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Summary

1. Seedlings near a conspecific adult might suffer increased mortality due to pressure from enemies such as belowground pathogenic fungi (Janzen-Connell Hypothesis), however variation exists among taxa such that some experience low levels of mortality. We hypothesized that seedlings close to adults might profit, rather than suffer, from belowground fungi, notably from mycorrhiza or decomposers, in particular near large adult trees and under a closely related canopy.

2. We planted oak seedlings in a temperate forest at different distances from adults and followed seedling mortality, budburst (early budburst permitting photosynthesis during the best light conditions) and leaf herbivory. We applied fungicide on half of the seedlings for 2 years to identify the net effect of belowground fungi on seedlings. We quantified seedling mycorrhization and fungal-driven decomposition of a neighbourhood-specific and an unspecific substrate, local oak leaves and cellulose, respectively. Finally, we related mycorrhization and decomposition to seedling performance.

3. We found that, in seedlings planted close to conspecific adults, belowground fungi had a negative net effect on seedlings: elimination of fungi, surprisingly, decreased herbivory. This effect could be due to oak leaf decomposition, which related to increased seedling herbivory and was higher near a conspecific adult. Under a closely related canopy, however, the net effect of belowground fungi on nearby seedlings became positive: elimination of fungi delayed budburst. This effect could be due to colonization by the ectomycorrhizal fungus *Cortinarius* sp., which related to accelerated budburst and was higher near a conspecific under a closely related canopy. Effects of belowground fungi on nearby seedlings were not dependent on the size of the conspecific and were species- but not lineage-specific.

4. Overall, our results suggest that a Janzen-Connell-like pattern, *i.e.* increased mortality of seedlings near conspecific adult, can be (i) induced by specialist decomposers increasing the nutritional quality of conspecific seedlings to herbivores, and (ii) offset by ectomycorrhizal fungal mutualists under a

closely related canopy. Coexistence among closely related adult trees appears to change interactions between adults and nearby conspecific seedlings from conspecific inhibition to conspecific facilitation.

Introduction

The fine-scale spatial structure of forest communities has essentially been attributed to natural enemies (Terborgh 2012). Natural enemies focus their attack on offspring near a conspecific adult, where the juvenile density is highest, leading to an increased mortality of offspring (Janzen-Connell Hypothesis, JCH, Janzen 1970; Connell 1971). Such a high mortality of conspecific offspring under the adult tree or where density of adults is high (Liu *et al.* 2015) allows establishment of heterospecifics, contributing to the maintenance of tree diversity in forests. JCH has been suggested for tropical forests, but appears to apply also in temperate forests (Comita *et al.* 2014), where belowground pathogenic fungi are an important group of enemies (Packer & Clay 2000; Masaki & Nakashizuka 2002; Reinhart & Clay 2009). However, Janzen-Connell patterns are sometimes weak (e.g. Packer & Clay 2000) and seedling survival may depend on processes other than enemy pressure. Weak enemy-induced mortality might suggest that some other factors replace or offset possible effects of enemies on seedlings. Specifically, supportive fungi have been overlooked (but see Liang *et al.* 2015 in tropical forests), even though most tree species depend on mycorrhization (Wang & Qiu 2006; Smith & Read 2008) and fungal-driven decomposition (Berg & McClaugherty 2008). Seedling mycorrhization and fungal-driven decomposition might be more important than possible enemy pressure. Mycorrhization and fungal-driven decomposition might have a particularly positive effect on seedlings near the conspecific adult provided that they both (i) improve seedling performance and survival and (ii) increase under the conspecific adult.

First, mycorrhization and fungal-driven decomposition might improve seedling performance and survival. In temperate forests, the dominant tree families, including Fagaceae, are related to ectomycorrhizal (EM) fungi (Wang *et al.* 2006) forming EM root tips. Shortly after germination, a simple assemblage of EM fungal species colonizes fine roots of seedlings (e.g. Nara 2008). EM fungi can enhance seedling performance and survival by improving water and nutrient uptake (Smith *et al.* 2008) and also by supplying the carbon required for inducing budburst in early season (Courty, Bréda & Garbaye 2007). Moreover, EM fungi can protect seedlings from their enemies, such as belowground pathogens (e.g. Martin-Pinto, Pajares & Diez 2006) and aboveground herbivores (Gange *et al.* 2005, but see Hartley & Gange 2009). Overall, seedling performance and survival may be improved by a high EM colonization, by a high EM fungal richness, and/or by particular EM fungal species (but see Gorzelak *et al.* 2015). In addition, fungal-driven decomposition of local litter, which results *inter-alia* from the activity of saprotrophic and mycorrhizal fungi (Dickie *et al.* 2014; Shah *et al.* 2016), may enhance seedling performance and survival by accelerating the availability of essential nutrients for seedlings through nutrient recycling (Berg *et al.* 2008).

Second, mycorrhization and fungal-driven decomposition might be particularly strong near conspecific adults. Effects of spatial proximity to adults on seedling mycorrhization have been little studied so far, except for the special case of adult residual trees. For such trees, it has been demonstrated that EM fungal richness increases on roots near the stem (Teste, Simard & Durall 2009 on *Pseudotsuga menziesii* from tropical environments). Also, EM fungal richness and colonization on tree seedlings outside closed forests increase near a residual tree (Teste *et al.* 2009) or a forest edge (Dickie & Reich 2005; Frank *et al.* 2009). Moreover, EM fungal species found close to conspecific adults differ from those at distance from adults (Dickie *et al.* 2005), and those at distance might not replace the services provided by those nearby. In fact, some EM fungi may be specific to particular tree species (Bruns, Bidartondo & Taylor 2002) or evolutionary lineages of tree hosts (Tedersoo *et al.* 2013). Overall, even within a closed forest, facilitation of seedlings by EM fungi might increase near a conspecific adult although this has not been studied. Similarly, fungal-driven decomposition could be more efficient near a conspecific adult. A litter is better decomposed in its original environment

(home-field advantage; Gholz *et al.* 2000; Veen *et al.* 2015), due to a specialization of the saprotrophs on the most abundant litter type (reviewed by Austin *et al.* 2014). Hence, near a conspecific adult where the monospecific litter fall and accumulate, the fungal-driven decomposition could increase.

High seedling mycorrhization and fungal-driven decomposition near conspecific adults might require particular conditions. First, a large adult tree has a larger root system, and could thus accumulate more mycorrhiza than smaller adults. Moreover, a large adult tree is mostly older, and affects the surrounding belowground fungi for a longer period than a small one. A large adult tree has thus had time to accumulate efficient EM fungi in its surrounding, and conspecific seedlings might profit. Similarly, a large adult tree has a larger crown and produce higher quantities of litter than a small adult: saprotrophic fungi may hence increasingly specialize on the locally accumulating litter of the large tree (home-field advantage). Second, a canopy in which closely related species dominate might have accumulated more efficient EM and saprotrophic fungi, for much the same reasons as a large individual adult (Cavender-Bares *et al.* 2009). Mycorrhization and fungal-driven decomposition might be particularly high when the conspecific adult is large or the canopy dominated by closely related species.

In this study, we investigated whether belowground fungi (EM fungi and fungal decomposers) have particular positive effects on seedlings near adults, opposite to effects proposed by the JCH. We used temperate oaks (*Quercus*) as model systems as they dominate many temperate forests, are attacked by both above- and below-ground enemies (e.g. Southwood *et al.* 2004) and strongly depend on EM associations (Wang *et al.* 2006). We focused on seedlings because the JCH is particularly applicable at this stage (Connell 1971; Hyatt *et al.* 2003; Comita *et al.* 2014). We applied a partly experimental approach: we planted standard seedlings into different adult neighbourhoods, studied decomposition of standard substrate and excluded fungi with fungicide. In contrast, we did not manipulate mycorrhiza and decomposers or their respective services separately, and evidence at these levels remains associational. We studied decomposition of the local oak litter within a given neighbourhood and of cellulose – the former is susceptible to home-field advantage and contributes most to local nutrient recycling, the latter is not. We followed seedling mortality and processes related to seedling

mortality: budburst delay and leaf herbivory. Seedling mortality increases with delayed budburst (Besford *et al.* 1996; Seiwa 1998), because it precludes photosynthesis during the best light conditions, prior to canopy closure in spring. Seedling mortality also increases due to high leaf herbivory (Wada, Murakami & Yoshida 2000), given the small leaf area of seedlings. We tested three groups of predictions: (i) Fungi mediate the relationship between seedlings and nearby conspecific adults, i.e. relationships between nearness to conspecific adults and seedling performance (mortality, budburst, herbivory) change after application of fungicide. Specifically, we predict that relationships between adults and nearby seedlings become positive when the conspecific adult is large or under a closely related canopy. We tested whether those relationships are restricted to a single species (disappeared if the nearby adult is heterospecific) or restricted to closely related species (disappeared if the nearby adult is a distantly related species). (ii) EM fungi and fungal-driven decomposition are related to increased seedling performance (characterizing seedling performance by low mortality, and low budburst delay or leaf herbivory, and characterizing EM fungi by total colonization, species richness, and prevalence of dominant taxa). (iii) Nearness to a conspecific adult relates to increased seedling EM and fungal-driven decomposition, and these relationships are strongest under a large conspecific adult or a closely related canopy. We tried to explain the net effects of fungi detected on seedlings near conspecific adults by either mycorrhization or fungal-driven decomposition.

Materials and methods

STUDY SITE

We conducted the study between 2013 and 2015 in the forest of Rennes, Brittany, France (48°12' N, 1°33' W; ca. 90 m altitude; 3,000 ha), an area characterized by an oceanic climate (mean annual temperature of 11.3 °C; cumulated annual rainfall of 836 mm). This forest is composed of two parcel types dominated by either oak (*Quercus petraea* hybridizing with *Quercus robur*) and beech (*Fagus sylvatica*), or by Scot's pine (*Pinus sylvestris*) interspersed with oaks and other Angiosperms. The other main tree species in the overstorey are *Betula pendula*, *Carpinus betulus* and also *Ilex*

aquifolium, *Castanea sativa*, *Ulmus minor*, *Abies alba*, *Alnus glutinosa*, *Sorbus torminalis* and *Rhamnus frangula* (details in Yguel *et al.* 2011), all typical for European temperate oceanic lowland forests.

We selected ten pairs of adult oak trees (*Q. petraea* or *Q. robur*, not strictly speaking distinct species and always partly hybridized) across the entire forest (used by Yguel *et al.* 2011; Appendix S1 for geographic distribution). Each pair had one tree in the more oak-dominated parcel and one in the more pine-dominated parcel, separated by distances of less than 150 m. Selecting adult oak trees in both types of parcels ensured a large range of dominances of close relatives in the canopy and of evolutionary proximities between seedlings and their adult neighbours. Such a blocked design offers the advantage of controlling for macroenvironmental variation such as soil types among blocks, i.e. in our case among tree pairs (Legendre *et al.* 2004).

EXPERIMENTAL DESIGN

Transplantation

In early January 2013, we harvested forty oak seedlings (*Q. petraea* or *Q. robur* or hybrids) around the adult oak tree located in the more oak-dominated parcel of each of the ten pairs (seedlings were too scarce in the more pine-dominated parcel). We chose seedlings carefully, harvesting seedlings of approximately the same height/age (ca. 1 year old) and morphology, with no signs of damage or pathology particularly on buds. Seedlings had no more acorn attached, ensuring seedlings were no more dependent upon seed reserves. We wanted to avoid importing fungi, notably EM fungi, from the harvested site and thus cut all fine roots of seedlings as close as possible to the taproot (EM fungi connect with fine roots to form ectomycorrhiza, and pre-experiments had shown that seedling rapidly re-establish such fine roots within days). This procedure also removed soil residuals. Seedlings were then immediately transplanted along a 5m transect based on the stem of each focal adult oak (Fig. 1), with two seedlings planted every 50cm and distant by 50cm. A transect length of 5m assured that the focal oak was usually the closest conspecific (although these adult neighbourhoods were measured separately, see below) while assuring also sufficient variation in

distance. We measured two variables of particular importance for a seedling at the ground of a forest: temperature (integrating sun exposure) and density of conspecific seedlings (Appendix S2).

Fungi exclusion

In order to assess the net effect of belowground fungi on seedlings, a fungicide excluding both pathogenic and supportive belowground fungi was applied. We used a fungicide composed of mancozeb (Dithane Paysage 75% mancozeb, Dow AgroSciences, Indianapolis, USA) because of its very broad spectrum of protection against pathogenic fungi and oomycetes (notice of manufacturer) and its inhibitory effect on EM fungi (Davies 2008), notably when diluted at 0.3% (Reddy & Natarajan 1995). At the time of transplantation and then every three months, we applied locally a fungicide treatment on the soil, at the base of ten seedlings located along each transect (one per distance, Fig. 1). Seedlings not treated with fungicide were watered, with an equivalent amount of water. We checked for a detrimental effect of the fungicide on seedling roots: we found no effect of fungicide application on the total number of tips ($F_{1,119}=0.0$, $P=0.99$), nor on the fine roots length ($F_{1,118}=0.2$, $P=0.66$). In the present study, the fungicide significantly reduced the EM colonization on oak seedlings ($F_{1,119}=7.3$, $P=0.0078$), but not the EM fungal richness ($F_{1,118}=0.03$, $P=0.86$) attesting its broad-spectrum action on EM fungi (see below for methodological details of fungal study). We admit that by applying a fungicide we manipulated only part of the community that is implicated in the decomposition process, notably no mesofauna or bacteria.

DESCRIPTION OF CLOSEST ADULT NEIGHBOURS

We measured the spatial distance of each transplanted seedling to the stem position of the closest conspecific and of the closest heterospecific adult tree (> 2m height). Distance to conspecific adult ranges from 0.3m to 5m (average 2.5m), and distance to heterospecific adult ranges from 0.2m to 6.8m (average 2.4m); note that these two measures are independent (-0.019; P=0.71, d.f.=398). We also measured the circumference at breast height of the closest con- and hetero-specific adult, and used this measure as a proxy of tree size. Tree size also tends to reflect tree age. Circumference at

breast height of conspecific adult ranges from 0.11 to 1.25m (average 0.64m), and from 0.01 to 2.46m (average 0.54m) for heterospecific adults. We took the average distance and circumference ('size' from hereon) when two adult trees were equally close to a seedling.

We determined evolutionary distances between oak seedlings and the spatially closest adult tree species, using distances established by Vialatte *et al.* 2010 and Yguel *et al.* 2011 (ranging from 0 to 140 Million Years Before Present, MYBP, Appendix S3 for the procedure). When two trees were equally close to a seedling, we calculated the respective mean evolutionary distance.

DESCRIPTION OF CANOPY COMPOSITION

We quantified the degree to which the canopy was dominated by species closely related to oaks. For a given focal adult oak, we determined relatedness to each adult neighbour, neighbours were mostly physically in contact with the focal crown, or at least the projections of crowns on the ground were overlapping (using the above-mentioned evolutionary distances; Appendix S3). We averaged these distances for each focal adult oak. We multiplied distance by -1 to get a measure of relatedness of the ambient canopy, consistent with the hypothesis. We refrained from merging information on tree size and on tree distance (spatial or evolutionary) into a single parameter as this would render impossible the distinction between statistical effects of size and distance.

MEASUREMENTS OF SEEDLING MORTALITY, BUDBURST DELAY AND LEAF HERBIVORY

We recorded 'seedling mortality' as well as all the below variables only the second year in order to avoid impacts that are due to the transplantation in year one. For seedling mortality, we considered the seedlings that died between winter 2013/2014 and 2014/2015. We considered seedlings as dead when they looked dry and brittle or were unearthed and broken at the base (the latter two often occurring to the seedlings being dry and brittle before).

We began surveying 'budburst delay' in January 2014 when all seedlings had closed buds, and ceased in late June when most of the buds had burst. We screened for a fully opened bud every 3 days until the end of May and approximately every week until the end of June. Five seedlings already

had leaves in January, but we excluded those seedlings from budburst analysis because their leaves could be the result of a late 2013 budburst rather than an early 2014 budburst. We defined the day zero of budburst as the date the first seedling had burst (5 March 2014, note that buds of adults burst later). Only seedlings that had burst during survey (between day zero and late June) were considered to avoid confounding budburst with mortality.

We recorded 'leaf herbivory' as the total amount of leaves fully or partially consumed throughout the vegetation period, as detailed in Appendix S4.

MEASUREMENTS OF MYCORRHIZATION AND MOLECULAR IDENTIFICATION OF EM FUNGAL TAXA

Two years after transplantation, just before beginning of budburst in April 2015, we gently uprooted the surviving seedlings (121/400), trying to collect their entire root system. In the same day, we brought these surviving seedlings to the lab and stored them at 4°C. We analyzed seedlings within 15 days of harvesting: fine roots of seedlings were cut, gently washed with tap water, and immediately screened under a binocular lens. For each seedling, we assessed the EM colonization on the entire collected root system, by counting the number of EM root tips out of the total number of undamaged tips; few tips looked dead (severely damaged) and were not counted. We distinguished ectomycorrhizae into morphological groups based on color and shape of EM root tips (Agerer 1987, but see Dickie *et al.* 2005) and determined colonization by each morphological group as root tips with a given morphological group vs. all root tips (referring to EM root tips lead to very similar results). We detected nine morphological groups, but only four were present on a sufficient number of seedlings for being statistically analyzed: '*black*', '*brown*', '*smooth white*' and *'white'* (Appendix S5 for pictures). Finally, we calculated the EM fungal richness as the number of morphological groups (using all of the nine morphological groups) on each seedling. We verified whether seedling mycorrhization was an effect of taproot depth or litter accumulation (excluding, obviously, seedlings treated by fungicide), and found that this was the case for the four EM fungal species (Appendix S6). We hence accounted for these variables when explaining the colonization by each of the four EM fungal species (see Data analysis below).

To identify the four dominant morphological groups of EM fungi, we used standard molecular protocols as outlined in Appendix S5. One morphological group could not be identified as a single species while the three others could: black=*Cenococcum geophilum*, smooth white=*Cortinarius* sp. and white=*Lactarius chrysorrheus* (identities=99.7, 99.1, 99.8% respectively).

MEASUREMENT OF FUNGAL-DRIVEN DECOMPOSITION

We studied the decomposition of two substrates (oak leaves and cellulose), which are primarily decomposed by fungi, in particular in the acid soils where fungi are the main decomposers (Biswas & Mukherjee 1994), albeit the precise identity of the decomposers is not important for our conclusions on spatial recruitment. First, we estimated fungal-driven decomposition of a neighbourhood-specific substrate, *i.e.* oak leaves sampled next to the seedlings. Oak leaves were sampled at the lower level of the Oi horizon (leaves at the surface of Oi being still intact and moved by wind), every 50cm from the stem of the focal adult oak up to 5m, between the two seedlings transplanted at each distance (Fig. 1). Leaves were scanned (Epson GT-20000; Epson, Suwa, JPN) and we determined the degree of decomposition of each oak leaf as the ratio between leaf area consumed by micro-organisms (areas appearing very light, almost transparent) and the total leaf area (areas quantified with Photoshop CS2 software, Adobe Systems Inc., San Jose, USA). Studied parameters on leaves are thus well representative of fungal-driven decomposition. Second, we estimated fungal-driven decomposition of standard substrate, cellulose, by using 200 Q-tips made out of organic cotton. We chose cellulose as it is a neutral substrate: carbon macromolecules without the specificities of oak litter, such a very high tannin contents and thick epidermis and high leave toughness. When cellulose is decomposed but oak litter not, this tentatively suggests abundance of generalist but scarcity of oak-specialist decomposers. We previously weighed Q-tips and then planted them vertically in the litter every 50cm from the stem of the focal adult oak up to 5m, between the two seedlings transplanted at each distance, in February 2013. The Q-tip head was approximately at 5cm depth. We harvested Q-tips 3 months later, they were gently washed to remove soil, placed in the oven at 60°C for 72h and then reweighed. We estimated cellulose decomposition as the ratio of dry weights after and before exposure. While the cellulose approach standardizes everything (substrate,

depth) and hence characterized a decomposition potential, the oak-leave approach purposefully treated decomposition of the local substrate under the local conditions of substrate accumulation and hence characterized a realized decomposition.

DATA ANALYSIS

We performed all statistical analyses using R version 3.1.1 (R Core Team 2014) and the lme4 package (Bates *et al.* 2015). As our experimental design used blocks, we worked with mixed models, always utilizing *tree pair* as random factor and including soil temperature and density of conspecific seedlings as covariables as they could influence seedling mortality, budburst delay, leaf herbivory, mycorrhization and fungal-driven decomposition. The adequacy of models to data was always checked graphically with residuals-vs-fitted values plot and QQ-plot of the residuals. Variance explained by each model was estimated by calculating the marginal R^2 (*i.e.* variance explained by fixed factors only) and the conditional R^2 (*i.e.* variance explained by both fixed and random factors) (Nakagawa & Schielzeth 2013).

In a preliminary analysis, we tested the relationship between budburst delay and leaf herbivory on seedling mortality using Generalized Linear Mixed Models (GLMMs), with a binomial error distribution. We then focused on testing our hypotheses:

(i) Fungi mediate the relationships between seedlings and nearby conspecific adults

We tested whether the net effect of belowground fungi on seedlings relates to the various characteristics of adult neighbourhood, which we specified as follows. First, we explained seedling mortality, budburst delay and leaf herbivory by: *distance to conspecific adult*, *fungicide* and the *distance to conspecific adult:fungicide* interaction term. For this purpose, we used a GLMM with a binomial error distribution to explain seedling mortality, and Linear Mixed Models (LMMs) to explain budburst delay and leaf herbivory.

Then, we tested whether the interactive effect of distance to conspecific and fungicide on seedlings changes either with the size of the conspecific or with dominance of closely related species in the canopy. To do so, we expanded the previous model by either *size* or by *relatedness to the*

canopy and the corresponding interaction terms, e.g.: *distance to conspecific adult*, *fungicide*, *size of the conspecific*, *size of the conspecific:fungicide*, and *distance to conspecific adult:size of the conspecific:fungicide*.

Finally, we tested whether fungal-mediated effects of adults on seedlings were species- or lineage-specific. To do so, we explained seedling mortality, budburst and leaf herbivory by alternative measure of distance: (a) *distance to heterospecific adult*, *fungicide* and the *distance to heterospecific adult:fungicide* interaction term; and (b) *evolutionary distance to the closest adult*, *fungicide* and the *evolutionary distance to the closest adult:fungicide* interaction term.

Here and below, we chose to work with multiple models rather than a single model incorporating all explanatory variables as such a 'super-model' would be overparameterized and existing relationships might be lost due to multicollinearity among variables.

(ii) EM fungi and fungal-driven decomposition are related to increased seedling performance

We explained seedling mortality by fungal-driven decomposition of local oak leaves or of cellulose (GLMM with a binomial error distribution). Note that we cannot explain mortality by mycorrhization as mycorrhization could only be assessed on surviving seedlings. We also explained budburst delay and leaf herbivory by either (a) EM colonization (total and by the four EM fungal species: *C. geophilum*, *Cortinarius* sp., *L. chrysorrheus* and '*brown*'); (b) EM fungal richness; and (c) fungal-driven decomposition (oak leaf and cellulose decomposition). Reciprocal transformation was necessary for budburst delay to approach normality and homoscedasticity. We included both seedlings treated and not treated by fungicide, as fungicide allows testing the effect of a wider range of mycorrhization on seedling budburst and leaf herbivory.

(iii) Nearness to a conspecific adult relates to increased seedling EM and fungal-driven decomposition, and these relationships are strongest under a large conspecific adult or a closely related canopy

We explained each variable characterizing EM colonization (total and by each of the four EM fungal species) by: distance to conspecific adult, using GLMMs with a binomial error distribution.

For this purpose, we only considered seedlings not treated by fungicide, and used data on how many root tips are or are not colonized. Variables of taproot depth and/or litter accumulation were added in the model if that significantly influenced the corresponding variable of mycorrhization (Appendix S6). Second, we explained EM fungal richness by *distance to conspecific adult*, using a GLMM with a Poisson error distribution, again considering only seedlings not treated by fungicide. Finally, we explained fungal-driven decomposition (either of local oak leaves or of cellulose) by *distance to conspecific adult*, using LMMs.

Then, we tested whether the effect of distance to conspecific adult depends on either the size of the conspecific or the dominance of closely related species in the canopy. To do so, we added into each previous model either *size of the conspecific adult* or *relatedness to the canopy* and the corresponding interaction term with distance, e.g. *distance to conspecific adult*, *size of the conspecific adult*, and the *distance to conspecific adult:size of the conspecific adult* interaction term.

We considered relationships at $R^2m<0.05$ as biologically non relevant and did not retain them (excluding 5 relationships at $R^2m=0.01$, 0.02 or 0.04).

Results

The dependent variables we will use below to test hypotheses showed strong variation in budburst delay (0-110 days, average 47 days) and leaf herbivory (0-100%, average 59%). Mortality was 66% after exclusion of the 44 seedlings that died the first year. Moreover, high seedling mortality related to late budburst ($z=2.94$, $P=0.0033$, $R^2m=0.074$, $R^2c=0.16$, n=171) and to high leaf herbivory ($z=3.33$, $P=0.00086$, $R^2m=0.082$, $R^2c=0.18$, n=177).

(i) FUNGI MEDIATED THE RELATIONSHIP BETWEEN SEEDLINGS AND NEARBY CONSPECIFIC ADULTS

Seedlings planted near conspecific adults suffered higher levels of herbivory than seedlings away from conspecifics. This relationship was likely mediated by fungi as seedlings treated with

fungicide tended to show the reverse relationship (interaction term in Fig. 2a and Appendix S7). In contrast, mortality or time to budburst did not differ between seedlings planted near conspecific adults and such away from conspecifics, whatever the fungicide application (Table S7a). The size of the conspecific adult was not related to mortality, budburst and leaf herbivory of the nearby seedlings, whatever the fungicide application (Table S7a). Under a closely related canopy, control seedlings planted near conspecific adults burst buds together with seedlings away from conspecifics, while fungicide-treated seedlings burst buds later when planted closer to conspecific adults (Fig. 2b). In a distantly related canopy, the relationships inversed (Fig. 2b). For herbivory and mortality, in contrast, we found no such statistical interactions between canopy relatedness, nearness to conspecific adult and fungicide treatment (Table S7a).

Nearness to heterospecific adults and the evolutionary distance to the nearest adult tree were related to seedling performance, but these relationships did not depend on fungicide application (Table S7b). Specifically, mortality decreased and budburst accelerated with nearness to heterospecific adult or with evolutionary distance to nearest adult; herbivory decreased with evolutionary distance to nearest adult, with or without fungicide.

(ii) EM FUNGI RELATED WITH REDUCED SEEDLING MORTALITY, ACCELERATED BUDBURST, AND HERBIVORY, BUT FUNGAL-DRIVEN DECOMPOSITION RELATED TO INCREASED LEAF HERBIVORY

We were not able to test a relationship between EM and seedling mortality as EM could only be assessed on surviving seedlings. Among seedlings alive at the end of the study period, for which we could measure EM, EM was related to budburst delay and leaf herbivory (Table 1). Specifically, a high colonization of seedlings by *Cortinarius* sp. related to early seedling budburst. Seedlings with different EM associations had different levels of herbivory. Specifically, a high total EM colonization of seedlings related to low leaf herbivory, but a high colonization of seedlings by *C. geophilum* related to high leaf herbivory.

Fungal-driven decomposition was related to leaf herbivory, but not to seedling mortality and budburst (Table 1): high rates of oak leaf decomposition were related to high levels of seedling leaf herbivory, but no such relationship was found for cellulose decomposition.

(iii) NEARNESS TO A CONSPECIFIC ADULT RELATED TO INCREASED SEEDLING EM AND FUNGAL-DRIVEN DECOMPOSITION, AND THESE RELATIONSHIPS WERE STRONGEST UNDER A LARGE CONSPECIFIC ADULT OR A CLOSELY RELATED CANOPY

Seedlings planted near a conspecific adult had a higher colonization by *L. chrysorrheus* (Fig. 3e). Oak leaves located near a conspecific adult were more decomposed (Fig. 3c), contrary to cellulose (Appendix S9). Relationships to nearness of adults depended on the size of adult and on the evolutionary relatedness of the canopy. Specifically, seedlings planted near a large conspecific adult had a lowest colonization by *C. geophilum* (Fig. 3a), but a highest colonization by '*brown*' EM fungi (Fig. 3b). Oak leaves located near a large conspecific adult were most decomposed (Fig. 3c), contrary to cellulose (Appendix S9). Under a closely related canopy, seedlings planted near the conspecific adult had a higher colonization by *Cortinarius s*p. (Fig. 3d) and *L. chrysorrheus* (Fig. 3e). In contrast, relatedness to the ambient canopy had no statistical effect on oak leaf and cellulose decomposition (Appendix S9).

Discussion

We found that seedlings tended to perform differently in different adult neighbourhoods, reflecting the impact of belowground fungi. Particularly, colonization by EM and fungal-driven decomposition of oak leaves were related to seedling performance and both were controlled by conspecific adults, as summarized in Fig. 4. Overall, we found weak, but existent, Janzen-Connell-like patterns of enemy pressure. However, correlative evidence suggests that these conspecific inhibitions resulted from the effect of adult trees on fungal-driven decomposition of neighbourhood-specific substrates rather than from a direct effect of adults on enemy abundance (Fig. 4). Moreover, conspecific facilitation appears to be caused by EM fungi in a canopy dominated by closely related

species. Both aspects, fungal-driven decomposition and evolutionary relatedness to the ambient canopy have been little or not considered in earlier studies.

LIMITATIONS OF OUR STUDY

Our study could present some limitations. First, we found no effect of fungi on seedling mortality, suggesting that ultimately fungi may have little effect. However, this would be a premature conclusion. While the observed total seedling mortality of 70% in two years appears impressive, almost all of the remaining seedlings are still to die as from hundreds or thousands of seedlings eventually only a single major adult remains (Leck, Parker & Simpson 2008). This major future mortality might be driven by the observed effects of fungi on processes related to seedling mortality (budburst and leaf herbivory). Overall, short-term studies have an interest in accounting also for processes related to seedling mortality, not just for mortality per se, in order to identify patterns during the first years of seedling development. Second, the explained variances of relationships tested were partly low. This, however, seems normal in studies on seedlings, which often do not show scatter of data points or r², or r² only across averages. The problem might be that compared to an adult, a seedling can only little integrate and 'average out' multiple impacts and hence tends to either flourish or perish. Third, we explained seedling budburst and leaf herbivory by mycorrhization. However, we cannot certify that mycorrhization is the cause of herbivory or budburst rather than the reverse, as we did not manipulate specifically seedling mycorrhization. This cause-or-effect uncertainty seems normal in such analyses (e.g. Sthultz *et al.* 2009; Barto & Rillig 2010).

SEEDLINGS PLANTED NEAR CONSPECIFIC ADULTS WERE LESS PERFORMANT – POTENTIALLY DUE TO FUNGAL-DRIVEN SPECIALIZED DECOMPOSITION

Albeit we found little effect of belowground fungi on plant survival, we did find an effect on insect herbivory. Specifically, near conspecific adults, seedlings suffered from fungi. Such a Janzen-Connell-like pattern has previously been demonstrated for some, albeit by far all, species in temperate forests (Bennett *et al.* 2017) and has mostly been explained by the activity of belowground pathogenic fungi around the conspecific adult (Packer *et al.* 2000; Masaki *et al.* 2002; Reinhart *et al.* 2009).

However, correlative evidence in our study suggests that fungi near adults seemed to affect seedlings by increasing leaf herbivory, and this increased leaf herbivory might be explained by fungal-driven decomposition, albeit we could not show effects on mortality (Fig. 4; we recall that some of the fungal decomposers might be EM fungi, Shah *et al.* 2016, or soil-borne pathogens, Hiruki & Teakle 1987). Fungal-driven decomposition might increase near the conspecific adult potentially because of a home-field advantage (Gholz *et al.* 2000; Veen *et al.* 2015): the litter is more efficiently decomposed in its original environment, due to a tendency of a local, effective specialization of the fungal decomposers on the most encountered litter-type (reviewed by Austin *et al.* 2014), notably through an increased match between decomposer physiology and litter chemistry (van der Wal *et al.* 2013), albeit other variables are important, too (Hobbie *et al.* 2006). We found correlative support for such specialization of decomposers for proximate conspecific adults given that close to adult trees decomposition rate was higher in the specific substrate than in the unspecific one (cellulose) (Appendix S9). Such a possible specialized litter-decomposition by fungi is consistent with earlier studies that have demonstrated specificity of fungal decomposers to particular litter species or litter chemistries, including to oaks or their high-tannins concentration (Gessner & Chauvet 1994; Baldrian & Valaskova 2008; Austin *et al.* 2014). The relationships between nearness to adults and decomposition require further investigation by litter-transplant experiments, but if confirmed such a specialized litter-decomposition activity near the conspecific adult could enhance nutritional quality of seedling foliage by increasing foliar nitrogen concentration, hence attracting and feeding herbivores (Kraft & Denno 1982; Herms 2002). Overall, we detected a Janzen-Connell-like pattern of aboveground herbivory, but induced by belowground fungi, supposedly by saprotrophs increasing nutritional quality of seedling for herbivores. To fully confirm and understand these relationships, future studies should measure leaf foliar chemistry near and far from conspecifics and should aim at selectively excluding saprotrophs or EM (as also EM might increase nutrient uptake and thereby the nutritional quality of seedlings for herbivores).

We detected no relationship between the size of the conspecific adult on fungal-mediated effects of adults on nearby seedlings. This result is consistent with Packer & Clay (2004) who found

no effect of the age of forest soils on seedling performance, but inconsistent with studies suggesting that bigger/older conspecific adults impose higher mortality of nearby seedlings and hence stronger Janzen-Connell patterns (Janzen 1970; see Packer *et al.* 2000 for tentative evidence). These authors proposed that old-growth adults may have accumulated pathogens for a longer time and, as old-growth adults are larger, they might attract and host more pathogens. This is consistent with our observation that nearness to a large adult related to increased litter decomposition, assuming that in the field many decomposers do show some effective specificity for particular litter species. Increased litter decomposition, in turn, related to increased leaf herbivory. However, our study has not revealed a net negative effect of fungi on seedlings near large conspecific adult as it might be expected (Bennett *et al.* 2017). A possible explanation is that large conspecific adults could have accumulated mutualists as much as they have accumulated enemies. For now, we cannot bring support to this hypothesis: we found that colonization by 'brown' EM fungi increased near a large adult (Fig. 3b), but 'brown' EM fungi had no effect on seedling budburst and leaf herbivory (Table 1). Overall, in our study system, we could not detect a significant difference between large and small adults in how they affect seedlings via fungi, possibly because accumulation of enemies and of mutualists mutually offset each other (Bennett *et al.* 2017).

IN A CANOPY DOMINATED BY CLOSE RELATIVES, ADULT OAKS APPEAR TO SUPPORT NEARBY OAK SEEDLINGS – VIA EM FUNGI

Under a canopy dominated by oaks and close relatives we observed conspecific facilitation: seedlings near a conspecific adult benefit from a positive net effect of fungi, if this adult was located under a closely related canopy. This result suggests that a lineage supports its adults supporting their conspecific offspring, hence increasing the fitness of the lineage members (whereas evolutionary relatedness between a seedling and its single nearest adult neighbours has no impact, consistent with Dickie *et al.* 2006). To our knowledge, such an effect has never been demonstrated before. We suggest that, due to a conservatism of biotic interactions, supportive fungi might accumulate as a tree lineage establishes within a canopy. Individual trees can thus more easily support conspecific seedlings via supportive fungi, which might offset a possible effect of pathogenic ones (in particular if

these supportive fungi are EM, Bennett *et al.* 2017). In our study system, seedlings near a conspecific adult were often more infected by *Cortinarius* sp., provided that this adult grew in a canopy dominated by close relatives (Fig. 4). This result is consistent with the idea that adult trees represent a reservoir of EM fungi from which nearby seedlings can be colonised (Frank *et al.* 2009) and with the observation of increased availability of mycorrhiza to seedlings in late successional stages (Dickie & Reich 2005). However, this result is inconsistent with Yguel *et al.* (2014) showing that an adult tree located in a canopy dominated by close relatives invests less into ectomycorrhiza compared to an isolated one which may depend on ectomycorrhiza to persist in a stressful environment.

The observed positive effect of fungi close to conspecific adults in a close related neighbourhood is consistent with our observations on EM. In our study system, high seedling colonization by *Cortinarius* sp. related to accelerated budburst. Such a facilitation of oak budburst in early season has already been shown for *Lactarius quietus*: albeit C dynamics as such had not been studied, correlative evidence suggests that this EM fungus acts as a saprotroph and provides the carbon required for leaf production, when photosynthetates are not yet available and oaks cannot meet their carbon requirement themselves (Courty *et al.* 2007). *Lactarius quietus* is a 'contact' exploration fungal type, which produces few external mycelia compared to *Cortinarius* sp., a 'medium-distance fringe' exploration taxa (Agerer 2001). It might be speculated that *Cortinarius* sp. might hence be even more capable of transferring carbon to seedlings (Deslippe *et al.* 2011) and thereby accelerating budburst (Courty *et al.* 2007). Overall, relatives might help adults supporting their seedlings via particular functional types of mycorrhiza.

HETEROSPECIFIC ADULTS DO NOT APPEAR TO AFFECT NEARBY SEEDLINGS VIA FUNGI, INDEPENDENT OF EVOLUTIONARY PROXIMITY

Contrary to conspecific adults, heterospecific adults, even if evolutionary proximate, showed no fungal-mediated effect on nearby seedlings. This result is inconsistent with Maron *et al.* (2011) who found that higher species diversity (i.e. more heterospecifics) correspond to a less negative impact of pathogenic belowground fungi. This result is also inconsistent with the phylogenetic Janzen-Connell effect (Liu *et al.* 2012), and suggests that such fungal-mediated interactions are tree-

species specific, but not lineage-specific (albeit the lineage may support adults in supporting conspecific seedlings). Surprisingly, seedlings planted near heterospecific (or evolutionary distant) adults were mostly more performant – but not due to fungi (Table S7b). Such species-specificity in adult-fungus-seedling interactions is unexpected, when considered that EM fungi are more generalists than specialists (Selosse *et al.* 2006). However, this concept of specificity in mycorrhizal interactions might drastically increase the role of these interactions for structuring tree-species communities. In fact, a key component in JCH is the specificity of the interactions: an enemy has a distinct preference for one or few among many available hosts, preferring a given adult tree attack nearby conspecific seedlings but less heterospecific seedlings, thereby enhancing forest diversity. If enemies were entirely generalists, seedlings of all species would die under adults, leading to a tree-free area. Overall, we detected that interactions between trees, seedlings, EM fungi and even fungal-driven decomposition show weak but significant patterns that are consistent with species-specificity, and are thus important to consider for explaining forest structure, as are the enemies.

Conclusion

We found that a conspecific adult has a negative fungal-mediated effect on nearby seedlings, a pattern already suggested by JCH. However, contrary to the mechanism proposed by JCH, this increased enemy pressure close to adults might result from fungal-driven specialized decomposition favouring nutritional quality of seedling foliage and hence attracting herbivore enemies, albeit the evidence remains associational (see Austin *et al.* 2014 and Gessner & Chauvet 1994, Baldrian & Valaskova 2008 for specialized decomposition). Moreover, we demonstrated that fungi near a conspecific adult can result in patterns opposite to JCH pattern, i.e. in conspecific facilitation, by enhancing seedling performance in a particular case: when the conspecific adult is located in a canopy dominated by close relatives. This adult-seedling support is likely mediated by EM fungi. Such spatial interactions between trees and seedlings appear to be mediated by tree-specific supportive fungi, which might influence forest structure no less than do enemy-mediated interactions. Future research may focus on the implications of our results for both forestry and evolutionary biology. Can the natural regeneration of oaks be favoured by a closely related adult canopy? And: If close related

adults support each other supporting their conspecific seedlings, does this ultimately select for maintaining a similar niche, *i.e.* phylogenetic niche conservatism?

Authors' Contributions

MD, VJ, CLL, AP conceived the ideas and designed methodology; MD, BB, TM collected the data; MD analysed the data; MD, VJ, AP led the writing of the manuscript. HK did the molecular analysis. All authors contributed critically to the drafts and gave final approval for publication.

Data Accessibility

Data Accessibility section: Data deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.hf83v, (Deniau et al., 2017).

References

Agerer, R. (1987) *Colour Atlas of Ectomycorrhizae*. Einhorn-Verlag, Schwäbisch, Gmünd.

Agerer, R. (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza*, **11**, 107-114.

Austin, A.T., Vivanco, L., Gonzalez-Arzac, A. & Pérez, L.I. (2014) There's no place like home? An exploration of the mechanisms behind plant-litter decomposer affinity in terrestrial ecosystems. *New Phytologist*, **204**, 307-314.

Baldrian, P. & Valaskova, V. (2008) Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiology Reviews*, **32**, 501-521.

Barto, E.K. & Rillig, M.C. (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. *Journal of Ecology*, **98**, 745-753.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1-48.

Bennett, J.A., Maherali, H., Reinhart, K.O., Lekberg, Y., Hart, M.M. & Klironomos, J. (2017) Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, **355**, 181-184.

Berg, B. & McClaugherty, C. (2008) *Plant Litter, decomposition, humus formation, carbon sequestration* (2nd edition). Springer-Verlag, Berlin.

Besford, R.T., Hand, P., Richardson, C.M. & Peppitt, S.D. (1996) Photoperiod effect on bud burst in *Prunus* is phase dependent: significance for early photosynthetic development. *Tree Physiology*, **16**, 491-496.

Biswas, T.D., Mukherjee, S.K. (1994). *Textbook of Soil Science* (2nd edition). Tata McGraw-Hill Publishing Company Limited, New Delhi.

Bruns, T.D., Bidartondo, M.I. & Taylor, D.L. (2002) Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integrative and comparative biology*, **42**, 352-359.

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A. & Kembel, S.W. (2009) The merging of community ecology and phylogenetic biology. *Ecology Letters*, **12**, 693-715.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., Beckman, N. & Zhu, Y. (2014) Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology*, **102**, 845-856.

Connell, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *Dynamics of Populations* (eds P. J. den Boer & G. R. Gradwell), pp. 298-312. PUDOC, Wageningen.

Courty, P.E., Bréda, N. & Garbaye, J. (2007) Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. *Soil Biology and Biochemistry*, **39**, 1655-1663.

Davies, F.T. (2008) Opportunities from down under: how mycorrhizal fungi can benefit nursery propagation and production systems. *Combined Proceedings International Plant Propagators' Society*, **58**, 539-548.

Deniau M, Jung V, Le Lann C, Kellner H, Béchade B, Morra T, Prinzing A (2017) Data from: Janzen-Connell patterns can be induced by fungal-driven decomposition and offset by ectomycorrhizal fungi accumulated under a closely related canopy. *Dryad Digital Repository*. Doi:10.5061/dryad.hf83v

Deslippe, J.R., Hartmann, M., Mohn, W.W. & Simard, S.W. (2011) Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. *Global Change Biology*, **17**, 1625-1636.

Dickie, I.A. & Reich, P.B. (2005) Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology*, **93**, 244-255.

Dickie, I.A., Oleskyn, J., Reich, P.B., Karolewski, P., Zythowiak, R., Jagodzinski, A.M. & Turzanska, E. (2006) Soil modification by different tree species influences the extent of seedling ectomycorrhizal infection. *Mycorrhiza*, **16**, 73-79.

Dickie, I.A., Koele, N., Blum, J.D., Gleason, J.D. & McGlone, M.S. (2014) Mycorrhizas in changing ecosystems. *Botany*, **92**, 149-160.

Frank, J.L., Anglin, S., Carrington, E.M., Taylor, D.S., Viratos, B. & Southworth, D. (2009) Rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal networks on *Quercus garryana*. *Botany*, **87**, 821-829.

Gange, A.C., Gane, D.R.J., Chen, Y. & Gong, M. (2005) Dual colonization of *Eucalyptus urophylla* S.T. Blake by arbuscular and ectomycorrhizal fungi affects levels of insect herbivore attack. *Agricultural and Forest Entomology*, **7**, 253-263.

Gessner, M.O. & Chauvet, E. (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, **75**, 1807-1817.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E. & Parton, W.J. (2000) Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology*, **6**, 751-765.

Gorzelak, M.A., Asay, A.K, Pickles, B.J. & Simard, S.W. (2015) Inter-plant communication through mycorrhizal networks mediates complex adaptive behavior in plant communities. *Annals of Botany Plants*, **7**, plv050.

Hartley, S.E. & Gange, A.C. (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annual Review of Entomology*, **54**, 323-342.

Herms, D.A. (2002) Effects of fertilization on insect resistance of woody ornamental plants: reassessing an entrenched paradigm. *Environmental Entomology*, **31**, 923-933.

Hiruki, C. & Teakle, D.S. (1987) Soil-borne viruses of plants. *Current Topics in Vector research*, vol 3 (ed. K.F. Harris), pp. 177-215. Springer-Verlag, New-York.

Hobbie, S.E., Reich, P.B., Oleksyn, J., Ogdahl, M., Zytkowiak, R., Hale, C. & Karolewski, P. (2006) Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology*, **87**, 2288-2297.

Hyatt, L.A., Rosenberg, M.S., Howard, T.G., Bole, G., Fang, W., Anastasia, J., Brown, K., Grella, R., Hinman, K., Kurdziel, J.P. & Gurevitch, J. (2003) The distance dependence prediction of the Janzen-Connell hypothesis: a meta-analysis. *Oikos*, **103**, 590-602.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *The American Naturalist*, **104**, 501-528.

Kraft, S.K. & Denno, R.F. (1982) Feeding responses of adapted and non-adapted insects to the defensive properties of *Baccharis halmifolia* L. *Oecologia*, **52**, 156-163.

Leck, M.A., Parker, T.V. & Simpson, R.L. (2008) *Seedling Ecology and Evolution*. Cambridge University Press, Cambridge.

Legendre, P., Dale, M.R.T., Fortin, M.J., Casgrain, P. & Gurevitch, J. (2004) Effects of spatial structures on the results of field experiments. *Ecology*, **85**, 3202-3214.

Liang, M., Liu, X., Etienne, R.S., Huang, F., Wang, Y. & Yu, S. (2015) Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology*, **96**, 562-574.

Liu, X., Liang, M., Etienne, R.S., Wang, Y., Staehelin, C. & Yu, S. (2012) Experimental evidence for a phylogenetic Janzen-Connell effect in a subtropical forest. *Ecology Letters*, **15**, 111-118.

Maron, J.L., Marler, M., Klironomos, J.N. & Cleveland, C.C. (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters*, **14**, 36-41.

Martin-Pinto, P., Pajares, J. & Diez, J. (2006) *In vitro* effects of four ectomycorrhizal fungi, *Boletus edulis*, *Rhizopogon roseolus*, *Laccaria laccata* and *Lactarius deliciosus* on *Fusarium* damping off in *Pinus nigra* seedlings. *New Forests*, **32**, 323-334.

Masaki, T. & Nakashizuka, T. (2002) Seedling demography of *Swida controversa*: effect of light and distance to conspecifics. *Ecology*, **83**, 3497-3507.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133-142.

Nara, K. (2008) Community developmental patterns and ecological functions of ectomycorrhizal fungi: implications from primary succession. *Mycorrhiza: state of the art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics* (ed A. Varma), pp. 581-599. Springer-Verlag, Berlin.

Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, **404**, 278-281.

Packer, A. & Clay, K. (2004) Development of negative feedback during successive growth cycles of black cherry. *Proceedings of the Royal Society of London B: Biological Sciences*, **271**, 317-324.

R Core Team (2014) R: A language and microenvironment for statistical computing. R Foundation for Statistical Computing, Vienna.

Reddy, M.S. & Natarajan, K. (1995) Effects of the fungicide Dithane M-45 on the growth and mycorrhizal formation of *Pinus patula* seedlings. *Soil Biology and Biochemistry*, **27**, 1503-1504.

Reinhart, K.O. & Clay, K. (2009) Spatial variation in soil-borne disease dynamics of a temperate tree, *Prunus serotina*. *Ecology*, **90**, 2984-2993.

Seiwa, K. (1998) Advantages of early germination for growth and survival of seedlings of *Acer mono* under different overstorey phenologies in deciduous broad-leaved forest. *Journal of Ecology*, **86**, 219-228.

Selosse, M.A., Richard, F., He, X. & Simard, S.W. (2006) Mycorrhizal networks: *des liaisons dangereuses*? *Trends in Ecology and Evolution*, **21**, 621-628.

Shah, F., Nicolas, C., Bentzer, J., Ellström, M., Smits, M., Rineau, F., Canbäck, B., Floudas, D., Carleer, R., Lackner, G., Braesel, J., Hoffmeister, D., Henrissat, B., Ahrén, D., Johansson, T., Hibbett, D.S., Martin, F., Persson, P. & Tunlid, A. (2016) Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytologist*, **209**, 1705-1719.

Smith, S.E. & Read, D.J. (2008) *Mycorrhizal symbiosis*. Academic Press, London.

Southwood, T.R.E., Wint, G.R.W., Kennedy, C.E.J. & Greenwood, S.R. (2004) Seasonality, abundance, species richness and specificity of the phytophagous guild of insects on oak (Quercus) canopies. *European Journal of Entomology*, **101**, 43-50.

Sthultz, C.M., Whitham, T.G., Kennedy, K., Deckert, R. & Gehring, C.A. (2009) Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species. *New Phytologist*, **184**, 657-667.

Tedersoo, L., Mett, M., Ishida, T.A. & Bahram, M. (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist*, **199**, 822-831.

Terborgh, J. (2012) Enemies maintain hyperdiverse tropical forests. *The American Naturalist*, **179**, 303-314.

Teste, F.P., Simard, S.W. & Durall, D.M. (2009) Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. *Fungal ecology*, **2**, 21-30.

Van der Wal, A. Geydan, T.D., Kuyper, T.W. & de Boer, W. (2013) A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews*, **37**, 477-494.

Veen, G.F., Freschet, G.T., Ordonez, A. & Wardle, D.A. (2015) Litter quality and environmental controls of home-field advantage effects on litter decomposition. *Oikos*, **124**, 187-195.

Vialatte, A., Bailey, R.I., Vasseur, C., Matocq, A., Gossner, M.M., Everhart, D., Vitrac, X., Belhadj, A., Ernoult, A. & Prinzing, A. (2010) Phylogenetic isolation of host trees affects assembly of local Heteroptera communities. *Proceedings of the Royal Society of London B: Biological Sciences*, **277**, 2227-2236.

Wada, N., Murakami, M. & Yoshida, K. (2000) Effects of herbivore-bearing adult trees of the oak *Quercus crispula* on the survival of their seedlings. *Ecological Research*, **15**, 219-227.

Wang, B. & Qiu, Y.L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, **16**, 299-363.

Yguel, B., Bailey, R., Tosh, D.N., Vialatte, A., Vasseur, C., Vitrac, X., Jean, F. & Prinzing, A. (2011) Phytophagy on phylogenetically isolated trees: why hosts should escape their relatives. *Ecology Letters*, **14**, 1117-1124.

Yguel, B., Courty, P.E., Jactel, H., Pan, X., Butenschoen, O., Murray, P.J. & Prinzing, A. (2014) Mycorrhizae support oaks growing in a phylogenetically distant neighbourhood. *Soil Biology and Biochemistry*, **78**, 204-212.

TABLES

Table 1 Seedlings were mostly positively related to EM, but negatively related to fungal-driven decomposition. (a) A high total EM colonization related to decreased leaf herbivory but a high colonization by *Cenococcum geophilum* related to increased leaf herbivory, *Cortinarius* sp. related to accelerated budburst; (b) EM fungal richness has no clear relationship to leaf herbivory; (c) fast oak leaf decomposition was related to high levels of seedling leaf herbivory, but not to mortality or budburst delay. Microenvironmental variables (soil temperature and density of conspecific seedlings) were included in each model, but not reported here (Appendix S8 for full models). 'Tree pair' was included in each model as random effect and was not tested. NA indicate relationships that could not be tested. One residual outlier was identified and excluded from budburst delay variable. Bold values represent statistical significance ((*) P < 0.1, * P < 0.05, ** P< 0.01, *** P <0.001).

|			Seedling mortality	Budburst delay (reciprocally transformed)	Leaf herbivory
			z value	*t* value	*t* value
	Total EM colonization		NA	-0.4 ns	**-2.9 **
	Cenococcum geophilum		NA	-0.1 ns	**2.5 ***
	Cortinarius sp.		NA	**2.1 ***	-0.7 ns
a	*Lactarius chrysorrheus*		NA	1.1 ns	1.0 ns
	'brown'		NA	1.4 ns	1.3 ns
		R²m, R²c	NA	0.16, 0.28	0.13, 0.21
		n		95	98
	EM fungal richness		NA	0.9 ns	**-1.8 (*)**
b		R²m, R²c	NA	0.09, 0.17	0.05, 0.14
		n		95	98
	Oak leaf decomposition		1.0 ns	-0.7 ns	**2.4 ***
	Cellulose decomposition		0.4 ns	-0.6 ns	1.4 ns
c		R²m, R²c	0.04, 0.18	0.06, 0.13	0.11, 0.11
		n	355	171	177

EM: ectomycorrhizal
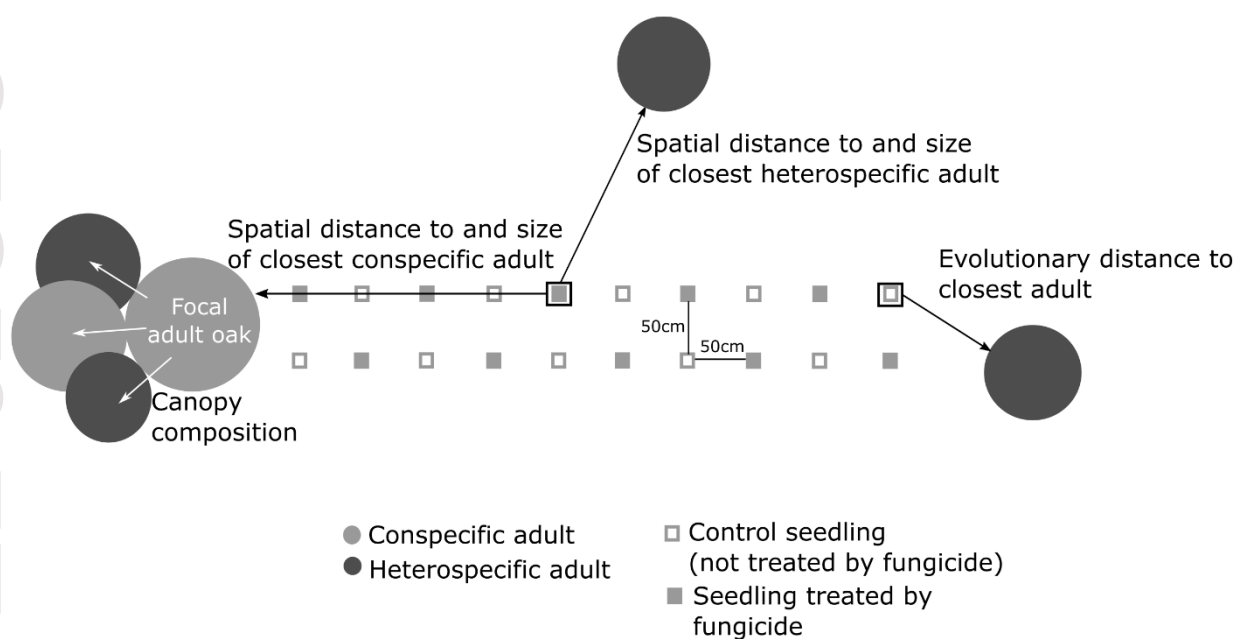
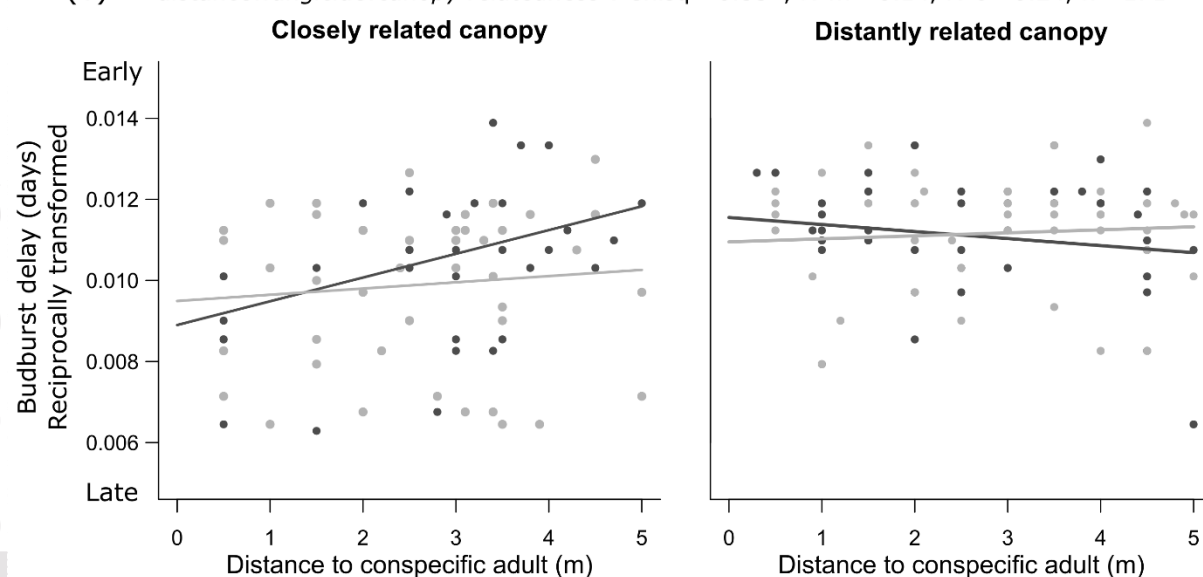Fig. 1. Experimental set-up (established for 20 focal oaks). See Methods for details.

Distance to conspecific adult

(a) *distance:fungicide* : Chisq= 4.30*,
R²m= 0.10, R²c= 0.13, n= 177
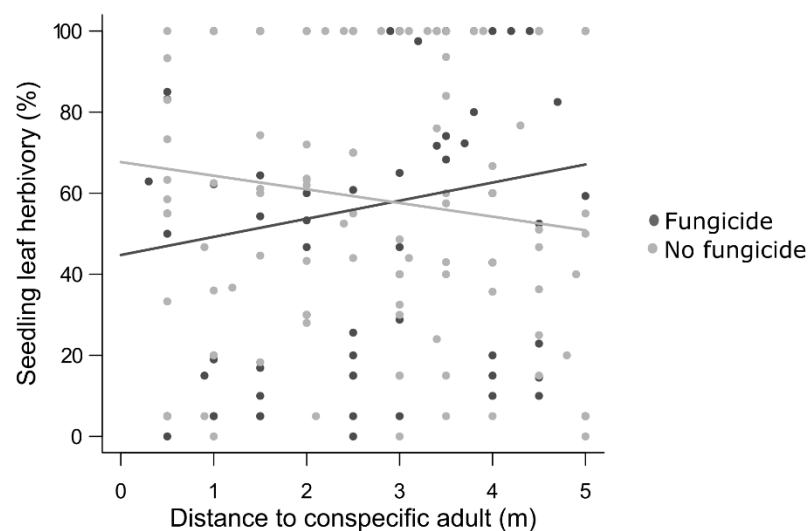
(b) *distance:fungicide:canopy relatedness* : Chisq= 6.35*, R²m= 0.14, R²c= 0.24, n= 171

Distance to heterospecific adult
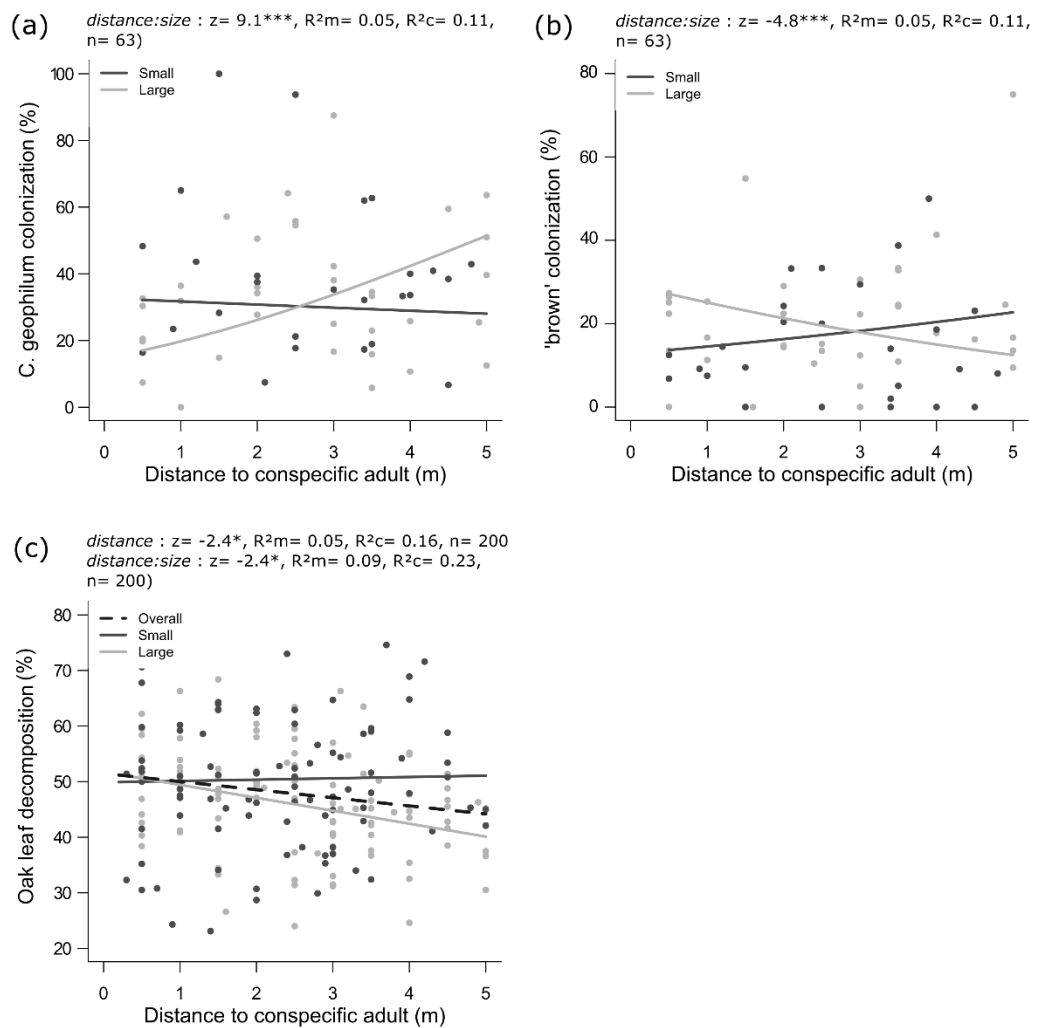
(none)

Evolutionary distance to the closest adult

(none)

Fig. 2. Significant fungal-mediated relationships between adult neighbourhood and seedlings
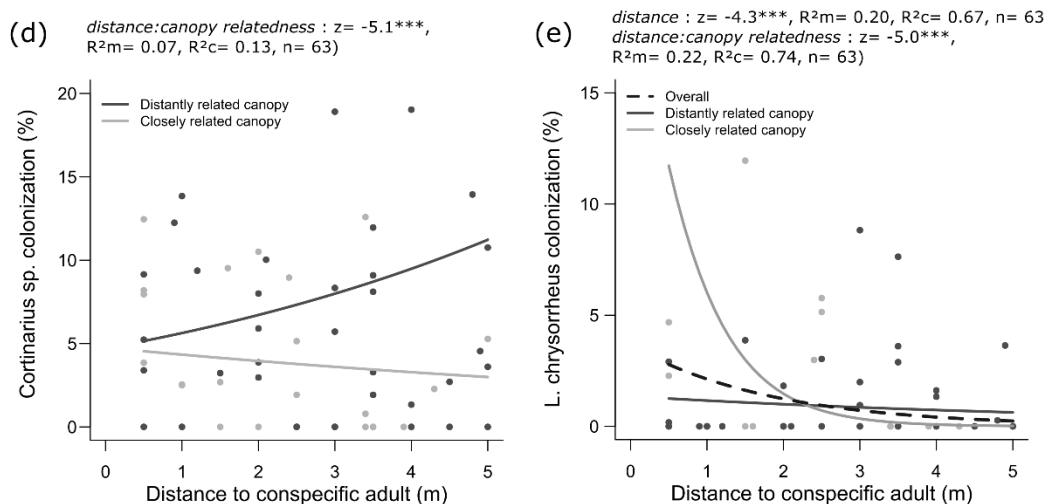
(Appendix S7 for complete results showing that the co-variables temperature or oak-seedling density

were rarely significant). Only conspecific adults had a fungal-mediated effect on nearby seedlings. (a)

Nearness to conspecific adult related to increased seedling leaf herbivory due to fungi; (b) nearness to a conspecific adult located in a canopy dominated by closely related species did not relate to budburst in the presence of fungi but to delayed budburst in their absence. The relationships tended to inverse in a canopy dominated by distantly related species (one residual outlier was identified and excluded from budburst delay variable). Dots correspond to observed values; lines correspond to values predicted by the models. *Relatedness to the canopy* was used as continuous variable in statistical tests, but categorized in two groups for visual representation using the median (distantly related canopy≥median, *i.e.* 51.03MYBP). Explanatory variables not shown in the figure but included in the model were set to their mean values.

Size of the conspecific adult

(a) *distance:size* : z= 9.1***, R²m= 0.05, R²c= 0.11, n= 63)

(b) *distance:size* : z= -4.8***, R²m= 0.05, R²c= 0.11, n= 63)

(c) *distance* : z= -2.4*, R²m= 0.05, R²c= 0.16, n= 200
distance:size : z= -2.4*, R²m= 0.09, R²c= 0.23, n= 200)

Relatedness to the ambient canopy

(d) *distance:canopy relatedness* : z= -5.1***, R²m= 0.07, R²c= 0.13, n= 63)

(e) *distance* : z= -4.3***, R²m= 0.20, R²c= 0.67, n= 63
distance:canopy relatedness : z= -5.0***, R²m= 0.22, R²c= 0.74, n= 63)

Fig. 3. Significant relationships between conspecific adults and seedling mycorrhization and fungal-driven decomposition, according to size of the conspecific or its relatedness to the

ambient canopy (Appendix S9 for complete results). (a) seedling colonization by *C. geophilum* decreased near large conspecific adult; (b) seedling colonization by 'brown' increased near large conspecific adult; (c) oak leaf decomposition increased near conspecific adult, notably when large; (d) seedling colonization by *Cortinarius* sp. increased near a conspecific adult under a closely related canopy; (e) seedling colonization by *L. chrysorrheus* increased near conspecific adult, notably when located under a closely related canopy. Dots correspond to observed values; lines correspond to values predicted by the models. Overall relationships were tested with models including *distance to conspecific* only; interaction terms were tested with models containing *distance to conspecific* and *size* or *canopy relatedness*, and the corresponding interaction term. *Size* and *canopy relatedness* were used as continuous in statistical tests, but categorized in two groups for visual representation using their median (large tree≥0.68 m and distantly related canopy≥51.03 MYBP). Explanatory variables not shown in the figure but included in the model were set to their mean values.
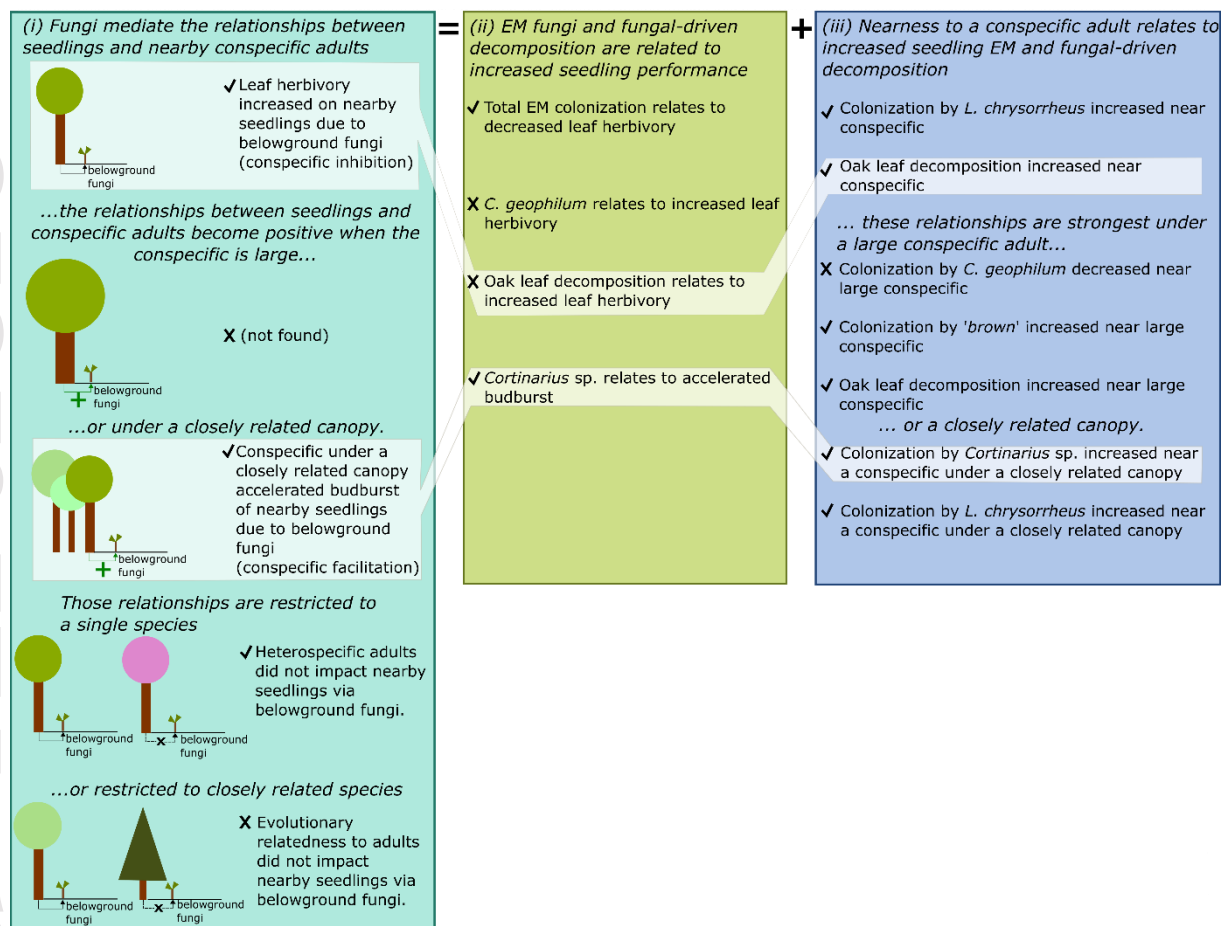
Fig. 4. Hypotheses on how seedlings may be affected by adult trees mediated via belowground fungi, notably mycorrhiza and decomposers, and the corresponding results (Hypotheses are explained by text in italics and figures; Results are explained by text in normal font)**.** Clear shading indicates consistency between the net fungi-mediated effect of adults on seedlings and the combination of effects of fungi on seedlings and adults on fungi. These mutually consistent results suggest that conspecific adults increased herbivory on nearby seedlings due to fungi, potentially fungal decomposers; and that conspecific adults located in a canopy dominated by close relatives accelerate budburst due to fungi, potentially *Cortinarius* sp. Note that while we experimentally excluded fungi and used standard seedlings, most of the ultimate mechanisms by which fungi might act or mediate the action of adult trees remain interpretation and had not been tested directly. Note also that restriction to a species/lineage is a gradual preference and refers only to the species/lineages present in the respective neighbourhoods.