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Research

Masting in whitebark pine (*Pinus albicaulis*) depletes stored nutrients

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Summary

- In masting trees, synchronized, heavy reproductive events are thought to deplete stored resources and to impose a replenishment period before subsequent masting. However, direct evidence of resource depletion in wild, masting trees is very rare. Here, we examined the timing and magnitude (local vs individual-level) of stored nutrient depletion after a heavy mast event in *Pinus albicaulis*.
- In 2005, the mast year, we compared seasonal changes in leaf and sapwood nitrogen (N) and phosphorus (P) concentrations and leaf photosynthetic rates in cone-bearing branches, branches that never produced cones, and branches with experimentally removed cones. We also compared nutrient concentrations in cone branches and branches that had never had cones between 2005 and 2006, and measured tree ring width and new shoot growth during 2005.
- During the mast year, N or P depletion occurred only in tissue fractions of reproductive branches, where photosynthetic rates were reduced. However, by the end of the following year, nutrients were depleted in all branches, indicating individual-level resource depletion. New shoot and radial growth were not affected by masting.
- We provide direct evidence that mast events in wild trees deplete stored nutrients. Our results highlight the importance of evaluating reproductive costs over time and at the individual level.

Introduction

A fundamental tenet in life history theory is that tradeoffs among vital rates in organisms arise because resources are finite and preferential allocation to a given function reduces resources available for other competing functions (Williams, 1966; Roff, 1992). In plants, for instance, costs of reproduction are very common (Obeso, 2002). Although limiting resources are the physiological basis of life history trade-offs, emphasis in the literature for wild plants has been on either documenting the demographic costs or quantifying several interrelated measures of proportional resource allocation to reproduction and subsequent effects on vegetative growth (see Obeso, 2002 for a review). By contrast, few studies have directly measured the degree to which reproduction depletes stored resources (i.e. measurements of storage) in wild plants (Cipollini & Stiles, 1991; Newell, 1991). Such data are especially relevant for long-lived, large plants that can store a significant amount of resources and retrieve them during times of demand (Chapin *et al.*, 1990). If storage pools are large enough to meet competing demands, the short-term costs of reproduction on growth may not be detected, although depletion of stored resources may lead to longer term costs. In this case, a more meaningful measure of the direct costs of reproduction is the degree of stored resource depletion (Reznick, 1985; Ehrlen & Van Groenendael, 2001).

Resource depletion is expected to be particularly high in masting species, which are characterized by the synchronous and intermittent production of large seed or fruit crops (Herrera *et al.*, 1998; Kelly & Sork, 2002; Crone *et al.*, 2011). Indeed, preferential resource allocation to reproduction during mast events has long been thought to deplete storage pools and to require a replenishment period with low reproductive output before the next mast event (Janzen, 1974; Harper, 1977; Rathcke & Lacey, 1985; Kelly, 1994; Newbery *et al.*, 2006). Consistently, resource depletion is a key assumption of theoretical models to explain masting patterns (Yamauchi, 1996; Satake & Iwasa, 2000; Masaka & Maguchi, 2001; Rees *et al.*, 2002; Satake & Bjørnstad, 2008). While depletion of stored resources has been shown in orchard species that are bred for increased fruit yield (Goldschmidt & Golomb, 1982; Brown *et al.*, 1995; Rosecrance *et al.*, 1998), empirical direct data for wild plants (Crone *et al.*, 2009) are very rare. Direct data on resource depletion in wild masting trees exists only for carbohydrates, and the evidence is mixed and species-specific, with no effects in some species (Hoch *et al.*, 2003) and tissue-specific depletion in other species (Miyazaki *et al.*, 2002; Ichie *et al.*, 2005). These mixed results

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likely reflect the fact that carbon is not necessarily the best currency to measure costs of reproduction (Ashman, 1994; Hemborg & Karlsson, 1998), particularly if plants compensate for carbon demands during reproduction (Reekie & Bazzaz, 1987a), or if carbon does not necessarily limit growth in the short term (Körner, 2003; Sala & Hoch, 2009).

Compared to carbohydrates, mineral nutrients, particularly nitrogen (N) and phosphorus (P), may be a more appropriate currency because they are usually limiting in the environment and because reproductive structures (particularly seeds and fruits) are generally nutrient enriched relative to vegetative biomass (Reekie & Bazzaz, 1987b). Convincing evidence for depletion of stored nutrients after mast events exists only for orchard trees (Goldschmidt & Golomb, 1982; Brown *et al.*, 1995; Rosecrance *et al.*, 1998). In wild trees, by contrast, such evidence is rare and more indirect. For instance, nutrient allocation to reproductive structures during mast events has been related to strongly phosphorus-depleted litter in some species (Newbery *et al.*, 1997), and to nutrient-limited flower primordia development in others (Han *et al.*, 2008). In both cases, reproduction is hypothesized to deplete within-tree nutrient reserves, but direct measurements were not available. By contrast, Yasumura *et al.* (2006) reported no nutrient-depleted litter after a mast event, although their results are equivocal due to limitations of the study design.

The assessment of costs of reproduction in trees is further complicated by the fact that trees are modular organisms where branches operate as semiautonomous units (Watson & Casper, 1984; Lovett-Doust & Lovett-Doust, 1988; Sprugel *et al.*, 1991) and costs of reproduction may vary depending on the modular level examined (Obeso, 1997). With respect to carbohydrate demands, branch autonomy has been shown to be species-specific, with some species showing complete autonomy (Hoch, 2005) and others relying to a variable degree on carbohydrate import from other parts of the tree (Obeso, 1998; Miyazaki *et al.,* 2002). However, branches cannot be indefinitely autonomous with respect to mineral nutrients. Depending on the overall nutrient demand for reproduction, nutrients may be drawn from branches bearing reproductive structures only (local depletion) or from other equivalent branches regardless of reproductive status (nonlocal depletion). Whether resource depletion is local vs nonlocal is likely to influence temporal patterns of reproduction. Under local resource depletion sexually mature branches that did not reproduce in a given year may have enough resources to reproduce the subsequent year. Therefore, individuals may be able to reproduce to some degree every year leading to lower inter-annual variation of reproductive output. By contrast, if reproduction in a given year causes all branches to become resource-depleted regardless of their reproductive status (nonlocal resource depletion), then we expect strong decreases of reproductive output after years with high seed set, which is the typical pattern in masting species. In spite of the fundamental, and often invoked, role of stored nutrients in wild masting trees, there are few convincing data on nutrient depletion in standing biomass during and after masting events, as well as on the degree to which costs of reproduction are local vs nonlocal.

Here, we document reproductive output and stored resource dynamics in *Pinus albicaulis* (whitebark pine), a high elevation masting pine. Specifically, we examine the timing (during and after a masting event) and magnitude (local vs nonlocal) of resource depletion. From the onset of an unusually heavy mast event in 2005, we compared seasonal changes in leaf and branch sapwood N and P concentrations and leaf photosynthetic rates in cone-bearing branches, branches not bearing cones, and branches where we removed cones. To test the timing and magnitude (local vs nonlocal) of resource depletion we compared nutrient concentrations in cone branches and branches that had never had cones. We also examined the consequences of masting for short-term growth by measuring new shoot growth during the mast event and tree ring growth from 1995 to 2010. Specifically, we addressed the following two main questions: does cone maturation deplete nutrients in reproductive branches (local-level depletion)? and, to what extent is nutrient depletion during and after the mast event local vs nonlocal (at the branch- vs the individual-level)? We also explored whether masting was associated with a short-term growth decline. Because whitebark pine produces very large, nutritious seeds (Lanner & Gilbert, 1994), we hypothesized that years of high reproductive output would incur significant nutrient costs with negative physiological consequences. We also hypothesized that if resource switching between reproduction and storage influences future reproduction, then nonlocal resource depletion should occur at some point after the mast event.

Materials and Methods

Study species

Pinus albicaulis Engelm. (whitebark pine) is a long-lived, stress-tolerant tree found in relatively cool climates of the northwestern US and southwestern Canada, usually at high elevations with relatively low rainfall (Weaver, 2001). Across much of its range *P. albicaulis* is an early successional species and it facilitates community development by mitigating an otherwise extreme environment (Tomback *et al.*, 2001). Trees reach reproductive age at *c.* 20–30 yr (McCaughey & Tomback, 2001). *Pinus albicaulis* is monoecious, and female cones, which are nonphotosynthetic, take 2 yr to develop. In Montana, male and female cones are visible as buds during spring (mid-June) of their first year, and wind pollination occurs in July, after which male cones senesce in early to mid-August (Weaver, 2001). Female cones are visibly expanded (but still small) by early July of their second year, and mature in late summer or early fall, that is, *c.* 18 months after initiation. Cones are nondehiscent and produce large, nonwinged seeds, very rich in nitrogen (N), phosphorus (P) and lipids (Lanner & Gilbert, 1994) which are an important food source for many animals, including the main seed disperser, Clark's nutcracker (*Nucifraga columbiana*), as well as grizzly bear (*Ursus arctos*) and red squirrel (*Tamiasciurus hudsonicus*; Tomback *et al.*, 2001). Like most other pine species, whitebark pine is a masting tree, although regional patterns of masting are highly variable (Crone *et al.*, 2011). *Pinus albicaulis* is undergoing broad species decline due primarily to the introduced white

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pine blister rust (*Cronartium ribicola)* which has reached epidemic status in many populations (Tomback *et al.*, 2001). In the past decade, a mountain pine beetle epidemic is also causing devastating mortality in high-elevation whitebark pine stands (Hicke & Logan, 2009). Restoration efforts for whitebark pine currently rely on seed collections from rust-resistant trees, but the physiological costs of seed production and their consequences for masting patterns are not known.

Site, experimental manipulations and sampling

We studied resource dynamics in relation to cone production during (2005) and after (2006) an unusually heavy mast event at a site located in the Flint Creek range of western Montana, USA (46.2°N and 113.3°W; 2300 m elevation). Female cones for this event were fertilized in the spring of 2004. Cone expansion, seed filling and final maturation occurred during the 2005 growing season. The site has a mixed stand dominated by whitebark pine and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). The understory is composed of a mixture of grasses and shrubs with a dominance of the small shrub *Arctostaphylos uva-ursis* (L.) Spreng. Mean annual air temperature (1990–2011) at the closest weather station (*c.* 12 km away; Warm Springs Snotel Site, Montana, *c.* 2360 m elevation) was 1.1°C, with mean January and July temperatures of − 6.7 and 12.2°C, respectively. Mean total annual precipitation was 572 mm. The specific site is located on a local plateau, where the sampled stand surrounds a relatively moist meadow with an ephemeral spring dissecting it.

In early July 2005, we selected nine trees based on accessibility for climbing. Tree diameter at breast height (DBH, 1.3 m) ranged from 19 to 54 cm. Trees were sufficiently spaced to prevent significant shading by neighbors. Some trees had multiple boles, a pattern that results from germination of cached seeds by Clark's nutcracker (Tomback *et al.*, 2001). In this case, sampling over time was limited to the same main bole. In July 2005, as soon as the site was accessible after snowmelt, we climbed the trees and selected eight upper-canopy, terminal branches per tree for sampling: five with cones and three without cones (see Table 1 for sampling details). We removed the cones from two of the five branches with cones, leaving three sets of branches (cone-bearing (C), removed cones (RC) and noncone bearing (NC)) for sampling over time: early July (only C and NC), late July and late August (C, NC and RC; Table 1). Branches of the different treatments were randomly assigned for sampling at each time (i.e. one branch of each treatment was harvested at each harvest period, except for the first sampling when cone removal was implemented and only C and NC branches were sampled). All branches assigned for late July and late August sampling (including NC) were protected with *c.* 15 × 15 cm wide and 50-cm long wire mesh cages to prevent predation by squirrels or nutcrackers once cones matured. Processing of late August cones led us to suspect that seeds were not yet completely mature, so we harvested one more set of C and NC upper-canopy branches per tree in late September. At this time, however, we did not have RC branches left for sampling. Because of the heavy mast event in 2005, it was not possible to find a set of nearby trees with no cones to sample.

In 2006 we sampled two classes of noncone-bearing branches for each tree: branches not bearing cones but that had produced cones in 2005 (C5), and branches that had never produced cones (NCE –no cones ever; Table 1). Like other stone pines (subgenus

Table 1 Sampling details and statistical analyses conducted showing the specific contrasts and subset of *Pinus albicaulis* (whitebark pine) samples used

Sampling details					Statistical analyses: type and subset of samples		
Year	Branch type	Description	Treatment	Sampling time	2005: C vs NC	2005: C vs RC	2005–2006: C5 vs NCE
2005	C	Bearing cones	C	Early July, Late July, Late August, Late September[a]	C: all dates	C: Late July, Late August	C5: Early July (E05), Late September (L05)
			RC: cones removed	Late July, Late August		RC: Late July, Late August	
	NC	Not bearing cones	na	Early July, Late July, Late August, Late September[a]	NC: all dates		NCE (subset of NC): Early July (E05; *n* = 4), Late September (L05; *n* = 6)
2006	C5	Bearing cones in 05, but not in 06	na	Early July, Late September			C5: Early July (E06), Late September (L06)
	NCE	Never produced Cones	na	Early July, Late September			NCE: Early July (E06), Late September (L06)

Except when indicated, sample size generally ranged from 8 to 9. The only exceptions were cases when limited amount sample for P analyses reduced sample size to 6 or 7 (two cases each). na, not applicable.
[a] A late September sampling of C and NC branches was added after the fact (see text).

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Strobus), *P. albicaulis* cones leave visible scars on branches (Crone *et al.*, 2011). Sampling took place as soon as the site was accessible (early July) and at the end of the season (late September). On some occasions, damage to caged branches (in 2005) or difficult access to cone branches reduced sample size from 9 to 8 trees.

Field measurements

At each sampling time in 2005, we harvested each of the treatment branches per tree for subsequent nutrient analyses. Photosynthetic rates were measured in early July (n = 6 trees), early August (n = 4 trees) and late August (n = 9 trees). Once on the ground, the cut end of each branch was immediately placed in a plastic bag with a moist paper towel and branches were kept illuminated. Shortly after harvesting, photosynthesis rates were measured in 1-yr-old needles and in older needles (generally 4 yr old) with a LICOR 6400 portable photosynthesis system (LICOR, Lincoln, Nebraska, USA) equipped with an LED light source. Based on light response curves for whitebark pine (Sala *et al.*, 2001) photosynthetically active radiation was kept at 1000 μmol m^{-2} s^{-1}. The CO_2 concentration in the chamber was kept at 400 μmol m^{-2} s^{-1} and temperature at 18°C (early July) or 20°C (subsequent samplings). Five needles were placed in a 2 × 3 cm chamber with care to prevent overlap. We verified that gas exchange was not negatively affected from the time branches were cut to the time they were measured (generally no > 20 min). Needle width was measured to calculate projected leaf area in the chamber. Photosynthesis rates were expressed on a projected leaf surface area. All harvested branches, including those used for gas exchange measurements, were kept in a cooler with ice until transport to the laboratory.

Cone counts on each sampled tree were done in late September from 2005 to 2009 with binoculars (cone counts by two separate observers were highly correlated; r^2 = 0.98). The number of seeds per cone and seed weight were also measured from 2007 to 2009 (cones were not caged in 2006 and were eaten by the time we harvested). In late September 2009, one increment core from each tree was taken for subsequent growth analyses. Cores were taken at breast height (1.3 m) from the north side with an increment borer.

Sample processing

For 2005 samples, once in the laboratory, we removed the most recent (1-yr-old), fully mature needle cohort (young) and the older, 4- to 5-yr-old cohort (old) per branch. Samples were oven-dried at 65°C for 72 h and ground to a fine power to pass a 0.25 mm mesh. For the late August and late September sampling times, we counted the number of cones on C branches, the number of seeds per cone, and measured the seed weight after removing the seed coat. Seeds from different cones per tree were pooled, oven-dried as before and ground with a mortar and pestle to a uniform paste for subsequent analysis. For the early July (early season) and late September (end of season) sampling times, a segment of each branch per tree between 9 and 13 mm of diameter was cut below the oldest needle cohort, the bark removed, and oven dried as before. Branch sapwood was ground with a Wiley mill and subsequently to a fine powder as before. Then 3 mg seed, 8 mg needle and 10 mg branch material were weighed into tin capsules for subsequent N content analysis at the Stable Isotope Laboratory of the University of California, Davis. Dried samples (50–100 mg) were sent to the Colorado State University Soil Plant and Water Testing Laboratory (Fort Collins, Colorado, USA) for P analyses. Occasionally, the limited amount of material for P analyses reduced sample size from nine trees to a minimum of five (one case) or six (two cases). Current yr shoot length and diameter was measured in late August and late September with a digital caliper as indicators of shoot growth. Because measurements for C and NC branches did not change from late August to September (paired t-test; $P > 0.05$), we used the late August data when we also had data for RC branches. Processing of the 2006 branches (needles and branch sapwood) was the same as for 2005, except that we did not measure seed traits and shoot extension.

Tree cores were mounted, sanded and ring width was measured to the nearest 0.01 mm with a dissecting scope using an eyepiece reticle calibrated against a stage micrometer. Cores were cross-dated manually because only 10 yr of growth was analysed.

Statistical analyses

We conducted three analyses of resource dynamics through time (Table 1). First, we compared the nutrient concentrations in needles of C and NC branches collected at four times during the 2005 growing season (mast year). In 2005 in the field we did not distinguish between NC branches that had never produced cones and those that had previously produced cones. Based on cone scars during the past 10 yr we later identified which of the NC branches had produced cones in the past and which had never produced cones (about half of each; Table 1). Nutrient concentrations and photosynthetic rates on any given date did not differ between branches that had previously had cones and branches that had never produced cones (equivalent to the NCE sampled in 2006; paired t-tests; $P > 0.05$ in all cases) and data were pooled. Second, we compared nutrient concentrations of C and RC branches collected in late July and late August 2005. (Due to the timing of cone removals, RC branches were not available for the late September sampling –see 'Site, experimental manipulations and sampling' in the Materials and Methods section). Third, to evaluate long-term consequences of mast-seeding, we analysed changes in N and P concentrations in branches over time: early July 2005 (before cone maturation), late September 2005 (after cone maturation), early July 2006, and late September 2006. Temporal changes were analysed for two groups (status) of branches (Table 1): (1) C5 branches: branches that produced mature cones in 2005. These included C branches sampled in 2005, and branches sampled in 2006 that did not bear cones but that had mature cones in 2005; (2) NCE (no cone ever) branches: branches that had never produced cones sampled in 2005 and 2006. NCE branches from 2005, were a subset of NC branches for which there were no signs of previous cone production based on cone scars (n = 4 and 6 for early July and late

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September, respectively; Table 1). In this third analysis, we use C5 branches to estimate 'local' effects of mast-seeding on branches that produced cones in 2005, and NCE branches to estimate 'nonlocal' effects of mast-seeding on resource concentrations in other parts of the tree.

All three analyses were implemented as linear mixed models with nutrient concentrations as the dependent variable, and time (harvest period), treatment (C vs NC, C vs RC, or C5 vs NCE), and time × treatment interactions as independent variables. To account for repeated sampling from individual trees, all analyses included tree identity as a random effect. Statistical significance of main effects and interactions was assessed using likelihood ratio tests of models with each term removed, relative to the full model (i.e. marginal effects). Following significant main effects or interactions, we calculated *post hoc* comparisons of specific group pairs using *t*-statistics (ratio of difference in means to standard error) for that pair in the full model. *Post hoc* comparisons were implemented in R (R Development Core Team, 2009) by re-running the full model with each treatment combination coded as a separate group, and one member of each pair set to be the reference group. Note that *P*-values for *t*-tests in mixed models are approximate (Baayen *et al.*, 2008).

Results

Reproductive output and resource status in 2005 before cone maturation

Cone production in 2005, the mast year, was much higher than that from 2006 to 2009 (Fig. 1). On average trees produced 125.6 ± 28.5 (SE) cones per tree in 2005 and 15.4 ± 3.3 cones per tree in 2006. Subsequently, cone production until 2009 did not exceed 25 cones per tree. One tree produced only 20 cones in 2005. However, it was included in all analyses because, although low, cone production in 2005 was higher than in any subsequent year (11, 0, 0 and 4 cones from 2006 to 2009, respectively). In 2005, C branches produced an average of 2.4 cones, with an average of 83.9 ± 23.0 seeds each, and an average seed weight (without the seed coat) of 119.4 ± 13.9 mg. On average, trees produced 1257.7 ± 285.4 g of seeds (without seed coat) in 2005. The number of seeds per cone, individual seed weight and total seed mass per tree were lower and quite variable in subsequent years, with a population average among years of 33.9 ± 16.4 seeds per cone, 46.9 ± 16.0 mg per seed, and 19.1 ± 9.7 g of seeds per tree. The population-level coefficient of variation (CV_p) was calculated based on the mean of the population annual means ($\bar{\chi}_p$) and the standard deviation (SD) of the annual means ($CV_p = SD/\bar{\chi}_p$; Koenig *et al.*, 2003). The CV_p of total seed mass and total seed number per tree during the study period was 1.85 and 1.56, respectively.

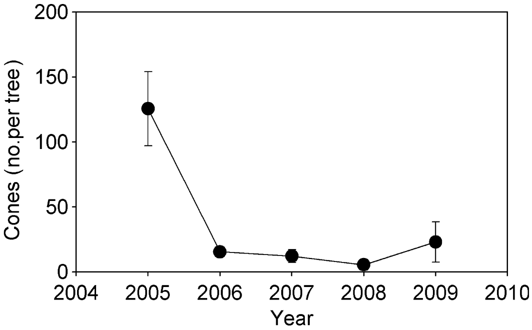

Fig. 1 Average number of cones per *Pinus albicaulis* (whitebark pine) tree from 2005 to 2009. $n = 9$, error bars are ± SE.

Average seed nitrogen (N) and phosphorus (P) concentrations (excluding coats) were 33.54 ± 1.96 and 7.95 ± 0.96 (SE) mg g^{-1}, respectively. Leaf and branch sapwood N and P concentrations in early 2005 were similar or higher in cone relative to no cone branches (Fig. 2; compare C5 vs NCE). Significantly higher values were found for N and P in branch sapwood (*post hoc* $t = -2.27$, $n = 11$, $P = 0.0317$; $t = -3.01$, $n = 11$, $P = 0.0058$, respectively). Values for N in young needles also tended to be higher, but the differences were not statistically significant at the 0.05 level (*post hoc* $t = -1.90$, $n = 11$, $P = 0.0680$).

Resource depletion, photosynthesis and growth

During 2005, N concentration in young needles of C branches decreased over time, while that of NC and RC branches increased (C vs NC, June, July, Aug & Sept harvests: treatment × time effect: $\chi^2 = 16.3$, df = 3, $n = 74$, $P = 0.001$; C vs RC, July & August harvests: $\chi^2 = 7.7$, df = 1, $n = 43$, $P = 0.006$; Fig. 3; Supporting Information Table S1). By contrast, seasonal dynamics of N concentration in old needles and P concentration in young and old needles did not differ between C and NC branches in 2005 ($\chi^2 < 2.3$, $P > 0.50$ for all treatment and treatment × time effects; Table S1). Seed mass and N concentration (without seed coat) did not differ between late August and late September, coinciding with no change of N in needles (Fig. 3). By contrast, there was a large significant increase of seed P ($t = -3.3$; df = 13; $P = 0.002$; Fig. 3), which coincided with a slight decrease in P concentration of old needles during the same period (albeit the time effect was not statistically significant when all periods were included in the analysis). Seasonal N depletion in young needles in 2005 coincided with significantly lower leaf photosynthetic rates in August in young needles of C branches relative to NC or RC (mixed model with random effect of tree; early August: $\chi^2 = 310.23$; df = 1, $n = 8$ samples, $P = 0.0014$; late August: $\chi^2 = 21.80$; df = 1, $n = 27$ samples, $P = < 0.001$; Fig. 4). Values for C and NC branches in early July differed less (Fig. 4), and were not statistically significant at the 0.05 level ($\chi^2 = 3.46$; df = 1, $n = 12$ samples, $P = 0.063$).

Nitrogen and P concentrations in all tissues generally declined from 2005 to 2006 (Fig. 2, Table 2). The time effect was significant for all fractions, although for P in young needles, an increase in early 2006 contributed to this effect. However, when the early 2006 sampling was excluded from the analysis, the time effect remained significant ($n = 38$, df = 2; $\chi^2 = 7.1$; $P = 0.029$) consistent with the decline from early 2005 to late 2006 in all other fractions. In 2005 (during the mast year), significant resource

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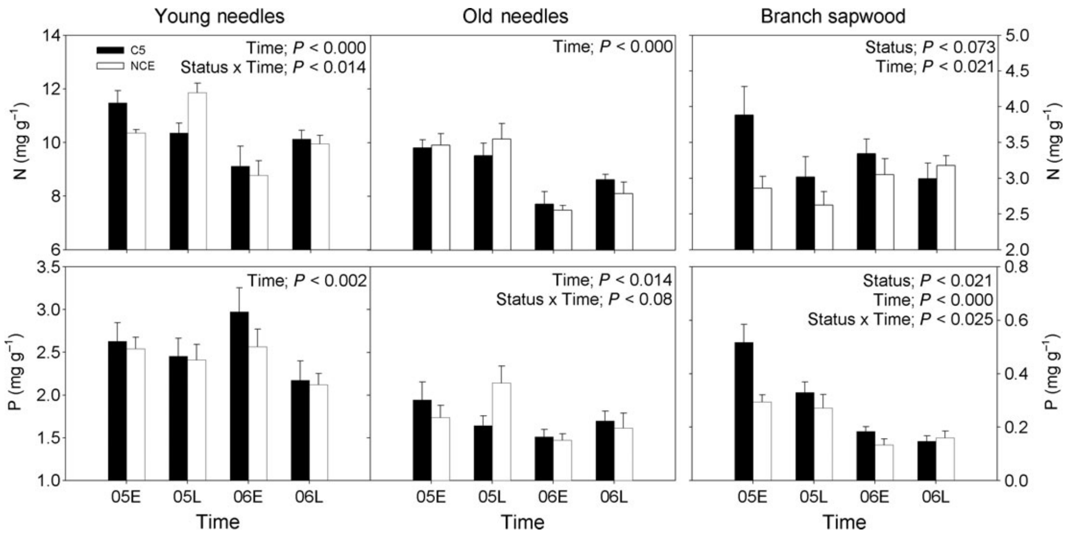

Fig. 2 Nitrogen (N) and phosphorus (P) concentrations in *Pinus albicaulis* (whitebark pine) young needles (fully mature 1-yr-old), old needles (4–6-yr old) and branch sapwood at four sampling times: July (early) 2005 (05E), September (late) 2005 (05L), July (early) 2006 (E06) and September (late) 2006 (06L). In each year, measurements were done in branches that produced cones in 2005 (C5; closed bars) and branches that had never produced cones (NCE; open bars). Error bars are ± SE. Significant effects of time, branch reproductive status and their interaction and the corresponding *P* values are indicated. Additional statistical details are in Supporting Information Table S1.

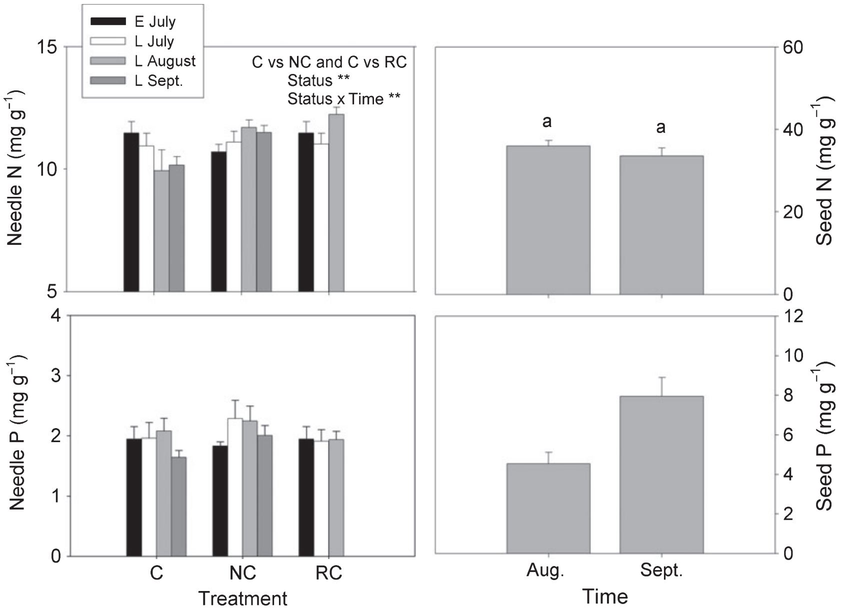

Fig. 3 Left panels: nitrogen (N) concentration in *Pinus albicaulis* (whitebark pine) fully mature 1-yr-old needles (upper) and phosphorus (P) concentrations in 4-yr-old needles (lower) in cone-bearing branches (C), branches not bearing cones (NC), and branches with cones experimentally removed (RC) at four times during the mast season of 2005: early July (black bars), late July (white bars), late August (light gray bars) and late September (dark gray bars). Right panels: N (upper) and P (lower) concentrations of seeds sampled in late August and late September. N ranges from 7 to 9 trees, except when limiting sample for P analyses reduced sample size to 5 or 6 (only one case each). Error bars ± SE. Significant main effects (see text for details) are indicated on left panels. Additional statistical details are in Supporting Information Table S1. Different letters on right panels indicate statistically significant differences between sampling times.

depletion (either N or P) from early to late in the growing season occurred only in tissue fractions of cone-bearing branches (C5), whereas by late 2006, significant depletion occurred in both previously cone-bearing (C5) and noncone-bearing (NCE) branches (Fig. 2; Tables 2, S2). This was reflected by significant time × status interactions for N in young needles: $\chi^2 = 10.6$, df = 3, $n = 60$, $P = 0.014$ and for P in branch sapwood: $\chi^2 = 9.4$, df = 3, $n = 57$, $P = 0.025$. There also was a marginal interaction effect for P in old needles ($\chi^2 = 6.8$, df = 3, $n = 52$, $P = 0.081$; Table S1). Nutrient concentrations in early and late 2006 did not differ between C5 and NCE branches.

By the end of 2005, growth of new shoots was similar for branches with cones, branches without cones but reproductively mature (i.e. that had cones in the past) and branches where cones had been experimentally removed (Fig. 5; linear mixed model with random effect of tree: $\chi^2 = 0.76$; df = 2, $n = 20$ observations, $P = 0.684$). Similarly, the mast event in 2005 was not associated with a radial growth decline (Fig. 6; linear mixed model with random effect of tree: $\chi^2 = 2.92$; df = 2, $n = 20$ observations, $P = 0.232$). Rather, tree ring width was relatively constant from 1994 to 2009, with the exception of a growth peak in 2003 followed by a decrease in 2004 (Fig. 6). Within individual trees, cone production from 2005 to 2009 was not related to tree ring width for the same year or the year after ($P > 0.05$ in all cases) and in most trees, tree ring width remained constant or increased in 2005 and 2006 relative to 2004 (only two trees showed a

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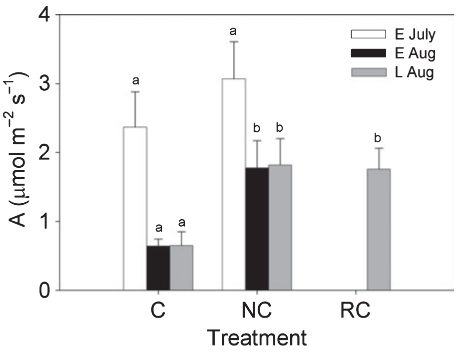

Fig. 4 Photosynthetic rates (*A*) of *Pinus albicaulis* (whitebark pine) 1-yr-old needles in branches bearing cones (C), no cones (NC), and branches where we removed cones (RC) in early July, early August and late August. n = 6, 4 and 9 trees per treatment, respectively. Error bars ± SE. Within a given date, different letters denote statistically significant differences at $P > 0.05$ (see text for details).

Table 2 Percentage decrease (i.e. amount lost) of nitrogen (N) and phosphorus (P) concentrations in late 2006 (relative to 2005) in different tissue fractions of *Pinus albicaulis* (whitebark pine) branches bearing cones in 2005 (C5) and branches that had never produced cones (NCE)

Nutrient	Tissue	C5	NCE
Nitrogen	Young Needles	13.6 (156.6)**	15.8 (186.8)^{a,***}
	Old Needles	18.4 (180.9)**	18.3 (181.0)***
	Branch Sapwood	22.9 (89.0)**	− 11.1^{ns}
Phosphorus	Young Needles	18.3 (48.2)*	16.5^{ns}
	Old Needles	15.7^{ns}	21.4 (14.6)^{a,*}
	Branch Sapwood	71.5 (37.0)***	45.9 (13.5)***

Negative values represent an increase. In parentheses are equivalent mg of N or P per 100 g tissue based on initial concentration and percent decrease.

[a] Indicates declines only from late 2005 to late 2006 (see Table S2).

P values (pairwise *post hoc* comparisons): *, $P \leq 0.1$; **, $P \leq 0.05$; ***, $P \leq 0.01$.

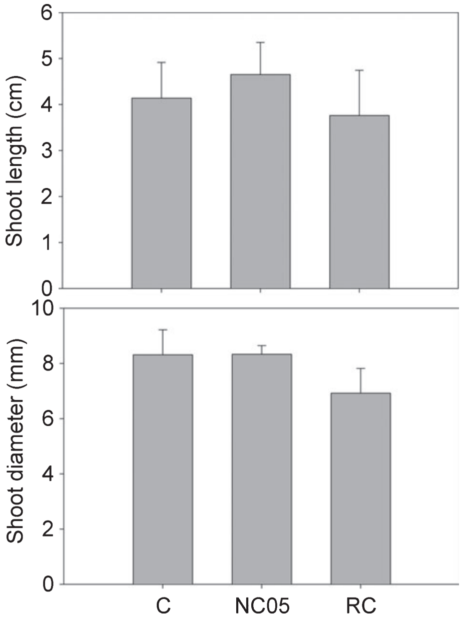

Fig. 5 Length and diameter of *Pinus albicaulis* (whitebark pine) new shoots produced in 2005 in cone bearing branches (C), reproductively mature branches that did not produce cones in 2005 (NC05) and branches with removed cones (RC). n = 9 for C and RC branches and 4 for NC branches. Bars indicate + SE.

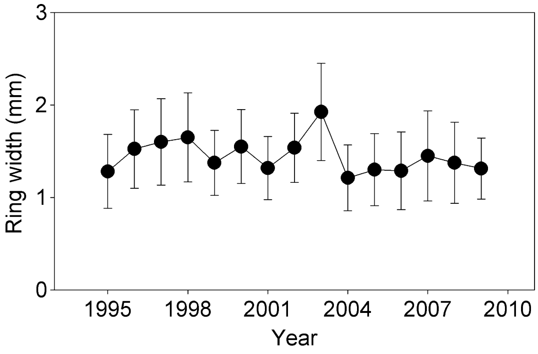

Fig. 6 *Pinus albicaulis* (whitebark pine) tree ring width measured at diameter at breast height from 1995 to 2009. Bars indicate ± SD, n = 9 trees.

decrease of tree ring width). Our short time series did not reveal any significant correlation between tree ring width and climatic variables.

Discussion

Although mast events have long been thought to cause a depletion of storage resource pools in plants (see the Introduction section), direct empirical data for wild trees is lacking (for nutrients) or inconclusive (for nonstructural carbohydrates). For whitebark pine, we show that a heavy mast event depleted stored nutrients. Initially (during the mast year), nutrients were depleted locally (in reproductive branches only), but by the subsequent year nutrient depletion was also observed at the individual level (in all terminal branches, regardless of their reproductive status). Our results provide direct evidence of a decline of stored nutrients after a mast event in a wild tree. This is consistent with data from orchard alternate-bearing trees (Goldschmidt & Golomb, 1982; Brown *et al.*, 1995; Rosecrance *et al.*, 1998), and suggests that nutrient depletion is not unique to species specifically bred for high crop yield. Results are also consistent with nitrogen-limited flower bud development after mast events (Han *et al.*, 2008) and with indirect evidence based on nutrient-depleted litter (Newbery *et al.*, 1997). Together, these results support the argument that mast events in wild trees impose a replenishment period before a subsequent mast event.

Nutrient depletion was based on concentrations, rather than total resource pools. Therefore, declines in concentration could, in principle, reflect dilution due to an increase of biomass. However, in our system, this is highly unlikely and probably unrealistic. Based on biomass allocation equations (Callaway *et al.*, 2000), the annual biomass increment of a 38-cm DBH whitebark pine tree (the average diameter of our sampled trees) is 1.4% of the total standing biomass. In the present study, decreases in nutrient concentrations ranged from 13% to 72% (Table 2), depending on the nutrient and tissue fraction, which is at least one order of magnitude greater than the average annual biomass increment.

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In whitebark pine, cone production in 2005 was unusually high. Similar cone crops in the region were recorded only once in the past 15–20 yr (Crone *et al.*, 2011; Interagency Grizzly Bear Study Team, unpublished). At our site, cone scar analysis also indicated that cone crops in the past 10 yr were low (Crone *et al.*, 2011). Accordingly, the CV_p of seed mass and seed number based on only 5 yr (1.85 and 1.56, respectively) are at the very high end of the CV_p range reported for other pines (maximum 1.8; Herrera *et al.*, 1998). Given the high cone crop in 2005 and the large and nutritious seeds of whitebark pine (Fig. 3; Lanner & Gilbert, 1994), the substantial nutrient depletion (between 14% and 72%, depending on the nutrient and tissue fraction; Table 2) is not surprising. Interestingly, depletion occurred incrementally over time: by the end of the masting season resource depletion was local (reproductive branches), but by the end of the next season nutrient depletion was at the individual level (in all terminal branches, regardless of their reproductive status during the mast event). This switch from local to nonlocal nutrient depletion suggests complex resource dynamics after masting in trees and highlights the importance of evaluating reproductive costs over time and at the individual level.

Depletion in cone-bearing branches during the 2005 mast season occurred only in tissues where nutrient concentrations before cone maturation were significantly higher than that in nonreproducing branches (young needles became N depleted and branch sapwood became N and P depleted). Cipollini & Stiles (1991) and Karlsson (1994) also reported higher resource concentrations in reproductive branches before fruit maturation. Because we did not sample before the mast event we cannot tell whether higher nutrients in reproductive branches contributed to cone initiation or the reverse. In any case, nutrient storage in cone-bearing branches was not sufficient to meet reproductive demands and by the subsequent season nutrients were depleted in all branches. A possibility is that the depletion in 2006 was caused by factors other than the mast event. However, this seems unlikely. First, 2006 was a year of near average annual precipitation (572 mm) but above average temperatures (2.8°C). This suggests that nutrient availability in 2006 was not limited by low water or cold temperatures. Second, radial growth data does not suggest any unusual growth pattern in 2006 that could have altered nutrient dynamics. On the contrary, in subalpine pines, tree ring growth is generally stimulated by warmer temperatures (Salzer *et al.*, 2009) as it appeared to be the case in 2003. Thus, it could be that the lack of growth stimulation in 2006 reflects nutrient limitations induced by masting in 2005. Our results are consistent with those of Han *et al.* (2008) suggesting that nutrient limitation impeded flower bud primordial development after a mast event. Newbery *et al.* (1997) also documented P-depleted litter after a mast event and hypothesized that reproduction depleted within-tree P reserves. However, direct evidence of nutrient depletion (in standing biomass) was not available in either case and alternative explanations are possible. For instance, litter-based inferences may be limited by the degree to which nutrient resorption offsets nutrient demands for reproduction and buffers changes in storage. Unless critical resource thresholds for reproduction are very low, the nutrient depletion we document suggests that a period of nutrient replenishment followed by appropriate climate cues is required before a subsequent mast event (Smaill *et al.*, 2011).

Most of the few studies on resource costs of mast-seeding in trees have focused on nonstructural carbohydrates and results have been mixed (Miyazaki *et al.*, 2002; Hoch *et al.*, 2003; Ichie *et al.*, 2005). This may reflect in part the degree to which current photosynthate production by foliage (and sometimes by reproductive structures) compensates for carbon demands by reproduction (McDowell *et al.*, 2000; Obeso, 2002; Ichie *et al.*, 2005). In contrast to nonstructural carbohydrates, nutrients are often considered a better currency to measure costs of reproduction in plants (Ashman, 1994; Hemborg & Karlsson, 1998). In trees, this is particularly true because carbon is often not limiting growth in the short term (Körner, 2003; Millard *et al.*, 2007; Sala & Hoch, 2009), as is the case for whitebark pine (Sala *et al.*, 2011). In addition, individual branches cannot be indefinitely autonomous with respect to nutrients (Sprugel *et al.*, 1991). Further, whitebark pine occurs in subalpine habitats (Tomback *et al.*, 2001) where short-term growth and reproduction are often nutrient- (Bowman *et al.*, 1993; Karlsson, 1994) but not carbon-limited (Hoch *et al.*, 2003; Hoch, 2005). The investment of leaf N to reproduction in cone-bearing branches at the cost of C assimilation via photosynthesis is consistent with nutrient- but not C-limited reproduction. In contrast to other results (Wheelwright & Logan, 2004), this apparent C cost did not affect new shoot or radial growth also suggesting that C does not limit growth in the short term. Current resource dynamics models to explain masting patterns are based on stored photoassimilates (Isagi *et al.*, 1997; Satake & Iwasa, 2000). Our results for whitebark pine indicate that these models should be interpreted more generally as dynamics of stored resources (carbohydrates and/or nutrients), depending on what resources limit reproduction in different species. For example, depletion of carbohydrates, but not nutrients, followed mast years in a mast-seeding perennial wildflower (Crone *et al.*, 2009) suggesting that carbon may be more limiting in herbaceous than in woody plants.

Relative N depletion was roughly similar in needles and branch sapwood while relative P depletion was greater in branch sapwood than in needles. However, much lower nutrient concentrations in branch sapwood translated to higher absolute N extraction from foliage relative to sapwood and roughly similar P extraction from both fractions (Table 2). Therefore, foliage contributed proportionally more N, but not P, to reproduction than branch sapwood, which may reflect a greater surplus of N relative to P. This is consistent with the proposed N storage function of the carboxylating enzyme rubisco in leaves of trees (Millard *et al.*, 2007), with P-limited growth in whitebark pine (Perkins, 2004), and with the importance of P acquisition for reproduction in other masting trees (Newbery *et al.*, 2006). Interestingly, a major peak of P allocation to seeds (but not N) was observed later in the season, which coincided with a slight decrease of P concentration in older needles from late August to late September. It may be that such ‘last minute’ allocation of P to seeds is a strategy to maximize P use or to prevent premature P

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loss to seed predators such as red squirrels (Tomback *et al.*, 2001). The role of foliage as a nutrient source for reproduction is consistent with the hypothesis that the unusual proportional increase of leaf biomass with tree size in whitebark pine is a strategy for nutrient storage for reproduction at the cost of water loss and carbon assimilation (Sala, 2006). The storage role of foliage is also consistent with increases in litter fall after mast seeding in Himalayan oaks (Singh *et al.*, 1990) and in *Nothofagus* species (Alley *et al.*, 1998). This is because nutrient re-allocation from foliage to reproduction could trigger leaf senescence if nutrient levels fall below critical thresholds to sustain a positive leaf carbon balance (Reich *et al.*, 2009).

Although nutrient costs of reproduction decreased photosynthetic rates in reproductive branches in 2005, short-term growth at the branch or the tree level was not affected. Rather, nutrient depletion was followed by reduced future reproduction. This pattern in whitebark pine is consistent with that in other species where resource switching during reproductive events occurs between storage and reproduction rather than between growth and reproduction (Stearns, 1989; Ehrlen & Van Groenendael, 2001). Apparently, at our site, factors other than reproduction influenced radial growth because no heavy mast event occurred since at least 1995 (Crone *et al.*, 2011), but tree ring growth decreased from a high peak in 2003, a year with higher (11.2°C) than average (9.5°C) June and July temperatures (but near average precipitation), to 2004, a year whose June and July temperature (9.47°C) and precipitation (30 mm) were near average (34 mm). These fluctuations probably reflect the combined effect of climatic and internal factors, although our short tree ring growth series did not reveal significant relationships between tree ring growth and climatic variables. Interestingly, mature cones in 2005 (mast year) were initiated following a warm year in 2003, which is consistent with previous findings that relatively high temperatures cue mast seeding in tree species (Selas *et al.*, 2002). Resource switching between storage and reproduction during reproductive events may operate in other mast-seeding tree species for which tradeoffs between growth and reproduction have not been detected (Despland & Houle, 1997; Yasumura *et al.*, 2006; Knops *et al.*, 2007).

True masting (Kelly, 1994), a distinct bimodal pattern of reproductive output with years of very high seed production interspaced between years of no or minimal reproduction, is rare in plants, and most species fall somewhere along a continuum from true masting to random variation in reproduction through time (Herrera *et al.*, 1998). In our study region, whitebark pines span a broad range of variability along this continuum (Crone *et al.*, 2011). Past studies (see review by Kelly & Sork, 2002) have tended to explain variability in masting in terms of variation in the costs of reproduction and the presence of external synchronizing factors such as pollen availability, drought and temperature. Recent work also indicates that the effect of some synchronizing climatic cues may be mediated via changes in nutrient availability (Smaill *et al.*, 2011). Our results further suggest that differences in mast-seeding could also be caused by the extent to which nutrient depletion is synchronized within individuals. For example, if costs of reproduction are moderate (e.g. where or when nutrient availability and resource storage is high) resource depletion may tend to be more localized (and therefore less synchronized within a tree) in which case subsequent reproduction may occur in branches that did not reproduce the year before, thus reducing annual variation in reproductive output. This is consistent with the prediction by Kelly & Sork (2002) that the coefficient of variation of seed fall should be lower in more productive habitats.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Details for the statistical comparisons of nutrient concentrations during 2005, and from 2005 to 2006

Table S2 *P*-values for the pairwise comparisons of N and P concentrations between early and late 2005 and between late 2005 and late 2006

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