

## **Antagonistic responses to crop diversification at two levels of fertiliser and herbicide application**

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### **Summary**

The use of pesticides and fertilisers in the last decades has led to numerous problems for mankind and the environment: polluted ground water, decreased soil quality, biodiversity loss and resistance of weed and pest species. To counteract these problems, conventional farming systems will need to become more sustainable. One approach is intercropping, which is common in traditional or low intensity farming, but not in conventional agriculture. Thus, little is known about responses of biotic interactions to intercropping under conventional management. In a cereal-legume intercropping experiment, we found that disease infection was highest in cereal sole crops and weed biomass was highest in legume sole crops, while herbivory was highest in intercrops. Management intensity had significant effects on pathogen infection, herbivory and weed biomass, but results differed between wheat-bean vs barley-pea cropping systems, showing that intercropping in conventional agriculture can reduce antagonistic patterns, but also that plant species must be specifically chosen for optimised performance with respect to reduced external inputs.

**Key words:** Cereal-legume intercropping, high and low management intensity, weed biomass, herbivory, disease

### **Introduction**

Growing demands for food, feed, and fuel from a growing world population result in large areas being transformed into agricultural systems. Today, already more than 40% of Europe's land area is used for agriculture (Eurostat, 2018). Most agricultural systems are intensively managed sole crop fields, which receive high inputs of synthetic fertilisers and pesticides to maximise yields. The massive use of pesticides (i.e. herbicides) has led to decreases in biodiversity components such as weeds, arthropods, and birds, which may result in dramatic effects across trophic levels, and to resistance in many weed and pest species (Gould *et al.*, 2018). However, as there is a lot of pressure to produce high-quality products, pesticides and fertilisers are still used in high quantities, continuing to pollute water systems, and to decrease soil quality (Foley *et al.*, 2011; Knapp & van der Heijden, 2018).

Farming practices therefore need to become more sustainable, while maintaining yield stability and increasing land use efficiency at reduced environmental impacts. One potential approach to change agriculture is intercropping, especially when it is extended not only to organic, but also to conventional farming. Intercropping is a practice where more than one crop species is cultivated on the same land, either simultaneously (mixed or strip intercropping) or with only partial overlap of growing periods (relay cropping). Intercropping has been shown to reduce disease incidence, pest outbreaks and weed infestation in agricultural systems (Hauggaard-Nielsen *et al.*, 2008; Letourneau *et al.*, 2011), which may reduce application of herbicides and fertilisers needed. Intercropping is, however, mostly used in organic farming. Consequently, there is little evidence about biotic interactions to crop diversification in intensively managed intercropping systems in Europe.

Within the framework of the pan-European DIVERSify project ([www.plant-teams.eu](http://www.plant-teams.eu)), we set up an intercropping trial in 2018 in Germany where we manipulated not only crop diversity and plant identity, but also management intensity (high vs low input) for cereal-legume sole and intercrops. We collected data on weed biomass, herbivory and disease incidence in a plot-scale experiment for wheat and faba bean sole crops and their intercrops in one field, and for barley and pea sole crops and their intercrops in a second field under high and low management intensity.

We hypothesise that weed biomass, disease and herbivory will be lower in intercrops than in sole crops, and we expect that there will be differences between the two types of management intensity. We assume that weed biomass, disease and herbivory will be reduced under high in comparison with low management intensity.

## Material and Methods

### *Field design*

We experimentally manipulated crop species, cultivars, crop density and management intensity (high vs low) in two separate fields with 96 plots each (1.5 m × 5 m) in a randomized split-plot design in Münster, Germany (51°58'29.8"N 7°34'02.7"E). Plots were sown with a sowing machine (Wintersteiger Plotseed S with Hege 80 carrier with Lemken single disk opener) on 23 April 2018, at a row spacing of 12.5 cm with 24 rows per plot at a sowing depth of 6 cm in sole crops and intercrops of spring wheat (*Triticum aestivum* L.) and faba bean (*Vicia faba* L.) in one field, and spring barley (*Hordeum vulgare* L.) and pea (*Pisum sativum* L.) in another field (Fig. 1). A 50/50 and a 75/25 replacement design was used for intercrops, where each species was sown at 50% and 75% (legume) and 25% (cereal) of the sole crop density, respectively (Table 1). Each field comprised two replicate blocks with management assigned at random to one half of each block. Every block was made of four columns, out of which two received no treatment (low input) and the other two received one herbicide spray (4.4 L ha<sup>-1</sup> Stomp Aqua, 455 g L<sup>-1</sup> Pendimethalin) and nitrogen fertiliser (70 kg N ha<sup>-1</sup>, high input). Crop diversity/cropping system (legume or cereal sole crop or intercrop) was assigned to random positions within each column, resulting in a total of N=192 plots. Within each plot, data collection was conducted on a standardised randomly allocated area of 1m<sup>2</sup> (with sufficient buffers to plot edges reduce edge effects). For data analysis, we only considered cereal and legume sole crops and their 50/50 intercrops (N=128).

### *Data collection*

The amount of herbivory and disease was monitored between 7 July and 26 July 2018 (field one: barley-pea) and between 28 June and 19 July 2018 (field two: wheat-bean) for five legume and four cereal plants chosen at random on one m<sup>2</sup> per plot. For every plant, the percentage of damaged (herbivory) or infected (disease) area was estimated visually. Weed biomass was harvested using scissors at one cm above ground between 23 July and 26 July 2018 (field one) and between 28 June and 19 July 2018 (field two) on a 40 cm × 40 cm area. Harvested plant material was oven-dried at 70°C for 72 h and weighted. Weed biomass was extrapolated to g m<sup>2</sup>.



Fig. 1. Experimental field in 2018 sown with sole crops and intercrops of wheat and faba bean (left hand side) and barley and pea (right hand side). Each field was divided into 96 plots á 1.5 m × 5 m. Jan Lehmann<sup>©</sup>.

Table 1. Overview of crop species, cultivars, country and breeders and sowing density (plants m<sup>2</sup> in sole crop) for the two fields used in 2018

Crop species	Cultivar	Country	Breeder	Plants m <sup>2</sup> sole crop	Field
<i>Hordeum vulgare</i>	Salome	Germany	Nordsaat	360	1
	Sunshine	Germany	Saatzucht J. Breun	360	1
<i>Pisum sativum</i>	Astronaute	Italy	Agroservice Spa	80	1
	Hardy	Italy	Agroservice Spa	80	1
<i>Triticum aestivum</i>	Cornetto	Denmark	Lantmännen Agro	440	2
	Tybalt	Germany	Saaten-Union	440	2
<i>Vicia faba</i>	Fuego	Germany	Norddeutsche Pflanzenzucht Hans-Georg Lembke KG	40	2
	Julia	Austria	Saatzucht Gleisdorf	40	2

### Statistical analysis

Proportion data (herbivory, disease) were logit transformed to calculate mean values per plant; the resulting mean values were inverse-logit transformed and then analysed using generalised linear mixed-effects models fit by penalised quasi-likelihood (package MASS, Venables & Ripley, 2002) with block, management and column as random effects; crop diversity/cropping system, management intensity and their interaction as fixed effects and binomial errors. Data on weed biomass were log-transformed and analysed using linear mixed-effects models (package nlme, Pinheiro *et al.*, 2019) with block, management and column as random effects and crop diversity/cropping system, management intensity and their interaction as fixed effects. Model predictions and confidence intervals (CIs) were derived using the effects package (Fox & Weisberg, 2018). Data analysis was done with the software package R (version 3.6.2, R Core Team, 2019) implemented in RStudio (version 1.2.5019).

### Results

Across both cropping systems (barley-pea and wheat-faba bean), herbivory was significantly higher in intercrops than in sole crops, whereas disease infection was significantly higher for high than for low intensity management. Weed biomass, on the other hand, was influenced by an interaction between crop diversity and management intensity and was higher in sole crops and for low intensity management (Table 2, Fig. 2).

Table 2. *Chi-square values, family distributions and levels of significance for generalised linear mixed-effects models fit by penalised quasi-likelihood on herbivory and disease and for linear mixed-effects model on weed biomass (log-transformed) vs explanatory variables (CD=crop diversity, MI=management intensity, and their interaction). Explanatory variables in **bold** mark significant differences with \*\*\*P<0.001, \*\*P<0.01 and \*P<0.05. Degrees of freedom=1 for all models*

Response	Explanatory	Family	$\chi^2$	Pr(> $\chi^2$ )	
Herbivory	<b>CD</b>	binomial	<b>5.173</b>	<b>0.023</b>	*
	MI		1.285	0.257	
Disease	CD	binomial	0.095	0.759	
	<b>MI</b>		<b>19.828</b>	<b>&lt;0.001</b>	***
Weed biomass	<b>CD</b>		<b>20.111</b>	<b>&lt;0.001</b>	***
	<b>MI</b>		<b>111.197</b>	<b>&lt;0.001</b>	***
	<b>CD:MI</b>		<b>4.49</b>	<b>0.034</b>	*

#### *Barley-pea cropping system*

For the barley-pea cropping system, herbivory was higher for high than for low management intensity, whereas differences between intercrops and sole crops were small. Disease infection was significantly higher under high than under low management intensity and was highest for barley sole crops and lowest for pea sole crops. Weed biomass was significantly influenced by an interaction between cropping system and management intensity (Table 3). Weed biomass was highest in pea sole crops, for both types of management intensity (Fig. 3a,b,c).

Table 3. Chi-square values, family distributions and levels of significance for generalised linear mixed-effects models fit by penalised quasi-likelihood on herbivory and disease and for linear mixed-effects models on weed biomass (log-transformed) vs explanatory variables (CS=cropping system, MI=management intensity, and their interaction) for systems made of a) barley-pea and b) wheat-bean. Explanatory variables in **bold** mark significant differences with \*\*\*P<0.001, \*\*P<0.01 and \*P<0.05

Cropping system	Response	Explanatory	Family	$\chi^2$	Df	Pr(> $\chi^2$ )	
a) Barley-pea	Herbivory	CS	binomial	2.399	2	0.301	
		MI		0.171	1	0.68	
	Disease	<b>CS</b>	binomial	<b>11.865</b>	2	<b>0.003</b>	**
		<b>MI</b>		<b>11.058</b>	1	<b>&lt;0.001</b>	***
		<b>CS:MI</b>		<b>35.569</b>	2	<b>&lt;0.001</b>	***
	Weed biomass	<b>CS</b>		<b>268.07</b>	2	<b>&lt;0.001</b>	***
		<b>MI</b>		<b>129.102</b>	1	<b>&lt;0.001</b>	***
<b>CS:MI</b>			<b>35.569</b>	2	<b>&lt;0.001</b>	***	
b) Wheat-bean	Herbivory	CS	binomial	5.194	2	0.075	
		<b>MI</b>		<b>16.259</b>	1	<b>&lt;0.001</b>	***
	Disease	<b>CS</b>	binomial	<b>7.679</b>	2	<b>0.022</b>	*
		<b>MI</b>		<b>13.538</b>	1	<b>&lt;0.001</b>	***
		<b>CS:MI</b>		<b>35.569</b>	2	<b>&lt;0.001</b>	***
	Weed biomass	<b>CS</b>		<b>21.209</b>	2	<b>&lt;0.001</b>	***
		<b>MI</b>		<b>92.486</b>	1	<b>&lt;0.001</b>	***

#### *Wheat-bean cropping system*

For wheat and bean cropping system, herbivory was higher for low intensity management, and higher for the intercrop than for both types of sole crops, although this was not significant. Disease was higher under high management intensity, except for bean sole crops, and was affected by cropping system as well. Disease was highest for wheat sole crops and lowest for bean sole crops. Weed biomass was affected by management intensity and by cropping system (Table 3) and was highest in low intensity bean sole crops (Figure 4a,b,c).

### **Discussion**

In the present study, herbivory was always higher in the intercrop than in the two corresponding sole crops, although differences were not significant. For barley-pea, herbivory was higher under high intensity management, while for wheat-bean, herbivory was higher under low intensity management. Results from biodiversity experiments in grasslands (Scherber *et al.*, 2006) have also shown that herbivory may increase with plant diversity, potentially resulting from higher abundance of herbivores in mixtures (Scherber *et al.*, 2010).

Other studies have shown contrasting results, where intercropping actually suppressed herbivores through natural enemy enhancement (Letourneau *et al.*, 2011), as varying habitat structure, food resources and microclimate may lead to higher numbers of predators or parasitoids. However, it should be noted that some of the most prominent studies (e.g. Root, 1973) did not compare sole crops and their respective intercrops only, but also manipulated crop configuration (fields vs strips/edges), making comparisons with our study difficult.

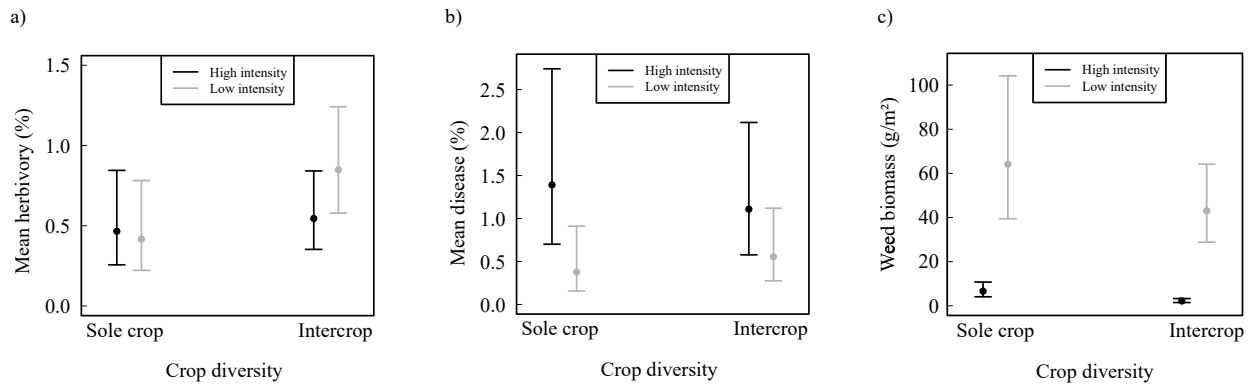


Fig. 2. Model predictions and 95% CIs for (generalised) linear mixed-effects models across cropping systems (barley-pea and wheat-faba bean) on a) herbivory, b) disease and c) weed biomass for sole crops and the intercrop for high (black) and low (grey) intensity management.

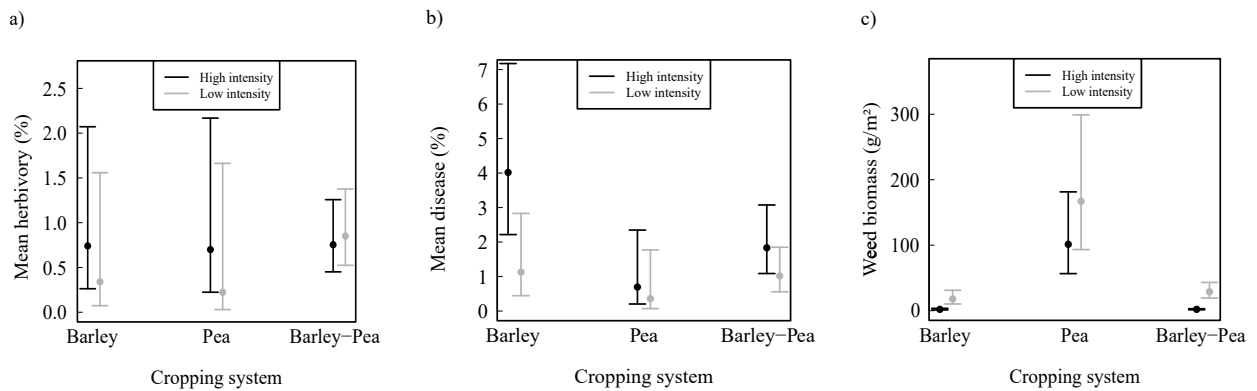


Fig. 3. Model predictions and 95% CIs for (generalised) linear mixed-effects models on a) herbivory, b) disease and c) weed biomass for cropping systems made of barley and pea for each sole crop and their intercrop for high (black) and low (grey) intensity management.

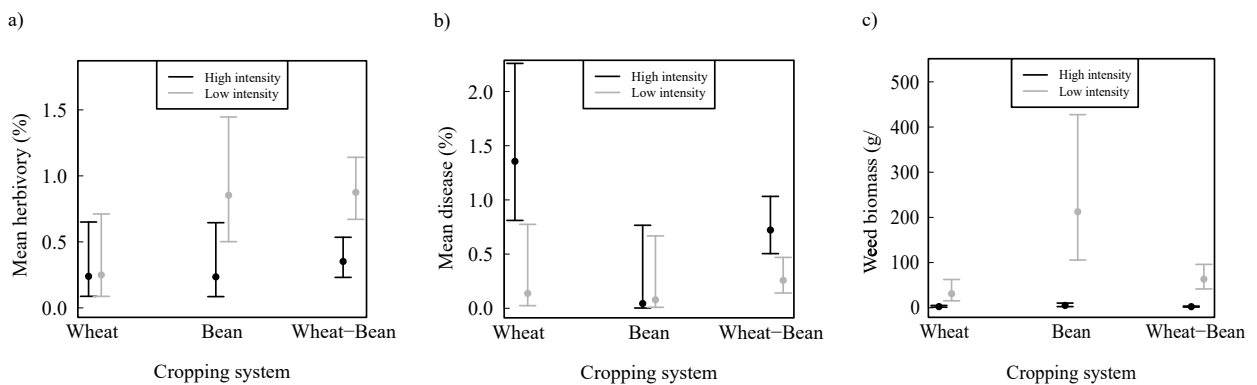


Fig. 4. Model predictions and 95% CIs for (generalised) linear mixed-effects models on a) herbivory, b) disease and c) weed biomass for cropping systems made of wheat and bean for each sole crop and their intercrop for high (black) and low (grey) intensity management.

In other studies, predator abundance or predation rate were not increased, underlining that effects from intercropping are variable and depend on many factors, such as climate/country, crop species and type of intercropping (Lopes *et al.*, 2016). Unfortunately, as 2018 was a dry and hot year in Germany, legumes in the intercrops did not grow as intended from the sowing density, and therefore legume proportion in the intercrop was lower than intended. Varying results regarding management intensity indicate that our small-scale experimental plots might have been too close together. Overall, as values of herbivory reported in our study were rather low, effects on yield can be regarded negligible.

For both cropping systems (barley-pea and wheat-bean), mean disease was higher under high than under low intensity management. Disease incidence was highest in cereal sole crops and lowest in legume sole crops, indicating that legumes, which were less infested, diluted overall disease incidence of the intercrop. One mechanism behind disease suppression through intercropping is the host dilution effect: as many pathogens (Puccinales such as *Puccinia striiformis* and *Uromyces fabae*) are dispersed by wind, it is likely that low host density in intercrops reduced the probability of spore deposition on hosts and thus the spread of the disease (Zhang *et al.*, 2019). Fertiliser increased the incidence of certain diseases, as increased nitrogen levels can lead to a denser canopy, which might in turn result in a microclimate benefitting pathogens (Zhang *et al.*, 2019). Overall, disease incidence was lower in intercropped cereals than in cereal sole crops (Hauggaard-Nielsen *et al.*, 2008), for all levels of fertiliser input (Zhang *et al.*, 2019), as also shown by our experiment. Weed biomass was always higher under low intensity management and was higher for legume sole crops than for the intercrop and cereal sole crops. Herbicide application (in high management intensity), of course, directly reduced weed biomass, although differences were small in pea sole crops. Both pea and faba bean have been reported to be weak competitors against weeds (Hauggaard-Nielsen *et al.*, 2008). Every part of the surface that is not occupied by crops can be used by weeds (Hauggaard-Nielsen *et al.*, 2008), explaining high weed biomass in our trial for pea and bean sole crops, which did not grow particularly well due to conditions already described. Growing legumes together with cereals results in a more competitive mixture, giving less space and resource availability to weeds.

Our results confirm that intercropping in conventional agriculture can reduce weed biomass or disease incidence. In our experiment, there were significant differences between the two farming systems (barley-pea *vs* wheat-bean), indicating that plant species need to be carefully selected for optimal crop performance. The extensive use of intercropping in high intensity agriculture can be an opportunity to reduce the amount of pesticides and fertiliser needed.

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