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Holm oak fecundity does not acclimate to a drier world

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Summary

- Climate change might impact tree fecundity by altering the relative influences of meteorological and physiological drivers, and by modifying resource investment in reproduction.
- Using a 13-yr monitoring of *Quercus ilex* reproduction in a rainfall exclusion experiment, we analysed the interactive effects of long-term increased aridity and other environmental drivers on the inter-annual variation of fecundity (male flower biomass, number of initiated and mature fruits).
- Summer–autumn water stress was the main driver of fruit abortion during fruit growth. Rainfall exclusion treatment strongly reduced the number of initiated and mature fruits, even in masting years, and did not increase fruit tolerance to severe drought. Conversely, the relative contribution of the meteorological and physiological drivers, and the inter-annual variability of fruit production were not modified by rainfall exclusion.
- Rather than inducing an acclimation of tree fecundity to water limitation, increased aridity impacted it negatively through both lower fruit initiation due to changes in resource allocation, and more severe water and resource limitations during fruit growth. Long-term increased aridity affected tree reproduction beyond what is expected from the current response to inter-annual drought variations, suggesting that natural regeneration of holm oak forest could be jeopardised in the future.

Introduction

Reproduction is a critical step of population demography and population adaptation to climate change through natural selection (Lefèvre *et al.*, 2014). The on-going climate change has been shown to impact the mean seed production of trees either positively (Allen *et al.*, 2014; Buechling *et al.*, 2016; Caignard *et al.*, 2017; Bogdziewicz *et al.*, 2020c; Shibata *et al.*, 2020), negatively (Mutke *et al.*, 2005), or to have no impact (Kelly *et al.*, 2013; Bisi *et al.*, 2016). Climate change is also suspected to affect the temporal patterns of tree reproduction (Hedhly *et al.*, 2009; Bogdziewicz *et al.*, 2020c; Shibata *et al.*, 2020). A global synthesis of plant reproductive patterns showed, for example, that seed production has become more variable over the last 115 yr (Pearse *et al.*, 2017), but other studies have reported opposite trends in beech and oak over the last decades (Bogdziewicz *et al.*, 2020c; Shibata *et al.*, 2020). Changes in inter-annual variability of seed production are important for reproductive success because the benefits of increased seed production could be lost if seed production also becomes less variable, thereby increasing predators' population density (Bogdziewicz *et al.*, 2020c). In such an uncertain context, understanding the drivers of tree reproductive success appears essential when predicting how forest natural regeneration may be affected by future climatic conditions.

In the Mediterranean region, it is predicted that temperatures will rise (Molina *et al.*, 2020), rainfall events will become more stochastic into extremes (IPCC, 2013; Lionello & Scarascia, 2018; Vogel *et al.*, 2020), and summer drought episodes will become more frequent and severe (Ruffault *et al.*, 2014; Cook *et al.*, 2020). Ecosystem rainfall manipulation experiments are powerful tools to anticipate how these changes might impact tree fecundity (Pérez-Ramos *et al.*, 2010, 2013; Bogdziewicz *et al.*, 2020a). Previous studies using rainfall exclusion experiments in Mediterranean *Quercus ilex* forests have reported the negative effects of increased water deficit on female flowers and total fruit biomass (Ogaya & Peñuelas, 2007; Rodríguez-Calcerrada *et al.*, 2011; Sánchez-Humanes & Espelta, 2011; Liu *et al.*, 2015; Gavinet *et al.*, 2019), although not all of them (Bogdziewicz *et al.*, 2020b). Some studies also reported negative effects on the mean seed biomass during years of high fruit production and an increased number of aborted fruits (Pérez-Ramos *et al.*, 2010). Conversely, little effect was observed on male flower biomass (Pérez-Ramos *et al.*, 2010; Gavinet *et al.*, 2019) while a reduction of the number of viable pollen grains has been found (Bykova *et al.*, 2018).

Precipitation reduction experiments, when carried out over a sufficiently long period, can disentangle the effect of the inter-annual drought variability from the long-term effect of (simulated) increased aridity, thereby enabling us to investigate whether trees may acclimate to long-term increased aridity or not (Estiarte *et al.*, 2016) and whether this response could be forecasted from short-term responses to episodic droughts. Here we

take advantage of a 17-yr long rainfall exclusion experiment to examine in detail how long-term increased aridity affects the different mechanisms involved in reproductive success from flower production to fruit maturation, and to determine whether these effects could be predicted from the effects of short-term droughts.

The drivers of seed production have particularly been investigated in masting species such as oaks, that is species showing irregular, but synchronised, fruit production among individuals of a population (Kelly & Sork, 2002). The proximate drivers of seed production and of masting are however still debated. Meteorological conditions, trees internal resource dynamics, mineral nutrient uptake, as well as their interactions, are all probably involved in inter-annual variations and synchrony in seed production (Tanentzap *et al.*, 2012; Crone & Rapp, 2014; Pearse *et al.*, 2016). In oaks, no environmental factor has yet been clearly identified as a reliable predictor of massive fruit production, both across and within species (Koenig & Knops, 2014; Pérez-Ramos *et al.*, 2015). Moreover, some controversies exist because correlations between environmental factors and fruit production are not necessarily indicative of a causal relationship (Kelly *et al.*, 2013; Pearse *et al.*, 2014; Bogdziewicz *et al.*, 2019). Detrimental weather events, such as drought or frost, can prevent flower production, fruit initiation or fruit maturation and have been called meteorological vetoes (Pearse *et al.*, 2016; Bogdziewicz *et al.*, 2018, 2019). In Mediterranean oaks, the water deficit during spring, summer and autumn might result in seed abortion and is considered as a major meteorological veto (Koenig & Knops, 2014; Pérez-Ramos *et al.*, 2015; Bogdziewicz *et al.*, 2017b, 2019). Whether tree acclimation to increasing aridity might modify this seasonal drought effect on tree fecundity remains however to be determined.

The dynamics of resources and their allocation to different tree functions are also commonly identified as drivers of reproduction and are themselves affected by meteorological conditions (Obeso, 2002; Barringer *et al.*, 2013). Schemes of resources allocation to reproduction are indeed central in explaining masting patterns (Pearse *et al.*, 2016). In addition, the nature of the resources limiting reproduction remains an important issue, as phosphorus, nitrogen and nonstructural carbohydrates are all suspected to limit reproduction and drive masting (Sala *et al.*, 2012; Miyazaki *et al.*, 2014; Allen *et al.*, 2017; Han & Kabeya, 2017; Fernández-Martínez *et al.*, 2019; Satake *et al.*, 2019). Reduced water availability might reduce the availability of all these important resources by reducing the tree photosynthesis and productivity (Misson *et al.*, 2009; Gavinet *et al.*, 2019), the root soil water uptake (Limousin *et al.*, 2009), and the rates of soil litter decomposition and nutrient mineralisation (Sardans & Peñuelas, 2007; Santonja *et al.*, 2017). As tree acclimation to drought is known to involve changes in biomass allocation among organs (Mencuccini, 2003; Poorter *et al.*, 2012; Martin-StPaul *et al.*, 2013; Gavinet *et al.*, 2019), tree fecundity is likely to be modified by drought effects on resource acquisition and allocation (Lauder *et al.*, 2019; Bogdziewicz *et al.*, 2020b).

Studies investigating the effects of both meteorological conditions and trees' internal resource dynamics on reproduction are scarce, and only a few have tried to disentangle their relative effects (Bogdziewicz *et al.*, 2017b; Fernández-Martínez *et al.*, 2017; Nussbaumer *et al.*, 2018; Moreira *et al.*, 2019). Three stages of reproduction seem particularly key for determining the number of mature fruits produced at the end of the season in *Quercus ilex*: female flower initiation and development that determines the number of receptive female flowers at pollination, fertilisation that determines fruit set, that is the number of fruits initiated, and fruit development until maturity (Sork & Bramble, 1993; Tsuruta *et al.*, 2011). Our aim in this study was therefore to identify the main drivers of fruit production at the key stages of the reproductive cycle of *Q. ilex* in a context of increasing aridity, and to disentangle their relative effects.

Using a 13-yr monitoring of *Q. ilex* reproduction in a long-term rainfall exclusion experiment set in a Mediterranean holm oak coppice, we aimed to answer the following questions:
(**1**) How does long-term rainfall reduction affect tree reproduction?
(**2**) Are the drivers of the inter-annual variability of fruit production modified by long-term rainfall reduction?
(**3**) Can tree reproduction acclimate to increasing aridity through changes in resource acquisition and allocation?

Materials and Methods

Experimental site and study species

The experiment was conducted in southern France (35 km northwest of Montpellier), on a flat area in the Puéchabon State Forest (43°44′29″N; 3°35′46″E, 270 m above sea level (asl)). This forest had been coppiced until the last clear cut in 1942. The evergreen *Quercus ilex* L. forms a dense canopy with a height of *c.* 5.5 m, a mean basal area of 26.5 m^2 ha^{-1}, a density of *c.* 4900 stems ha^{-1} and a leaf area index (LAI) of 2.2. The evergreen species *Buxus sempervirens*, *Phyllirea latifolia*, *Pistacia terebinthus* and *Juniperus oxycedrus* compose a sparse understory shrubby layer with *c.* 25% cover. The bedrock is a hard Jurassic limestone and the soil is extremely rocky with *c.* 75% of stones and rocks in the top 0–50 cm and 90% below. The stone-free fraction of the soil within the 0–50 cm layer is a homogeneous silty clay loam (38.8% clay, 35.2% silt and 26% sand), leading to a field capacity of 210 mm. The area has a Mediterranean-type climate with a mean annual temperature of 13.5°C (on-site meteorological station, 1990–2019), the coldest month being January (6.0°C mean daily temperature) and the hottest month July (22.4°C mean daily temperature). The mean annual precipitation is 953 mm with a range of 578–1549 mm (1990–2019). Rainfall mainly occurs during autumn and winter with *c.* 80% between September and April.

The dominant species, *Quercus ilex* L. or holm oak, is an evergreen wind-pollinated monoecious tree that usually flowers in May in the study area. The male inflorescences, called catkins, bear *c.* 20–25 staminate flowers (Yacine & Bouras, 1997; Gómez-Casero *et al.*, 2004). Female pistillate flowers mature a few days after male flowers. Fertilisation occurs in late June to early July, leading to the initiation of fruit (acorn) growth. Fruits

achieve their maturation in November–December (Yacine & Bouras, 1997). In *Q. ilex*, two to three cohorts of leaves produced in different years usually coexist on the branches (Limousin *et al.*, 2012). Floral initiation period remains unknown in *Q. ilex*, but sporadic observations of second flowering during summer and autumn led us to suspect that staminate flowers might be initiated in early summer, as observed in *Q. alba* (Merkle *et al.*, 1980).

Experimental design of the rainfall exclusion

In March 2003, a throughfall exclusion experiment was set up on three replicated blocks located 50–100 m apart one from the other and situated on a flat forested area with no lateral flow. Each block comprised two contiguous 10 × 10 m plots subjected to either control precipitation or throughfall exclusion (further named 'dry treatment'). For the throughfall exclusion treatment, the experimental plot was equipped with parallel 14 m long and 0.19 m wide PVC gutters hung below the canopy with a slope, between 1 m and 2 m height, to cover 33% of the ground area under the tree canopy within the 10 × 10 m plot and a 2 m buffer zone. Taking into account rainfall interception and stemflow, the net input of precipitation was reduced by 27% compared with the control plots (Limousin *et al.*, 2008). The experimental design reduced significantly the surface soil water content (García de Jalón *et al.*, 2020), and induced significantly more negative tree water potentials in most summers since treatment installation in 2003 (Bykova *et al.*, 2018). In the control plots, identical gutters were installed upside down to homogenise albedo and understorey micro-climate without reducing precipitation inputs. Stand density and mean diameter at breast height (DBH) did not differ significantly between throughfall exclusion and control plots at the start of the experiment, with, respectively, 5930 and 6430 stems ha^{-1}, and 6.5 and 7.6 cm DBH (Gavinet *et al.*, 2019). In total, 178 and 193 *Q. ilex* trees, were, respectively, included in the three replicated blocks with control and dry treatments.

Reproduction, growth and phenology data

Stem DBH was inventoried every year in winter from 2003 to 2019 for all the stems with a DBH > 2 cm and converted into aboveground wood biomass (stem and living branches) using an allometric equation calibrated on the study site (Rambal *et al.*, 2004):

$$Biomass\,(g) = 191.6 \times DBH^{2.171} \qquad \text{Eqn 1}$$

Wood biomass increment at the plot scale was calculated by summing annual biomass increment of the trees. Relative plot biomass increment of the year was calculated as the plot aboveground biomass increment divided by the plot biomass.

Litterfall was collected monthly from 2003 to 2019 in 12 litter traps per plot placed regularly on a grid over the gutters at a height of 2 m and representing a total collecting area of 1.1 m^2 per plot. Litterfall was oven dried at 60°C for 3 d, separated into leaves, wood, flowers and fruits and weighed. Fallen fruits were further categorised into three different categories and counted from 2007 to 2019: (1) aborted (not completely or maldeveloped seeds, with length < 13 mm and/or diameter < 7 mm), (2) insect infested (having signs of larvae predation, such as gnaw marks or holes), and (3) mature (attaining mature seed size). The number of initiated fruits was calculated as the sum of the three categories. Annual litterfall was calculated as the sum of monthly litterfall from 1 May of the year until the 30 April the following year to consider all the flowers and fruits produced during one reproductive season. Fruit number (initiated and mature), fruit biomass and male flower biomass produced per year and per plot were divided by the plot-estimated aboveground biomass so that investment in reproduction was comparable among blocks. These variables are therefore expressed per kg of aboveground biomass from this point forward.

The phenology of leaves and catkins was monitored weekly during the season on six trees and three ramets per tree in each treatment of one of the blocks where scaffolds allowed access to branches from the top of the canopy. A median date of catkin anthesis was calculated for each year and the mean length of the period between anthesis and flower senescence was 9 ± 4 d. We therefore considered a 25-d pollination period comprising 8 d before median anthesis (2 SD), and 17 d after median anthesis (mean flowering period + 2 SD).

Meteorological variables, water stress modelling and carbon fluxes data

Continuous meteorological data were collected by a weather station located in a clearing < 100 m away from the experimental plots. For each year, precipitation during pollination was calculated as the sum of daily precipitation during the 25 d of the pollination period and was considered similar for the two treatments because it affected airborne pollen above the gutters. The number of days of torrential rain, previously identified as a potential determinant of mature acorn production in *Q. ilex* (Pérez-Ramos *et al.*, 2010), was determined as the number of days between July and November (period between fruit initiation and fruit maturity) during which daily rainfall exceeded 76 mm (threshold determined as the 0.99 percentile value of daily precipitations between July and November from 1984 to 2019).

Tree water stress, assessed by the predawn leaf water potential (Ψ_{lpd}), was simulated at a daily time scale using the water balance module of the SIERRA vegetation model (Mouillot *et al.*, 2001) calibrated and validated on our experimental site by Cabon *et al.* (2018). Soil parameters were kept constant for the two treatments, the LAI was reduced by 18% in the dry treatment relatively to the 2.2 value in control to reflect the lower leaf production in this treatment (Gavinet *et al.*, 2019), and the net precipitation input to the dry treatment was reduced by 27% compared with the control (Limousin *et al.*, 2008). Model performance was evaluated against 11 yr of discrete predawn leaf water potential measurements (described in Bykova *et al.*, 2018) and was similarly good in the two treatments (control treatment: $R^2 = 0.85$, RMSE = 0.58 MPa; dry treatment: $R^2 = 0.89$,

RMSE = 0.57 MPa). Daily absolute values of simulated Ψ_{lpd} were summed over the period from April to June to calculate the water stress index (WSI; defined by Myers, 1988) during the period of flower development and fertilisation, and from July to November to calculate the WSI during the period of fruit development and maturation. WSI was also calculated for the summer (July–September) of the previous year, which corresponds to the suspected period of initiation of male flowers.

Eddy covariance fluxes of CO_2 between the ecosystem and the atmosphere were measured continuously during the experiment using a three-dimensional sonic anemometer and a closed path infrared gas analyser set up at the top of a 12-m-high tower located near the experimental plots (see (Allard et al., 2008) for details). Processing schemes of FLUXNET have been used for filling data gaps and partitioning net ecosystem productivity (NEP) into gross primary productivity (GPP) and ecosystem respiration R_{eco} (Reichstein et al., 2005; Papale et al., 2006). Half-hourly values of GPP were summed over the period from July to November as an estimate of ecosystem-scale carbon assimilation during fruit development and maturation.

Leaf chemical analyses

From 2007 to 2018 (except 2013), leaves from the top canopy of the 6–8 trees per treatment in the scaffold-equipped block, where we also monitored phenology and fruit growth, were collected in winter (mid-November to mid-February, depending on years) oven dried at 60°C for 3 d and ground for chemical analyses. Leaves were previously sorted between leaves produced during the current year (<1 yr old) and leaves produced the year before. Nitrogen (N) mass-based concentration was determined by thermal combustion, using a Flash Smart NC Elemental Analyser (Thermo Fisher Scientific, Waltham, MA, USA) and phosphorus (P) mass-based concentration was measured colorimetrically using the molybdenum blue method. A mean N and P winter leaf concentration for each leaf cohort was calculated from 2007 to 2018 (except for missing P data in 2013 and 2017, and N data in 2013 and 2018).

Variables selection and statistical analyses

The four main reproduction variables that we studied are the biomass of male flowers, the number of initiated fruits, the fruit abortion rate and the number of mature fruits.
• First, we tested for a treatment effect on each reproductive variable, by running generalised linear mixed models (GLMMs) using the R package LME4 (Bates et al., 2015) with treatment as a fixed effect, and block and year as random effects. We used negative binomial distribution with a log link for count variables to account for over-dispersion issues.
• Second, we did an extensive review of the literature to identify all the factors potentially driving the inter-annual variability of Q. ilex fruit production (summarised in Table 1). Due to the large number of potential explanatory variables and of time periods on which they can be considered, and to the high collinearity among some weather variables, we narrowed our analyses based on existing knowledge about the main drivers of fruit production

in Q. ilex. We only considered the factors that we could associate to a physiological mechanism, and therefore excluded variables such as the difference of annual temperature between current and previous year (Koenig et al., 2016) or January minimum temperature (García-Mozo et al., 2007). We excluded the factors which had a very large temporal coverage, such as annual rainfall (Alejano et al., 2008), evapotranspiration between January and August (Bogdziewicz et al., 2017b) or precipitations during the 10 months before fruit maturation (Ogaya & Peñuelas, 2007), or a very large spatial coverage, such as the remote sensing enhanced vegetation index (Camarero et al., 2010; Fernández-Martínez et al., 2015). We then attributed the explanatory variables to each period of the reproductive cycle according to the time of the year they cover. When some variables were too strongly correlated to one another (such as temperature, rainfall and water stress in autumn), we chose to include in the model selection only the WSI that is the variable that best integrates the physiological state of the tree.
• Third, to determine the set of variables that best explained the inter-annual variability of the four reproductive response variables, we applied a model selection procedure on the full models including all the variables that we had previously selected, and in which variance inflation factors never exceeded 3 as recommended by Zuur et al. (2010).

The four models are described in Table 2, indicating both the variables retained after model selection and the variables excluded by model selection. The model with the best empirical support was selected by minimising the corrected AIC for small sample size (AICc) using the R package MUMIN (Bartoń, 2019). The best models exhibited a ΔAICc > 2 from others for all the reproduction variables, except for the number of initiated fruits for which we chose the most parsimonious model (ΔAICc = 0.6 with the one including male flower biomass). We proceeded in two steps. First, we ran the full models (GLMMs with block as random factor) on the control treatment only to identify the main drivers of inter-annual variability in tree fecundity. Second, we ran the selected model, with the same characteristics (response variables, model distribution and random effect), on both the control and dry treatments to determine the impact of long-term increased aridity on the trees' responses to seasonal drivers (Table 2). As fixed effects, we included the variables retained by model selection for each response variable (except for GPP, which was not measured in the dry treatment), treatment and the interactions between treatment and all the other variables. We then simplified the model by sequentially removing the insignificant terms, starting with the weakest and least significant interaction.

To determine whether the production of initiated fruits and of male flowers was impacted by nitrogen and phosphorus concentration in leaves during the winter before bud break, we used data from the two cohorts of leaves and the two treatments. We fitted GLMMs with a negative binomial distribution for the number of initiated fruits produced per kg of aboveground biomass and with a Gaussian distribution for male flower biomass per kg of aboveground biomass. Treatment, the standardised nutrient concentration and their interaction were included as fixed effects.

All statistical analyses and visual representations were conducted using the software R v.3.6.1 (2019) and the GGPLOT2

Table 1 Summary of the main meteorological and physiological explanatory variables mentioned in previous publications affecting *Quercus ilex* reproductive success.

Stage of the reproductive cycle	Explanatory variable proposed in previous publications	Effect +/− [Reference]	Explanatory variable included for model selection
Male flower initiation [Summer of previous year?]	Water stress	− ?	July–September WSI of the previous year
	Negative autocorrelation	− ?	Male flower biomass of the previous year
			Total fruit biomass of the previous year
Female flower initiation [unknown]	Previous acorn crop (negative autocorrelation)	− (mitigated by weather) [1]	Total fruit biomass of the previous year
	Water stress	− ?	July–September WSI of the previous year
Male flower development [April]	Warm spring	+ [1]	April mean temperature
	Secondary growth	+ ?	Annual plot relative biomass increment
Female flower development and pollination [April–May]	April air humidity	− [2]	Precipitations during pollination
	Pollen quantity	+ [2, 3] ns [1, 4]	Male flower biomass
	Warm spring	+ [1]	Mean temperatures of April and of May
	Minimum temperature in May	− [2]	
	Delayed phenology	+ [4]	na (but related to spring temperature)
Fertilisation and fruit initiation [June]	Spring drought	− [2, 4, 5, 6]	April–June WSI
	Spring rainfall	+ [3, 7]	
	Mean temperature in June	+ [3]	June mean temperature
	Spring secondary growth	ns [8]	Annual plot relative biomass increment
Fruit growth and maturation [July–November]	Drought in summer and/or early autumn	− [5, 7, 9, 10, 11]	July–November WSI
	Temperature in summer or early autumn	− [12]	
	Autumn rainfall	+ [3, 7]	
	Torrential rain	− [5]	Number of days of torrential rain
	Autumn secondary growth	− [8]	Annual plot relative biomass increment
	Carbon assimilation	+ ?	July–November GPP

Variables are assigned to a stage of the reproductive cycle. The periods corresponding to each stage of the reproductive cycle are specified based on the knowledge of *Q. ilex* reproductive biology and our observations. ?, hypothetical direction of the effect; GPP, gross primary production; na, nonapplicable; ns, nonsignificant; WSI, water stress index.
[1] Bogdziewicz *et al*. (2017b); [2] García-Mozo *et al*. (2012); [3] García-Mozo *et al*. (2007); [4] Fernández-Martínez *et al*. (2012); [5] Pérez-Ramos *et al*. (2010); [6] Fernández-Martínez *et al*. (2015); [7] Alejano *et al*. (2008); [8] Martín *et al*. (2015); [9] Carevic *et al*. (2010); [10] Espelta *et al*. (2008); [11] Liu *et al*. (2015); [12] Montserrat-Martí *et al*. (2009).

Table 2 Details of the structure of generalised linear mixed models on which model selection was applied to retain the set of variables that best explain the reproductive responses in *Quercus ilex*.

Response variable	Model distribution	Variables of the full model retained and excluded after model selection	Random effect
Male flower biomass per kg of aboveground biomass	Gaussian	Retained: Total male flower biomass of the previous year, July–September WSI of the previous year	Block
		Excluded: Total fruit biomass of the previous year, relative biomass increment, mean temperature of April	
Number of initiated fruits per kg of aboveground biomass	Negative binomial	Retained: Relative biomass increment, June mean temperature, precipitations during pollination	Block
		Excluded: Total fruit biomass of the previous year, male flower biomass, April mean temperature, May mean temperature, April–June WSI, July–September WSI of the previous year	
Abortion rate between initiation and maturity	Binomial	Retained: Number of initiated fruits, relative biomass increment, July–November WSI, July–November GPP	Block
		Excluded: Number of days of torrential rain	
Number of mature fruits per kg of aboveground biomass	Negative binomial	Retained: Number of initiated fruits, July–November WSI, July–November GPP	Block
		Excluded: Relative biomass increment, number of days of torrential rain	

For each reproductive stage the distribution of the model, the variables retained and excluded by model selection, and the random effect are presented. GPP, gross primary production; WSI, water stress index.

package (Wickham, 2009). Marginal (R^2m) and conditional (R^2c) were calculated with the package MuMIn according to Nakagawa's method (Nakagawa & Schielzeth, 2013) to estimate the variance explained by fixed effects and fixed plus random effects, respectively.

Results

Treatment effect on tree water stress and reproductive variables

Water stress (measured by WSI) of spring (April–June), summer–autumn (July–November) and summer (July–September) of the previous year were significantly increased by 20.6%, 16.8% and 13.4%, respectively, in the rainfall exclusion treatment compared with the control (Supporting Information Fig. S1). Rainfall exclusion significantly reduced the male flower biomass, the number of initiated fruits and the number of mature fruits, while it significantly increased the fruit abortion rate (Fig. 1a–d). Rainfall exclusion had, however, no impact on leaf nutrient concentrations in winter (Table S1).

Inter-annual variability of reproduction

The number of initiated fruits and the biomass of male flowers were less variable among years (mean CVp 42% and 57%, respectively, in control treatment), than the number of mature

fruits (CVp 126% in control treatment) and the rate of fruit abortion between initiation and maturity (Fig. 2a–d; Table S2). Rainfall exclusion did not affect any of these coefficients of variation (Fig. 3).

The mean mass of mature fruits did not differ between treatments (Table S2; Fig. S2a). There was no trade-off between mean mature fruit mass and mature fruit number. By contrast, there was a positive relationship between the two variables: years of high fruit production were also years during which mature fruits were heavier (Fig. S2b).

Male flower biomass

According to model selection, male flower biomass was best explained by (in order of importance) the male flower biomass of the previous year and the summer WSI of the previous year that both had a negative effect. The two variables explained collectively 22% of the variance, which was less than the variance explained by the random effect (Table 2; Fig. 4a). The effect of the rainfall exclusion on male flower biomass remained negative when combined with other variables (Fig. 4b).

Number of initiated fruits

The number of initiated fruits in the control plots was best explained by (in order of importance) cumulative rainfall during pollination, which had a negative effect, wood biomass increment
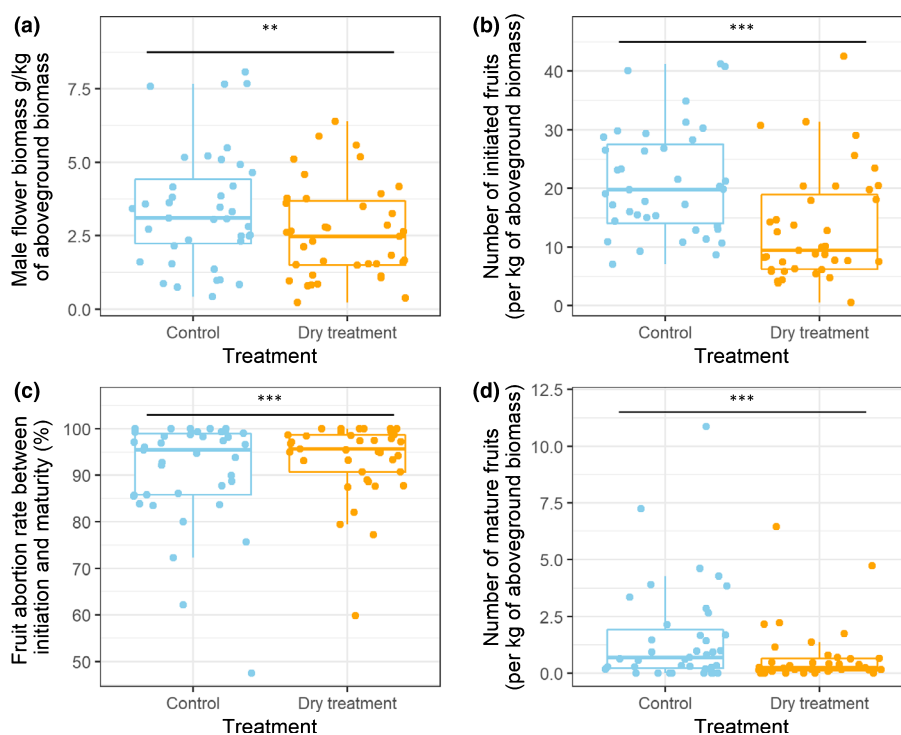

Fig. 1 Effect of the rainfall exclusion treatments on *Quercus ilex* (a) male flower biomass, (b) number of initiated fruits, (c) fruit abortion rate, and (d) number of mature fruits. *P*-values correspond to generalised linear mixed models with year as a random effect (**, $P < 0.01$; ***, $P < 0.001$). The boxplots represent the three quartiles with the median depicted as a solid thick horizontal line. The whiskers extend towards 1.5 times the inter-quartile range. Each dot represents the value for one plot in 1 yr (points have been jittered horizontally and vertically to avoid overplotting).
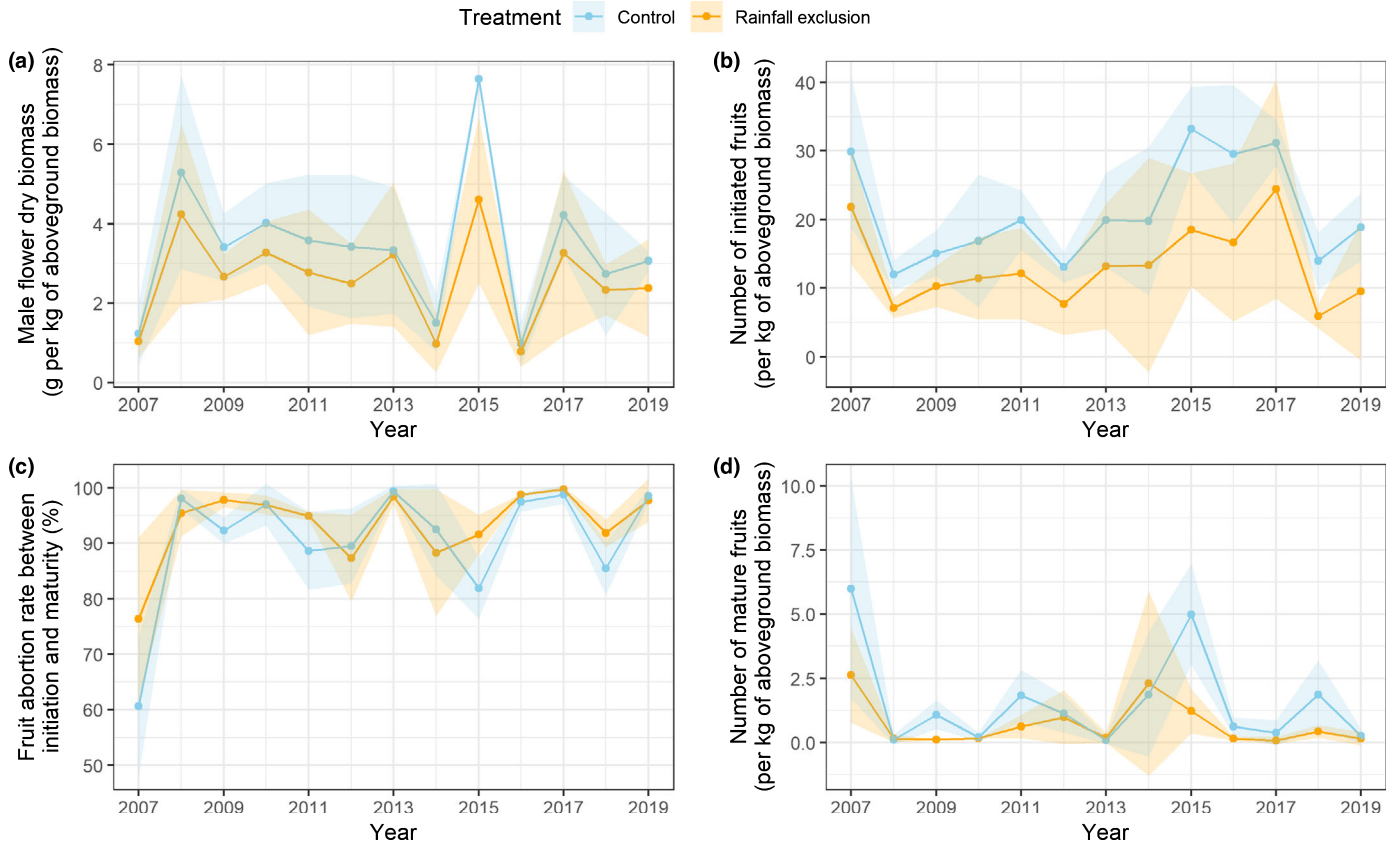
Fig. 2 Mean inter-annual variability (± SD) of the production of (a) male flower biomass, (b) number of initiated fruits, (c) fruit abortion rate and (d) number of mature fruits in the control (blue) and dry (orange) treatments in *Quercus ilex*. Points indicate the mean value of the 12 traps for each plot and each year.
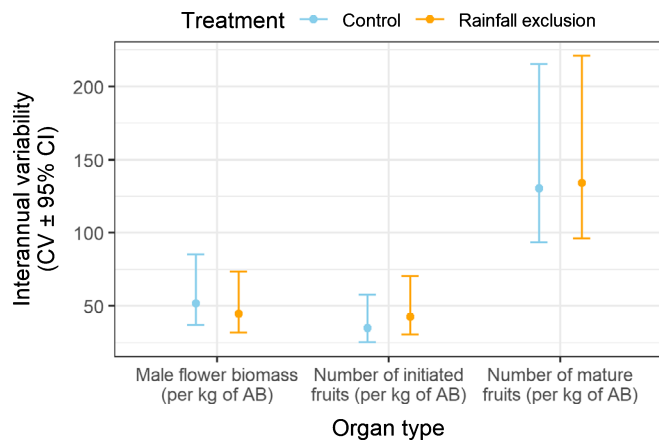

Fig. 3 Treatment effect on the inter-annual variability (population coefficient of variation) of *Quercus ilex* mean male flower biomass, mean number of initiated fruits and mean number of mature fruits among the three plots per treatment. AB, aboveground biomass; CI, confidence interval.

and mean June temperature, which both had positive effects (Table 2; Fig. 5a). The variance explained by the fixed effects was *c.* 39% which is larger than for the male flowers. In addition, contrary to the male flowers, the number of fruits was very little explained by random effects. There was no trade-off between wood biomass increment and reproduction standardised by the plot biomass. By contrast, years during which large amounts of wood biomass were produced were also years during which a large number of fruits were initiated. Interestingly, neither the fruit crop of the previous year, the male flower biomass nor the spring WSI were retained by model selection as strong predictors of the number of initiated fruits (Table 2). The long-term rainfall exclusion treatment had an additional strong negative effect on fruit initiation (Fig. 5b). There was no interaction between wood biomass increment and the rainfall exclusion effect.

Abortion rate between fruit initiation and maturity

Fruit abortion rate between initiation and maturity was best explained by (in order of importance) July–November WSI, which had a positive effect, and by July–November GPP, the number of initiated fruits and wood biomass increment which had a negative effect, with all the fixed effects explaining 21% of the variance (Table 2; Fig. 6a). Years during which trees initiated a large number of fruits tended to be years during which fruits survived better. As WSI and the number of initiated fruits were already strongly affected by the dry treatment (Figs 1b, S1f), the latter did not have a significant effect on abortion when tested in addition to these two variables (Fig. 6b). However, treatment and July–November WSI interacted significantly (Fig. 6b), indicating that trees from the dry treatment experienced less fruit
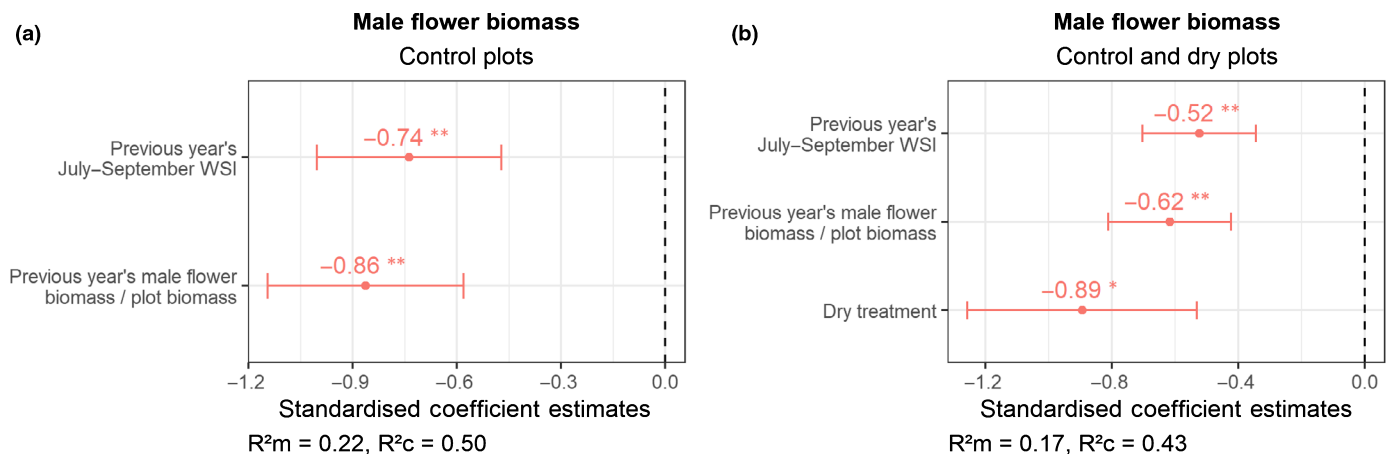
Fig. 4 Generalised linear mixed models standardised estimates ± SE and associated *P*-values (*, *P* < 0.05; **, *P* < 0.01) of the variables explaining *Quercus ilex* male flower biomass per kg of aboveground biomass (a) after model selection in the control plots for 2007–2019 and (b) with the variables retained by model selection in (a) plus the dry treatment effect in control and dry plots. Interactions between treatment and all covariables were first included, and nonsignificant interactions were sequentially removed from the model starting with the least significant ones. We report marginal (R^2m) and conditional (R^2c) R^2 for the reduced final model. WSI, water stress index.
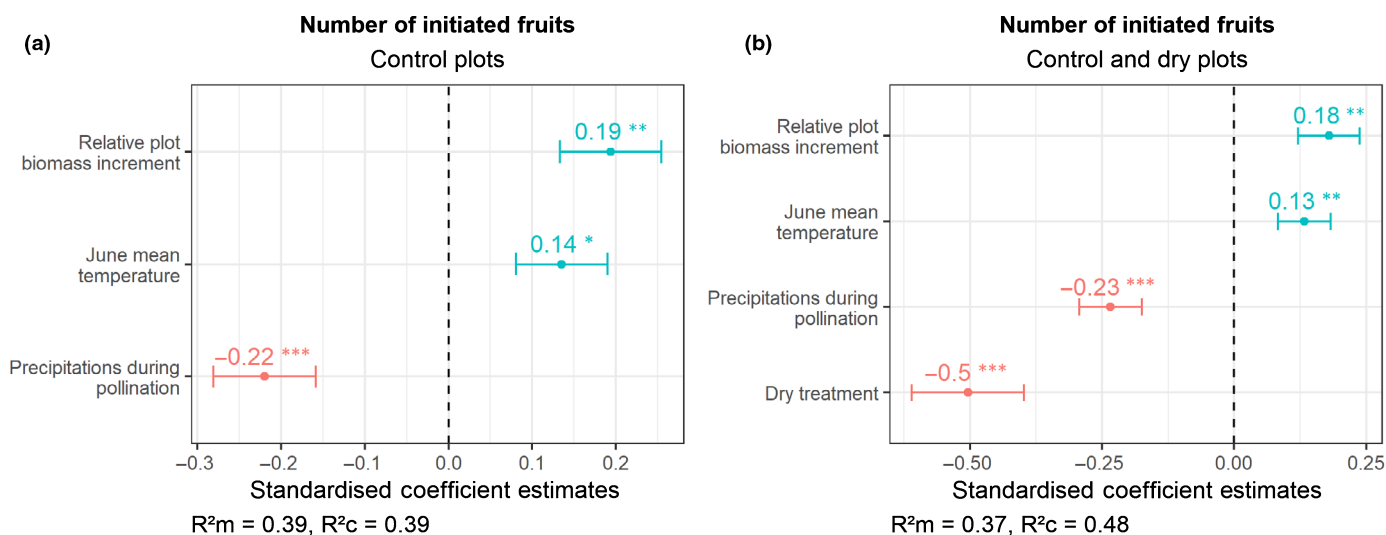

Fig. 5 Generalised linear mixed models standardised estimates ± SE and associated *P*-values (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001) of the variables explaining the number of initiated fruits per kg of aboveground biomass in *Quercus ilex* (a) after model selection in control plots for 2007–2019, and (b) with the variables retained by model selection in (a) plus dry treatment effect in control and dry plots for 2007–2019. Interactions between treatment and all covariables were first included, and nonsignificant interactions were sequentially removed from the model starting with the least significant ones. We report marginal (R^2m) and conditional (R^2c) R^2 for the reduced, final model.

abortion than control trees during years with low water stress in summer and autumn (Fig. S3a,b).

Number of mature fruits

The number of mature fruits was best explained by (in order of importance) the number of initiated fruits that had a positive effect, July–November WSI that had a negative effect, and July–November GPP that had a positive effect (Table 2; Fig. 7a). Our model explained as much as 65% of the variance in mature fruits, probably as a result of the inclusion of the number of initiated

fruits as a predictor of the number of mature fruits. However, the final number of mature fruits was driven more by the rate of abortion than by the number of initiated fruits (Fig. S4a,b). The rainfall exclusion treatment did not affect the number of mature fruits when tested in addition to the number of initiated fruits and summer–autumn WSI (Fig. 7b).

Effect of leaf nutrients content

Male flower production and the number of initiated fruits were both positively related to winter nitrogen concentration in old
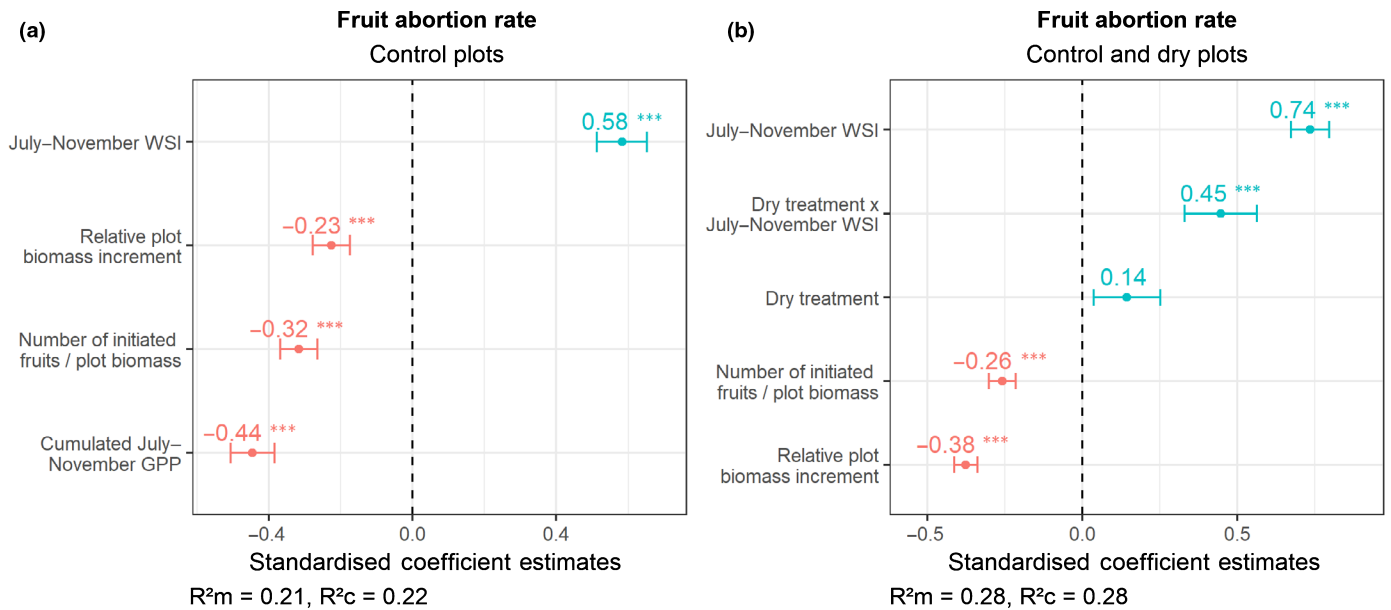
Fig. 6 Generalised linear mixed models standardised estimates ± SE and associated *P*-values (***, *P* < 0.001) of the variables explaining fruit abortion rate between initiation and maturity in *Quercus ilex* (a) after model selection in control plots for 2007–2019 and (b) with the variables retained by model selection in (a) plus the dry treatment effect in control and dry plots. GPP was not included in this last model, as it was not measured in the dry treatment. Interactions between treatment and all covariables were first included, and nonsignificant interactions were sequentially removed from the model starting with the least significant ones. The positive interaction between July–November WSI and dry treatment reflects the fact that trees from the dry treatment experienced less fruit abortion than control trees during years with lower water stress in summer and autumn. Note that in the driest years both treatments showed 100% fruit abortion (see Supporting Information Fig. S3b). We report marginal (R^2m) and conditional (R^2c) R^2 for the reduced, final model. GPP, gross primary production; WSI, water stress index.
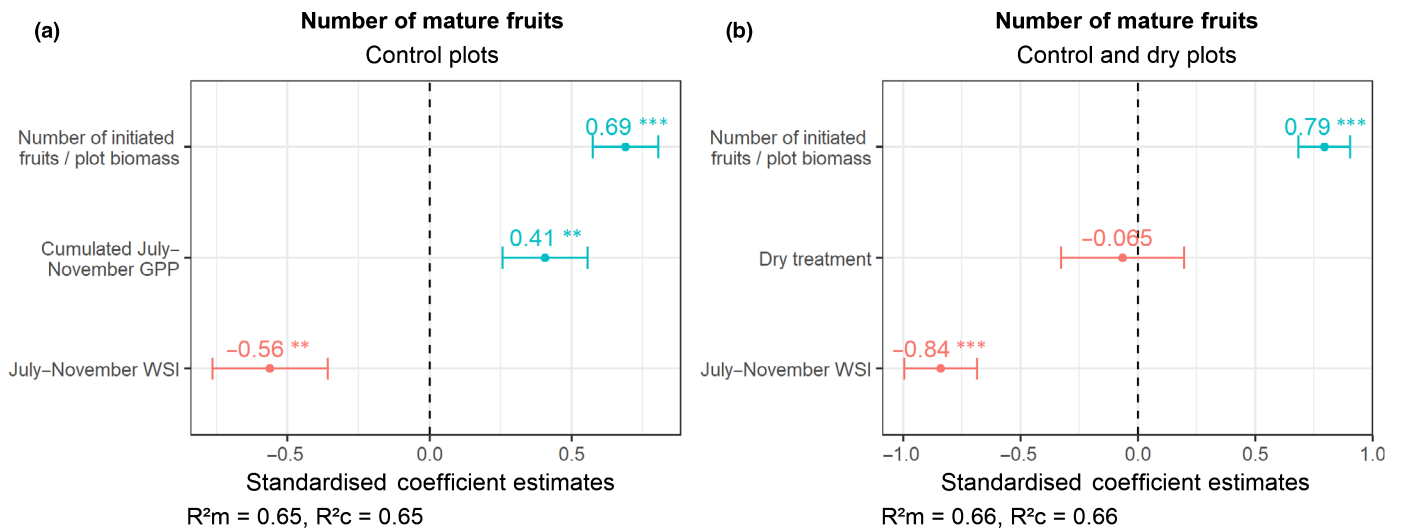

Fig. 7 Generalised linear mixed models standardised estimates ± SE and associated *P*-values (**, *P* < 0.01, ***, *P* < 0.001) of the variables explaining the number of mature fruits per kg of aboveground biomass in *Quercus ilex* (a) after model selection in control plots for 2007–2019 and (b) with the variables retained by model selection in (a) plus the dry treatment effect in control and dry plots. GPP was not included in this model as it was not measured in the dry treatment. Interactions between treatment and all covariables were first included, and nonsignificant interactions were sequentially removed from the model starting with the least significant ones. We report marginal (R^2m) and conditional (R^2c) R^2 for the reduced, final model. GPP, gross primary production; WSI, water stress index.

leaves (cohort produced 2 years before) (Table 3). They were not correlated to nitrogen concentration in young leaves, nor with phosphorus concentrations. There were no interactions between treatment and nutrient concentrations. Conversely, the total biomass of fruits produced during summer and autumn (i.e. aborted and mature fruits) and the treatment had no effect on the nitrogen and phosphorus concentration of the leaves the following winter in either treatment (Table S1).

Table 3 Standardised estimates ± SE and associated statistical significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) of the generalised linear model relating fruit initiation and male flower production to treatment and leaf nutrient concentration during the preceding winter (from 2007 to 2018) in *Quercus ilex*.

Response variable	Fixed effects		
	Treatment	$[N]_{n-1 \ leaves}$	$[P]_{n-1 \ leaves}$
Number of fruits produced a year n	**−0.93 ± 0.13***	−0.03 ± 0.08	0.11 ± 0.08
Flower biomass produced a year n	0.06 ± 8.1	0.68 ± 0.5	−0.53 ± 0.5
	Treatment	$[N]_{n-2 \ leaves}$	$[P]_{n-2 \ leaves}$
Number of fruits produced a year n	**−0.94 ± 0.13 ***	**0.18 ± 0.07**	−0.03 ± 0.07
Flower biomass produced a year n	0.12 ± 0.7	**1.0 ± 0.4***	−0.19 ± 0.4

Nutrient concentration was measured in young leaves produced in the preceding spring ($n − 1$ leaves) and old leaves produced 2 yr before ($n − 2$ leaves). Interactions between treatment and all covariables were first included, and nonsignificant interactions were sequentially removed from the model starting with the least significant ones (none of them were significant).

Discussion

Increased aridity has detrimental effects on flower production, fruit set and fruit growth

The long-term experimental reduction of precipitation by 27% increased water stress during flower development and fruit initiation in spring, fruit growth in summer and autumn, and male flower initiation in summer. Increased aridity significantly decreased the production of male flowers, the number of initiated and mature fruits, and increased fruit abortion during summer and autumn. Consequently, the final fruit crop under rainfall exclusion was reduced due to two cumulative effects, that is a lower number of initiated fruits in spring and a higher rate of fruit abortion in summer and autumn. Our results confirmed those from other rainfall exclusion experiments in *Q. ilex* forests, in which a 15% rainfall exclusion reduced both the number of initiated fruits and of mature acorns (Sánchez-Humanes & Espelta, 2011), as well as the biomass of male flowers and fruits (Ogaya & Peñuelas, 2007; Liu *et al.*, 2015).

Conversely, the long-term precipitation reduction did not modify the inter-annual variability in seed production, which can result from variation in either flower production, fruit initiation rate or fruit abortion rate (Pearse *et al.*, 2016). In our study, the number of mature fruits was driven more by fruit abortion than by fruit initiation and our results confirmed that *Q. ilex* is a 'fruit maturation masting' species (Sork & Bramble, 1993; Espelta *et al.*, 2008; Bogdziewicz *et al.*, 2019), that is that fruit crop is determined more by what happens after fruit set than before it. With a three-fold higher CVp for the number of mature fruits than for initiated fruits, the difference of inter-annual variability

among reproduction stages was larger here than in previous studies on Mediterranean oaks (Espelta *et al.*, 2008; Pérez-Ramos *et al.*, 2014). In any case, our experimental reduction of precipitation did not modify the relative variability of either flower production or fruit abortion, and it did not increase the inter-annual variability of seed production. This result therefore does not support the hypothesis proposed by some authors that inter-annual variability of fruit production should increase in more stressful long-term conditions (Espelta *et al.*, 2008; Bogdziewicz *et al.*, 2018; Wion *et al.*, 2020).

Several studies have shown that fruit abortion rate was strongly influenced by water stress during summer and autumn (Alejano *et al.*, 2008; Espelta *et al.*, 2008; Carevic *et al.*, 2010; Pérez-Ramos *et al.*, 2010; Liu *et al.*, 2015). Alejano *et al.* (2008) suggested that water stress may act as a climatic veto when leaf water potential is lower than −3.5 MPa, which is close to the turgor loss point of *Q. ilex* (Tognetti *et al.*, 1996). In our study, the 2 yr during which fruit production was the highest (2007 and 2015) were years during which the simulated water potential never reached this threshold in any treatment (in 2015) or only for a few days (in 2007). However, the same condition was also met during other years and did not result in a high fruit crop. A low summer water stress therefore appears as a necessary, but not sufficient, condition for reproductive success.

The fruit abortion rate was the only reproductive variable to exhibit an interactive effect between the dry treatment and summer–autumn water stress (Fig. 6). Fruit abortion had a greater sensitivity to water stress in the dry treatment than in the control, suggesting that increased aridity worsens the effect of summer drought on fruit growth. Although this greater sensitivity to water stress in the dry treatment translated into a lower abortion than in the control in favourable years, it did not allow for better fruit development in dry years and fruit production remained lower in the dry treatment irrespective of the drought conditions.

Fruit set has also been shown to be limited by pollination success, which depends on meteorological conditions during pollination because rainfall during flowering washes pollen out of the atmosphere and alters pollination success (García-Mozo *et al.*, 2007). As our experiment was not designed to manipulate rainfall over the tree canopy, we cannot estimate how less precipitation in spring may affect fruit initiation due to pollen washing. Nevertheless, in a previous study, we found that rainfall exclusion decreased viable pollen production by the trees (Bykova *et al.*, 2018), which may reduce pollen availability in the future. Previous studies on oaks have observed a pollen limitation effect on fruit production, but only in low tree density savannah-like landscapes (García-Mozo *et al.*, 2007, 2012; Pesendorfer *et al.*, 2016), and not in dense forests (Fernández-Martínez *et al.*, 2012; Bogdziewicz *et al.*, 2017b). Finally, pollination may be positively affected by warmer spring temperatures in Mediterranean oaks because they favour flowering synchrony and thereby the efficiency of pollination (Koenig *et al.*, 2015; Bogdziewicz *et al.*, 2017b), and fertilisation (Sork *et al.*, 1993; Cecich & Sullivan, 1999) possibly through a positive influence on pollen tube growth (Hedhly *et al.*, 2009). Our results validated the positive effect of June temperature, during the fertilisation period, on the

number of initiated fruits, as previously observed by García-Mozo *et al.* (2007), but not an effect of the temperatures during flowering (April–May).

Increased aridity has additive carry-over effects on male flower production and fruit set

Experimentally increased aridity had long-term effects on tree allocation to reproductive organs in our experiment. This was revealed by the strong treatment effect on the initial fruit set which is highly correlated to female flower biomass and on male flower biomass, that could not be explained merely by an increased water stress during critical periods of the reproductive cycle. Indeed, the treatment effect on male flower biomass remained significant in addition to the previous year WSI effect, and seasonal water stress had no significant effect on fruit initiation (Figs 4, 5). This means that the effect of experimentally increased aridity was additive with the other drivers, so that trees in the dry treatment always produced less male flowers and initiated less fruits than the control trees, independently of the meteorological conditions. Conversely, the long-term increased aridity did not interact with the meteorological drivers of fruit production and therefore did not modify their hierarchy of importance in explaining the inter-annual variation in fecundity. Increased aridity therefore lowered the intercept of the relationship between reproduction and climatic conditions but without affecting the slope (no interaction). Therefore, the sensitivity of the reproductive organs to temperature and seasonal drought remained similar across treatments while the overall capacity to allocate to reproduction was diminished by increased aridity. In other words, the dry treatment reduced fruit production more strongly than what we would predict from a 27% decrease in precipitation with the model adjusted on the control treatment data. Consequently, we argue that *Q. ilex* acorn production may be more affected by increasing aridity in the future than what current inter-annual variations would lead us to forecast.

We suggest that this additive effect of increased aridity might be induced partly by the carry-over effects of previous summer water stress on bud development. In oak species, primary growth is largely determined by preformed organs inside the buds (Fontaine *et al.*, 2000; Alla *et al.*, 2013). Bud enlargement (Montserrat-Martí *et al.*, 2009), leaf primordia initiation (Alla *et al.*, 2013) and male floral primordia initiation (Merkle *et al.*, 1980) have all been shown to occur during the previous growing season and to be potentially affected by summer drought. Our results indeed show a significant effect of previous year WSI on male flower production, and previous studies of the same experiment found a 1-yr lagged effect of drought on leaf production (decreased leaf number per growth unit) (Limousin *et al.*, 2012; Gavinet *et al.*, 2019), and a lower number of viable pollen grains (Bykova *et al.*, 2018). Female flowers, contrary to other organs, were not affected by water stress of the previous summer. However, because they are generally initiated on the larger shoots bearing numerous leaves (Alla *et al.*, 2012), their lower number in the dry treatment might be caused indirectly by the reduced number of ramifications and leaves per shoot in this treatment (Limousin *et al.*, 2012).

Fewer resources are allocated to reproduction under increased aridity

In our study, years during which conditions were favourable for growth were also favourable for fruit production. Fruit crop and fruit abortion rate in the control treatment were, respectively, positively and negatively correlated to cumulated GPP from July to November. This result is consistent with previous studies showing that the carbon used for fruit development is provided mainly by recent uptake of photo-assimilates (Hoch *et al.*, 2013; Ichie *et al.*, 2013; Han *et al.*, 2016), and it suggests that *Q. ilex* acorn production might be limited by the availability of carbon resources. Other studies conducted in temperate forest ecosystems on *Q. petraea* or *F. sylvatica* have, however, failed to observe such a relationship between GPP and seed production (Delpierre *et al.*, 2016; Mund *et al.*, 2020). In the Mediterranean context of our study, where the ecosystem carbon uptake is driven by water availability in summer (Rambal *et al.*, 2014), GPP and acorn production are both largely influenced by water stress. In spite of this, the WSI and GPP were both retained by model selection as significant drivers of the annual fruit crop and fruit abortion rate (Figs 6, 7), thereby suggesting an effect of carbon resources on acorn production, in addition to the effect of water availability. The greater sensitivity of the fruit abortion rate to summer–autumn WSI in the dry treatment could be partly explained by a reduced availability of recently assimilated carbon given that leaf carbon assimilation and ecosystem GPP are reduced in this treatment compared with the control (Misson *et al.*, 2009; Limousin *et al.*, 2010).

Our results also show that biomass increment was positively related to the number of initiated fruits per kilogram of aboveground biomass and negatively related to the fruit abortion rate, thereby indicating an absence of causal trade-off between growth and reproduction. In masting Fagaceae, massive fruit crops have been either associated to reduced growth (Pearse *et al.*, 2016) or increased growth (Pérez-Ramos *et al.*, 2010; Fernández-Martínez *et al.*, 2015; Bogdziewicz *et al.*, 2020b), although these relationships might not be causal (Knops *et al.*, 2007; Knops & Koenig, 2012), or not associated to growth at all (Yasumura *et al.*, 2006; Pulido *et al.*, 2014). The absence of trade-off between growth and reproduction in *Q. ilex* might be explained by a temporal lag between stem and fruit growth. *Q. ilex* stem growth takes place mostly in spring and early summer, before the drought and concomitantly to flower development and fruit initiation, but also in autumn when water availability is restored after the drought (Fig. S5; Methods S1). Stems and fruits are therefore in competition for resources mainly in autumn, consistently with Martín *et al.* (2015) who observed a negative correlation between acorn production during mast years and autumn stem growth, but not spring growth. Here, we observed no interactive effect between stem growth and the treatment, but the biomass increment and treatment were both retained by model selection as significant drivers of fruit initiation and abortion (Figs 5, 6). This means

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that for a given allocation to wood growth, the allocation to fruits was lower in the rainfall exclusion treatment. Our results therefore confirm an earlier conclusion by Bogdziewicz *et al.* (2020b) that increased aridity modifies the relative allocation to growth vs reproduction in *Q. ilex*, although in our case the allocation to fruits was more impacted by aridity than the allocation to wood. In our experiment, stand aboveground net primary productivity was reduced by 11% in the dry treatment compared with the control but, while wood production was not significantly impacted by the dry treatment, acorn mean biomass production was reduced by as much as 34% over the course of our experiment (Gavinet *et al.*, 2019). This suggests that short-term maintenance of tree functioning is prioritised over reproduction under increased aridity, raising concerns about long-term persistence of *Q. ilex* populations under climate change as well as on its ability to colonise newly favourable areas.

Male flower biomass and the number of initiated fruits were both positively correlated to mean nitrogen concentration in 2-yr-old leaves sampled during the winter before. These old leaves usually fall in early summer, shortly after new leaves and flowers have been produced. Our results therefore suggest that nitrogen used for flower production might be remobilised from old leaves before they fall (Cherbuy *et al.*, 2001) and that male flowers development might be limited, in part, by nitrogen availability. There is increasing evidence that stored nitrogen or phosphorus contributes to masting events (Sala *et al.*, 2012; Ichie & Nakagawa, 2013; Miyazaki *et al.*, 2014; Han & Kabeya, 2017; Satake *et al.*, 2019), and fertilisation experiments in oak forests have sometimes increased acorn production (Bogdziewicz *et al.*, 2017a), although not always (Brooke *et al.*, 2019). Conversely, the absence of an interactive effect between rainfall exclusion and leaf nutrient content on flower and fruit production suggests that the long-term increased drought effect was not mediated by nutrient limitation.

Conclusion

Taken together our results have important implications for forecasting the response of tree reproduction to increasing aridity because they suggest that this may affect tree reproduction beyond what is expected from the current response to seasonal drought. Under the drier conditions simulated by our experiment, trees initiate fewer flowers and fruits, and these fruits are less likely to withstand summer drought and reach maturity. We interpreted the strong negative effect of increased aridity on fruit production as caused by more severe water stress during fruit development, stronger limitations by resources and changes in resources allocation, all suggesting that holm oak forest natural regeneration could be jeopardised in a drier future.

Author contributions

ILR, IC and J-ML conceived and designed the study; J-MO and J-ML collected data; FM developed the water stress model; ILR and JG analysed data; ILR led the writing. All authors contributed critically to the drafts and gave final approval for publication.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Treatment effect on the water stress index of spring, summer of the previous year and summer–autumn of the current year.

Fig. S2 Mean mature fruit mass according to year, treatment and the number of mature fruits produced.

Fig. S3 Relationship between summer–autumn water stress index and fruit abortion rate according to treatment.

Fig. S4 Effects of the number of initiated fruits and the fruit abortion rate on the number of mature fruits.

Fig. S5 Intra-annual variations of phenological phases, fruit and stem growth, water potential, precipitations, gross primary production and temperature.

Methods S1 Fruit growth and stem growth measurements.

Table S1 Effect of fruit biomass production and treatment on leaf nutrient concentration the following winter.

Table S2 Population Coefficient of Variation (CVp) for male flower biomass, numbers of initiated and mature fruits and mean fruit mass.

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