual water yield following die-off. Post-mortality, annual water yield increases from pre-mortality flows (solid line) could be caused primarily by increased peak-flows (dashed line) or by increased low flows (dotted line). Peak flow increases are more likely following die-off, especially for watersheds where flows are dominated by snowpack dynamics (Brown *et al.*, 2005; Carver *et al.*, 2009a,b). Loss of species that play a unique hydrological role in the seasonality of watershed flows could alter seasonal patterns of ETI, and by extension water yield, affecting annual totals through increased low flows (Ford and Vose 2007).

Table III. Research priorities to address key uncertainties that currently limit development and testing of hypotheses regarding the ecohydrological consequences of tree die-off.

Research priority	Description
Follow-up catchment studies	More catchment studies of mortality-affected watersheds using existing gauge data are needed for a wide variety of climates, forest types, and affected species. The extent of recent die-off makes it probable that such results already exist from currently monitored watersheds, or could be obtained from re-starting measurements at previously instrumented watersheds.
Persistence of effects over ecological trajectories	Hydrological and ecological monitoring post-mortality are needed to determine the persistence of die-off hydrological effects and their dependence on ecological changes. Such studies should also examine how ecological changes following mortality depend on post-mortality hydrological changes so that ecohydrological feedbacks can be considered.
ETI partitioning	Research partitioning the stand-level effects of ETI into its E, T, and I components following tree die-off could be done through experimentally killing trees within an intensely instrumented eddy covariance tower footprint to compare ETI components before, initially after treatment, and following post-mortality ecological responses.
Flowpath partitioning of indirectly affected components	Investigation should also focus on flowpath partitioning of the lesser-studied indirect effects of tree mortality such as saturation excess and Hortonian overland flow, subsurface flow, and groundwater recharge, and groundwater flow following tree die-off.
Modelling large ungauged areas to scale hydrological effects	More modelling assessments are needed that incorporate existing and new empirical data to scale hydrological effects across large areas. Such models should be coupled with atmospheric models to better understand the comprehensive water budget and runoff dynamic effects of tree die-off at sub-continental scales.

contrast, when mortality occurs without a change in precipitation but is primarily driven by pest or pathogen outbreak, we expect ecohydrological effects will be more similar to harvest responses. Given that tree die-off could ultimately affect a very large proportion of the world's forested area (Allen *et al.*, 2010; Gonzalez *et al.*, 2010), we expect there will be substantial cumulative hydrological effects. Consequently, building on the hypothesis

tree presented, we suggest research priorities in four key areas (Table III). These include specific aspects related to follow-up catchment studies in watersheds that have experienced recent mortality; evaluation of the persistence of such effects over subsequent ecological trajectories; flow path partitioning investigations, including ETI partitioning; and improved modelling of large ungauged areas (all detailed in Table III).

In conclusion, episodes of regional-scale tree die-off around the world have been recently documented and likely provide a glimpse into the range of potential hydrological responses that may accompany die-off. Increased die-off is projected to occur with climate change, necessitating a rapid improvement in our ability to understand and predict how tree die-off affects watersheds. In particular, although we know tree mortality directly affects the E and T components of ETI though canopy cover loss and can alter the snow accumulation and melt dynamics of a watershed, how the net direct effects of die-off will be translated through changes in the water balance to indirect effects on soil moisture status, groundwater recharge dynamics, and streamflow volume and timing are unclear and require future research. The direct and indirect hydrological consequences of tree die-off in combination with temperature and precipitation shifts may exceed the hydrological effects of climate change alone, and for some regions could present risks to water resources. Post-mortality successional dynamics will ultimately determine if short-term die-off impacts to hydrological function will persist, be diminished, or altered further over longer time scales. Although management options for preventing die-off may be limited, development of policies to mitigate potential effects and restore watershed function will depend on a better understanding of the hydrological consequences of tree die-off. The hypotheses and research priorities we present provide a roadmap to help address this emerging core challenge in ecohydrology.

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APPENDIX C: TEMPERATURE SENSITIVITY OF DROUGHT-INDUCED TREE MORTALITY PORTENDS INCREASED REGIONAL DIE-OFF UNDER GLOBAL-CHANGE-TYPE DROUGHT

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, Villegas JC, Breshears DD, Zou CB, Troch PA, Huxman TE. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America*. 106:7063-7066.

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Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought

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Large-scale biogeographical shifts in vegetation are predicted in response to the altered precipitation and temperature regimes associated with global climate change. Vegetation shifts have profound ecological impacts and are an important climate-ecosystem feedback through their alteration of carbon, water, and energy exchanges of the land surface. Of particular concern is the potential for warmer temperatures to compound the effects of increasingly severe droughts by triggering widespread vegetation shifts via woody plant mortality. The sensitivity of tree mortality to temperature is dependent on which of 2 non-mutually-exclusive mechanisms predominates—temperature-sensitive carbon starvation in response to a period of protracted water stress or temperature-insensitive sudden hydraulic failure under extreme water stress (cavitation). Here we show that experimentally induced warmer temperatures ($\approx 4^\circ\text{C}$) shortened the time to drought-induced mortality in *Pinus edulis* (piñon shortened pine) trees by nearly a third, with temperature-dependent differences in cumulative respiration costs implicating carbon starvation as the primary mechanism of mortality. Extrapolating this temperature effect to the historic frequency of water deficit in the southwestern United States predicts a 5-fold increase in the frequency of regional-scale tree die-off events for this species due to temperature alone. Projected increases in drought frequency due to changes in precipitation and increases in stress from biotic agents (e.g., bark beetles) would further exacerbate mortality. Our results demonstrate the mechanism by which warmer temperatures have exacerbated recent regional die-off events and background mortality rates. Because of pervasive projected increases in temperature, our results portend widespread increases in the extent and frequency of vegetation die-off.

biosphere–atmosphere feedbacks | drought impacts | global-change ecology | *Pinus edulis* | carbon starvation

Global change assessments and supporting research have largely focused on how vegetation will respond to incremental changes in the central tendency of climate variables, but the most dramatic vegetation shifts are likely to result from changes in climate extremes altering patterns of disturbance events arising from hurricanes, freezes, fires, and droughts (1, 2). The effects of drought on vegetation under warmer conditions can be severe, as highlighted by recent regional-scale woody-plant die-off across the southwestern United States (3–6) and around the globe (7–10). Worldwide, many coniferous tree species are experiencing widespread, historically unprecedented mortality, mainly as a result of drought and the eruption of tree pests, such as bark beetles (1, 3, 7–9, 11–16). Consequent impacts of regional tree die-off could include reduction in habitat for wildlife, enhanced opportunities for invasion by exotic species, formation of novel communities, alterations to the hydrologic

cycle, and temporal disruptions in ecosystem goods and services (2, 3, 17, 18). In addition, extensive tree die-off could impact regional carbon (C) budgets, reducing ecosystem potential to sequester C and increasing C losses through enhanced soil respiration rates (19–22). Drought-induced tree mortality not only alters C fluxes but also modifies water and energy fluxes between the atmosphere and land surface (3, 20, 23, 24). The consequences of potentially large releases of C from the biosphere to the atmosphere due to widespread mortality could contribute to further warming (19–21, 25). Small drought-induced increases solely in background mortality rates may even be sufficient to alter regional C budgets (13, 16, 26).

Drought-induced tree mortality is a pivotal vulnerability of vegetation to climate change, yet our understanding of, and ability to predict, tree mortality is astonishingly poor. Drought-induced tree mortality is difficult to predict because it is a nonlinear threshold process (1, 19, 27). Recent observational studies have raised concern that warmer temperatures could be amplifying the effects of drought on tree mortality both for background rates of mortality and for regional die-off events (3, 16). Yet experimental assessment of whether warmer temperatures associated with drought exacerbate tree mortality is lacking for any tree species, and therefore tree mortality has only been predicted in response to a simple metric of accumulated dry conditions (28, 29). The sensitivity of tree mortality to temperature is dependent on which of 2 non-mutually-exclusive mechanisms predominates: (i) carbon starvation, whereby trees close stomata to keep safe levels of xylem pressure, stopping most photosynthesis, and rely on stored carbohydrates to support the metabolic costs of maintaining tissue; or (ii) catastrophic hydraulic failure, whereby trees maintain stomatal conductance during drought to continue photosynthesizing, but run the risk of xylem pressures suddenly exceeding cavitation thresholds beyond which air bubbles block transport of stem water (30–32). Both hypotheses are interrelated with biotic agents, such as bark beetles and associated fungi, and carbon starvation would preclude production of the photosynthate necessary for tree defense, thereby increasing susceptibility to biotic agents (9, 33). Because respiration rates increase with temperature (34), carbon

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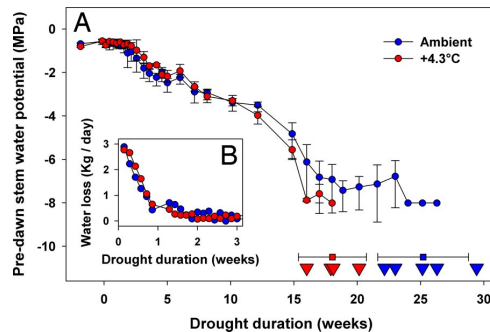


Fig. 1. Water relations progression and death dates. (A) Predawn stem water potential (circles), death dates (triangles), and death date means (squares) of piñon pines during simulated drought under ambient (blue) and elevated (red) temperatures. Error bars are standard errors. (B) Water loss from a subset of trees in A.

starvation should be highly sensitive to temperature, whereas hydraulic failure should not.

We experimentally investigated the temperature sensitivity of drought-induced mortality in *Pinus edulis*, a piñon pine tree that has exhibited regional-scale die-off in response to recent drought (3, 4) and which has been evaluated in observational and modeling studies (30, 31, 35). We transplanted small, reproductively mature piñon pines into the environmentally controlled Biosphere 2 facility to grow them in either near-ambient or warmer (+4.3 °C) temperatures, and then imposed a drought treatment that completely excluded water from selected trees until all “drought” trees in both temperature treatments died.

Results and Discussion

All drought trees in the warmer treatment died before any of the drought trees in the ambient treatment (on average 18.0 vs. 25.1 weeks, $P < 0.01$; Fig. 1A). This 28% shortening in time to mortality was not reflected in a water balance difference (Fig. 1B). Indeed, predawn stem water potential measurements documented no differences in xylem pressure between drought treatments at any time during the experiment ($P = 0.88$, Fig. 1). Because water potential is highly correlated with the level of air embolism occluding water transport in the xylem (30, 32, 36), we saw no evidence of catastrophic loss of hydraulic transport as a primary driver of differences between treatments. Catastrophic loss in this species has been consistently observed at a stem water potential of -6 MPa (36). Therefore, we suggest that higher respiratory loads associated with warmer temperatures incited differences in mortality, reflecting carbon starvation, not sudden hydraulic failure as the causal mechanism required to predict tree mortality differences in a future warmer world. Such results are consistent with inferences from recent observational and modeling assessments (30, 31, 35).

During the experiment, we also measured leaf-level exchange of CO_2 before dawn to estimate respiratory load and during the middle of the day to follow photosynthetic patterns. After the start of drought treatment, photosynthesis declined rapidly and similarly in both temperature treatments, approaching zero by the third week of the experiment in drought trees (Fig. 2A). Initially, instantaneous rates of respiration were similar among drought trees in both temperature treatments, but they diverged during the third and fourth weeks of drought (Fig. 2B). An analysis of time-integrated respiration revealed that trees in the elevated-temperature drought treatment consumed C reserves faster than trees in the ambient drought treatment (Fig. 2C), reflecting the increased C cost for maintenance of tissue under

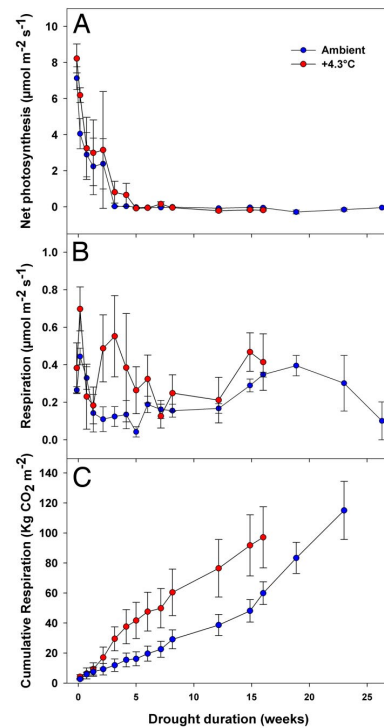


Fig. 2. Leaf carbon exchange progression. (A and B) Instantaneous midday net photosynthesis (A) and predawn respiration (B) of piñon pines during simulated drought under ambient (blue) and elevated (red) temperatures. Error bars are standard errors. (C) Cumulative time-integrated respiration costs of piñon pines during simulated drought under ambient (blue) and elevated (red) temperatures. Error bars are standard errors calculated by following standard methods for summation.

warmer temperatures (34). Mean time-integrated cumulative respiration just before mortality for drought trees did not differ significantly between temperature treatments ($P = 0.57$). Combined, our results provide experimental evidence that piñon pines attempted to avoid drought-induced mortality by regulating stomata and foregoing further photosynthesis but subsequently succumbed to drought due to carbon starvation, not sudden hydraulic failure. Importantly, we isolate the effect of temperature from other climate variables and biotic agents and show that the effect of warmer temperature in conjunction with drought can be substantial.

Our results imply that future warmer temperatures will not only increase background rates of tree mortality (13, 16), but also result in more frequent widespread vegetation die-off events (3, 35) through an exacerbation of metabolic stress associated with drought. With warmer temperatures, droughts of shorter duration—which occur more frequently—would be sufficient to cause widespread die-off. In our calculation of a 103-year record of regional drought duration for piñon, widespread mortality occurred only once, during a 6-month (26.1-week) drought in 2002 (Fig. 3A). By fitting a curve to the frequency distribution of regional drought duration, we estimated that our observed 28% acceleration in mortality with warmer temperatures indicated that a shorter, ≈ 4 -month (18.7-week) drought would cause widespread mortality. Therefore we estimated that a 4.3 °C increase in temperature corresponded to a 5-fold increase in the

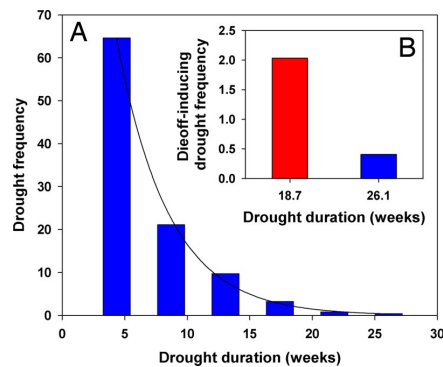


Fig. 3. Drought frequency and die-off projections. (A) Duration and frequency of drought events from a 103-year record of regional climate. (B) A comparison of the frequency of a widespread die-off causing drought from the 103-year record (26.1-week regional drought, blue), and under a warmer-temperature, accelerated mortality scenario (18.7-week regional drought, red) for the Four Corners region.

frequency of mortality-inducing events (Fig. 3B). This projection is conservative because it is based on the historical drought record and therefore does not include changes in drought frequency, which is predicted to increase concurrently with warming (2, 37–39). In addition, populations of tree pests, such as bark beetles, which are often the proximal cause of mortality in this species and others, are also expected to increase with future warming (7, 9, 38).

Our results demonstrate that future warming will exacerbate regional die-off (3, 35) and elevate background mortality rates (13, 16) independently of other changes in ecosystem water balance. The high degree of temperature sensitivity we have documented in drought-induced mortality for piñon pine needs to be assessed for other widespread, dominant tree species. The temperature sensitivity we document highlights the need to improve model predictions and could profoundly alter assessments of climate change impacts, which continue to reveal increasingly dangerous risks (40), including those for ecosystem function, species distributions, energy fluxes, hydrological processes, and perhaps most importantly C fluxes (17, 19–22, 25, 35, 39, 41, 42). Our results underscore the critical importance of understanding temperature sensitivities associated with the mechanisms that trigger plant mortality and drive vegetation change and their implications for assessments of climate change impacts and consequent land surface–atmospheric feedbacks. Most importantly, because increased temperature is among the most widespread and least uncertain climate projections (37–39), our results portend widespread increases in the extent and frequency of vegetation die-off.

Materials and Methods

We selected reproductively mature piñon pines (*P. edulis*) from a ranch near Ojitos Frios, NM (35.5177°N, 105.3337°W, 2,050 m above sea level) with similar allometry, an average height of 1.7 m (range: 1.3–2.4 m tall), and an average root-collar-diameter of 6.5 cm (range: 3.5–11 cm) that were isolated (nearest-neighbor canopy-to-canopy distances > 1 m) and were not in rocky areas or eroded rills. After transport to Biosphere 2, we placed trees in 0.5-m-diameter, 100-L containers. Where minor soil additions were needed, we added a soil with similar texture, organic C, and nitrogen content. Twenty trees were randomly distributed into 2 conditions at Biosphere 2: temperatures that approximately tracked mean ambient conditions for piñon pine (weekly mean minima of 10.9 to 20.8 °C and maxima of 22.8 to 34.2 °C), vs. those elevated consistently by an average of 4.3 °C. Mean weekly relative humidity was kept constant between treatments and varied from 34% to 78%, resulting in mean vapor pressure deficits of 1.18 kPa for the ambient treatment (weekly mean range: 0.35–1.85 kPa) and 1.51 kPa for the warmer treatment (weekly mean range: 0.56–2.64 kPa).

Initially high volumetric soil water content (20–30%) was maintained for all trees by daily watering (confirmed with 20-cm ECH₂O probes, Decagon Devices). Irrigation was curtailed for 5 randomly selected trees on February 9, 2008, in each temperature treatment, while the remaining 5 trees in each continued to be watered. Weighing scales (Industrial Commercial Scales) were placed under 3 drought trees in each temperature treatment to record water loss gravimetrically. We measured predawn plant water potentials before and during the experiment on excised twigs from the south side of the tree canopy by using a pressure chamber (PMS Instruments). We curtailed water potential measurements at –8 MPa because previous research documented complete loss of hydraulic conductivity in *P. edulis* at branch water potential of –6 MPa (36). We measured midday photosynthesis and predawn respiration by using an LI-6400 portable infrared gas analyzer (LI-6400, LI-COR Biosciences). Time-integrated cumulative respiration costs were calculated by multiplying instantaneous predawn rates by the period in seconds each measurement represented. These time periods began and ended at the halfway point in time between each sampling date. Trees were checked weekly for signs of needle browning and declared dead when 90% of their canopy foliage turned brown.

We estimated a relevant regional drought distribution with climate data (Western Regional Climate Center, www.wrcc.dri.edu) by using 1 station each from Arizona, Colorado, New Mexico, and Utah with an ≈100-year record and in an elevation range which includes *P. edulis* (1,300–2,100 m) (Table S1). We averaged monthly precipitation totals (January 1905 to July 2008, excluding months with >10 days missing data) and defined drought months as those where total precipitation was <50% of the long-term monthly mean, consistent with the recent die-off (3). To project our temperature sensitivity on the historical record we fit a negative exponential function to the probability distribution of regional drought, yielding: drought frequency = $183.5 \times e^{-0.2408 \times (\text{drought duration})}$. The recent, widespread mortality-causing drought was the only 6-month regional drought (26.1-week) on the record, whereas during the same period, there were more than 5 drought events exceeding 4.3 months (18.7 weeks) in duration.

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Supporting Information

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Table S1. Metadata for climate stations used to estimate the frequency of historical regional drought duration

Station	Period of record	Latitude	Longitude	Elevation, m
Blanding, UT	1905–2008	37°37'N	109°28'W	1,841
Bloomfield, NM	1905–2008	36°40'N	107°58'W	1,768
Jerome, AZ	1905–2008	34°45'N	112°07'W	1,601
Mesa Verde, CO	1922–2008	37°12'N	108°30'W	2,125

APPENDIX D: NON-STRUCTURAL LEAF CARBOHYDRATE DYNAMICS OF
PINUS EDULIS DURING DROUGHT-INDUCED TREE MORTALITY SUPPORT
ROLE FOR CARBON METABOLISM IN MORTALITY MECHANISM.

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Summary

- Vegetation change is projected to increase with global change, potentially altering ecosystem function and climate feedbacks. However, physiological mechanisms underlying plant mortality remain unclear, particularly the roles of carbon starvation and xylem cavitation as proximate causes.
- We report analysis of foliar non-structural carbohydrates (NSC) and associated physiology from a previous experiment where earlier drought-induced mortality of *Pinus edulis* at elevated temperatures exhibited higher cumulative respiration. We predicted faster NSC decline for warmed trees than for ambient-temperature trees.
- Foliar NSC in droughted trees declined by 30% through mortality and was lower than in watered controls. NSC decline resulted primarily from decreased sugar concentrations. Starch initially declined, and then increased above pre-drought concentrations prior to mortality. Although there was no temperature effect on foliar total NSC and sugar at mortality, starch concentrations ceased declining and increased earlier for warmer droughted trees.
- Reduced foliar NSC during drought through mortality indicates a role for C metabolism in temperature-sensitive mortality differences. Carbohydrates were not completely exhausted at mortality, possibly due to feedback inhibition of photosynthesis and maintenance of NSC for non-metabolic functions. Drought mortality appears related to carbon dynamics concurrent with increasing hydraulic stress, at least in *P. edulis* and perhaps physiologically similar species.

Keywords: tree mortality, carbon starvation, hydraulic failure, mortality mechanism, biosphere-atmosphere feedbacks, drought impacts, global change, non-structural carbohydrates

Introduction

Widespread tree mortality associated with drought, increased temperatures, and tree pest/ pathogen outbreak is an emerging threat of global change now documented across all six forested continents (Allen et al., 2010). Recent die-off from tree mortality in western North America is now sub-continental, affecting over 605,000 km² of coniferous forests from Mexico to Alaska (Allen et al., 2010). The consequences of tree mortality include impacts to community composition, stand structure, and ecosystem function (Allen & Breshears, 1998; Breshears et al., 2005; Koepke et al., 2010; Carnicer et al., 2011; Royer et al., 2011). These changes in the landscape affect biological diversity (Gitlin et al., 2006; Sthultz et al., 2009), wildlife habitat (Klenner & Arsenault 2009), carbon cycling (da Costa et al., 2010; Metcalfe et al., 2010), ecosystem services (Breshears et al., 2011), hydrological function (Guardiola-Claramonte et al., 2011; Adams et al., 2012), and susceptibility to invasion by undesirable exotic species (Kane et al., 2011). Widespread tree mortality is a potentially positive feedback that could accelerate global change through the loss of terrestrial carbon sinks and canopy cover influences on landscape energy balance (Breshears & Allen, 2002; Chapin et al., 2007; Field et al., 2007; Bonan et al., 2008; Kurz et al., 2008a, 2008b; Rotenberg & Yakir,

2010). Despite these threats, no model yet exists that can mechanistically predict tree mortality in response to a changing climate (Fisher et al., 2010; McDowell et al., 2011).

There is great interest in understanding the physiological processes underlying mortality of trees. Two mechanisms have been proposed to explain drought-induced tree mortality: hydraulic failure and carbon starvation (Hacke et al., 2000; Hacke & Sperry 2001; McDowell et al., 2008). Hydraulic failure from drought could occur if high xylem tension causes catastrophic cavitation of the vasculature by air embolism, impeding the flow of water and leading to a loss of turgor pressure that results in cell death. Carbon starvation could occur when stomatal limitations to photosynthesis, associated with preventing xylem failure during drought, reduce carbohydrate availability to support respiratory demands, leading to loss of metabolic capabilities. (McDowell et al., 2011). In the case of carbon starvation, both the immediate production of photosynthate and the availability of stored energy reserves may be important in regulating the time to mortality (Sala et al., 2010). Mortality by both mechanisms can be exacerbated by a number of internal and external factors, including tree pests and/or pathogens (McDowell et al., 2008; Kane & Kolb 2010).

An initial experimental test of these mechanisms was conducted inside the environmentally-controlled Biosphere 2 glasshouse with transplanted piñon pine (*Pinus edulis*) trees under two temperature regimes (Adams et al., 2009a). Trees in the warmer treatment died 28% (7 weeks) earlier than ambient-temperature trees (Adams et al., 2009a). Xylem tensions increased similarly for trees under both temperatures to a threshold sufficient to cause complete cavitation, as inferred from a hydraulic

vulnerability curve developed for this species (Linton et al., 1998), indicating that hydraulic failure occurred prior to mortality (Adams et al., 2009a). However, this threshold was reached in trees of both temperature treatments at the same time, and did not explain time-to-mortality differences between these two treatments. Trees in both treatments ceased photosynthesis in response to drought immediately. Prior to mortality all trees had cumulatively respired equivalent amounts of CO₂, but elevated-temperature trees respired this CO₂ in a significantly shorter period of time than ambient-temperature trees. Therefore, temperature-sensitive differences in survival during drought were linked to temperature-sensitive carbon metabolism—a result consistent with the carbon starvation hypothesis. Adams et al. (2009a) were challenged for not directly measuring stored carbohydrate resources and for failing to consider other research showing increased carbohydrate storage during drought (Leuzinger et al., 2009; Sala, 2009; Sala et al., 2010; Piper, 2011).

Subsequent conceptual assessments have led to a sophistication of initially proposed hypotheses, positing that tree drought mortality is a complex process and can occur by multiple mechanisms that are highly interrelated and hierarchical (Adams et al., 2009b, 2009c; Sala, 2009; Sala et al., 2010; McDowell & Sevanto, 2010; McDowell, 2011; McDowell et al., 2011). For example, carbon starvation could occur from a reduction of tree carbohydrate reserves below a survival threshold during severe drought, or if these resources become inaccessible through inhibition of stored starch conversion to sugar (mobilization failure), or if high xylem tension prevents generation of positive hydrostatic pressure flow in the xylem (phloem failure; Münch, 1930; Minchin &

Lacointe, 2005; Höltta et al., 2006; Knoblauch & Peters, 2010; Sala et al., 2010; McDowell et al., 2011). Additionally, non-structural carbohydrates (NSC) are also actively maintained in plant tissue for use in cryoprotection and desiccation protection (Obendorf, 1997; Ogren et al., 1997; Bansal & Germino 2008, 2009; Bansal et al., 2011), as well as signaling between cells and tissues (Chiou & Bush, 1998; Lalonde et al., 1999; Smeekens, 2000). The functional dynamics of NSC in these processes could complicate the relationship between pools of carbohydrates and metabolic fluxes as water stress progresses (Ryan, 2011; Zeppel et al., 2011, Sala et al., 2012).

Although there are a few cases where tree NSC declined in response to non-lethal drought (Körner, 2003; Sayer & Haywood, 2006), observations of increased NSC during drought are much more common in the literature (Körner, 2003; Würth et al., 2005; Sala and Hoch, 2009; Galvez et al., 2010). Increased NSC likely results when growth and growth respiration demand for C is reduced faster than photosynthesis, a response known as sink limitation (Körner, 2003). Sink limitation may initially seem incompatible with drought-induced tree mortality by carbon starvation, a source limitation to survival (Leuzinger et al., 2009; Sala, 2009; Gruber et al., 2012). However, mechanisms underlying drought mortality likely differ from growth responses to non-lethal water stress due in part to the time-scales of response from different physiological processes (McDowell & Sevanto 2010; Hoffman et al., 2011). Current conceptual models hypothesize that during drought, trees will initially experience sink limitation and increased NSC pools, but if drought persists, then source limitation will occur and NSCs will decrease or will become inaccessible through mobilization and/or phloem failure

prior to death (Sala et al., 2010; McDowell et al., 2011). In several recent studies of lethal drought, carbohydrates were diminished for some species, but not others: relative to survivors, NSC was lower for drought-killed *Pinus sylvestris* in Spain (Galiano et al., 2011) and for near-dead seedlings of *Nothofagus nitida*, but not for *Nothofagus dombeyi*, in Chile (Piper, 2011), nor mature *Populus tremuloides* in Colorado, USA (Anderegg et al., 2012).

Here we present an analysis of NSC from leaf samples collected during the piñon pine drought mortality experiment at Biosphere 2 (Adams et al., 2009a) to more fully investigate potential mechanisms of tree drought-induced mortality and address previously raised concerns about aspects of Adams et al. (2009a). Notably, our study is the first repeated measurement of tree NSC response during drought through mortality for trees under two temperature regimes. We hypothesized that NSC would be reduced during drought, and that NSC trends would reflect changes in cumulative respiration such that elevated-temperature trees would experience a faster decline in foliar carbohydrates than ambient-temperature trees.

Materials and Methods

The Biosphere 2 piñon pine mortality experiment was conducted with small (mean height of 1.7m), reproductively mature trees transplanted from northern New Mexico into 100-L containers. Estimating from the dimensions of these pots and field measurements of *Pinus edulis* roots (Foxx and Tierney, 1987; Tierney and Foxx, 1987), transplanted trees had on average 7% of their lateral root spread and 39% of their vertical

root spread. Ten trees were randomly assigned to two areas of the glasshouse set to near-ambient or elevated (by $\sim 4^{\circ}\text{C}$) temperatures (for full description of the experiment, see Adams et al., 2009a). After four months of irrigation to promote acclimation to these temperatures following transplant, we withheld water from five of the trees (randomly selected) in each temperature treatment. One droughted tree in the elevated temperature treatment accessed water during the experiment and was excluded from further analysis, resulting in a sample size of 5 ambient droughted trees, 4 elevated-temperature droughted trees, and 5 watered controls at each temperature. The drought treatment resulted in mortality, defined as 90% foliar browning, which occurred within 1-2 weeks after the first signs of foliar browning for trees in both temperature treatments.

Physiological measurements and collection of foliar material were periodically made on the south facing side of all trees throughout the time-course of the experiment. On the same day as foliar sampling, we measured pre-dawn stem water potential (Ψ_{pd}) by pressure chamber (PMS Instruments, Albany, OR, USA), CO_2 exchange with an LI-6400 portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA), before dawn to quantify respiration (R) and at midday for net photosynthesis (A). We measured stomatal conductance (g_s) from ~ 1 hour after sunrise to sunset with a Model SC-1 leaf porometer (Decagon Devices, Pullman, WA, USA). For sampling of foliar tissue, we collected the most recent mature needles from a single branch on each tree, usually the same used in water potential measurements. Needles were dried at 70°C and ground to a powder fine enough to pass a 40-mesh screen (.42 mm sieve). Foliar material was analyzed for NSC, defined as starch and soluble sugar (glucose, fructose, and sucrose), following the

enzymatic digest and UV spectrophotometry methods modified from Hoch et al. (2002, Supporting Information). Any NSC or component value result less than zero was excluded from further analysis.

We calculated NSC differentials by subtracting the NSC, starch, and soluble sugar content values for droughted trees from watered-tree means in each temperature treatment for each sampling date. Diurnal measurements of stomatal conductance were interpolated to 10 time intervals following Fritsch and Carlson (1980) that were used to calculate daily means. Time series data, including NSC totals, components, and differentials, stomatal conductance, photosynthesis, and respiration, were analyzed using repeated measures ANOVA (SPSS 19.0, IBM Corporation, $\alpha = 0.05$). Mean total NSC, sugar, and starch in droughted trees initially and at time of mortality for each tree were also analyzed with repeated measures ANOVA. For all repeated measures ANOVA tests, when sphericity assumptions were violated (determined by Mauchly's test for sphericity), we used the Huynh-Feldt correction to degrees of freedom of the F-statistic to determine the significance of results (Huynh and Feldt, 1976). Note that measurements ceased earlier for tree in the elevated-temperature drought treatment because these trees died earlier (Adams et al., 2009).

We used structural equation modeling (SEM) to analyze causal relationships among water potential, gas exchange, and NSC constituents as a series of multiple linear regressions for each independent variable to calculate error terms and standardized partial correlation coefficients (SPSS 19.0, IBM Corporation, $\alpha = 0.05$, Supporting Information). We analyzed the model separately for data from trees in each of the four treatments:

ambient watered control, elevated-temperature watered control, ambient-temperature drought, and elevated-temperature drought. Additionally, we analyzed the correlation of cumulative respiration (Adams et al., 2009a) with total NSC, sugar, and starch for droughted trees in both temperature treatments separately and pooled (SPSS 19.0, IBM Corporation, $\alpha = 0.05$)

Results

At the start of the drought experiment, foliar total NSC concentrations were similar for both droughted and watered trees (means 5.55 ± 0.33 and 4.96 ± 0.38 % dry mass, respectively, $p > 0.05$). For droughted trees in both temperature treatments we found a significant decline in NSC over the course of the experiment (Fig. 1, $p < 0.05$) and as a pairwise contrast between initial and at-mortality values (Fig. 2, $p < 0.05$). Mean NSC at mortality was 3.88 ± 0.49 % dry mass, a reduction of 30% from initial measurements (Fig. 2). The decline in total NSC in both analyses was related to a decrease in soluble sugar concentration, despite an apparent net increase in starch concentration ($p < 0.05$). There was no effect of temperature on the declines in total NSC and sugar concentration ($p > 0.05$, Fig 1, 2). However, post-hoc analysis of starch trends show an initial decline in values followed by a significant increase in starch at week 12 for warmer droughted trees and week 17 for ambient droughted trees ($p < 0.05$, Fig. 1). A significant time x temperature interaction observed in the starch analysis indicates this trend reversal, from a decline to an increase, occurred earlier in the warmer drought treatment than in the ambient drought treatment ($p < 0.05$).

Foliar NSC of watered controls doubled during the course of the experiment, and was greater than drought tree means in weeks 6, 8, 12, 17, and 22 ($p < 0.05$, Fig. 1). For sugar, we also observed a significant water x time interaction ($p < 0.05$), although trends in sugar did not differ greatly between droughted trees and watered controls (Fig. 1). Sugar content in droughted trees was greater than watered controls at week 8, but was significantly lower than watered trees at weeks 15 and 22 ($p < 0.05$). Similar to the trend in total NSC in watered controls, starch concentrations increased in watered controls by between five and eight times the initial values ($p < 0.05$) during the course of the experiment. Foliar starch content was greater in watered controls than droughted trees at weeks 6, 8, 15, 17, and 19 ($p < 0.05$). There was no significant temperature effect on watered control tree NSC, sugar, or starch. When calculated as a differential (watered – droughted, within each temperature treatment), there were no differences between the temperature treatments in total NSC, and sugar, or starch content ($p > 0.05$). Total NSC and starch differentials increased over time ($p < 0.05$), but sugar differentials were unchanged ($p > 0.05$).

Repeated measures analysis revealed significant changes over time for Ψ_{pd} , g_s , A , and R ($p < 0.001$). There were no differences in Ψ_{pd} , g_s , A , and R between temperature treatments for droughted trees ($p > 0.05$), although when quantified cumulatively R was higher in warmer droughted trees (Adams et al., 2009a). Differences between watered controls and droughted trees were found for Ψ_{pd} , g_s , A ($p < 0.05$), but not R ($p > 0.05$).

For watered control trees in both temperature treatments, SEM revealed positive relationships between Ψ_{pd} and g_s ($p < 0.05$, Fig. 4). Ambient watered trees showed a

significant inverse effect of A on g_s , while for warmer watered trees there were positive relationships between A and g_s , and a positive effect of A on sugar concentration ($p < 0.05$). Relationships found with SEM in droughted trees were similar between temperature treatments, where a positive effect of g_s on Ψ_{pd} , positive relationships between g_s and A, and a positive effect of A on sugar concentration were observed ($p < 0.05$). Inverse effects of sugar on starch and Ψ_{pd} on R were seen in ambient droughted trees, but not warmer droughted trees ($p < 0.05$). Cumulative respiration was correlated positively with starch concentration for ambient droughted trees ($R = 0.36$, $p < 0.05$), but not with total NSC or any other NSC component when the analysis was conducted separately on temperature treatments (Fig S1). When droughted tree data was pooled from both temperature treatments, cumulative respiration was inversely correlated with sugar ($R = -0.30$, $p < 0.05$) and positively correlated with starch ($R = 0.35$, $p < 0.05$), but not correlated with total NSC ($p > 0.05$, Fig S1 and S2).

Discussion

Foliar carbohydrate resources were diminished during drought as foliar NSC declined by 30% through mortality (Fig 1, 2). This decline in the foliage of droughted trees was related to a reduction in sugar concentration, despite an increase in starch. Both droughted and watered trees had similar NSC at the start of the experiment, but as the growing season progressed, foliar NSC increased greatly for watered control trees. Increased starch concentration in watered trees was the primary driver of differences in total NSC between watered and droughted trees. Together, the results that

NSC declined for droughted trees through mortality, and was much lower in droughted trees than in watered controls suggests an important role for carbon metabolism in the physiological mechanism of mortality in this species. This should not be interpreted to diminish the potential role for hydraulic failure in the mortality mechanism, since droughted trees in both temperature treatments trees also reached the threshold for catastrophic xylem dysfunction (inferred as $\Psi_{pd} > -6$ MPa) prior to mortality (Adams et al. 2009a). However, there were no temperature differences in hydraulic function that predict the differences in time-to-mortality interpretable within a mechanistic framework.

Despite the link between cumulative respiration and reduced survival time under elevated, relative to ambient, temperatures, we did not observe an earlier reduction in NSC pools in elevated-temperature trees. While this may suggest that neither hydraulic failure, nor foliar carbon metabolism entirely explained the observed temperature-sensitive difference in time-to-mortality during drought, the change in the dynamics of the NSC components through time (decline in sugar, decline-followed-by-increase in starch through time) are indicative of shifts in carbon metabolism driven by temperature which are related to the ability of plants to maintain positive carbon status at the primary sites of metabolic activity (Adams et al., 2009a). Our ability to partition the physiological cause of mortality is limited by the overall experimental design and the assumption of patterns of translocation among other plant organs. Improved assessment of carbohydrate status could be made through sampling NSC in woody sink tissues (stems and roots) further from the sites of C fixation in the foliage (Landhäusser and Lieffers, 2012). However, sampling these tissues can cause cavitation and potentially

exacerbate tree stress, interfering with the primary objective of experimentation and the quantification of temperature differences in time-to-mortality. Creative experimental design will be required to further evaluate these metabolic and hydraulic processes.

Although we saw a 30% decline in foliar NSC in droughted trees, it was not completely depleted at mortality (Fig. 2). While the decline in total NSC was related primarily to a decline in sugar concentrations, just less than 50% of initial sugar still remained in the needles of droughted piñon pines at mortality. This result shows that although carbohydrate resources were diminished during drought, they were not entirely exhausted at mortality. Moreover, although starch initially declined in droughted trees, starch was higher at mortality than pre-drought levels, a result that does not appear consistent with carbon starvation by reserve exhaustion, but instead may reflect a lack of sugar translocation (Sala et al., 2010). The mechanism for feedback inhibition of photosynthesis leading to starch accumulation is fairly well known; foliar starch accumulation in the chloroplast occurs when sugar sink strength is low in the cytoplasm and RuBP regeneration is low (Kozlowski and Pallardy, 1997; Myers et al., 1999). Development of low sink strength in the droughted trees could have resulted from reduced phloem function, preventing the export of sugars to other tree tissues. Thus RuBP regeneration was limited by a lack of P translocator exchange with sugar, leaving starch synthesis as the only path available to any assimilated or re-assimilated C. Our observation of increased foliar starch concentration concurrent with the lack of complete sugar depletion could indicate that reduced C sink strength in other tree tissues due to insufficient water for growth lead to a decrease in phloem flow and sugar export from the

foliage as mortality approached. This result, highlights the role of mobilization, translocation and phloem function during the mortality process as high xylem tension disrupted whole-plant processes and affects C sink strength in non-foliar tissues (Sala et al., 2010, McDowell et al., 2011). Distinguishing between the causes of reduced C sink strength is a future challenge in resolving the mechanism of tree mortality.

Another potential explanation for the lack of complete depletion of foliar NSC could be related to maintenance of sugars for non-metabolic functions that can lead to the decoupling of carbohydrate pools from the external carbon fluxes (Hill et al., 2011; Ryan, 2011; Zeppel et al., 2011; Sala et al., 2012). In studies of subalpine conifer seedlings at treeline, photosynthesis and respiration only explained ~40-50% of the variation in NSC (Bansal & Germino 2008, 2009, 2010a), and NSC dynamics during the growing season were not attributed to growth (Bansal & Germino 2010b). Instead, researchers suspected environmental influences on active maintenance of sugars for cryoprotection were linked to NSC dynamics (see also Strimbeck et al., 2008). Sugars also aid in desiccation tolerance during drought through osmotic adjustment and stabilization of membranes and proteins, and their active maintenance for such protection in leaf tissue is well-recognized (Obendorf, 1997; Ogren et al., 1997; Nelson & Bartles, 1998; Murakozy et al., 2002; Oliver et al., 2011; Sergeant et al., 2011). In addition, the use of sugar for signaling of gene expression between tissues also contributes to its maintenance for non-metabolic functions (Koch, 1996; Chiou & Bush, 1998; Lalonde et al., 1999; Smeekens, 2000; Hill et al., 2011). An actively maintained threshold in sugar may have contributed to the lack of complete carbohydrate exhaustion in piñon pines (Fig. 1), despite respiration demands

and a lack of supply from photosynthesis (Fig. 3). Fully resolving the interrelated contribution of hydraulic failure and carbon starvation to drought mortality will require improved understanding of the active control of NSC (Fig. 3b). Without estimates of NSC thresholds for maintenance of osmoprotection and signaling, the contribution of carbon starvation to mortality may not be easily determined.

Previously published results from this experiment showed that trees in the warmer drought treatment died approximately 30% faster, and had higher cumulative respiration than the ambient-temperature droughted trees (Adams et al., 2009a). Therefore, we hypothesized that NSC would decline earlier in warmer droughted trees than in the ambient droughted trees. However there was no difference in total NSC and sugar decline between droughted temperature treatments, indicating that the respiration difference was not reflected in changes to foliar NSC, but likely due to a component that we did not have the ability to mass balance. Moreover, there was no temperature difference in NSC of watered control trees. The only significant difference in NSC trends between temperature treatments was that starch increased earlier for warmer than ambient droughted trees (Fig. 1), which could indicate earlier onset of mobilization constraints and phloem functional changes, as discussed above. Correlation of cumulative respiration with total NSC and components was weak, and only significant for starch in ambient droughted trees or when data were pooled across temperatures for sugar and starch (Fig. S1 and S2). Correlations with cumulative respiration were negative for sugar and positive for starch, consistent with droughted tree trends over time during the experiment (Fig. 1).

There were also no temperature differences in transpiration or photosynthesis as these fluxes declined to near-zero prior to mortality in droughted trees (Fig. 3). Therefore maintenance of equivalent foliar total NSC and sugar pools despite differences in respiration, but not photosynthesis, suggest changes to internal tree NSC dynamics. Potential explanatory processes include translocation of NSC between other tree tissues and leaves, and conversion of other carbon macromolecules into NSC (Hoch, 2007). Additionally, changes in the concentrations of other compounds within the leaf can cause NSC concentrations to shift even though absolute abundance of NSC remains constant (Bansal and Germino 2008). For these processes to explain similar NSC in all droughted trees during drought, they would have to occur differently between the ambient and warmer droughted trees. If the increase in foliar starch in droughted trees was the result of mobilization/phloem failure, then its earlier occurrence in warmer droughted trees could explain similar decline in sugar and total NSC despite respiration differences between droughted tree temperature treatments.

SEM of tree physiology during the experiment revealed that respiration was decoupled from photosynthesis for both watered controls and droughted trees at both temperatures. The positive interrelationship between Ψ_{pd} and g_s in watered control trees suggests that the physiology of these trees was sensitive to moisture, although A was not limited by g_s in ambient watered trees (Fig 4). Sugar content responded positively to increased A in warmer watered trees, but not in ambient watered trees. For droughted trees, the interrelationship between g_s and A was stronger than for watered controls. However, the lack of an effect of Ψ_{pd} on g_s in droughted trees is likely a result of the

linear nature of the model: low Ψ_{pd} had a strong threshold-like influence on g_s (Fig 3). Photosynthesis was positively related to sugar concentration in droughted trees at both temperatures. However, the inverse relationship between sugar and starch content observed in the repeated measures analysis of trends (Fig 1) was only significant for ambient droughted trees in the SEM analysis (Fig 4).

Expanding physiological insight into the mechanism of drought-induced tree mortality from carbon exchange dynamics, we show that foliar carbohydrate resources were diminished, but not completely exhausted, for piñon pine during drought through mortality. Our results and those of Adams et al. (2009a), illustrate that the mechanism of tree drought mortality can be complex, incorporating multiple interrelated physiological processes, but is consistently predicted from patterns of carbon metabolic response to temperature. Since xylem tensions in droughted trees in both of our temperature treatments simultaneously reached a threshold inferred to cause complete cavitation, hydraulic failure, while likely related to driving mortality, does not appear to explain the temperature response of these trees (Adams et al., 2009a). Rather, temperature sensitivity of mortality appears related to differences in cumulative respiration. Although we did not detect temperature differences in the decline, foliar NSC was reduced by 30% in droughted trees, while NSC increased in watered control trees, results which also support a role for carbon metabolism in the drought mortality mechanism of these trees. Additionally, the functional dynamics of starch depletion and accumulation by treatment highlight temporal differences in carbon metabolism potentially driving mortality sensitivity to temperature. We acknowledge that our results were limited by constraints

in our experimental design which led to a lack of NSC assessment in stems and roots, where carbon starvation by reserve exhaustion is more likely (Landhäusser and Lieffers, 2012). Further physiological resolution of drought-induced mortality mechanisms will likely require increased experimental sophistication to determine active NSC maintenance, account for NSC translocation among tissues, link temperature sensitivity to physiological mechanism, and assess trends in whole plant NSC during drought through mortality. Consequently, temperature sensitivities need to be further evaluated for more tree species while simultaneously pursuing drought-induced mortality mechanisms. Nonetheless, our results support and refine the conclusions of Adams et al. (2009a) of a role of carbon metabolism in drought mortality, at least for *Pinus edulis* and perhaps in other physiologically similar species.

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Figure Legends

Figure 1. Foliar non-structural carbohydrates for piñon pines during the experiment for trees under ambient (blue) and warmer ($\sim+4^{\circ}\text{C}$, red) temperatures that received no water (drought, circles) or continued to be irrigated (controls, squares). Mean values for total non-structural carbohydrates (NSC) and component soluble sugar and starch content are shown on the left, quantified as a percentage of total tissue dry mass. Droughted tree differentials, calculated by subtracting droughted tree values from watered tree means, for total NSC and components are shown on the right as percentage points. Asterisks indicate significant differences in repeated measures ANOVA ($p < 0.05$) between the watered control and drought treatment means. Error bars are standard errors.

Figure 2. Mean foliar non-structural carbohydrates (NSC) at the start of the experiment and at mortality for trees under ambient and warmer ($\sim+4^{\circ}\text{C}$) temperature treatments. Error bars are standard errors for total NSC. At both temperatures, total NSC and sugar concentrations were lower at mortality than initially, while starch content was higher at mortality than initially ($p < 0.05$). There were no temperature differences in this analysis ($p > 0.05$).

Figure 3. Physiological responses of piñon pines during the experiment for trees under ambient (blue) and warmer ($\sim+4^{\circ}\text{C}$, red) temperatures that received no water (drought, circles) or continued to be irrigated (controls, squares). Responses are shown for pre-

dawn stem water potential (Ψ_{pd}), leaf stomatal conductance (g_s), mid-day net photosynthesis (A), and pre-dawn respiration (R). Error bars are standard errors. For droughted trees only, Ψ_{pd} , A, and R data were previously published in Adams et al. (2009a).

Figure 4. Structural equation modeling of the relationships between pre-dawn water potential (Ψ_{pd}), stomatal conductance (g_s), net photosynthesis (A), sugar and starch content, and pre-dawn respiration (R). Relationships are shown for watered control trees and droughted trees at ambient and elevated ($\sim+4^\circ\text{C}$) temperatures. Values alongside arrows are standardized partial correlation coefficients which indicate the strength and direction of relationships. Positive relationships are indicated with solid lines and inverse relationships with dashed lines. Values in italics adjacent to variables indicate the error terms of the multiple regression in which that variable was the dependent variable. Significant partial correlation coefficients are shown adjacent to arrows ($p < 0.05$).

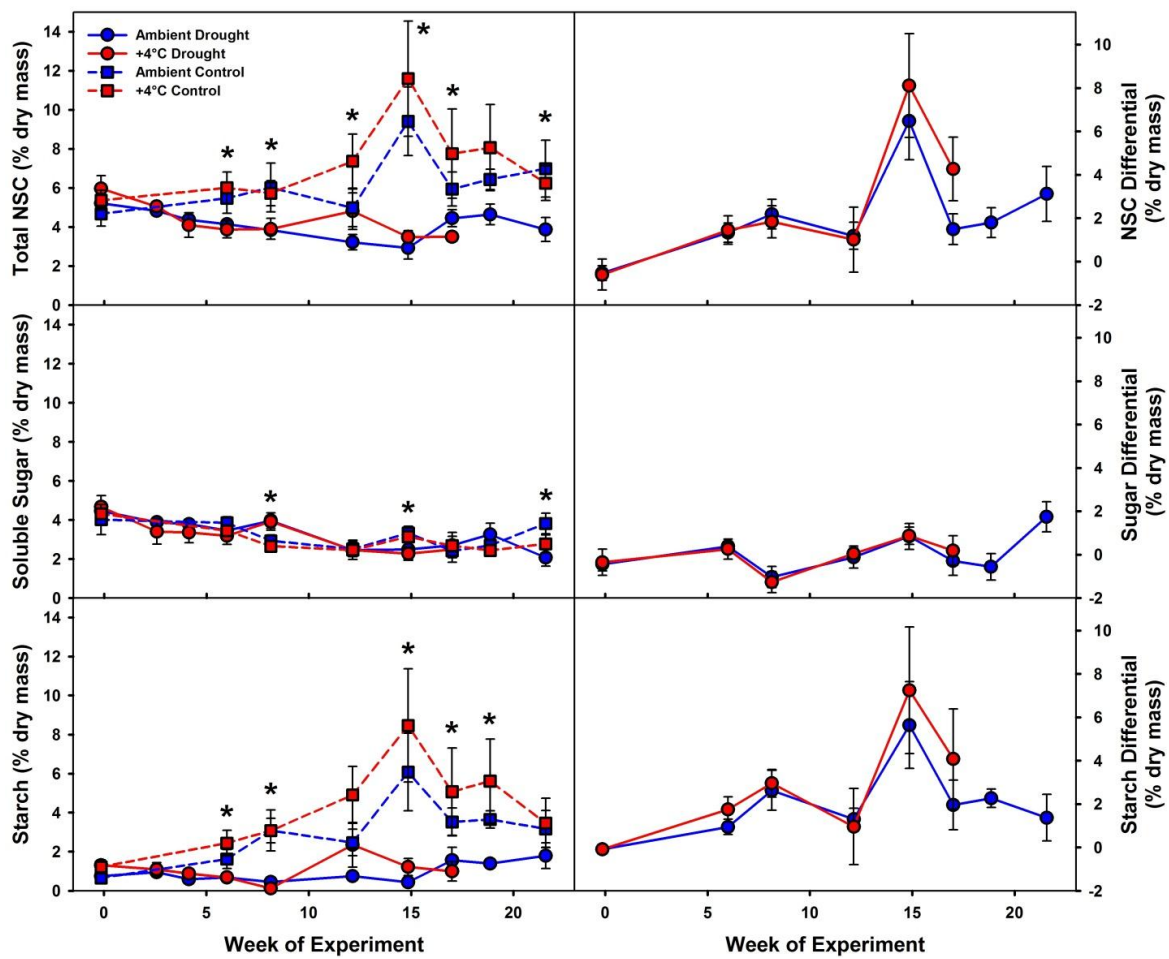


Figure 1.

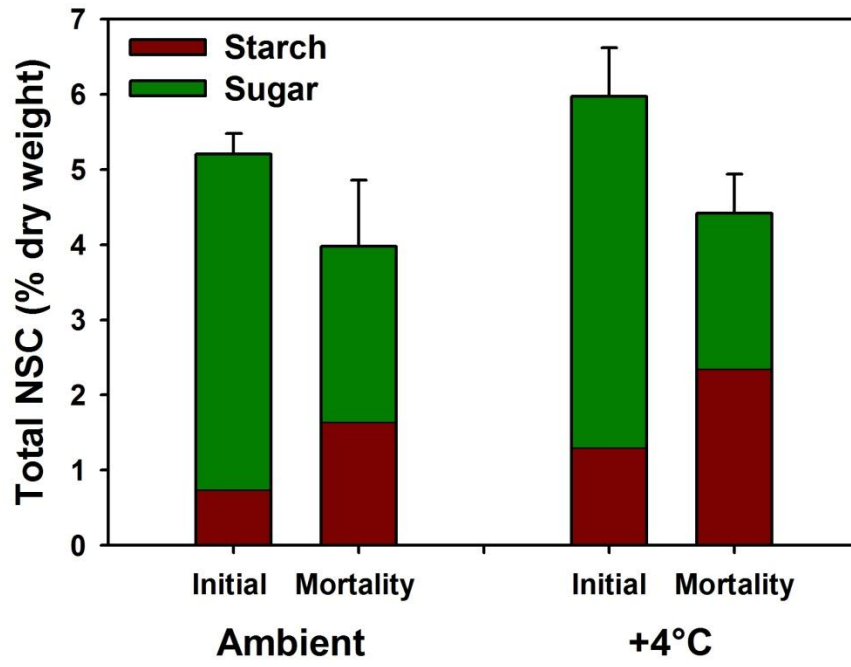


Figure 2.

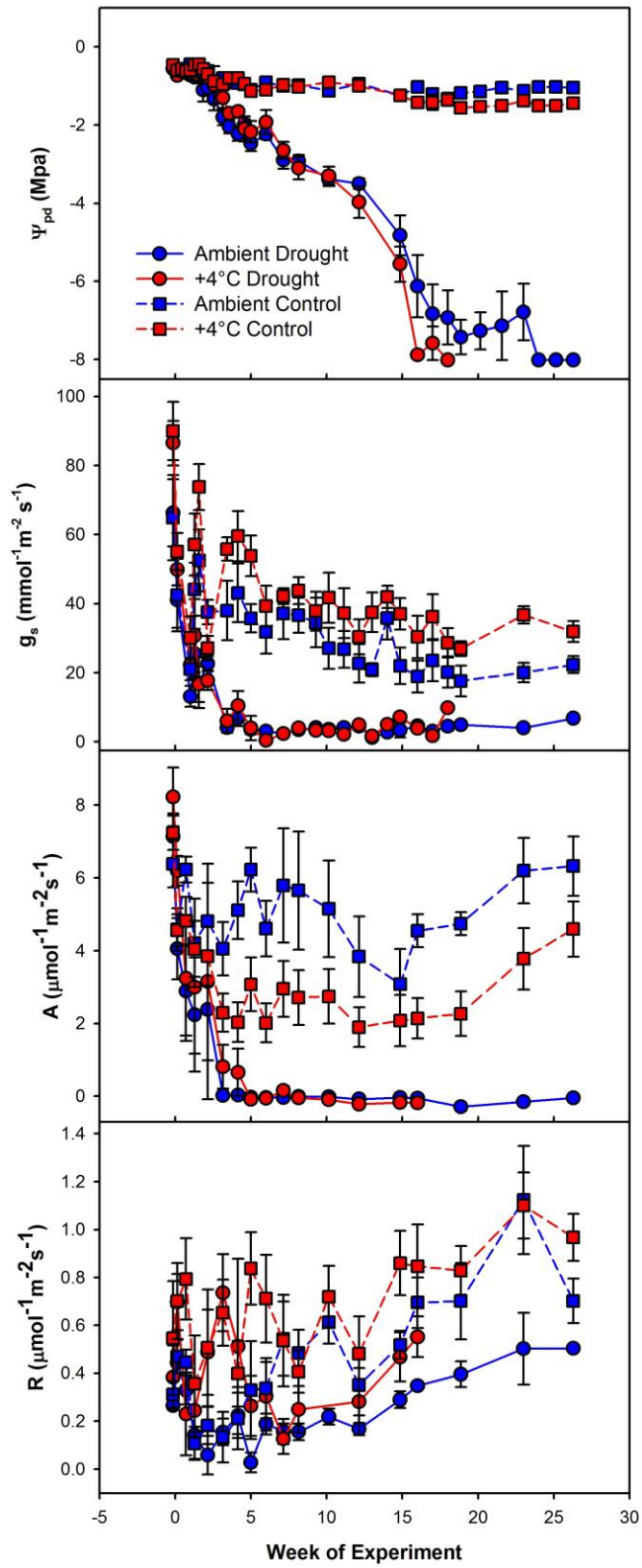


Figure 3.

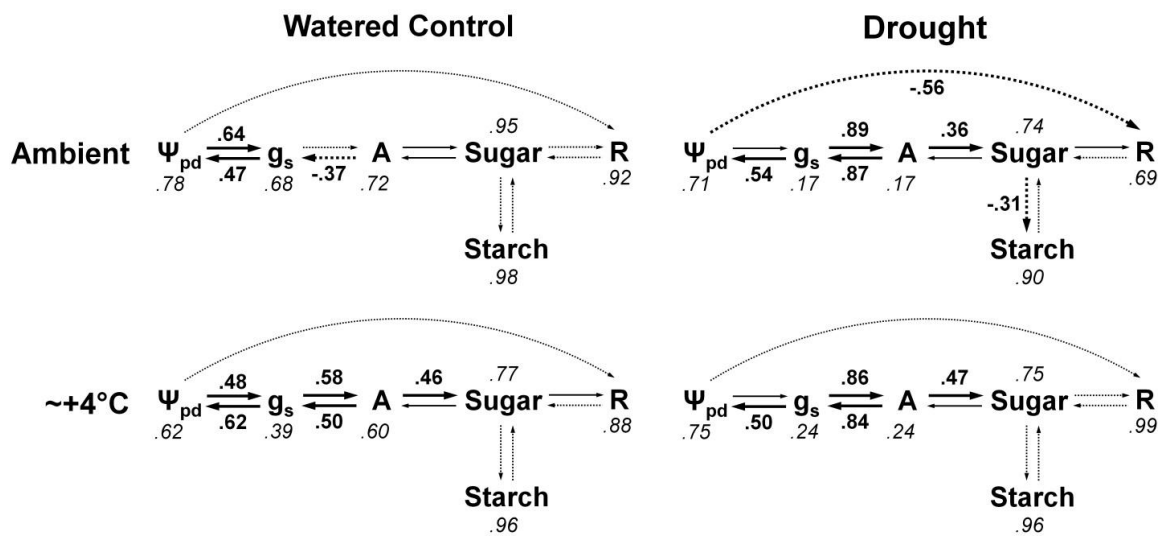


Figure 4.

Supporting Information

Materials and Methods

We analyzed non-structural carbohydrates following the methods of Hoch et al. (2002). We ground the needles to a fine powder, added 2 ml water to a ± 0.1 mg subsample, heated to 100°C with steam for 30 min to extract soluble sugars, and then treated each with invertase, phosphoisomerase, and glucose hexokinase to convert sucrose, fructose, and glucose to 6-Phosphogluconate (Sigma Diagnostics, St. Louis, MO, USA). Oxidation of the soluble sugars to 6-Phosphogluconate resulted in an equimolar reduction of NAD to NADH, increasing absorbance of the solution at 340 nm, which was measured using a spectrophotometer (Synergy Microplate Reader, Bio-tek Instruments, Winooski, VT, USA). The original sample (powder plus water) was then additionally treated with a high activity fungal alpha amylase from *Aspergillus oryzae* (Clarase G-Plus, Genecore International, Rochester, NY, USA) to metabolize starch to glucose. The solution was re-analyzed for total NSC (starch and soluble sugars) with the procedure described previously. Starch was calculated as total NSC minus soluble sugars. Each trial plate run included duplicates of each sample, standards included pure starch, sucrose, glucose, as well as internal plant standards that had known starch ranging from zero to 50% dry mass and had been cross-referenced to other labs. All NSC data were normalized for dry mass of needle tissue.

Structural equation modeling (SEM) has been used in ecology to analyze species interactions (Schemske & Horvitz, 1988), functional life history trade-offs across plant species (Shipley et al., 2006), the effect of climate variation on net ecosystem exchange

(Huxman et al., 2003), and relationships among water potential, nutrient content, stomatal conductance and photosynthesis (Geber & Dawson, 1997; Bassow & Bazzaz, 1998; Ignace & Huxman 2009). For our model, we integrated relationships in plant physiology where causality is well-established: between water potential and stomatal conductance (Kriedemann et al., 1972; Farquhar & Sharkey, 1982; Nobel 1991), stomatal conductance and photosynthesis (Farquhar & Sharkey, 1982; Farquhar et al., 1982), photosynthesis and carbohydrate pools (Wardlaw, 1990; Luxmoore et al., 1995; Körner, 2003), carbohydrates and respiration (Wardlaw, 1990; Luxmoore et al., 1995), and water potential and cell growth (Lockhart 1965; Kroeger et al., 2011; Tardieu et al., 2011). Dependent and independent variables, including pre-dawn water potential (Ψ_{pd}), stomatal conductance (g_s), net photosynthesis (A_n), sugar content, starch content, and pre-dawn respiration (R), were used in the multiple linear regressions used to construct the structural equation model as follows (dependent variable with independent variable[s]): Ψ_{pd} with g_s ; g_s with Ψ_{pd} , A_n ; A_n with g_s and Sugar; Sugar with A_n , Starch, and R; Starch with Sugar; and R with Sugar and Ψ_{pd} .

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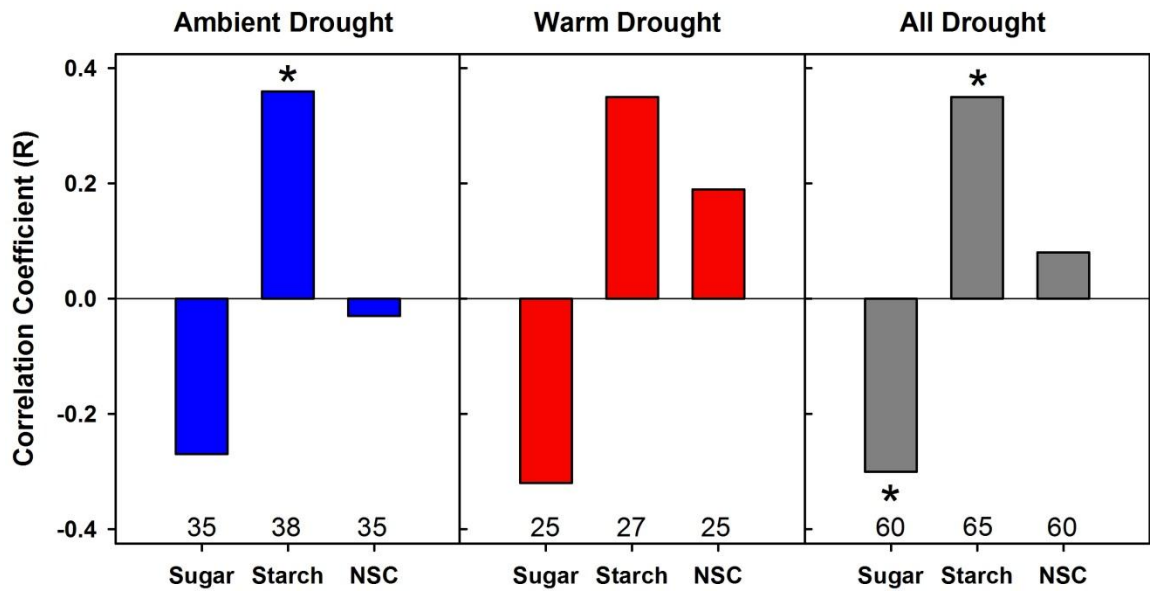


Figure S1. Correlation of Cumulative respiration with total non-structural carbohydrates and component sugar and starch concentration for ambient, warm (~+4°C), and all droughted trees pooled from both temperatures. Significant relationships are indicated with an asterisk. Numbers below bars indicate sample size.

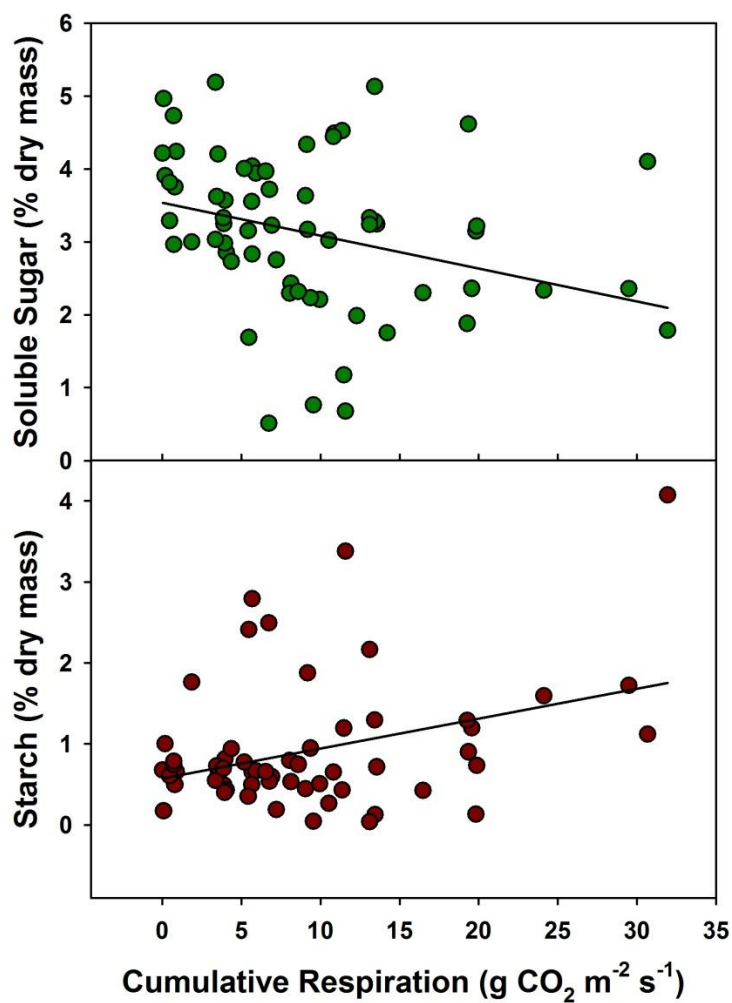


Figure S2. The relationship between cumulative time-integrated respiration and foliar sugar and starch concentrations for droughted trees at both ambient and elevated ($\sim+4^{\circ}\text{C}$) temperatures. Statistics for these relationships are shown in Figure S1.

APPENDIX E: DROUGHT-INDUCED PINUS SEEDLING MORTALITY HASTENS
PROGRESSIVELY WITH TEMPERATURE AS REFLECTED IN RESPIRATION
DURING STOMATAL CLOSURE

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Abstract

Extensive tree die-off in forested ecosystems has recently been documented around the world in association with drought and warmer temperatures. Such events are expected to become more frequent with future climate change, potentially altering biogeochemical and hydrologic cycles, with implications for the land-surface feedback on climate and ecosystem services for society. Seedling establishment is a key demographic process, and its response to drought and temperature changes will be a critical determinant of the long term consequences of such forest mortality events. However, the temperature sensitivity of drought-induced mortality in seedlings to a range of higher temperatures has not been widely evaluated. Here we used growth chambers to experimentally assess the temperature sensitivity of drought-induced mortality for tree seedlings of two species—the more drought-tolerant *Pinus edulis* and the less drought-tolerant *Pinus ponderosa*, both of which have experienced recent, large-scale tree die-off. Drought-induced mortality in both pine species was progressively affected by increased

temperature, accelerating time-to-mortality during drought by 4.4 % per °C for *P. edulis* and 3.6 % per °C for *P. ponderosa* across 4 temperature treatments spanning a range of 7.7°C. Notably, while *P. edulis* showed greater drought resistance, outlasting *P. ponderosa* at all temperatures, *P. edulis* also demonstrated a higher degree of within-species temperature- sensitivity to drought than *P. ponderosa*. For both species, earlier mortality during drought was associated with a greater effect of temperature on respiration, particularly during the period of stomatal closure prior to mortality. Under elevated temperatures, more frequent, shorter droughts become sufficient to increase the risk tree mortality such that episodes of seedling mortality are expected to increase from future climate warming alone, even without an increase in drought frequency. This vulnerability of seedlings to drought at higher temperatures suggests that seedlings may not offset adult mortality and that woodland and forest communities may undergo substantial future changes.

Introduction.

Increased tree mortality due to drought, elevated temperatures, pest and/or pathogen infestation is emerging as a growing concern regarding the potential response of the terrestrial biosphere to contemporary climate change. Episodes of tree mortality have now been documented in many natural and managed forests of the world (Allen et al. 2010). Elevated tree mortality has been observed from the tropics to high latitudes, in both wildland and agroforestry settings, associated with increased rates of background mortality (van Mantgem et al., 2009, Carnicer et al., 2011, Peng et al. 2011), infestations

that only affect a single tree species in a diverse forest (Ford and Vose, 2007), and widespread landscape and regional die-off of a majority of dominant canopy trees (Allen et al., 2010, Hicke et al., 2012). Extensive tree die-off has affected 600,000 km² of *Pinus* species across western North America, including 12,000+ km² of *P. edulis* in the Southwest US, and 50,000 km² of *Eucalyptus* species in Australia in diverse landscape settings (Breshears et al., 2005, Bentz et al., 2009, Fensham et al., 2009, Allen et al., 2010). Tree mortality has exceeded 90% loss of overstory individuals of affected species at some sites in each of these die-offs. The ecological impact of these changes in forest structure has implications for ecosystem services and land-surface dynamics that affect climate.

The small and large-scale consequences of tree mortality depend on the direction and trajectory of the demographic response following this disturbance (Suarez and Kitzeberger 2008, Brown et al., 2010, Hicke et al., 2012, Adams et al., 2012). Tree mortality that exclusively affects overstory trees and leaves understory components unaffected potentially facilitates rapid recovery to pre-mortality forest structure and function (Axelson et al., 2009, 2010, Brown et al., 2010, Collins et al., 2011). On the other hand, if the recruitment process is also affected by the same climate- or insect-related perturbation that kills overstory trees, the ecosystem consequences of tree mortality may be long-lasting (Mueller et al., 2005, Gitlin et al., 2006). The wide range of possible community responses to tree die-off, which includes recovery of the pre-mortality forest, shifts in composition, and conversion to non-forest, that will ultimately determine the persistence of mortality effects, highlights the need to evaluate the

vulnerability of seedlings to the disturbances that cause overstory die-off (Suarez and Kitzberger, 2008).

The severity of plant mortality that occurs during drought has been linked to the temperature dependence of ecophysiological and ecohydrological processes. Observation of drought effects in the southwestern US found that a regional drought in the late 1950s caused only localized tree mortality at the dry, lower forest ecotones (Allen and Breshears, 1998), but a similar drought during a period of unusually high temperatures was associated with a widespread die-off of *P. edulis* (Breshears et al., 2005) that was reflected in substantial and protracted water stress in affected trees (Breshears et al., 2009). A subsequent drought experiment found that a temperature increase of $\sim 4.3^{\circ}\text{C}$ caused a 28% acceleration of time-to-mortality with ~ 20 year-old *P. edulis* trees (Adams et al., 2009). Similarly, an experiment with Australian *Eucalyptus sideroxylon* demonstrated that a $\sim 4^{\circ}\text{C}$ increase in temperature caused trees to die $\sim 15\%$ earlier during lethal drought (Chaszar et al., *in review*). Increasing background mortality rates observed in western North America in recent decades were associated with increasing temperatures and drought (van Mantgem et al., 2009). Despite the important influence of temperature on tree mortality, its effects on tree survival during drought have only been studied in a few species. Additionally, studies of tree mortality across a range of elevated temperatures are critically needed.

We experimentally assessed the temperature sensitivity of time-to-mortality and associated ecophysiological processes under drought across a range of temperatures for two widespread species of pine seedlings, *P. edulis* and *P. ponderosa*. We hypothesized

seedling drought mortality would occur sooner with warming, and that since *P. edulis* and *P. ponderosa* co-occur in the southwest US at the lower forest boundary ecotone between the piñon-juniper woodland and ponderosa pine forest, *P. edulis* would persist longer during drought than *P. ponderosa* in a common setting. However, despite *P. edulis* and *P. ponderosa* differing in their ecological resistance to drought (evidenced by their phytogeography), both have a similar drought tolerance strategy of reducing stomatal conductance to avoid cavitation until wetter conditions return (Piñol and Sala, 2000, Simonin et al., 2007, McDowell et al., 2008, 2010, 2011). For such species, drought-induced mortality has been hypothesized to occur predominately by ‘carbon starvation’, whereby reduced stomatal conductance curtails photosynthesis and leads to death when internal carbon resources are exhausted in (or become inaccessible to) maintenance respiration (McDowell et al. 2008), recognizing that this response may be interrelated with hydraulic failure associated with water stress (McDowell et al. 2011). Elevated temperatures should increase respiration rates, potentially hastening death by this mechanism (Adams et al., 2009). We hypothesized that time to mortality would hasten with magnitude of increase in temperature. In addition, we hypothesized that species differences in time-to-mortality during lethal drought across a range of temperatures would be a function of initial physiological responses to elevated growth temperature, prior to the onset of drought; specifically, that reduced photosynthesis and increased respiration, driven by elevated temperatures during drought would lead to earlier mortality. In addition, we hypothesized that larger seedlings would show reduced

survival during drought, consistent with field observations of higher mortality of larger *P. edulis* seedlings (Mueller et al., 2005).

Materials and Methods.

We obtained pine seedlings in “cone-tainers” (height 21 cm, volume: 175 ml) of two species (*P. edulis* and *P. ponderosa*) from the Colorado State Forest Service Nursery (Fort Collins, CO) in March 2010. Seedlings were kept in growth chambers (Conviron, Winnipeg, Manitoba, Canada) at 25° C during the day and 10° C at night under ~600 $\mu\text{mol m}^{-2}\text{sec}^{-1}$ PPFD of PAR, a slightly higher than mean daily PPFD of ~430-450 $\mu\text{mol m}^{-2}\text{sec}^{-1}$ observed in a *P. ponderosa* forest (Dore et al., 2010). We transplanted seedlings into treepots (height: 36 cm, volume: 2.8 L, Stuewe & Sons, Tangent, OR) in September 2010 with potting soil (Sunshine Mix #4, SunGrow Horticulture, Bellevue, WA). In December 2010, we placed 7 randomly selected seedlings of each species, under each of 4 temperature treatments. Ambient temperatures used in the experiment were modified from data for a piñon-juniper woodland / ponderosa pine forest ecotone, using average daily maximum and minimum temperatures from early July 2009 and 2010 that were obtained from a station located in a piñon-juniper woodland near Valle, Arizona. To adjust daily temperatures to the higher, cooler ecotone conditions we used the difference in monthly maximum and minimum temperature between the station data and the mean of 20 years of interpolated July PRISM data (<http://www.prism.oregonstate.edu>, PRISM group, Corvallis, OR) for a point at the nearby ecotone, a reduction of ~2.4°C (25/11°C day/night). This resulted in an ambient treatment of 25/11°C day/night. The three

elevated temperature treatments were set above this baseline, resulting in treatments elevated by +3.6, +5.9, and +7.7°C for day and night.

Seedlings were watered to saturation for the last time on April 4, 2011, after which water was withheld. For a designated subset of seedlings, 3 per species per temperature treatment, we repeatedly measured CO₂ and H₂O gas exchange, before dawn (respiration) and mid-day (net photosynthesis, stomatal conductance), weekly on the same set of seedlings with an LI-6400 Portable Photosynthesis system fitted with a 6400-22L lighted conifer chamber (LiCor, Lincoln, NE). Measurements were corrected by projected total removed needle area of measured foliage harvested following the conclusion of the experiment. We calculated the period of stomatal closure, the number of weeks when mid-day stomatal conductance was < 0.01 mol m⁻²sec⁻¹ prior to mortality, and the mean respiration rate during this period (R_s), for each seedling.

Seedling foliage color was checked 3 times per week, and seedlings were declared dead when we observed 90% foliar browning, following Adams et al. (2009). Foliar browning typically took 1-2 weeks from initiation before reaching the 90% threshold. Survival data was inadvertently lost for 2 *P. ponderosa* pine seedlings, 1 each in the ambient and +3.6°C treatments, reducing sample size in each of these treatments to 6 seedlings for *P. ponderosa*. We harvested dead seedlings, separating foliar, aboveground woody (stem), and belowground woody (root) tissues. All material was dried at 70°C for 72 hours before determining dry mass. Mean seedling total dry mass was 7.6g (0.42 SE) for *P. edulis* and 8.1 g (0.34 SE) for *P. ponderosa*.

Data were analyzed using ANOVA and repeated measures ANOVA using IBM SPSS Statistics (SPSS, Inc., Amonk, NY) and Fisher's LSD for post-hoc testing. In three cases (time-to-death, initial respiration, and R_s) where ANOVA conditions of equal variance were violated (Levene's test, $p < 0.05$), we used regression to analyze for species differences across temperature treatments and analyzed for species x temperature interaction with a test for common slope in SMATR (Falster et al., 2006, Warton et al., 2006).

Results.

Patterns of mortality

Elevated temperature progressively reduced time-to-mortality during drought for both pine species across the entire temperature range studied ($R^2 = 0.29$, $F_{1,52} = 21.648$, $p < 0.001$, Fig. 1). Across temperature treatments, *P. edulis* seedlings persisted longer than *P. ponderosa* seedlings ($F_{1,52} = 21.648$, $p < 0.001$). However, a test for common slope revealed that time-to-mortality for *P. edulis* declined faster across increasing temperatures than that for *P. ponderosa* ($F_{1,52} = 5.294$, $p < 0.05$), a result analogous to a significant temperature x species interaction in an ANOVA. Mean acceleration of time-to-mortality (relative to control) with rising temperature across all treatments was 4.4 %/°C for *P. edulis* and 3.6 %/°C for *P. ponderosa*.

Initial pre-drought physiological patterns

Mean initial pre-drought stomatal conductance, one week after watering, ranged between $0.04 - 0.10 \text{ mol m}^{-2}\text{sec}^{-1}$ for *P. edulis* and $0.04 - 0.11 \text{ mol m}^{-2}\text{sec}^{-1}$ for *P. ponderosa* and was higher at higher temperatures ($F_{3,16} = 4.366$, $p < 0.05$, Fig. 2A). When pooled across temperature treatments there was no species difference in initial conductance ($F_{1,16} = 0.130$, $p > 0.05$), nor was there a significant species x temperature interaction ($F_{3,16} = 0.392$, $p > 0.05$). Mean initial pre-drought net photosynthesis was $4.4 - 5.6 \mu\text{mol m}^{-2}\text{sec}^{-1}$ for *P. edulis* and $2.6 - 4.7 \mu\text{mol m}^{-2}\text{sec}^{-1}$ for *P. ponderosa*, and did not vary across the four temperature treatments when species were pooled ($F_{3,16} = 5.486$, $p > 0.05$; Fig. 2B). Species differences were significant, where initial net photosynthesis was greater in *P. edulis* than *P. ponderosa* for the ambient, $+3.6^{\circ}\text{C}$, and $+5.9^{\circ}\text{C}$ treatments ($F_{1,16} = 15.935$, $p < 0.01$) by 40 %. There was no significant temperature x species interaction in initial net photosynthesis ($F_{3,16} = 3.468$, $p > 0.05$). In contrast, there was no species difference in initial respiration rate ($F_{1,16} = 2.275$, $p > 0.05$) and a test for common slope indicated no significant interaction between temperature and species prior to the drought ($F_{1,22} = 0.071$, $p > 0.05$). Mean initial pre-drought respiration was varied from $0.37 - 0.90$ in *P. edulis* and $0.39 - 0.96$ in *P. ponderosa* and was higher with increasing temperature ($R^2 = 0.41$, $F_{1,22} = 15.006$, $p < 0.01$, Fig. 2C). From measurements of CO_2 exchange, a complex needle carbon balance was associated with the different species and temperature treatments—with the difference in net photosynthesis and respiration being the greatest for *P. edulis* at ambient temperatures and least for *P. ponderosa* at the highest temperature treatment. Additionally, from stomatal conductance differences and the patterns in leaf carbon assimilation and

respiration, highly individualistic water-use efficiencies resulted between treatments that were greatest for *P. edulis* in the +5.9°C temperature treatment and lowest for *P. ponderosa* in the +3.6°C treatment.

Physiological dynamics during drought

During drought, stomatal conductance declined to near zero for all trees prior to mortality ($F_{7,112} = 30.128$, $p < 0.001$), and this decline occurred more rapidly for seedlings at higher temperatures ($F_{21,112} = 2.293$, $p < 0.001$, Fig. 3). The rate of decline was similar for both species ($F_{7,112} = 1.52$, $p > 0.05$) and there was no significant time x temperature x species interaction ($F_{21,112} = 0.539$, $p > 0.05$). Additionally, there were no overall differences in stomatal conductance between species ($F_{1,16} = 0.438$, $p > 0.05$) or among temperatures ($F_{3,16} = 3.043$, $p > 0.05$).

Net photosynthesis also declined to near zero for all trees during drought, prior to mortality ($F_{7,112} = 43.741$, $p < 0.001$) and they declined more rapidly for seedlings under elevated temperatures ($F_{21,112} = 4.007$, $p < 0.001$, Fig. 3). Across the time-course, photosynthetic rates were higher in *P. edulis* than *P. ponderosa* ($F_{1,16} = 16.61$, $p < 0.05$) but overall temperature differences were not significant ($F_{3,16} = 0.293$, $p > 0.05$). There were no significant time x species ($F_{7,112} = 0.908$, $p > 0.05$) or time x temperature x species interactions ($F_{21,112} = 0.925$, $p > 0.05$), such that the difference in assimilation patterns of might strongly influence needle carbon balance. Respiration rates declined during the lethal drought for all seedlings ($F_{7,112} = 17.512$, $p < 0.001$), and, notably, did so more rapidly with increased temperature ($F_{21,112} = 1.839$, $p < 0.001$, Fig 3.). This

decline in respiration did not differ between species ($F_{7,112} = 1.286$, $p > 0.05$), nor was there a significant time x temperature x species interaction ($F_{21,112} = 0.828$, $p > 0.05$). Generally, respiration rates were higher at higher temperatures ($F_{3,16} = 12.799$, $p < 0.001$), but there was no overall species effect ($F_{1,16} = 3.451$, $p > 0.05$). As such, the initial conditions associated with seedling behavior as a function of temperature and species differences at the start of the experiment was a good descriptor of the overall dynamics associated with physiological processes during the period of mortality, as we hypothesized.

Relating temperature, time-to-mortality, ecophysiology and seedling characteristics

Length of the pre-mortality period of stomatal closure (stomatal conductance $< 0.01 \text{ mol m}^{-2}\text{sec}^{-1}$) was highly dependent on temperature, decreasing as temperature increased ($F_{3,16} = 5.726$, $p < 0.01$, Fig. 4a). The duration of this period in *P. edulis* seedlings was longer than in *P. ponderosa* seedlings ($F_{1,16} = 13.057$, $p < 0.01$), yet there was no significant interaction between species and temperature ($F_{3,16} = 0.135$, $p > 0.05$). Mean needle R_s increased with growth temperature ($R^2 = 0.32$, $F_{1,22} = 8.698$, $p < 0.01$, Fig 4b.) and was higher in *P. ponderosa* than in *P. edulis* ($F_{1,22} = 8.554$, $p < 0.01$). Additionally, there was no difference between species in the slope of R_s across temperatures ($F_{1,22} = 0.254$, $p > 0.05$). Time-to-mortality decreased with increasing R_s across seedlings at all temperatures ($R^2 = 0.65$, $F_{1,22} = 40.952$, $p < 0.001$) and this relationship did not differ between species ($F_{1,22} = 0.92$, $p > 0.05$, Fig. 4c). Thus, differences in carbon loss by respiration during this period of intense stress described

differences in seedling drought tolerance dynamics and predicted the associated differences in known drought resistance based on phytogeography, as we hypothesized.

Interestingly, although total dry seedling mass did not differ between species ($F_{1,46} = 1.763$, $p > 0.05$) or among temperature treatments ($F_{3,46} = 1.763$, $p > 0.05$), *P. ponderosa* seedlings with more mass, whether in foliage, aboveground, or total, died faster during drought ($R^2 = 0.39, 0.37, 0.30$, $F_{1,24} = 15.179, 14.005, 10.339$, $p < 0.01$; Table 1).

Discussion.

Seedling time-to-mortality for both *P. edulis* and *P. ponderosa* was highly sensitive to elevated temperature during experimentally induced drought (Fig. 1): time-to-mortality declined across a wide range of increased temperatures, and, importantly, each progressive increase in temperature exacerbated drought stress in the seedlings. These results are consistent with previous work using larger trees, which showed significant temperature sensitivity of physiological processes and time-to-mortality (Adams et al., 2009, Chaszar et al., *in review*). The current study is notable in that it is perhaps the first to show that temperature-sensitive reductions in time-to-mortality during drought continue to progress across a range of elevated temperatures, whether for seedlings or trees. Additionally, our results provide insight into the linkages between ecological patterns and ecophysiological processes. *P. ponderosa* seedlings were more susceptible to drought than *P. edulis* seedlings across all temperatures, consistent with their geographical ranges and observations of greater drought tolerance in *P. edulis*

(Adams and Kolb, 2004) and higher tree mortality at the lower forest boundary in *P. ponderosa* during the 1950s drought (Allen and Breshears 1998). Despite having similar physiological drought tolerance strategies, differences in carbon metabolism associated with temperature provided insight into temperature and species differences in time-to-mortality.

Although both species exhibited progressive temperature sensitivity in time-to-mortality as hypothesized, surprisingly time-to-mortality declined more with increasing temperature for the more-drought tolerant *P. edulis* than for the less drought-tolerant *P. ponderosa* (Fig. 1). This indicates that although seedling drought resistance was greater in *P. edulis* than *P. ponderosa*, drought mortality of *P. edulis* was more temperature-sensitive than that of *P. ponderosa*, and implies species differences in compensatory dynamics of temperature and drought stress resistance. A compensatory response in adult trees would be consistent with observations of similar mortality (~15%) of co-occurring *P. edulis* and *P. ponderosa* at the lower forest boundary ecotone following the 2002/2003 drought (Koepke et al., 2010), a drought notable for its high temperatures (Breshears et al., 2005, Weiss et al., 2009).

As hypothesized, the temperature sensitivity of gas exchange under well-watered conditions was predictive of drought resistance difference of the two pine species and of the differences in time-to-mortality between them (Fig. 2). One week after watering, net photosynthesis of *P. ponderosa* was lower than that of *P. edulis*, and was highly sensitive to temperature. Initial stomatal conductance increased in seedlings of both species with temperature (Fig. 2). When stomatal conductance and photosynthetic rates are

considered together, two responses emerge: a lower water-use efficiency (WUE) in *P. ponderosa* than *P. edulis*, and that WUE in *P. edulis* was decreased with increased temperature, but that of *P. ponderosa* was not. Both responses are predictive of *P. edulis* having a higher drought resistance but greater sensitivity of mortality to temperature. Initial respiration also increased similarly for both species with increasing temperature, and when considered with photosynthetic response, indicated the potential for greater C balance stress in *P. ponderosa* (Fig. 2). These differences and sensitivities to temperatures were consistent with shifts in C metabolism as a key driver of differential mortality during drought (McDowell et al., 2008), likely in conjunction with interrelated hydraulic stress (McDowell et al. 2011), both within and among treatments and species.

Trends in gas exchange during the drought demonstrated a similar drought tolerance strategy and a high degree of temperature sensitivity in both pine seedlings (Fig 3). Seedlings reduced stomatal conductance and photosynthesis to near-zero during drought, consistent with observations of drought response in larger trees (Piñol and Sala, 2000, Simonin et al., 2007, Adams et al., 2009, McDowell et al., 2011). Although respiration declined during drought, consistent with a reduction in growth metabolism (Ayub et al. 2011), the decline in photosynthetic rate was greater, leading to a negative carbon balance in seedlings prior to mortality. Stomatal conductance, photosynthesis, and respiration started higher, declined faster, and finished lower for seedlings at higher temperatures (Fig. 3). The duration of the stomatal closure period was substantially shorter with increased temperature, and between species, with *P. edulis* outlasting *P. ponderosa* by 4.3 weeks on average (Fig 4a). Respiration during stomatal closure was

low, but still temperature-sensitive and much higher for *P. ponderosa* than *P. edulis*. Notably, at higher temperatures across species, reduced persistence at stomatal closure was associated with higher respiration rate during this period (Fig 4a, b), a result consistent with a role for C balance in the mortality of both species (McDowell et al., 2008, Adams et al., 2009, McDowell et al., 2011). Moreover, higher respiration during stomatal closure was correlated with a reduced time-to-mortality in both species (Fig 4c).

Seedling survival and gas exchange were highly sensitive to temperature during drought, demonstrating that seedlings are at least as vulnerable to same climate-related die-off that affected overstory trees of these species (Allen and Breshears 1998, Breshears et al. 2005, Mueller et al., 2005, Gitlin et al., 2006) and perhaps others (Suarez and Kitzberger, 2008, Galiano et al., 2010). Following the 2002/2003 drought and bark beetle outbreak in the southwest US, surveys showed mortality in ~25-80% of adult and ~50-80% of seedling *Pinus ponderosa* (Gitlin et al., 2006) and where ~80% of overstory *P. edulis* trees died, in ~25-45% of *P. edulis* seedlings (Mueller et al., 2005). Shading by overstory nurse trees reduces microsite temperature and is important for seedling establishment and survival in drought-prone forests (Callaway et al., 1996, Mueller et al., 2005). In northern Arizona, after the 1996 drought ~2% of nursed and ~20% of open-growing *P. edulis* seedlings were killed, and after the 2002/2003 drought mortality was ~25% of nursed and ~45% of open-growing seedlings (Mueller et al., 2005). Overstory mortality increases the solar radiation reaching the understory and land surface, a loss of shading facilitation that increased temperatures in the understory, and could exacerbate seedling stress in subsequent droughts (Royer et al., 2010, 2011, 2012). This would be in

addition to the temperature rises expected with global change, increasing the vulnerability of seedlings in drought-prone regions. In such communities, recovery of pre-mortality tree species composition and stand structure could be inhibited by lack of overstory shading, potentially with persistent ecosystem consequences.

Since elevated temperatures reduce time-to-mortality, future temperature increases with global change can be assumed to reduce the duration of a regional drought sufficient to cause significant mortality. In a similar study, reduced time-to-mortality in larger *P. edulis* trees from a 4.3°C temperature increase was used to project a 5-fold increase in the number of regional droughts per century that were sufficient to cause widespread die-off (Adams et al., 2009). Following the same methods, we calculated a 100-year frequency distribution of regional drought duration using precipitation data from stations at the southwest US, lower forest boundary ecotone (Western Regional Climate Center). The shape of the drought duration frequency distribution (Fig. 5a) results in the projection that incremental increases in temperature could cause large shifts in the number of seedling mortality events (Fig. 5b). An increase of 3.6°C was projected to add 1-2 seedling die-off events per century, but a 7.7°C increase in temperature could lead to 7-8 additional seedling die-off events per century. Successful regeneration events at these lower forest communities are already rare (Savage et al., 1996, Barger et al., 2009). Although such projections are simplistic, they are also conservatively based on the historical drought frequency distribution, given that future drought frequency is expected to increase with climate change (IPCC, 2007, Jentsch et al., 2007, Adams et al., 2009).

Tree mortality alters ecological interactions among species, affecting plant community composition, wildlife habitat, and biogeochemical cycles (Mueller et al., 2005, Gitlin et al., 2006, Klenner and Arsenault, 2009, Clow et al., 2011, Kane et al., 2011). Forests exert a strong effect on earth system processes though their influence on the terrestrial carbon sink, land surface energy balance, and hydrological cycling (Bonan, 2008, Chapin et al., 2008). Thus widespread tree mortality caused by increased temperatures is a potential positive feedback of global climate change (Adams et al., 2010). Ecological and biosphere-atmosphere consequences of tree mortality are most likely where tree recruitment is also affected by drought (Suarez and Kitzberger, 2008, Brown et al. 2010, Adams et al., 2012). Importantly, our results demonstrate that seedling survival and gas exchange during drought is highly and progressively sensitive across a range of elevated temperatures (i.e., not just in contrast to a single elevated temperature) for two widespread and ecologically important species of *Pinus*. This highlights how risks to forests increase as temperatures continue to rise. We found that drought resistance in *P. edulis* exceeded *P. ponderosa*, although time-to-mortality was more temperature sensitive for *P. edulis*, results which were both reflected in the temperature sensitivity of seedling gas exchange responses. Reduced time-to-mortality leads to large increases in the number of projected seedling mortality events with each progressive increase in temperature. Our results highlight that a successional return to pre-mortality tree cover should not be assumed in regions where tree mortality is episodic and driven by drought, especially considering increased future temperatures and drought frequency with global climate change. More field surveys of post mortality tree species

recruitment are needed in mortality-affected forests to confirm this experimental result. Quantifying the persistence of tree mortality effects on ecosystem function and atmosphere-land surface interactions is critical for predicting the water, energy, and carbon budgets consequences of this disturbance, which include feedbacks to the climate system that could contribute to further global change. In conclusion, our study illustrates how progressive temperature increases hasten drought-induced mortality in two key *Pinus* species differentially, and in a way that is reflected in carbon metabolism. The results have direct implications for recruitment in post-die-off ecosystems with respect to vegetation dynamics and their associated land-surface atmosphere fluxes.

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Table 1. The relationships of total and component tissue dry mass, and root:shoot ratio, to time-to-mortality during drought for *Pinus* seedlings. Aboveground mass is the sum of foliage and stem mass. Simple linear regressions of mass variables with survival were pooled across temperature treatments. The symbol “ns” indicates that the relationship was not statistically significant ($p > 0.05$). For *P. ponderosa*, significant negative slopes for total, aboveground, and foliage mass indicate that reduced time to mortality (weeks) was associated with increased seedling mass (g).

Component	<i>P. edulis</i>			<i>P. ponderosa</i>		
	R²	p-value	slope	R²	p-value	slope
Total	ns	ns	ns	0.30	< 0.01	-0.93
Aboveground	ns	ns	ns	0.37	< 0.01	-1.64
Foliage	ns	ns	ns	0.39	< 0.01	-1.86
Stem	ns	ns	ns	ns	ns	ns
Roots	ns	ns	ns	ns	ns	ns
Root: Shoot	ns	ns	ns	ns	ns	ns

Figure Legends

Figure 1. Time-to-mortality during drought of two pine species, *P. edulis* and *P. ponderosa*, under 4 temperature treatments. Linear regression was calculated from all individuals, not from means, for *P. edulis* ($R^2 = .60$, $p < 0.001$, $n=28$) and *P. ponderosa* ($R^2 = 0.44$, $p < 0.001$, $n=26$). A test for common slope found species differences across temperatures ($p < 0.001$). Error bars are standard errors.

Figure 2. Net photosynthesis (A_{net}), stomatal conductance (g_s), and respiration (R) one week after watering, prior to the onset of drought. Letters indicate significant differences among treatments for A_n and g_s from ANOVA post-hoc Fisher's LSD. Due to unequal variance among treatments, R was analyzed with linear regression and a test for common slope which indicated a positive effect of temperature on R ($R^2 = 0.41$, $p < 0.01$) and no species difference ($p > 0.05$). Error bars are standard errors.

Figure 3. Net photosynthesis (A_n), stomatal conductance (g_s), and respiration (R) for seedlings of two pine species under 4 temperature treatments during lethal drought. Error bars are standard errors.

Figure 4. a. The duration of stomatal closure prior to mortality for pine seedlings under four temperature regimes. Stomatal closure was defined as the continuous period prior to mortality for which stomatal conductance was less than $0.01 \text{ mol m}^{-2}\text{sec}^{-1}$. Letters indicate significant differences among species and treatments from ANOVA post-hoc

LSD ($p < 0.05$). b. Mean respiration rate during that period of stomatal closure (R_s). Due to unequal variance among treatments, R_s was analyzed with linear regression and a test for common slope which indicated a positive effect of temperature on R ($p < 0.01$) and no species difference ($p > 0.05$). Error bars are standard errors. c. The relationship between (R_s) and time-to-mortality during drought for seedlings of *P. edulis* and *P. ponderosa* ($R^2 = 0.65$, $p < 0.05$).

Figure 5. A simple projection of seedling mortality events from the historical drought frequency distribution. a. This regional drought duration frequency distribution for the lower forest boundary in the southwest US shows the number of drought events, periods with less 25% of monthly mean precipitation, in the 100 year record (gray bars). The 2002/2003 drought known to have caused seedling mortality in both *P. edulis* and *P. ponderosa* lasted 10 months regionally (black bar). An exponential curve was fitted to the frequency distribution ($R^2=0.96$, $p > 0.001$), and the shift in relative seedling survival during drought with increased temperature is projected for *P. edulis* (red triangles) and *P. ponderosa* (blue circles) using experimental results. b. The additional number of droughts per 100 years sufficient to cause seedling drought mortality in each species calculated from the curve fitted to the frequency distribution for three elevated temperature scenarios.

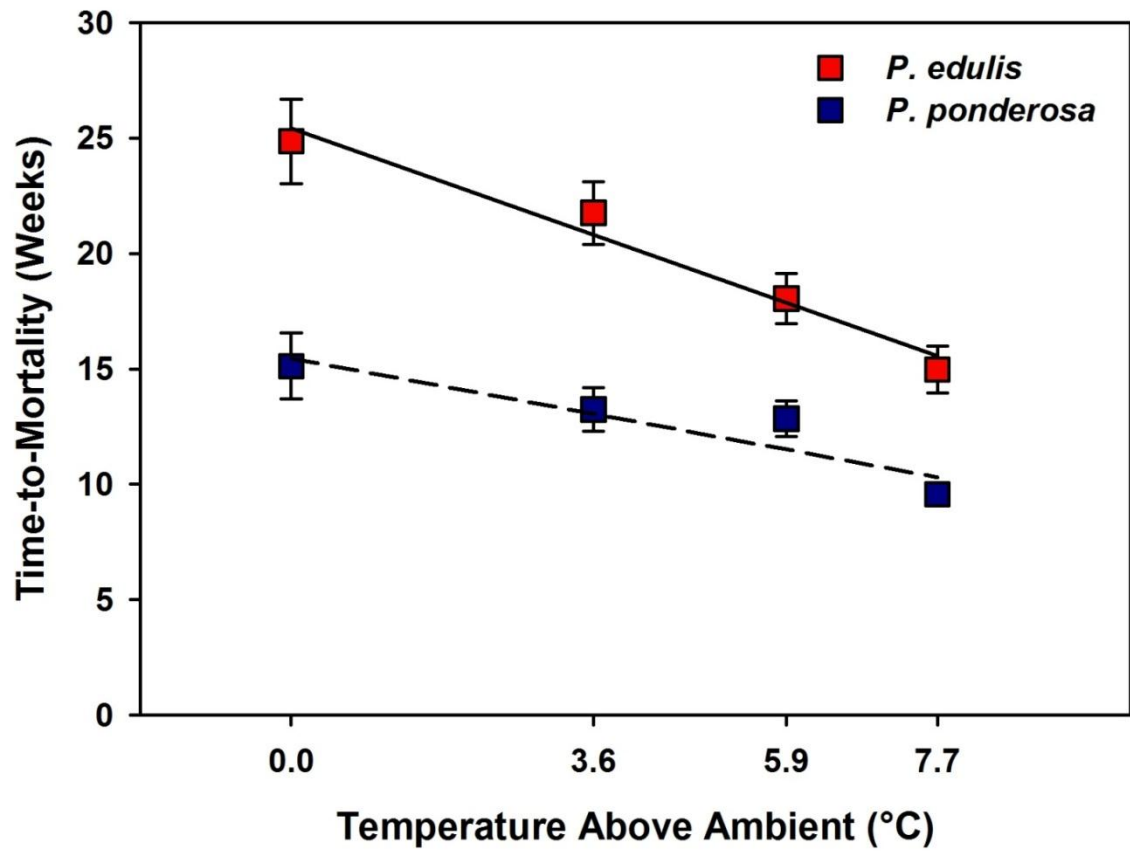


Figure 1.

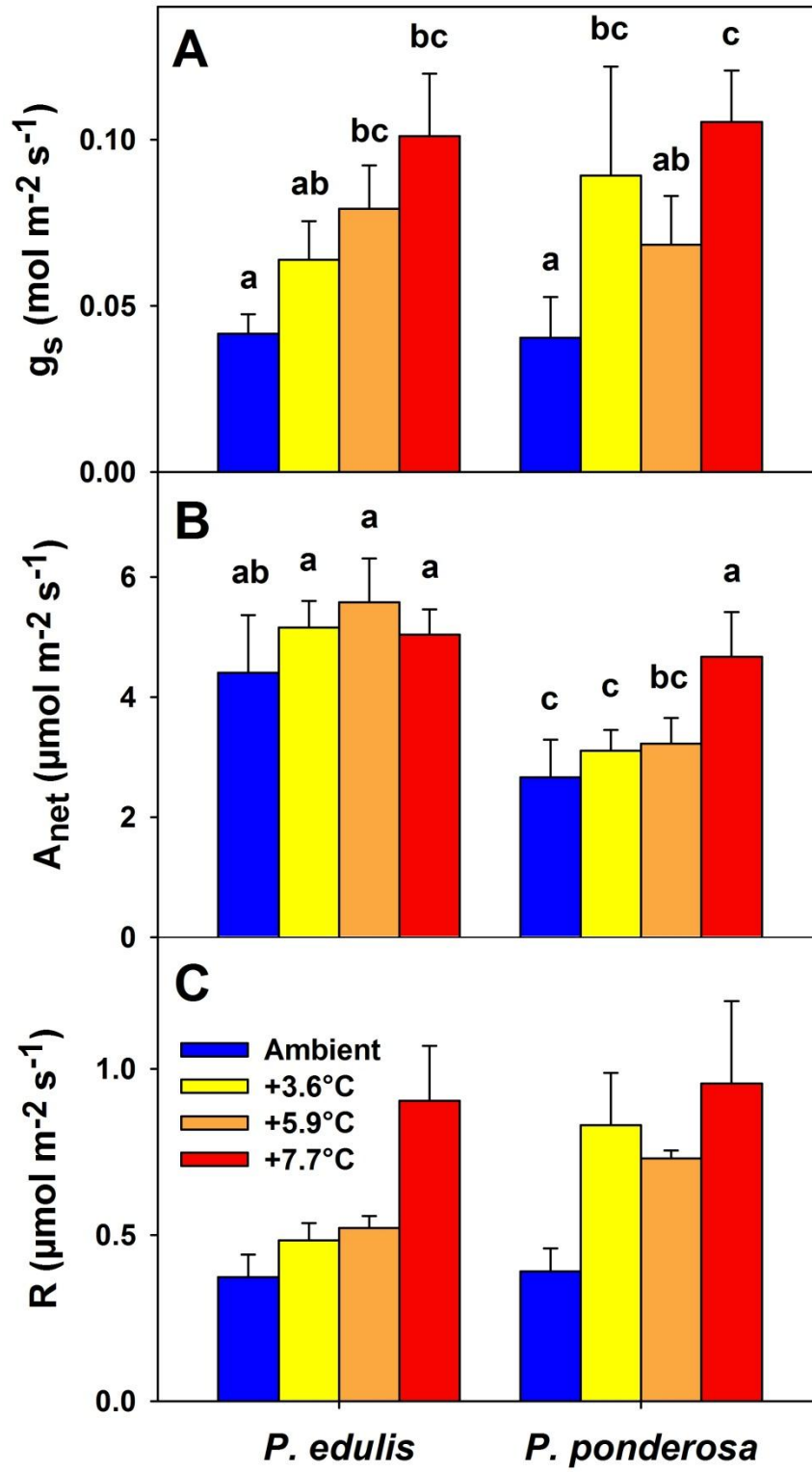


Figure 2.

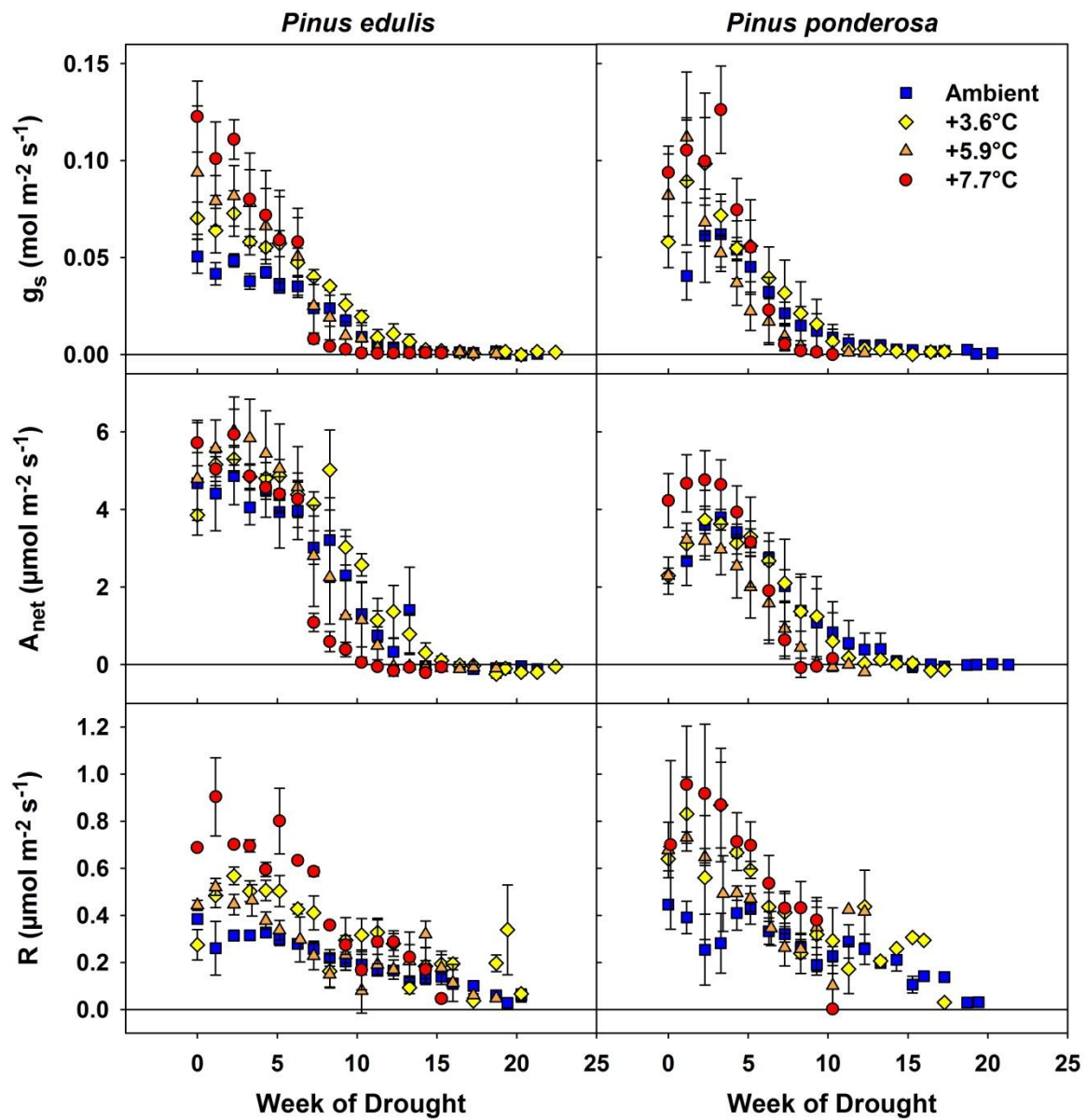


Figure 3.

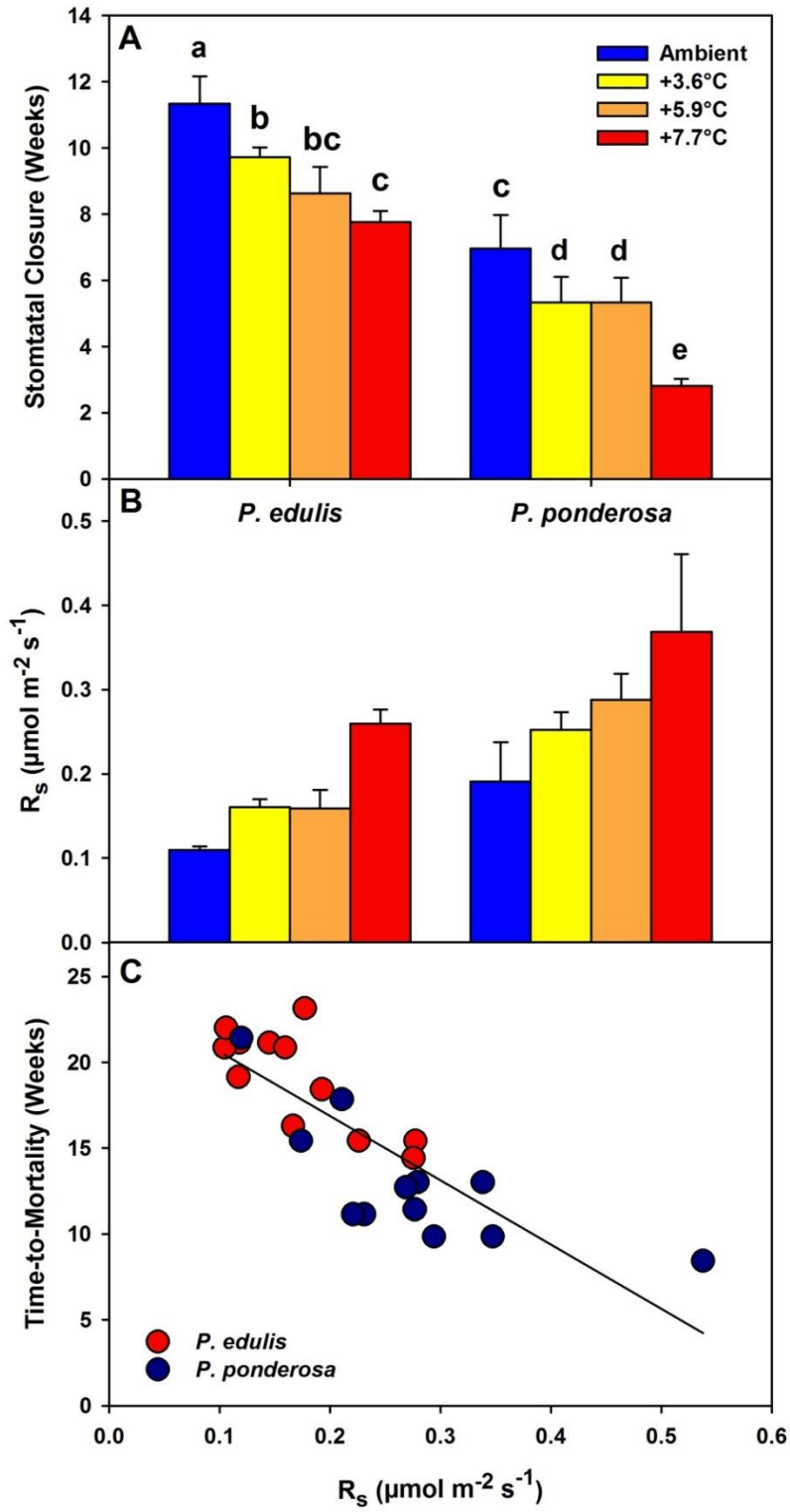


Figure 4.

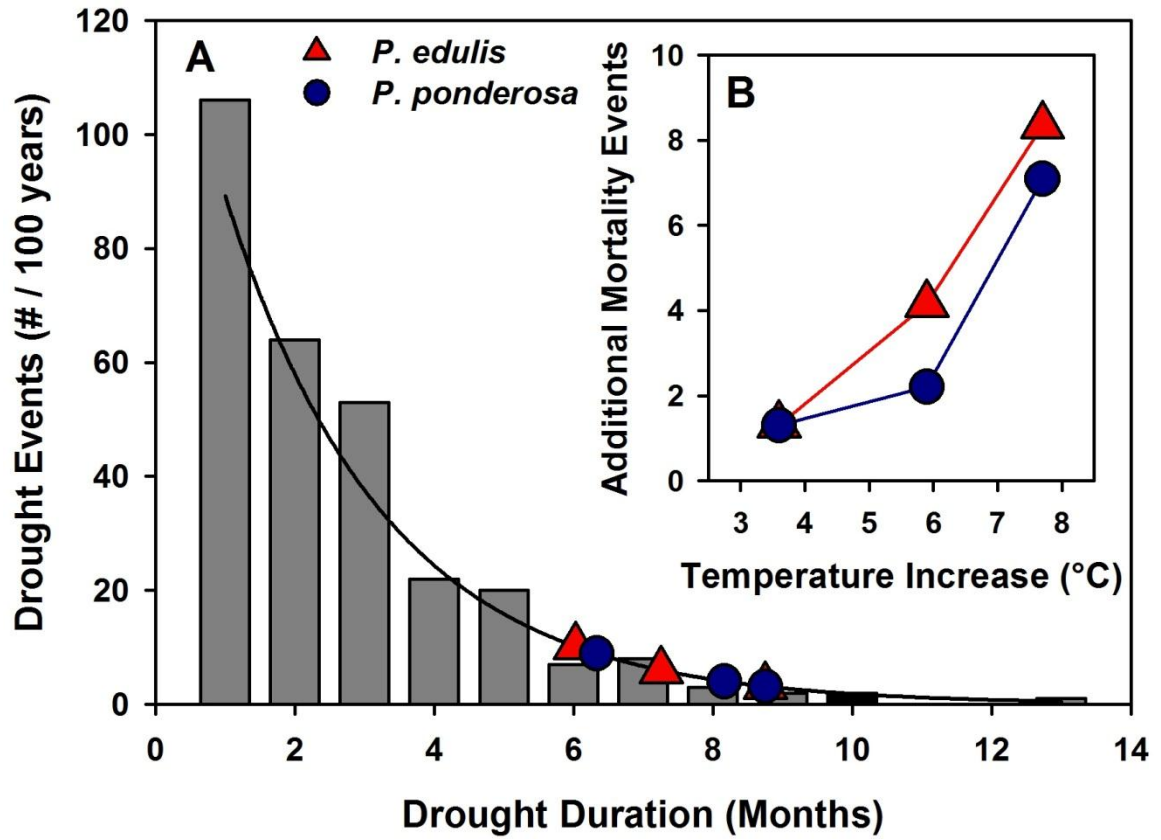


Figure 5.