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Reproduction alternation in trees: testing the resource depletion hypothesis using experimental fruit removal in *Quercus ilex*

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Summary

The key stones of resource budget models to explain mast seeding are that fruit production depletes tree stored resources, which become subsequently limiting to flower production the following year. These two hypotheses have, however, rarely been tested in forest trees. Using a fruit removal experiment, we tested whether preventing fruit development would increase nutrient and carbohydrates storage and modify allocation to reproduction and vegetative growth the following year.

We removed all the fruits from nine adult *Quercus ilex* trees shortly after fruit set and compared, with nine control trees, the concentrations in nitrogen, phosphorus, zinc, potassium and starch in leaves, twigs and trunk before, during and after the development of female flowers and fruits. The following year, we measured the production of vegetative and reproductive organs as well as their location on the new spring shoots.

Fruit removal prevented the depletion of nitrogen and zinc in leaves during fruit growth. It also modified the seasonal dynamics in zinc, potassium and starch in twigs, but had no effect on reserves stored in the trunk. Fruit removal increased the production of female flowers and leaves the following year, and decreased the production of male flowers. Our results show that resource depletion operates differently for male and female flowering, because the timing of organ formation and the positioning of flowers in shoot architecture differ between male and female flowers.

Our results suggest that nitrogen and zinc availability constrain flower production in *Q. ilex*, but also that other regulatory pathways might be involved. They strongly encourage further experiments manipulating fruit development over multiple years to describe the causal relationships between variations in resource storage and/or uptake and male and female flower production in masting species.

Keywords

Resource allocation, nutrients, mast seeding, flowering, architecture, non-structural carbohydrates, photosynthesis, organogenesis

Introduction

Flowering, the first step of plant sexual reproduction, results from a chain from floral induction, by which a vegetative meristem is converted into a floral meristem, to floral initiation and development until anthesis. This chain of processes is regulated by internal factors (e.g. hormones, resource levels), environmental cues (e.g. temperature, photoperiod) and environmental vetoes (i.e. environmental conditions occurring at a precise stage of the reproductive cycle that compromise either the integrity of the reproductive organs or their development, e.g. frost, drought, biotic stress; Allen et al. 2017). While the determinants of flowering intensity have been well described in fruit crop trees (Monselise and Goldschmidt 1982, Samach and Smith 2013, Sharma et al. 2019), they remain poorly known in forest trees although they are essential to understand what drives tree fecundity in general, and mast seeding in particular (Crone and Rapp 2014). Indeed, many forest and fruit tree species reproduce following a behaviour of massive and synchronous seed production that alternate with one or more years of negligible production, also called masting or mast seeding (Silvertown 1980, Kelly and Sork 2002). These masting patterns are expected to be modified by the on-going climate change. However, predictions of tree reproduction under unprecedented climatic conditions are still limited by our lack of knowledge of the physiological mechanisms behind masting and of the respective roles of resource availability and weather conditions on seed production (Hackett-Pain and Bogdziewicz 2021).

As reproduction is considered a costly function for plants (Obeso 2002), most hypotheses for masting patterns, and more generally reproduction alternation in trees, imply a regulation of resources allocated to reproduction. This assumption is notably based on the commonly observed pattern of negative autocorrelation between past and current intensity of seed production in forest masting species (Koenig and Knops 2000, Koenig et al. 2016, Moreira et al. 2019), and in fruit crop trees (Jonkers 1979, Monselise and Goldschmidt 1982, Samach and Smith 2013). This negative autocorrelation pattern is usually attributed to resource limitation and a reproductive trade-off among years.

In particular, the resource depletion (or storage) hypothesis proposes that trees' reserves are depleted during mast years and that the tree needs to stock resources for several years before it can invest strongly in reproduction again (Sork et al. 1993, Sánchez-Humanes et al. 2011, Pesendorfer et al. 2016, Pearse et al. 2016, Bogdziewicz et al. 2018, Schermer et al. 2019). The resource depletion hypothesis, together with the assumption that storage level during floral induction determines flowers abundance in the following spring (Monks et al. 2016, Funk 2017), is a key stone in the resource budget models that are designed to reproduce masting behaviour within forest tree population (Satake and Iwasa 2002, Crone and Rapp 2014, Venner et al. 2016, Abe et al. 2016). However, the explicit relationships between inter-annual variability in resource availability and floral induction and initiation, which

determine the potential number of flowers and thus the potential number of fruits, have been largely unexplored (Crone and Rapp 2014, Miyazaki et al. 2014, Allen et al. 2017, Han and Kabeya 2017). Depletion of stored resources has been historically mostly shown in fruit crop species (Goldschmidt and Golomb 1982, Brown et al. 1995, Rosecrance et al. 1998) that are selected and managed for high and regular fruit production. Recent correlative studies in forest tree species have added evidence of resource depletion induced by masting for nitrogen (N), phosphorus (P) and non-structural carbohydrates (NSC) depending on the species (reviewed in Pearse et al. 2016 and Han and Kabeya 2017). In a remarkable study on *Fagus crenata*, Miyazaki et al. (2014) also showed that the expression of genes involved in floral induction was increased under high nitrogen availability (naturally or following fertilisation). Yet, in many species, we still miss basic understanding of how resources are involved in flower and seed production, of whether resources consumed by current reproduction are indeed limiting future flowering, and of which resource or combination of resources is involved.

One experimental approach can be to prevent fruit growth, which is the most resource-demanding phase of the reproductive cycle, by harvesting all fruits early in their development on some trees (Obeso 2002, Bogdziewicz et al. 2020) and determine whether their resource levels at the end of the reproductive season are higher than in control trees. Flower and fruit thinning is a common agronomic practice in commercial fruit crops in order to reduce alternate bearing patterns and increase fruit size and quality (Coneva and Cline 2006). Flower and fruit removal experiments have thus mainly been realised in fruit crop species (Reig et al. 2006, Verreyne and Lovatt 2009, Samach and Smith 2013, Fernandez et al. 2018), but also in herbaceous plants (Crone et al. 2009), to determine how it impacted resource storage and further flowering. To our knowledge, only two studies used such experiment in forest trees (Sala et al. 2012, Santos-del-Blanco and Climent 2014), both realised on *Pinus* species, and none quantifying resources in N, P and NSC after whole tree fruit removal.

Our objective in this study was to investigate how preventing fruit development and maturation would affect the resource availability and allocation to flowering the following year in *Quercus ilex*, an evergreen species belonging to the most commonly studied masting taxon of oak. We had no *a priori* on which resource (N, P, K, Zn or NSC) would be most limiting to reproduction and in which compartment storage and depletion would preferentially occur. By removing all the fruits of a group of nine *Quercus ilex* trees shortly after fruit set and comparing them to a control group that could develop their fruits during summer, we aimed at testing the two following hypotheses:

Hypothesis 1: Resources that could not be allocated to fruit development will be stored in the trees while photosynthetic carbon uptake will be downregulated due to a reduced sink activity because

primary and secondary growth are mostly finished in *Q. ilex* at the time of fruit set (Lempereur et al. 2015, Le Roncé et al. 2021).

Hypothesis 2: Fruit removal will increase male and female flower production the following spring because resource stored would up-regulate floral induction.

Materials and methods

Study species and area

Quercus ilex L. is an evergreen Mediterranean species that reproduces by masting and is regularly considered as a model species in studies on mast reproduction (e.g. Perez-Ramos et al. 2010; Bogdziewicz et al. 2017; Bogdziewicz et al. 2019; Le Roncé et al. 2021). It is a wind-pollinated monoecious tree that usually flowers in May in the study area (southern France). The male inflorescences, called catkins, bear around 20-25 staminate flowers (Yacine and Bouras 1997, Gómez-Casero et al. 2004). Catkins develop in the axils of lower leaves of the current-year shoot or in separate buds bearing only catkins. Female pistillate flowers mature a few days after staminate flowers and female inflorescence can bear one to six pistillate flowers. Fertilization occurs late June to early July, leading to fruit initiation. Fruits, called acorns, achieve their maturation in November-December (Yacine and Bouras 1997).

The experimental plot is located in Montpellier, France (latitude: 43.64°N, longitude: 3.86°E, altitude: 76 m). The climate is Mediterranean with a mean annual rainfall of 629 mm and mean annual temperature of 15.2°C. The soil of the field site is a rendzina-like silty clay soil, with a pH of 8 and a depth varying from 150 to 200 cm. The trees used for the experiment were planted in 1998 from fruits collected from nearby natural populations. Trees were on average (\pm SD) 3.6 ± 0.5 meters tall with a mean basal area of 52 ± 28 cm² (at 0.4 m) during the experiment and no difference in basal area between groups (Table S1).

Experimental setting

The experiment took place in 2018, which was an intermediate year in terms of fruit production for *Quercus ilex* in the area, i.e. neither a mast seeding year nor a year with important reproductive failure (Le Roncé et al. 2021). In 2018, mean temperature was 15.8°C, minimum temperature -6.3°C, maximum temperature 38.3°C, total precipitation 1135 mm, and precipitation during the period of acorn development, from June to November, was 467 mm. In spring 2018, 24 trees were selected for

the experiment. Out of the 24 trees, 6 did not set any fruit in June (the naturally “No Fruit” (NF) group), and the 18 others were randomly divided in two groups of 9 trees each. In the first group, all the fruits from the 9 trees were removed between the 12th and the 19th of June 2018 (the “Removed Fruit” (RF) group) and the second group was used as control (Fig. 1). The trees of the naturally “No Fruit” (NF) group did not set female flowers the subsequent year, in spring 2019.

On each tree of the “Removed Fruit” and control groups, 20 shoots that had set a fruit in June 2018 were randomly selected on different parts of the tree crown, although often in the upper part where fruiting shoots were mostly located. On all trees from the three groups (NF, RF, control), 8 shoots that had not set a fruit in 2018 were also randomly selected in the upper part of the crown of each tree. The shoot is defined here as the growth unit of the current year (or the spring and summer growth units in cases when two bud flushes occurred due to polycyclism).

In order to estimate fruit development success in the control group, the number of fruits was counted after fruit set in June 2018 and shortly before fruit maturity (the 19th of October or the 8th of November 2018 depending on fruit maturation phenology) to estimate fruit development success on the twenty shoots that had set fruits.

Photosynthesis after fruit removal

In order to estimate the fruit removal effect on photosynthesis, we measured leaf gas-exchange on current-year leaves adjacent to the fruits in control trees or that had been adjacent to one fruit before fruit removal in RF trees. We measured gas-exchange on two leaves per tree of 14 trees (7 trees of the control group and 7 trees of the RF group, 28 leaves in total). Leaf gas exchange was measured between June 28th, 2018 and July 4th, 2018, after the spring leaves had reached maturity and 2 weeks after fruit removal. Measurements were carried out with two portable photosynthesis systems (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with a light source (6200-02B LED, Li-Cor). Leaves were first acclimated in the chamber for more than 20 min at ambient temperature and relative humidity, ambient CO₂ concentration (400 ppm) and a saturating photosynthetic photon flux density (PPFD) of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Shoot growth and production of vegetative and reproductive organs the year following fruit removal

In Spring 2019, we randomly selected 4 to 15 shoots per tree in the control and RF groups among the 20 shoots that had set a fruit and were followed in 2018 (Fig. 1). On each of these shoots and on each

of the 8 shoots that had not set fruits, we counted the number of shoots that were produced in spring 2019, and counted the number of leaves, catkins and female flowers on each of these shoots. For each of these organs, we also noted the node rank on which they occurred (Barthélémy and Caraglio 2007; Peyhardi et al. 2017). The 2019 spring shoots could be composed exclusively of catkins (in this case, there is no twig), of both leaves and flowers (catkin or female inflorescence), or exclusively of leaves. Therefore, each node could be composed either by catkins only, by catkins at the axil of one leaf, by one leaf only, by one female inflorescence at the axil of one leaf, or rarely by one female inflorescence only. On each tree, we collected two of the catkins produced by the 2019 shoots, if existing (1 tree did not produce catkins in spring 2019). On each catkin, we counted the number of staminate flowers. On July 19th, 2019, we counted the number of initiated fruits (female flowers that had developed into small fruits, Fig. 1) on each of the 2019 spring shoots. The fruit set was calculated for each shoot as the ratio between the number of initiated fruits and the number of female flowers supported by the shoot. We planned to monitor fruit growth and maturation in summer and autumn 2019 but an early and extreme heat wave damaged significantly the leaves and fruits on June 28th, 2019 when air temperature reached a maximum of 45.5°C. Therefore fruit growth in 2019 could not be followed after this event.

Tissues sampling

In order to estimate the effect of fruit removal on tree reserves, we sampled tree tissues before fruit removal in spring 2018 shortly before bud break (16th of April), shortly after fruit removal in summer 2018 once spring leaves had reached maturity (4th of July, between two and three weeks after fruit removal in the RF group), long after fruit removal in winter 2019 after last fruit fall (7th of January 2019) and in spring 2019 shortly before bud break (8th of April). The different sampling sessions are described in Fig. 1.

On each tree and at each sampling date, two small sun-exposed branches close to shoots that bore a fruit (in the control group) or had borne a fruit (in the RF group) or random shoots (in the NF group) were cut. For each branch, leaves and shoots produced during the current year (less than 1 year old) were separated from leaves produced one or two years before, called 1- and 2-year-old leaves and 1- and 2-year-old shoots hereafter. Leaves and shoots of the two sampled branches and of the same age cohort were pooled together within each sample type. Trunk sapwood samples were taken at 30 cm above the ground with a 1-cm wide corer in spring 2018 and winter 2019. Sapwood was sampled in the first cm depth past the cambium and the bark and phloem were removed. We sampled sapwood of the trunk only twice in order to avoid frequent damages to the trees. In order to estimate

the nutrient content of female organs at different stages of the reproductive cycle, fruits were collected on nearby trees after fruit set in June 2018 (3 trees, 85 ± 10 fruits collected per tree) and at fruit maturity in November 2018 (3 trees, 6 ± 1 fruits collected per tree). Female flowers were collected in May 2019 (5 trees, 128 ± 36 flowers collected per tree). All leaf, shoot, trunk, flower and fruit samples were micro-waved for 30 seconds shortly after collection to stop enzymatic activity, oven-dried at 60°C for three days and grinded to a fine powder prior to the chemical analyses (Quentin et al. 2015).

Chemical analyses

The carbon (C) and nitrogen (N) concentrations were determined by thermal combustion of 2 mg of ground sample using a Flash Smart NC Soil Elemental Analyzer (ThermoFisher Scientific, USA). The phosphorus (P) concentration was measured colorimetrically using the molybdenum blue method (Grimshaw et al. 1989). Eighty mg of ground sample, 8 mL of HNO_3 and 2 mL of H_2O_2 were mixed and the mixture was heated at 175°C for 40 min using microwaves (Ethos One, Milestone SRL, Italy). After this mineralization step, the sample was diluted to a total of 50 mL. A hundred μL of sample, 100 μL of NaOH, 50 μL of mixed reagent (emetec tartar and ammonium molybdate solution) and 50 μL of ascorbic acid were mixed directly in a 96 well microplate. After 30 min at 40°C , the reaction was completed, and the P concentration was measured at 720 nm using a microplate reader (Victor Nivo S, PerkinElmer, Singapore). Following the mineralization step (i.e. the same as for P analysis), potassium (K) and zinc (Zn) concentrations were measured using an atomic absorption spectrometer (AAS, iCE 3000 series, ThermoScientific, China). Phosphorus analysis were conducted on all tissues, except on female flowers and fruit.

Non-structural carbohydrates (NSC) were analysed following the protocol described by Wong (1990) and adapted according to Hoch et al. (2002). NSCs are defined here as low-molecular-weight sugars (glucose, fructose and sucrose) plus starch. Ten to 12 mg of ground material were boiled in 2 mL of distilled water for 30 min. After centrifugation, an aliquot of 200 μL was treated with Invertase and Isomerase from baker's yeast (Sigma-Aldrich, St Louis, MO, USA) to degrade sucrose and convert fructose into glucose. The total amount of glucose (sugars) was determined photometrically at 340 nm in a 96-well microplate photometer (HR 7000; Hamilton, Reno, NE, USA) after enzymatic conversion to gluconate-6-phosphate (hexokinase reaction, hexokinase from Sigma Diagnostics, St Louis, MO, USA). The total amount of NSC was measured by taking 500 μL of the extract (including sugars and starch) incubated with a fungal amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich) for 15 h at 49°C to digest starch into glucose. Total glucose (corresponding to NSC) was determined photometrically as

described above. The concentration of starch was calculated as NSC minus free sugars. Pure starch and glucose, fructose and sucrose solutions were used as standards, and standard plant powder (Orchard leaves; Leco, St Joseph, MI, USA) was included to control the reproducibility of the extraction. Because all samples were run in a single laboratory with no change in protocol during the processing of samples, issues with the comparison of results across methods or laboratories were avoided (Quentin et al. 2015). NSC analyses were conducted on tissues sampled in spring 2018, summer 2018 and winter 2019.

Statistical analyses

All statistical analyses and visual representations were conducted using the software R version 3.6.1 (R Core Team 2019) and the packages *ggplot2* and *interactions* (Wickham 2016, Long 2019). We used the following packages for data analysis: *lme4*, *car* and *multcomp* (Hothorn et al. 2008, Bates et al. 2015, Fox and Weisberg 2019). We considered our level of significance as $p\text{-value} < 0.05$.

The relationship between the number of fruits per tree and the tree basal area was studied within the RF group for which we had a reliable estimation of the total number of initiated fruits. We tested the significance of the relationship with a Spearman correlation on un-transformed data and with a Pearson linear correlation on log-transformed data. The effects of fruit removal, of the sampling date (before or after the development of female organs) and of their interaction on nutrient (N, P, K, Zn) and starch concentrations in leaves, twigs and trunk were tested with fifteen linear mixed effect models (5 chemical resources x 3 tissues). We used organ cohort (1 yr-old and 2-yr old) nested within tree identity as a random factor for models explaining resources concentrations in leaves and twigs, and tree identity as a random factor for models explaining resources concentrations in trunk. The effect of experimental fruit removal on photosynthesis was tested with a linear mixed effect model with tree identity as a random effect.

The effect of fruit removal on the production of vegetative and reproductive organs the year following the treatment was tested for each shoot reproductive status (shoots that had set a fruit in 2018 and shoots that had not) with seven generalised linear mixed effect models (GLMMs): three models related to shoots and leaves (the production of spring shoots in 2019 and the numbers of leaves and nodes per spring shoot in 2019) and four models related to reproductive organs (the numbers of female flowers and catkins, the fruit set and the mean number of initiated fruits per spring shoot in 2019). We fitted GLMMs with a Gaussian distribution for the response variables presenting a normal error-structure (number of staminate flowers per catkin), with a negative binomial distribution and its log link function for count data (number of 2019 spring shoots per 2018 shoot, numbers of nodes, leaves, female flowers, catkins, initiated fruits per 2019 spring shoot) and with a binomial distribution

and its logit link function for dichotomous response variables (fruit set). We used 2018 shoot identity nested in tree identity as random effect in models explaining the numbers of nodes, leaves, female flowers and catkins per 2019 spring shoot, and tree identity as a random effect for other models at 2018 shoot scale. We used as fixed effects the fruit removal treatment, and as covariable the 2018 shoot diameter, as well as their interaction. 2018 shoot diameter was standardized prior to analysis to compare models estimates between variables. For each response variable, we then applied a simplification of the model by sequentially removing the insignificant interaction terms. Marginal (R^2_m) and conditional (R^2_c) R-squares were calculated with the package MuMIn (Bartoń 2019) according to Nakagawa's method (Nakagawa and Schielzeth 2013) to estimate the variance explained by fixed effects and fixed plus random effects, respectively. Within treatments, the effect of the reproductive history (i.e. whether the shoots had set a fruit or not in 2018) on the numbers of leaves, female flowers and catkins per spring shoot and on the mean number of initiated fruits per spring shoot were studied with a negative binomial distribution, reproductive status and 2018 shoot diameter as fixed effects, and 2018 shoot identity nested in tree identity as random effect.

Results

On the nine trees from the RF group, we removed respectively 28, 82, 129, 131, 178, 213, 653, 798 and 3994 fruits. The number of fruits removed was strongly correlated to the tree basal area (Spearman $\rho = 0.83$, $p = 0.008$; Pearson on log-transformed data $R=0.85$; $p = 0.004$; Appendix S1). On the nine trees of the control group, 56 ± 20 % (mean \pm SD) of the fruits reached maturity in 2018.

Tissues resource content, allocation and seasonal dynamics

Before bud break in spring 2018, concentrations in N, P, K, Zn and starch in any tissue did not differ between the three groups (Table S1). At the time of fruit removal, initiated fruits weighted less than 10 % of their mean final dry mass and contained less than 25 % of their final content of C, N, K and Zn in mature fruits (Fig. S1). Nutrient concentrations in N, K, Zn and P were highest in flowers (catkins or female flowers) and were progressively diluted in acorns during fruit enlargement and maturation (Fig. S2). Starch concentration was highest in old wood tissues of the trunk and 2-yr-old twigs, whereas conversely, nutrient concentrations were the lowest in the trunk (Fig. S2). In 2018, total acorn production represented 5.8% of the total aboveground biomass production (estimation for a 10-cm tree, see Appendix S1), and acorns represented respectively 11.5 %, 4.5% and 1.6% of the tree aboveground allocation of K, N and Zn (Appendix S1). Seasonal patterns of nutrients and starch

concentrations were generally similar in leaves and branches from different cohorts but varied depending of organs (Fig. S3).

Effect of fruit removal on resource contents and photosynthetic activity

Changes in nutrient and starch concentrations between spring 2018 (before bud break) and winter 2019 (after fruit fall) were not significantly affected by the fruit removal treatment, whether these changes were quantified as the difference in concentration between the two dates or as the final concentration relative to the initial (Table S2). A notable exception was the starch concentration that was more strongly depleted between the two dates in the RF treatment than in the control. Significant interactions between the treatment and the sampling date were observed for the concentrations of N in leaves, Zn in leaves and twigs, K in twigs, and starch in twigs (Table 1, Fig. 2). Concentrations of N in leaves, and Zn in leaves and branches decreased during fruit growth in control trees, i.e. trees bearing fruits, but not in RF trees (Fig. 2A, 2B and 2C). Concentrations of K and starch of the two cohorts of twigs differed between the control and RF trees prior fruit set, but this difference vanished after fruit growth (Fig. 2D and 2E). Leaves close to a fruit in control trees showed photosynthetic rates not significantly different than leaves that had been close to a fruit in RF trees (Fig. S4, N = 29, Type II Wald Anova: $\chi^2 = 2.0$, $p = 0.2$).

Effect of fruit removal on the production of vegetative and reproductive organs the following year

All the variables, except the number of fruits initiated in 2019, were significantly correlated to the 2018 shoot diameter (positively for the number of leaves and of nodes, negatively for the number of catkins) (Table 2). Fruit removal did not affect the number of ramifications produced the following year (spring shoots produced in 2019 per 2018 shoot, Table 2 and Table S3). However, the number of 2019 spring shoots was positively related to the diameter of 2018 shoots (Table 2 and Table S3), and the relationship was stronger in shoots on which fruits were removed compared to shoots on which fruits developed (Table 2). Fruit removal increased the number of female flowers produced in 2019 whatever the reproductive status of the shoots (i.e. whether they had or not set fruits in 2018) (Fig. 3A; Table 2 and Table S3). Female flower production was also positively correlated to the 2018 shoot diameter (Table 2 and Table S3). Fruit removal decreased the number of catkins and increased the number of fruits initiated in 2019 and the number of leaves, although these effects were significant only in shoots that had not initiated fruits in 2018 (Fig. 3B, 3C, 3D; Table 2; Tables S2, S3). Fruit removal did not affect

the number of staminate flowers per catkin (N = 127, Type II Wald Anova: $\chi^2 = 0.01$, p = 0.9 and $\chi^2 = 0.48$, p = 0.5, respectively).

Effect of fruit removal on shoot architecture the following spring

The study of shoot architecture showed that catkins were present mainly at the basal part of the shoots, on nodes 1 to 4 (Fig. 4), and that leaves and female flowers presented a mode around nodes 4 to 6 (Fig. 4, Fig. S5). We never observed shoots bearing catkins at a higher node rank than female flowers. In both RF and control trees and whatever the shoot type, the number of female flowers was strongly correlated to the number of leaves (Fig. S6). Qualitatively, changes in the relative occurrence of leaves, female flowers and catkins between RF and control trees occurred mainly between Node 2 and Node 6 (Fig. 4, Fig. S5).

Discussion

Limited evidence in favour of the resources depletion hypothesis

This study stems from the negative temporal autocorrelations generally observed in the fecundity of masting species and the widely accepted assumptions that reproduction has a cost on future reproduction (Obeso 2002) and that the masting pattern is due to resources depletion. In support to this assumption, we found that preventing fruit development and maturation increased female flower production the following year. Besides, we observed an increase in the number of female flowers both on shoots that had bear fruits and shoots that had not, which suggests that allocation to reproduction in *Q. ilex* is regulated at the crown scale rather than the shoot scale. In forest trees, only one study, conducted on *Pinus halepensis* (Santos-del-Blanco and Climent 2014), showed a positive effect of seed removal on female cone production the following spring. However, this effect is better documented in fruit crop trees, such as olive, peach and apple tree (Reig et al. 2006, Haouari et al. 2013, Fernández et al. 2015, Haberman et al. 2016), although it is not systematically observed (e.g. no effect in almond tree nor in grapevine; Vaillant-Gaveau et al. 2014, Fernandez et al. 2018).

Concentrations of nitrogen and zinc in leaves and twigs were significantly depleted in trees bearing fruits but not in the fruit removal treatment (Fig. 2). This demonstrates that *Q. ilex* fruits are a significant sink of nitrogen and zinc during their development although they represented only 4.5% and 1.6% of the immobilization of these resources at the tree scale (Appendix S1). These nutrients can

be supplied to the fruits from the nearby leaves and twigs, as observed here, because leaves are usually the main pool of N storage in evergreen species (Cherbuy et al. 2001, Millard and Grelet 2010, Palacio et al. 2018). They may also come from farther pools such as stems, trunk and big roots when these sources are removed by defoliation (Le Roncé et al. 2020). The importance of nitrogen in *Q. ilex* reproduction has been highlighted in several studies showing that foliar nitrogen content is lower in reproductive shoots compared to vegetative shoots (Alla et al. 2013), or that interannual variations in foliar nitrogen is correlated with the number of initiated fruits (Le Roncé et al. 2021). Similarly, Callahan et al. (2007) noted an increase in acorn production in N-fertilized oak forest plots, and interannual variations in foliar nitrogen have been linked to flower production in *Fagus crenata* (Miyazaki et al. 2014, Satake et al. 2019). Foliar zinc concentration has also been associated positively with fruit production in European forest trees, and negatively related to fruit production variability (Fernández-Martínez et al. 2017). Leaves of fruit bearing shoots have lower zinc concentration than non-bearing shoots in almond tree (Saa et al. 2018) and many fruit crop trees increase their fruit production in response to zinc fertilization (Hafeez 2013, Zhang et al. 2014). This might be explained by the involvement of the Zn-finger transcription factors in the development of floral tissues in some plant species (Hafeez 2013). The increased flower production in trees where fruits were removed compared to control trees could thus be explained by the maintained high resources of nitrogen and zinc in winter. Fruit removal was also found to modify the potassium dynamics in shoots (Fig. 2), which is consistent with the fact that acorns mobilized 11.5% of total potassium for only 5.8% of the total biomass. Conversely, phosphorus concentrations were not affected by fruit removal, contrarily to observations by Sala et al. (2012) in reproductive branches of *Pinus albicaulis* on which cones had been removed during a mast year. This different finding might be due to a difference in soil P availability between the two studies, a stronger P limitation to reproduction in conifers than in oaks, or the fact that phosphorus limitation might only become apparent in mast seeding years.

Although nitrogen and zinc concentrations decreased significantly more in trees bearing fruits, this did not translate into significant differences in their concentrations between the two treatments at the end of the reproductive season. Similarly, inter-annual variations in fruit production in a nearby *Q. ilex* forest had no effect on N and P concentrations in leaves the following winter (Le Roncé et al. 2021). We calculated that N and Zn consumption by acorns represented only 4.5% and 1.6%, respectively, of what was used for aboveground biomass production. Nutrient consumption by fruits might thus be negligible in regard to the whole tree storage, as also suggested by a study applying a resource budget model to *Q. ilex* reproduction (Bogdziewicz et al. (2019). This conclusion would thus be contradictory with the general assumption of the resource storage hypothesis and the empirical support it has found in deciduous species such as *F. sylvatica*, *Q. petraea*, and *Q. robur*, in which leaf N

and P contents were observed to decrease with increasing fruit production (Nussbaumer et al. 2021). However, the fact that fruit removal, even during a non-mast year, affected nitrogen and zinc concentrations demonstrates nevertheless that fruits are strong sinks of these two nutrients.

Starch reserves were not depleted more in control than in RF trees, consistently with previous reports that fruit production in oaks is supported by current photosynthesis rather than by stored NSC (Körner 2003; Hoch et al. 2013). Conversely, the decrease in starch concentrations in all organs from spring to winter, which is commonly reported in evergreen species (Hoch et al. 2003), was even more pronounced in the RF trees (Fig. 2E, Fig. S3). This seems surprising considering that fruit removal did not downregulate photosynthetic activity, contrary to what has sometime been shown in peach, pistachio and olive trees (Vemmos 1999, Proietti 2000, Haouari et al. 2013, Andrade et al. 2019), and that most of the primary and secondary growth in *Q. ilex* occurs before July (Lempereur et al. 2015, Le Roncé et al. 2021). This result suggests a strategy of homeostatic NSC levels in response to fruit removal, as observed in response to drought in multiple species (Schönbeck et al. 2018; Dickman et al. 2019). While a slight increase in starch concentration was seen in spring after fruit removal, maybe due to the sudden change in energy demand, starch concentrations in winter were restored to the same level in the two treatments, independently on fruit production. The carbohydrates produced in summer and autumn that were neither used by fruits nor by vegetative growth have probably been translocated to other parts of the trees, and most likely to the collar and roots where important NSC reserves are stored (Loescher et al. 1990, Kozłowski 1992).

Fruit removal affects differently the allocation to male and female flowering

Our results suggest that the physiological mechanisms of masting depend on the timing of development of the male and female reproductive organs. The extension of a shoot is followed in many species by the formation of a resting terminal bud consisting of a set of partially developed organs and of one to several groups of meristems (Guédon et al. 2006). The primordial organs, called preformed organs, usually complete their development later, during the growth period. A shoot may also grow in length by developing neoformed organs, i.e. a shoot portion or set of organs, each of which differentiates at the time of shoot growth (Guédon et al. 2006). *Q. ilex* has preformed buds (Montserrat-Martí et al. 2009). A recent study on its close relative *Q. suber* showed that male flowers are preformed and induced in the early summer of the previous year, whereas female flowers are most likely induced during the vegetative flush in spring (Sobral et al. 2020). Similarly, the basal part of the shoots is preformed in *Q. ilex* while their distal part is very likely neoformed (Y. Caraglio, personal communication). Fruit production and shoot basal area are positively correlated in *Q. ilex*, because

large shoots can provide more water, carbon and nutrients to fruits (Alla et al. 2012), but also because female flowers are formed at higher node position and thus generally on the longest and largest shoots. We observed no effect of fruit removal on the number of 2019 spring shoots, probably because bud set had already taken place at the time of fruit removal in June, so the number of buds and future shoots was already determined. Conversely, we observed that fruit removal in late June 2018 modified the frequency of occurrence of preformed male flowers and leaves at the basal part of the 2019 shoots, which suggests that the preformation of catkins and first leaves in *Q. ilex* takes place in summer, after fruit initiation.

Fruit removal upregulated female flower and leaf production in 2019 shoots, whether they were borne by shoots that had set fruits in 2018 or the usually less vigorous shoots that had not (Alla et al. 2012). Female flower production was positively correlated to leaf production in all shoot types, thereby suggesting a developmental constraint linking leaves and female function at the shoot scale. This is supported by the fact that female flowers are formed at higher node positions along the shoot than male flowers which always occupy the first 5 to 6 nodes on the shoot (Fig. 4, Fig. S5). Since node formation proceeds acropetally, this implies that male flowers are always formed earlier than female flowers. Fruit removal might thus first downregulate male flower preformation during the summer, and later upregulate female flower and leaf preformation in the winter and early spring. Our results thus suggest some competition in sex allocation at the shoot scale. Different timings of allocation to male flowers and to female flowers could thus generate desynchronization of resource allocation between the two sexes. This highlights the need to characterize the link between male and female allocation, and the weather and resource cues affecting this link in order to understand oak masting.

Conclusion

This study is one of the first to manipulate fruiting intensity at the whole tree scale in a forest tree species and to measure the effects of this manipulation at a fine scale, i.e. shoots and organs levels. It showed that even in a year of moderate reproduction, fruit development inhibited female flower induction in *Q. ilex* at both the shoot and the crown scale and that fruit development impacted nitrogen and zinc concentrations. Although changes in allocation to organs (female and male flowers and leaves), both in quantity and nature, and changes in nutrient dynamics could not be compared over several years, our results suggest that flower and fruit production in *Q. ilex* might be zinc and nitrogen limited, but also that other regulation pathways might be involved. It would, therefore, be instructive to repeat the experiment in contrasting years, especially years with mast fruiting. Further experiments manipulating fruiting intensity are thus needed to determine whether there is a causal

link between variation in resource storages and/or uptake and flowering (Allen et al. 2017), and which resource decline after reproduction prevents flower induction the following spring (Crone and Rapp 2014). Indeed, inhibition of future flowering by current fruit development might not be mediated merely by depletion of stored resources, but also by other processes such as secretion of hormones inhibiting floral induction, which is the case of gibberellins in apples, for example (Bangerth 2009, Haberman et al. 2016, Pearse et al. 2016, Sharma et al. 2019). The hypothesis of hormonal regulation of flowering should also be explored as a complement to regulation by resources, because from an evolutionary point of view and to efficiently control the dynamics of fruit consumers (satiation hypothesis), it may be crucial for trees not to have mast-seeding two years in a row (Rees et al. 2002), even if reserves allow it.

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Author Contribution

ILR, IC, JML and SV conceived and designed the study; ILR, ED and IC collected the data in the field; ED, ILR, LS and AG performed the chemical analyses; ILR analysed the data; ILR, IC, JML and SV interpreted the results; ILR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Figures captions

Figure 1. Diagram summarising the experimental protocol.

Figure 2. Model interactions between treatment and sampling date (before and after the development of fruits) observed for concentrations of nitrogen (N) in leaves (A), zinc (Zn) in leaves (B) and twigs (C), potassium (K) in twigs (D) and starch in twigs (E). These figures illustrate the significant interactions detected in the GLMMs described in Table 1. Full big points and error bars represent an average value and its associated confidence interval for each date and treatment, and smaller points the individual data. Significant differences between treatments within each date are indicated in black ($p^* < 0.05$, "n.s."= non-significant). Significant differences between dates for each treatment are indicated in blue for the control and orange for the removed fruit treatment ($p^* < 0.05$, "n.s."= non-significant). Results that support the resource depletion hypothesis are highlighted with a gray background.

Figure 3. Number of A) female flowers, B) leaves, C) catkins and D) initiated fruits per 2019 spring shoot according to the treatment, control or whole-tree fruit removal in 2018, and to the reproductive status of the supporting 2018 shoot. Full black points indicate group mean. Within shoot reproductive status in 2018, the effect of the fruit removal treatment is indicated in black ($* p < 0.05$, "n.s." = non-significant, corresponding to the GLMMs in Table 2 and Table S2). Within each treatment, the effect of shoot reproductive status in 2018 is indicated in blue for the control and orange for the removed fruit (RF) trees ($* p < 0.05$, "n.s."= non-significant). Results that support an increased allocation to female flowers and fruits after fruit removal are highlighted with a gray background.

Figure 4. Relative frequency of the different organs (leaves, female inflorescences and catkins) at each node rank of the 2019 spring shoots in control and RF trees. Increment in node rank indicates location of the node from the base toward the apex of the shoot. The vertical dotted line represents the node 5, which is approximatively the mode for leaves and female flowers. Female inflorescences frequency is also presented alone in Fig. S4 with higher precision.

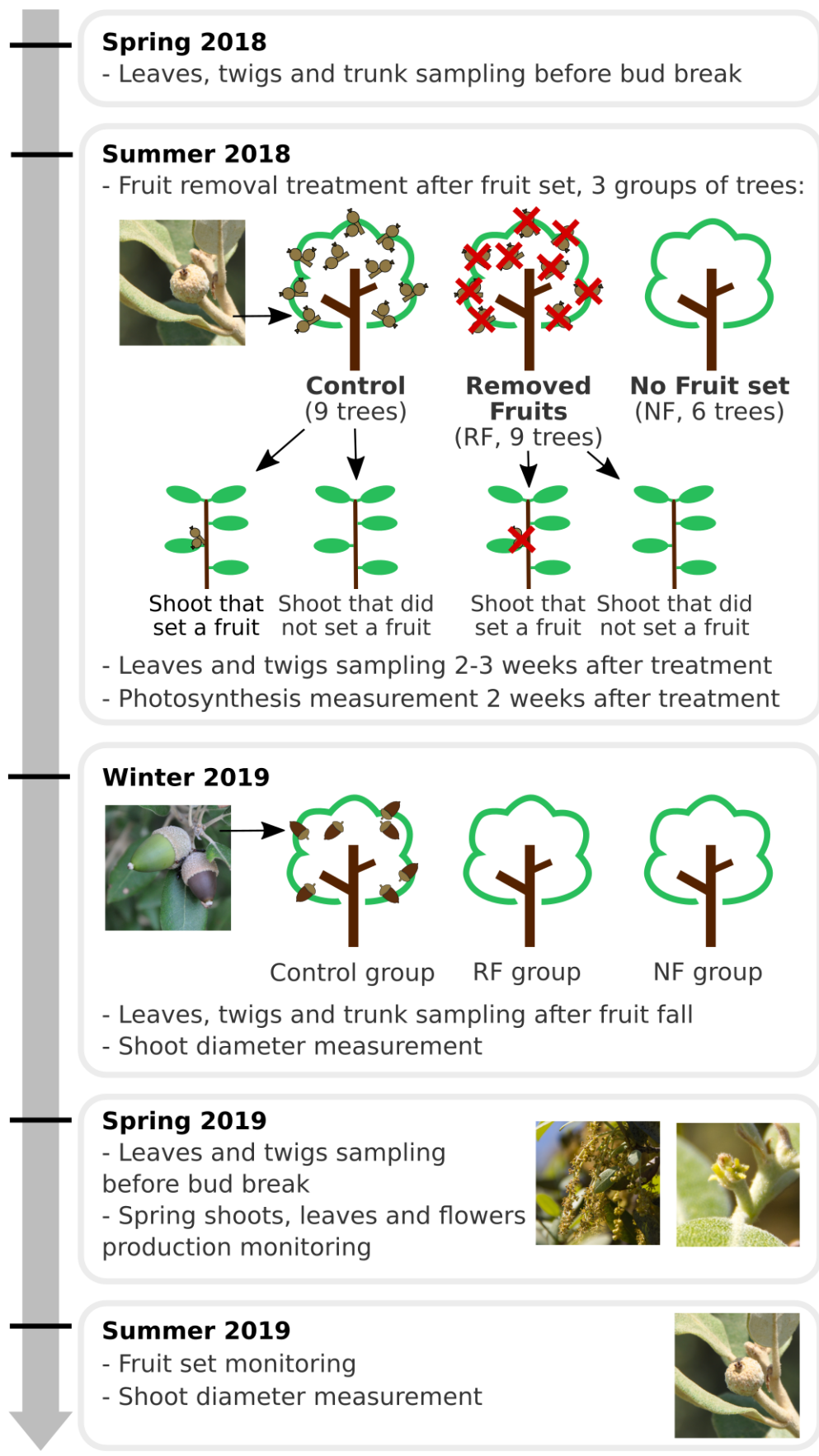


Figure 1

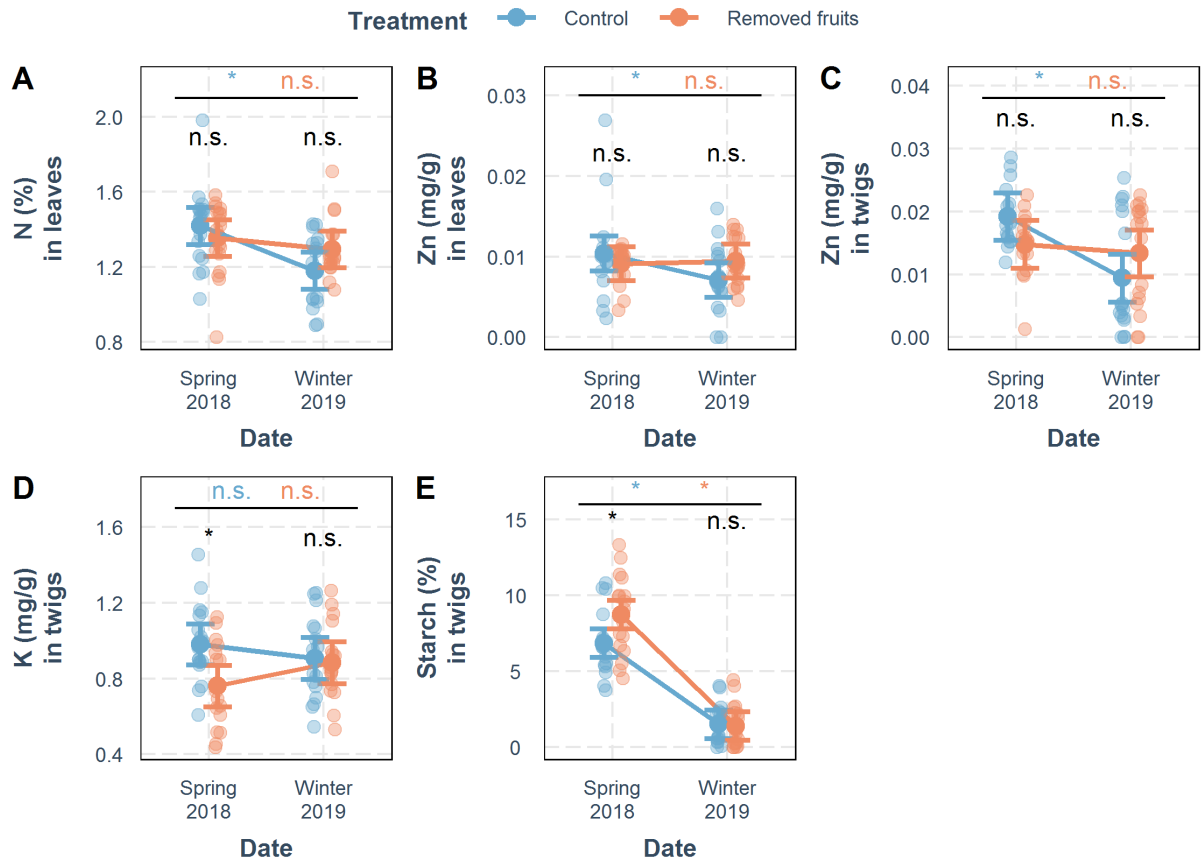


Figure 2

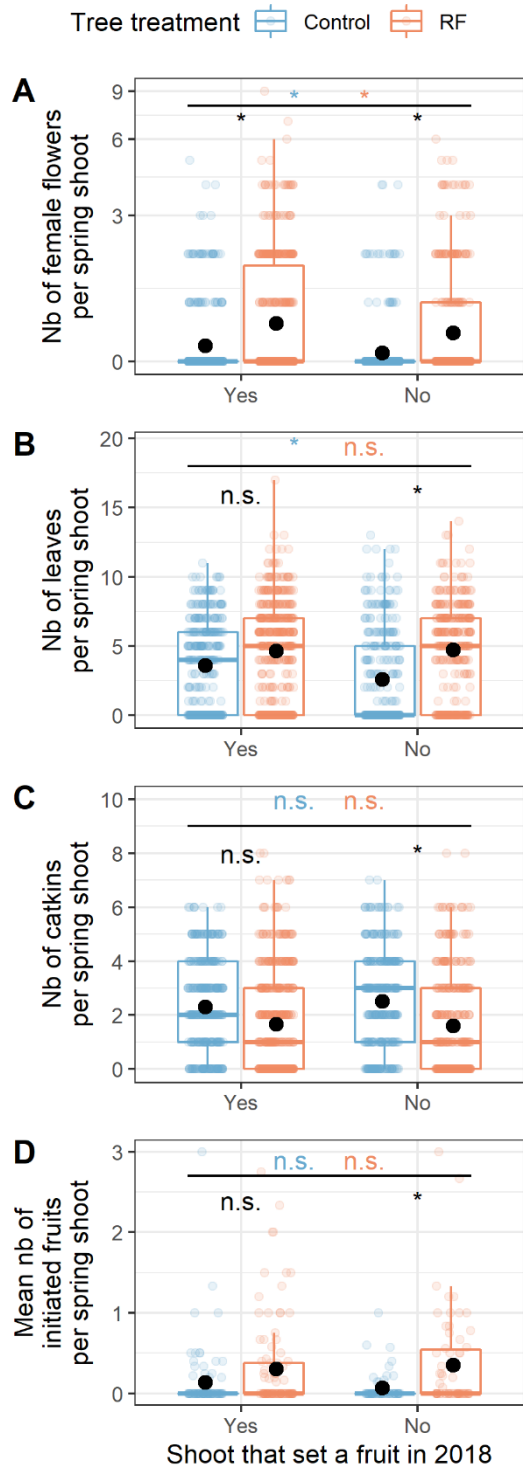


Figure 3

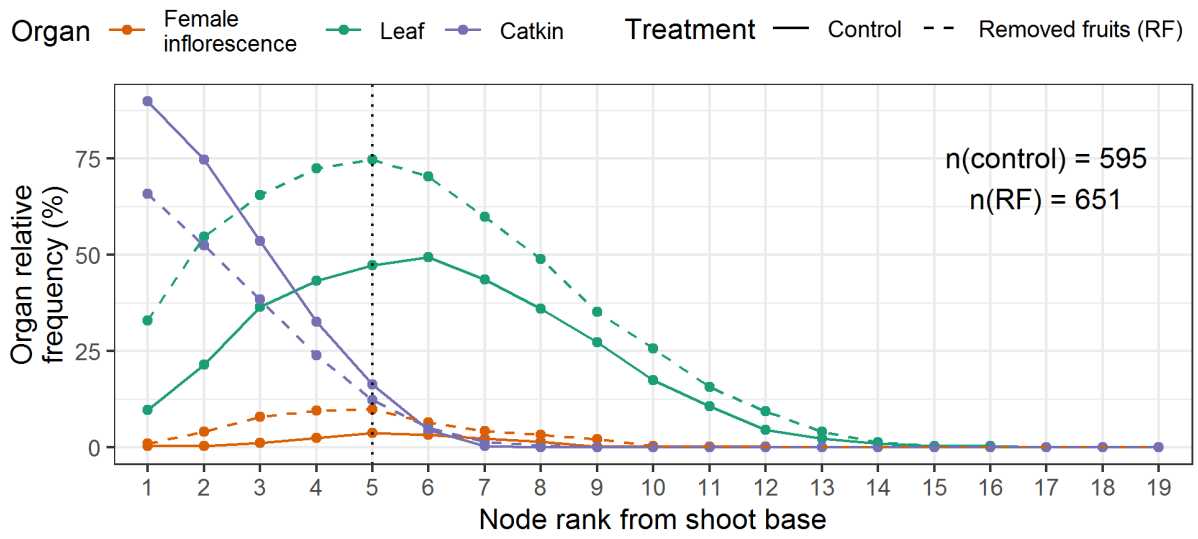


Figure 4.

Table 1. Summary of GLMMs testing the effects of fruit removal, of the sampling date (winter 2019 vs spring 2018, i.e. before and after the development of flowers and fruits) and of their interaction on nitrogen, phosphorus, zinc, potassium and starch concentrations in leaves (1 and 2-yr old), twigs (1 and 2-yr old) and trunk. We report number of observations, standardised estimates \pm SE and p-values for general treatment (removed fruit (RF) compared to control (C)), date and interaction effects. Characters in bold font refer to significant effects ($p < 0.05$) and stars to levels of significance ($0.05 < p^* < 0.01$; $0.01 < p^{**} < 0.001$; $p^{***} < 0.0001$). Model significant interactions are plotted in Fig. 2.

Response variable	Tissue	Number	Standardised estimate \pm SE of the predictor		
			Treatment (RF – C)	Date (Winter 2019 – Spring 2018)	Treatment x Date (RF – C: Winter – Spring)
[Nitrogen] (%)	Leaves	70	0.09 \pm 0.06	-0.15 \pm 0.04^{***}	0.13 \pm 0.06 *
	Twigs	72	-0.01 \pm 0.05	0.24 \pm 0.04^{***}	0.10 \pm 0.06
	Trunk	36	0.03 \pm 0.16	0.35 \pm 0.14 ^{**}	-0.02 \pm 0.19
[Phosphorus] (mg.g ⁻¹)	Leaves	69	0.03 \pm 0.05	0.008 \pm 0.03	0.03 \pm 0.04
	Twigs	70	-0.04 \pm 0.1	-0.18 \pm 0.06 ^{**}	0.02 \pm 0.09
	Trunk	35	0.009 \pm 0.08	-0.12 \pm 0.06 *	-0.03 \pm 0.09
[Zinc] (mg.g ⁻¹)	Leaves	70	0.002 \pm 0.001	-0.002 \pm 0.0008	0.003 \pm 0.001 *
	Twigs	70	0.003 \pm 0.003	-0.006 \pm 0.001 ^{***}	0.006 \pm 0.002 ^{**}
	Trunk	36	0.001 \pm 0.002	0.001 \pm 0.002	0.003 \pm 0.003
[Potassium] (mg.g ⁻¹)	Leaves	70	0.15 \pm 0.26	1.31 \pm 0.18 ^{***}	0.11 \pm 0.25
	Twigs	69	-0.05 \pm 0.07	0.03 \pm 0.04	0.14 \pm 0.05 *
	Trunk	36	0.02 \pm 0.10	0.10 \pm 0.06	-0.09 \pm 0.08
[Starch] (%)	Leaves	70	-0.23 \pm 0.61	-4.11 \pm 0.30^{***}	-0.74 \pm 0.42
	Twigs	72	0.17 \pm 0.62	-6.34 \pm 0.34^{***}	-1.42 \pm 0.48 ^{**}
	Trunk	36	0.47 \pm 1.12	-5.97 \pm 0.60^{***}	-1.47 \pm 0.84

Table 2. Summary of GLMMs testing the effect of fruit removal and of 2018 shoot diameter in shoots that had set fruits in 2018 on: the production of spring shoots in 2019, the numbers female flowers, catkins, leaves and nodes per spring shoot in 2019, on fruit set in 2019 and on the mean number of initiated fruits per spring shoot after fruit set in 2019. For each model, interactions between treatment and diameter were first included, and non-significant interactions were removed from the model. We report the number of observations, standardised estimates \pm SE and p-values for treatment comparisons (control (C), removed fruit (RF)), diameter and interaction effects, marginal (R^2_m) and conditional (R^2_c) R^2 for the reduced, final model. Characters in bold font refer to significant effects ($p < 0.05$) and stars to levels of significance ($0.05 < p^* < 0.01$; $0.01 < p^{**} < 0.001$; $p^{***} < 0.0001$).

Response variable	Number	Standardised estimate \pm SE of the predictor			R^2_m (R^2_c)
		(Effect estimated)			
		Treatment (RF – C)	2018 shoot diameter	Treatment x Shoot diameter (RF – C x slope)	
Number of female flowers	812	0.81 \pm 0.38 *	0.21 \pm 0.10 *	n.s.	0.10 (0.41)
Number of catkins	812	-0.81 \pm 0.54	-0.17 \pm 0.07 *	n.s.	0.12 (0.25)
Number of leaves	812	0.25 \pm 0.29	0.23 \pm 0.06 ***	n.s.	0.07 (0.51)
Number of nodes	812	0.08 \pm 0.10	0.11 \pm 0.03 ***	n.s.	0.06 (0.27)
Number of 2019 spring shoots	184	0.02 \pm 0.14	0.37 \pm 0.05 ***	0.21 \pm 0.09 *	0.29 (0.45)
Fruit set in 2019	181	-0.04 \pm 0.45	0.08 \pm 0.11	n.s.	0.001 (0.14)
Mean number of initiated fruits per 2019 spring shoot	181	0.72 \pm 0.49	0.22 \pm 0.19	n.s.	0.08 (0.25)

Supplementary Material

Table S1. Mean values (\pm SD) of tree basal area and of nitrogen (N), phosphorus (P), zinc (Zn), potassium (K) and starch concentrations in 1 and 2-yr old leaves, 1 and 2-yr old twigs, and trunk across the three groups of trees before fruit removal treatment in spring 2018, and of 2018 shoots basal area in winter 2019. Difference between groups was tested with linear models for tree basal area and nutrient concentrations, and with linear mixed effects models for shoot basal area with Tree identity as random effect. Different letters refer to significant differences between defoliation treatments ($p < 0.05$, Tukey post hoc tests).

Variable	Control (n = 9 trees)	Removed fruit (n = 9 trees)	Naturally no fruits (n = 6 trees)	N
Tree basal area (mm ²)	49.9 (20.1)a	52.5 (24.4)a	54.7 (43.0)a	24
Basal area of shoots that did set a fruit in 2018 (mm ²)	3.2 (1.2)a	3.6 (1.8)a	-	354
Basal area of shoots that did not set a fruit in 2018 (mm ²)	3.1 (1.6)a	3.8 (1.6)a	4.4 (2.6)a	191
[N] 1-yr old leaves (%)	1.39 (0.17)a	1.44 (0.14)a	1.45 (0.17)a	24
[N] 2-yr old leaves (%)	1.45 (0.26)a	1.27 (0.2)a	1.46 (0.31)a	23
[N] 1-yr old twigs (%)	0.99 (0.24)a	0.85 (0.34)a	0.91 (0.42)a	24
[N] 2-yr old twigs (%)	0.96 (0.1)a	0.83 (0.13)a	0.92 (0.16)a	24
[N] trunk (%)	0.25 (0.5)a	0.3 (0.6)a	0 (0)a	24
[Starch] 1-yr old leaves (%)	4.43 (2.14)a	4.74 (1.47)a	5.02 (1.77)a	24
[Starch] 2-yr old leaves (%)	4.95 (2.49)a	5.96 (1.83)a	4.47 (1.41)a	23
[Starch] 1-yr old twigs (%)	5.41 (1.08)a	7.15 (2.04)a	6.88 (3.3)a	24
[Starch] 2-yr old twigs (%)	8.29 (1.85)a	10.32 (1.94)a	7.94 (3.21)a	24
[Starch] trunk (%)	7.15 (4.16)a	9.39 (1.35)a	9.31 (5.45)a	24
[P] 1-yr old leaves (mg.g ⁻¹)	0.68 (0.09)a	0.65 (0.1)a	0.65 (0.05)a	23
[P] 2-yr old leaves (mg.g ⁻¹)	0.61 (0.2)a	0.65 (0.11)a	0.62 (0.15)a	23
[P] 1-yr old twigs (mg.g ⁻¹)	0.89 (0.18)a	0.83 (0.35)a	0.67 (0.17)a	23

[P] 2-yr old twigs (mg.g ⁻¹)	0.9 (0.36)a	0.84 (0.29)a	0.74 (0.03)a	24
[P] trunk (mg.g ⁻¹)	0.47 (0.19)a	0.52 (0.26)a	0.4 (0.2)a	24
[Zn] 1-yr old leaves (mg.g ⁻¹)	0.0094 (0.0047)a	0.0085 (0.0024)a	0.0082 (0.0022)a	23
[Zn] 2-yr old leaves (mg.g ⁻¹)	0.0114 (0.007)a	0.0098 (0.002)a	0.01 (0.0083)a	23
[Zn] 1-yr old twigs (mg.g ⁻¹)	0.0202 (0.0044)a	0.0148 (0.0068)a	0.0182 (0.007)a	23
[Zn] 2-yr old twigs (mg.g ⁻¹)	0.0182 (0.0048)a	0.0144 (0.0028)a	0.0174 (0.008)a	24
[Zn] trunk (mg.g ⁻¹)	0.0052 (0.0068)a	0.0035 (0.0062)a	0.003 (0.0037)a	24
[K] 1-yr old leaves (mg.g ⁻¹)	0.66 (0.11)a	0.78 (0.19)a	0.7 (0.17)a	23
[K] 2-yr old leaves (mg.g ⁻¹)	0.69 (0.17)a	0.63 (0.16)a	0.6 (0.12)a	23
[K] 1-yr old twigs (mg.g ⁻¹)	1.06 (0.22)a	0.78 (0.26)a	0.85 (0.39)a	23
[K] 2-yr old twigs (mg.g ⁻¹)	0.9 (0.16)a	0.74 (0.2)a	0.83 (0.18)a	24
[K] trunk (mg.g ⁻¹)	0.37 (0.22)a	0.5 (0.31)a	0.38 (0.22)a	24

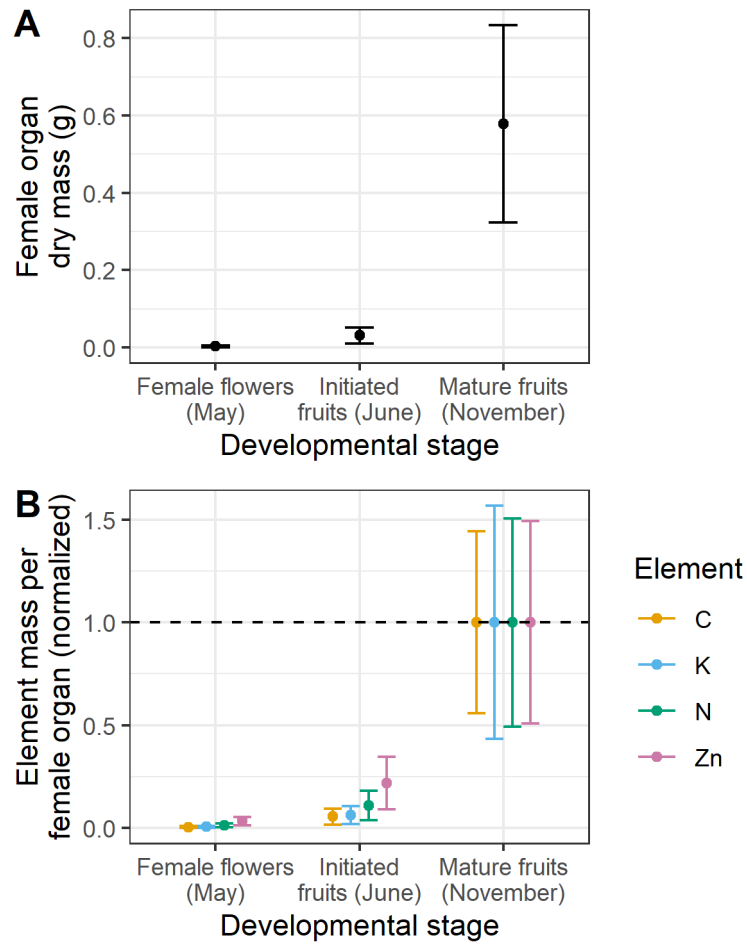


Figure S1. Variation of A) mean \pm SD dry mass of female organs and B) normalized mean \pm SD element mass for carbon (C), potassium (K), nitrogen (N) and zinc (Zn) in dry female organs depending on developmental stage in May (flowers at anthesis, $n = 5$ trees), June (initiated fruits at the time of fruit removal, 3 trees) and (mature fruits and cupules, 3 trees).

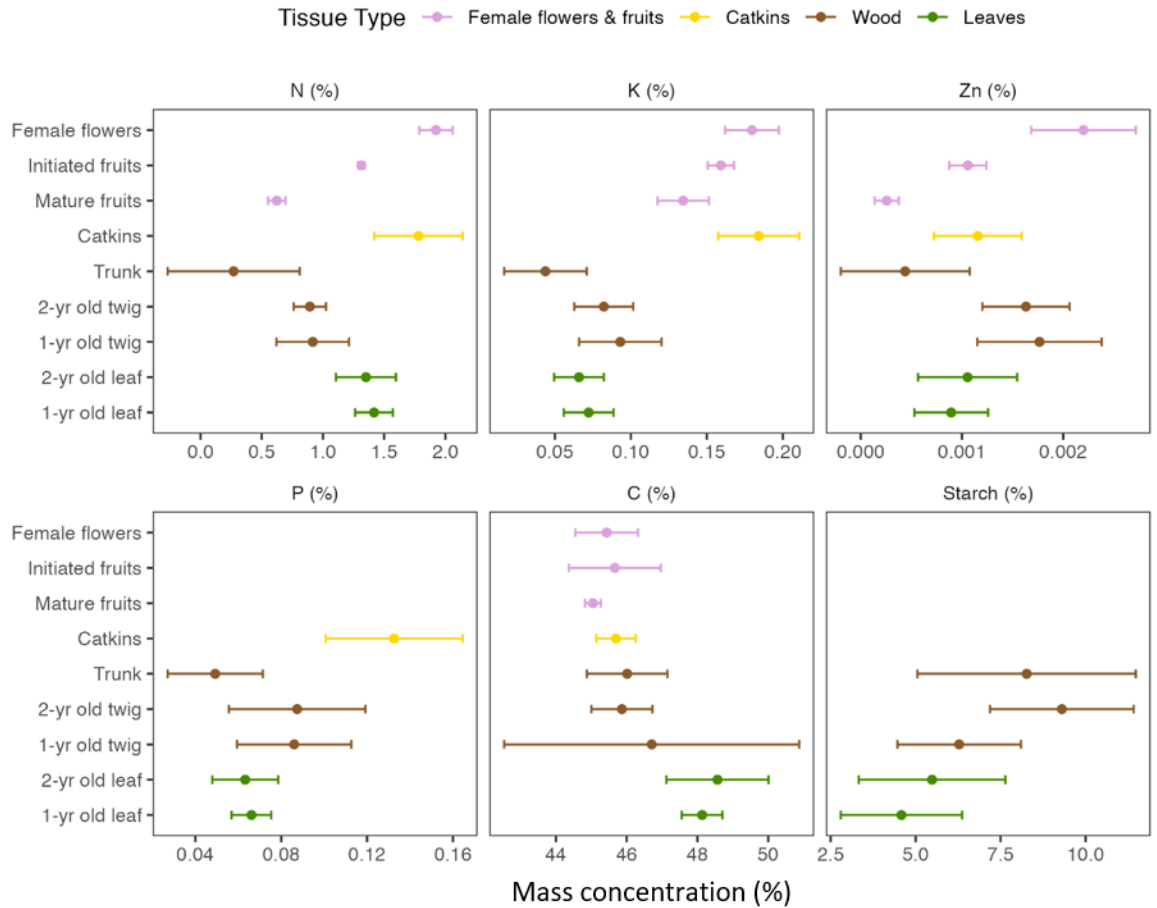


Figure S2. Mass concentration (mean \pm SD) of nitrogen (N), potassium (K), zinc (Zn), phosphorus (P), carbon (C) and starch in various tree organs: female flowers at anthesis ($n = 5$ trees, May 2019), initiated fruits ($n = 3$ trees, June 2019), mature fruits ($n = 3$ trees, Fall 2019), catkins ($n = 14$ trees from the control and defruited treatment in Spring 2019), wood and leaves ($n = 18$ trees from the control and the defruited treatment, Spring 2018).

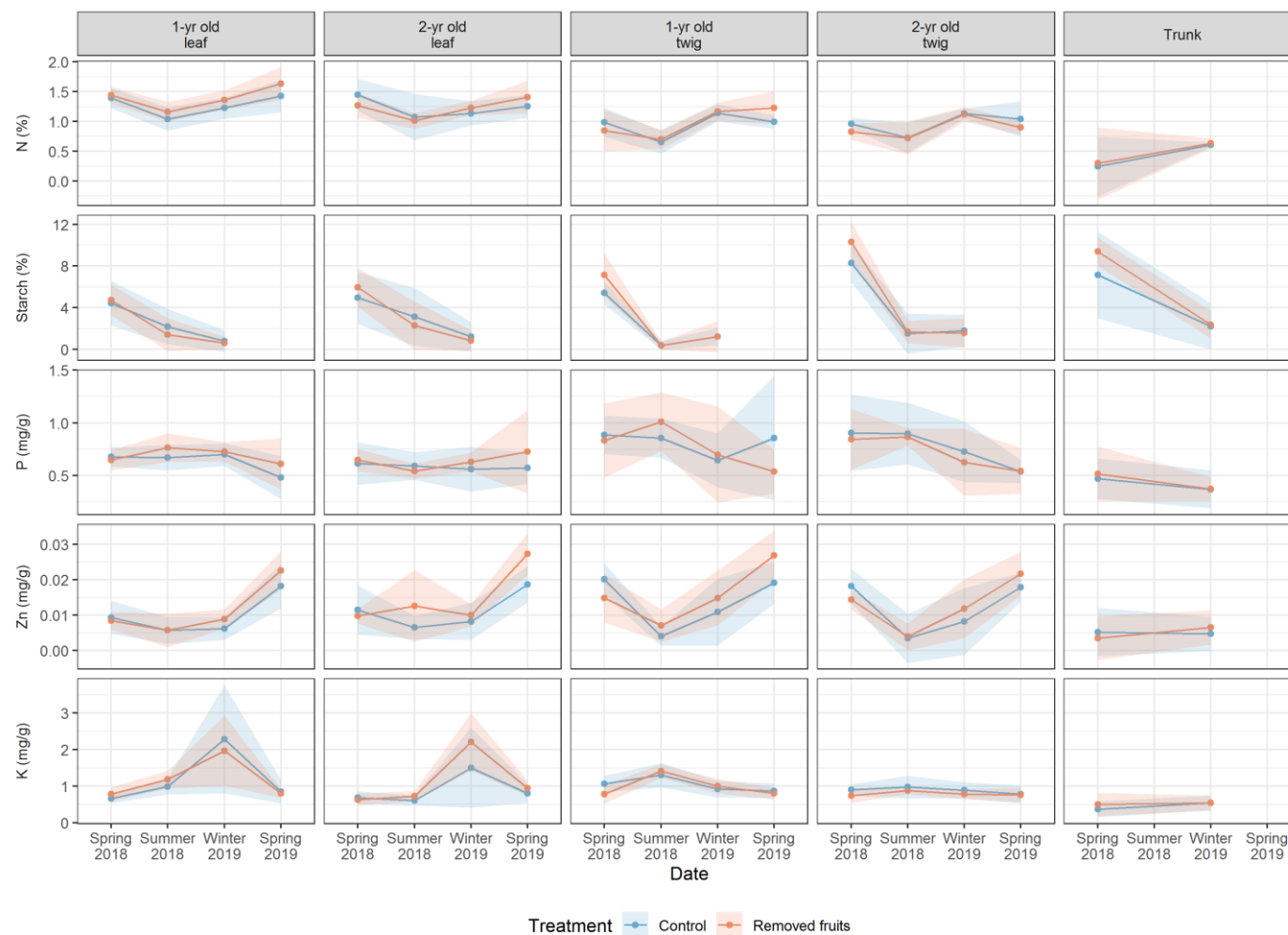


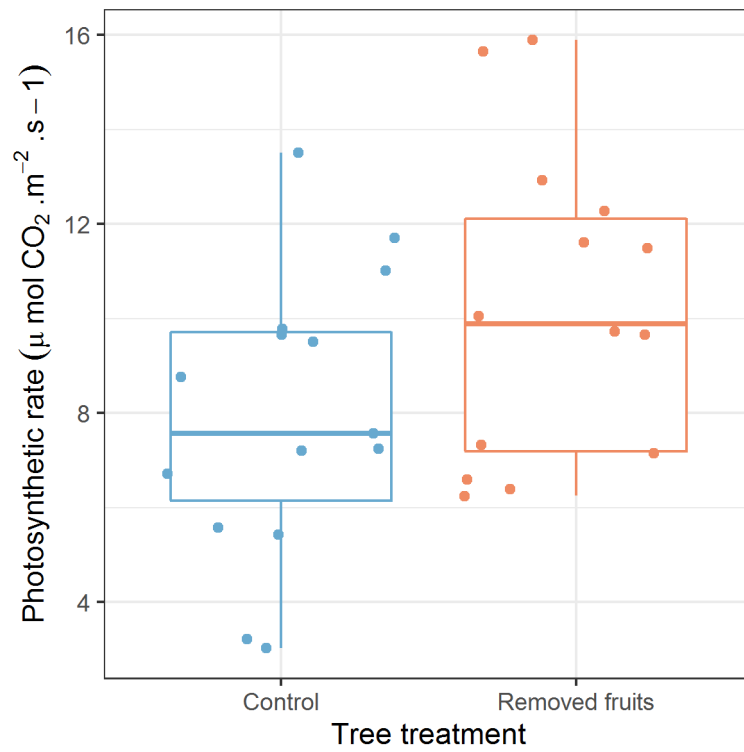
Figure S3. Seasonal dynamics of tissues concentrations (mean \pm SD) in nitrogen (N, top panels), starch (upper middle panels), phosphorus (P, middle panels), zinc (Zn, lower middle panels) and potassium (K, bottom panels) in less than 1-yr old (top left panels) and less than 2-yr old (middle left panels) leaves, in less than 1-yr old (middle panels) and less than 2-yr old (middle right panels) twigs and in trunk (top right panels); depending on the treatment applied to the trees (N = 9 tree in each group).

1 **Table S2.** Summary of the GLMMs testing the effect of fruit removal on differences, between spring
 2 2018 and winter 2019, in nutrient concentrations (called “Difference” in the table) and nutrient
 3 concentrations relative to the initial concentration in spring 2018 (called “Ratio” in the table). In each
 4 model, the analysed organs (1 and 2-yr old leaves, 1 and 2-yr old twigs and trunks) were included as a
 5 random effect. Characters in bold font refer to significant effects ($p < 0.05$) and stars to levels of
 6 significance ($0.05 < p^* < 0.01$). N = nitrogen, K = potassium, Zn = zinc.

Response variable	Number	Standardised estimate \pm SE of treatment effect
Difference in [N]	35	-0.006 \pm 0.23
Ratio in [N]	35	.033 \pm 0.29
Difference in [K]	34	0.04 \pm 0.02
Ratio in [K]	34	0.54 \pm 0.37
Difference in [Zn]	34	0.0006 \pm 0.0002
Ratio in [Zn]	34	1.18 \pm 0.78
Difference in [Starch]	35	-1.53 \pm 0.61 *
Ratio in [Starch]	35	-1.18 \pm 0.10
Difference in [P]	33	0.005 \pm 0.01
Ratio in [P]	33	0.15 \pm 0.12

7

8



9

10 **Figure S4.** Effect of treatment on the photosynthetic rate of leaves measured soon after fruit removal
 11 in the early summer 2018 (non-significant, $p < 0.05$).

12

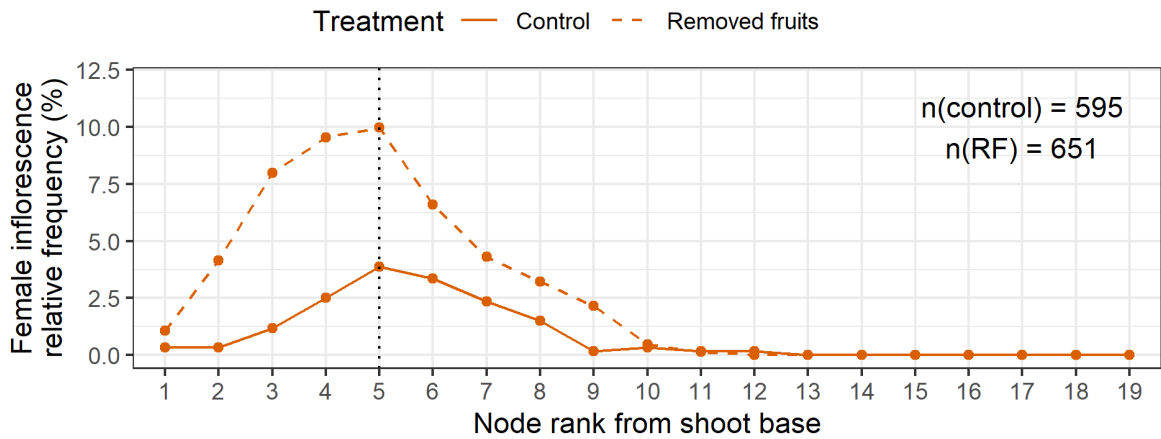
13 **Table S3.** Summary of GLMMs testing the effect of fruit removal and of 2018 shoot diameter in shoots
 14 that had not set a fruit in 2018 (and were adjacent to shoots that had undergone fruit removal in
 15 defruited trees) on: the production of spring shoots in 2019, the numbers female flowers, catkins,
 16 leaves and nodes per spring shoot in 2019, on fruit set in 2019 and on the mean number of initiated
 17 fruits per spring shoot after fruit set in 2019. For each model, interactions between treatment and
 18 diameter were first included, and non-significant interactions were removed from the model. We
 19 report the number of observations, standardised estimates \pm SE and p-values for treatment
 20 comparisons (control (C), removed fruit (RF)), diameter and interaction effects, marginal (R^2_m) and
 21 conditional (R^2_c) R^2 for the reduced, final model. Characters in bold font refer to significant effects ($p <$
 22 0.05) and stars to levels of significance ($0.05 < p^* < 0.01$; $0.01 < p^{**} < 0.001$; $p^{***} < 0.0001$).

Response variable	N	Standardised estimate \pm SE of the predictor			R^2_m (R^2_c)
		(Effect estimated)			
		Treatment (RF – C)	2018 shoot diameter	Treatment x Shoot diameter (RF – C x slope)	
Number of female flowers per 2019 spring shoot	655	1.47 \pm 0.66 *	0.60 \pm 0.18 ***	n.s.	0.22 (0.62)
Number of catkins per 2019 spring shoot	655	-0.93 \pm 0.47 *	-0.10 \pm 0.07	n.s.	0.14 (0.78)
Number of leaves per 2019 spring shoot	655	1.13 \pm 0.49 *	0.27 \pm 0.09 **	n.s.	0.19 (0.74)
Number of nodes per 2019 spring shoot	655	0.24 \pm 0.08 **	0.09 \pm 0.03 **	n.s.	0.10 (0.26)
Number of 2019 spring shoots per 2018 shoot	131	-0.12 \pm 0.13	0.32 \pm 0.05 ***	n.s.	0.28 (0.41)
Fruit set in 2019	123	0.42 \pm 0.38	-0.31 \pm 0.14 *	n.s.	0.03 (0.05)
Mean number of initiated fruits per 2019 spring shoot	123	1.51 \pm 0.60 *	0.50 \pm 0.21 *	n.s.	0.30 (0.37)

23

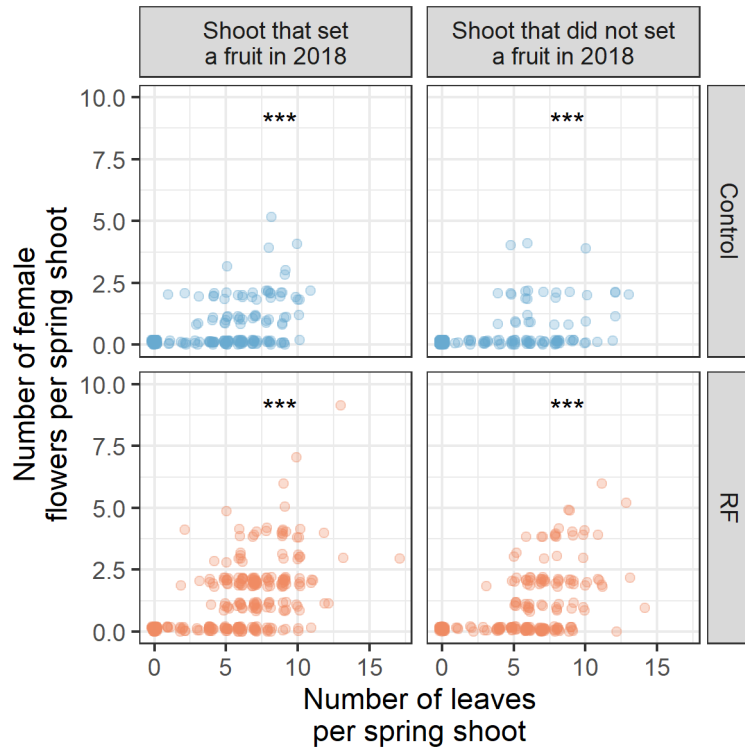
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26

27 **Figure S5.** Female inflorescences relative frequency at each node rank of 2019 spring shoots in control
28 and RF trees (detail from Fig. 4). Increment in node rank indicates location of the node from the base
29 toward the apex of the shoot. The vertical dotted line represents the node 5, which is approximately
30 the mode for leaves and female flowers.



31

32 **Figure S6.** Relationship between the number of female flowers and the number of leaves per spring
 33 shoot in the control group (upper panels) and the removed fruit group (RF, lower panels) and on shoots
 34 borne by a 2018 shoot that had set a fruit (left panels) and by shoots that had not set fruit in 2018
 35 (right panels). “***” indicates the p-value associated to the relationship for each combination of
 36 treatment group and shoot reproductive status in 2018 (GLMMs with negative binomial distribution
 37 and 2018 shoot identity nested in tree identity as a random effect, $p^{***} < 0.001$).

38

39 Appendix S1. Resource allocation to acorns

40 Resource investment in acorns compared to resource investment in other tree compartments was
41 estimated for the trees from the control group in 2018.

42 The stem diameters of the trees from the control group were measured in spring 2018 and again in
43 autumn 2022 to calculate the average growth over the 2018-2022 five-year period. Mean annual stem
44 diameter increment was $2.0 \pm 1.4 \text{ mm y}^{-1}$ (mean \pm SD) and was strongly correlated to the tree diameter
45 ($R^2 = 0.61$; $p = 0.022$, RMSE = 0.77). Diameter increment is related to diameter as follows:

$$46 \text{ Diameter increment (mm y}^{-1}\text{)} = 0.540 \times \text{Diameter (cm)} - 1.813 \quad (\text{Eq. S1})$$

47 The biomass of stems and branches of each tree was calculated using the allometric equations for
48 *Quercus ilex* published by Canadell et al. (1988). Biomass increment from 2018 to 2019 was calculated
49 as the difference between the biomass of these compartments calculated with the diameter measured
50 in 2018 and the diameter estimated in 2019 using Eq. S1. For the diameter range of the trees in our
51 study, the allometric equations published by Canadell et al. (1988) are in very close agreement with
52 those published by Rambal et al. (2004) for *Q. ilex* trees in the vicinity of Montpellier (Slope=0.99;
53 $R^2=0.995$; $p < 0.001$, RMSE = 1440 g DM for the relationship between the predictions of wood biomass
54 with the two equations). We chose the allometric equations of Canadell et al. (1988) because they
55 separated the biomass of stem wood and branch wood, contrary to Rambal et al. (2004).

56 The total leaf biomass of the trees calculated with the allometric equation of Canadell et al. (1988) was
57 also in agreement with predictions of the allometric equation published by Rambal et al. (2004),
58 although it tended to yield slightly higher values (Slope=1.16; $R^2=0.999$; $p < 0.001$, RMSE = 200 g DM
59 for the comparison of Canadell et al. (1988) versus Rambal et al. (2004)). For consistency with wood
60 biomass estimations, we used the equation from Canadell et al. (1988). Based on leaf demography
61 data from a nearby site (Limousin et al. 2012), we considered that 47% of the leaves supported in the
62 tree crowns were older than 1-yr. Therefore, the annual leaf production for every tree was calculated
63 as 53% of the total leaf biomass calculated from the allometric relationships.

64 The number of acorns produced in 2018 was calculated from an allometric relationship (Eq. S2)
65 between tree stem diameter and the number of acorns initiated in spring, which we had been counted
66 on trees of the RF (removed fruits) group:

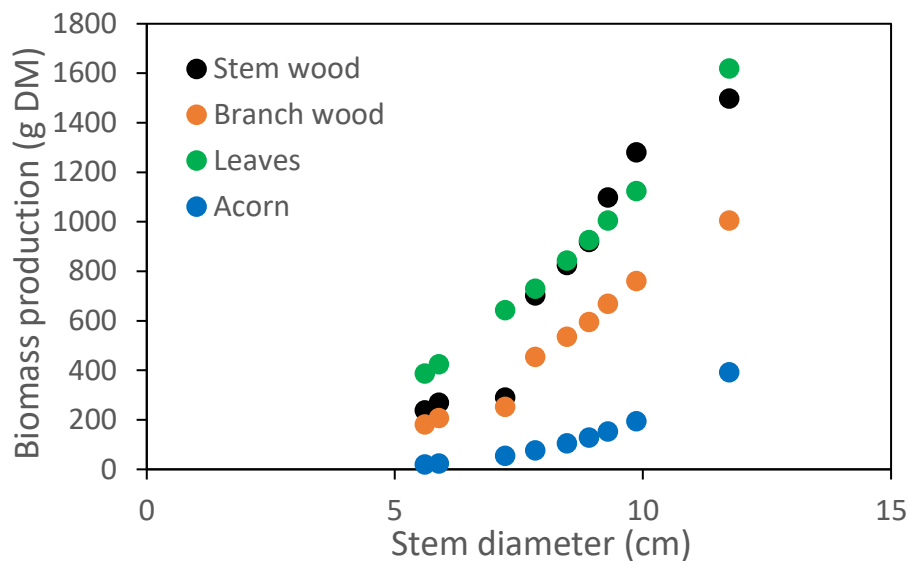
$$67 \text{ Nb of acorns initiated} = 0.033 \times \text{Diameter}^{4.03} \quad (R^2=0.72; p = 0.004, \text{RMSE} = 1034) \quad (\text{Eq. S2})$$

68 Considering that the average mass of a mature acorn in our experimental site was 0.58 g DM in
69 November 2018, and ignoring the fruit abortion during the summer, the maximum mass of acorns
70 potentially produced by the trees in 2018 according to their size was (Eq. S.3):

$$71 \text{ Maximum mass of acorns (g)} = 0.0192 \times \text{Diameter}^{4.03} \quad (\text{Eq. S3})$$

72 Note that approximately 44% of the acorns initiated in 2018 aborted before reaching maturity,
73 consequently the mass of acorns calculated with Eq. S3 represents the potential maximum allocation
74 to acorns and not the actual acorn biomass produced that particular year.

75 Using the above allometric equations and the concentrations in nitrogen, potassium and zinc
76 measured in the different compartments (Table S1, Fig. S1, Fig. S2), we calculated the relative
77 investments that the acorns represented in comparison to the total aboveground biomass production.
78 The fraction of biomass allocated to acorn production was strongly dependent on tree size and was
79 higher in larger trees (Fig. S7). We thus normalized our results for a tree with a stem diameter of 10
80 cm, which corresponds approximately to the average diameter of dominant *Q. ilex* trees in typical
81 coppices of the French Mediterranean region.



82
 83 **Figure S7.** Relationship between stem diameter in cm and the biomass production allocated to stem
 84 wood, branch wood, leaves and acorns in g of dry matter (g DM) for the trees of the control group.

85
 86 For a tree with a stem diameter of 10 cm, maximum biomass allocation in acorns in 2018 represented
 87 5.8 % of the total aboveground biomass production (Table S4). This is in very good agreement with the
 88 long-term average in the nearby Puéchabon forest, where acorn production was observed to account
 89 for 7.1% ($\pm 4.1\%$) of the annual aboveground net primary production between 2004 and 2017 (Gavinet
 90 et al. 2019). The slightly lower value in our experiment reflects the fact that the year 2018
 91 corresponded to an intermediate year in terms of acorn production.

92 According to this calculation, in 2018, acorns production represented 5.8 % of the aboveground
 93 biomass production but mobilized respectively, 11.5 %, 4.5% and 1.6% of the annual tree aboveground
 94 allocation in potassium, nitrogen and zinc. This proportion was always minor compared to the
 95 resources invested in leaves and wood. Nevertheless, a large fraction of total potassium was invested
 96 in acorns compared to their fraction of the biomass production. This suggests that potassium might be
 97 limiting acorn production in most years when total acorn biomass is larger.

98
 99 **Table S4.** Proportion of resource allocation in stem wood, branch wood, leaves and acorns for an
 100 average *Q. ilex* tree with a 10 cm diameter during the year 2018. Allocation is reported in biomass or
 101 mass of N, K and Zn and in % of the annual total.

	Total	Stem wood	Branch wood	Leaves	Acorns
Biomass (g DM)	3552	1392	799	1155	206
<i>Biomass allocation (%)</i>	100	39.2	22.5	32.5	5.8
N (g)	28.6	3.5	7.8	16.1	1.3
<i>N allocation (%)</i>	100	12.2	27.2	56.1	4.5
K (g)	2.33	0.52	0.78	0.76	0.27
<i>K allocation (%)</i>	100	22.1	33.6	32.8	11.5
Zn (mg)	34.0	7.2	15.3	10.9	0.5
<i>Zn allocation (%)</i>	100	21.3	45.2	32.0	1.6

102

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