Crown defoliation decreases reproduction and wood growth in a marginal European beech population.

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Summary

1. Although droughts and heatwaves have been associated to increased crown defoliation, decreased growth and a higher risk of mortality in many forest tree species, their impact on tree reproduction and forest regeneration still remains understudied. Flowering, pollination and seed maturation processes are expected to be affected by prolonged water stress, eventually resulting in reproduction failure. However, inter-individual variations in growth and reproduction responses to drought-induced dieback could favor long-term adaptation at population level.

2. We investigated the relationships between individual crown defoliation, wood growth and reproduction in a drought-prone population of European beech, *Fagus sylvatica*. We used a spatially explicit mating model and marker-based parentage analyses to estimate effective female and male fecundities of 432 reproductive trees, which were also monitored for radial growth and crown defoliation over nine years.

3. Female and male fecundities markedly varied among individuals, more than did growth. Both female fecundity and growth decreased with increasing crown defoliation and competition and increased with size. Male fecundity only responded to competition, and tended to decrease with increasing competition. On average, the decline in female fecundity with increasing defoliation was nearly twice less strong as that of growth. Moreover, the negative effect of defoliation on female fecundity was size-dependent, with a slower decline in female fecundity with increasing defoliation for the large individuals. Finally, a tradeoff between growth and female fecundity was observed in response to defoliation, suggesting that some defoliated individuals can maintain significant female fecundity at the expense of reduced growth.

4. *Synthesis*. Our results suggest that while decreasing their growth, the large defoliated trees still contribute to reproduction through pollination and seed production. This non-coordinated decline of growth and fecundity at individual-level in response to water stress could compromise the evolution of drought-resistance traits at population level, and increase forest tree vulnerability.

Key-words:

Crown defoliation, dieback, female fecundity, male fecundity, radial growth, *Fagus sylvatica*, Mixed Effect Mating Model, vulnerability, water stress

Introduction

Shifts in rainfall patterns and increasing temperatures associated with climate change are likely to cause widespread forest decline in regions where droughts are predicted to increase in duration and severity (Allen *et al*., 2010; Park Williams *et al.*, 2013). Prolonged droughts and high temperatures have been extensively associated to decreasing tree growth and forest productivity (Zhao & Running, 2010; Zimmermann *et al.* 2015), increasing crown defoliation and leaf fall (Dobbertin, 2005; Galiano, Martínez-Vilalta, & Lloret, 2011) together with higher risks of tree mortality (Adams *et al*., 2017; Allen *et al*, 2010; Anderegg, Kane, & Anderegg, 2013), insect outbreaks and wildfire (Park Williams *et al.*, 2013). By comparison, their impacts on tree sexual reproduction and forest regeneration remain largely under-documented, despite the critical importance of reproduction for the maintenance, demography and adaptation of drought-prone populations at the rear-edge of species distribution (Hampe & Petit, 2005). Here, we consider sexual reproduction globally, including all the stages from floral initiation to the production of mature seeds.

Water stress induced by the combination of prolonged droughts and high temperatures is expected to directly affect plant sexual reproduction through altered reproductive phenology (i.e. the timing of flowering and fruiting), a higher risk of pollen abortion or pollination failure, a shorter seed maturation cycle and a higher risk of seed abortion (Bykova *et al*., 2012; Hedhly, Hormaza, & Herrero, 2009; Zinn, Tunc-Ozdemir, & Harper, 2010). Moreover, indirect effects are also expected: for instance, by decreasing photosynthetic activity, drought-induced leaf fall may reduce the amount of stored resources to invest in reproduction of the next year (Obeso, 2002). Water stress induced could also shift patterns of resource allocation and act like a cue stimulating higher reproductive effort and reduced growth (Lee, 1988; Bréda *et al.*, 2006; Pulido et al., 2014; Wiley, Casper, & Helliker, 2017). Overall, the net effects of water stress on the quantity and quality of seeds production thus appear difficult to predict, and are likely to vary among species, populations and individuals. These effects may additionally vary and interact among years. Moreover, many tree species show an intermittent production of large seed crops synchronized across a population (i.e., masting), triggered both by weather and plant resource status (Pearse, Koenig & Kelly, 2016). The possible effect of drought on reproduction could thus sometimes be masked by the strong inter-annual fluctuations of seed production due to masting.

Experiments manipulating drought and/or temperature *in situ* can clarify their impacts on reproduction, but only few results are available so far, particularly for long-lived plants. The impacts of water stress on reproductive phenology have been increasingly reported in the last decades, with contrasting outcomes. Using experimental warming, Sherry *et al.* (2007)

demonstrated divergent responses of reproductive phenology for grass species: some species advanced their flowering and fruiting phenology before the peak of summer heat while other species started flowering after the peak temperature and delayed their reproduction. By manipulating temperature during pollen dispersal and germination, Flores-Rentería *et al.* (2018) demonstrated negative impacts of high temperatures on male reproduction, particularly on pollen viability of a coniferous species, *Pinus edulis*. Bykova *et al.* (2018) also showed that water deficit increases pollen abortion and thus decreases pollen production in *Quercus ilex*. In *Quercus ilex*, Pérez-Ramos *et al.* (2010) showed that reduced water ability increased the rate of acorn abortion, while Sanchez-Humanes & Espelta (2011) showed that increased drought reduces acorn production in coppice. Also, in *Quercus ilex*, Pulido et al. (2014) did not find evidence that drought enhances resource allocation to reproduction and suggested on the contrary that the negative individual correlation between growth and female reproduction observed in controlled conditions disappears under limited resources (including water stress). In parallel, these drought-manipulation experiments generally demonstrated that growth decreases (e.g. Delaporte et al. 2016; Lempereur et al., 2015) and crown defoliation increases (e.g. Galiano, Martínez-Vilalta, & Lloret, 2011) in response to increasing water stress.

Other experimental evidences of the impact of biotic and abiotic stresses on reproduction and growth can be found in the literature on forest seed orchards and fruit trees orchards. Among the cultural practices allowing early and abundant flowering, water stress is used to enhance flower initiation in conifers, while hot, dry summers are reported to induce abundant seed crops in both conifers and broadleaved species (Meilan, 1997). Another practice relies on circumferential girdles (the removal of a swath of the bark, down to the phloem, around the entire stem), which are associated to reduced vegetative growth and increased fruiting (Bonnet-Masimbert & Webber, 1995). Finally, pruning (the reduction of crown leaf area) is also recommended to favor reproductive development while reducing vegetative growth in fruit trees (Karimi et al., 2017).

Taken together, these results suggest that the relationship between reproduction and growth in response to water stress needs further investigations. Environment-manipulation experiments typically use a limited number of individuals in controlled conditions to characterize how environment affect the physiological mechanisms underlying this relationship (eventually testing for individual effects, e.g. Camarero, et al. 2015). Besides these ecophysiological approaches, we also need population and evolutionary ecology approaches, investigating how the relationship between growth and reproduction vary among individuals and if these variations are a function of their vulnerability to drought. As post-dispersal processes and recruitment patterns may compensate the decline in seed production in drought-prone populations (Barbeta *et al.*, 2011), it is important to focus not only on the stage of seed and pollen production, but to target the whole regeneration process. Due to the crucial role of reproduction on the evolutionary dynamics, two extreme

hypotheses can be proposed for the long-term response of a population to water stress, assuming that crown defoliation is a proxy of drought vulnerability: (H1) if crown defoliation and growth decline are combined at individual level with a proportional decline in fecundity, then the individuals which are the most vulnerable to drought should contribute less to regeneration; consequently, assuming that at least some of the traits underlying vulnerability to drought are under genetic control, the population could evolve traits improving its adaptation to drought. (H2) alternatively, if at individual-level, fecundity does not decline, or even increases while defoliation increases and growth declines, the individuals which are the most vulnerable to drought should contribute more to regeneration, and the population could evolve traits compromising its adaptation to drought.

We focus here on the European beech (*Fagus sylvatica* L.), a major broadleaf tree species considered to be vulnerable to summer drought. Several studies showed a decline in beech growth at the species' southern range limit (Jump, Hunt, & Peñuelas, 2006; Piovesan *et al.*, 2008), and even in central Europe (Zimmermann et al., 2015). Moreover, in one site at the species' southern range limit, where beech has shifted attitudinally upwards by ca. 70 m since 1945, increased discoloration and defoliation of crowns were reported at lower elevations and interpreted as a sign of declining health (Peñuelas & Boada, 2003). *Fagus sylvatica* is a monoecious, wind-dispersed species, and reproductive trees produce beech nuts irregularly during mast years. Hacket-Pain, Lageard, & Thomas (2017) showed that drought years were associated to both reduced reproduction and growth, while during non-drought years, both masting and high growth could be observed. By contrast, Bréda *et al.* (2006) reported increased seed production associated to leaf fall in high drought years, even though this relationship between crown defoliation and fruit production may not be directly causal.

This study investigates the relationships between individual health, growth and fecundity in 432 individuals of rear-edge natural population of *Fagus sylvatica* in Southern France, where crown defoliation and mortality are being surveyed since 2004. We assumed that recurrent defoliation is related to the physiological stress induced by water stress and symptomatic of a declining health (Bréda et al. 2006, Petit et al., in prep). We used molecular markers and parentage analyses to estimate effective, relative female and male fecundities, which integrate the success of pollination and germination processes cumulated from 2002 to 2012. Growth over the same period was assessed through inventory data, completed by ring-width measurements. We analysed the relationships between crown defoliation, growth and fecundity by (1) comparing the decline in growth and fecundity associated with defoliation and (2) investigating the correlation between growth and fecundity in response to defoliation.

Material and methods

Study site

Located in southern France and bordering Spanish Cataluña, the Massane forest is situated on the foothills of Eastern Pyrenees (Figure 1B). This coastal range, called Albera massif, covers about 19,000 ha on the French territory. The Massane forest National Nature Reserve (42° 28' 41" N, 3° 1' 26" E) was created in 1973. It covers 336 ha on the highest part of the Massane valley, from 600 to 1,127 m a.s.l., and is only around 5 km far from the Mediterranean Sea. The site is under a meso Mediterranean climate influence (Quezel et Medail, 2003: mean annual temperature = 11.95°C; annual precipitations = 1164.9 mm, monitored on site since respectively 1976 and 1960; Figure S1A). This site is one of the of the French beech location the most prone to water stress (Figure S1B).

More than half of the Reserve is constituted of an old grown forest, where no logging operation has been performed since at least 1886. The canopy is dominated by European beech (*Fagus sylvatica* L.*)*, in mixture with downy oak (*Quercus pubescens* Willd.), maples (*Acer opalus* Mill., *Acer campestris* L., *Acer monpesulanum* L.) and holly (*Ilex aquifolium* L.). A 10 ha fenced plot was remote from any cow grazing since 1956. All trees from this protected plot are intensively monitored since 2002.

Adult seed-tree inventory and phenotyping

This study was conducted on two circular-shaped plots (as classically used in parentage analyses) covering 0.17 ha in total, where all the 683 alive adult beeches were mapped and collected for genetic analyses in 2012 (red dots on Figure 1). Although beech reproduction is mostly sexual, vegetative reproduction may occasionally occur, with the production of stump shoots resulting in multiple stems (i.e. several ramets for a single genet). In obvious cases of vegetative reproduction (ie root-connected stems), we sampled only the biggest ramet of each genet for genetic analyses.

Only a subset of the 683 alive beeches were included within the protected plot and monitored since 2002 (filled dots on Figure 1). The monitoring consisted first in measuring tree size as the diameter at breast height (DBH) in 2002 and 2012. We also computed a quadratic measure of size, the basal area ($BA=\pi*DBH^2$). Individual growth was measured by the relative increment in basal area (herein BAI) between 2002 and 2012, as estimated by: $BAI=\pi(DBH^2_{2012}-DBH^2_{2002})/4$.

The presence of dead branches and leaves was recorded each year between 2004 and 2012 as a qualitative measure (1=presence; 0=absence). We used the sum of these nine annual defoliation scores (herein DEF) as an integrative, qualitative ordered measure combining the recurrence of defoliation, and the ability to recover from defoliation.

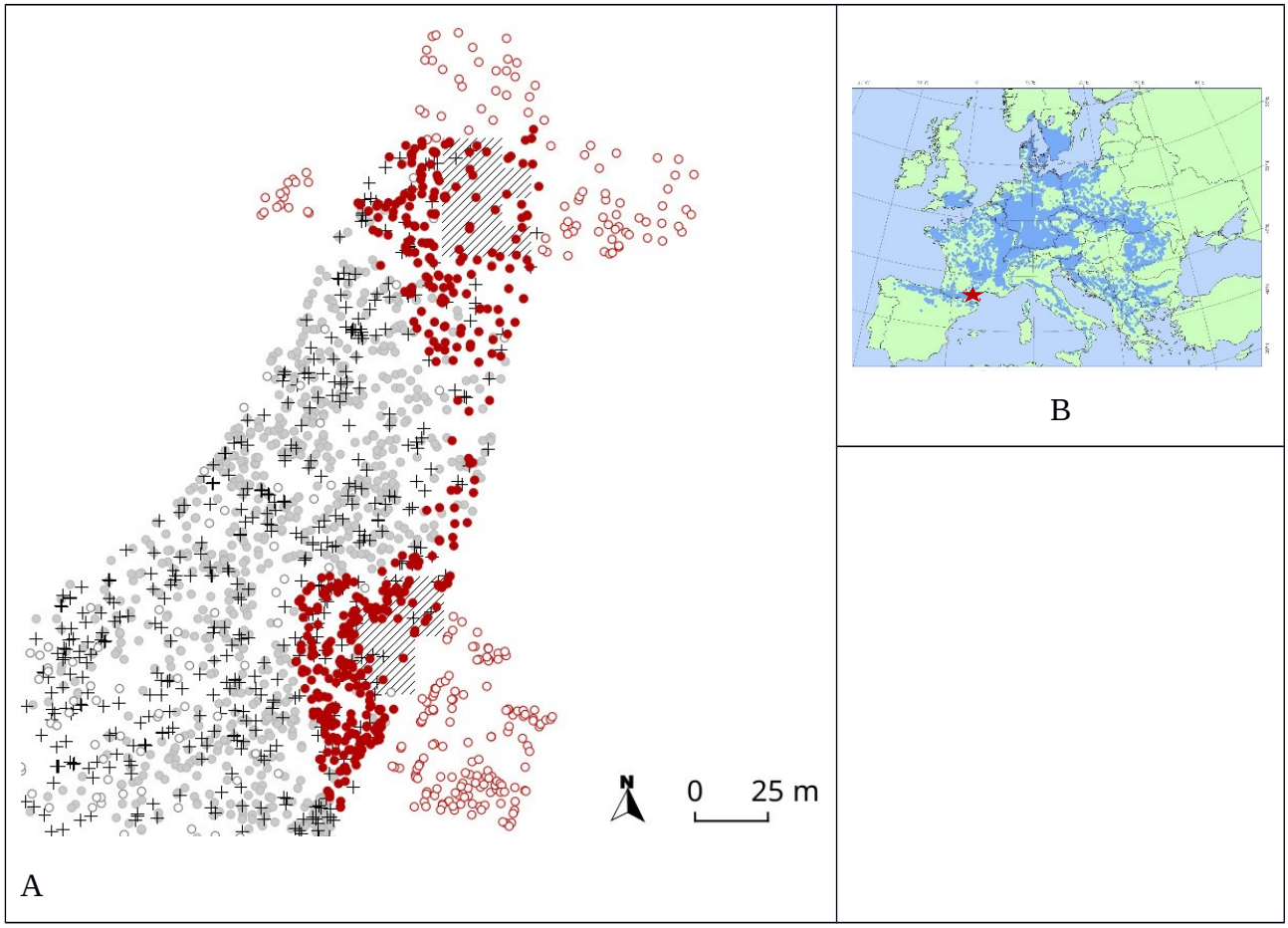

Figure 1: Study site and (A) sampling design: red filled dots (●) represent the 432 beech trees for which individual fecundity, growth and health decline were assessed. Hatched squares represent the seedlings patches used to estimate fecundity through parentage analyses and spatially explicit mating models. Red empty dots (○) represent the 240 beech trees outside of the protected area which were included in the fecundity analyses (but not phenotyped for growth and health decline). Grey dots (●) and crosses (+) represent other beeches within the protected area not included in the fecundity analyses either because they were far from sampled seedlings (●) or because they were dead in 2012 (+). Empty dots (○) represent other species within the protected area. (B) Study site location (red star) on European Beech distribution map (source Euforgen).

The conspecific local density (herein $Dens_{dmax}$) was estimated as the number of beech neighbors found within a radius of *dmax* around each mother-tree. We also used the Martin-Ek index (Martin & Ek, 1984) to quantify the intensity of competition on a focal individual *i*. This index (herein $Compet_{dmax}$) accounts simultaneously for the diameter and the distance of each beech competitor *j* to the competed individual *i*:

$$\mathrm{Compet}_{d\,\max} = \frac{1}{\mathrm{dbh}_i} \sum_{j=1}^{n} d\max \mathrm{dbh}_j \exp\left[\frac{-16\, d_{ij}}{\mathrm{dbh}_i + \mathrm{dbh}_j}\right] \qquad \text{(equation 1)}$$

where d_{bhi} and d_{bhj} are the diameter at breast height (in cm) of the competed individual i and of competitor j (any adult tree of any species with $dbh_j > dbh_i$), n_{dmax} the total number of competitors in a given radius d_{max} (in m) around each individual i, and d_{ij} the distance between individuals i and j. We computed a total of 20 $Dens_{dmax}$ variables and 20 $Compet_{dmax}$ variables, by considering *dmax* values between 1 and 20 m with a 1m-step. The $Dens_{dmax}$ on the one hand and the $Compet_{dmax}$ variables on the other hand were strongly and positively correlated with each other's, but loosely correlated among them (Figure S2).

Offspring sampling and genotyping

To estimate adult fecundity, we sampled 365 seedlings established amidst the 683 genotyped adult beeches (shaded quadrats on Figure 1). Two cohorts of seedlings were sampled exhaustively within a selected number of quadrats at the center of each circular plot: 165 "young" seedlings germinated in spring 2012, and 200 "old seedlings" germinated from spring 2011 back to spring 2001 (age was estimated using annual bud scars). In this study, the two seedlings cohorts were mixed, in order to estimate cumulated reproduction from 2001 to 2012.

The genotypes of the 683 alive adult beeches and 365 seedlings were scored at a combination of 18 microsatellite loci (Table S1). DNA extraction, PCR amplifications and genotype scoring with a MegaBACE 1000 sequencer were performed using the conditions described by Oddou-Muratorio, *et al.*, (2018). The total number of alleles observed in each cohort was greater than 95 (Table S1). Adult genotypes revealed 7 pairs of clones among the adult beeches. We checked that these clones were always spatially clustered, and kept only one ramet for each genet in the MEMM analyses (i.e. 676 adult beeches).

Inference of male and female relative fecundities: MEMM analyses

Male and female fecundities were jointly estimated with the pollen and seed dispersal kernels in a Bayesian framework implemented in the MEMMseedlings program (Oddou-Muratorio, *et al.*, 2018). MEMMseedlings is one of the recently developed full-probability mating models based on naturally established seedlings (see also Burczyk *et al.*, 2006; Goto *et al.*, 2006; Moran & Clark, 2011; Oddou-Muratorio & Klein, 2008). Briefly, these approaches rely on a Spatially Explicit Mating Model (SEMM) where each seedling can generally originate either (i) from a mother tree located outside the study site (implying seed immigration) or (ii) from a mother tree located within the study site. The latter case includes three possible origins of the fertilizing pollen: (i) pollen immigration, (ii) selfing, or (iii) pollination by a male tree located within the study site.

The main advantage of the SEMM implemented in MEMMseedlings is to estimate individual male and female fecundities together with the pollen and seed dispersal kernels and mating system parameters, so that SEMM-estimate of fecundity are not biased by the confounding

effects of spatial and sampling designs (the arrangement of male/female parents and sampled seedlings). The approach bypasses parentage assignation and focuses instead on the fractional contribution of all adults, either as female or as male parent, to each seedling. For instance, the probability π_{Sij} of each sampled female tree j to contribute to the seedling pool at the spatial location of seedling i is modeled as:

$$\pi_{S_{\mathrm{ij}}} = \frac{F_{\mathrm{Fj}}\theta_s\left(d_{\mathrm{ij}}\right)}{\sum_{l:\mathrm{mother}} F_{\mathrm{Fl}}\theta_s\left(d_{\mathrm{il}}\right)} \quad \text{(equation 2)}$$

where F_{Fj} and F_{Fl} are the female fecundities of mother j and l, respectively; d_{ij} and d_{il} are the distances between seedling i and mother j and l, respectively; and θ_s is the seed dispersal kernel. In hermaphroditic species such as beech, each focal reproductive individual l is considered as a male with male fecundity F_{Ml} and a female with female fecundity F_{Fl}. When considering a couple of parents, the closest one is not automatically assumed to be the mother, but the jointly estimated dispersal distributions usually indicate that seed dispersal is likely to be more limited than pollen dispersal.

All the possible parents around the seedlings should theoretically be included in SEMM analyses. This is why we included 240 adult beeches outside the protected plot among the 676 parent trees. However, the long-term inventory within the protected plot showed that 62 dead beeches could not be collected for genetic analyses (Figure 1), with 55 of them already dead in 2010. These “missing” parents within plot could theoretically bias the estimation of fecundities. However, our hypothesis in that biases primarily occur in the estimated rates of pollen and seed immigration (not used in this study), and remain negligible on the estimated fecundities of the sampled parent trees.

Note that the fecundity estimates provided by the SEMM are related but not equivalent to the traditional resource-based estimates of female (i.e. the biomass/number of ovules, seeds, ovuliferous flowers or fruits) and male fecundity (i.e. the biomass/number of pollen grains or staminate flowers). First the latter estimate the resources allocated by each plant to reproduction while the former can only estimate a relative amount of pollen or seeds produced by each plant as compared to other plants. Second, the resource-based estimate is a pre-dispersal evaluation of seed and pollen production while the SEMM estimates an effective amount of pollen achieving successful fertilization, and of seeds achieving successful germination. In consequence, SEMI-based estimates of fecundities account for individual effects (either maternal or genetic) that act independently on location to modify the success of mating, seed maturation or germination, or early survival during the post-dispersal processes preceding the sampling stage (Oddou-Muratorio *et al.*, 2018).

For the estimation, we accounted for typing errors at microsatellite loci, with two possible types of mistyping: in the first type, the allele read differs only by one motif repeat from the true allele with a probability P_{err1}, while in the second type, the allele read can be any allele observed at this locus with a probability P_{err2}. We considered a mixture of the two error types, with $P_{err1} = 0.01$ and $P_{err2} = 0.01$. We ran 10 Markov chain Monte Carlo (MCMC) of 10,000 steps, each with additional 500 first MCMC steps as burn-in, checked that the different chains converged to the same value visually, and them combine the 10 chains together. Individual female (F♀) and male (F♂) fecundities were summarized by their median value across the 100,000 iterations.

Adult subsampling for dendrochronological analyses

We selected 90 trees within the protected plot for which we sampled cores to measure ring-width. These 90 trees were chosen to represent contrast in terms of defoliation and female fecundity (Figure S3). Cores were extracted in February 2016 at 1.30 m above ground. After preparation by sand, cores were scanned at high resolution (1200 dpi). Boundary rings were read using CooRecorder v 9.0. Ring width were transcribed, individual series were checked for missing rings and dating errors and mean chronologies were calculated using Cdendro 9.0 (CDendro 9.0 & CooRecorder 9.0; Cybis Elektronik & Data AB. Sweden). Using the sum of ring width increments between 2002 and 2012 (Σrw), the growth of the 90 individuals between 2002 and 2012 was estimated as: $BAI_{wood}=\pi((DBH_{2002}/2+\Sigma rw)^2-DBH^2_{2002}/4)$.

Statistical analyses of the ecological drivers of growth and fecundities

Our objective herein was to test whether defoliation significantly affected individual growth and female/male fecundity. For each response variable independently (i.e., growth as measured by BAI, and fecundities as estimated with MEMM), we considered the following full linear model:

$$\text{BAI or F♀ or F♂} = DEF + DBH_{2002} + Compet_{dmax} + Dens_{dmax}$$

$$+ DEF{:}DBH_{2002} + DEF{:}Compet_{dmax} + DEF{:}Dens_{dmax} \quad \text{(equation 3)}$$

where all the predictors are quantitative variables (Table S2). Besides the target defoliation factor (DEF), this model includes one size-related factor (DBH_{2002}), and two competition-related factors ($Compet_{dmax}$ and $Dens_{dmax}$). Size and competition are considered here as “nuisance” parameters, susceptible to blur the signal between defoliation, growth and reproduction. Therefore, we want equation 3 to include a minimal number of such predictors. Density and competition index can both be relevant to capture competition effect on growth or fecundity, and moreover, their influence may vary with the distance up to which competitors are accounted for. Therefore, we first selected the best $Compet_{dmax}$ and the best $Dens_{dmax}$ terms for each response variable independently using the model described by equation 3 without interaction terms, and retaining the d_{max} values

leading to the highest R^2. Then, we included interactions terms (the three last terms in equation 3) to investigate specific effects of defoliation depending on individual size or on the level of competition. For each response variable, we selected the most parsimonious model based on the AIC using the procedures 'lm' and 'step' in R 3.3 (R Core Team 2018).

The model was fitted on 432 focal adult beech trees within the protected plot (Figure 1) for which BAI was estimated from inventory data. All response variables were log-transformed to approach Gaussian distribution and to account for the higher variance associated to higher fecundity or higher growth. Interaction effects were visualized with the package 'jtools' (Long, 2018).

Collinearity resulting from correlations between predictor variables is expected to affect statistical significance of correlated variables by increasing type II errors (Schielzeth, 2010). To evaluate this risk, we computed variance inflation factors (VIF) associated to each term retained in the best model with R package 'car' (Fox & Weisberg 2011). Moreover, we used a model similar to equation (3) to investigate the effects of tree size and competition on defoliation :

$$DEF=DBH_{2002}+Compet_{dmax}+Dens_{dmax}+ DBH_{2002}:Compet_{dmax}+ DBH_{2002}: Dens_{dmax} \text{ (equation 3')}$$

Statistical analyses of the joint defoliation effects on female fecundity and growth

Our objective herein was to focus on the two variables (growth and female fecundity) responding to defoliation (see results), and to investigate how the relationship between these two variables varied with defoliation. We first compared the effects of defoliation on female fecundity vs growth after centering and normalizing fecundity and growth, and by using the best models fitted with equation 3 to estimate the effect of defoliation on these transformed variables.

Then, we investigated the individual correlation between raw relative female fecundity and growth for non-defoliated trees (DEF=0) versus defoliated trees (DEF>0). Note that a part of these correlations may be due variation in size and/or competition among individuals. Moreover, they do not account for the quantitative nature of DEF.

To overcome these limitations, we further investigated the trade-off between growth and female fecundity using the following linear model:

$$F♀ =BAI + DEF+DBH_{2002}+Compet_{dmax}+Dens_{dmax}$$

$$+DEF:BAI + DEF:DBH_{2002}+ DEF:Compet_{dmax}+ DEF: Dens_{dmax} \text{ (equation 4)}$$

where BAI and the interaction between BAI and DEF are added to the model described by equation (3) above.

Results

Patterns of covariation of defoliation, tree size and competition

Recurrent crown defoliation was overall limited in the 432 individuals, with 95 trees with a non-null DEF-value (mean=0.36, Table S2). Defoliation increased with tree size ; the significant interaction between DBH_{2002} and competition (mediated by Comp19) or density (mediated by Dens20) reflected a stronger effect of size on defoliation as competition increased (Figure S4).

Inter-individual variations in relative fecundities and growth

The distributions of relative female and male individual fecundities (F♀ and F♂) estimated by MEMM were strongly L-shaped (Figure 2A). Female fecundities varied from 0.03 to 32.44 (median=0.42, mean= 1, sd= 2.78), while male fecundities varied from 0.17 to 21.16 (median= 0.48, mean =1, sd= 1.86).

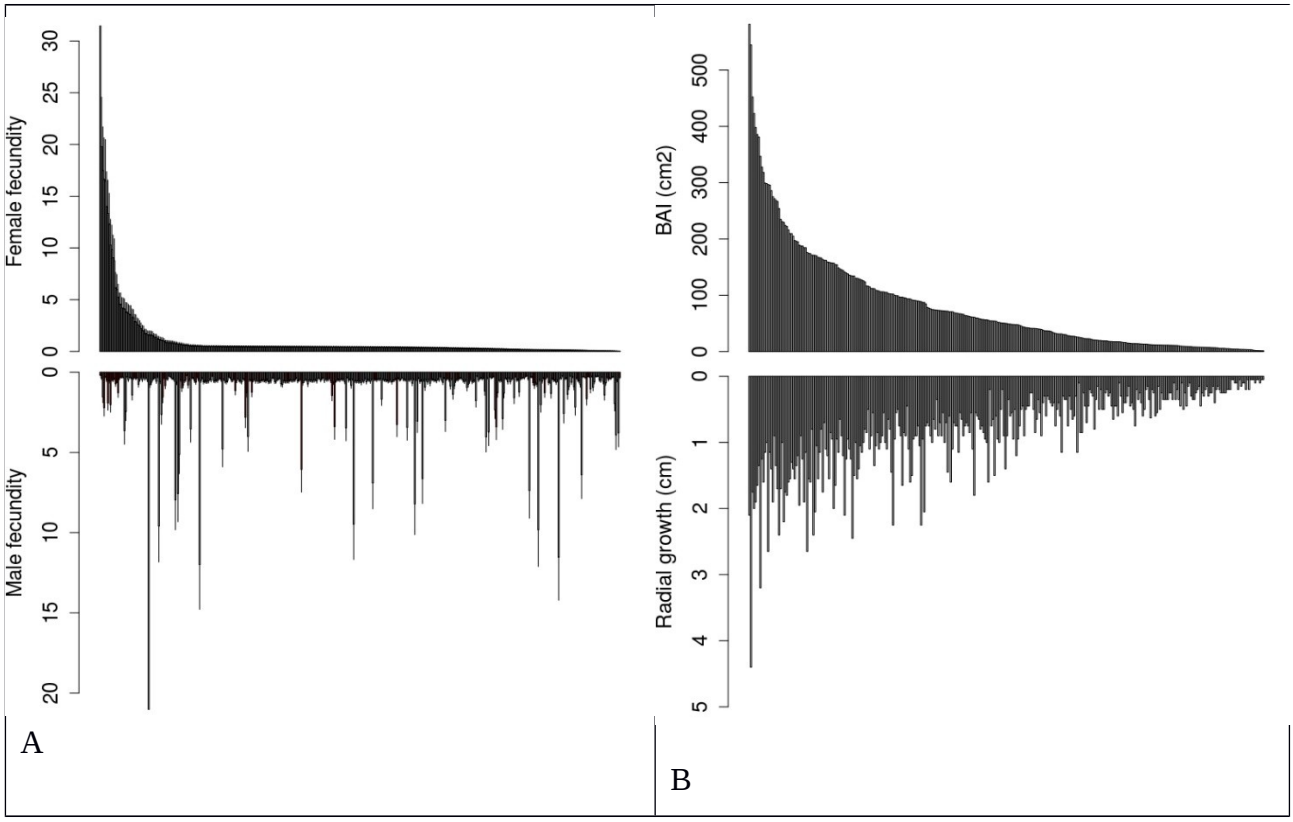

Figure 2: Distribution of individual (A) relative female (top) and male (bottom) fecundities estimated with MEMM, and (B) absolute growth estimated by BAI (top) or radial growth (bottom) for the 432 adult trees. Parents on the x-axis are ranked in decreasing order of female fecundity (A) or BAI (B).

By comparison, the distribution of growth values was less L-shaped than those of fecundity (Figure 2B). In the data set of 432 adult trees, where cumulated growth from 2002 to 2012 was

estimated through inventory data, radial growth varied from 0 to 4.4 cm (median=0.45, mean= 0.60, sd= 0.62), while BAI varied from 0 to 581.22 cm^2 (median=23.98, mean= 61.58, sd= 86.87).

In the subset of 90 cored trees, where cumulated growth from 2002 to 2012 was estimated through ring width data, radial growth varied from 0.17 to 2.70 cm (median=0.97, mean= 1.03, sd= 0.57), while BAI varied from 7.8 to 805.89 cm^2 (median=126.30, mean= 180.07, sd=172.7). Moreover, for these 90 cored trees, the correlation between inventory-based and ring-width-based radial growth was 0.84 (p-value<0.001), while the correlation between inventory-based and ring-width-based BAI was 0.68 (p-value<0.001). The lower correlation for BAI values was due to the largest trees, for which inventory data generally underestimated growth (Figure S5).

Ecological drivers of fecundities and growth

Defoliation, size and competition overall explained a significant part of the variation in growth (57%) and female fecundity (12%), while competition alone was found to marginally explain a small part of the variation in male fecundity (<1%). In the whole data set of 432 individuals, the most parsimonious model showed that female fecundity (F♀) significantly decreased with defoliation and competition (mediated by Compet10), while it increased with DBH_{2002} and density (mediated by Dens10; Table 1A). Moreover, the interaction between DEF and DBH_{2002} was significant, reflecting a weaker negative effect of defoliation on female fecundity as tree size increased (Figure 3A). By contrast, male fecundity (F♂) was only marginally (and negatively) affected by competition (mediated by Dens5, Table 1B). Finally, growth (as measured by BAI) significantly decreased with defoliation and competition (mediated by Compet7), and increased with DBH_{2002} and density (mediated by Dens10; Table 1C). By contrast with female fecundity, no interactions between defoliation and size were detected on growth. Centering and normalizing F♀ and BAI, the average decline in F♀ (measured in standard unit of trait) was -0.06 versus -0.10 for BAI (using the best models fitted for each response variable).

Joint defoliation effects on female fecundity and growth

The raw F♀s and BAIs were significantly and positively correlated in the 337 non-defoliated trees ($cor_{F♀\text{-}BAI\text{-}nondef}$ = 0.31, pval<0.001), but not in the 95 defoliated trees ($cor_{F♀\text{-}BAI\text{-}def}$=0.13, pval=0.2).

The linear model for F♀ including BAI as a predictor (equation 4) allowed to disentangle the respective effects of defoliation, size and competition on the relationship between female fecundity and growth. In addition to previous effects (equation 3), a significant interaction between BAI and defoliation was detected (Table 2): F♀ overall decreased with increasing defoliation, but this decrease was faster and stronger for trees with a higher BAI (Figure 3B). The complex interaction between BAI, DEF and DBH_{2002} on F♀ resulted in a defoliation-dependent tradeoff between growth

and female fecundity: F♀ of the non-defoliated trees (Figure 3C, left panel) increased with BAI (no tradeoff), whereas F♀ of the most defoliated trees (Figure 3C, right panel) decreased with increasing BAI (tradeoff). Moreover, F♀ of small trees (Figure S6, left panel) always decreased in response to increasing defoliation, whatever their BAI, whereas the female F♀ of large trees (Figure, right panel) could increase in response to increasing DEF, at the expense of reduced BAI.

Table 1. Analysis of variance table for (A) *F♀:* female fecundity (B) *F♂*: male fecundity and (C) BAI: basal area increment, in response to ecological determinants included in equation (3). We show the results of the most parsimonious model: its adjusted R^2, the type III sum of squares (SSQ) and degree of freedom (df) associated to each term. For each predictor, we give the estimate of its effect, the standard error (S.E.) and associated t and p-value. Variance inflation factors (VIF) were computed with R package CAR. All the response variables were log-transformed. Results are based on the whole data set of 432 individuals for *F♀* and *F♂*, and on the 341 with non-null BAI for BAI.

Predictor	***R²***	**SSQ**	**df**	**Estimate**	**S.E.**	**t**	**p-value**	**VIF**
(A) log(F♀)	*0.12*						*<0.001*	
DEF		9.33	1	-0.349	0.111	-3.148	0.002	4.13
DBH_2002		11.97	1	0.013	0.004	3.564	<0.001	2.19
Compet10		7.9	1	-0.033	0.011	-2.896	0.004	1.43
Dens10		7.46	1	0.010	0.003	2.815	0.005	1.35
DEF:DBH_2002		7.28	1	0.006	0.002	2.780	0.006	4.61
residuals		401.29	426					
(B) log(F♂)	*0.004*						*0.097*	
Dens5		1.695	1	-0.01	0.01	-1.662	0.10	-
residuals		263.854	430					
(C) log(BAI)	*0.57*						*<0.001*	
DEF		6.21	1	-0.427	0.15	-2.837	0.005	6.78
DBH_2002		114.35	1	0.042	0.00	12.173	<0.001	1.94
Compet7		40.35	1	-0.119	0.02	-7.231	0.000	1.47
Dens14		4.00	1	0.004	0.00	2.278	0.023	1.49
DEF:Dens14		2.82	1	0.004	0.00	1.913	0.057	6.83
residuals		258.49	335					

Table 2. Analysis of variance table for female fecundity, in response to ecological determinants included in equation (4). Results are based on the whole data set of 432 individuals. See Table 1 for legends.

Predictor	***R²***	**SSQ**	**df**	**Estimate**	**S.E.**	**t**	**P-value**	**VIF**
	0.13						*<0.001*	
BAI		28.867	1	0.001	0.001	0.773	0.440	3.495
DEF		0.020	1	-0.384	0.112	-3.433	0.001	4.641
DBH		9.785	1	0.011	0.004	2.582	0.010	3.914
Compet10		8.113	1	-0.032	0.011	-2.828	0.005	1.449
Dens10		8.604	1	0.009	0.003	2.704	0.007	1.350
BAI:DEF		1.376	1	-0.001	0.001	-2.091	0.037	9.096
DEF:DBH		8.977	1	0.009	0.003	3.488	0.001	15.516
Residuals		397.339	424					

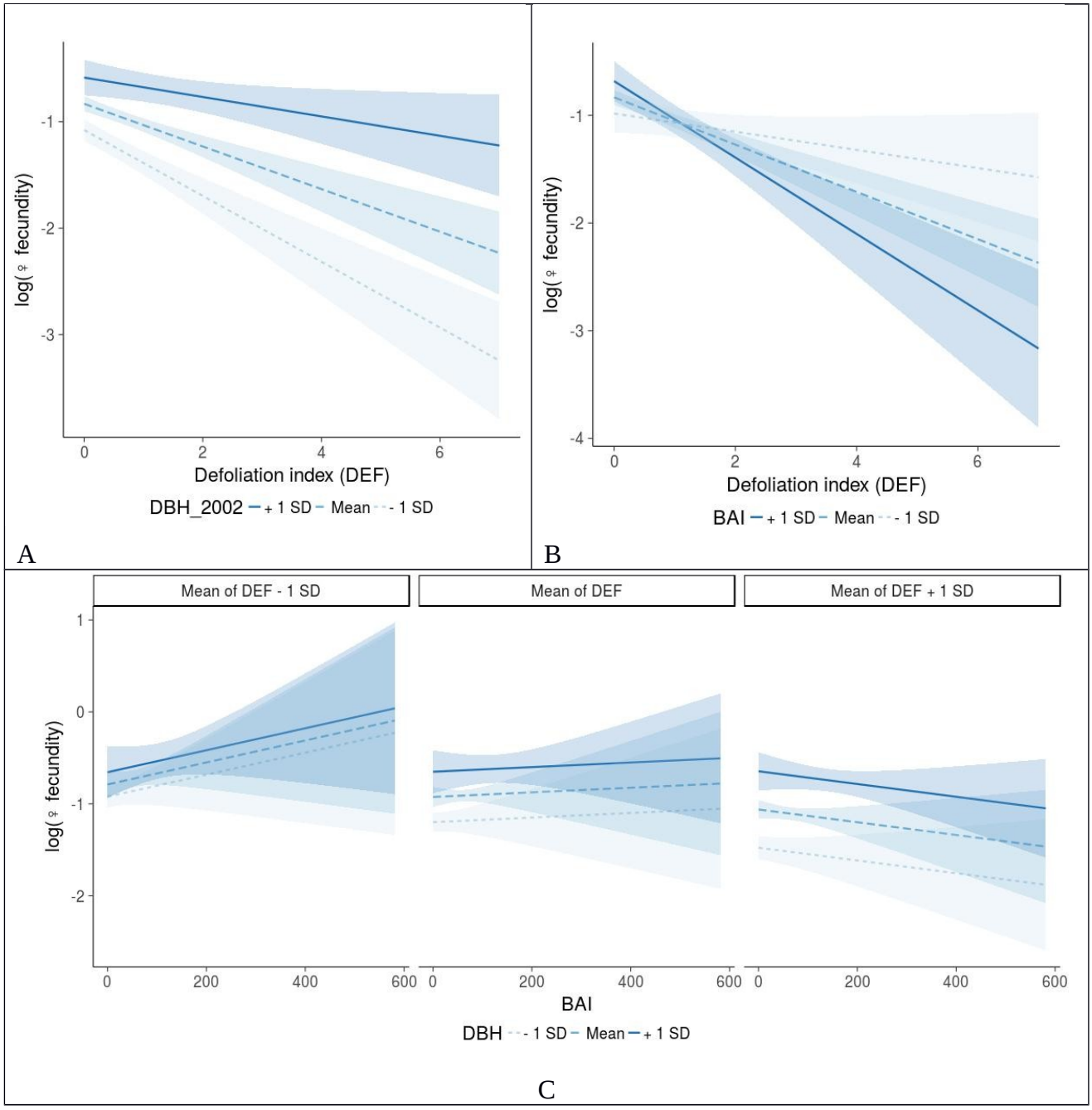

Figure 3: Interaction plots for (A) DEF and DBH_{2002} effects on female fecundity (B) DEF and BAI effects on female fecundity, (C) BAI, DBH_{2002} and DEF effects on female fecundity. Regression lines are plotted for 3 values of each moderator variable, corresponding to +/- 1 standard deviation from the mean. Confidence interval at 80% are shown around each regression line.

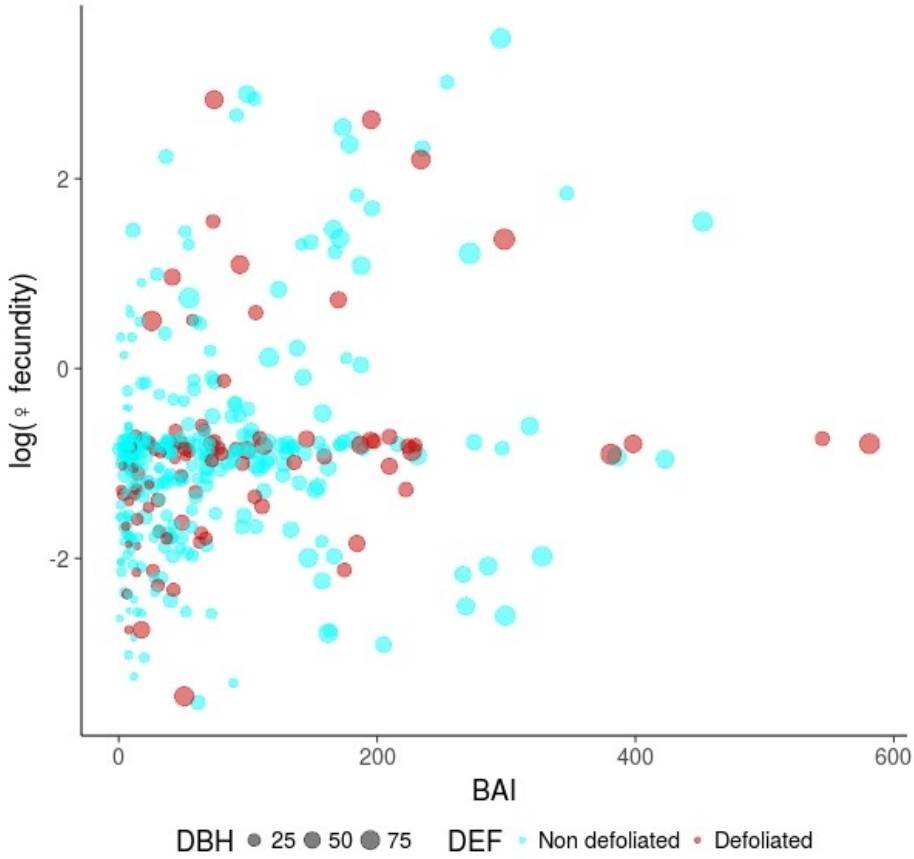

Figure 4: Correlation between growth, measured by the Basal Area Increment (BAI) and female fecundity (F♀), plotted on a log scale. The size of the dots is proportional to tree diameter (DBH_{2002}).

Discussion

By investigating the coordination between defoliation, female/male fecundity and wood growth at the individual scale within a large, natural, drought-prone population, this study brings significant new insights on the response to water stress of a major European tree species, *F. sylvatica*. We show that crown defoliation significantly decreased wood growth and female fecundity, but not male fecundity. A tradeoff between growth and female fecundity was observed in response to defoliation, suggesting that some defoliated individuals can maintain significant female fecundity at the expense of reduced growth. The consequences of these results on short-term evolutionary dynamics of the studied population are discussed.

The coordination of crown defoliation, wood growth and reproduction in response to water stress.

The observed coordination between increasing crown defoliation and decreasing wood growth is consistent with the temporal sequence of ecophysiological processes involved in the

response of trees to water stress. During summer, low precipitation and high evaporative demand due to high temperatures and vapor-pressure increase water stress, which leads trees to close stomata, in order to reduce transpiration and protect the integrity of the hydraulic system by maintaining water potentials above irreversible embolism thresholds. In turn, this stomatal response decreases photosynthesis, which directly impacts wood growth by limiting cell division and elongation of wood cells due to carbon limitation (Lempereur *et al.*, 2015). Post-drought stomatal closure can prolong the decrease in photosynthesis and potentially affect carbon storage (Bréda *et al.*, 2006), which may lead to a decrease in radial growth in subsequent years. Under severe drought, some branches can experience hydraulic failure or undergo carbon starvation, which leads to leaf fall. Leaf fall can then in turn have a negative effect on radial growth, first by decreasing photosynthesis and thus carbon availability in the years following defoliation. Secondly, leaf fall can induce allocation shifts that reduce the priority of growth relative to other sinks such as reserves storage, as observed in black oak (Wiley, Casper, & Helliker, 2017). For these non-mutually exclusive reasons, related to either carbon- or sink- limitation of growth, a negative effect of crown defoliation on growth is often observed, especially on beech (Delaporte et al., 2016, our study).

Although seed production is recognized as being resource-limited in plants (Lloyd & Bawa, 1984), the ecophysiological processes involved in the response of tree sexual reproduction to drought and high temperature are less well characterized than those involved in wood growth response. The observed negative effect of crown defoliation on female but not male fecundity is consistent with the expected decrease in photosynthesis and thus in carbon availability induced by leaf fall, and with the expected higher resource-limitation of female fecundity (costly nut-seeds) as compared to male fecundity in beech (Lloyd & Bawa, 1984 ; Obeso, 2002). The second expectation is also supported by the marked increase in female fecundity (but not in male fecundity) with increasing tree size and decreasing competition. Moreover, recent studies showed that many tree species use mainly current photosynthates to maturate their fruits, while flowers are produced from old carbon storage (Hoch, Siegwolf, Keel, Körner, & Han, 2013; Ichie *et al.*, 2013). Altogether, these results suggest that beech reproduction is less limited by the carbon resources needed for producing and maturating flowers than by those required for seed maturation. Although nitrogen storage and remobilization is usually a limiting resource for seed production, and particularly masting (Han & Kabeya, 2017), this may not be the case in our study site, where cow grazing could favor nitrogen enrichment.

However, the mitigating effect of increasing tree size on decreasing female fecundities for defoliated trees suggests that other processes than resource-limitation could be involved. Of course, we cannot exclude that the qualitative notation of crown defoliation used in our survey, as the presence/absence of major dead branches or foliage, overestimates the impact of defoliation for the

largest trees: indeed, a single dead branch may proportionally less compromise the resource-budget of a large tree as compared to a small tree. However, such interactions between size and defoliation were not observed on tree growth, where defoliation had similar negative effects on the growth of large and small trees. Alternatively, crown defoliation could shift the allocation of carbon to reproduction above a given tree size. Besides the literature on forest seed orchards and fruit trees orchards, one of the rare studies supporting such hypothesis is that of Wiley, Casper & Helliker (2017), who experimentally defoliated black oak, a tree species which maturates its acorns over two years. Recovery following defoliation was showed to involve substantial allocation shifts, with carbohydrate storage and already initiated reproduction cycles (i.e. maturation of 2-year acorn) being favored relative to growth and new reproductive cycles (i.e. flowering and production of new 1-year acorn). The prioritization of the allocation to fruit maturation versus flowering may however not be generalizable to species with a shorter, one-year long reproductive cycle.

The observed negative effects of crown defoliation on growth and female fecundity support our initial hypothesis that defoliation is related to the physiological stress induced by water stress, and is symptomatic of a declining health. Moreover, in a companion study in the same forest (Petit et al., in prep), crown defoliation was shown to enhance the risk of mortality, with size-dependent effect. The mortality risk of smaller trees (DBH <40 cm) increased with defoliation, while defoliation did not influence the mortality of larger trees. Overall, future work should include ecophysiological approaches to investigate the dynamics of carbon allocation to storage, growth and reproduction, by controlling for tree size.

Defoliation induced a trade-off between growth and reproduction

Several studies tested the existence of a negative correlation between growth and reproduction at the individual level, as a signature of the possible trade-off between these functions. The key assumption underlying this trade-off is that reproduction is costly and competes with growth for resources (Koenig & Knops, 1998; Obeso, 2002; Thomas, 2011). By contrast, the absence of correlation is usually interpreted as independence between these functions in terms of resource pool (Knops, Koenig, & Carmen, 2007; Obeso, 2002; Pulido et al., 2014). A trade-off between growth and reproduction was already found for *Fagus sylvatica* (Hacket-Pain, Friend, Lageard, & Thomas, 2015; Lebourgeois et al., 2018). More precisely, Hacket-Pain et al. (2017) found for beech that masting years (i.e. high seed production) are negatively correlated with growth and this trade-off is more pronounced during drought years due to resource scarcity. We found here a trade-off between growth and reproduction for defoliated trees, possibly due to their lower capacity to accumulate resource. By contrast, the positive relationship between growth and reproduction found in non-defoliated trees is likely to result from their higher photosynthetic capacity leading to a higher

resource level. These individuals could thus insure reproduction and growth with independent resource pool, as it was found also for *Fagus* genus (Yasumura, Hikosaka, & Hirose, 2006). The correlation between reproduction and growth is thus linked to the level of resource, a trade-off being present only under limiting resources (Obeso, 2002; van Noordwijk & de Jong, 1986).

Our results overall suggest that in the studied population, some large, defoliated individuals can maintain a high female fecundity, at the expense of reduced growth. This response supports the hypothesis of a shift in the allocation of carbon to reproduction above a given tree size, and suggest that reproduction could be favored relative to growth as observed by Wiley, Casper & Helliker (2017).

Long-term consequences for population adaptive response to drought stress

The large defoliated trees favoring their female fecundity at the expense of reduced growth are thus likely to contribute to regeneration through female function. Moreover, as male fecundity was found to be insensitive to crown defoliation, the less competed defoliated trees also contribute to reproduction through male function. Altogether, our results suggest that the trees which are the most sensitive to drought may contribute substantially to regeneration. However, it is still unclear how this contribution may compromise the adaptation of the population to drought.

Assuming that the recurrence of defoliation is related to the physiological stress induced by water stress, a first missing information relates to its role in the coordinated response to drought of beech and on its demographic consequences. Indeed, two main ecological strategies for drought response are well described in plants: 1) a water economy strategy, where plants maintain low growth rates and low rates of gas exchange during droughts, and 2) a water uptake strategy, where plants have a more rapid instant growth through higher rates of gas exchange when water is available, typically spring in Mediterranean climate, allowing them to complete important biological functions before drought onset (Arntz & Delph, 2001). These two strategies rely on different combinations of physiological, morphological or phenological trait values (in particular those related to hydraulics and carbon-storage). Bontemps et al. (2017) demonstrated the co-existence of these two strategies in a drought-prone population of beech and showed a higher reproductive output of the water uptake strategy. In this context, defoliation could be one of the traits involved in the water uptake strategy, allowing the maintenance of the water balance after drought onset. Indeed, if defoliated trees are characterized by higher xylem vulnerability but also higher hydraulic conductivity, the more a tree is efficient for transpiration and photosynthesis, the more it is vulnerable to drought (Cochard, Lemoine, & Dreyer, 1999). However, recurrent defoliation across years is also likely to induce early mortality (Petit et al., in prep). Thus, the

benefits of the water uptake strategy should ideally be integrated over tree lifetime and by comparison with the water economy strategy.

Assuming that defoliation is one of the traits involved in strategies of beech response to drought, a second missing information relates to the genetic basis of sensitivity to drought in beech. The inter-individual variation in the traits defining water uptake or water economy strategies is shaped both by environmental and genetic variation, where the importance of the latter remaining largely unknown in most tree species (Hamanishi & Campbell, 2011). Micro-environment shapes a significant part of the variation in defoliation in the studied population, with the largest and most competed individuals being more susceptible to defoliation.

Assuming that some of the traits involved in the water-uptake strategy are under genetic control, a third missing information is the absolute contribution of these defoliated individuals to the regeneration, in terms of total amount of seedlings effectively recruited. If many seedlings are produced both by defoliated and non-defoliated individuals, we can expect that density-dependent mortality will eliminate some of these seedlings during the early stage of recruitment, which is known to have the potential to reshuffle effective individual contributions (Gerzabek, Oddou-Muratorio, & Hampe, 2017).

Conflict of interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article.

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Author Contributions

SOM, EM, and HD designed the study. JAM, JG and CH phenotyped and mapped the trees and collected samples with the help of EM. ML performed the genotyping, and estimated fecundities with SOM. CP performed the wood core analyses. SOM analyzed the data and drafted the manuscript. VJ, CP, HD, EM contributed to improve the manuscript.

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Data availability

Data (genotypes, phenotypes, location) and statistical scripts in R are available from the corresponding author, and will be made available in an open data repository in the next future. The color distinguishes defoliated (dark red) from non-defoliated (light blue) trees.

Supplementary Tables and Figures for the manuscript "Crown defoliation decreases reproduction and wood growth in a marginal European beech population", by Sylvie Oddou-Muratorio, Cathleen Petit, Valentin Journé, Matthieu Lingrand, Jean-André Magdalou, Christophe Hurson, Joseph Garrigue, Hendrik Davi, Elodie Magnanou

Table of Contents

Table S1: Genetic data characteristics for the adult and offspring population.....2
Table S2: Size, competition and defoliation variables measured in adults beeches.....3
Figure S1 Climate characteristics of the study site.....4
Figure S2 : Patterns of covariation among competition index (the CX's) and density (the DX's) computed in radius a different size (X=1 to 20 m) around each focal beech.....6
Figure S3: Sampling design for the 90 cored individuals.....7
Figure S4: Effect of size and competition on defoliation (equation 3').....8
Figure S5: Relationship between growth estimated from ring-width and inventory data.....10
Figure S6: Interaction plots for DEF , BAI, and DBH_{2002} effects on female fecundity.....11

Table S1: Genetic data characteristics for the adult and offspring population.

For each SSR marker, N is the total number of scored individuals, %M the percentage of missing data. Na is the number of alleles, AR allelic richness (estimated for a sample of 36 individuals), Ne the effective number of alleles ; He the expected heterozygosity. Exclusion probabilities for maternity (PE-1P) and parentage (PE-PP) are also given with their cumulated values over 21 loci on the last line.

Marker	N	%M	Na	AR	Ne	He	PE-1P	PE-PP
Csolfagus_19	956	8.8%	12	8.07	5.69	0.82	0.52	0.17
Csolfagus_7	726	30.7%	6	5.04	4.50	0.78	0.63	0.27
F1_15	928	11.5%	18	9.83	4.77	0.79	0.56	0.18
Fs3_4	821	21.7%	4	2.69	2.02	0.50	0.87	0.69
Sfc0007	854	18.5%	7	5.21	4.20	0.76	0.65	0.29
Sfc1143	746	28.8%	10	7.05	3.17	0.68	0.70	0.31
Csolfagus_25	906	13.5%	6	4.73	2.17	0.54	0.85	0.53
Csolfagus_29	908	13.4%	5	4.04	2.68	0.63	0.79	0.45
Csolfagus_31	742	29.2%	11	6.65	3.17	0.68	0.72	0.36
Csolfagus_6	1025	2.2%	10	6.66	4.23	0.76	0.63	0.27
Fi05	297	71.7%	7	5.36	1.99	0.50	0.86	0.54
Mfc7	915	12.7%	7	5.76	1.98	0.49	0.86	0.50
sfc061	559	46.7%	13	9.72	5.19	0.81	0.54	0.18
concat14_A_0	689	34.3%	6	5.15	3.50	0.71	0.70	0.35
DE576	552	47.3%	6	4.55	3.16	0.68	0.74	0.41
DUKCT_A_0	920	12.2%	5	4.97	2.66	0.62	0.78	0.41
DZ447_A_0	1019	2.8%	6	5.46	3.70	0.73	0.68	0.31
EEU75	891	15.0%	9	5.26	1.91	0.48	0.88	0.55
EJV8T	1001	4.5%	8	4.53	2.61	0.62	0.80	0.50
EMILY_A	869	17.1%	6	5.50	3.06	0.67	0.74	0.39
ERHIBI_A_0	718	31.5%	6	3.58	2.75	0.64	0.80	0.49
Mean	811.5	22.6%	8.0	5.7	3.29	0.66		
Cumulated							0.001	$5.8\ 10^{-10}$

Table S2: Size, competition and defoliation variables measured in adults beeches.

Trait code	Trait	Unit		432 trees	90 cored trees
DBH2002 (cm)	Diameter in 2002	cm	Mean (sd)	25.7 (18.9)	39.5 (14.5)
			Median	22.4	34.2
			Min-max	2.5-95.2	18.5-95.2
Compet5	Competition index within 5 m	-	Mean (sd)	3.9 (3.5)	1.90 (1.1)
			Median	3	1.7
			Min-max	0– 23.1	0.03-4.6
Compet10	Competition index within 10 m	-	Mean (sd)	6.6 (5.0)	3.7 (1.2)
			Median	5.2	3.7
			Min-max	0.8 – 30.5	0.8-6.5
Compet15	Competition index within 15 m	-	Mean (sd)	8.05 (5.5)	4.9 (1.2)
			Median	6.5	5
			Min-max	0.6 – 30.8	1.9-7.7
Compet20	Competition index within 20 m	-	Mean (sd)	9.47 (7.0)	6.9 (6.0)
			Median	7.1	5.8
			Min-max	2.5 – 34.0	2.6-33.9
Dens5	Nb of neighbors within 5 m	-	Mean (sd)	9.7 (5.3)	8.1 (5.1)
			Median	10	7.5
			Min-max	0-24	1-22
Dens10	Nb of neighbors within 10 m	-	Mean (sd)	34.0 (15.6)	29.6 (15.3)
			Median	33	26.5
			Min-max	4-70	5-65
Dens15	Nb of neighbors within 15 m	-	Mean (sd)	75.1 (34.1)	8.1 (5.1)
			Median	72	57
			Min-max	11-166	14-124
Dens20	Nb of neighbors within 20 m	-	Mean (sd)	132.9 (60.3)	116.9 (55.0)
			Median	135	103
			Min-max	20-263	28-219
DEF	Defoliation index	-	Mean (sd)	0.37 (0.9)	0.7 (1.0)
			Median	0	0
			Min-max	0-7	0 -4

Figure S1 Climate characteristics of the study site.

Figure S1A: Climatic diagram at La Massane representing the sum of monthly precipitations (P, blue barplot) and the average monthly temperature (T, continuous black line). The error bars on the P barplot and the dashed lines around the T continuous line show the confidence interval at 95% of monthly values, based on the variation observed from 1976 to 2015

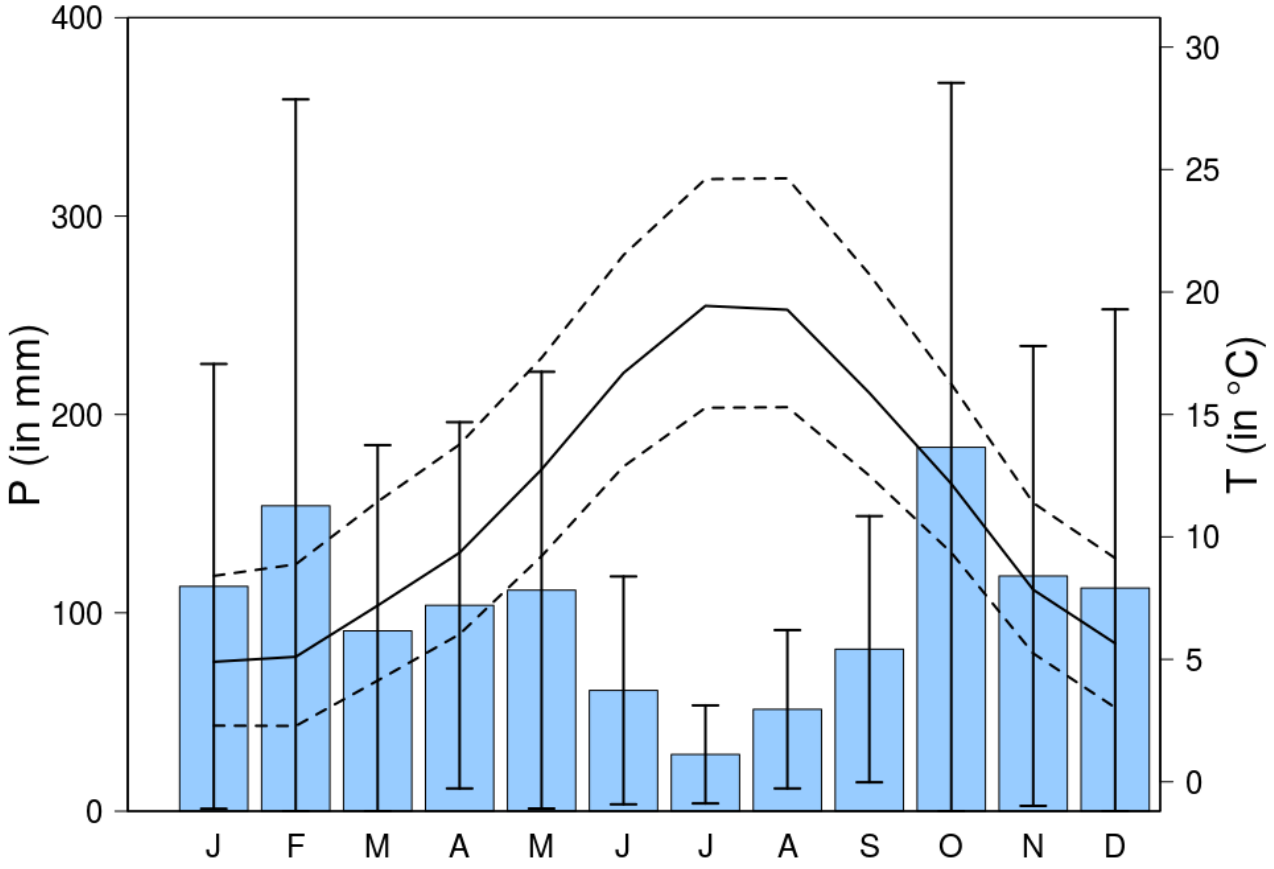


Figure S1B: Position of La Massane (as the magenta triangle) on French beech bioclimatic niche (green crosses). Left: presence of beech (green crosses) according to French Inventory data (IFN). We used the meteorological Safran data base for the period 1958-2015 (collected on a 8km square grid represented by black empty circles) to draw the bioclimatic niche graph (right), as depicted by mean annual temperature (MAT) and the sum of summer precipitation (PRECsummer). Note that the magenta triangle correspond the the Safran point the closest from La Massane, and not to the climate data monitored on site as in Figure S1.

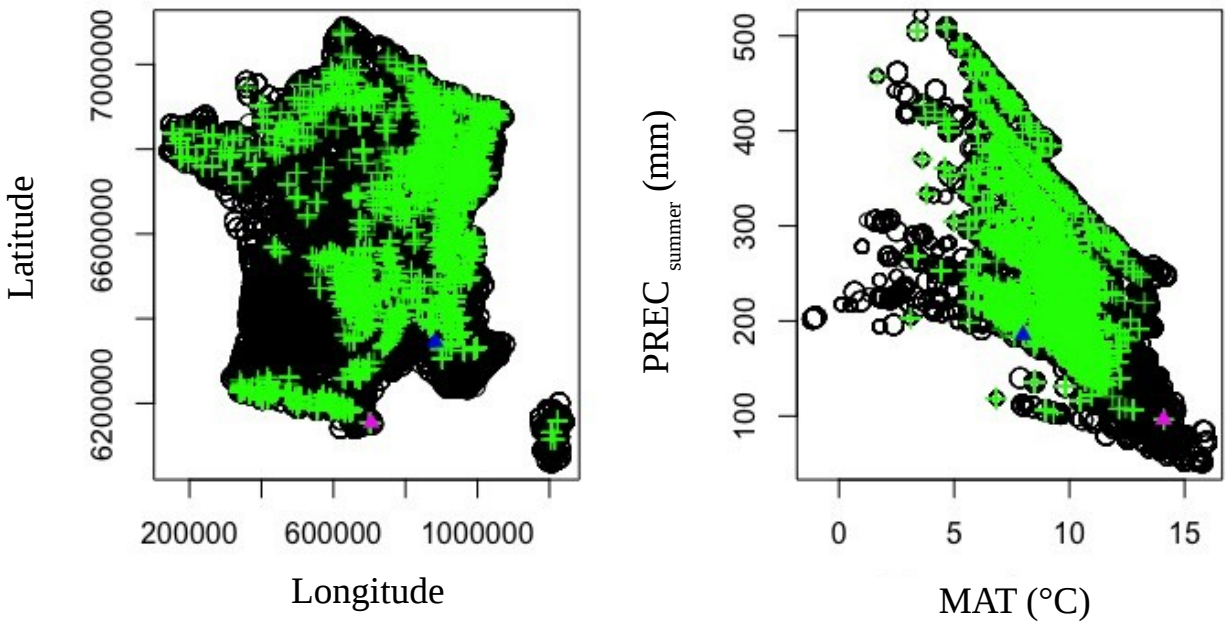


Figure S2 : Patterns of covariation among competition index (the CX's) and density (the DX's) computed in radius a different size (X=1 to 20 m) around each focal beech.

The left plot shows the variables projection onto the Principal Component Analysis plane define by the two first axis. The right plot shows the pairwise correlations between variables.

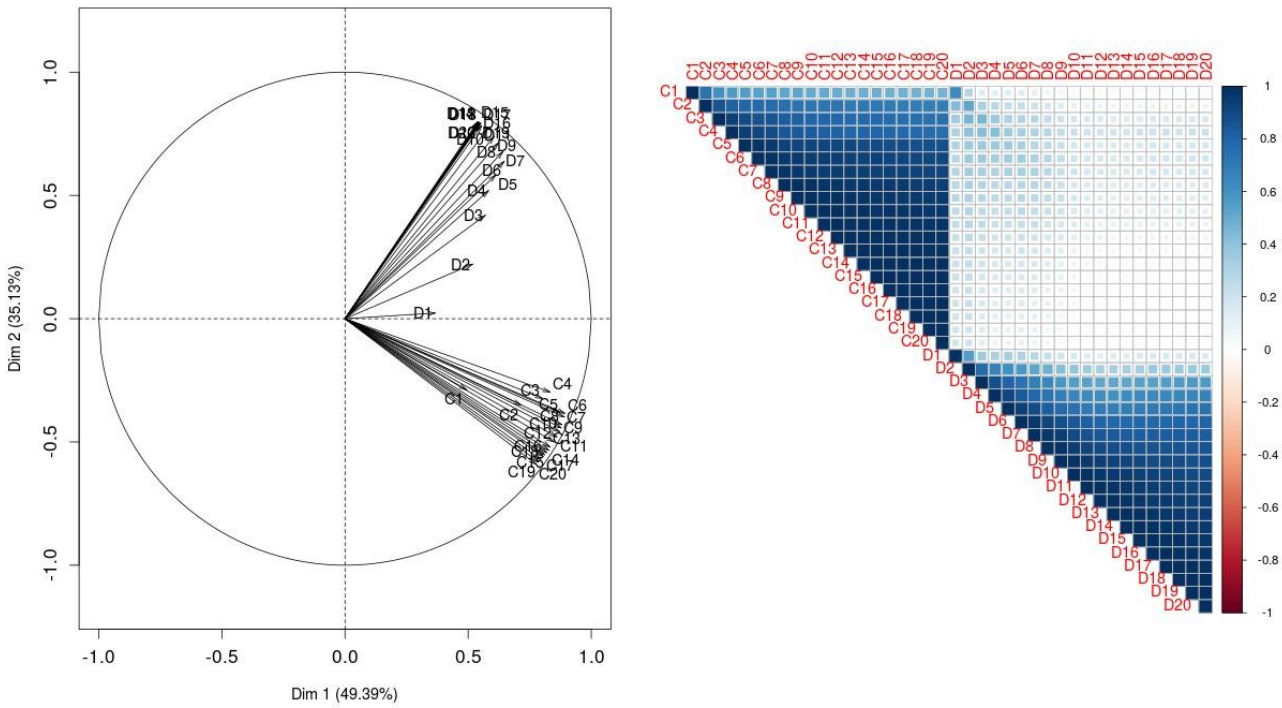
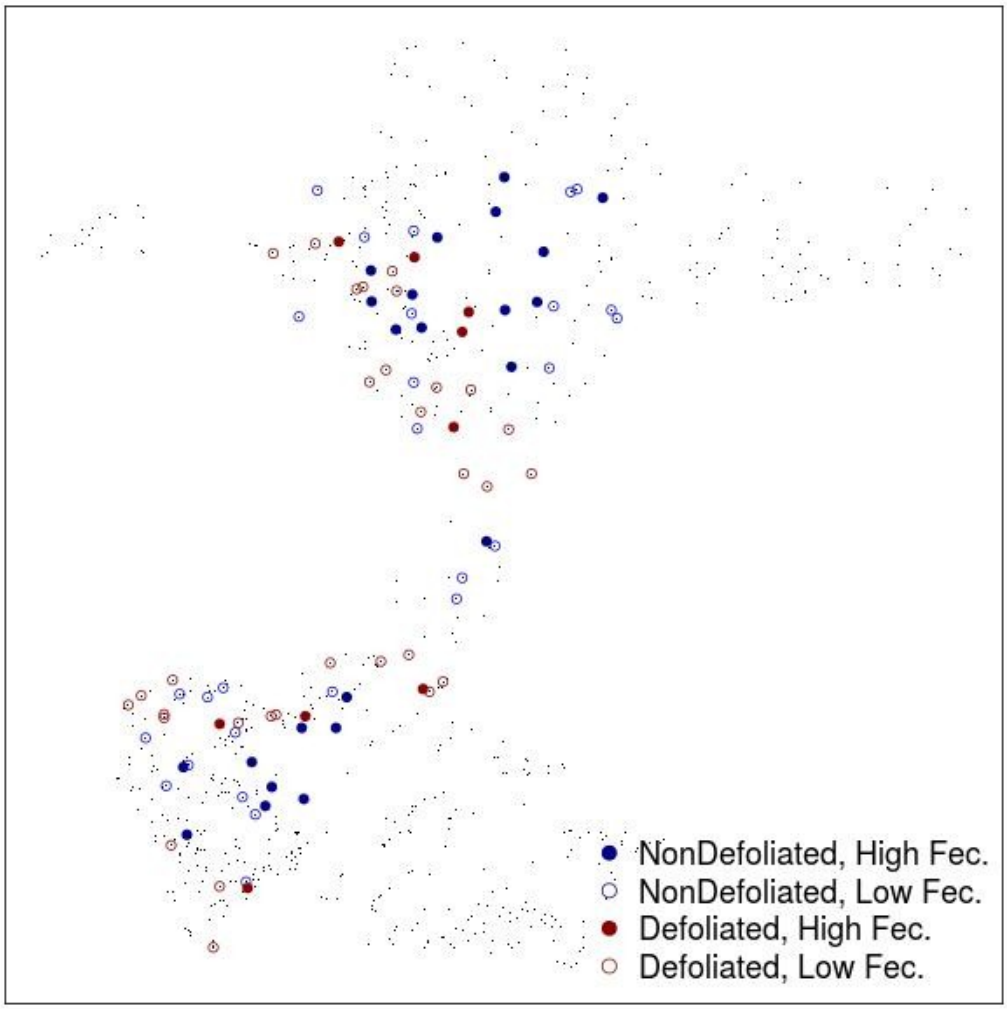


Figure S3: Sampling design for the 90 cored individuals

A. Map of the cored trees per category. Points represent all the 683 adult alive beeches**.**



B. Samplig size, fecundity and defoliation per category

Category	**# indiv.**	**Female fecundity**	**Defoliation**
Non-defoliated, High fecundity	23	2.1 (2.2)	0 (0)
Non-defoliated, Low fecundity	27	0.16 (0.16)	0 (0)
Defoliated, High fecundity	9	1.1 (1.6)	1.4 (0.73)
Defoliated, Low fecundity	31	0.13 (0.051)	1.7 (0.98)

Figure S4: Effect of size and competition on defoliation (equation 3').

A. Analysis of variance table of the model described by equation 3': the adjusted R^2 was 0.28. For each term, we give the type III sum of squares (SSQ) and degree of freedom (df), and for each predictor, the estimate of its effect, the standard error (S.E.) and associated t and p-value. Variance inflation factors (VIF) were computed with R package CAR.

	SSQ	df	Estimate	S.E.	t	P-value	VIF
DBH	29.34	1	0.137	0.200	0.686	0.028	5.49
Compet19	4.97	1	-0.010	0.004	-2.212	0.059	2.69
Dens20	17.06	1	-0.015	0.008	-1.896	0.156	3.38
DBH:Compet19	28.18	1	-0.002	0.001	-1.420	0.000	2.99
DBH:Dens20	12.87	1	0.002	0.000	6.046	0.000	4.02
residuals	223.52	426	0.000	0.000	4.953		

B. Interaction plot showing regression lines of defoliation against DBH for three levels of B1-competition or B2-density, corresponding to +/- 1 standard deviation from the mean. Confidence interval at 80% are displayed around each regression line.

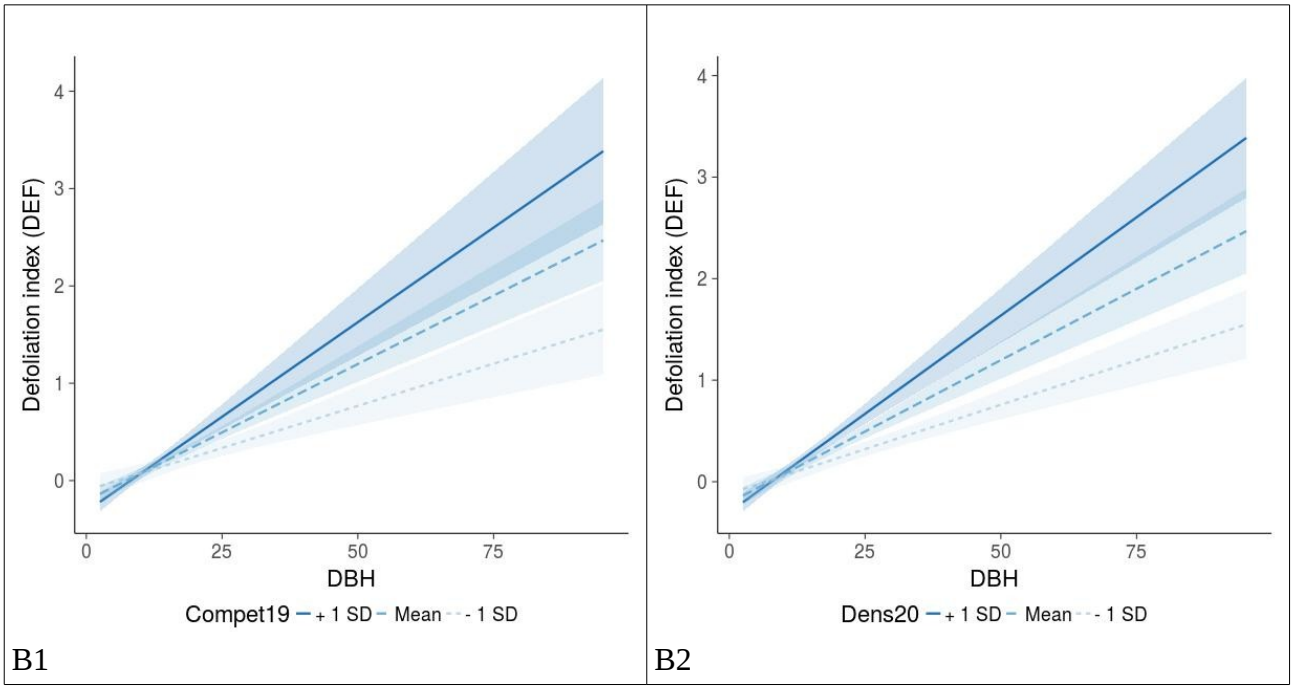


Figure S5: Relationship between growth estimated from ring-width and inventory data.

The graph on the left plots the cumulated radial growth from 2002 to 2012 respectively estimated from ring-width (x-axis) and inventory (y-axis). The graph on the right plots the cumulated basal area increment from 2002 to 2012 (BAI) respectively estimated from ring-width (x-axis) and inventory (y-axis) , based on the 90 cored trees. The correlation between estimates in showed on each graph

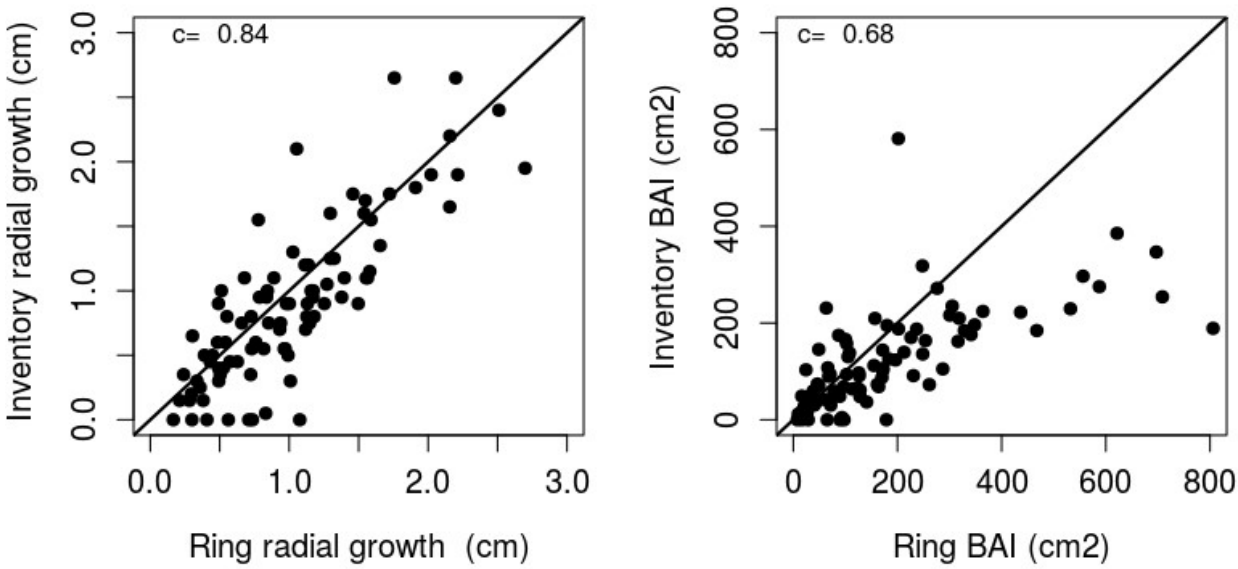
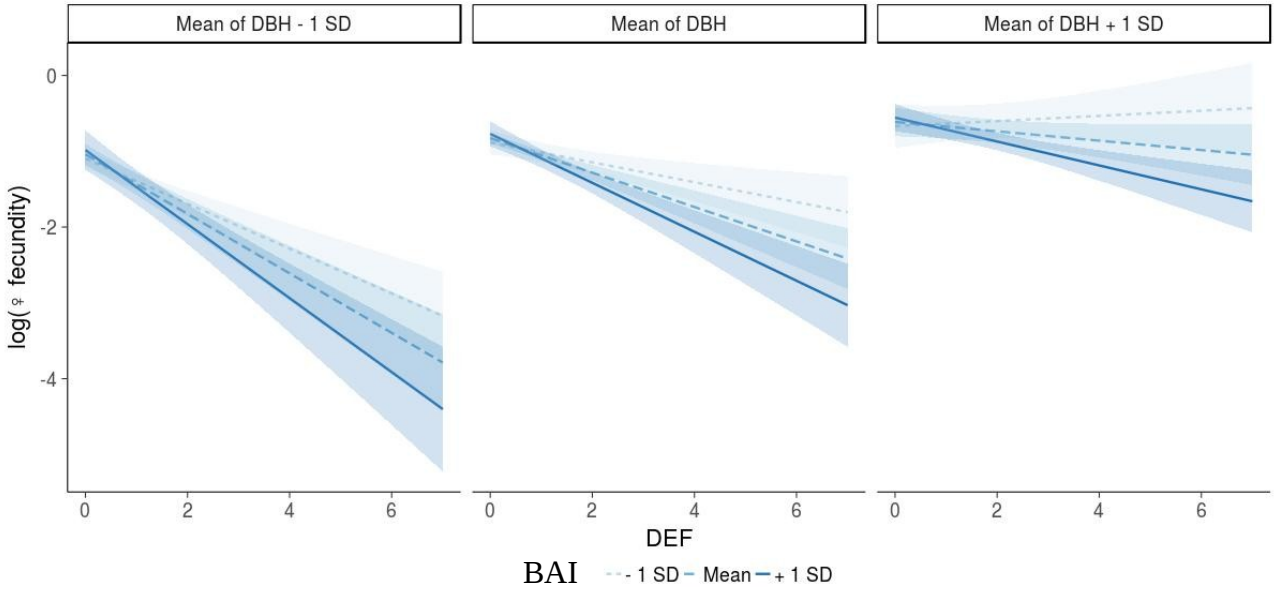



Figure S6: Interaction plots for DEF , BAI, and DBH_{2002} effects on female fecundity.
Regression lines are plotted for 3 values of each moderator variable, corresponding to +/- 1 standard deviation from the mean. Confidence interval at 80% are shown around each regression line.