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Plant neighbours can make or break the disease transmission chain of a fungal root pathogen

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Summary

- Biodiversity can reduce or increase disease transmission. These divergent effects suggest that community composition rather than diversity *per se* determines disease transmission. In natural plant communities, little is known about the functional roles of neighbouring plant species in belowground disease transmission.
- Here, we experimentally investigated disease transmission of a fungal root pathogen (*Rhizoctonia solani*) in two focal plant species in combinations with four neighbour species of two ages. We developed stochastic models to test the relative importance of two transmission-modifying mechanisms: (1) infected hosts serve as nutrient supply to increase hyphal growth, so that successful disease transmission is self-reinforcing; and (2) plant resistance increases during plant development.
- Neighbouring plants either reduced or increased disease transmission in the focal plants. These effects depended on neighbour age, but could not be explained by a simple dichotomy between hosts and nonhost neighbours. Model selection revealed that both transmission-modifying mechanisms are relevant and that focal host–neighbour interactions changed which mechanisms steered disease transmission rate.
- Our work shows that neighbour-induced shifts in the importance of these mechanisms across root networks either make or break disease transmission chains. Understanding how diversity affects disease transmission thus requires integrating interactions between focal and neighbour species and their pathogens.

Introduction

The positive relationship between plant biodiversity and productivity (Cardinale *et al.*, 2012; Grace *et al.*, 2016) was initially attributed to belowground resource complementarity among plant species (Tilman, 2001; Barry *et al.*, 2019) and the dominance of productive plant species (i.e. the 'selection effect'; Loreau & Hector, 2001). However, an alternative hypothesis related to pathogens has been gaining ground in the last decade. This hypothesis states that root pathogens accumulate in monocultures compared to species-rich communities, leading to increased productivity in mixtures as pathogens are 'diluted' (Maron *et al.*, 2011; Schnitzer *et al.*, 2011; Bever *et al.*, 2015; Cappelli *et al.*, 2020). Although pathogen dilution seems to be a general pattern in diverse communities (Keesing *et al.*, 2010), the opposite effect – pathogen amplification – has also been reported (Power & Mitchell, 2004; Halliday *et al.*, 2017). Understanding these divergent effects of diversity on pathogen accumulation and disease pressure, requires a better understanding of pathogen transmission in diverse communities (Keesing *et al.*, 2006; Ampt *et al.*, 2019; Collins *et al.*, 2020).

Belowground transmission of plant pathogens is often considered to be a function of the density of host plants (Burdon & Chilvers, 1982; Burdon *et al.*, 2006). Belowground, as well as aboveground, the distance between conspecific host plants is in general smaller in monocultures compared to mixtures. However, recent studies on aboveground pathogens indicate that there are additional effects of neighbouring plants in diverse plant communities. For example, the presence or abundance of particular neighbour species either increased or decreased fungal pathogen infestation or damage in mixed forest communities (Hantsch *et al.*, 2014; Setiawan *et al.*, 2014; Field *et al.*, 2020). Yet, for belowground pathogens the effects of neighbouring plants have rarely been addressed (Otten *et al.*, 2005; Cook *et al.*, 2007). Both aboveground and belowground it matters if the neighbour is a host, a nonhost or an asymptomatic host species for a certain pathogen (Roberts & Heesterbeek, 2020). Although asymptomatic host species do not display any disease symptoms upon colonization of their tissue by a pathogen (Malcolm *et al.*, 2013), their roots can act as 'bridges' for the pathogen to the next susceptible individual plant (Termorshuizen, 2014; Palma-Guerrero *et al.*, 2021). Hence, their presence in the community could

potentially increase pathogen transmission. In addition, nonhost species, which are often treated as 'neutral' players in plant epidemiology, can also affect pathogen transmission. For example, some nonhosts may actively reduce pathogen transmission by secreting antifungal compounds belowground (Bednarek & Osbourn, 2009; Baetz & Martinoia, 2014; Yang *et al.*, 2014) or attracting antagonists of the pathogen (Berendsen *et al.*, 2018; Stringlis *et al.*, 2018). A fundamental understanding of the mechanisms by which neighbouring plant species alter belowground pathogen transmission in diverse plant communities is needed to predict the effects of plant diversity on disease dynamics.

Belowground transmission of many fungal root pathogens, including economically important species such as *Rhizoctonia solani*, primarily occurs via hyphal growth from the roots of an infected plant to those of a susceptible plant (Stacey *et al.*, 2001; Raaijmakers *et al.*, 2009). This transmission results in the characteristic disease patches often observed in agricultural monocultures. Infections resulting from hyphal growth between plants are usually referred to as 'secondary infections', while 'primary infections' are those that arise from individual infectious propagules in the soil, for example at the beginning of a growing season. (Gilligan & Kleczkowski, 1997; Gilligan, 2002). Infected plants serve as a nutrient source for the pathogen and these nutrients enable further hyphal growth on the root surface or through the soil. Therefore, each successful secondary infection may increase

the likelihood of the fungal pathogen finding and infecting its next host sooner (Garrett, 1970; Stacey *et al.*, 2001; Simon *et al.*, 2014) (Fig. 1a). If this mechanism is operating, successful pathogen transmission between plants will be a self-reinforcing process, with each new secondary transmission event being more likely than the last.

Another mechanism that affects pathogen transmission is the susceptibility of a host plant, which often depends on the ontogenetic stage of the plant (Develey-Rivière & Galiana, 2007). If resistance of the plant to a pathogen increases with age as plant development progresses, as has been observed for many seedling pathogens including *R. solani*, this increase in resistance may outpace the growth of the pathogen and thus limit its transmission (Kleczkowski *et al.*, 1996; Otten *et al.*, 2003) (Fig. 1b). Alternatively, Bailey *et al.* (2000) hypothesized that increased root intermingling between host plants with plant age might increase belowground pathogen transmission. The net effect of these mechanisms on pathogen transmission may not only differ between host plant species but may also depend on the presence of neighbouring plants and the host plant's interactions with its neighbours.

Here, we test (1) whether different neighbour species can alter the disease transmission of a fungal root pathogen in two host plant species; and (2) whether neighbours affect the role of transmission-modifying mechanisms (i.e. self-reinforcing increases in
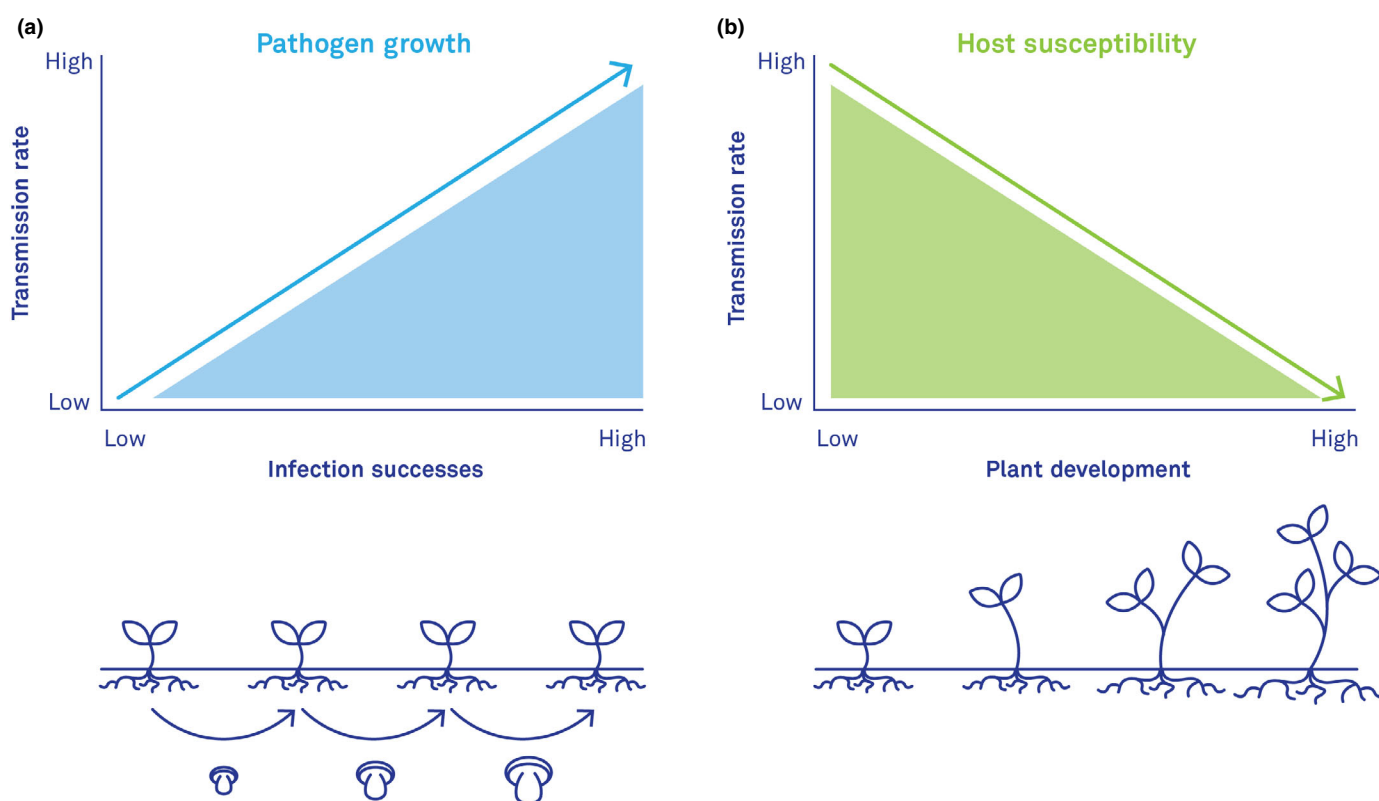
Fig. 1 Conceptual overview of hypothesized mechanisms that modify fungal root pathogen transmission. Belowground pathogen transmission via hyphal growth between host plants can depend on (a) the infection successes of the pathogen, i.e. the infection history of the pathogen (here: the number of successful transmission events from a single disease focus), which results in more available nutrients from host tissue for pathogen growth, and/or (b) the ontogenetic development of the host plants, which can increase host resistance (i.e. reduce host susceptibility). Note that the relationships as depicted are not necessarily linear and our models allow for plant development to be linked with either decreased or increased transmission.

the infection success of the fungal pathogen and increases in plant resistance during development). We performed experiments with focal host plant and neighbour plant combinations across (1) two focal plant species; (2) four neighbour species; and (3) two ages for all neighbour species. For all these combinations, we determined the effects of host identity, neighbour identity and neighbour age on disease transmission of *R. solani*, which causes damping-off disease in seedlings (Anderson, 1982; González García *et al.*, 2006). In addition, we developed stochastic models to test the relative importance of the two transmission-modifying mechanisms in mixed plant communities. Together, our experimental and modelling approaches reveal how neighbours affect belowground disease transmission and the underlying mechanisms. This integrated approach is needed to understand whether a neighbour makes or breaks disease transmission chains.

Materials and Methods

Study system

Our fungal root pathogen was *R. solani* (AG4-HGI, strain CBS 124 594 from the Westerdijk Fungal Biodiversity Institute, the Netherlands, originally isolated from *Coprosma repens*). *Rhizoctonia solani* is a common pathogen in many crops (Anderson, 1982), but also occurs in grasslands (Thornton, 1956; Hannula *et al.*, 2017; Mommer *et al.*, 2018). *Rhizoctonia solani* isolates can be classified into subgroups called 'anastomosis groups' (based on the ability of hyphae to fuse), which differ in ecological traits such as host range (Ogoshi, 1987). Anastomosis group AG4-HGI is known to infect several dicot crops, including lettuce, melon, and beans (Kuramae *et al.*, 2003; Van Beneden *et al.*, 2009; Nerey *et al.*, 2010). *Rhizoctonia solani* was maintained on 1/5 potato dextrose agar (15 g agar-agar (Carl-Roth, Karlsruhe, Germany) and 4.89 g potato dextrose broth (Difco, Sparks, MD, USA) l^{-1}) at 20°C in a dark climate room.

We used four common European perennial grassland species: the forbs *Leucanthemum vulgare* and *Plantago lanceolata* and the grasses *Anthoxanthum odoratum* and *Festuca rubra*. These species frequently occur together in European mesotrophic grasslands (Schaminée *et al.*, 1996; Rodwell, 1998) and have been found to experience negative plant soil feedback that is likely due to soil biota (Hendriks *et al.*, 2013). Furthermore, in the Wageningen biodiversity experiment, which included these four species, the root-associated fungal community showed evidence of host specificity and dilution of fungal root-pathogens (Mommer *et al.*, 2018). Seeds were obtained from native seed supplier Cruydt-Hoeck (Nijeberkoop, the Netherlands).

All seeds were surface sterilized in bleach (12% for *P. lanceolata*, 2% for all other species) to successfully remove microbial contaminations for 20 min, followed by rinsing with demineralized-water (H_2O) four (*P. lanceolata*) or three (all other species) times. Seeds were germinated on moist filter paper in Petri dishes with demineralized-H_2O (23°C constant, 16 h : 8 h light : dark, 170 μmol m^{-2} s^{-1} at plant level, relative humidity (RH) 75%). Once radicles appeared the Petri dishes were stored at 4°C for a maximum of 1 wk until use.

Experimental set-up

We tested the effect of neighbour identity and age on disease transmission in two focal host plant species. In a pilot experiment with the same set-up as described later for our main experiment ($n = 10$, three months before the main experiment), we determined that the two forbs *L. vulgare* and *P. lanceolata* sustained *R. solani* disease transmission, regardless of interplant distance (1, 2, or 4 cm), while the two grasses *A. odoratum* and *F. rubra* did not (Supporting Information Methods S1; Fig. S1; Table S1). Therefore, *L. vulgare* and *P. lanceolata* were used as focal host species in the main experiment, and all four species were used as neighbours. For neighbour age, we either used seedlings of the same age as the focal host seedlings or seedlings that were 10 d older. This age difference was chosen based on a pilot experiment with *A. odoratum* as neighbour species and *P. lanceolata* as focal species ($n = 15$), which showed that 10 d older neighbours reduced disease transmission in *P. lanceolata*, while neighbours of the same age did not (Fig. S2). We planted seedlings in a row in sandy soil (organic matter (OM): 4.8%, pH 6.60, N-NO$_3$: 84 mg kg^{-1}, P-PO$_4$: 0.20 mg kg^{-1}, 0.01 M calcium chloride ($CaCl_2$) extraction) in rectangular plastic containers (5 cm × 20 cm × 5 cm). Each row contained seven seedlings of the focal host species with 2 cm interplant distance (Fig. 2a). In between each pair of focal host seedlings, three neighbour seedlings were placed (Fig. 2b,c). Neighbour seedlings that were 10 d older than the focal host seedlings were planted in the containers 10 d before the focal host seedlings. We used focal rows without neighbours as a positive control (with *R. solani*) and as a mock control (without *R. solani*).

The containers were placed in larger trays with transparent covers to reduce evapotranspiration and were kept in a climate chamber (23°C constant, 16 h : 8 h light : dark, 170 μmol m^{-2} s^{-1} at plant level, RH 75%). The containers were arranged in a randomized block design, in which each block contained four large trays that together included one replicate of each treatment and each positive and negative control. The containers were randomly assigned to each large tray. The experiment had a full factorial design consisting of two focal host species × four neighbour species × two neighbour ages = 16 treatments, plus four controls (two focal host species without neighbours, with and without the fungus), each replicated 15 times and thus arranged in 15 blocks.

Three days after planting, missing seedlings were replaced with seedlings with fully developed cotyledons that germinated on glass beads. Four days after planting, the first seedling of each row was inoculated with *R. solani*. An agar plug (5 mm diameter) with actively growing mycelium of *R. solani* was added with the mycelial side directly adhering to the primary root of the seedling. For mock controls (without *R. solani*), a sterile agar plug was used. All containers were watered with 10–20 ml demineralized-H_2O as needed every 2 d throughout the experiments. Disease transmission between the focal host seedlings was measured every 2 d for 17 days post inoculation (dpi) as the distance from inoculation point to the most distal focal seedling in the row with aboveground damping-off disease symptoms (i.e. black hypocotyl base, hypocotyl and leaf rot, wilting, and collapse). No disease was observed in any of the mock control
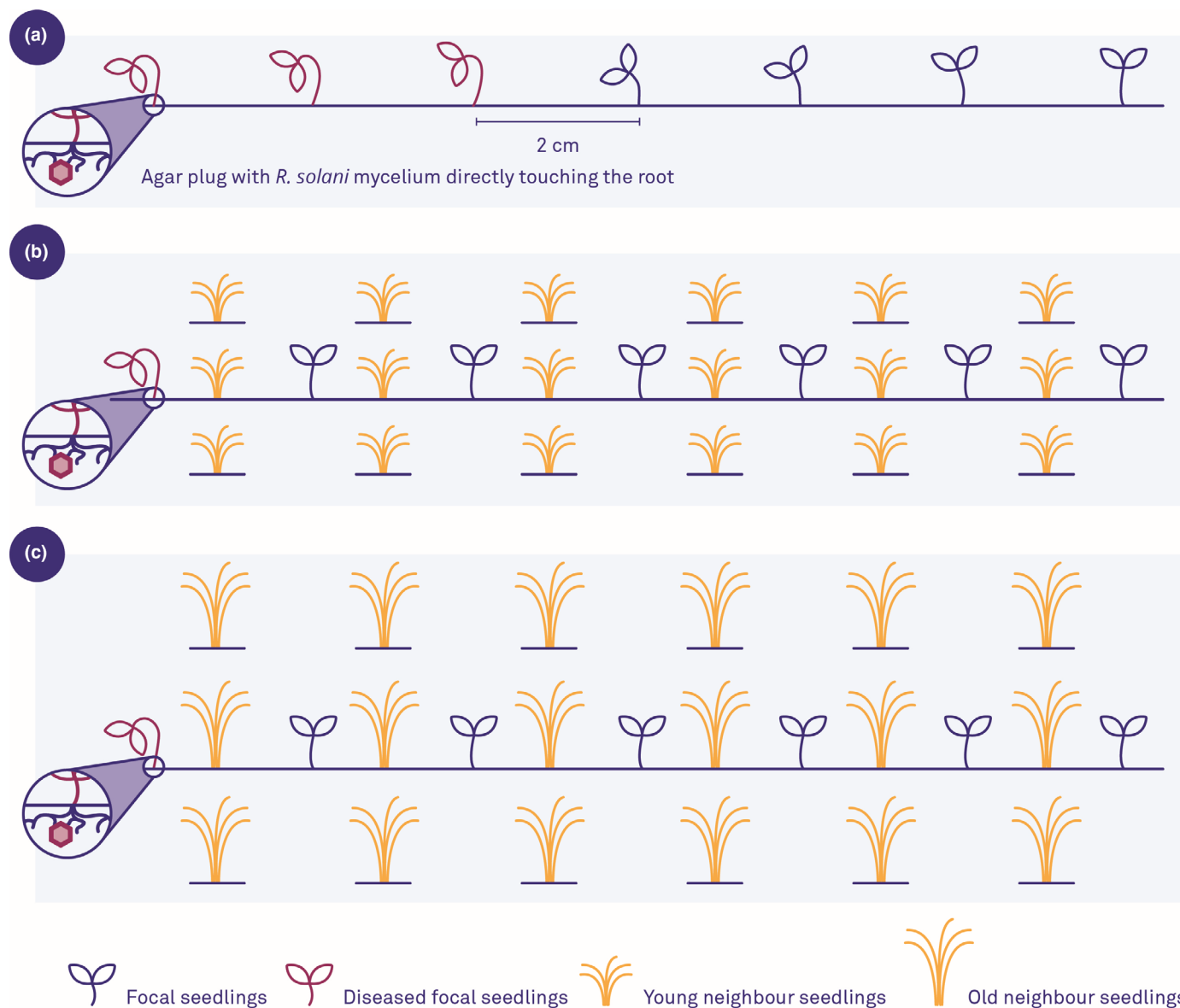
Fig. 2 Overview of disease transmission experimental set-up. Each panel (a–c) represents the design of a single experimental row, consisting of seven 'focal' host seedlings (either *Leucanthemum vulgare* or *Plantago lanceolata*) with 2 cm interplant distance, without (a) or with young (planted on the same day as the focal seedlings) (b) or old (planted 10 d earlier than the focal seedlings) (b) neighbour 'barriers' of three seedlings (either *L. vulgare*, *P. lanceolata*, *Anthoxanthum odoratum* or *Festuca rubra*) in between each pair of focal host seedlings. The first focal seedling in each row was inoculated with an agar plug with *Rhizoctonia solani* mycelium directly touching the root. Disease transmission between focal host seedlings was measured for 17 d after inoculation based on aboveground damping-off disease symptoms in focal host seedlings (example of disease transmission shown in (a), with red seedlings indicating diseased individuals).

replicates. In addition, we used a toothpick-baiting technique in a subset of our treatments (Methods S1), to test whether the spread of *R. solani* through soil was consistent with the observed disease transmission as measured by visible disease symptoms in the focal plants.

Data analysis

First, we assessed whether focal host identity affected host susceptibility at inoculation by testing whether the infection success (binary variable) of the inoculated seedling of each row without

neighbours at 4 dpi differed between focal host species with a chi-squared (χ^2) test of independence.

As a measure of disease transmission in the focal hosts, we calculated the area under the disease progress curve (AUDPC) per row as:

$$\text{AUDPC} = \sum_{i=1}^{u-1} \frac{(y_i + y_{i+1})}{2} \times (t_{i+1} - t_i)$$

where t_i are the timepoints in days after inoculation, u is the total number of time points at which measurements were made, and y_i are the measurements of the distance in centimetres to the

furthest diseased seedling (Gómez Expósito *et al.*, 2015). The AUDPC is an integrated measure of the extent (i.e. number of plants infected) of disease transmission (Hiddink *et al.*, 2005), if there is a clear correlation between the occurrence of the pathogen and the occurrence of symptoms (see Fig. S1).

Differences in disease transmission between the rows without neighbours (positive controls) of the two focal host species were tested using a general linear model with AUDPC as the dependent variable and focal host identity as the independent variable. Tray nested in block or block alone were not included in this final model as they were was not significant as a random effect in a linear mixed effects model (Likelihood Ratio Test: tray nested in block vs block : $\chi^2(1) < 0.001$, $P = 1$; block vs no block: $\chi^2(1) = 0.14$, $P = 0.71$; NLME package, (Pinheiro *et al.*, 2020)). To be able to compare the effects of neighbours on disease progress in the two focal host species, we calculated the change in disease transmission (ΔAUDPC) as the difference in AUDPC between each replicate of the rows with neighbours and the average AUDPC without neighbours of the respective focal host species. A linear mixed effects model (NLME package, (Pinheiro *et al.*, 2020)) was used to analyse ΔAUDPC with the focal host identity (*L. vulgare* or *P. lanceolata*), neighbour identity (*L. vulgare*, *P. lanceolata*, *A. odoratum* and *F. rubra*), and the neighbour age (same age or 10 d older) as fixed factors and block as a random effect. Tray nested in block was not included in this final model as the addition of tray was not significant as a random effect (Likelihood Ratio Test model with tray nested in block vs block as random effect: $\chi^2 (1) = 0.74$, $P = 0.39$). Assumptions for normality of residuals and homogeneity of variance were met after allowing for varying variance components for the interaction between focal host identity and neighbour identity. To further investigate the interactions between neighbour identity and the other explanatory variables, we constructed separate models for each neighbour identity with focal host identity, neighbour age and their interaction as fixed factors and block as

random effect. To test for differences between specific neighbour treatments and the rows without neighbours, we tested whether the ΔAUDPC estimates of the levels of significant factors differed from 0 with *post hoc t-tests* with Holm–Bonferroni adjustment for multiple comparisons (EMMEANS package, (Lenth, 2020)). Replicates in which the inoculated seedling did not develop disease symptoms (focal host species *L. vulgare*: 8/135, *P. lanceolata*: 5/135), or where seedlings were missing after inoculation ($n = 10$ out of 270 rows), were excluded from all AUDPC analyses. The final sample sizes are shown in Fig. 3. Nonsignificant interactions were removed from our final models. All data analysis was performed in R (v.3.6.3, (R Core Team, 2020)).

Modelling

Description of models We developed a set of stochastic models to investigate the effect of neighbouring plants on the transmission-modifying mechanisms in disease transmission in our experimental rows. In our experiments, there is always a row of focal-species plants, with or without neighbours, and disease status is determined only for the focal host species. In our modelling, we attempt to understand how the neighbours – or lack thereof – affect disease transmission-modifying mechanisms between plants of the focal host species. We therefore model only the focal host species and attempt to estimate the probability that when one plant becomes diseased the disease will be transmitted to the next plant in the row. In the null model (Model 1), this probability of transmission between pairs of focal host plants is fixed. In the more complex models, the probability of transmission can vary over space and time (Table 1). These alternative models incorporate the following mechanisms: positive feedback of transmission on the transmission probability due to resource acquisition by the fungus (Model 2), effects of plant development on transmission (Model 3), or both of these effects (Model 4).
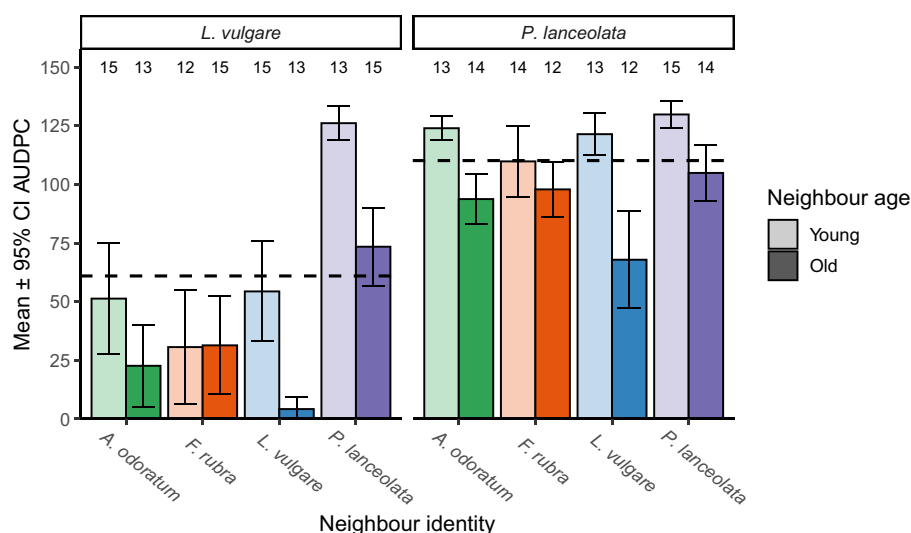
Fig. 3 Effects of neighbour identity and age on disease transmission (measured as area under disease progress curve (AUDPC)) in seedlings of the two focal host species (panels) in the main experiment. Dashed lines show average disease transmission without neighbours colour indicates neighbour identity as in Figs 4, 5. See also Fig. 4 for disease progress curves and Table 1 and Supporting Information Table S4 for stats. Bars show mean AUDPC ± 95% confidence interval (CI). Numbers above bars indicate sample size.

Table 1 Overview of transmission-modifying mechanisms that affect disease transmission probability in Models 1–8. Models 5–8 and their corresponding results are only described in the Supporting Information (Methods S1; Tables S2, S3).

Model	Infection success	Plant development	Heterogeneity in susceptibility	Parameters
1				ρ_0
2	x			ρ_0, ψ
3		x		ρ_0, γ
4	x	x		ρ_0, ψ, γ
5			x	α, β
6	x			ρ_0, ψ, ω
7		x		ρ_0, γ, Δ
8	x	x		$\rho_0, \psi, \omega, \gamma, \Delta$

Furthermore, we included an extension of our null model, which assumes that there is stochastic variation in the focal plant susceptibility to disease (Model 5). In addition, more complex models (Models 6–8) in which the mechanisms incorporated in Models 2–4 commence their action at a specific time point were also considered. Models 6–8 and their corresponding results are only described in the Methods S1, as they were not well supported due to their higher complexity (Table S2).

Model 1. Our null model has a fixed transmission probability between adjacent pairs of plants, implying a fixed susceptibility of all plants and a fixed disease transmission potential over time. The model predicts, in discrete time (days), the sequential infection of individual plants in a row (i.e. a transmission chain). In the experiments, only rows in which the first focal plant became infected are considered, and likewise here we assume the first focal plant is always infected. Once a plant has become infected it stays infectious to its adjacent noninfected neighbour throughout the experiment and infection can maximally progress by one focal plant per day (consistent with the maximum progress in the experiments). Thus, I is the infected focal plant furthest away from the initially infected source plant on day t, in a row with a total of R focal host plants. For the null model, the probability that the infection will spread to the next plant by day $t + 1$ follows a Bernoulli distribution, such that: $P = \rho^n (1 - \rho)^n$ where the binary transmission outcome $n \in \{0, 1\}$ and ρ is the probability of transmission. When $I_t = R$, the infection has reached the end of the row and no further transmission is possible.

Model 2. Under this model, successful infections provide more nutrients for the hyphal growth of *R. solani* increasing the transmission probability. Therefore, the potential for transmission depends on recent pathogen performance, increasing because of the successful transmission between plants (i.e. positive feedback). Model 2 links the probability of transmission to the number of infection successes that have already occurred, such that:

$$\rho_t = \rho^{e^{-\psi I}}$$

where $\psi \in [0, \infty)$ is a constant that determines the effect size. In practice, $\psi \in [0, 10]$ was used to avoid computational problems,

with the upper limit being well above expected values as determined by initial model fitting.

Model 3. Under this model, seedlings can become more resistant to *R. solani* with age, thereby decreasing transmission probability over time (Kleczkowski *et al.*, 1996). By contrast, transmission probability can also increase over time due to increased root growth and root intermingling (Bailey *et al.*, 2000). Therefore, the transmission probability ρ can vary due to changes in the resistance of the focal host plants over time or differences in the potential of neighbouring plants to reduce or increase disease transmission over time. Model 3 therefore lets the transmission probability depend on time to reflect changes due to plant development and independent of disease incidence. Under this model, ρ therefore can become either smaller or larger over time, such that:

$$\rho_t = \rho^{e^{-\gamma t}}$$

where $\gamma \in (-\infty, \infty)$ is a constant that determines the effect size and direction ($\gamma < 0$ reduces ρ and $\gamma > 0$ increases ρ over time). We again limited the range to $\gamma \in [-10, 10]$ for computational reasons.

Model 4. Models 2 and 3 were also combined, to allow the transmission probability ρ to vary due to both changes in the pathogen's transmission potential due to recent infection successes and the focal plant resistance or root growth, such that:

$$\rho_t = \rho^{e^{-\psi I - \gamma t}}$$

Model 5. As an alternate null model hypothesis, the transmission probability ρ may vary between pairs of focal host plants in the row. This model captures natural heterogeneity in focal plant susceptibility, heterogeneity in neighbour potential to reduce or amplify disease transmission, or both. Such heterogeneity in host susceptibility has been shown to reduce transmission of both viral and fungal pathogens in animals and plants (Dwyer *et al.*, 1997; Cook *et al.*, 2007). Under this model, a realization x of ρ is drawn from the beta distribution such that $P(\rho = x) = \frac{x^{\alpha-1}(1-x)^{\beta-1}}{B(\alpha, \beta)}$, where α and β are the shape parameters and $B(\alpha, \beta)$ is the beta function. The variation introduced by this model is stochastic and random with respect to infection successes and plant development.

Model evaluation and model fitting To evaluate model predictions for a given set of model parameters, we used both a numerical (only Models 1–4) and iterative approach (all models). To evaluate the models numerically, we used difference equations to predict the frequency f at which the jth plant in that row was the furthest infected plant of the total R focal host plants, e.g. for Model 1 for the first plant in the row $f_{1,t+1} = f_{1,t} - \rho f_{1,t}$, for the last plant $f_{R,t+1} = f_{R,t} + \rho f_{R-1,t}$, and for all intermediate plants in the row $f_{j,t+1} = f_{j,t} - \rho f_{j,t} + \rho f_{j-1,t}$. To evaluate the models iteratively, we performed 10 000 simulations of a

transmission chain, with the number of new plants infected being drawn using the rbinom() function in R, which generates pseudo-random variates, and determined the frequency at which each plant in the row was the furthest infected plant. For both approaches, each transmission chain consisted of 17 d with $R = 7$ and $I_0 = 1$ (i.e. inoculation of first seedling in experiment). Disease transmission started from t_3 onwards, because of the initial lag in the experiment between moment of inoculation (t_0) and first disease symptoms.

To estimate parameters for model selection we used a stochastic hill-climbing algorithm: a random set of parameter values was chosen as a starting point, one randomly chosen parameter was varied, and the new set of parameter values was accepted if model fit improves. We determined the multinomial log likelihood by comparing the predicted and observed frequency at which each position in the row was the furthest pathogen spread for each day in the experiment, and then summed the multinomial log likelihoods over all days with observations to obtain a single value of model fit. We used the Laplace law of succession (Chew, 1971) to determine the model prediction for $f_{i,t}$, to avoid missing likelihoods due to frequencies of 0 or 1, and this approach therefore employs pseudo-likelihoods. The search algorithm was repeated 1750 times per model per row type, for both numerical and iterative model evaluation. The total number of searches required to ensure that multiple searches converged on the best result was estimated based on an initial set of searches. To account for stochastic variation in model predictions using the iterative approach, the parameter estimates from the search with the lowest pseudo-NLL were used to run the same model 100 times with 10 000 simulations each with these parameter values. From these replicates the mean pseudo-NLL was calculated as an overall estimate of the model fit with these parameter values. The Akaike Information Criterion (AIC) was used for model selection.

Finally, we obtained confidence intervals (CIs) for the model parameter estimates. Due to computational limitations, we only did so (1) for the best-supported model for each row type; and (2) using the numerical approach to evaluate the model. For each combination of focal host identity, neighbour identity and neighbour age ('row type') we tested which model had the most support from our experimental observations based on the AIC. ΔAIC ($AIC_{min} - AIC_{model\ i}$) > 2 was considered a significantly better fit. When ΔAIC < 2 we considered the most parsimonious model as the best supported. For the best supported model for each row type, we used bootstrapping ($n = 1000$) to obtain 95% percentile CIs for all parameters. From the total set of bootstrapped samples, we used the parameter estimates to calculate transmission probability on day 3 ($\rho_{t=3}$), i.e. at the start of transmission, given the applicable model, thereby taking into account ρ_0 as well as any effect size parameters. To compare parameter values between row types, we constructed pairwise comparisons by calculating the pairwise difference between parameter estimates for all bootstrap samples and obtaining the 95% CI of this difference. We considered two parameter estimates significantly different if the 95% CI of their difference did not contain 0.

In the main text we report results based on the numerical evaluation of the Models 1–4, whereas results from iterative

evaluation, including the more complex Models 6–8, are reported in Tables S2, S3.

Results and discussion

Neighbour identity and neighbour age alter disease transmission

Susceptibility to *R. solani* damping-off disease was similar for both focal plant species (i.e. the host species in which transmission was assessed) of our study, the forbs *P. lanceolata* and *L. vulgare* ($\chi^2(1) = 0.00$, $P = 1.00$). We found that 93% of the inoculated seedlings (i.e. the first focal seedling in each row, Fig. 2a) of these two species developed disease symptoms in the rows without neighbours present. However, the transmission of *R. solani* damping-off disease was significantly higher in *P. lanceolata* rows than in *L. vulgare* rows without neighbours present ($F_{1,27} = 12.80$, $P < 0.01$; Figs 3, 4; Fig. 2 for experimental set up).

The presence of neighbours in the rows of focal host plant species significantly affected disease transmission in the focal host seedlings (i.e. the seedlings of either host species in which transmission was assessed, see Fig. 2b,c). These effects depended on focal host identity, neighbour identity and neighbour age (Table 2). *Plantago lanceolata* neighbours increased disease transmission in both focal host species, but only at the younger age (i.e. when planted on the same day as the focal host seedlings; young neighbours with *L. vulgare*: $t_{14} = 9.81$, $P < 0.001$; with *P. lanceolata*: $t_{14} = 4.06$, $P < 0.01$; Figs 3, 4; Table S4). When *P. lanceolata* neighbours were older than the focal host seedlings (i.e. planted 10 d earlier than the focal host seedlings), disease transmission in both focal host species did not differ from the control without neighbours (old neighbours with *L. vulgare*: $t_{14} = 1.99$, $P = 0.13$; with *P. lanceolata*: $t_{14} = -1.27$, $P = 0.22$; Figs 3, 4). The increase in disease transmission with young *P. lanceolata* neighbours was larger with *L. vulgare* than with itself as focal host species (significant age × focal interaction; Table S4); this may be due to the fact that the disease transmission in *P. lanceolata* was already higher than in *L. vulgare*.

The other three neighbour species (the host *L. vulgare* and two nonhost grasses *A. odoratum* and *F. rubra*) decreased disease transmission in most cases (Figs 3, 4; Table S4). *Leucanthemum vulgare* and *A. odoratum* neighbours that were older than the focal host seedlings significantly decreased disease transmission in both focal host species (old *L. vulgare*: $t_{14} = -8.42$, $P < 0.001$; old *A. odoratum*: $t_{14} = -5,47$, $P < 0.001$; Figs 3, 4; Table S4), while young neighbours did not affect disease transmission (young *L. vulgare*: $t_{14} = 0.43$, $P = 0.68$; young *A. odoratum*: $t_{14} = 0.46$, $P = 0.65$; Figs 3, 4; Table S4). The transmission-reducing effect of *A. odoratum* was stronger for focal host *L. vulgare* than for focal host *P. lanceolata* (focal host identity: $F_{1,38} = 8.07$, $P < 0.01$; Figs 3, 4; Table S4), whereas the neighbour effect of *L. vulgare* did not differ between focal host species ($F_{1,36} = 4.03$, $P = 0.05$; Figs 3, 4; Table S4). *Festuca rubra* neighbours decreased disease transmission only for focal host *L. vulgare* (focal host identity: $F_{1,36} = 6.3$, $P < 0.05$; *post hoc*:
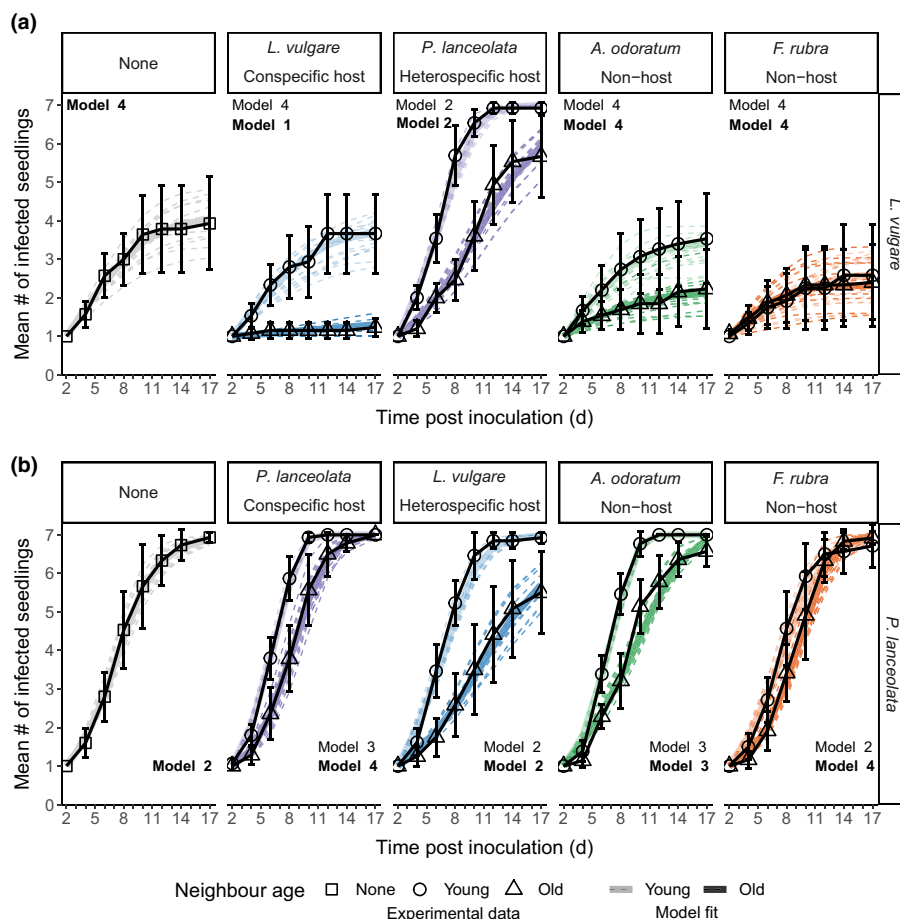
Fig. 4 Disease transmission of *Rhizoctonia solani* in host seedlings depends on focal host identity and neighbours. Experimental observations and model fits of disease curves (mean of furthest infected focal seedling over time) for both focal host species: (a) *Leucanthemum vulgare* and (b) *Plantago lanceolata*, without and with different neighbour types (horizontal panels). Experimental data in black symbols (squares = young neighbours, triangles = old neighbours; mean ± 95% confidence interval, see Fig. 3 for sample sizes) and solid, thin black lines. Model fits in coloured lines (colour indicates neighbour identity as in Figs 3, 5), based on model parameter estimates from fit of best-supported model (label, plain = young, bold = old neighbours) to full dataset per treatment (wide, solid line) and on 15 randomly drawn bootstrap samples (thin, dashed lines) to indicate prediction accuracy. See also Fig. 3 (experimental area under the disease progression curve) and Table 1 and Supporting Information Table S1 for experimental stats.

Table 2 Neighbour effects on disease transmission depend on focal host identity, neighbour identity and neighbour age.

Predictor	df	F	P
Focal host identity	1,192	4.11	<0.05
Neighbour identity	3,192	42.94	<0.001
Neighbour age	1,192	86.49	<0.001
Focal host identity × Neighbour identity	3,192	17.24	<0.001
Neighbour identity × Neighbour age	3,192	4.93	<0.01

ANOVA results for linear mixed effects model of the effects of host identity, neighbour-identity and -age on disease transmission (measured as difference in area under the disease progression curve compared to control without neighbours (ΔAUDPC), see the Materials and Methods section and Supporting Information Fig. S3). Nonsignificant interactions were removed from the final model. Type III sum of squares were used.

L. vulgare $t_{14} = -3.2$, $P < 0.05$; *P. lanceolata*: $t_{14} = -0.39$, $P = 0.70$; Figs 3, 4; Table S4), both when older and the same age as the focal host seedlings (neighbour age: $F_{1,36} = 1.86$, $P = 0.18$;

Figs 3, 4; Table S4). In general, disease transmission was consistent with the spread of *R. solani* through soil, as confirmed through toothpick-baiting (Fig. S3; Table S5; Methods S1). Together, our experimental data revealed both a difference in disease transmission between the two focal host species and both increasing and reducing effects of neighbouring plants on disease transmission. Our results indicate that although differences in total host density may drive effects of host neighbours, these effects strongly depend on host neighbour identity and age.

Transmission-modifying mechanisms depend on interactions with neighbours

To elucidate the interactive effects of focal host identity and neighbours on disease transmission, we developed stochastic models that specify the different mechanisms underlying disease transmission. By comparing support for the models and parameter estimates, we evaluated the evidence for the two transmission-modifying mechanisms in our experimental data (see the

Materials and Methods section; Fig. 1; Table 1). In our null model the transmission probability (ρ) between each pair of focal host plants is a constant (Model 1). In the more complex models (Fig. 1), the transmission probability is allowed to change because (1) infection success provides more nutrients to the pathogen for growth (Model 2, with extra scaling parameter ψ); (2) host plant development over time increases resistance to the pathogen (Model 3, with extra scaling parameter γ); or (3) both (Model 4).

The best-supported model differed between the two focal host species without neighbours (Tables 3, S6, *L. vulgare*: Model 4, Figs 4(a), 5(a); *P. lanceolata*: Model 2, Figs 4b, 5b) revealing two differences in the role of transmission-modifying mechanisms in the focal host species. Transmission probability increased with infection success in both focal host species but this effect was significantly larger in *P. lanceolata* than in *L. vulgare* ($\Delta\psi$ 95% CI (0.02,1.4)), suggesting that *P. lanceolata* seedlings provided more nutrients to the pathogen than those of *L. vulgare*. In support of this hypothesis, we observed that *P. lanceolata* had higher root biomass and length than *L. vulgare* (Fig. S4). Across both focal species, our data thus provide proof of principle for the self-reinforcing transmission mechanism. Most likely, this mechanism is particularly relevant for fungal pathogens that spread through soil via mycelial growth. Moreover, the transmission probability only decreased with plant development in *L. vulgare* (Fig. 5c; Table S7), which suggests that *L. vulgare* resistance increased with age. The latter observation was confirmed in an additional experiment where the infection probability of *L. vulgare* seedlings decreased with their age, while the infection probability did not change for older *P. lanceolata* seedlings (Fig. S5).

The development of resistance in *L. vulgare* outpaced the disease transmission to such an extent that transmission halted before the end of the row (i.e. a plateau in the disease curve: Fig. 4a). This is consistent with epidemics of *R. solani* in monoculture crops (Gilligan *et al.*, 1997) and epidemiological models (e.g. Otten *et al.*, 2003; Cook *et al.*, 2007), which often show a characteristic decrease in secondary transmission rate due to development of host resistance. Together, our results show that the lower disease transmission in *L. vulgare* compared to *P. lanceolata* is due to differences in the strength of both transmission-modifying mechanisms between host species.

In the presence of neighbouring plants, the best-supported models were often different models from those best-supported when without neighbours (Tables 3, S6). This already indicates that neighbours change the transmission-modifying mechanisms of the focal host species.

The role of transmission-modifying mechanisms in disease transmission in *L. vulgare* was mainly affected by the other host species *P. lanceolata*. With *P. lanceolata* neighbours, the transmission solely depended on the infection success of the fungal pathogen (Model 2, infection success of the fungal pathogen; Table 3; Figs 4a, 5a), while the effect of plant development, which reduced disease transmission in *L. vulgare* without neighbours, disappeared. Thus, *P. lanceolata* neighbours increased the disease transmission rate in *L. vulgare* to such an extent that the transmission outpaced the development of disease resistance in the focal hosts. In contrast, *L. vulgare* neighbours that were older than the focal individuals decreased the disease transmission probability in *L. vulgare* to almost zero (Fig. 5a). Therefore, no transmission-modifying mechanisms could be detected (Model

Table 3 Neighbour effects on transmission-modifying mechanisms depend on focal host identity.

| Focal host identity | Neighbour | | | ΔAIC = AIC$_{null}$ − AIC$_{model\ i}$ | |
	Identity	Age	Type	Infection success	Plant development
Leucanthemum vulgare	None			**−28.40**	**−6.22**
	Leucanthemum vulgare	Young	Conspecific host	**−17.40**	**−4.32**
	Leucanthemum vulgare	Old	Conspecific host	2.00	−0.70
	Plantago lanceolata	Young	Heterospecific host	**−2.20**	1.98
	Plantago lanceolata	Old	Heterospecific host	**−42.64**	−7.54
	Anthoxanthum odoratum	Young	Nonhost	**−44.24**	**−4.88**
	Anthoxanthum odoratum	Old	Nonhost	**−22.44**	−0.14
	Festuca rubra	Young	Nonhost	**−64.92**	0.64
	Festuca rubra	Old	Nonhost	**−48.04**	**−5.44**
Plantago lanceolata	None			**−48.74**	−2.66
	Plantago lanceolata	Young	Conspecific host	−14.82	**−20.40**
	Plantago lanceolata	Old	Conspecific host	**−36.28**	**−25.00**
	Leucanthemum vulgare	Young	Heterospecific host	**−5.68**	1.46
	Leucanthemum vulgare	Old	Heterospecific host	**−36.66**	−3.26
	Anthoxanthum odoratum	Young	Nonhost	−15.16	**−35.06**
	Anthoxanthum odoratum	Old	Nonhost	−3.78	**−8.82**
	Festuca rubra	Young	Nonhost	**−31.70**	−2.34
	Festuca rubra	Old	Nonhost	**−25.82**	**−29.82**

Support for transmission-modifying mechanisms as compared to null model: ΔAIC (AIC$_{null}$ − AIC$_{model\ i}$) < −2 indicates support for a model with a transmission-modifying mechanism. Best-supported model indicated in bold text and grey shading (none: null model, infection success only: Model 2, plant development only: Model 3, both: Model 4). In cases where several models provide an equally good fit (ΔAIC (AIC$_{model\ i}$ − AIC$_{model\ j}$) < 2), the most parsimonious model was considered the best supported. ΔAIC for Model 4 not shown. See Supporting Information Table S6 for complete model selection.
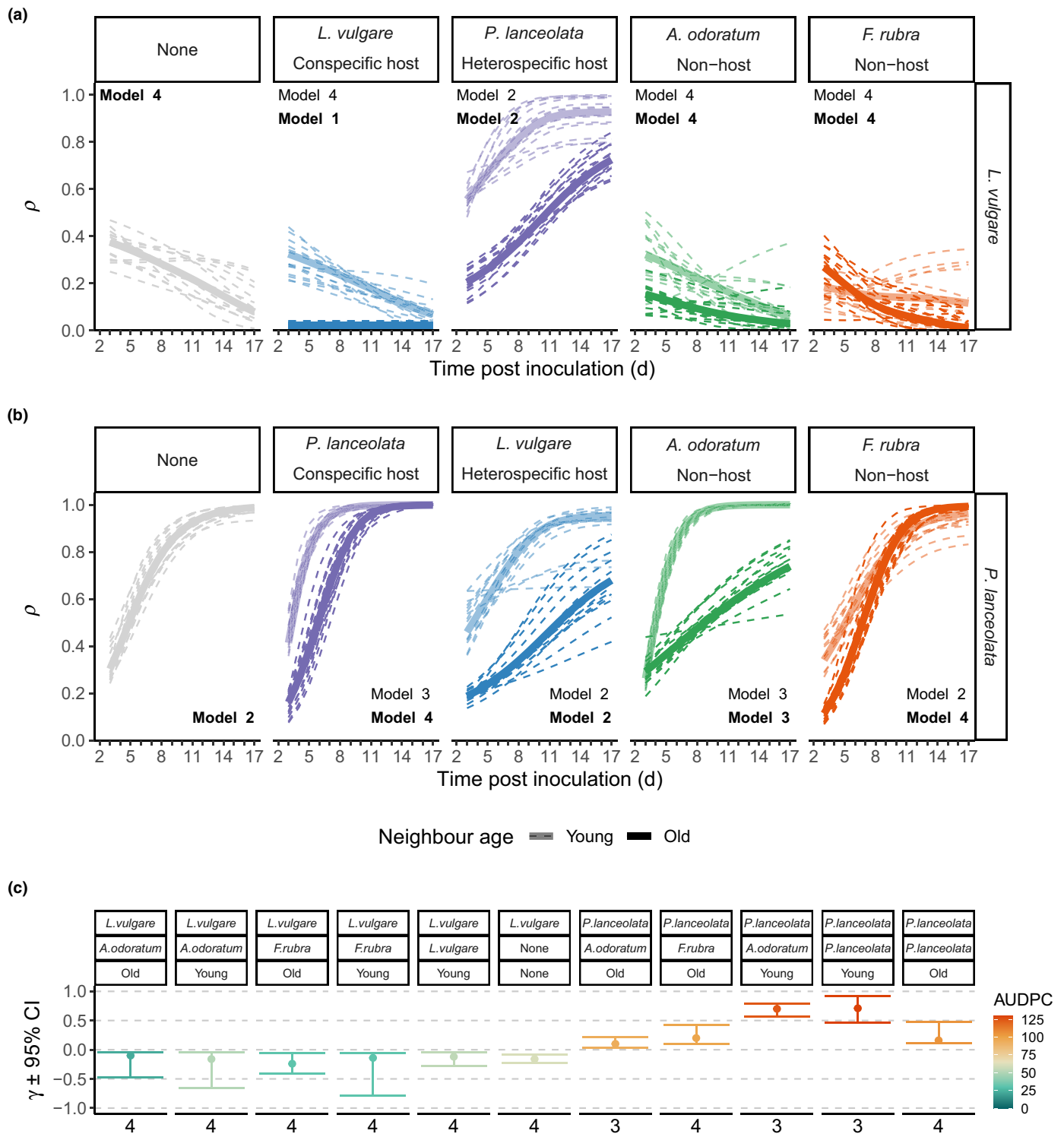
Fig. 5 Modelling reveals that disease transmission-modifying mechanisms depend on focal host identity and neighbour effects. (a, b) Disease transmission model predictions of disease transmission probability (ρ) over time for both focal host species ((a) *Leucanthemum vulgare*; (b) *Plantago lanceolata*), without and with different neighbour types (horizontal panels; light colour = young, dark colour = old neighbours). Predictions are based on model parameter estimates from fit of best supported model (label, plain = young, bold = old neighbours) to full dataset per treatment (wide, solid line) and on 15 randomly drawn bootstrap samples (thin, dashed lines) to indicate prediction accuracy. (c) Plant development effect size parameter γ only for treatments (horizontal panels) with significant transmission-modifying effect of plant development (Model 3 or Model 4 as best supported model). Positive γ values indicate an increase in ρ over time, negative γ values a decrease in ρ over time. Estimate from fit to full dataset per treatment ± 95% bootstrap percentile confidence interval (CI). Colour scale represents area under disease progress curve from experimental data. See Supporting Information Tables S7, S8 for model parameter estimates and pairwise comparisons.

1, i.e. fixed transmission probability, Table 3; Figs 4a, 5a). Young neighbours of the same species (i.e. *L. vulgare*) did not change the role of transmission-modifying mechanisms in disease transmission in *L. vulgare* (Model 4; Figs 4(a), 5(a); Tables 3, S7, S8) and neither did nonhost neighbours *A. odoratum* and *F. rubra* (Model 4; Figs 4(a), 5(a); Tables 3, S7, S8), despite the reduction of the transmission by the nonhost neighbours (all but young *A. odoratum*). Whether other putative transmission-reducing mechanisms by neighbouring plants, such as alteration of root growth, root architecture or disease resistance of the focal plants, play a role was not investigated here and will be an intriguing avenue for future studies.

While plant development did not play a role in disease transmission in *P. lanceolata* without neighbours, plant development played an important role when *P. lanceolata* was with most neighbours (all neighbours except *L. vulgare* or young *F. rubra*, Figs 4 (b), 5(b); Table 3). However, rather than finding that plant development reduced disease transmission probability, as shown for *L. vulgare* (Fig. 5c), we found that plant development enhanced disease transmission probability in *P. lanceolata* with neighbours (Fig. 5c; Table S7). Because it is unlikely that the *P. lanceolata* host plants become more susceptible to *R. solani* over time (Gibson *et al.*, 1999; Develey-Rivière & Galiana, 2007), we argue that disease transmission in *P. lanceolata* is enhanced by a more extensive root network over time, leading to enhanced contact between focal host individuals (Bailey *et al.*, 2000; Leclerc *et al.*, 2013). Conspecific host neighbours (i.e. *P. lanceolata*) directly contribute to this root contact network, enhancing the effect of plant development on disease transmission probability in *P. lanceolata* (Model 3, plant development; Figs 4(b), 5(b,c); Tables 3, S7, S8).

The development of a root contact network in *P. lanceolata* enabled disease transmission to overcome the potential transmission-interfering effects of the nonhosts. When the nonhost neighbour *A. odoratum* (Model 3, plant development; Figs 4(b), 5(b); Tables 3, S6) was older than the focal host individuals, and decreased the transmission in *P. lanceolata*, the transmission-modifying effect related to plant development was smaller than with young nonhost *A. odoratum* neighbours (young vs old *A. odoratum* neighbours: Δγ 95% CI (0.42,0.71); Fig. 5 (c); Table S8). Notably, when the nonhost neighbour *F. rubra* was older than the focal host individuals, it also induced the transmission-modifying effect related to plant development, although here infection success also affected transmission probability in *P. lanceolata* (Model 4; infection success of the fungal pathogen and plant development; Figs 4(b), 5(b,c); Tables 3, S7). This may indicate a transition towards plant development as the sole transmission-modifying mechanism, as was found with the other nonhost neighbour *A. odoratum*. The transmission-modifying mechanisms indicate that a firm root network of the host species is important for disease transmission. However, the effect of the neighbouring nonhost on disease transmission was also related to their age and thus likely the size of their root system. This combination will affect how intensely nonhost roots are intermingled with host roots (Kesanakurti *et al.*, 2011; Frank *et al.*, 2015), the amount and composition of potential antifungal

root exudates (Yang *et al.*, 2014; Li *et al.*, 2018; Schulz-Bohm *et al.*, 2018), and the antagonistic effects of the nonhost rhizosphere community (Berendsen *et al.*, 2012; Lange *et al.*, 2015; Stringlis *et al.*, 2018).

Older host neighbours decrease transmission through delayed onset of transmission

Irrespective of changes in the role of transmission-modifying mechanisms, neighbours also affected the onset of disease transmission, measured as the initial transmission probability ($\rho_{t=3}$) in our models. Specifically, we found that in both host species, older host neighbours significantly decreased the initial transmission probability ($\rho_{t=3}$) compared to host neighbours of the same age as the focal hosts (Fig. 5a,b; Table S8), which cascaded into significantly decreased disease transmission rates over time, a common consequence of the nonlinear nature of epidemics (Gilligan, 2002). In addition, a slower onset of transmission may allow host seedlings to develop resistance, further decreasing disease transmission (Kleczkowski *et al.*, 1996; Otten *et al.*, 2003), as we observed for focal species *L. vulgare* in our experiment. The intriguing finding that a neighbouring host species (i.e. *L. vulgare*) can strongly reduce transmission when it is older may be related to its development of resistance with age. Our additional experiment indicates that, at inoculation, the older *L. vulgare* neighbours would already have been *c.* 60% less susceptible than the focal hosts (Fig. S5). As the mechanism of developmental resistance to *R. solani* is not yet fully understood and likely not ubiquitous across plant species (Bateman *et al.*, 1969; Reddy, 1980; Yang *et al.*, 1992), we cannot point to a specific transmission-reducing mechanism in play at this moment. Nonetheless, these findings highlight that variability in plant developmental stages within a host population may contribute to pathogen dilution in diverse communities (Neher *et al.*, 1987; Dwyer *et al.*, 1997; Kauffman & Jules, 2006).

Conclusions

We demonstrate that neighbouring plants can positively and negatively affect disease transmission of the fungal root pathogen *R. solani*. These divergent neighbour effects were not explained by a simple distinction between hosts and nonhosts, highlighting that functional characterizations within hosts and nonhosts are needed to understand diversity-disease relationships. Our comprehensive approach, which included 18 root networks differing in plant community composition, shows that differential disease transmission in such root networks is driven by shifts in the relative importance of two different underlying mechanisms: (1) pathogen infection success increasing disease transmission; and (2) the development of plant resistance decreasing disease transmission. These shifts reveal that the importance of these two mechanisms that steer belowground fungal disease transmission is determined by the interaction between the identity and developmental stage of the neighbours and the focal plants. This interaction thus determines whether the roots of the species interfere with or connect the nodes in a plant community's root network.

In other words: the root network determines the possible 'routes' of pathogen transmission and thereby makes or breaks the transmission chains. Plant community composition may therefore determine which pathogen traits are under selection locally, whilst the presence of a pathogen can shape community composition (Bever *et al.*, 2015), as plants may have higher reproduction success in highly diverse communities with root networks that suppress pathogen transmission. Unravelling these interactive effects of plants and pathogens on the mechanisms underlying belowground pathogen transmission will be crucial to understand the disease-diversity relationship.

Author contributions

EAA, LM, JvR, AJT and JMR designed the project. EAA, JvR and LM performed the experiments. EAA, MPZ and LM designed the stochastic models. EAA analysed the experimental and modelling data. EAA, JvR, MPZ and LM wrote the draft of the manuscript. All authors discussed the results and contributed substantially to the writing of the manuscript.

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Data availability

The experimental data that were used for model fitting are available on GitHub at: https://github.com/ElineAmpt/Neighbour_transmission_modelling. All other data that support the findings of this study are available from the corresponding author upon reasonable request. Code availability: the source code of R for model fitting is available on GitHub at: https://github.com/ElineAmpt/Neighbour_transmission_modelling.

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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 *Rhizoctonia solani* disease transmission (measured as area under disease progress curve) in pilot experiment with conspecific seedling rows of the four focal plant species.

Fig. S2 *Rhizoctonia solani* disease transmission (measured as area under disease progress curve) in pilot experiment with young or old *Anthoxanthum odoratum* neighbours.

Fig. S3 *Rhizoctonia solani* progress through seedlings and soil.

Fig. S4 Root biomass and length of *Plantago lanceolata* and *Leucanthemum vulgare* rows without neighbours and without *Rhizoctonia solani*.

Fig. S5 Development of disease resistance with age is different between host species.

Methods S1 Assessment of *Rhizoctonia solani* growth through soil, statistical analysis of pilot experiment and description of complex models.

Table S1 Disease transmission in monoculture rows of four focal plant species at three interplant distances in pilot experiment.

Table S2 Model selection: fit of simple (Models 2, 3 and 4) vs complex (Models 6, 7 and 8) models with the same transmission-modifying mechanism(s).

Table S3 Model selection using the iterative approach including Model 5 (heterogene susceptibility).

Table S4 Effects of focal host identity and neighbour age on disease transmission rate per neighbour identity.

Table S5 Comparison of *Rhizoctonia solani* progress through seedlings and soil.

Table S6 Model selection for effect of neighbour identity and age on disease transmission in both focal host species.

Table S7 Parameter estimates of best supported model per treatment.

Table S8 Pairwise comparisons of best supported model parameter estimates between treatments.

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