Revival of traditional agricultural systems – A multidisciplinary on-farm survey of maize-bean intercropping reveals unexpected competition effects on bean.

Noa Vazeux-Blumental[1], Laura Mathieu[1], Théo Trabac[2], Carine Palaffre[3], Bernard Lagardère[3], Maryse Carraretto[4], Cyril Bauland[1], Martine Le Guilloux[1], Christine Paysant Le Roux[5,6], José Caïus[5,6], Anne Marmagne[7], Jérôme Enjalbert[1], Timothée Flutre[1], Edith Le Cadre[8], Virginie Parnaudeau[8], Daniel Muller[2], Yvan Moënne-Loccoz[2], Domenica Manicacci[1], Maud I. Tenaillon[1]

[1] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-sur-Yvette, France

[2] Université Claude Bernard Lyon 1, CNRS, INRAE, VetAgro Sup, UMR5557 Ecologie Microbienne, 69622, Villeurbanne, France

[3] INRAE, Unité expérimentale du maïs, 40390, Saint Martin de Hinx, France

[4] Université de Toulouse, Université Toulouse, CNRS, EHESS, ENSFEA, INU Champollion, LISST, 31058, Toulouse, France

[5] Université Paris-Saclay, CNRS, INRAE, Université Evry, IPS2, 91190, Gif-sur-Yvette, France

[6] Université Paris Cité, CNRS, INRAE, IPS2, 91190, Gif-sur-Yvette, France

[7] INRAE, AgroParisTech, Université Paris-Saclay, IJPB, 78026, Versailles, France

[8] I NRAE, Institut Agro Rennes-Angers, SAS, 35042, Rennes, France

ORCID IDs

N.V.-B.: 0009-0001-1971-1019; L.M.: 0009-0003-4083-1298; C.P.: 0000-0002-8427-1726; C.B.: 0000-0001-9916-2744; C.P.-L.: 0000-0003-2046-6492; J.C.: 0000-0001-8692-042X; A.M.: 0000-0003-3466-9477; J.E.: 0000-0003-0408-2253; T.F.: 0000-0003-4489-4782; E.L.C.: 0000-0001-5859-5682; V.P.: 0000-0002-4442-6703; D.M.: 0000-0002-6619-4691; Y.M.-L.: 0000-0002-9817-1953; Do.M.: 0000-0002-6779-113X; M.I.T.:0000-0002-0867-3678;

Correspondance

Domenica Manicacci, Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-sur-Yvette, France

Email: domenica.manicacci@inrae.fr

Maud Tenaillon, Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-sur-Yvette, France

Email: maud.tenaillon@inrae.fr

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Societal Impact Statement

Cereal-legume intercropping is a promising strategy for sustainable agroecosystems that leverages the biological complementarities between species, reducing the need for chemical inputs while enhancing field biodiversity. Here, we focused on maize-bean intercropping, which is experiencing a revival in modern conventional agricultural settings. We found a trend towards an increased soil bacterial diversity in intercropping. But competition between the two crops dominated and primarily impacted yield and gene expression in beans. Despite lower yield, bean seeds exhibited greater size. Our study indicates that implementing variety selection of both species and adapting farming practices is essential to fully harness the potential of intercropping.

Summary

- Cereal-legume intercropping is a promising strategy for sustainable agroecosystems. The traditional intercropping of maize and bean is experiencing a revival in some modern agricultural settings, such as in southwestern France, where maize hybrids are intercropped with the commercialized Tarbais bean. We conducted on-farm surveys and a field assay to address the following questions: How do intercropping and sole cropping impact yield, nutrient uptake and rhizosphere bacterial assemblages? Do positive or negative interactions between maize and bean dominate in intercropping? What is the effect of intercropping on plant transcriptomics?
- We recorded farming practices, conducted yield and nutrient measurements, and characterized soil bacterial assemblages to compare sole-cropped maize and beans with intercropped plants. We also set up a controlled field assay to extend this comparison to plant gene expression differences.
- Intercropping was associated with a trend towards increased bacterial diversity. The cropping system significantly influenced agronomic traits, with frequent farm-by-cropping system interactions underscoring the critical role of farming practices. Competition dominated maize-bean intercropping, with 34 negative correlations among the 47 significant ones between maize and bean traits. This competition had effects on both yield and nutrition but primarily impacted beans, which produced fewer but bigger/heavier seeds. Transcriptomic results concurred with these findings revealing no differentially expressed genes in maize but 5,070 in beans under competition.
- Overall, our findings suggest that beneficial interactions between the two crops are hindered, underscoring the importance of carefully considering partner varieties and farming practices in the revival of traditional agricultural systems.

Keywords

agroecology, bacterial metabarcoding, cereal-legume mixture, competition, plant nutrition, RNAseq

1. INTRODUCTION

Conventional agriculture mainly relies on simplified cropping systems, with a single crop species grown per field (sole cropping) and environmental uniformization through the use of chemical inputs to ensure high yields. Given the current environmental challenges, there is a growing concern for more sustainable agroecosystems based on ecological regulations (Beillouin 2021; Fréville et al. 2022; Gaba et al. 2015). In this context, mixed intercropping (thereafter intercropping), where several crops are grown simultaneously with no distinct row arrangements, relies on biological complementarities and beneficial species interactions that diminish inputs requirement (Bedoussac et al. 2015). This is the case for cereal-legume intercrops whose success relies on their complementarity, which results primarily from the capacity of legumes (*Fabaceae*) to assimilate nitrogen fixed by symbiotic rhizobia. By mixing cereals (*Poaceae*) with legumes, the need for nitrogen-based fertilizers is reduced as compared to sole-cropped cereals. Cereals promote symbiotic nitrogen fixation as they deplete soil nitrogen accessible for legumes but also through their root exudation, stimulating nodulation and rhizobia symbiotic activity (Rodriguez 2020; Li et al. 2016a). Cereal-legume interactions can yield additional benefits. First, improved resource utilization may occur through complementarity of their aerial and root architecture. This has been demonstrated at the root level in maize-bean-squash intercropping (Zhang et al. 2014), where complementarity increases biomass production in N-deficient soils. Second, facilitative processes involving soil acidification by legume root exudates, which locally increases the availability of inorganic phosphorus in the soil, may benefit the cereal crop as exemplified in maize-faba bean (Li et al. 2007; Zhang et al. 2016), maize-chickpea (Li et al. 2004), wheat-faba bean (Li et al. 2016b), and wheat-common bean (Li et al. 2008) intercropping. Third, weed reduction can result from enhanced competitive abilities of the crop mixture, which decreases the need for herbicides (Poggio 2005). Fourth, transfer of N and P may be accrued between plant species interconnected through arbuscular mycorrhizal fungi (Johansen and Jensen 1996; Zhang et al. 2019). Fifth, the recruitment of plant growth-promoting rhizobacteria, such as phloroglucinol-producing *Pseudomonas* on cereal roots, can promote plant growth and provide control over various root pathogens (Mazzola 2004).

Although beneficial interactions between cereals and legumes are well-documented from an agronomic perspective, the genetic and molecular determinants of their interactions, as well as their impact on soil ecology, remain poorly explored (Becker et al. 2023). This is particularly true for the intercropping of maize with climbing common bean, where the maize stalk supports the climbing bean, thereby promoting its exposure to light. Yet this association is of major interest, for both economic and historical reasons. Economically, maize is the second most widely grown crop in the world and common bean is the most widely used legume for direct human consumption. Historically, both crops were domesticated in Mexico (Bitocchi et al. 2013; Matsuoka et al. 2002) and their association within the same cropping system dates back to 7,000 to 4,400 years BP (Zizumbo-Villarreal and Colunga-GarcíaMarín 2010). This cropping system, central to the iconic “milpa”, featured maize, squash, and

beans—collectively known as the "three sisters"—and was the predominant multi-cropping subsistence system in Mesoamerica. Notably, the practice of intercropping maize and bean has spread beyond its region of origin, such as on small farms in sub-Saharan Africa (Nassary et al. 2020) and in various traditional European farming systems (Vazeux-Blumental et al. 2024). Thus, the intercropping of maize and bean appears to have been introduced and maintained as these crops spread beyond their centers of domestication.

While being abandoned in favor of sole cropping during the 20th century, this practice has persisted on small-scale subsistence farms in many parts of the world and has recently experienced a revival in some modern agricultural settings. An example is the Tarbais bean in southwestern France, where maize and bean were introduced in the 18th century and widely intercropped, becoming an essential part of the culinary heritage (Bonnain-Dulon and Brochot 2004). However, during the 1950s, the cultivation of beans considerably declined due to the intensification of agriculture and the introduction of maize hybrids, i.e. maize varieties mostly used for animal feed and developed through crosses of inbred lines, significantly increasing yield and supporting industrial farming. As beans were not suitable for mechanization, their cultivation around Tarbes (Hautes-Pyrénées area) decreased from approximately 12,000 hectares at the beginning of the 20th century to around 55 hectares by 1970 (Bonnain-Dulon and Brochot 2004). It was not until 1986 that the Tarbais bean was developed to diversify local agriculture and enhance farming profitability, thereby promoting a local crop used in traditional dishes, such as the cassoulet stew. Tarbais bean obtained a quality label and a protected geographic indication in 2000, which makes it an emblematic crop in the region, with high added value. Along with the increase in acreages of Tarbais bean, several farmers reintroduced intercropping with maize as an alternative to the use of plastic nets in sole-cropped beans (Figure 1).

The objective of our study was to gain a deeper understanding of the functioning and potential benefits of intercropping maize and common bean. We focused on the Tarbais-maize system, which represents a revival of a traditional farming system using modern varieties and, most often, conventional farming practices (notably relatively high levels of nitrogen fertilization). While these practices may not be ideal for leveraging beneficial interactions between crops (Fréville et al. 2022), we hypothesized that maize-bean intercropping could still offer benefits. Due to the lack of research on this particular intercropping, we adopted a descriptive multidisciplinary approach combining agronomy, microbial ecology, and transcriptomics to answer three main questions: How do intercropping and sole cropping impact yield, nutrient uptake and rhizosphere bacterial assemblages? Do positive or negative interactions between maize and bean dominate in intercropping at the phenotypic level? What is the effect of intercropping at plant transcriptomic level? We recorded farming practices, performed agronomic and nutrient measurements on plants collected from both intercropped and sole-cropped plots in farmers' fields, and we examined bacterial communities and corresponding soils in the rhizosphere. Because the detection of molecular signatures of intercropping requires a controlled environment with

150 replicates, we established a controlled field assay in which we compared both agronomic traits and gene
151 expression between sole crop and intercropping of Tarbais bean and two maize hybrids.
152
153 **2. MATERIALS AND METHODS**
154
155 **2.1 On-farm analyses in the Tarbes region**
156
157 **2.1.1 Farms and farming practices**
158 We focused on the Tarbes region (Figure S1) located in southwestern France. In order to compare sole
159 and intercropping, we identified multiple farms that practiced at least one of the two sole cropping
160 (maize or bean) in addition to intercropping, seven of which agreed to participate to this study. In this
161 region, most farmers use the recent "Label Rouge" bean variety Alaric, an inbred line derived from
162 crosses among local landraces registered in the European Community's varietal catalog in 1999. The
163 Lapujole variety, registered in 2014, was derived from Alaric by introgression of an anthracnose
164 resistance allele from scarlet runner bean (*Phaseolus coccineus*). Both varieties are therefore genetically
165 similar (hereafter we used Tarbais bean to designate both). Among the seven farmers included in our
166 study, five used the Tarbais bean and two farmers used local bean landraces of their own. Several maize
167 varieties were grown, most of which were modern hybrids (Dataset S1). A single farmer (F4) used a
168 local maize landrace. Only two farms (F6 and F7) practiced the three cropping systems (sole-cropped
169 bean, sole-cropped maize and maize-bean intercropping).
170 Farming practices in each farm were recorded (Dataset S1). The sowing dates for the different
171 farms ranged from April 15th to June 10th. Since manual harvest of beans requires wide space between
172 rows, sole-cropped maize was cultivated at a much higher density (8 plants per m^2) compared to both,
173 intercropped maize and bean (2.5 plants per m^2 for each of the species) and sole-cropped bean (2.5 plants
174 per m^2). Except for the organic farm F4, with low N-fertilization (50 kg N ha^{-1}) for sole-cropped maize
175 and no fertilization for intercropped maize and bean), N-fertilizer input was on average 224 N units ha^{-}
176 ^{1} for sole-cropped maize, 130 N units ha^{-1} for sole-cropped bean, and 135 N units ha^{-1} for intercropped
177 maize and bean. Farm F4 did not practice irrigation. For the other farms, irrigation along the growing
178 season was, on average, 181 mm for sole-cropped maize, 150 mm for sole-cropped bean, and 117 mm
179 for intercropped maize and bean.
180
181 **2.1.2 Plant and soil sampling for agronomic analyses**
182 All plant and soil samples were collected before harvest in October 2017, from 16 fields (one to three
183 fields per farm, Dataset S1). In each field, we randomly sampled three plots (located > 5 m apart) that
184 served as replicates in the analyses. Each plot consisted of 4 to 5 (sole cropping) or 7 to 10
185 (intercropping) plants each. Among the 16 fields, seven fields corresponded to intercropping (for a total
186 of 21 plots), five to maize sole cropping (for a total of 15 plots) and four to bean sole cropping (for a

total of 12 plots). Given that we collected maize and bean separately from the intercropping plots, these 48 plots led to a total of 69 plant samples of 4 to 5 pooled plants each (with the exception of one plot of 3 plants). In addition, we retrieved about 500 g of soil from each plot to determine soil properties.

2.1.3 Soil properties determination

We measured 13 variables on soil samples taken at 5-15 cm depth (Dataset S2). Analyses were performed at the Laboratoire des sols d'Arras (INRAE) accredited by the French Accreditation Committee (Cofrac). Samples were first gently dried at 40°C, then sieved and ground at 2 mm. Soil texture was determined according to LAS-SOL-0302 procedure with 5-fraction granulometry without decarbonation (g kg^{-1}). From this first sample, a second representative sample was obtained and further ground at 250 µm (ISO 11464) for analyses requiring finer grinding. Finally, ammoniacal (Am, $NH4^{+}$) and nitric (Ni, $NO3^{-}$) nitrogen were quantified on all fresh samples (i.e., without pooling samples; Dataset S3), and samples from the same field, albeit different plots, were pooled for the subsequent analyses (Dataset S3).

Sampling and sieving were carried out after destruction of the organic matter by hydrogen peroxide ($H2O2$). The soil water pH was determined (soil:water ratio of 1:5) according to NF EN ISO 10390. The Metson Cationic Exchange Capacity (Metson 1957) was determined following the procedure NF X 31-130. Total organic carbon and nitrogen were determined by heat combustion (1000°C) on 250 µm particles obtained from grinding the 2-mm dried soil, with decarbonation performed for the former. Calcium carbonate content was measured from $CO2$ release after acidification of the sample by HCl according to NF EN ISO 10693.

2.1.4 Plant phenotyping

All phenotypic and nutrient measures on plants were performed by pooling all plants from a same plot. In addition to the number of plants per sample, we measured 22 (for maize) or 24 traits (for bean, Table 1). Note that we separated vegetative (including maize cobs and bean pod shells) from reproductive (kernels for maize or seeds for beans, referred to as "propagules" in Table 1) parts. Propagules were counted per plant, but also per ear for maize and per pod for bean – referred to as "sets" in Table 1). To measure plant nutrient parameters related to nitrogen and carbon content, we ground the samples into a fine and homogeneous powder. A subsample (0.8–1.2 mg) of each powder sample was assayed for total N and C contents and ^{15}N abundance using the FLASH 2000 Organic Elemental Analyzer coupled to an isotope ratio mass spectrometer (ThermoFisher Scientific, Waltham, MA). The N and C concentrations (N% and C%) of each sample were expressed as mg (of N or C) per 100 mg of dry weight for both vegetative tissues (NV, CV) and propagules (NP, CP). We also computed N and C content per plant both for vegetative parts (NV_p, CV_p) and propagules (NP_p, CP_p) by multiplying NV, CV, NP, CP with

the dry weight per plant, which generated four additional variables. Collected plant phenotypic data are given in Dataset S4.

2.1.5 Plant nutrient simulations

To assess the effect of intercropping on the nitrogen cycle, we used the N mineralization as a proxy during presence of the intercropping in fields. Accordingly, we simulated soil nitrogen mineralization (kg $N.ha^{-1}$) in the agricultural plots using Nsim (Parnaudeau et al. 2012). Nsim includes an agronomic model that estimates the potential soil N mineralization according to the characteristics of the soil (Clivot 2019), and uses soil water content and daily temperatures recorded in the Tarbais region in 2017 to calculate the effective N mineralization from organic matter. Soil water content is itself a function of soil characteristics, weather and canopy conditions. We used as input parameters: calendar dates of sowing and harvest as well as the irrigation and nitrogen supply (Dataset S1); because mineral N in the soil was not measured at the beginning of the experiment, the previous harvest values were used as default values at the beginning of the simulations. We also considered soil texture as determined by soil analyses. Since bean parameters were not available for Nsim, we parametrized the model using default parameters for pea (*Pisum sativum*).

2.1.6 Isotope analysis

The ^{15}N abundance was calculated as atom% (percentage of total N) and defined as $\%^{15}N=100 \times (^{15}N)/(^{15}N + ^{14}N)$ (Marmagne et al. 2022). We determined $^{15}N/^{14}N$ ratio (*d15N*) for the vegetative parts (*d15NV*) and propagules (*d15NP*) of maize and bean using the ^{15}N abundance method as discussed in Shearer and Kohl (1988), with the following equation:

$d15N = 1000[(\%^{15}N - \%^{15}N_{atm})/(\%^{15}N_{atm})]$

where $\%^{15}N_{atm}$ is the percentage of ^{15}N in the atmosphere, a constant value of 0.3663.

The $^{13}C/^{12}C$ ratio (*d13C*) was calculated for the vegetative parts (*d13CV*) and propagules (*d13CP*) of maize and bean using the following equation:

$d13C = 1000[(^{13}C/^{12}C)/(^{13}C/^{12}C_{atm})-1]$

where $^{13}C/^{12}C_{atm}$ is the actual ratio of ^{13}C to ^{12}C of the atmosphere, a constant equal to 0.011142.

We estimated symbiotic nitrogen fixation of the bean from the *d15N* by calculating the percent nitrogen derived from atmosphere (*d15Ndfa*) for the vegetative parts (*d15NVdfa*) and the propagules (*d15NPdfa*) separately, using the following equation (Shearer et al. 1980):

$d15Ndfa = 100[(d15N_{non.fix} - d15N_{fix})/(d15N_{non.fix} - B)]$

where $d15N_{non.fix}$ is the *d15N* of a non-fixing plant such as maize. We therefore estimated it from the average value of maize *d15N* between the replicates of each farm. B = $d15Nfix$ when plant N only comes from the atmospheric N (when plants are grown in N-free soil). Here, we took the B value of *d15N* common bean equal to -1.988. This value was proposed by Farid and Navabi (2015) as the

258 average of *d15N* measurements of a total of 20 randomly-selected bean genotypes (Peoples et al.
259 2002).
260
261 **2.1.7 Statistical analysis on soil characteristics and plant phenotypic traits**
262 We employed ANOVA (R package "stats") to examine whether the 13 soil property variables, simulated
263 nitrogen mineralization (Nsim), irrigation and N fertilization (Nin) varied according to farm and
264 cropping systems (sole-cropped bean, sole-cropped maize or maize-bean intercropping). Since cropping
265 conditions were not replicated within farms, we were unable to test the interaction between those factors.
266 Therefore, we utilized a linear model including farm and cropping system as fixed effects. Note that the
267 farm effect incorporates differences in the plant varieties, in farming practices and in soil characteristics.
268 We assessed the plant phenotypes of the maize and bean separately, initially employing Principal
269 Component Analysis (PCA) and ellipse plots for graphical analysis using the factoextra R package
270 where ellipses represent the 95% confidence interval (https://github.com/kassambara/factoextra).
271 Subsequently, we examined the farm and cropping system effects on plant phenotypes through ANOVA.
272 To assess the interaction between farm and cropping system for each crop, we restricted our analysis to
273 farms practicing both sole cropping and intercropping (F3, F5, F6, F7 for beans, and F1, F2, F4, F6, F7
274 for maize), and used the three plots per field as replicates to test the farm-by-cropping system interaction.
275 To address multiple testing, we used PCA plots to determine effective number of independent
276 traits in the data sets as the number of principal components required to explain more than 90% of the
277 variation in soil, bean and maize, respectively. Each *P* value was adjusted in accordance, i.e. multiplied
278 by the corresponding number of components.
279 To investigate the type of interaction between maize and bean plants, we examined correlations
280 between phenotypic traits of both species using intercropping data, correcting for multiple testing
281 through False Discovery Rate (FDR; Benjamini and Hochberg 1995) with a threshold at 20%. Negative
282 correlations are expected in case of competition between species, while positive correlations suggest
283 beneficial interactions.
284 We did not compute the Land Equivalent Ratio, often used to compare production in
285 intercropping versus sole cropping (Mead and Willey 1980), because the farming practices differed
286 between cropping conditions.
287
288 **2.1.8 Soil processing for metabarcoding**
289 Metabarcoding analysis of the soil bacterial community was carried out using 39 composite samples
290 from the three plots in each of the 16 fields. Non-rhizospheric samples were collected from each of the
291 16 fields, while rhizospheric samples were taken separately for each crop in the seven intercropped
292 maize and bean fields (14 samples), in addition to those from the sole-cropped fields (9 samples).
293 Composite samples of non-rhizosphere soil and rhizosphere soil (for each crop) were taken at 5-15 cm
294 depth. For rhizosphere soil, 2 or 3 plants of each species were studied per plot (6-9 plants of each species

295 per field) and soil in contact with roots was taken from 2 to 3 different roots per plant. Therefore, soil
296 from roughly 18 roots of each species was sampled per field (~1 g per composite sample) with a sterile
297 scalpel. Likewise, non-rhizosphere soil was taken from 2 to 3 root-free soil samples in each of the 3
298 plots (~1 g non-rhizosphere soil per composite sample).
299 Each composite sample was placed in a 2 ml ZR BashingBead Lysis tube containing beads and
300 ZR stabilization reagent (Zymo Research, Irvine, CA), and was mixed immediately in the field using a
301 bead-beating TerraLyzer lysis system (Zymo Research) for 30 s. DNA extraction was carried out in the
302 laboratory using the FastDNA kit (MP Biomedicals, Santa Ana, CA), following the manufacturer's
303 instructions. We sequenced 16S rRNA genes using Illumina MiSeq technology (2 × 300 bp).
304
305 **2.1.9 Operational Taxonomic Units assignation and analysis**
306 Sequence trimming and quality control (removal of sequences < 150 bp or with ambiguous base calls)
307 were performed by MR DNA (Shallowater, TX). The remaining sequences were denoised, Operational
308 Taxonomic Units (OTUs; arbitrarily defined with a threshold of 3% divergence) identified, and chimeras
309 removed. Final OTUs were taxonomically classified using BLASTn against RDPII
310 (http://rdp.cme.msu.edu). OTU quantification and taxonomic assignation are given in Tables S5 and S6.
311 We used datasets without singletons (i.e., sequences found a single time among the 39 sequenced
312 samples) to generate rarefaction curves.
313 Data analysis was primarily conducted using the phyloseq R package (McMurdie and Holmes
314 2013). At each taxonomic level, we visualized the 10 most abundant taxa in the entire dataset using
315 percentage normalization and assessed the proportion of these OTU sequences for each condition. For
316 other analyses, we utilized the rarefy_even_depth function to normalize sample sizes based on the
317 smallest sample size. We computed alpha diversity indices (Observed diversity, Chao1) that represent
318 measures of biodiversity within individual samples. The Observed diversity index simply counts the
319 number of unique taxa observed in a sample, while the Chao1 index estimates the true richness of the
320 sample, taking into account the presence of rare taxa. These indices were calculated using the
321 plot_richness function on the normalized data. Additionally, Unifrac distances, a measure of
322 dissimilarity between microbial communities, were computed between samples and then visualized
323 using Non-metric MultiDimensional Scaling (NMDS) plots. Permutational Multivariate Analysis of
324 Variance (PERMANOVA) was employed to test differences among conditions using the adonis2
325 function from the vegan R package (https://github.com/vegandevs/vegan), with sample distances as
326 input. The unique and common OTUs between conditions were determined on non-normalized data
327 using Venn diagrams created with the limma package (Ritchie et al. 2015). Furthermore, variations
328 among conditions were assessed using Between Class Analysis (BCA).
329 We relied on differential analysis based on OTU count using negative binomial (a.k.a. Gamma-
330 Poisson) distribution and Wald significance tests to identify discriminant OTUs (with FDR Q value)

between sole cropped and intercropped conditions for maize and bean taken separately. This analysis was performed using the DESeq2 R package (https://github.com/thelovelab/DESeq2).

Subsequently, we compared OTU composition among soil types and farming practices using co-inertia analysis. The RV coefficient between the two datasets was computed, and its significance was tested using Monte-Carlo tests with 10,000 replicates. Both BCA and co-inertia analyses were conducted using the ade4 R package (https://adeverse.github.io/ade4/).

2.2 Experimental assays of maize-bean intercropping in Saclay

2.2.1 Experimental design and field management

To assess the effect of maize-bean intercropping at the transcriptomic level, we set up a controlled field experiment in Saclay in 2022 (France). A single bean genotype (B, the Tarbais variety Alaric) was associated with two maize hybrids, DG0801 (M1, INRAE hybrid used as a control variety for low-nitrogen trials in southwestern France) and LG30.275 (M2, commercial hybrid used by INRAE as a control variety in northern France). Each of the three genotypes (B, M1, M2) was grown in sole cropping along with the two possible intercropping (M1B and M2B) in a three-blocks randomized design. Each block therefore contained five randomized plots with 160 cm between plots and two stand types: sole and intercropping. Each plot encompassed two rows 5.2m length of 12 plants for sole cropping with 24 cm intra-row spacing and 80 cm inter-row spacing, and similarly for intercropping albeit with 12 maize and 12 beans per row. Bean and maize plants were sown at a similar density as practiced on-farm for intercropping, that is 2.36 plants m^{-2} for both sole cropping and intercropping conditions (meaning that plant density is doubled for intercropping plots).

The soil was prepared before sowing by adding nitrogen fertilization in line with the farming practices recommended by the Tarbais bean cooperative (residual nitrogen of 26 kg N ha^{-1} after winter and application after sowing of 75 kg N ha^{-1} of nitrogen in the form of 27% ammonium nitrate). Such nitrogen supply corresponded to a grain yield target of 30% of their potential for commercial maize hybrids. No other chemical treatment was applied. Sowing occurred on April 28th 2022 for maize. Beans were sown after maize emergence on May 24th 2022, and the seeds were inoculated with a mix of symbiotic rhizobia (LEGUMEFIX Phaseolus Inoculant, Legume Technology LTD, Nottingham, UK). Stakes were used for support in bean sole cropping. Note that we encountered a drought with only 94.5 mm of cumulated rainfall for the three first months of the growing season (188 mm 20 years average). Manual irrigation was applied once during the course of the experiment.

2.2.2 Sampling and phenotyping

Leaf sampling was performed on the morning of July 11th, 2022, with a collection minimized to ensure physiological consistency across plants. This date coincided to the flowering stage for M2, an advanced vegetative stage for M1, and an early flowering stage in beans where nodules are likely most active

(Peña-Cabriales and Castellanos 1993). We pooled 1 leaf per plant for 10 randomly-selected plants per plot, for a total of 21 samples (9 sole-cropping samples i.e. 3 plots × 3 blocks, and 12 intercropping samples i.e. 2 plots × 3 blocks × 2 species; Dataset S7). For maize, we sampled 11th leaf, and for bean, we chose the terminal leaflet of the last fully developed leaf on each plant. We retained 2 cm^2 of the central portion of the blade without the midrib from the 10 leaves. Samples were flash-frozen and stored at -80°C. Traits measured on maize and bean plants are given in Dataset S8.

2.2.3 Quantification of gene expression with QuantSeq 3'RNA-seq

Total RNAs were extracted from 100 mg of tissue with the Nucleospin RNA plant kit (Macherey-Nagel, Düren, Germany) following the manufacturer instructions. Extracted RNAs were checked for purity and quantity with a bioanalyzer (Agilent, Santa Clara, CA). Barcoded libraries were constructed using QuantSeq 3'RNA-Seq Library Prep Kit FWD for Illumina (Lexogen, Vienna, Austria) with Unique Molecular Identifiers (UMIs) to reduce amplification biases, and sequencing was conducted by the POPS transcriptomics platform (IPS2, Université Paris-Saclay). QuantSeq 3'RNA-seq produce a single read per transcript close to the 3′ end of polyadenylated RNAs. From 3.4 to 17.4 million 75-bp reads were obtained per sample (Dataset S7).

Pre-processing steps and read counts included deduplication of reads based on UMIs with UMI-tools v1.0.1 (Smith et al. 2017). Reads with UMI base quality score falling below 10 were removed. We performed removal of adapter sequences and quality trimming with BBduk from the BBmap suite (v38.84, https://sourceforge.net/projects/bbmap/) with the options k=13 ktrim=r useshortkmers=t mink=5 qtrim=r trimq=10 minlength=30. The nf-core/rnaseq pipeline version 3.10.1 (Ewels et al. 2020; Patel et al. 2024, https://doi.org/10.5281/zenodo.7505987) was then used, as implemented with "nextflow" version 22.10.6 (Di Tommaso et al. 2017). Fastq files were sub-sampled with fq version 2022-02-15 (https://github.com/stjude-rust-labs/fq) and strandedness was automatically inferred using Salmon version 1.4.0 (Patro et al. 2017). Trimmed reads were then mapped using STAR (v2.7.9a, Dobin et al. 2013) with default parameters~~,~~ on the Zm-B73-REFERENCE-NAM-5.0 maize reference genome (https://phytozome-next.jgi.doe.gov/info/Zmays_Zm_B73_REFERENCE_NAM_5_0_55) and on the *Phaseolus vulgaris* v2.1 bean reference genome (https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1). Quantification was then made with Salmon version 1.4.0, and the file "salmon.merged.gene_counts.tsv" was used for subsequent analysis.

2.2.4 Differential gene expression in sole cropping and intercropping

From the raw read counts, we verified reproducibility among replicates (blocks) by computing R^2. We removed the library B1_M2B1_1, which was identified as R^2 outlier in the two comparisons involving blocks 2 and 3 (Figure S2). We used the standard procedure proposed by EdgeR (Lun et al. 2016) for filtering and normalization. In order to compute scaling factors for each library, we used the Trimmed

Mean of M-values (TMM) normalization method (Robinson and Oshlack 2010) implemented in EdgeR (function calcNormFactors) with the following parameters: min.count=10, min.total.count=15, large.n=3, min.prop=0.6. We subsequently performed MultiDimensional Scaling (MDS) on the resulting normalized counts to compute pairwise distances between libraries.

In order to identify differentially expressed (DE) genes, we further used EdgeR to estimate the trended dispersion parameters (function estimateDisp), fit a quasi-likelihood negative binomial generalized log-linear model (glmQLFit), and employed a quasi-likelihood F-test as described in Lun et al. (2016) (function glmQLFTest) to compute contrasts and associated *P* values for each transcript. The *P* values were then adjusted to control the FDR at 5% (Benjamini and Hochberg 1995). First, using only the intercropped beans, we tested the maize genotype effect by contrasting M1 vs M2. Second, we decomposed the bean expression variance into a cropping system (sole vs intercropping) effect and a block effect. As for maize, we decomposed the expression variance into a cropping system effect, a genotype effect, a block effect and a (cropping system-by-genotype) interaction. Just like for beans, DE genes and adjusted *P* values were identified from the sole vs intercropping contrast.

3. RESULTS

3.1 Soil properties and farming practices

We examined soil properties among farms. Overall, the soils of the 16 fields were all loamy but their contents varied from 17.1% to 30.0% for clay, from 36.1% to 62.0% for silt and from 10.3% to 44.8% for sand (Figure S3). We found significant differences among farms for 6 of the 13 soil variables (Dataset S9) and variability between fields was mainly due to soil fertility variables such as total N content (N), organic matter content (OM), C:N ratio (CN) and Cation Exchange Capacity (CEC), as exhibited on the first principal component of the PCA (Dim1, Figure 2a).

Among the three variables related to farming practices (Nsim, Nin and irrigation), the simulated soil nitrogen mineralization from organic matter (Nsim) and the N fertilization (Nin) differed among farms (Dataset S9). Notably, the only organic farm in our panel (F4) stood out as an outlier along the first principal component of the PCA, suggesting superior soil fertility as expected from the longer crop rotation and high pre-sowing manure inputs in this farming system (Figure 2b). Conversely, farm F6 (which used the highest quantity of nitrogen fertilizers; Dataset S1) displayed low values for fertility soil variables (Figure 2). Note that Nsim was positively correlated with N, OM and CEC ($r > 0.63$) while it was negatively correlated with irrigation ($r = -0.79$, $P = 0.0003$, Dataset S10). Additionally, Nsim exhibited a negative correlation with N fertilization ($r = -0.27$, $P = 0.037$), as expected, indicating that soils with greater soil mineralization require less fertilization.

440 **3.2 Soil bacterial communities associated with plants are shaped by agronomic practices and**
441 **cropping system.**
442 We described rhizospheric and non-rhizospheric bacterial communities. A total of 4,632,917 16S rRNA
443 sequences were obtained. Their abundance varied from 80,394 to 160,393 sequences (Dataset S5).
444 Sequence assignation resulted in 12,171 bacterial OTUs, classified at seven taxonomic levels (Dataset
445 S6). When combining data from all farms, the most prevalent phyla were *Proteobacteria*,
446 *Actinobacteria*, *Bacteroidetes*, *Chloroflexi* and *Firmicutes* (Figure S4).
447 Interestingly, we observed no significant difference in alpha diversity between maize and bean.
448 However, intercropping conditions displayed a trend toward higher alpha diversity (Observed OTUs and
449 Chao1 indexes) irrespective of the rhizosphere/non-rhizosphere status for both maize and bean soils
450 (Figure S5). This increase in diversity under intercropping conditions was significant for soils associated
451 with maize only (FDR Q = 0.014). The observed diversity of non-rhizosphere soil in intercropping
452 conditions appeared to be intermediate between the non-rhizosphere soils of intercropped maize and
453 intercropped bean.
454 We examined distances between samples considering all OTUs (Figure S6) and revealed a closer
455 proximity among samples originating from the same farm (farm effect at $P < 0.001$ in PERMANOVA,
456 Dataset S11), regardless of soil conditions (rhizosphere vs non-rhizosphere), plant species (maize vs
457 bean), or cropping system (intercropping vs sole cropping). OTUs whose counts differed among
458 samples, however, separated rhizospheric from non-rhizospheric samples with a clearer pattern for beans
459 than for maize (Figure 3). The second axis of the BCA differentiated samples by cropping system for
460 both crops (Figure 3).
461 Co-inertia analysis unveiled a significant association between OTU composition and the 13 soil
462 variables, irrigation, N input, and Nsim (correlation coefficient RV = 0.58; P = 0.025, Figure S7). The
463 first dimension of soil PCA (Figure 2) exhibited a negative contribution to the first dimension of co-
464 inertia (-0.93) while the second dimension of soil PCA contributed positively to the second dimension
465 of co-inertia (0.93). The first two axes of the co-inertia accounted for 61.2% of the total inertia (Figure
466 S7). Notably, Irrigation and Nsim presented the highest values of canonical weights (respectively 0.45
467 and -0.43, Dataset S12) that defined the second axis of co-inertia, indicating that these two variables
468 highly impacted OTU composition. F4 and F6 emerged as the most distinct farms on the co-inertia plot
469 along the first axis (Figure S7). Interestingly, F4 was the only organic farm in our sample, applying the
470 lowest level of mineral inputs (Dataset S1) while displaying superior soil fertility (Figure 2).
471 Finally, Wald multiple comparison tests carried out with individual OTUs indicated that *Vibrio*,
472 *Acinetobacter*, *Pseudonocardia alaniniphila*, *Hydrogenophaga palleronii* and *Pseudoxanthomona*
473 *koreensis* were more abundant in sole-cropped than intercropped bean rhizosphere, while
474 *Janthinobacterium lividum* was more abundant in the intercropped bean rhizosphere (Figure S8). In the
475 maize rhizosphere, *Actinospica*, *Sphingomonas mali, Ktedonobacter robiniae* (previously bacterium
476 SOSP1-30) and *Alcaligenes* were more abundant in sole-cropped samples while *Thermoactinomyces*

and *Bdellovibrio* were more abundant in intercropped samples (Figure S9). These last results need however to be taken with caution as the data are scarce and significance is, in some instances, driven by a single data point.

3.3 Cropping system impacts maize agronomic traits

We examined the effect of the cropping system on maize agronomic traits using a multivariate analysis. We found that the first principal component explained 40.9% of the variation and was strongly associated with yield-related variables, including propagule dimensions and number (Dim 1, Figure 4a). This principal component correlated positively with nitrogen fertilization (Nin, r = 0.77, $P < 0.01$) and irrigation (r = 0.68, $P < 0.01$), and negatively with simulated nitrogen mineralization (Nsim, r = -0.78, $P < 0.01$). In addition, different fields of a given farm tended to cluster together along the first principal component, which suggests that farming practices and local soil properties primarily determined maize yield (Figure 4b). The ellipse plot further revealed a clear distinction between sole (M) and intercropped maize (IC) along this axis (Figure 4b).

The difference between cropping systems was stable across farms for three traits, intercropping being associated to higher values of two N-nutrition related traits (NP and *d15NV*) and lower values of kernel weight per ear (Sw), regardless of the farm (Figure 5, Dataset S13). The percentage of carbon in vegetative parts (CV), although showing significant farm-by-cropping system interaction, exhibited consistently smaller values in intercropping than in sole cropping (Figure S10). For eight other traits, the difference between cropping systems varied among farms (i.e., significant farm-by-cropping system interactions, Dataset S13).

3.4 Cropping system impacts bean agronomic traits

We examined the effect of the cropping system on bean agronomic traits. The first two principal components explained over 59% of the variation (Figure 4c). The first principal component was associated with yield and nutrition traits (Pw, NbS, NbP, PS, CP_p, CV_p, NP_p, Vw), while the second one was linked to propagule size (Pwi, Ple and Psu). Sole cropped (B) and intercropped (IC) beans were clearly separated along the first principal component (Figure 4d). Intercropping negatively affected yield components across all farms, although the intensity varied, as indicated by significant farm-by-cropping system interactions (Figure S11). This included vegetative weight (Vw), seed weight (Pw), the number of seeds per plant (NbP), and the number of pods per plant (NbS). A similar pattern was observed for traits related to nitrogen and carbon content per plant, such as CV_p, CP_p, and NP_p (Figure 5, Dataset S13). Interestingly, as compared to sole cropping, intercropped beans displayed higher values for seed size traits (Ple, Pwi, Psu) as well as for nutrition-related traits (NV, CP) across all farms (Figure 5, Dataset S13).

514 **3.5 Competition prevails in maize-bean intercropping**

515 Correlations between maize and bean traits were computed to explore the interplay between the two
516 crops, positive correlations being expected under facilitation and negative correlations under
517 competition. Of the 528 correlations (22 traits for maize and 24 traits for bean), 47 were significant
518 among which 34 were negative and 13 were positive (Figure S12), pointing towards competition rather
519 than beneficial interactions. Considering the subset of traits impacted either in the maize or the bean by
520 intercropping (Figure 5), we found that maize traits related to nitrogen nutrition (*d15NV*; *d15NP*; CV_p;
521 Table 1) and vegetative dry weight (Vw) were negatively correlated both with yield-related traits (NbS,
522 Pw, Sw, Tpw, NbP) and two nutrition-related traits (CP_p, NP_p) measured in bean (Table 1). These
523 results indicate inter-species competition for resources such as light, water and mineral N needed for
524 nitrogen assimilation and CO_2 fixation through photosynthesis, resulting in reduced bean yield.
525 Conversely, the nitrogen percentage in vegetative parts (NV) of bean was positively correlated with four
526 maize traits (Pw, NbP, CP_p, *d13CP* ; Table 1). This most likely resulted from an indirect effect linked
527 to the lower seed production—less nitrogen mobilized into the reproductive organs—in intercropped
528 beans.

529

530 **3.6 Controlled field assay shows that species competition affects bean transcriptome**

531 We conducted a controlled field assay in INRAE plant breeding station of Saclay (northern France) to
532 further assess the impact of intercropping on plant gene expression. Agronomic measures revealed that
533 6 of 13 yield and development traits exhibited lower values in intercropped bean as compared to sole-
534 cropped bean, i.e. total seed mass per plant (reduced by 87%; Figure 6a), number of seeds per plant
535 (reduced by 89%), number of pods per plant (reduced by 89%), width of the main leaflets (reduced by
536 19%), foliage density (reduced by 56%), and plant height (reduced by 59%; Figure 6b) (Figure S13).
537 Although the two maize varieties strongly differed for all yield and development traits (except for the
538 number of ears per plant and the number of lodged plants), they were both unaffected by the cropping
539 condition (Figure 6c,d and Figure S14).

540 We investigated transcriptomic changes in sole cropping versus intercropping stands. First, we
541 tested differences in maize gene expression between genotypes M1 and M2. We found clear distinctions
542 between the two genotypes on the first axis of the MDS plot, that accounted for 66% of the total
543 variation; Figure 7a). We further investigated differentially expressed (DE) genes, with the prediction
544 that their number would be higher in beans than in maize, since bean phenotypes (but not maize
545 phenotypes) were severely affected by the cropping condition. Following our prediction, we detected no
546 DE genes between sole-cropped and intercropped maize plants. In bean, we identified only 11 DE genes
547 between M1B and M2B and therefore concluded on the near absence of impact of maize variety on bean
548 transcriptome. We thus considered M1B and M2B as biological replicates for the detection of DE genes.
549 Out of 14,993 bean genes, nearly a third (5,070 genes) were DE between cropping conditions (first MDS
550 axis, Figure 7b).

4. DISCUSSION

Intercropping systems are gaining in popularity, appreciated for their low-input requirements as well as their yield in low-input settings. Such positive effects result from beneficial plant-plant interactions (Fréville et al. 2022). In this study, we undertook a multidisciplinary approach to get insights into maize-bean intercropping, a practice that was recently revived in southwestern France for the cultivation of the Tarbais bean. The primary strength, yet limitation, of this study lies in the direct sampling of plants from on-farm fields. This approach introduces numerous confounding factors such as maize variety, inputs, abiotic and biotic environmental factors, that are challenging to control. Here, these factors were collectively captured as the "farm" effect.

Soil analyses revealed notable differences in fertility and pH across farms (Dataset S9), while soil texture remained relatively consistent (Figure S3). Globally, we observed a positive correlation between nitrogen mineralization by microorganisms and soil fertility, i.e., with total N content ($r = 0.64$; $P < 0.01$) and organic matter content ($r = 0.64$; $P < 0.01$), alongside a negative trend with nitrogen fertilizer application. These correlations driven by the organic farm suggest that farmers compensate for poor soil fertility with nitrogen inputs, and they also highlight the intricate connections between farming practices, soil fertility, and the capacity of microorganisms to release nutrients into the soil.

While it is well known that bulk bacterial communities are shaped by various soil variables—including texture (Girvan et al. 2003), nitrogen content (Zeng 2016), pH (Lauber et al. 2008), and water (Li 2021), the rhizosphere communities are also shape by crop genotypes (Bouffaud et al. 2016) and physiology, which collectively determine the compounds released in the immediate vicinity of roots as exudates (Ulbrich et al. 2022). Due to the complexity of interactions, studying the impact of intercropping on bacterial composition remains challenging (Duan et al. 2024). Here, we found a trend towards higher diversity in soil samples obtained from intercropping than from sole cropping (significant in maize; Figure S5), a pattern observed in sugarcane–soybean intercropping (Malviya et al. 2021). Interestingly, alpha diversity levels of maize and bean soils were not different, meaning that the greater diversity observed in intercropped maize more likely results from a complementarity effect rather than a selective effect (Loreau and Hector 2001). The combination of root exudates released by both crops may indeed enhance the diversity of the associated bacterial communities. In addition, we found in bean that two of the OTUs that discriminated the cropping systems include plant growth-promoting rhizobacteria (Figure S8). The *Acinetobacter* spp., which were predominant in sole-cropped bean rhizosphere, comprise strains that can produce indole-3-acetic acid (IAA), siderophores, gibberellin, antibiotics, biosurfactants/bioemulsifiers and solubilize phosphate, potassium, and zinc (Rokhbakhsh-Zamin et al. 2011). Also, *Janthinobacterium lividum* was more abundant in the bean rhizosphere when intercropped with maize. Interestingly, strains of this species can inhibit a number of soil-borne pathogens, including *Pythium ultimum*, *Rhizoctonia solani* and *Rhizoctonia oryzae*, and display biocontrol activity in the wheat rhizosphere (Yin et al. 2021).

In cereal-legume intercropping, cereal roots develop more quickly, utilizing nitrogen before legumes thereby stimulating nodule formation and nitrogen fixation in beans (Hauggaard-Nielsen et al. 2008). Although we did not conduct a detailed examination of root nodules, initial observations revealed no nodules on the bean in topsoil. This is likely caused by the sampling timing (close to harvest), which is not ideal to make such observations, but nevertheless suggests limited symbiotic nitrogen fixation, despite the nodulation capability of Tarbais beans (Laguerre et al. 2006). This could be due to two factors: the non-restrictive nitrogen fertilization conditions, which can inhibit nodulation (Voisin et al. 2002), or the absence of the specific rhizobial strains needed for nodulation in the soil (Yan et al. 2014). Because we evidenced presence of *Rhizobium leguminosarum* (Dataset S6), involved in bean nodulation, our findings suggest that years of conventional practices of maize monoculture without a Fabaceae cover crop may have diminished the potential benefits of plant-microbial interactions in these fields.

In order to assess the potential beneficial interactions between maize and bean in intercropping, we examined the effect of intercropping on plant nutrition and development. Pairwise correlations between traits of the two crops revealed that most significant correlations were negative, indicating that competition outweighed beneficial interactions. Notably, yield components in both crops were negatively affected by intercropping, with beans experiencing greater reductions compared to sole cropping situations (Figures S10, S11 and S12). Interestingly, however, bean seed size traits (length, width and surface) were larger in intercropped than sole cropping, indicating that competition with maize translates into beans producing fewer pods and seeds but larger seeds. This pattern illustrates the trade-off in resource allocation between fecundity and seed size, the latter providing offspring with more resources (Aarssenand and Keog 2002) and therefore a potential competitive advantage during early development, as shown in other species (Houssard and Escarré 1991). In intercropping, maize also displayed increased nitrogen acquisition in kernels and higher ^{15}N abundance in vegetative tissues (Figure 5), suggesting an enhanced capacity of maize to mobilize soil nitrogen – the elevated ^{15}N levels indicate that maize primarily relies on soil nitrogen. This contrasts with bean, which showed a higher nitrogen percentage in vegetative tissues (Figure S12). The reduced capacity of certain beans to compete with maize for soil nitrogen (Dawo et al. 2009) likely explains their lower yield. In this setting, nitrogen fixation was limited, as evidenced by no significant increase in nitrogen fixation in intercropping. Enhanced nitrogen in vegetative parts of beans may hence simply reflect a reduced demand for nitrogen in seeds due to fewer seeds being produced, and a lower dilution of nitrogen assimilates. These findings suggest that competition between species dominates the observed resource allocation patterns, with maize benefiting more from shared soil nitrogen resources while beans face limitations.

Finally, our field assay in Saclay provided a first glimpse on the characterization of the molecular signatures of maize-bean intercropping (Becker et al. 2023). Our results show that competition in intercropping was much stronger than what we observed on-farm. It is interesting to note that under such increased competition, the burden falls on the bean and resulted into a strong decline in

yield (~ 87%) and vegetative development as compared to sole cropping beans. Note that early growth of bean remained unaffected. In contrast, maize showed no difference between cropping conditions across all measured traits. Interestingly, gene expression analysis concurred with phenotypic data, revealing no differentially expressed genes in maize but 5070 DE genes in beans, which indicated a complete rewiring of bean gene expression when suffering drastic competition.

5. CONCLUSION

Despite the intrinsic limitations of field surveys in farmers' settings, including confounding effects and scarce sampling, our results demonstrate that intercropping leads to plant competition that significantly impacts the agronomic traits of maize and bean in the Tarbes region. However, this impact is highly dependent on farming practices. Key factors such as crop variety, plant row arrangement and density, soil properties and farm inputs that affect soil fertility and microbial composition play critical roles in shaping these outcomes. Differences observed between on-farm surveys and the field experiment, for instance, illustrate the potential importance of maize genotype and environmental conditions, such as soil composition and drought, in driving plant competition dynamics. These findings underscore the pivotal role of farming practices and soil fertility in mediating species interactions, extending even to the diversity of bacterial soil communities. Interestingly, intercropping tends to foster more diverse microbial communities compared to sole cropping, a potentially beneficial effect for soil health. Despite this advantage, intercropping remains characterized by strong interspecific competition, particularly to the detriment of bean. This competition negatively affects both gene expression and yield-related traits in bean. However, a notable outcome is the production of larger seeds by intercropped beans compared to those grown in sole cropping—an attribute that could be valued by farmers as larger seed size may be a criterion for premium labels, guaranteeing higher market prices and stable demand.

This interdisciplinary study paves the way for recommendations on maize-bean intercropping and outlines several research perspectives. First, the genetic basis of beans in the Tarbes region is particularly narrow and the two bean varieties have been selected without explicit consideration of their symbiotic N-fixation potential in soils of this area, where rhizobial inoculation is not customary. Second, beans are the primary focus in this system, and selecting an appropriate maize variety is crucial. The ideal maize partner should be robust enough to support the beans but not overly competitive. Hybrids optimized for sole cropping and mineral nitrogen absorption are unsuitable in this context. Molecular studies, such as those conducted here but expanded to other growth stages, plant organs, and varieties, could help identify the metabolic pathways involved and guide partner selection using available genetic resources. Third, the study encourages a reevaluation of the criteria for the success of this intercropping system. Beyond productivity, traits such as the nutritional quality of seeds and/or their specific characteristics could be shaped by consumer preferences, while acknowledging the positive impacts of intercropping on the agroecosystem.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare that are relevant to the content of this article.

DATA AVAILABILITY STATEMENT

Raw reads from metabarcoding data have been deposited in the DDBJ/EMBL/GeneBank under accession number PRJNA 1112577 and similarly for raw reads from QuantSeq 3'RNA-seq under accession number PRJNA 1115135. Raw phenotypic data as well as input files to run the code were deposited on recherche.data.gouv.fr with the DOI https://doi.org/10.57745/BZMPT9. Codes for RNAseq and phenotypic data analyses are available at recherche.data.gouv.fr with the DOI https://doi.org/10.57745/BZMPT9.

SUPPORTING INFORMATION

This paper contains a supporting information file with 14 supplementary figures 13 supplementary datasets.

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920 **FIGURE LEGENDS**

921

922 **FIGURE 1.** The local Tarbais bean intercropped with maize (left) or as a sole crop (right).

923

924 **FIGURE 2.** Principal Component Analysis (PCA) computed on 13 soil variables per field. The two first
925 axes are shown. (a) Correlation circle of the 13 soil variables with three supplementary variables added
926 to the PCA (in blue), i.e. Nsim (which estimates soil nitrogen mineralization), Irrigation, and Nin
927 (nitrogen units supplied during the growing season). (b) Projection of fields with colors indicating farms
928 and symbols the cropping system, i.e. maize sole cropping (M), bean sole cropping (B) or maize-bean
929 intercropping (IC). Variable names are indicated in Dataset S2.

930

931 **FIGURE 3.** Between Class Analysis (BCA) computed on 11,586 OTUs. Samples are named according
932 to rhizosphere (R) or non-rhizosphere (NR) soil, followed by maize (M) or bean (B), and then sole
933 cropping (SC) or intercropping (IC). Projection on the first two dimensions are presented, with the
934 corresponding percentages of total inertia explained by these axes.

935

936 **FIGURE 4.** Principal Component Analysis (PCA) on maize and bean phenotypic and nutrition
937 variables. The first two PCs computed from 22 maize variables (Table 1) are shown with corresponding
938 correlation circle (a) and data projection (b). Similarly, for beans, the first two PCs computed on 24
939 variables (Table 1) are shown with corresponding correlation circle (c) and data projection (d). The three
940 variables Irrigation, Nin (N input) and Nsim (simulated soil mineralization) shown in blue (a and c)
941 were added to the PCA. In b and d, colours indicate farms, symbols the cropping system with
942 corresponding ellipses, i.e. sole-cropped maize in b (M, dashed lines), sole-cropped bean in d (B, dashed
943 line), intercropped maize in b and intercropped bean in d (IC, full lines).

944

945 **FIGURE 5.** Effect of the cropping system on maize and bean phenotypic and nutrition variables. A two-
946 way ANOVA model with interaction was used to test the effects of the Farm and the Cropping system
947 (sole cropping versus intercropping) on phenotypic and nutrition variables (Table 1). Significant *P*
948 values for the cropping system effect are indicated by *, ** and *** for $P < 0.05$, $P < 0.01$ and $P <$
949 0.001, respectively. Pink asterisks indicate significance of tests performed on maize traits and green
950 asterisks those for bean traits. Asterisks are underlined when the value is higher in intercropping than in
951 sole cropping system. Variables (see Table 1 for full details) impacted by intercropping are *d15NV* (delta
952 15N among total N of the Vegetative parts), NV (Percent Nitrogen in Vegetative parts), CV (Percent
953 Carbon in Vegetative parts), CV_p (Per plant Carbon content in Vegetative parts), Vw (Vegetative parts
954 dry weight per plant), Pw (Propagule dry weight per plant), TPw (Thousand Propagules dry weight),
955 NbP (Number of Propagules produced per plant), NbS (Number of Sets of propagules per plant), Sw
956 (Average propagule weight per Set), Ple (Average Propagule length), Pwi (Average Propagule width),

957 Psu (Average Propagule surface), NP (Percent Nitrogen in Propagules), CP (Percent Carbon in
958 Propagules), CP_p (Per plant Carbon content in propagules) and, NP_p (Per plant Nitrogen content in
959 propagules).
960
961 **FIGURE 6.** Development and yield of maize and bean plants under sole cropping and intercropping in
962 the field assay at Saclay. Stripcharts display the total seed weight per bean plant (a) and the height of
963 bean plants (b), the total kernel weight per maize plant (c) and the height of maize plants (d). Blue dots
964 represent the average value in each of the three blocks and horizontal lines indicate the means. Data are
965 shown in a and b for sole-cropped maize genotype 1 (M1), sole-cropped maize genotype 2 (M2), maize-
966 bean intercropping (M1B and M2B), and in c and d for sole-cropped bean (B), and maize-bean
967 intercropping (M1B and M2B). Pictures illustrate sole-cropped beans (e) and intercropped beans and
968 maize (f).
969
970 **FIGURE 7.** MDS plots of distances between gene expression profiles in sole-cropped and intercropped
971 maize and bean. The distances observed between samples in the two MDS plots approximate the typical
972 $\log_2$ fold changes between the samples. MDS plots were computed from 16,021 genes for maize samples
973 (a) and 14,993 genes for bean samples (b). Colors and labels indicate the cropping system and the
974 sample, for sole-cropped maize of the two different genotypes (M1 and M2), sole-cropped bean (B), and
975 maize-bean intercropping (M1B and M2B). The last digit in the labels indicate the corresponding block
976 (1, 2, or 3). The percentage of variation explained by each dimension is shown.

TABLE 1. Description of phenotypes and nutrient variables measured on plants. [a]: only measured for the bean.

Variable names	Variable description
Plants	Number of Plants per sample
Vw	Vegetative parts dry weight per plant (g)
Pw	Propagule dry weight per plant (g)
NbP	Number of Propagules produced per plant
Ple	Average Propagule length – measured on 50 propagules (mm)
Pwi	Average Propagule width – measured on 50 propagules (mm)
Psu	Average Propagule surface – measured on 50 propagules (mm^2)
d15NP	Delta ^{15}N among total N of the Propagules
d13CP	Delta ^{13}C among total C of the Propagules
d15NV	Delta ^{15}N among total N of the Vegetative parts
d13CV	Delta ^{13}C among total C of the Vegetative parts
NP[a]	Percent Nitrogen (measured in dry matter) in Propagules
CP[a]	Percent Carbon (measured in dry matter) in Propagules
NV[a]	Percent Nitrogen (measured in dry matter) in Vegetative parts
CV[a]	Percent Carbon (measured in dry matter) in Vegetative parts
NP_p	Per plant Nitrogen content (g) in propagules
CP_p	Per plant Carbon content (g) in propagules
NV_p	Per plant Nitrogen content (g) in Vegetative parts
CV_p	Per plant Carbon content (g) in Vegetative parts
NbS	Number of Sets of propagules (ears or pods) per plant
PS (=NbP/NbS)	Average number of Propagules per Set – ears or pods
Sw (=Pw/NbS)	Average propagule weight per Set – ears or pods (g)
TPw (=1000xPw/NbP)	Thousand Propagules dry weight (g)
d15NVdfa[a]	N in the vegetative parts derived from symbiotic fixation (for bean only)
d15NPdfA[a]	N in the propagules derived from symbiotic fixation (for bean only)

Figure 1.

Figure 2.

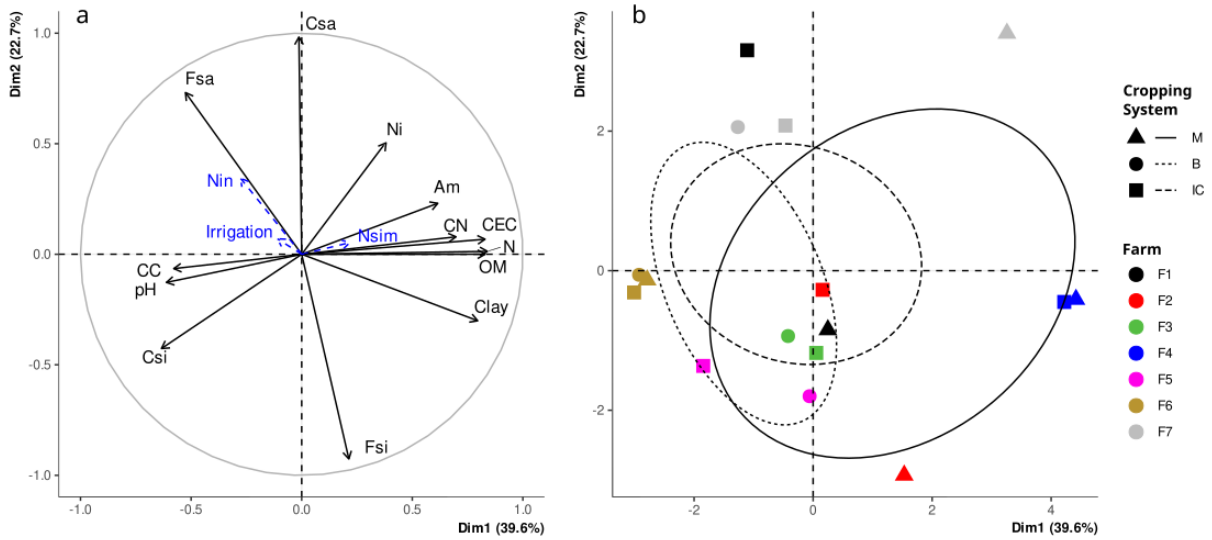

a
Csa
Fsa
Ni
Nin
Am
Irrigation
Nsim
CN
CEC
N
OM
CC
pH
Clay
Csi
Fsi
Dim1 (39.6%)
Dim2 (22.7%)
b
Cropping System
M
B
IC
Farm
F1
F2
F3
F4
F5
F6
F7

Figure 3.

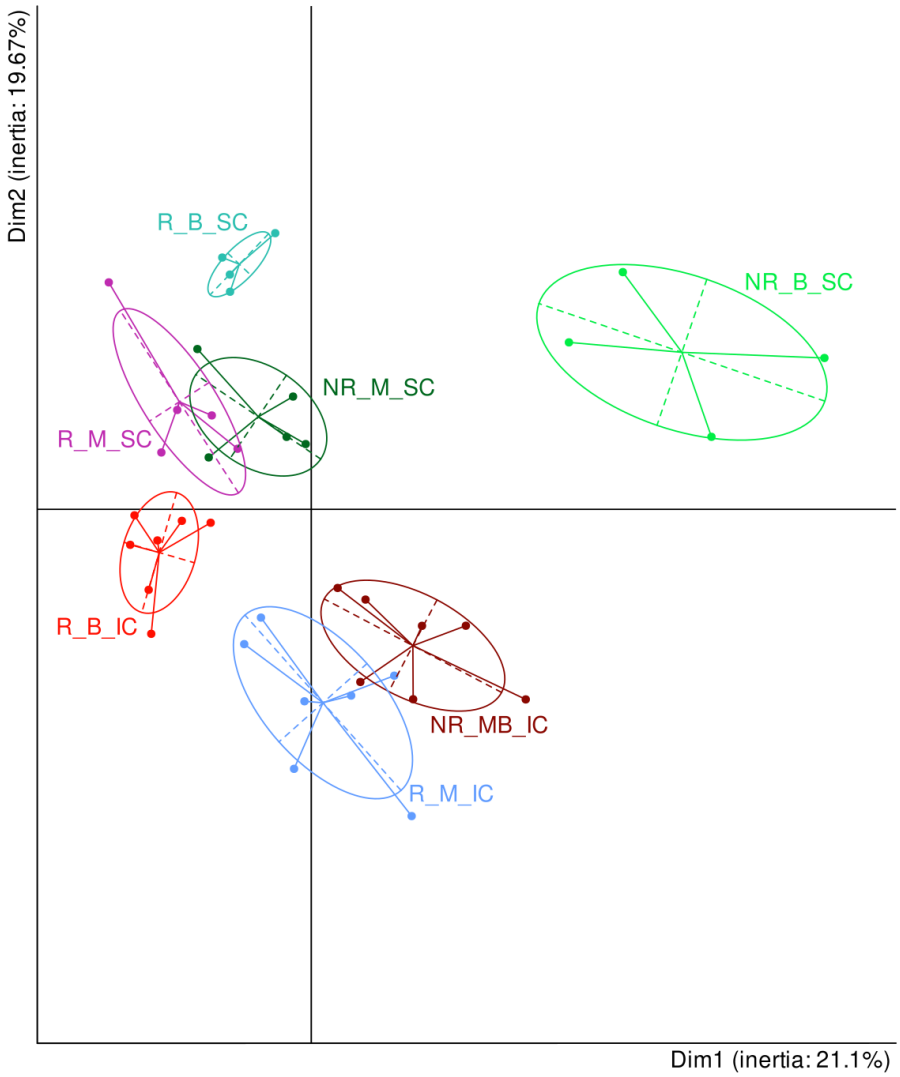

Dim2 (inertia: 19.67%)
R_B_SC
NR_B_SC
NR_M_SC
R_M_SC
R_B_IC
NR_MB_IC
R_M_IC
Dim1 (inertia: 21.1%)

Figure 4.

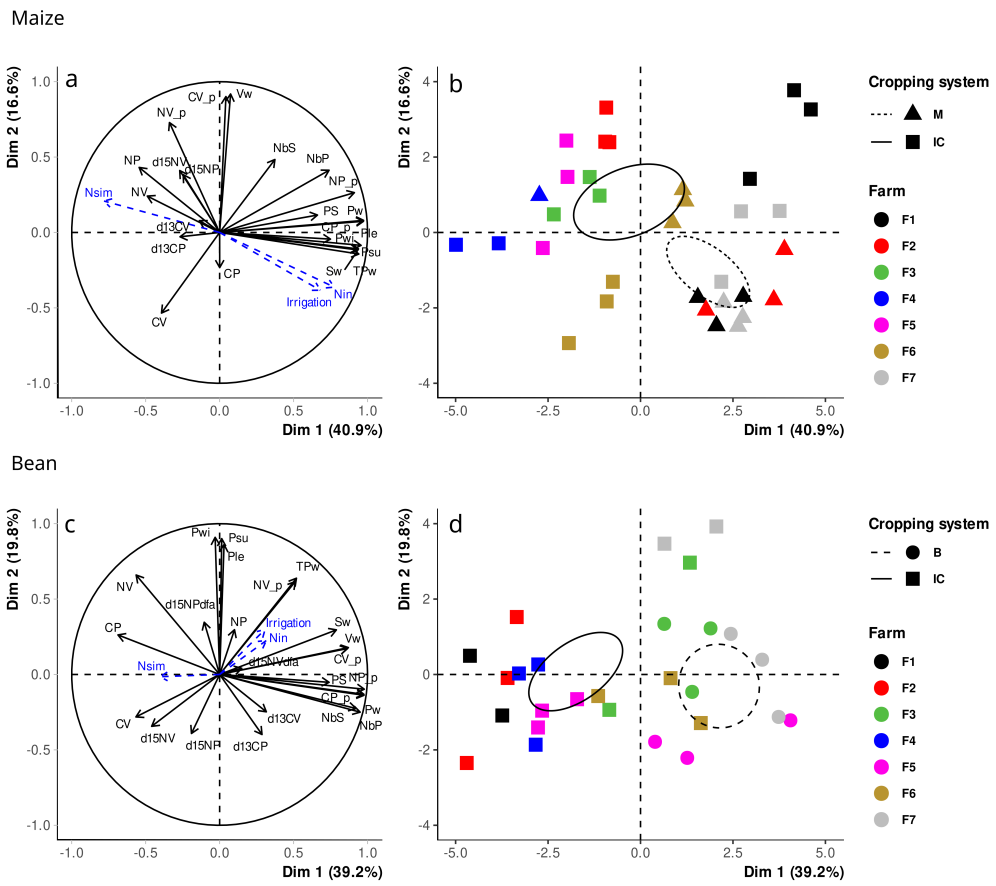

Maize
a
b
Dim 1 (40.9%)
Dim 2 (16.6%)
CV_p
Vw
NV_p
NbS
NbP
NP
d15NV
d15NP
NP_p
Nsim
NV
PS
Pw
d13CV
CP_p
Ple
d13CP
Psu
CP
Sw
TPw
Irrigation
Nin
CV
Cropping system
M
IC
Farm
F1
F2
F3
F4
F5
F6
F7
Bean
c
d
Dim 1 (39.2%)
Dim 2 (19.8%)
Pwi
Psu
Ple
TPw
NV
NV_p
d15NPdfa
CP
NP
Irrigation
Nin
Sw
Vw
Nsim
d15NVdfa
CV_p
PS
NP_p
CP_p
Pw
NbP
CV
d15NV
d15NP
d13CV
d13CP
NbS
B
IC

Figure 5.

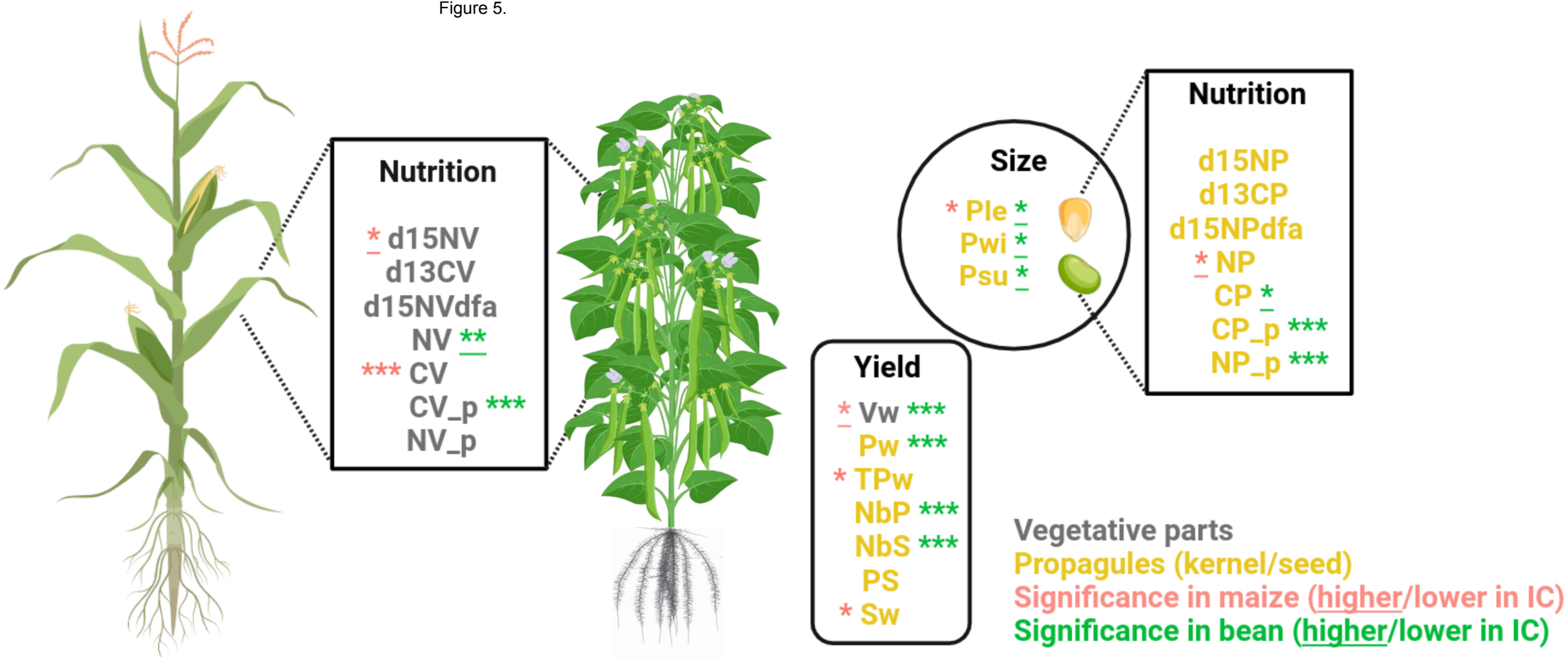

Nutrition
* d15NV
d13CV
d15NVdfa
NV **
*** CV
CV_p ***
NV_p
Size
* Ple *
Pwi *
Psu *
Nutrition
d15NP
d13CP
d15NPdfa
* NP
CP *
CP_p ***
NP_p ***
Yield
* Vw ***
Pw ***
* TPw
NbP ***
NbS ***
PS
* Sw
Vegetative parts
Propagules (kernel/seed)
Significance in maize (higher/lower in IC)
Significance in bean (higher/lower in IC)

Figure 6.

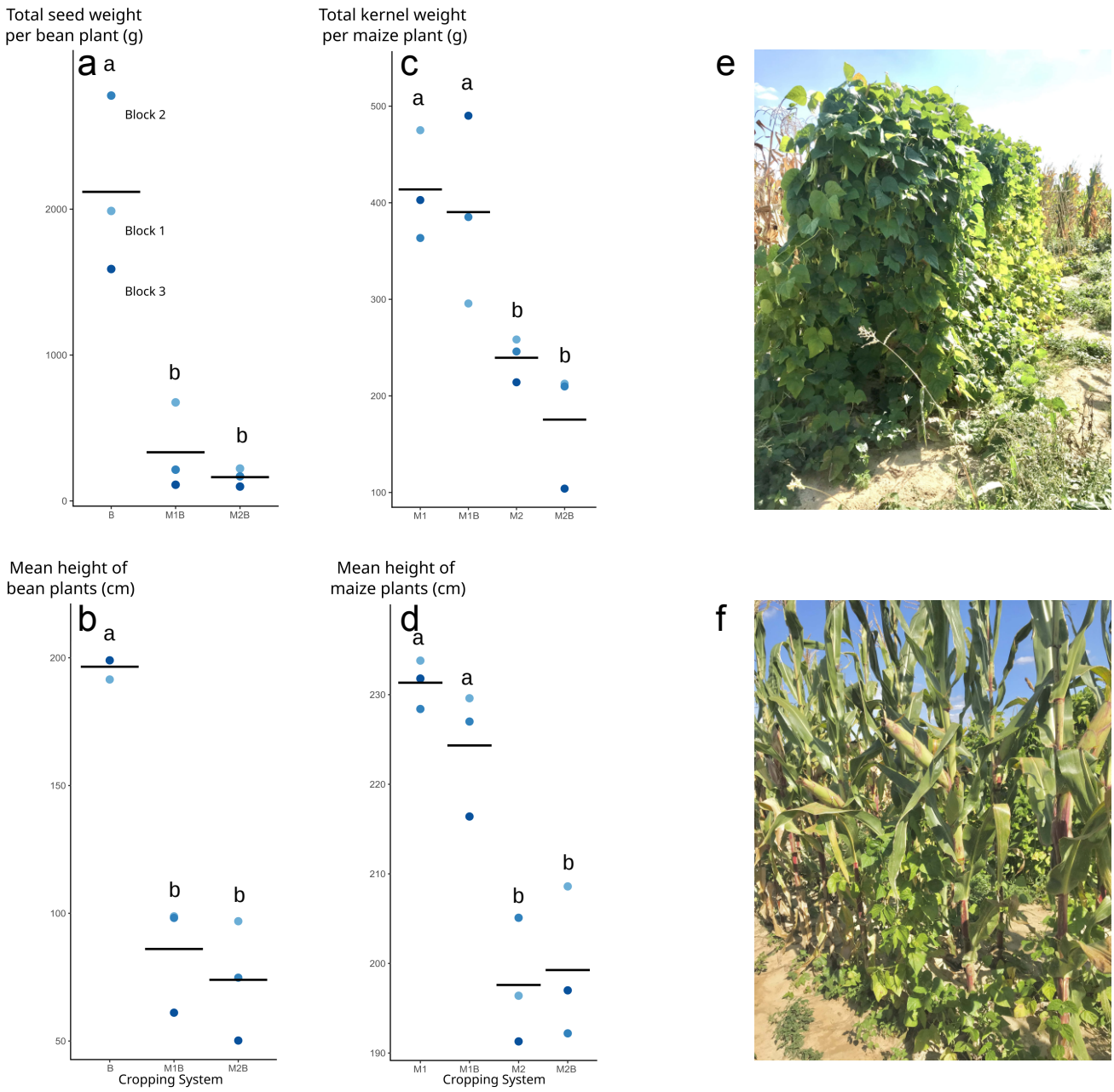
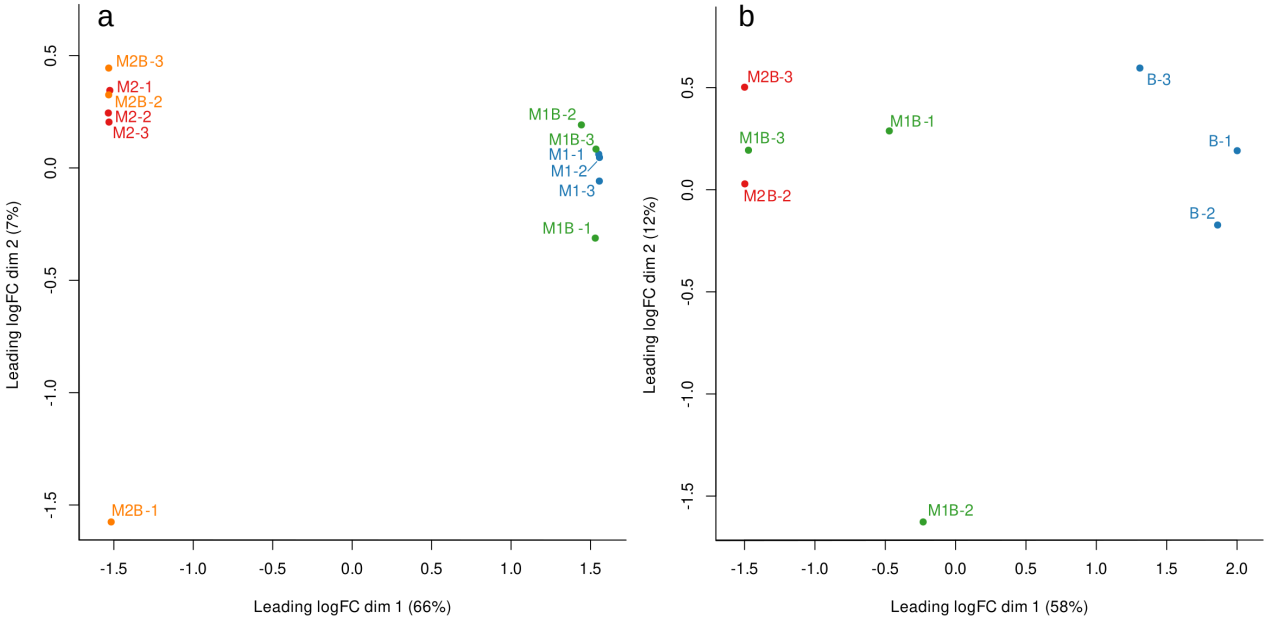

Total seed weight per bean plant (g)
a
a
Block 2
Block 1
Block 3
b
b
2000
1000
0
B
M1B
M2B
Total kernel weight per maize plant (g)
c
a
a
b
b
500
400
300
200
100
M1
M1B
M2
M2B
e
Mean height of bean plants (cm)
b
a
b
b
200
150
100
50
B
M1B
M2B
Cropping System
Mean height of maize plants (cm)
d
a
a
b
b
230
220
210
200
190
M1
M1B
M2
M2B
Cropping System
f

Figure 7.


a
M2B-3
M2-1
M2B-2
M2-2
M2-3
M1B-2
M1B-3
M1-1
M1-2
M1-3
M1B-1
M2B-1
Leading logFC dim 2 (7%)
Leading logFC dim 1 (66%)
b
M2B-3
B-3
M1B-1
M1B-3
B-1
M2B-2
B-2
M1B-2
Leading logFC dim 2 (12%)
Leading logFC dim 1 (58%)