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Retrospective approach to dieback in Pinus sylvestris through the analysis of carbon and oxygen stable isotope composition in tree ring cellulose

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Since the end of last century, forest stress and dieback have spread around the world, pointing to climate change as catalyst of such phenomenon (Allen et al. 2010 *For. Ecol. Man.*). In some cases, dieback affects selectively a subset of individuals in a forest stand, suggesting differential susceptibilities to the stress factors causing tree decline. Understanding the physiological factors underlying forest dieback, as well as the particular adaptations allowing some trees to survive, may be crucial to delineate mitigation strategies. The aim of this study was to compare the historical record of healthy and declined trees in a *Pinus sylvestris* L. stand, before and after a dieback episode occurring just after a strong thermal contrast in autumn 2001. The stand is located in the Gúdar Range (Iberian System, Spain), at an altitude of 1690 m.a.s.l, with an average temperature of 8.6ºC and an annual precipitation of 546 mm. In this area, a progressive increase in annual temperature has been recorded during the last decades. We hypothesised that the adjustment to these new conditions should be detected in tree-ring archives (radial growth, carbon -Δ^{13}C- and oxygen -δ^{18}O- isotopes in wood cellulose), and that this adjustment would differ in healthy and declined trees. For this purpose, we compared 1) the previous tree-ring record of surviving and dying trees of similar age and 2) the post-dieback tree-ring record of surviving trees showing different levels of defoliation. Following a multi-scale approach, we determined Δ^{13}C and δ^{18}O in (i) tree slices at breast height with annual resolution (1975-2004), (ii) tree slices from the apex separating early and late wood (1990-2004) and (iii) tree cores from surviving trees in which each tree-ring was divided into four sections (2000-2006). We observed a decline in radial growth (mainly early wood) in both tree groups, being faster in declined trees, which showed a higher growth in the 70s and 80s. During this period, Δ^{13}C records suggested a progressive increase in water-use efficiency, although stronger in declined than in healthy trees. Comparing Δ^{13}C and δ^{18}O, the two isotopes were negatively correlated in declined trees but not in healthy ones, further supporting a stronger stomatal limitation of susceptible trees. Similarly, radial growth was positively associated with Δ^{13}C only in declined trees, which also showed a greater response to climate variables. Among the surviving trees, the ones with symptoms of decline (defoliation) showed increased Δ^{13}C (and lowered water-use efficiency) just after the dieback episode, whereas healthy trees showed lower Δ^{13}C. Overall, our results indicate that a differential history in terms of water-use determined the fate of individuals during the dieback event. Post-dieback response of defoliated trees, which inverted pre-dieback trends, could be either due to an uncoupling of regulation mechanisms in senescing needles or to a faster recovery (at the leaf level) of highly defoliated trees due to their lower water demand.

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