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Intercropping cereals with faba bean reduces plant disease incidence regardless of fertilizer input; a meta-analysis

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Abstract Ecological intensification of agriculture calls for ecological mechanisms to replace anthropogenic inputs. Cereal/legume intercropping increases yields due to species complementarities, it produces high protein food and feed, and it reduces the need for artificial N fertilizer because legumes fix N biologically. In addition, intercropping has the potential to suppress plant diseases, but its efficacy for disease suppression in

cereal/legume mixtures has not been well characterized quantitatively. Here we conducted meta-analysis to quantify the disease suppressive effect of intercropping cereals with legumes at different levels of N fertilizer. Intercropping reduced disease incidence (measured by the odds ratio of disease occurrence) by 45% on average. This reduction was significant ($P < 0.01$) for four out of six studied pathogens: yellow rust (*Puccinia striiformis* f.sp. *tritici*) and mildew (*Blumeria graminis*) in wheat (*Triticum aestivum*), and chocolate spot (*Botrytis fabae*) and Fusarium wilt (*Fusarium oxysporum*) in faba bean (*Vicia faba*). Disease reduction was marginally significant for yellow rust in barley (*Puccinia striiformis* f.sp. *hordei*) ($P < 0.10$) and not significant for bean rust (*Uromyces fabae*). The reduction in disease incidence was greatest during the early stages of epidemics. N fertilizer strongly increased the incidence of powdery mildew of wheat, but it did not affect the incidence of the other diseases and it did not affect the effectiveness of intercropping as a management strategy for disease control. While nitrogen input increased powdery mildew incidence in both sole and intercropped wheat, the incidence was lower in the intercropped than sole wheat at all levels of N input. The disease suppressive effect of intercropping on wheat powdery mildew or any other disease was not affected by the amount of nitrogen fertilizer. The results show that intercropping has a substantial and consistent effect on disease incidence in cereal/faba bean mixtures across studies, but is not sufficient to provide complete disease control. Intercropping is therefore best used as a component in an integrated approach for managing plant diseases.

Chaochun Zhang and Yan Dong contributed equally to this work.

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Keywords Intercropping · Disease control · Meta-analysis · Nutrient management

Introduction

Pests and diseases are a major cause of crop losses, estimated at 10–16% of global agricultural productivity (Chakraborty and Newton 2011). Achieving effective control of pests and diseases is therefore important for food security (Wiik 2009; Savary et al. 2012). Diversification of crops at field and landscape level has been shown to suppress diseases, but the level of suppression varies substantially between studies (Zhu et al. 2000; Mitchell et al. 2003; Garrett et al. 2009; Turnbull and Hector 2010; Boudreau 2013). There is a need for quantitative synthesis to ascertain the extent to which crop diversity reduces disease, and assess the practical usefulness of this strategy in modern high input agricultural systems.

Intercropping is the cultivation of two or more crop species in the same field at the same time. Intercropping is used by smallholder farmers across the world to increase yield per unit area and reduce the risk of crop failure (Willey 1985; Vandermeer 1998; Zhang and Li 2003; Li et al. 2009; Martin-Guay et al. 2018). Intercropping has an average land equivalent ratio of slightly above 1.2 across the world, indicating that attaining the output of a unit area of intercrop would require 20% more land if sole crops were used (Yu et al. 2015). Thus, adoption of intercropping provides substantial land savings (Martin-Guay et al. 2018). Moreover, intercropping can improve soil quality (Cong et al. 2015) and it suppresses diseases (Trenbath 1993; Zhu et al. 2000; Boudreau 2013) and pests (Tooker and Frank 2012). Intercropping provides a possible pathway for ecological intensification, i.e. achieving high crop yields and improving the environment by using ecological mechanisms to reduce anthropogenic inputs in agriculture (Bommarco et al. 2013).

In practical intercropping, smallholder farmers usually combine crop species with complementary growth patterns and patterns of resource requirement over time or space (Lithourgidis et al. 2011). However, reductions in plant diseases and pests have been recorded also for variety mixtures, i.e. through intra-specific diversity (Zhu et al. 2000; Tooker and Frank 2012). Zhu et al. (2000) reported 94% reduction in plant disease in intercropping of two rice genotypes in Yunnan. Other

studies on intercropping for disease control have usually achieved lower rates of disease suppression, but experiments assessing the plant disease reducing effect of species mixtures have never been synthesized quantitatively through meta-analysis to provide an overarching analysis. There is a need for critical analysis of literature to synthesize the existing knowledge and ascertain the average levels of disease reduction attained in intercropping, its variability across studies, and factors affecting disease suppression.

Mixtures of cereals and legumes are widely used in traditional smallholder agriculture (Zhang and Li 2003) and they also attract interest in modern agriculture because they can reduce the need for synthetic nitrogen fertilizer input in farming systems (Pelzer et al. 2012; Bedoussac et al. 2015). The majority of studies on yield increase by intercropping have been done using mixtures of cereals and legumes (Yu et al. 2016) and disease suppression has also been most widely studied for cereal/legume mixtures (Boudreau 2013).

Cereal/faba bean mixtures are widely cultivated for food in southern China, particularly in the province of Yunnan where many studies on disease suppression by intercropping have been conducted over the last 20 years (Chen et al. 2007; Li et al. 2009). However, no synthesis has been made of the data in these studies, and their results are largely not available in the international literature because the original sources are in Chinese. Most of the studies on disease control by intercropping in Yunnan include nitrogen treatments to test the hypothesis that high rates of nitrogen fertilizer would increase plant disease and reduce the effectiveness of intercropping for disease control. Intensive farming relies largely on nitrogen fertilizer, and there is increasing concern on the fact that overuse nitrogen may exaggerate the yield losses due to the enhanced disease severity (Lv et al. 2005; Zhang et al. 2008), and thus increase the use of pesticides (Tilman et al. 2002). Analysing the interaction between the effects of intercropping and N fertilizer on plant disease is therefore pertinent. Very little work on these interactions has been published in English, but there is a substantial body of knowledge in the Chinese literature, particularly from the southern province of Yunnan, where intercropping is actively used by farmers (Hong et al. 2017).

Here, we synthesize the published information on disease suppression by intercropping in cereal/faba

bean mixtures in Yunnan. We quantify the effect of intercropping on important wheat diseases caused by *Puccinia striiformis* f. sp. *tritici* and *Blumeria graminis*, and in barley by *Puccinia striiformis* f. sp. *hordei*, as well as three major pathogens in field bean: *Botrytis fabae*, *Uromyces fabae* and *Fusarium oxysporum* f. sp. *fabae*. The results demonstrate large and consistent reductions in disease incidence across pathogen taxa, including three aerially spread plant pathogens (rusts and mildew), but also a soil-borne pathogen (*Fusarium*).

Methods

Data collection

A literature search was performed in Web of Science Core Collection and the China National Knowledge Infrastructure (CNKI) using the terms “disease”, “intercropping”, “nitrogen fertilizer” or “N fertilizer”, in various combinations in the fields for title, keywords and abstract. The search was originally done in 2010 and updated in May, 2018. We excluded review papers, studies without replicates, studies without quantitative data on disease incidence, or studies that did not report the rate of N fertilizer. The search resulted in 26 publications on a variety of systems, but with a dominance of cereal/legume mixtures. To reduce heterogeneity in the data, and because of the expected environmental benefit of an integration of legumes in cropping systems, we focused the current analysis on cereal/legume mixtures. The resulting dataset included 17 publications (supplement S2). It comprised seven peer-reviewed publications, two book chapters, and eight MSc and PhD theses (all in Chinese). Some publications reported the results of several experiments, and the total number of experiments was equal to 46. All selected publications turned out to be studies conducted in the province Yunnan in China (this was not a selection criterion but an outcome). Yunnan province is located in the southwest of China, and the high humidity in the region favours diseases. Many intercropping studies were conducted in this province following the work of Zhu et al. (2000) that showed the suppressive effect of rice variety mixtures on rice blast. The suppressive effect of

cereal/legume intercropping was rarely studied in other Chinese regions such as the Northwest of China, due to their semi-arid conditions unfavourable for disease (Zhang and Li 2003).

In our dataset, cereal crops were wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) (in two of the 26 papers), and the legume crop was in all instances faba bean (*Vicia faba*); these species are grown in most cereal/legume intercropping systems in Yunnan province. All the selected studies shared several common experimental design characteristics (supplement S3). In short, each experiment included three treatments: a sole cereal (wheat or barley), a sole faba bean, and an intercrop of these species. The most common intercrop configuration comprised an alternation of a cereal strip consisting of six rows and a faba bean strip consisting of two rows (Fig. S4, S5). This configuration is also common amongst local farmers. In all publications, plot sizes varied from 30 to 200 m². In 37 out of 46 experiments, disease observation data were collected in three replicate plots. In all other experiments, the number of replicates was unknown but always equal to or higher than three. The number of nitrogen fertilizer levels was three or four, including in most cases an unfertilized treatment, conventional fertilizer amount (varying among studies), a reduced rate (often 50% of conventional) and an increased rate (often 150% of conventional). No fungicides or insecticides were used in any of the experiments.

Disease incidence

Disease incidence was determined at five random positions in each plot (approximately in an X pattern across the plot) (Fig. S6) and at each sampling position the diseases were recorded on three faba bean plants or on one wheat or barley plant. In experiments including three replicates, each disease incidence observation was thus derived from a total of $3 \times 5 \times 3 = 45$ faba bean plants or $3 \times 5 = 15$ bunches of wheat or barley. All leaves of the observed plants were inspected to score disease presence or absence. Disease incidence (*I*) was calculated at the replicate level as the number of infected leaves in the plot divided by the number of surveyed leaves in the plot, and then averaged over replicates. In the case of

Fusarium wilt, 30 plants per plot were scored, and the presence of wilting symptoms on the plant was recorded. Dates of disease observation were classified in three time periods: early season when disease symptoms just start to be detectable, mid-season, 20 days after the first observation when disease incidence is rapidly increasing, and late season, 20 days after the second observation. Disease observation times were tailored to the different pathogens, depending on their dates of the first appearance in the field (Supplement S3).

Disease incidence data were converted to logits for further analysis (Agresti 2002; Zadoks and Schein 1979), y :

$$y = \text{logit}(x/100) = \ln\left(\frac{x}{100-x}\right) \quad (1)$$

where x is disease incidence (proportion, in %), $x/(100-x)$ are the “odds” of disease incidence (i.e. the ratio of the proportion of infected leaves to the proportion of healthy leaves), and y is the logit of disease incidence. The logit is the natural logarithm of the Odds (here, the Odds of disease occurrence). This function is commonly used to transform variables taking values in the range 0–1 (e.g., proportions computed from binomial data). Such variables cannot be directly related to a linear model because a linear combination of covariates can take any real value, and there is no guarantee that the values predicted by the linear model will be in the range 0–1. The logit function maps proportions from the range (0, 1) to the entire real line. Thus, the logit transformation removes the 0–1 range restriction, and the transformed data can then be directly related to any linear combination of covariates. Logits are back-transformed to incidence (%) as $x = \frac{100 \times e^y}{1 + e^y}$. Incidence observations equal to 0 or 100 were set equal to 0.1 and 99.9, respectively, to avoid singularities when calculating logits.

The collected publications contained data on six diseases, two of them infecting wheat (powdery mildew, *Blumeria graminis* (DC.) Speer, and yellow rust, *Puccinia striiformis* f. sp. *tritici*), one infecting barley (yellow rust, *Puccinia striiformis* f. sp. *hordei*), and three of them infecting faba bean (chocolate spot, *Botrytis fabae*, bean rust, *Uromyces fabae*, and Fusarium wilt, *Fusarium oxysporum* f. sp. *fabae*).

Disease control effectiveness

Within each block, we calculated the difference in y between the plants of a given species in intercrop plots and those in the sole crop plots:

$$\begin{aligned} \Delta y &= \text{logit}(x_{IC}/100) - \text{logit}(x_M/100) = \ln\left(\frac{x_{IC}}{100-x_{IC}}\right) - \ln\left(\frac{x_M}{100-x_M}\right) \quad (2) \\ &= \ln\left(\frac{\left(\frac{x_{IC}}{100-x_{IC}}\right)}{\left(\frac{x_M}{100-x_M}\right)}\right) \end{aligned}$$

where x_{IC} is disease incidence in the intercrop and x_M is disease incidence in the sole crop.

Δy is also known as the log of the odds ratio (Agresti 2002; Borenstein et al. 2009). The log odds ratio is frequently used in meta-analysis when the data represent proportions of diseased individuals (Borenstein et al. 2009). Analyses of the disease control effect of intercropping were conducted using Δy as response variable. The disease control effectiveness (CE) was calculated from Δy as $CE = 100(1 - e^{-\Delta y})$, where $e^{-\Delta y}$ is the ratio of the odds of disease in the intercrop versus the sole crop. Positive values of CE indicate that intercropping reduces relative disease risk, while negative values indicate that intercropping increases relative risk. As all the considered diseases are specific to either the cereal or the legume species, disease in only one of the sole crops was considered as a reference to calculate Δy .

Explanatory variables and statistical analysis

Δy was analysed using linear mixed effects models (Table 1), considering three explanatory variables (Table S1): pathogen species (categorical with six levels), observation time (categorical with three levels), fertilizer level (categorical with four levels) and N dose. Seven linear mixed effects models were formulated (Table 1). All seven models included nested random effects reflecting the effects of factors associated with publications, and within publication the effect of study (site \times year) (Pinheiro and Bates 2000; Zuur et al. 2009; Yu et al. 2015). The optimal random effects structure (publication and study as nested random effects) was identified using Akaike’s Information Criterion (Zuur et al. 2009). A Box-Cox transformation was applied to normalize Δy and an optimal exponent for this transformation was selected using the

Table 1 Mixed-effect models fitted to analyse relationship between log disease odds ratio and pathogen species, time of observation, and N fertilizer

Model	Equation
1	$\Delta y_{ijk} = \beta_0 + a_i + b_{ij} + \epsilon_{ijk}$
2	$\Delta y_{ijk} = \beta_{\text{time}}(\text{time}_{ijk}) + a_i + b_{ij} + \epsilon_{ijk}$
3	$\Delta y_{ijk} = \beta_{\text{disease}}(\text{disease}_{ijk}) + a_i + b_{ij} + \epsilon_{ijk}$
4	$\Delta y_{ijk} = \beta_{\text{disease}}(\text{disease}_{ijk}) + \beta_{\text{time}}(\text{time}_{ijk}) + a_i + b_{ij} + \epsilon_{ijk}$
5	$\Delta y_{ijk} = \beta_{\text{NlevelCereal}}(\text{NlevelCereal}_{ijk}) + a_i + b_{ij} + \epsilon_{ijk}$
6	$\Delta y_{ijk} = \beta_{\text{NlevelLegume}}(\text{NlevelLegume}_{ijk}) + a_i + b_{ij} + \epsilon_{ijk}$
7	$\Delta y_{ijk} = \beta_{\text{Ntotal}} N_{\text{total } ijk} + a_i + b_{ij} + \epsilon_{ijk}$

(Seven models were fitted to data. All are mixed effects models accounting for fixed effects (disease, time of observation, N fertilizer) and random effects (publication, study (disease) within publication, and multiple observations within studies). Model 1 was used to describe the mean and variability of log disease odds ratio between intercropping and sole cropping (Fig. 1). Model 2 was used to describe differences between observation times in the effect of intercropping (Fig. S1). Here $\beta_{\text{time}}(\text{time}_{ijk})$ can take three values, depending on the time of observation. The values describe the average effect of intercropping on disease incidence at three times of observation (early, middle or late). Model 3 was used to describe the overall effect of intercropping on six different diseases (Fig. 2d). Here $\beta_{\text{disease}}(\text{disease}_{ijk})$ describes the average effect of intercropping on the incidence of each of six diseases (wheat powdery mildew (WPM), wheat yellow rust (WR), barley rust (BR), faba bean chocolate spot (FBLP), faba bean rust (FBR), faba bean wilt (FBW)). Model 4 was used to describe the effect of intercropping on six diseases at three different observation times (Fig. 2a–c). Here $\beta_{\text{disease}}(\text{disease}_{ijk})$ and $\beta_{\text{time}}(\text{time}_{ijk})$ are the vectors with the same meanings of those in the Model 3 and the Model 2, respectively. Model 5 was used to describe the effect of intercropping on four levels of nitrogen fertilizer applied to the cereal strip (Fig. 3a), while model 6 does the same for fertilizer applied to the legume (Fig. 3b). Here $\beta_{\text{NlevelCereal}}(\text{NlevelCereal}_{ijk})$ or $\beta_{\text{NlevelLegume}}(\text{NlevelLegume}_{ijk})$ is the vector with four elements representing the average effect of intercropping on the incidence at each of four N levels (no N application, half of the optimal N level, N dose recommended in practice, N dose 50% higher than the recommended dose) given cereal or legume crop, respectively. Model 7 was used to describe the effect of intercropping varying with N dosage. The indices $i, j,$ and k represent publication ID, study ID and observation number, respectively. Study refers to the combination of crop species and disease within the publication. In all mixed effect models, a_i is a random publication effect; b_{ij} is a random study effect nested within the i^{th} publication. Both a_i and b_{ij} are assumed normally distributed with constant variances. ϵ_{ijk} is a residual random error assumed normally distributed with constant variance. The variance terms, a_i, b_{ij} and ϵ_{ijk} were all assumed independent)

Shapiro test (Royston 1982). The normality of the residuals of the selected model was checked graphically (Fig. S7) (Zuur et al. 2009).

To study the effect of intercropping and N input on disease incidence, we analysed the logit-transformed disease incidence (y). This variable was related to three explanatory variables using three linear mixed regression models including N fertilizer dosage, cropping system (categorical with two levels) and observation time (categorical with three levels) as independent variables (Table 2). All these four models were optimally structured with respect to random effects as described for models for Δy . Furthermore, the logit-transformed disease incidence (y) was further optimized for analysis by applying the Box-Cox transformation to normalize y (Royston 1982). The optimal exponent for this transformation was found by maximizing the P value of Shapiro’s test for departure from normality of residuals. The normality of the residuals of the selected model was checked graphically (Fig. S8).

All analyses were performed in R (R Core Team, 2015) using the R function lme for fitting linear mixed effects models (R package nlme; Pinheiro and Bates 2000).

Table 2 Mixed-effect models fitted to analyse relationship between log disease odds and N dose and cropping systems

Model	Equation
1	$y_{ijk} = \beta_0 N_{\text{total } ijk} + a_i + b_{ij} + \epsilon_{ijk}$
2	$y_{ijk} = \beta_{\text{CS}}(\text{CS}_{ijk}) + \beta_{\text{Ntotal}} N_{\text{total } ijk} + a_i + b_{ij} + \epsilon_{ijk}$
3	$y_{ijk} = \beta_{\text{CS}}(\text{CS}_{ijk}) + \beta_{\text{NCereal}} N_{\text{cereal } ijk} + a_i + b_{ij} + \epsilon_{ijk}$
4	$y_{ijk} = \beta_{\text{CS}}(\text{CS}_{ijk}) + \beta_{\text{NLegume}} N_{\text{legume } ijk} + a_i + b_{ij} + \epsilon_{ijk}$

(Four models were fitted to data. All are mixed effects models accounting for fixed effects (cropping system and N fertilizer) and random effects (publication, study (disease) within publication, and multiple observations within studies). Model 1 was used to describe overall effect of nitrogen rate on log disease odds. Model 2 was used to describe a regression of log disease odds against nitrogen dosage in two cropping systems across all times (Fig. S2) or at each observation time (Fig. S3). Here $\beta_{\text{CS}}(\text{CS}_{ijk})$ describes the log disease odds of two cropping systems (intercropping and sole cropping). Model 3 or Model 4 was used to describe the regression of log disease odds in two cropping systems against nitrogen dosage given specifically to cereal in the cereal strip (Fig. 4a, b), or N given to the legume in the legume strip (Fig. 4c), respectively. The indices $i, j,$ and k represent publication ID, study ID and observation number, respectively. Study refers to the combination of crop species and disease within the Publication. In all mixed effect models, a_i is a random publication effect; b_{ij} is a random study effect nested within the i^{th} publication. Both a_i and b_{ij} are assumed normally distributed with constant variances. ϵ_{ijk} is a residual random error assumed normally distributed with constant variance. The variance terms, a_i, b_{ij} and ϵ_{ijk} were all assumed independent)

Results

Disease control effectiveness of the intercropping

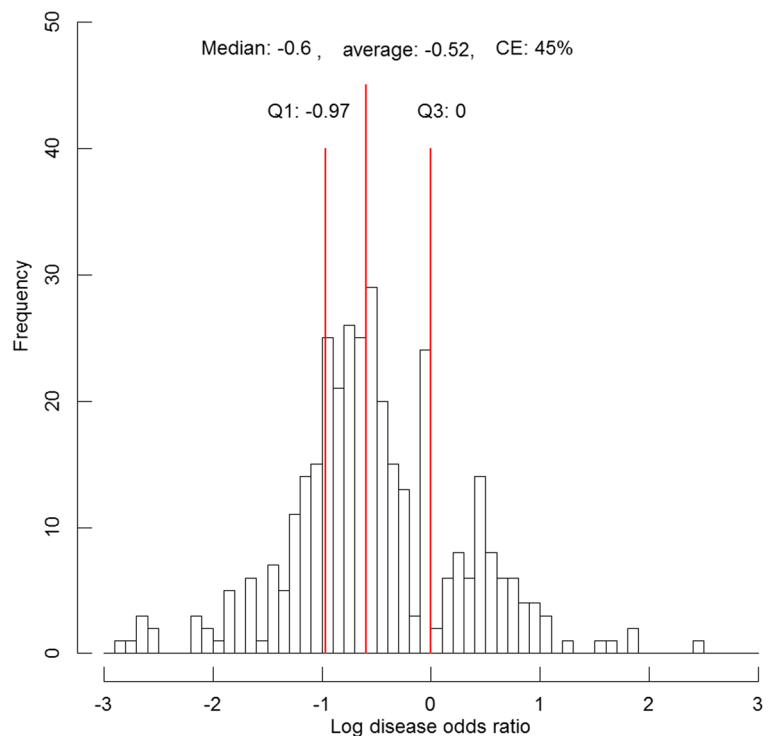
The disease odds ratio in the intercrop and the sole crop was measured with the equation: $\frac{x_{IC}}{100-x_{IC}} / \frac{x_M}{100-x_M}$, where x_{IC} is the measured incidence of disease in the intercrop and x_M is the measured incidence of disease in the sole crop (IC for intercropping and M for Sole crop) (Fig. 1). The median log of the disease odds ratio was equal to -0.60 and was highly variable over studies, experiments within studies, and treatments within experiments (Fig. 1; model 1 in Table 1). About 75% of the log odds ratios were negative, and this result indicated that disease incidence is reduced in intercropping in most cases, and intercropping gave an average reduction in relative disease risk of $CE = 45\%$ (Fig. 1).

The effect of intercropping on disease incidence varied over the season (model 2; Table 1 Fig. S1), with a greater control effect during early ($\Delta y = \beta_{\text{time}}(\text{early}) = -0.407$, $CE = 33\%$, $P < 0.001$; model 2; Table 1) and mid-season ($\Delta y = \beta_{\text{time}}(\text{mid}) = -0.305$, $CE = 26\%$, $P < 0.001$; model 2; Table 1) than during late season ($\Delta y = \beta_{\text{time}}(\text{late}) = -0.118$, $CE = 11\%$, $P = 0.055$; model 2;

Table 1) (Fig. 2). Intercropping reduced the odds of yellow rust of wheat by 28% ($\Delta y = \beta_{\text{disease}}(\text{WR}) = -0.332$, $P < 0.001$; model 3; Table 1) and of powdery mildew of wheat by 21% ($\Delta y = \beta_{\text{time}}(\text{WPM}) = -0.240$, $P = 0.008$; model 3; Table 1) according to the mean estimates obtained from the fitted mixed-effect models (Fig. 2). The meta-analysis demonstrated no significant effect of intercropping on barley rust ($P = 0.508$), possibly due to the small number of studies with barley as the cereal. A 31% reduction in the odds of disease was observed for chocolate spot of faba bean ($\Delta y = \beta_{\text{disease}}(\text{FBLP}) = -0.376$, $P < 0.001$; model 3; Table 1) and a large (39%) but only marginally significant effect on bean rust ($\Delta y = \beta_{\text{disease}}(\text{FBR}) = -0.488$, $P = 0.080$; model 3; Table 1). Intercropping reduced the odds of Fusarium wilt in faba bean by 72% ($\Delta y = \beta_{\text{disease}}(\text{FBW}) = -1.28$, $P < 0.001$; model 3; Table 1).

For none of the six diseases did we find an effect of N fertilizer rate on the control effectiveness of intercropping (Fig. 3; models 5 and 6; Table 1). N input dosage did not significantly affect the control effectiveness (CE) across all diseases ($P = 0.478$; model 7; Table 1), and neither influenced the CE of any of the six diseases ($P = 0.759$ for wheat powdery mildew, $P = 0.277$ for wheat yellow rust,

Fig. 1 Frequency distribution of the relative odds of disease in intercropping as compared to sole cropping, using 351 data records, originating from 46 experiments on the effects of intercropping on plant diseases as a function of nitrogen level, and 86 crop species-pathogen combinations within experiments (i.e. on average 1.87 crop species-pathogen per experiment). Horizontal axis represents (Δy), i.e. the log of the disease odds ratio, or – equivalently - the difference in logit of incidence of disease in the intercrop and the sole crop (Eq. 1). Vertical lines indicate the first quartile (Q1), median and the third (Q3) quartile of the intercropping effect. CE is the control effectiveness of intercropping, calculated as $CE = 100(1 - e^{\Delta y})$



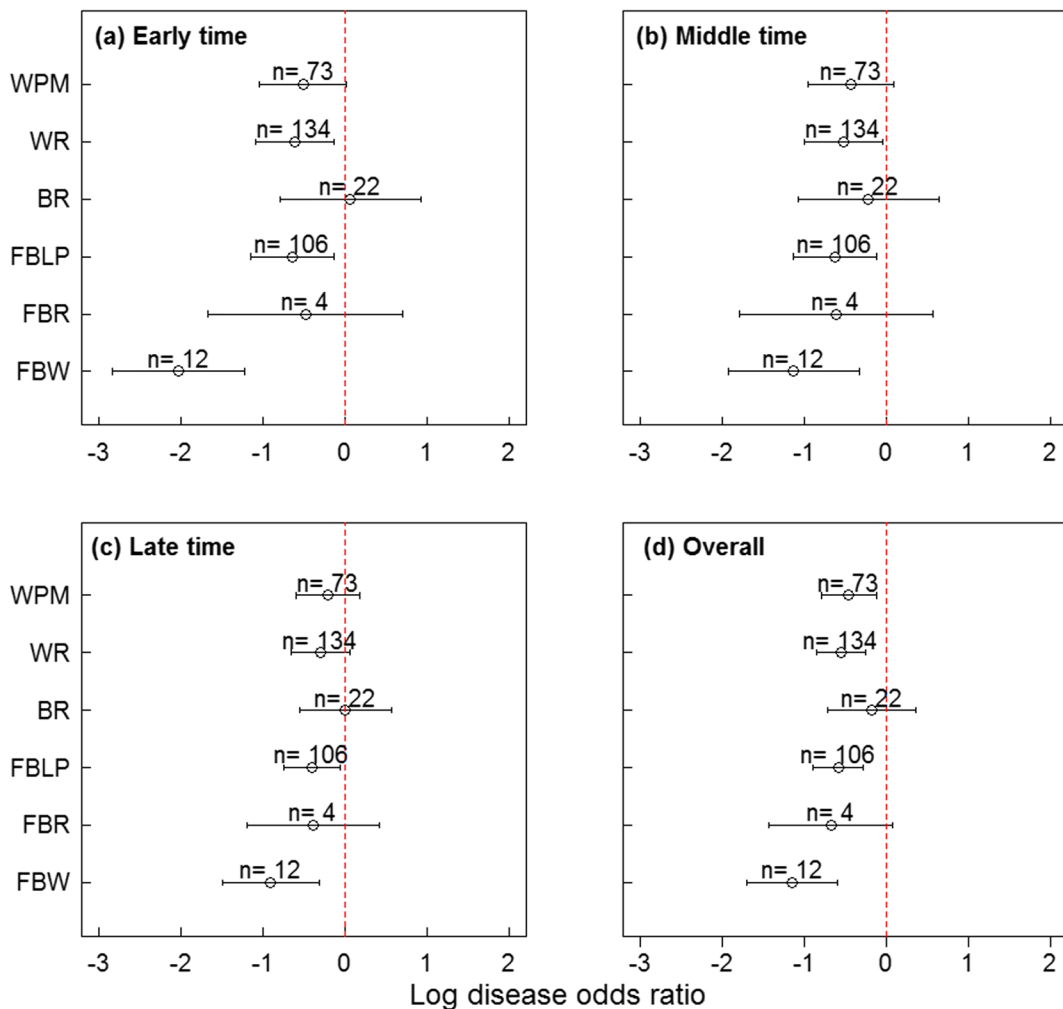


Fig. 2 Relative incidence of disease in intercropping as compared to sole crop for six plant diseases and during three phases of the epidemic: early (**a**), mid (**b**) and late (**c**) and overall (**d**). Abbreviations for diseases: WPM: wheat powdery mildew (*Blumeria graminis* (DC.) Speer); WR: wheat yellow rust (*Puccinia striiformis* f. sp. *tritici*); BR: barley rust (*Puccinia striiformis* west f. sp. *hordei*); FBLP: faba bean chocolate spot (*Botrytis fabae*);

$P = 0.280$ for barley rust, $P = 0.581$ for faba bean chocolate spot, $P = 0.888$ for faba bean rust, and $P = 0.914$ for faba bean wilt). We therefore conclude that intercropping suppresses plant disease consistently across dosages of N fertilizer.

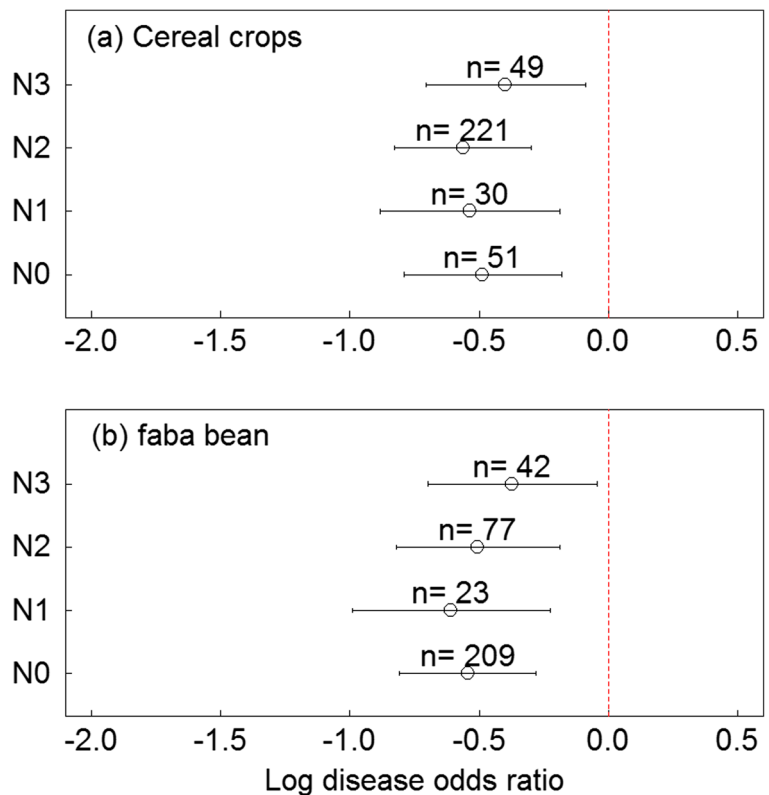
Disease incidence affected by intercropping and nitrogen

Plant disease incidence increased significantly with N fertilizer input ($P = 0.014$; model 1; Table 2), with a marginal effect on intercrops but no effect on sole

FBR: faba bean rust (*Uromyces viciae fabae*); FBW: faba bean Fusarium wilt (*Fusarium oxysporum* f. sp. *fabae*). Times of observation are detailed in Supplementary materials S2. Horizontal axis (Δy) represents the log of the disease odds ratio, i.e. the difference in logit (incidence) in the intercrop and the control treatment (sole crop) (Eq. 2). Error bars represent 95% confidence intervals. n indicates the number of records in the database for a given disease

crops (Fig. S2; model 1; Table 2). The effect of N dose on disease incidence differed with the time of survey (Fig. S3; model 1; Table 2). Disease incidence at the mid time increased significantly with N fertilizer, but the incidences at both early and late observation times were not significantly affected by N fertilizer input. Higher doses of N fertilizer significantly increased wheat Powdery Mildew incidence in both sole crops and intercrops (Fig. 4a; model 2; Table 2), but N fertilizer did not influence the incidence of other diseases (Fig. 4b, c; models 3 and 4; Table 2).

Fig. 3 Relative incidence of disease in intercropping as compared to sole crop at four levels of nitrogen fertilizer input: N0, no nitrogen; N1, half the recommended rate; N2, recommended rate; N3, recommended rate + 50%. Horizontal axis (Δy) represents the log disease odds ratio, i.e. the difference in $\text{logit}(\text{incidence})$ in the intercrop and the control treatment (sole crop) (Eq. 2). Error bars represent 95% confidence intervals. n indicates the number of records in the database for a given disease



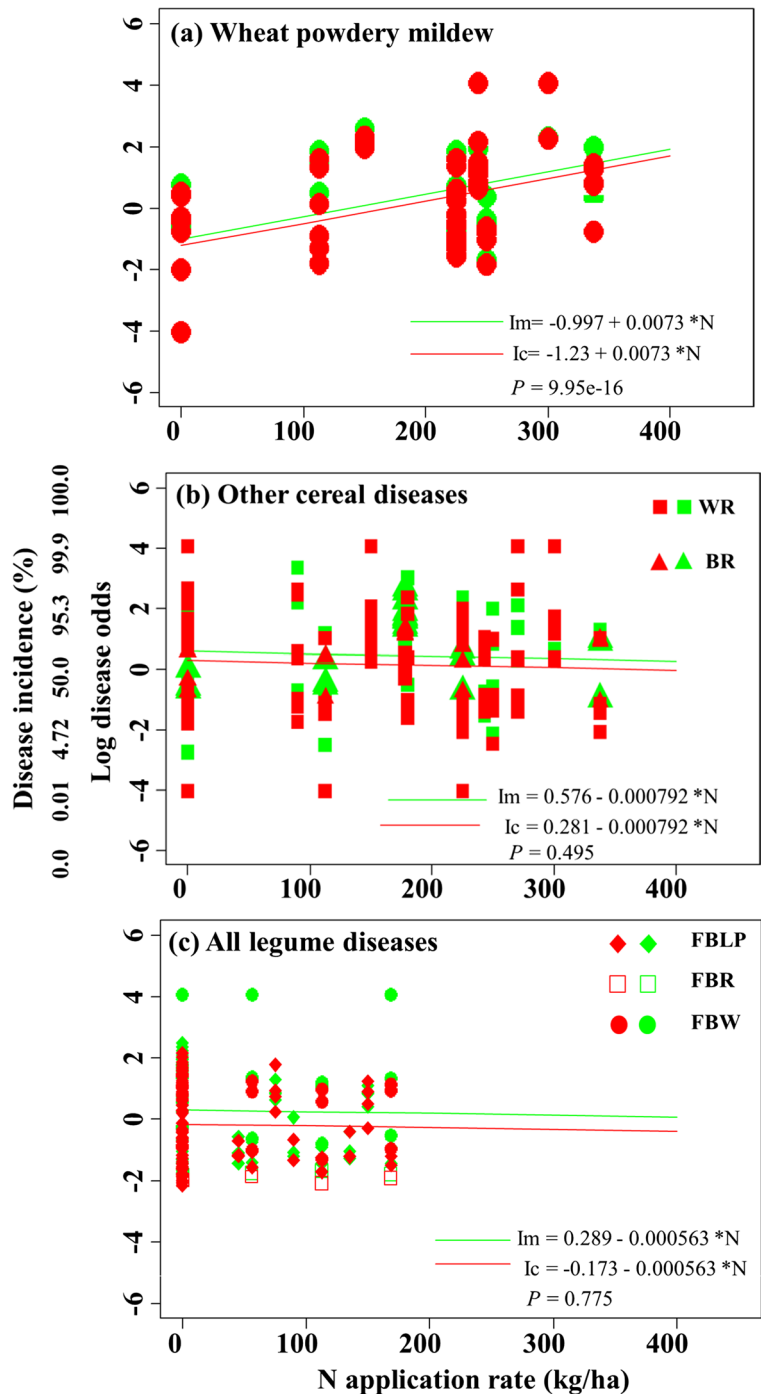
Discussion

Results of this meta-analysis show that intercropping consistently and substantially suppresses plant diseases. The overall average disease reduction was 33% ($\pm 4\%$). The level of disease suppression varied between pathogens. The average control effect was 34% ($\pm 7\%$) for *P. striiformis* in wheat, 20% ($\pm 9\%$) for *B. graminis* in wheat, and insignificant for *P. striiformis* in barley. Control effectiveness in field bean was 39% ($\pm 7\%$) for *B. fabae*, 36% ($\pm 27\%$) for *U. fabae*, and 51% ($\pm 21\%$) for *F. oxysporum*. The first five diseases are caused by air-borne obligate pathogens, and the last one is induced by a soil-borne facultative pathogen. The evidence confirms that greater host functional divergence, as the result of intercropping, leads to disease reduction (Mitchell et al. 2003; Garrett et al. 2009; Keesing et al. 2010; Dassou and Tixier 2016). Although the disease reduction levels reported here are both statistically significant and biologically relevant, they do not lead to a complete control of the diseases. This indicates that intercropping may not be suitable as a standalone solution, but it could well be used in an integrated pest

management approach in which it may be combined with other management techniques such disease resistant cultivars, sanitation measures, diversified rotations, and fungicides.

Boudreau (2013) lists five mechanisms for disease suppression by intercropping: (1) interference with spore dispersal by wind or rain (as related to barrier effects of companion crops and host dilution in space), (2) interference with vectoring of disease, (3) alterations in micro-climate, (4) changes in host physiology and resistance, and (5) direct pathogen inhibition. Four of the five diseases that showed suppression in intercrops in this meta-analysis are dispersed by air, and it is highly plausible that host dilution in intercrops in these cases reduced the efficiency of spore deposition on hosts and the rate of spread of disease. This explanation is consistent with the greater reductions during early season, as a slower epidemic (resulting from lower dispersal efficacy) can catch up with a faster epidemic in later season (Skelsey et al. 2005). Results of this study are difficult to reconcile with a possible role for altered microclimate. All of the studied pathogens except *F. oxysporum* require leaf wetness for spore

Fig. 4 Effect of nitrogen fertilizer on plant disease incidence on sole and intercropped wheat and faba bean. Green symbols show disease incidence (logit values; Eq. 1) in sole crops while red symbols show disease incidence on the same species in an intercrop. Panel A shows a strong and highly significant increase in the incidence of powdery mildew (*Blumeria graminis*) in sole and intercropped wheat. Panel B shows absence of a significant effect of nitrogen fertilizer on the incidence of yellow rust in wheat (WR; *Puccinia striiformis* f.sp. *tritici*) and yellow rust in barley (BR; *Puccinia striiformis* f.sp. *hordei*). Panel C shows absence of a significant effect of nitrogen fertilizer on three diseases in faba bean: leaf spot (FBLP; *Botrytis fabae*), rust (FBR; *Uromyces fabae*) and wilt (FBW; *Fusarium oxysporum* f.sp. *fabae*). In all three panels, there was a significant effect of intercropping on the intercept ($P < 0.05$) while there was no significant effect on the slope. Only *B. graminis* responded to nitrogen level. Fitted models have a common slope (after testing for difference in slope between sole cropping and intercropping, which was in all cases not significant)



germination and infection. It is difficult to see how mixing a cereal with field bean would lower leaf wetness in both species. Hence, it seems not so likely that changed microclimate plays a role. The studied diseases are not vectored, and hence interference with vectors is not involved. Direct

inhibition seems also unlikely for the aerially dispersed pathogens. Alterations in host physiology and resistance cannot be completely ruled out; however, this hypothesis does not need to be invoked because the level of disease suppression by intercropping in this meta-analysis is consistent with

levels of disease suppression that have been found in models of plant disease epidemics in mixtures that take into account only the host dilution effect (Mundt 2002; Skelsey et al. 2005). Hence, the explanation for intercropping effect on plant disease that is most plausible for the aerially spread pathogens in this meta-analysis is the host dilution effect.

Fusarium wilt in faba bean is a soil-borne disease. Intercropping had greater control effectiveness on this disease than on the five aerially dispersed pathogens (Fig. 2d). Evidence is accumulating that soil-borne diseases can be affected by biotic interactions in the rhizosphere that are driven by root exudates (Ren et al. 2008; Philippot et al. 2013; Xu et al. 2015; Jiang et al. 2017). Root exudates contain an array of primary and secondary plant metabolites that can attract, deter, or kill belowground microbes (Weston and Mathesius 2013; Strehmel et al. 2014; van Dam and Bouwmeester 2016). A recent study showed that plants roots excrete lipids that promote the growth of mycorrhizal and plant pathogenic fungi (Jiang et al. 2017). Thus, the working mechanisms of intercropping might differ fundamentally between aerially dispersed pathogens and pathogens that affect the below-ground plant parts.

Nitrogen fertilizer is commonly regarded as a key force driving disease occurrence due to its effect on plant nutritional status and the suitability of the plant for pathogen multiplication (Hoffland et al. 1999; Snoeijers et al. 2000; Dordas 2008). Additionally, greater N application may cause a denser canopy, which results in a more favorable microclimate for infection (Jensen and Munk 1997; Simón et al. 2003; Neumann et al. 2004). In this data-set, nitrogen affected only the incidence of powdery mildew in wheat, and the incidence of other diseases was not affected. Here we found that intercropping was an effective disease suppressive measure for five out of six considered pathogens at all N fertilization levels, indicating that the disease suppressive effect may be exploited in high input agriculture.

Knowing the extent to which intercropping reduces disease is critical for end-users to raise interest in this ecological approach for disease management and to reduce where possible reliance on chemical pesticides. Our quantitative synthesis of experiments under smallholder conditions with zero fungicide input in cereal/legume systems provides strong evidence of the suppressive effect of intercropping, and demonstrates the

interest of including this approach within an integrated pest management framework.

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Author's contributions YD and CZ did the literature search, extracted data and conducted the analysis. CZ, YD, YY, DM and WW designed methods for statistical meta-analysis and interpreted results. All authors contributed to formulation of research aims and overall approach, discussed outcomes and approved the submission. CZ, YD, DM and WW wrote the paper.

Compliance with ethical standards

Conflict of interests The authors declare no conflicts of interests.

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