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Abstract

Maintaining yield when reducing inputs is one prime objective of sustainable agriculture. In this context, cereal-legume intercropping is a practice that can achieve increased yield under low-input conditions through the complementary use of abiotic resources and facilitation mechanisms. Many management options exist to design cereal-legume intercropping systems, among which the choice of the species intercropped and the level of nitrogen (N) fertilization are essential.

In this study, we collected the results of 35 field experiments across Europe of cereal-grain legume intercrops that combined various intercropped species and N fertilization levels. We first assessed the intensity of the biodiversity effect and its components in unfertilized intercrops. Then, we focused on a subset of systems to analyze how N fertilization influenced biodiversity effects on three intercrops (durum wheat / pea, soft wheat / pea and durum wheat / faba bean). The biodiversity effect represents the gap between observed and expected yield of a mixture. The complementarity effect is the performance of mixtures relative to the performance of the component monocultures. The selection effect captures the extent to which a species with a high monoculture yield dominate a mixture at the expense of the other intercropped species.

Our results confirmed an overall positive biodiversity effect under unfertilized conditions and various climate conditions (0.86 ± 0.04 t.ha -1). Complementarity effect was the main driver as it represented 76% of the biodiversity effect, confirming intercropping as a useful practice in low-input systems. N fertilization lowered the complementarity effect in durum wheat / pea intercrops, did not influence these effects in soft wheat / pea intercrops and increased only the selection effect in durum wheat / faba bean intercrops. These results highlight the need for a sufficiently competitive legume in intercrops when N fertilizers are applied in order to avoid too much disruption of plant-plant interactions.

Introduction

From 1960-2000, the use of fertilizers, irrigation and pesticides mitigated effects of climatic hazards, soil heterogeneity and pest pressure, and had a large and positive impact on crop yield (Tilman et al. 2002). More recently, especially in Europe, the growing trend of reducing inputs in agricultural systems, due to environmental and social concerns, and the climatic uncertainty caused by climate change have increased the variability in cropping conditions compared to that of the intensive agriculture practiced in the late 20th century. To reduce the negative consequences of climatic uncertainty and continue to produce enough food while reducing the use of inputs (Sadras and Denison 2016), a promising avenue is to favor functional complementarity of abiotic resource use and biological regulations between plants by designing innovative agricultural practices and systems (Duru et al. 2015). This can be achieved by selecting relevant plant phenotypes (Lynch 2019) and/or using positive biodiversity effects through plant mixtures, also known as the biodiversity-ecosystem function (BEF) effects (Brooker et al. 2021).

Positive BEF effects on ecosystem services have been widely studied in natural communities (Cardinale et al. 2012), and interest in using them in cropping systems has increased in the past several years (Gurr et al. 2016; Martin-Guay et al. 2018; Brooker et al. 2021). Analyzing the diversity-productivity relationship enables the effect of biodiversity on primary production of a given system to be estimated and can divide it into complementarity and selection effects (Loreau and Hector 2001). The former measures the effect due to niche complementarity and/or facilitation, while the latter measures the effect due to the dominance of a given species that fits well with the growth environment. Thus, BEF effects should be viewed as resulting from particularly positive specific interactions rather than explaining underlying processes themselves (Maier 2012). As Brooker et al. (2021) highlight, a collaboration gap between BEF scientists and crop scientists has led to a poor understanding of "the operation of positive diversity effects in intensive agricultural systems" and thus of how to enhance them.

In agricultural systems, plant diversity can be promoted by a range of intercropping practices (i.e., combining at least two crop species in the same field for most of their growing periods), which may improve crop yield (Li et al. 2020a). Several mechanisms can, for example, improve nitrogen (N) acquisition by the intercrops, including complementary distribution of roots in soil volumes (Postma and Lynch 2012), use of distinct forms of N in soils (McKane et al. 2002) and fixation of atmospheric N_2 by one species in the intercrop (Jensen et al. 2020). In a context of input reduction, the use of N_2-fixing legumes is particularly promising. In Europe, this has been widely demonstrated in low-input cereal-legume intercrops, with an increase in total yield and cereal grain quality compared to those of sole crops (Bedoussac et al. 2015). However, supplying too much N fertilizer can cause the cereal to dominate the legume, which decreases positive plant-plant interactions in intercropping systems (Pelzer et al. 2012). Thus, the extent to which N fertilization can be used without compromising BEF effects in such systems remains unclear. More particularly, while recent meta-analyses and reviews generally agree upon positive BEF effects when multiple experiments are assessed, the results of individual experiments have high variability (Bedoussac et al. 2015; Gurr et al. 2016; Raseduzzaman and Jensen 2017; Martin-Guay et al. 2018). Few recent studies underline a positive effect on intercrops' yield, via temporal niche differentiation (Yu et al. 2016; Dong et al. 2018; Li et al. 2020b).

In this study, using a database of 35 field experiments (Fig. 1) from five European countries, we first assessed the intensity of the biodiversity effect in winter and spring cereal-grain legume intercrops under unfertilized conditions. Then, focusing on a subset of three winter intercrops – durum wheat (*Triticum turgidum* L.) / pea (*Pisum sativum* L.), soft wheat (*Triticum aestivum* L.) / pea and durum wheat / faba bean (*Vicia faba* L.) – we tested the influence of two levels of N fertilization (moderate and high) on the biodiversity effect depending on the intercropped species considered.

Fig. 1. Example of a field experiment of winter wheat / pea intercrops (and their corresponding sole crops) conducted at the ARVALIS experimental station, near Angers, France (Photograph courtesy of C. Naudin, ESA, France).

Materials and methods

1. Field experiments

To estimate the net biodiversity effect on intercrop productivity in a wide range of environmental conditions, we collected results from 35 factorial experiments conducted in five countries (France, Denmark, Italy, Germany, and the United Kingdom; Fig. 2A), as detailed hereafter.

We used the following criteria to include set of experiments in our database: (1) grain yield was measured for both species in sole- and intercropping conditions, (2) different species and genotypes were used among cereal and legumes, and (3) a given mixture was observed at least in two locations.

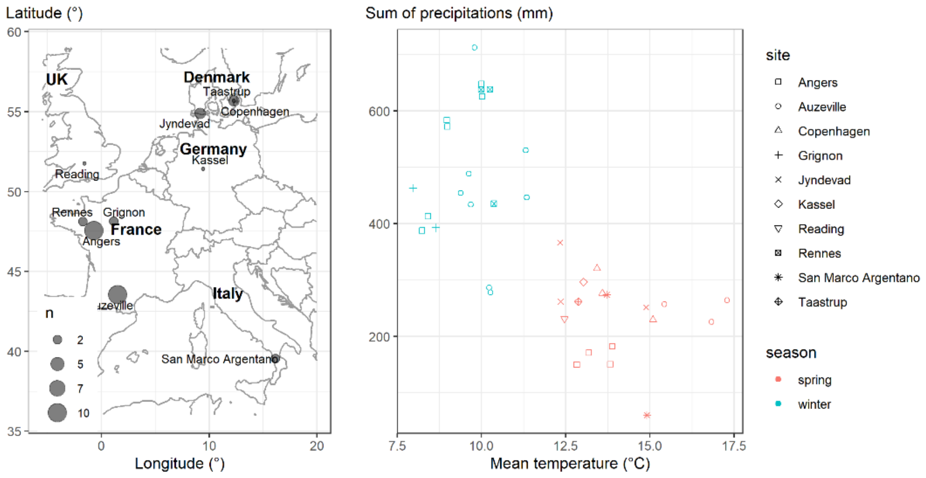

Fig. 2. Location and main climatic features of the experiments. Panel A displays the number of experiments conducted at each location (different years and cropping systems). Panel B displays the sum of precipitation (mm) as a function of mean temperature (°C) during the crop cycle, with spring and winter crops encoded by colors, and experiment location encoded by symbols.

1.1. Environmental conditions

Climate conditions of each experiment were characterized using the following variables retrieved from the NASA POWER API: the sum of precipitation (mm) and mean temperature (°C) during the crop cycle (from sowing to harvest dates). The experiments were separated into two groups: winter crops, which had higher precipitation (280-712 mm) and lower mean temperature (6.8-11.3°C) during the crop cycle, and spring crops, which had lower precipitation (60-366 mm) and higher mean temperature (12.3-17.3°C) (Fig. 2B).

1.2. Agricultural management

All experiments included cereal-grain legume intercrops of two annual crop species and their corresponding sole crops for which grain yield ($t.ha^{-1}$) was measured at harvest. Cereals and legumes were each represented by three species: barley (*Hordeum vulgare* L.), durum wheat and soft wheat for the cereals and faba bean, lentil (*Lens culinaris* L.) and pea for the legumes (Table 1). In the database, 39% and 61% of the intercrops were spring or winter crops, respectively. Intercropped species were sown and harvested at the same time. The sowing dates ranged from March 11 to May 3 for spring crops and from October 25 to December 15 for winter crops. The harvest dates for all crops ranged from June 6 to August 23.

Table 1. Description of the 35 cereal-legume experiments analyzed in this study. The *Type* column defines if the experiment is carried on conventional (C) or organic (O) farming.

Intercropped species (cereal / legume)	**Country**	**Year(s)**	**Soil water capacity (mm)**	**Soil texture (clay-silt-sand, %)**	**Type**	**N treatments ($kg.ha^{-1}$)**	**Mixture design**	**Spatial arrangement**	**No genotypes (cereal / legume)**	**Relative density in intercrop (cereal / legume)**	**References**
Spring barley / fababean	Denmark	2001, 2002, 2003	173	24-29-47	O	0	substitutive	within row	2-1	0.5-0.5	(Gaudio et al., 2021; Hauggaard-Nielsen et al., 2008; Knudsen et al., 2004)
		2001, 2002, 2003	119	4-9-87	O	0	substitutive	within row	2-1	0.5-0.5	
Spring barley / pea	Denmark	2001, 2002, 2003	173	24-29-47	O	0	substitutive	within row	2-2	0.5-0.5	(Gaudio et al., 2021)
		2001, 2002, 2003	119	4-9-87	O	0	substitutive	within row	2-2	0.5-0.5	
		2003	173	24-29-47	O	0	substitutive, additive	alternate row	1-1	0.5-0.5, 0.5-1	
	France	2002	124	6-15-79	C	0	additive	alternate row	1-1	0.33-1	
		2003	124	6-15-79	C	0-130	substitutive, additive	alternate row	1-1	0.5-0.5, 0.5-1	
		2003, 2004	94	21-40-39	O	0	substitutive, additive	alternate row	1-1	0.5-0.5, 0.5-1	(Gaudio et al., 2021; Hauggaard-Nielsen et al., 2008, 2009; Launay et al., 2009)
	Germany	2004	176	51-29-20	O	0	substitutive, additive	alternate row	1-1	0.5-0.5, 0.5-1	(Gaudio et al., 2021)
	Italy	2003, 2004	169	22-36-42	O	0	substitutive	alternate row	1-1	0.5-0.5	(Gaudio et al., 2021; Hauggaard-Nielsen et al., 2008, 2009; Launay et al., 2009)
	United Kingdom	2003	142	49-32-19	O	0	substitutive, additive	alternate row	1-1	0.5-0.5, 0.5-1	
Spring soft wheat / lentil	France	2015	135	10-8-82	O	0	substitutive, additive	within row	2-4	0.5-1, 0.33-1, 0.3-0.7, 0.17-1	
		2016	187	18-48-34	O	0	substitutive, additive	within row	2-4	0.5-1, 0.33-1.3, 0.33-1, 0.3-0.7, 0.17-1.3, 0.17-1	

Winter durum wheat / fababean	France	2010	187	18-48-34	C	0-60-80-140	substitutive, additive	alternate-, within row	1-1	0.5-0.5, 0.67-0.5, 0.67-1, 0.33-0.5	
		2011	187	18-48-34	C	0	substitutive	alternate row	1-1	0.5-0.5	
		2011	187	18-48-34	C	0-140	substitutive	alternate-, within row	1-1	0.5-0.5	
		2012	135	10-8-82	C	0	substitutive	within row	3-4	0.5-0.5	(Kammoun, 2014)
		2013	187	18-48-34	C	0	substitutive	within row	3-4	0.5-0.5	
Winter durum wheat / pea	France	2006	187	18-48-34	C	0-100-180	substitutive	alternate row	1-1	0.5-0.5	(Bedoussac and Justes, 2010a, 2010b)
		2007	135	10-8-82	C	0-60-80-140	substitutive	alternate row	4-1	0.5-0.5	
		2012	135	10-8-82	C	0	substitutive	within row	3-5	0.5-0.5	(Kammoun, 2014)
		2013	187	18-48-34	C	0-140	substitutive	within row	3-5	0.5-0.5	
		2015	135	10-8-82	C	0	substitutive, additive	within row	1-4	0.5-0.5, 0.5-1	
Winter soft wheat / fababean	France	2018	169	22-36-42	O	0	additive	within row	8-2	0.7-0.75	
Winter soft wheat / pea	France	2010	205	11-54-35	C	0-45-90-140	substitutive, additive	within row	1-1	0.5-0.5, 0.33-0.66, 0.7-0.5	(Pelzer et al., 2016)
		2017	205	11-54-35	C	0	substitutive, additive	within row	1-2	0.5-0.5, 0.5-1, 0.15-1, 0.05-1	
		2007	83	20-38-42	C	0-30-45	substitutive	within row	1-1	0.5-0.5	(Gaudio et al., 2021; Naudin et al., 2010, 2014)
		2008	83	20-38-42	C	0-30-45-60-90	substitutive	within row	1-1	0.5-0.5	
		2017	197	19-49-32	O	0	additive	within row	8-3	0.5-0.75, 0.5-1	
		2018	169	22-36-42	O	0	additive	within row	8-3	0.5-0.75, 0.5-1	
		2006	94	21-40-39	O	0	substitutive	within row	1-1	0.5-0.5, 0.3-0.7	
		2007	94	21-40-39	O	0-30	substitutive	within row	1-1	0.5-0.5, 0.7-0.3	
		2008	94	21-40-39	O	0-35-72	substitutive	within row	1-1	0.5-0.5, 0.7-0.3	(Gaudio et al., 2021)
		2009	94	21-40-39	O	0-40	substitutive	within row	1-1	0.5-0.5, 0.7-0.3	

In the database, 54% of the intercrops were grown in a substitutive design (i.e., the sum of the relative sowing densities of the two species intercropped equals 1), while 46% were grown in an additive design (i.e., the sum of relative sowing densities exceeds 1). A species' relative density is its sowing density in the intercrop relative to that in its reference sole crop. Consequently, the database contained 199 sole crop experimental units and 307 intercrop experimental units (site x year x mix of genotypes x relative densities x N treatment), of which 140 were in an additive design and 167 in a substitutive design. Depending on the experiment, each experimental unit was replicated 2-8 times.

Additional details on experimental designs and management practices are reported in the reference publications of 33 of the 35 experiments (Knudsen et al. 2004; Corre-Hellou et al. 2006; Hauggaard-Nielsen et al. 2008, 2009; Launay et al. 2009; Bedoussac and Justes 2010a, b; Naudin et al. 2010, 2014; Pelzer et al. 2016; Tang et al. 2016; Viguier et al. 2018; Gaudio et al. 2021).

2. Estimating the biodiversity effect on intercrop performance

For each experimental unit, grain yield ($t.ha^{-1}$) was measured for each species. We calculated the biodiversity effect (BE, Loreau and Hector 2001) as the observed grain yield minus expected grain yield in intercrops (Eq. 1):

$$BE = (YO_C + YO_L) - (YE_C + YE_L) \text{ (Eq. 1)}$$

where YO_C and YO_L are the observed yield of the cereal and legume grown in intercrop, respectively, and YE_C and YE_L are the expected yield of the cereal and legume grown in intercrop, respectively.

Expected yield was estimated from the yield of the species in sole crop weighted by its scaled relative density in intercrop (Eq. 2; Li et al. 2020a):

$$YE_C = M_C \frac{RD_C}{RD_C+RD_L} \text{ and } YE_L = M_L \frac{RD_L}{RD_C+RD_L} \text{ (Eq. 2)}$$

where M_c and M_L are the yield of the cereal and legume in sole crop, respectively, and RD_C and RD_L are the relative density of the cereal and legume in intercrop, respectively. Grain yield in sole crops and intercrops is calculated as the mean from each replicate of every experimental units, within each experiment.

As mentioned, the biodiversity effect can be divided into a selection effect (SE, Eq. 3) and a complementarity effect (CE, Eq. 4) (Loreau and Hector, 2001; Li et al. 2020a):

$$SE = \frac{1}{2} \times \left(\left(\frac{YO_C}{M_C} - \frac{RD_C}{RD_C+RD_L} \right) - \left(\frac{YO_L}{M_L} - \frac{RD_L}{RD_C+RD_L} \right) \right) \times (M_C - M_L) \text{ (Eq. 3)}$$

$$CE = \frac{M_C+M_L}{2} \times \left(\frac{YO_C}{M_C} - \frac{RD_C}{RD_C+RD_L} + \frac{YO_L}{M_L} - \frac{RD_L}{RD_C+RD_L}\right) = M \times (LER - 1) \text{ (Eq. 4)}$$

These formulas, used to compute selection and complementarity effects, are only valid in bispecific mixtures.

The first term of Eq. 3 calculates the difference in increase or decrease in yield between the two species intercropped, while the second term calculates the difference between their sole crop yields. Thus, a positive selection effect means that the species with the higher yield in sole crop has a higher relative increase in yield in intercrop (i.e., benefits more from intercropping).

Into the equation for the complementarity effect (Eq. 4), we introduced the classic Land Equivalent Ratio, which is used to calculate land-use efficiency (LER = $Y_C/M_C + Y_L/M_L$; Willey and Rao 1980). Thus, the complementarity effect equals the Land Equivalent Ratio minus 1, multiplied by M, the mean yield in sole crops.

3. Experimental design, data processing and analysis

The data were curated and formatted in a database. The data were ordered, reshaped and homogenized using the collection of R packages *tidyverse* (Wickham et al. 2019).

The dataset was unbalanced (i.e., groups had different numbers of observations) because the experiments collected were conducted for different purposes and examined many factors (e.g., N fertilization, intercrop design) (Table 1). Thus, the influence of several of the factors on the biodiversity effect and its components could not be analyzed, especially due to the lack of certain treatments in some experiments and to the nesting of factors. For example, only 12 of the 35 experiments tested N fertilization levels, or the species effect also included site and year effects (e.g., spring barley / faba bean intercrops were grown only in Denmark, so they could not be analyzed properly). The statistical analysis performed was adjusted in response to this unbalanced structure.

We first investigated the overall behavior of mean biodiversity, complementarity and selection effects within the unfertilized cereal-legume intercrops in the 35 experiments, and the correlation between the biodiversity effect and each of its components. Thus, our goal was to assess the influence of N fertilization on the biodiversity effect and its components. N fertilization ranged from 0-180 kg $N.ha^{-1}$, which we split into three levels: null, moderate (30-80 kg $N.ha^{-1}$) and high (> 80 kg $N.ha^{-1}$). A factorial design was then defined between the species intercropped and these levels of N fertilization. The subset of our database with a factorial design of species and N fertilization levels corresponded to three intercrops: durum wheat / pea, soft wheat / pea and durum wheat / faba bean (70 experimental units, among which 62 are in substitutive design, all located in France, Table 1). Durum wheat / pea and durum wheat / faba bean intercrops were grown in experiments with moderate and high levels of N fertilization, while soft wheat / pea intercrops were grown only with a moderate level of N fertilization.

The effect of N fertilization on the biodiversity effect and its components in intercrops was assessed using the Bayesian approach. Bayesian inference is based on reallocating credible values for a parameter (posterior distribution) given prior knowledge (prior distribution) and the adequacy of the data to the model (likelihood). The Bayesian approach provides information about the probability of a hypothesis being true given the data (P(hypothesis|data)). Bayesian estimation for the difference in group means (Kruschke 2018) is an alternative to the classic Student's *t* test to compare the means of two groups. This method calculates a posterior distribution for the mean differences between the two groups and derives a 95% highest density interval (HDI), which is defined as the 95% most credible values of the parameter. We performed Bayesian estimation for the difference in mean values of components of the biodiversity effect between N-fertilized (moderate and high) and unfertilized treatments for each of the three intercrops. The null hypothesis (H0) was defined as equal mean biodiversity effect components for N-fertilized and unfertilized intercrops. We applied the following decision rule to the position of the 95% HDI: reject H0 if the 95% HDI excludes 0 but do not reject H0 if it includes 0.

All indicator calculations and statistical analyses were performed with R software, v. 4.0.0 (R Core Team 2020). Bayesian statistical analyses were performed using the R package *BEST* (Kruschke and Meredith 2020).

4. Definition of references for fertilized legumes

A common assumption when calculating indicators to compare the performance of intercrops to that of sole crops is that N is not a limiting resource for legumes and does not influence their yield (e.g., Pelzer et al. 2012). To test this hypothesis, we performed Bayesian estimation for the difference in group means between N-fertilized and unfertilized legume sole crops. The database contained only three experiments (i.e., 11 experimental units) in which legume sole crops were N-fertilized, because the experiments we collected were designed to conform to agronomic practices of farmers, who rarely fertilize legume sole crops (Magrini et al. 2016). The Bayesian estimation confirmed that N fertilization had no significant influence on the yield of legume sole crops. Given this result and the lack of data on N-fertilized legume sole crops, we used the unfertilized legume sole crops as a reference when calculating the biodiversity effect and its components in all experimental units.

Results and discussion

1. Distribution of the biodiversity effect and its components in unfertilized intercrops

On the whole dataset, the mean (± 1 standard error) yield gain in unfertilized intercrops equaled 0.86 ± 0.04 t.ha $^{-1}$ (1.04 ± 0.01 t.ha $^{-1}$ for additive designs and 0.68 ± 0.00 t.ha $^{-1}$ for substitutive designs) for a mean total intercrop yield of 3.54 ± 0.08 t.ha^{-1} (Fig. 3A). These results highlight an increase in the yield of cereal-legume intercrops in most experimental units under unfertilized conditions compared to those of the corresponding sole crops, which agrees with results of several studies (Pelzer et al. 2012, 2014; Yu et al. 2016) and confirms the ability of intercropping to increase grain yield in low-input farming systems (Bedoussac et al. 2015).

However, the increase in yield observed was influenced by the cropping conditions used as references to calculate the biodiversity effect. The unfertilized cereal sole crops used as references had lower grain yield (3.2 ± 0.08 t.ha^{-1}, all cereals pooled) than cereals grown under conventional farming conditions, which are always N fertilized (i.e., a mean grain yield of 6.1 t.ha^{-1} for the cereals of interest in the five European countries considered for the period covered by the experiments (Food and Agriculture Organization of the United Nations; http://faostat.fao.org/)). Thus, the low yield observed for the unfertilized cereal sole crops contributed greatly to the positive biodiversity effect estimated (Garnier et al. 1997).

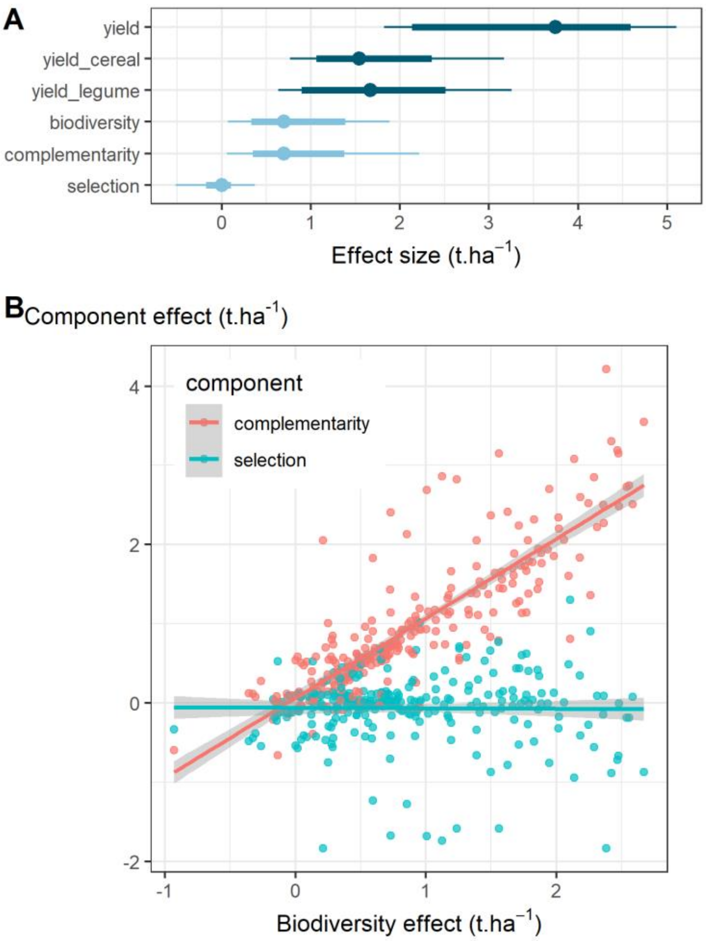

Fig. 3. **(A)** Distribution of unfertilized cereal-legume intercrop yield and biodiversity effect ($t.ha^{-1}$). Points represent the median, broad lines represent the interquartile range, and thin lines represent the [0.1, 0.9] quantile interval. **(B)** Correlation between biodiversity effect ($t.ha^{-1}$) and complementarity effect ($t.ha^{-1}$) or selection effect ($t.ha^{-1}$) in unfertilized cereal-legume intercrops. Grey zones represent the 95% confidence interval for the linear regressions. Data used: whole dataset (n = 263)

The biodiversity effect was strongly and positively correlated with the complementarity effect ($r = 0.86$, $p < 10^{-15}$), but it was not correlated with the selection effect ($r = -0.01$, $p = 0.87$) (Fig. 3B). Thus, the complementarity effect was the main driver of the yield gain in unfertilized cereal-legume intercrops, meaning that positive plant-plant interactions (i.e., facilitation and / or niche complementarity) rather than the dominance of one of the species increased intercrop yields (Pelzer et al. 2012). However, caution is needed when distinguishing complementarity causes (e.g., niche partitioning, facilitation) of the resulting complementarity effect (Barry et al. 2019). To quantify the relative importance of these processes, specific measurements would be needed, such as symbiotic N_2 fixation to reflect differences in N use between cereals and legumes, or a lodging score to quantify mechanical facilitation (e.g., Podgórska-Lesiak and Sobkowicz 2013). As Brooker et al. (2021) highlight, explicitly distinguishing facilitation and niche partitioning would help when applying new analytical and conceptual frameworks to design intercrops. Nevertheless, differences in N use in cereal-legume intercrops is a well-known process in which the more competitive cereal usually takes disproportionally more soil mineral N than the legume, which is forced to compensate by increasing symbiotic N_2 fixation (Rodriguez et al. 2020). In a low-input context, this complementarity of N use enables cereals in intercrops to have higher grain yield and quality than cereals in sole crops.

The complementarity effect contributed 76% of the biodiversity effect when the latter was positive (i.e., in 94% of the experimental units), but it contributed only 36% when the latter was negative (i.e., in 6% of the experimental units). In the few cases in which we observed a yield loss in intercrops, the relative contributions of complementarity and selection were reversed: -0.05 ± 0.02 and -0.16 ± 0.02 $t.ha^{-1}$, respectively. In these cases, the total yield of intercrops were lower than those of corresponding sole crops because the competition between cereals and legumes exceeded the complementarity effect (also reported by Pelzer et al. (2016) for soft wheat / pea intercrops and Baxevanos et al. (2017) for oat / pea intercrops).

2. Influence of N fertilization on the biodiversity effect and its components

The biodiversity effect and its components were altered by N fertilization, which is a key practice in agricultural systems. While the biodiversity effect was positive in 100% of the unfertilized experimental units of the data subset considered (i.e., factorial designs of species and N fertilization levels), the percentage of experimental units with a positive biodiversity effect decreased with N fertilization (i.e., 92% and 67% of the experimental units under moderately and highly N-fertilized conditions, respectively) (Fig. 4). Overall, the total intercrop yield increased with N fertilization (4.16 ± 0.18, 5.09 ± 0.24 and 4.62 ± 0.21 t.ha^{-1} under unfertilized, moderately and highly N-fertilized conditions respectively); specifically, mean grain yield decreased for legumes (2.23 ± 0.12, 1.88 ± 0.19 and 1.84 ± 0.16 t.ha^{-1} under unfertilized, moderately and highly N-fertilized conditions respectively) but increased for cereals (1.93 ± 0.20, 3.21 ± 0.23 and 2.78 ± 0.15 t.ha^{-1} under unfertilized, moderately and highly N-fertilized conditions respectively) with N fertilization (Fig. 4). The same pattern was observed for the complementarity effect, which was positive in 96%, 83% and 56% of the experimental units under unfertilized, moderately and highly N-fertilized conditions, respectively. Conversely, the percentage of experimental units with a positive selection effect increased with N fertilization: 25%, 71% and 61% of the experimental units, under unfertilized, moderately and highly N-fertilized conditions, respectively. Thus, N fertilization tends to decrease positive plant-plant interactions within cereal-legume intercrops by acting on the balance between the two intercropped species to the benefit of the cereal (Pelzer et al. 2012).

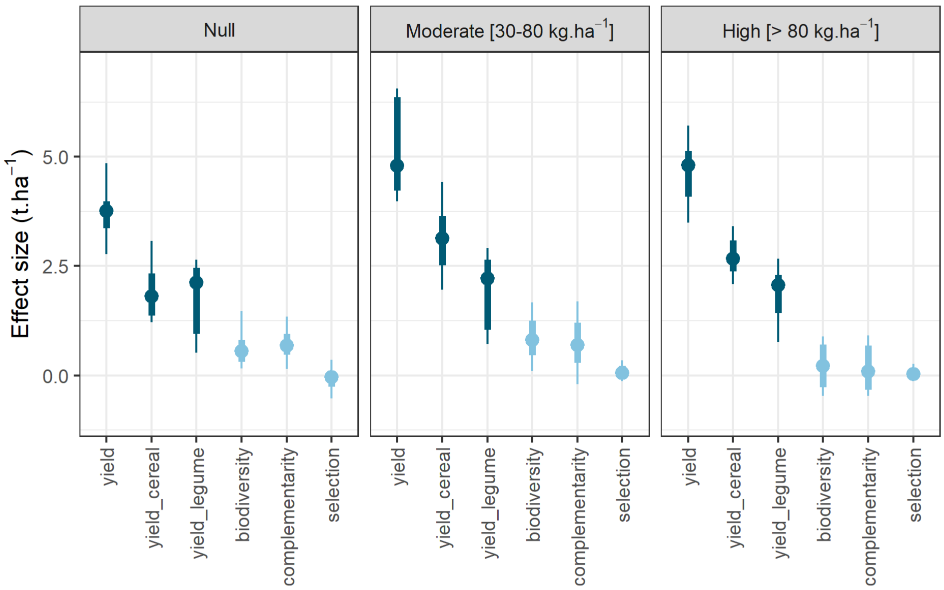

Fig. 4. Distribution of cereal-legume intercrop yield, cereal and legume yield (t.ha^{-1}) and the biodiversity effect (t.ha^{-1}) as a function of nitrogen fertilization level. Points represent the median, broad lines represent the interquartile range, and thin lines represent the [0.1, 0.9] quantile interval. Data used: Experiments with a factorial design of species and N fertilization levels (n = 82)

The effect of N fertilization on the biodiversity effect and its components depended on the species intercropped (Fig. 5). In durum wheat / pea intercrops, even moderate N fertilization decreased the biodiversity effect significantly by 66% compared to that under unfertilized conditions. This moderate

N fertilization increased the selection effect significantly by 0.21 $t.ha^{-1}$ (99.1% of the posterior values for the difference in group means between N-fertilized and unfertilized conditions were positive), while the complementarity effect decreased by 0.65 $t.ha^{-1}$ (99.1% of the posterior values for the difference in means were negative). These effects were emphasized under highly N-fertilized conditions (Fig. 5). When focusing on the yield of both species intercropped, N fertilization disadvantaged the legume, since pea yield decreased by a mean of 37% under N-fertilized conditions compared to that under unfertilized conditions, while the opposite was observed for durum wheat, whose yield increased by a mean of 94%. These results could explain the shift in complementarity and selection effects for durum wheat / pea intercrops between N-fertilized and unfertilized conditions. This behavior is usually highlighted in existing literature related to cereal-legume intercrops (e.g., Naudin et al. 2010). Under N-fertilized conditions, selection effect increases because durum wheat has a competitive advantage over the legume (Mariotti et al. 2009; Duchene et al. 2017). Our results showed, however, that choosing a different cereal or legume species can change this effect.

When soft wheat replaced durum wheat in wheat / pea intercrops, N fertilization did not influence the biodiversity effect or its components (Fig. 5). Because the cereal and legume yields tended to increase slightly with N fertilization, the latter did not disrupt the balance between the two species (Table 2). Based on the soil and climate conditions considered, the level of N fertilization (45 kg $N.ha^{-1}$) was probably too low, compared to usual N fertilization rates in conventional agriculture, to increase the yield of one or both species significantly, unlike that of durum wheat / pea intercrops (60-140 kg $N.ha^{-1}$).

Finally, in durum wheat / faba bean intercrops, N fertilization did not influence the biodiversity effect or its complementarity effect, but it did increase the selection effect significantly by 0.3 $t.ha^{-1}$ and 0.2 $t.ha^{-1}$ under moderately and highly N-fertilized conditions, respectively (95.5% and 95.2% of posterior values for the difference in group means were positive, respectively) (Fig. 5). This increase was due to an increase in durum wheat yield, since faba bean yield changed little in intercrops as N fertilization increased. This behavior contrasts with that of pea yield when intercropped with durum wheat: pea yield decreased as N fertilization increased. Height and biomass differences between two intercropped species have been shown to influence their yields (Gaudio et al. 2021). Since the faba bean is taller and larger than the pea (Guinet et al. 2018), it showed greater competitive ability (but whether aboveground for light capture or belowground for nutrient and water acquisition remains to be tested), which explains the lack of shift in the biodiversity effect observed in durum wheat / faba bean intercrops.

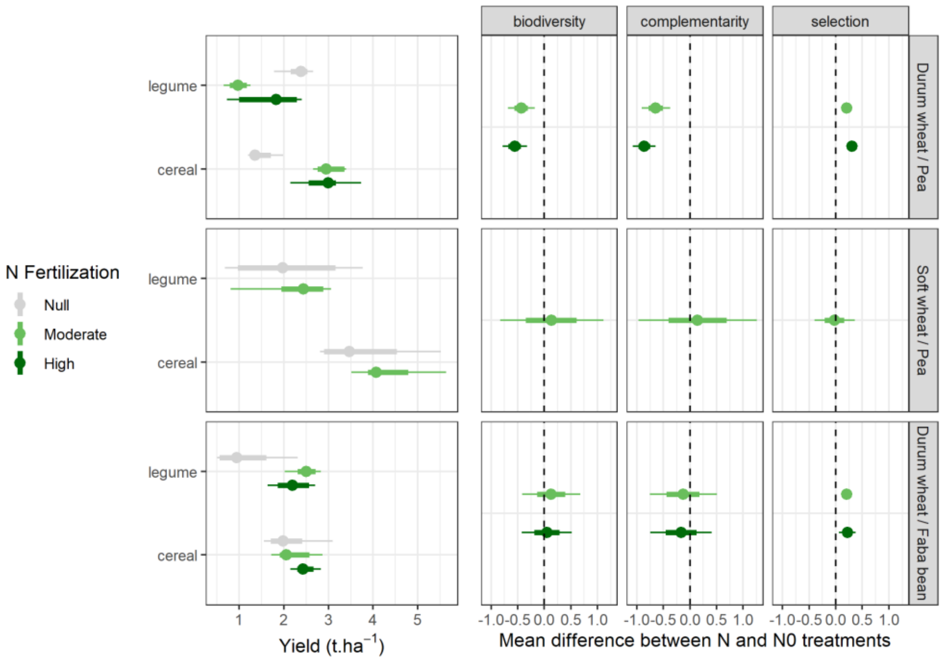

Fig. 5. Distribution of cereal and legume yields (t.ha^{-1}) in three cereal-grain legume intercrops (durum wheat / pea, soft wheat / pea and durum wheat / faba bean) as a function of nitrogen (N) fertilization level: null, moderate (30-80 kg N.ha^{-1}) and high (> 80 kg N.ha^{-1}). For the three intercrops, posterior distributions of the difference in mean of the biodiversity effect between the two N-fertilized (moderate and high) and unfertilized (N0) treatments is illustrated (t.ha^{-1}), with dashed lines representing the null value of the posterior difference in means. Points represent the median, broad lines represent the interquartile range, and thin lines represent the [0.1, 0.9] quantile interval. Data used: Experiments with a factorial design of species and N fertilization levels (n = 82).

3. Pathway to applications

Because cereal-legume intercrops are used mainly to decrease the use of agricultural inputs, most are managed without synthetic inputs. In this way, our study confirmed an increase in productivity under a wide range of unfertilized cropping conditions, with a balance between the two species intercropped (i.e., no species clearly dominated), although the increase depends on the species intercropped (Cheriere et al. 2020). N fertilization can disrupt this balance, shifting positive plant-plant interactions to a dominance of the cereal at the expense of the legume (e.g., in durum wheat / pea intercrops). This shift appeared at moderate N fertilization levels and even led to lower productivity of intercrops than that of sole crops at the high N fertilization levels applied to wheat sole crops in conventional agriculture (> 100 kg N.ha^{-1}).

It would thus be interesting to identify the level of moderate N fertilization that provides benefits from positive effects of intercropping and positive plant-plant interactions, while increasing the total yield by increasing the cereal yield, as farmers often perform in winter intercrops (Verret et al. 2020). Because this N level is likely to differ among species, future research should focus on the interaction between N fertilization and the intercrop species chosen. For instance, recent meta-analysis (Li et al. 2020b) shows high advantages of N fertilization on mixtures including maize (Zea mays L.).

In our study, only one combination of species x N fertilization had a positive interaction on yield (i.e., durum wheat / faba bean intercrops): cereal yield increased and legume yield remained the same, while in durum wheat / pea intercrops, legume yield decreased. Thus, our results suggest that the legume chosen can be a management mechanism, with the idea that the legume should be sufficiently competitive to counterbalance the increased competition from the N-fertilized cereal (Duchene et al. 2017). Probably, it is the balance of competition between the two components rather than competitiveness of the legume that matters. However, we also observed that the cereal yield stagnated if the N fertilization level was not sufficient (e.g., soft wheat / pea intercrops). Thus, the optimal N fertilization level should depend on the proportion of legume biomass in the intercrop (Naudin et al. 2010). As highlighted by other studies, the species chosen are a relevant mechanism for controlling intercrops' yield (Cheriere et al. 2020) and suitability for the cropping environment in which they grow (Baxevanos et al. 2017). Finally, it is worthwhile to recall that many barriers to adoption of intercrops in Europe exist, beyond the scope of this article, such as. technical and economical ones (Bonke et al. 2020). Different possibilities (e.g., better communication of scientific results, breeding adapted to intercrops) exist to overcome these barriers (Meynard et al. 2018) and allow intercrops to be more widely cultivated.

Conclusion

This study highlights that the complementarity between intercropped species is the main driver of the positive biodiversity effect on the performance of cereal-legume intercrops under diverse cropping conditions. If the biodiversity effect depended instead mainly on the selection effect (i.e., if one intercropped species strongly dominated), growing the dominant species alone would be more practical agronomically, which would shift the balance towards sole crops.

While multiple meta-analyses and reviews highlighted the overall yield gain in intercrops, analysis and tools to derive specific management recommendations for farmers from this general knowledge are still lacking (Brooker et al. 2021). We argue that it may be counterproductive to emphasize that biodiversity has this broad beneficial effect while the specific positive interactions between pairs of species and even more so, cultivars, remain to be identified (Maier 2012).

The key question remains how to secure complementarity while intensifying or increasing productivity. When focusing on the response of complementarity processes to N fertilization, we found that behavior differed depending on the species chosen. We highlighted that N fertilization does not always depress complementarity processes as long as the legume species can also benefit from it. Therefore, such shifts in balance need to be understood through the prism of community ecology to develop the use of intercrops in a wider range of agricultural systems besides low-input agriculture.

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Declarations

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- Conflicts of interest/Competing interests (include appropriate disclosures): The authors declare that they have no conflict of interest
- Ethics approval (include appropriate approvals or waivers): the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards
- Consent to participate (include appropriate statements): not appropriate
- Consent for publication (include appropriate statements): not appropriate
- Availability of data and material (data transparency): the datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
- Code availability (software application or custom code): not applicable
- Authors' contributions (include appropriate statements): “Funding acquisition: PC, NH, NG; data collection and formatting: NG, RM, PC; data analysis: RM, NH; writing original draft: RM, NG, PC, NH; writing, review and editing: all co-authors”

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