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Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk

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Abstract

Forest mortality constitutes a major uncertainty in projections of climate impacts on terrestrial ecosystems and carbon-cycle feedbacks. Recent drought-induced, widespread forest die-offs highlight that climate change could accelerate forest mortality with its diverse and potentially severe consequences for the global carbon cycle, ecosystem services, and biodiversity. How trees die during drought over multiple years remains largely unknown and precludes mechanistic modeling and prediction of forest die-off with climate change. Here, we examine the physiological basis of a recent multiyear widespread die-off of trembling aspen (*Populus tremuloides*) across much of western North America. Using observations from both native trees while they are dying and a rainfall exclusion experiment on mature trees, we measure hydraulic performance over multiple seasons and years and assess pathways of accumulated hydraulic damage. We test whether accumulated hydraulic damage can predict the probability of tree survival over 2 years. We find that hydraulic damage persisted and increased in dying trees over multiple years and exhibited few signs of repair. This accumulated hydraulic deterioration is largely mediated by increased vulnerability to cavitation, a process known as cavitation fatigue. Furthermore, this hydraulic damage predicts the probability of interyear stem mortality. Contrary to the expectation that surviving trees have weathered severe drought, the hydraulic deterioration demonstrated here reveals that surviving regions of these forests are actually more vulnerable to future droughts due to accumulated xylem damage. As the most widespread tree species in North America, increasing vulnerability to drought in these forests has important ramifications for ecosystem stability, biodiversity, and ecosystem carbon balance. Our results provide a foundation for incorporating accumulated drought impacts into climate–vegetation models. Finally, our findings highlight the critical role of drought stress accumulation and repair of stress-induced damage for avoiding plant mortality, presenting a dynamic and contingent framework for drought impacts on forest ecosystems.

Keywords: biosphere–atmosphere interactions, climate change, ecosystem shift, forest mortality, vegetation model, xylem cavitation

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Introduction

Tree death is a fundamental, yet poorly understood ecological process (Franklin *et al*., 1987). Tree mortality has substantial leverage on ecological community composition and structure, ecosystem processes such as hydrologic and nutrient cycling, and provision of ecosystem services (Bonan, 2008; Anderegg *et al*., 2012a). Along with recruitment and growth rates, mortality rates greatly influence forest ecosystem carbon sequestration and cycling (Van Mantgem & Stephenson, 2007; Kurz *et al*., 2008; Pan *et al*., 2011). Increased tree mortality rates have recently been observed, particularly in association with drought and temperature stress, in tropical, temperate, and boreal forests in recent decades (Phillips *et al*., 2009, 2010; Van Mantgem *et al*., 2009; Peng *et al*., 2011). Drought-induced widespread tree die-off events have been documented around the globe and are expected to increase with climate change in coming decades (Allen *et al*., 2010).

Our physiological understanding of how trees die during drought is remarkably limited, hindering our ability to mechanistically model tree mortality. Only recently have studies began to examine physiological mechanisms of drought-induced tree die-off. Growing appreciation of the interdependencies of carbon and hydraulic changes during tree mortality emphasizes

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the need to examine complex and interconnected pathways to tree death (Sala *et al*., 2010; McDowell *et al*., 2011; Anderegg & Callaway, 2012; Anderegg *et al*., 2012b). Furthermore, recent research documenting lags in tree mortality between drought stress and mortality rates and extended multiyear tree mortality following drought in multiple ecosystems (e.g., Bigler *et al*., 2007; Hogg *et al*., 2008; Phillips *et al*., 2010; Worrall *et al*., 2010), even when adequate conditions return, highlights an important role of the accumulation of stresses and repairs at seasonal and interannual timescales. Based on extensive plot networks (Phillips *et al*., 2010) or repeated measurements of plots from the 1950s through present (Van Mantgem *et al*., 2009; Peng *et al*., 2011) in multiple ecosystems, lagged and multiyear periods of tree mortality may in fact be more common than abrupt mortality in many ecosystems, and thus are crucial for modeling forest mortality with climate change. Such lags and extended mortality pose a major challenge to hypotheses of drought-induced mortality typically framed for mortality *during* drought (e.g., McDowell *et al*., 2008).

We examine the physiology of lagged and multiyear tree mortality from the perspective of a tree as a complex organism. As exemplified in the medical literature, it is useful to think of death of such an organism as a system failure (Bernat *et al*., 1981; Anderegg *et al*., 2012c). This is quite different from a lethal event at the cellular level such as the lysis of a cellular membrane with a clear threshold. In fact, individual cells of a complex multicellular organism may go on living for some time after essential systems fail and the organism dies (e.g., Mims, 1998). Whether a tree lives or dies in response to a drought event ultimately depends on many interacting factors and processes that may come into play in the plant's responses to maintain the integrity of its hydraulic system as it is poised between the dry soil and the even dryer atmosphere. Complex organisms will generally have an array of regulatory mechanisms to keep critical systems operating within appropriate bounds and mechanisms to repair damage that may occur when these bounds are exceeded (Brodribb, 2009; Zwieniecki & Holbrook, 2009). These further complicate attempts to define thresholds for mortality and highlight that understanding the accumulation and repair of damage may be critical to modeling tree mortality with climate change (Anderegg *et al*., 2012c).

We examine here the temporal dynamics of physiological changes underlying a recent, drought-induced widespread die-off of trembling aspen (*Populus tremuloides*), a clonal tree species (Worrall *et al*., 2008, 2010; Michaelian *et al*., 2011; Anderegg *et al*., 2012b). This mortality, termed sudden aspen decline (SAD), was triggered by a severe drought coupled with elevated temperatures, considered a 'climate change-type drought' (Worrall *et al*., 2010; Anderegg *et al*., 2012b). SAD has been estimated to affect more than 17% of Colorado aspen forests (Worrall *et al*., 2010), and has documented ecological and climatological consequences, including substantial biomass loss to carbon emissions (Michaelian *et al*., 2011; Huang & Anderegg, 2012) and declines in understory plant species diversity (Anderegg *et al*., 2012d).

The drought that triggered SAD was a single episode from 2000 to 2003, yet aspen die-off commenced in 2004–2005 and has continued through 2011 (Anderegg *et al*., 2012b). SAD both lagged the initial drought and continued even after drought conditions were alleviated (Worrall *et al*., 2010). Considerable previous research has documented that SAD is not primarily induced by pathogens or pests (Worrall *et al*., 2008, 2010; Anderegg *et al*., 2012b) and is unrelated to changes in nonstructural carbohydrate reserves (Anderegg, 2012; Anderegg *et al*., 2012b), but is associated with embolism of branches and roots (Anderegg *et al*., 2012b). Yet this association between xylem embolism and SAD related mortality does not answer multiple questions that are critical to our understanding of the physiology that underlies SAD and our ability to mechanistically model similar forest mortality events. Because xylem embolism can eventually be either repaired or overcome through processes such as growth (Brodribb *et al*., 2010) or refilling (Zwieniecki & Holbrook, 2009), drought-induced embolism alone cannot explain how hydraulic damage can kill trees more than 8 years after drought stress is relieved. Explanations of this mortality lag must invoke an amplifying feedback, by which initial drought damage continues to harm tree hydraulic function once adequate soil moisture conditions have returned. One such possible feedback is that, due to irreparable damage such as root mortality, documented in SAD (Worrall *et al*., 2008), trees that will eventually die suffer increased water stress (i.e., xylem tensions) during subsequent years. Another possibility is that initial drought stress permanently reduces growth, restricting a damaged tree's ability to recover hydraulic function (e.g., Brodribb *et al*., 2010). A third explanation is that initial drought-induced embolism increases a tree's vulnerability to future and previously survivable drought stress (i.e., similar xylem tensions produce higher rates of embolism in dying trees than in healthy trees) through a process such as cavitation fatigue (Hacke *et al*., 2001).

In this study, we examine the ability of hydraulic properties to predict interyear stem mortality. We then consider observational and experimental evidence of accumulated hydraulic changes that could mediate

lagged and multiyear aspen die-off. We ask: (1) can hydraulic properties predict interyear probability of stem mortality and (2) what multiyear hydraulic changes could mediate lagged and multiyear mortality? Specifically, we test whether differential water stress, differential accumulated growth of xylem, or differential vulnerability of xylem vessels to cavitation can explain accumulated hydraulic differences between trees that lived and trees that died.

Materials and methods

Study area

We examined temporal changes in aspen water stress, hydraulic performance, and mortality/dieback in SAD-affected areas over 2010–2011 growing seasons, 8 years after peak drought intensity, in the San Juan National Forest, Colorado, USA. Aspen forests in the region experienced some of the most severe SAD in western Colorado (Worrall *et al*., 2008). The San Juan National Forest has an mean annual temperature of 3.2 ° C and mean annual precipitation 508 mm with a bimodal precipitation regime – precipitation falling either as snow during November–May or as monsoonal summer rains during July–September (Huang & Anderegg, 2012). Hot and dry seasonal drought usually occurs during early summer (June–July) and represents peak water stress for the aspen forest.

Research sites

We located seven aspen clones that displayed a gradient of stand mortality. This gradient spanned from a healthy area to a SAD area that was less than 100 m away (see Anderegg *et al*., 2012b for site descriptions). These clones allowed *in situ* examination of temporal dynamics in hydraulic performance and physiological changes between surviving and dying groups of stems within the same (clonal) individual and site. For all analyses except the logistic regression model, we examined two categories of ramets – SAD affected (defined as ramets with >50% visually assessed crown dieback) and healthy (<20% crown dieback). All methods of crown dieback measurements were the same as previous research in these forests (Worrall *et al*., 2010; Anderegg *et al*., 2012b).

Interyear stem mortality

To examine whether hydraulic performance could predict interyear probability of stem mortality, we tracked crown dieback and measured hydraulic conductance in 36 ramets of all categories of crown dieback between August 2010 and July 2011. We used logistic regression to estimate the probability of a ramet dying (100% crown dieback) between August 2010 and July 2011 based on the native basal area-specific xylem conductance of distal branch networks. In August 13–14, 2010, we collected branch distal networks (branch + petioles + leaves) from the 36 ramets longer than 10 cm from midcanopy between 12:00 and 14:00 hours. We immediately misted segments and placed them in dark plastic bags, keeping them continually hydrated and dark during transportation, and transported them to the lab the next day. We recut all exposed segment ends and cut all attached leaves in half perpendicular to petiole attachment under water. We calculated conductance using the vacuum method described in Kolb *et al*. (1996) using distal branch networks (including leaf petioles), which captures hydraulic failure in the entire path length from branch to twig to petiole, standardizing by branch basal area (yielding basal area-specific conductance as mg/s*Pa*mm^2). Native conductance is relevant both because it is a measure of the tree's ability to transport water *in situ* and because it should theoretically integrate all of the elements of hydraulic differences as a system failure described above. For this analysis, we explicitly broadened our sampling beyond the two classifications of ramets described above to include ramets that were visually healthy, but located in a largely SAD-affected area. Because logistic regression does not provide a typical R^2 goodness-of-fit estimate, we present a commonly used 'pseudo-R^2' analogue based on log-likelihood but scaled from 0 to 1, Nagelkerke R^2, to evaluate how well the model fits the data (Long, 1997).

Xylem tension measurements

We assessed seasonal levels of plant water stress, measured by xylem tension, in five of the selected clones during June 17–19, July 16–18, and August 17–19, 2011. This period captures annual peak water stress in late June and July and amelioration of water stress in August 2011. Weather conditions were generally clear, sunny, with temperatures between 16 and 19 ° C (measured *in situ* by Campbell Scientific CS107 temperature probe) on the day of and several days before sampling. Within each clone, we randomly selected three ramets per class (healthy vs. dying) and sampled two randomly selected branches per ramet. Because forest canopy height precluded use of pole clippers, we collected midcanopy branch networks by severing them with a shotgun and 7.5 ounce birdshot. We immediately misted branches and placed them in a dark plastic bag. Within 5 min of collection, we subsampled a small twig (<3 mm diameter) more than 30 cm and two distal forks from the initial branch break, and measured the twig in a Scholander-type pressure chamber (PMS Instruments, Corvalis, OR). We typically took ca. 3 twigs per branch to check consistency and stability of measurements. We measured branch xylem tensions before dawn (03:00–05:00 hours: generally considered lowest daily tensions where plant water is equilibrated with soil water absent nighttime transpiration), and at midday (12:00–14:00 hours: generally considered highest daily tensions due to transpiration).

Growth and vessel diameter measurements

To examine whether changes in growth, a prominent multiyear effect of drought, could partially account for differences in conductivity, we measured branch growth and vessel diameter in healthy and SAD-affected ramets in the same five clones. We examined the branch growth increment from 1998

to 2011 via measuring the annual growth ring width. The age of branches used for the measurements varied between 18 and 30 years; thus, all the branches were fully developed and well established before the drought period in 2000–2003. 40 μm-thick branch cross-sections were prepared using a sliding microtome (SM2400; Leica Microsystems, Wetzlar, Germany) and observed with a light microscope (DM3000; Leica) under 25× magnification. The growth rings could be clearly distinguished except for the few oldest rings close to the center of the stems. The ring width was measured with image analysis software (ImagePro Plus v. 6.1; Media Cybernetics, Silver Spring, MD, USA) and normalized by the total xylem diameter for each individual branch. Nine branches per treatment from a June 2011 sampling were analyzed for both healthy and SAD-affected treatments. Subsequently, vessel diameters were measured in growth rings from years 2003 and 2010 observed at 100× magnification. At least 350 vessels in total were measured per growth ring for both healthy and SAD ramets. The mean values were calculated for six branches per treatment.

Vulnerability curve methods

Drought may also affect the vulnerability of xylem vessels to subsequent cavitation over multiyear timescales, placing limits on the effectiveness of cavitation repair and restoration of hydraulic conductivity. We examined changes in xylem vulnerability in these mortality-gradient sites via the standard vulnerability curve technique (Sperry & Saliendra, 1994), which measures percent loss conductivity (PLC) of a branch sample as a function of artificially induced xylem tension/pressure. Vulnerability curves were determined on 12 branches per treatment using either the air injection method (Sperry & Saliendra, 1994) or centrifuge method (Alder *et al*., 1997) to generate cavitation-inducing pressure differences within branches. Stems were flushed of embolism via vacuum infiltration prior to conducting vulnerability curves, but we calculated the maximum conductivities needed for the calculation of PLC from both native and flushed conductivities to ensure that flushing did not bias our results. We validated these lab measured vulnerability curves by plotting *in situ* measurements of xylem tension (above) against basal area-specific hydraulic conductivity before and after vacuum infiltration (giving percent loss of conductivity), using previously described methods (Sperry *et al*., 1988), to generate a vulnerability curve of *in situ* stems. We also measured the vulnerability curve via air injection of two clones directly adjacent to ponds (which local forest experts attested held water during the 2000–2003 drought) to examine the vulnerability of clones unlikely to have been stressed by the severe drought.

Mature forest drought experiment

We also monitored the hydraulic performance of trees recovering from water stress after an experimentally imposed drought. We followed hydraulic performance and health of 24 mature ramets during and after experimental drought. We subjected two adjacent, but mechanically separated via trenching (12 × 18 m) plots of a mature aspen clone to drought via rainfall exclusion from June to August 2010 (Anderegg *et al*., 2012b). Clear troughs drained downhill away from the plots and covered ca. 50% of the plot surface area in each treatment. Twelve mature ramets were included in each treatment. We perforated small holes in the control treatment's troughs to allow water to pass through unimpeded while still creating the same microenvironment in the soil as the drought treatment. This experiment created significant water stress over the course of the growing season (Anderegg *et al*., 2012b). We measured xylem tensions and hydraulic conductivity (as above in vulnerability curve analysis) on 5 ramets per treatment as above during July 2010, July 2011, and July 2012.

Statistics

We analyzed time-series and repeated-measures data (xylem tensions, loss of conductivity, and vulnerability curves) with ANOVA or repeated-measures ANOVA, after checking assumptions of sphericity with Mauchley's test of sphericity. We examined direct group comparisons (vessel diameters, growth) with Student's *t*-tests after checking for assumptions of normality with a Shapiro–Wilkes test.

Results

We first asked whether native hydraulic conductance of distal branch networks could predict interyear mortality between 2010 and 2011. Twelve of 36 ramets died between August 2010 and July 2011, including both ramets that were visibly dying and visibly healthy at the onset of the study in 2010. Logistic regression based on August 2010 native branch distal conductance captured the probability of ramet mortality between August 2010 and July 2011 with high accuracy (Nagelkerke $R^2 = 0.64$, $P < 10^{-6}$; Fig. 1). This suggests that

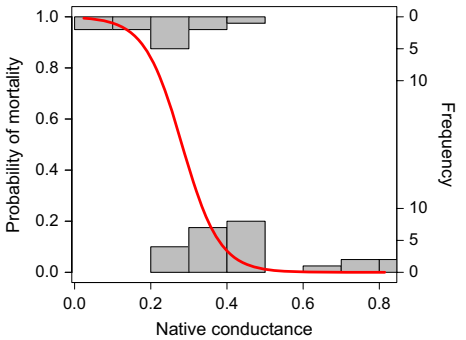

Fig. 1 Probability of ramet mortality between August 2010 and July 2011 ($N = 12$ died; $N = 36$ studied) as predicted by basal area-specific branch hydraulic conductance (red; $mg*mm^{-2}*Pa^{-1}*s^{-1}$) in August 2010. Histograms show dead (top) and living (bottom) ramets' hydraulic conductance as of July 2011.

standing differences in hydraulic conductivity are directly tied to probability of mortality, even 7 years following drought stress.

We then sought to determine whether these extant differences in conductivity were influenced by differential water stress in dying stems, perhaps through amplifying feedbacks such as root mortality. Predawn and midday xylem tension measurements conducted in two classifications of stems (dying and healthy) in five clones over the 2011 growing season revealed that SAD and healthy ramets did not experience significantly different xylem tensions (repeated-measures ANOVA, $F_{1,4} = 0.63$, $P = 0.47$; Fig. 2). Thus, dying and healthy stems did not appear to experience different levels of water stress during typical seasonal water stress, as in June and July 2011.

We tested whether accumulated differences in xylem vessel diameter and growth as indicators of construction and performance of newly grown xylem could influence extant hydraulic disparities between dying and healthy stems. We found that xylem vessel diameter was smaller during 2003 compared with 2010 when combining both SAD and healthy branches (Student's *t*-test: $t = 2.18$, $P = 0.04$), but it was not significantly different in SAD ramets compared with healthy ones in 2003 (Student's *t*-test: $t = 0.82$, $P = 0.42$; Fig. 3a). We found significantly decreased branch growth in SAD ramets for 3 years following the peak drought severity in 2002 (ANOVA: $F = 6.67$, $P = 0.01$; Fig. 3b). Yet branch increment recovered in dying stems by 2007 and was not different through 2010.

Given that dying trees were neither growing less xylem nor experiencing higher water stress, we then tested whether the same amount of water stress was more damaging to dying trees than to healthy trees

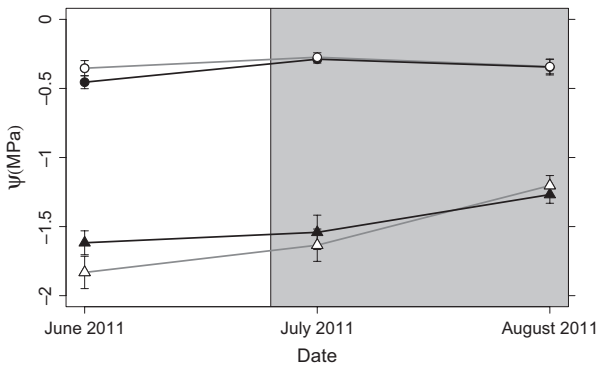

Fig. 2 Branch xylem tensions (mean ± SE) prior to dawn (circles) and at midday (triangles) of healthy (open; $N = 15$) and SAD-affected (filled; $N = 15$) ramets during the growing season of 2011. Shading indicates beginning of monsoonal rainy season in 2011.

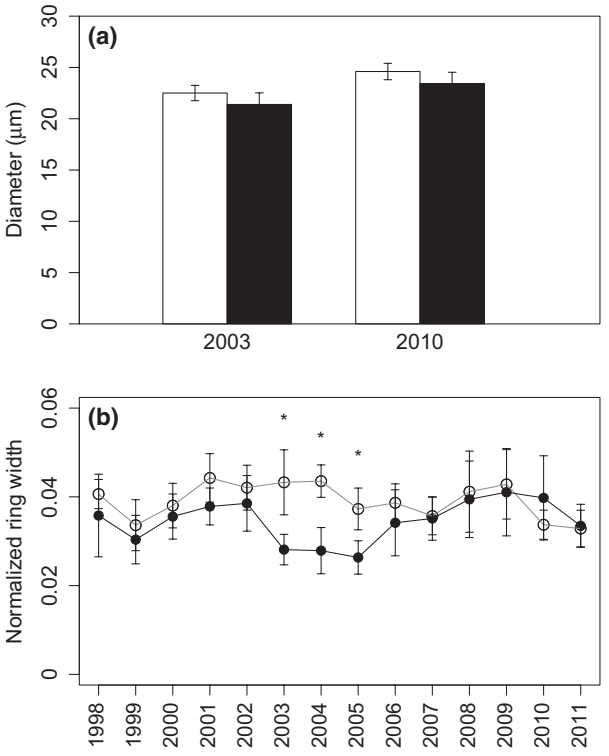

Fig. 3 (a) Vessel diameter (mean ± SE) of healthy (white; $N = 9$) and SAD-affected (black; $N = 9$) ramets in 2003 following drought and in an average nondrought year, 2010. (b) Branch ring width (mean ± SE) standardized to xylem diameter of healthy (open circles; $N = 9$) and SAD-affected (filled circles; $N = 9$) ramets from 1998 to 2011.

(i.e., an increase in vulnerability to water stress). Xylem vulnerability to embolism was significantly higher in SAD branches than in healthy branches (repeated-measures ANOVA: $F_{1,4}$=17.2, $P = 0.001$; Fig. 4a). Both air injection and centrifuge vulnerability curve methods yielded largely similar results. Differences in vulnerability were apparent in hydraulic conductivity as well (Figure S1) and did not depend on whether the maximum conductivity baseline was flushed or native hydraulic conductivity (Figure S2). The xylem pressure needed to yield 50% loss of conductivity was −1.0 MPa in SAD ramets, compared to −2.3 MPa in healthy ramets (Fig. 4a). Native *in situ* hydraulic conductivity and xylem tensions assessed in healthy and dying ramets over the 2011 growing season largely tracked the vulnerability curves produced in the lab (Fig. 4b, Figure S3), indicating cavitation fatigue occurs *in situ* as well, although SAD branches had substantially lower conductivity in general. Surprisingly, even visually healthy ramets appeared to exhibit increased vulnerability (Fig. 4c). Vulnerability of never-stressed clones was close to previously published estimates for aspen vulnerability to drought (24), whereas vulnerability of

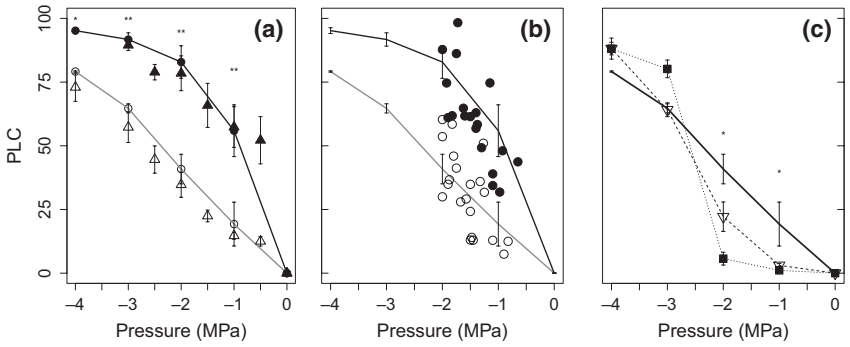

Fig. 4 (a) Percent loss of branch conductivity (mean ± SE) as a function of xylem tension/pressure applied through two methods, air injection (circles; pressure divided by −1) and centrifuge (triangles), of healthy (open; $N = 6$) and SAD-affected (filled; $N = 6$) ramets. (B) Percent loss of branch conductivity measured in native clones as a function of *in situ* xylem tension measurements in healthy (white; $N = 15$) and SAD-affected (black; $N = 15$) ramets. Lines indicate vulnerability curves from air injection in (A). (C) Percent loss of branch conductivity (mean ± SE) as a function of air injection pressure of healthy ramets (line; $N = 6$), unstressed clones near ponds (downward triangles; $N = 6$), and previously published work on aspen ramets (filled square; Hacke *et al*., 2001).

apparently healthy aspen clones was higher than that of never-stressed clones at lower xylem tensions (Student's *t*-test, −1 MPa: $t = -3.6$, $P = 0.03$; −2 MPa: $t = -5.97$, $P = 0.002$) (Fig. 4c).

Lastly, experimental drought produced similar effects to those seen in SAD, both in terms of lagged mortality and hydraulic physiology, although with relatively small sample sizes ($N = 12$ per treatment). As was observed in SAD, we found lags in significant crown dieback in artificially droughted ramets. No differences in crown dieback between treatments were observed while water was excluded in 2010; moderate differences were observed in 2011 1 year after water exclusion ceased (Student's *t*-test, $t = -1.73$, $P = 0.1$); and significant differences were observed by 2012 (Student's *t*-test, $t = -2.28$, $P = 0.03$; Fig. 5). Drought ramets experienced significantly higher midday xylem tensions (i.e., more negative pressures) during experimental drought (July 2010; Student's *t*-test, $t = 2.13$, $P = 0.02$), but xylem tensions in droughted ramets and control ramets were nearly identical in subsequent years where they varied with typical seasonal rainfall. Drought ramets exhibited increases in PLC during experimental drought (July 2010). These differences remained after experimental drought stress had abated (but typical seasonal water stress still occurred) in 2011, and grew even larger in 2012 with substantial seasonal water stress (repeated-measures ANOVA: $F_{1,6} = 1.98$, $P = 0.01$; Fig. 5). In 2011, however, previously drought-stressed ramets had significantly higher PLC than control ramets (*post hoc t*-test, $t = 1.89$; $P = 0.04$). Thus, despite experiencing identical conditions following the 2010 experiment and similar xylem tensions, droughted ramets reveal strong signs of increased vulnerability to cavitation and accompanying lagged mortality (Fig. 5).

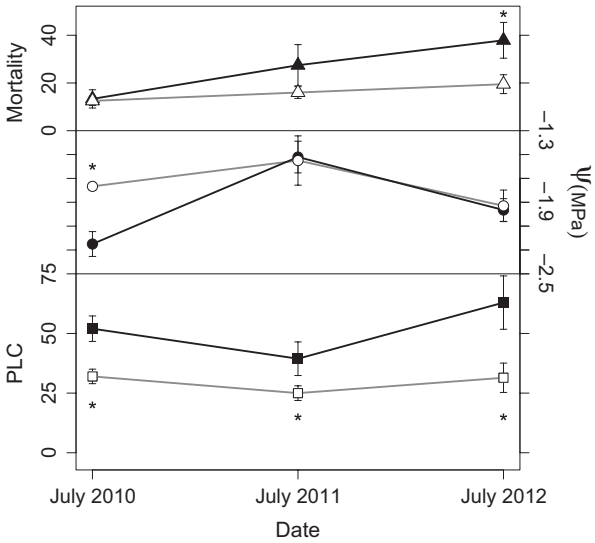

Fig. 5 Average crown mortality (%; mean ± SE), midday xylem tensions (MPa; mean ± SE), and percent loss of branch conductivities (%, mean ± SE) in experimental drought of drought stressed in 2010 (black; $N = 12$) and control (white; $N = 12$) ramets from July 2010 to July 2012.

Discussion

We document here that multiyear and accumulated hydraulic changes appear to play a critical role in widespread, drought-induced aspen die-off. Standing differences in hydraulic performance largely determined interyear stem mortality, independent of crown status. We tested whether differences in water stress, growth, or hydraulic vulnerability likely mediated hydraulic performance. Seasonal changes in xylem tension indicate that dying ramets do not experience different levels of water stress during typical seasonal drought.

Similar xylem tensions were observed in droughted stems compared with control stems for 2 years following experimental drought as well. How dying trees maintain similar xylem tensions – perhaps through stomatal regulation or concurrent root and leaf mortality such that root-to-leaf ratios are maintained – is a fruitful future research direction. Root mortality certainly occurs during SAD (Worrall *et al.*, 2008; Anderegg *et al.*, 2012b) and may play a role in within-year feedbacks to water stress, but the balance and timing of root vs. crown mortality are key unknowns.

Branch-increment and vessel diameter data provide insight into the trajectory of postdrought growth and its hydraulic consequences. Vessel diameters were smaller in both SAD-affected and healthy branches in 2003 and were not significantly different in 2010, suggesting that lasting hydraulic changes were not likely mediated by vessel diameter. Branch growth decreased for 3 years following the drought and may have affected hydraulic performance, but it eventually recovered with extended nondrought conditions. These processes may be determined by the carbon status of the tree, as declines in growth and carbon uptake have been documented in experimental drought in aspens (Anderegg, 2012; Anderegg & Callaway, 2012), and thus are prominent hydraulic–carbon interconnections to test. Our findings present clear evidence that some physiological processes such as declines in growth lag drought, but appear to be reversible and play a somewhat minor role in determining standing differences in hydraulic performance in this species.

Although SAD-affected ramets experienced similar levels of xylem tension, vulnerability curves revealed that their vulnerability to cavitation from tension had increased sharply. This phenomenon whereby previous cavitation damage leads to increased vulnerability to cavitation in subsequent stresses is known as 'cavitation fatigue' (Hacke *et al.*, 2001). Cavitation fatigue is supported by conductivity changes in the vulnerability curve analysis (Fig. 4a, Figure S1), measured *in situ* xylem tensions and cavitation (Fig. 4B, Figure S3), increased PLC in the subsequent years in experimental drought ramets despite similar xylem tensions (Fig. 5; see below), and it has been demonstrated in aspen branches in lab experiments previously (Hacke *et al.*, 2001). These substantial differences in vulnerability occurred alongside exceptionally low conductivities in SAD branches, even after flushing to refill embolism, in comparison with healthy branches, possibly due to heartwood formation (Figures S1 and S3). Experimental drought results indicated that lags in mortality/dieback were highly associated with a shift toward increased vulnerability to cavitation in drought ramets. Our results reveal that cavitation fatigue likely plays an important role in mediating multiyear hydraulic changes during this forest die-off.

The increased drought susceptibility of visually healthy aspen ramets compared with not previously stressed clones near ponds and previously published data suggests that these forests may be at increased risk from future droughts, even of lesser severity. Drought-driven increases in susceptibility to subsequent drought have been documented in conifer species in this region as well (Mueller *et al.*, 2005), and could play a role in accelerating rates of tree mortality documented across the United States (Van Mantgem *et al.*, 2009) and Canada (Peng *et al.*, 2011). Our results provide a physiological mechanism through which accumulated damage can lead to future increased vulnerability in this species.

We document here a major role of accumulated hydraulic changes in this widespread forest die-off. These changes decrease transport within a tree, and do not appear to be reversible, even with restoration of normal climatic conditions. These accumulated changes in hydraulic performance, which we term hydraulic deterioration, are associated with continued tree mortality from SAD due to vulnerability to previously tolerable seasonal water stress up to 9 years following severe drought. Logistic regression drawing on the accumulated hydraulic deterioration represented a ramet's probability of mortality across years. This regression is one of the first relationships to directly capture ongoing forest mortality via observed physiological mechanisms, but further work is needed to validate this preliminary finding across a range of regions and environmental conditions. If supported, it could provide guidance for a mechanistic basis of tree mortality in future vegetation–climate models of this species.

This hydraulic deterioration does not exclude other processes, which may be occurring as well, including branch scarring and elevated insect attack which could further damage ramets' hydraulic systems (Marchetti *et al.*, 2011; Anderegg & Callaway, 2012). Indeed, increases in fungal pathogens documented in SAD areas (Marchetti *et al.*, 2011) might impair plant hydraulics as well. Yet these hydraulic changes have substantial influences on tree's function as a system, reconcile previous physiological evidence with mortality lags (Hogg *et al.*, 2008; Worrall *et al.*, 2010), and appear to underlie ongoing mortality. Our results highlight that multiyear feedbacks, repair of damage, and accumulated stress may be critical in understanding forest vulnerability to drought. While the clonal nature of trembling aspen complicate our ability to generalize these results, similar lagged and multiyear tree death from drought has been observed in conifer species in the Rocky Mountains (Bigler *et al.*, 2007), across the

western United States (Van Mantgem *et al*., 2009), across boreal Canadian forests (Hogg *et al*., 2008; Peng *et al*., 2011), and tropical Amazonian forest plots (Phillips *et al*., 2010). Moreover, recent results from a multiyear drought experiment in a tropical rainforest in southeast Asia documented similar long-term declines in hydraulic conductance, evidence that the mechanisms elucidated here may operate more broadly (Schuldt *et al*., 2011).

The hydraulic deterioration studied here is specific to one widespread forest-type dominated by a single species. Nonetheless, our results demonstrate that hydraulic changes can accumulate over multiple years, and the ability and limits of repair of drought-induced damages could be as critical as the severity of the damages themselves. If true in other ecosystems, such complex interactions of mechanisms would preclude a simple threshold for modeling mortality in vegetation models. In particular, shifts in tree species' vulnerability to drought may be important for accurately modeling the response of forests to drought and climate change. Current dynamic global vegetation models (DGVMs) consider physiological properties of plant functional types (PFTs) to be constant over time (e.g., Cox *et al*., 2000), yet a single extreme event may have altered the properties of these aspen forests up to a decade later. Improved understanding of the drivers and definition of mortality from processes such as hydraulic vulnerability and repair could be important to incorporate into models to better forecast the future of forest ecosystems under climate change.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Xylem area-specific hydraulic conductivities (Ks; kg s^{-1} MPa^{-1} m^{-1}) as a function of imposed xylem pressure (MPa) via centrifuge in SAD (black; $N = 6$) and healthy (white; $N = 6$) branches. Ks presented at far right is the native Ks prior to vacuum infiltration.

Figure S2. Vulnerability curves of healthy (white; $N = 6$) and SAD (black; $N = 6$) branches presented as Percent Loss of Conductivity (PLC) as a function of imposed xylem pressure (MPa). (A) Vulnerability curve where PLC is calculated from flushed (embolism removed) hydraulic conductivity. (B) Vulnerability curve where PLC is calculated from native (*in situ* embolism still present) hydraulic conductivity.

Figure S3. Basal-area specific hydraulic conductivity (Ks; kg s^{-1} MPa^{-1} m^{-1}) as a function of xylem tension (MPa) in healthy (white; $N = 15$) and SAD (black, $N = 15$) ramets, combined over June–August 2011 sampling events.