A multidisciplinary on-farm survey of maize-bean intercropping highlights key considerations for reviving traditional agricultural systems.

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Abstract

- 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 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 at the phenotypic level? What is the effect of intercropping at the transcriptomic level?
- We recorded technical itineraries, conducted yield and nutrient measurements, and characterized soil bacterial assemblages via metabarcoding to compare sole cropped maize and beans with intercropped plants. We also set up a controlled field assay to quantify differentially expressed (DE) genes between cropping systems.
- We observed an increase in bacterial diversity in intercropping and a significant impact of the cropping system on agronomic traits, with frequent farm-by-cropping system interactions. Competition dominated maize-bean intercropping, with 56 out of 90 significant negative correlations between maize and bean traits. The bean displayed lower yield in intercropping but greater seed size, weight, nitrogen and carbon percentages. From the controlled field assay, we found that competition primarily affected the beans and their transcriptome, with nearly 30% of DE genes detected while none were found in maize.
- Overall, our findings suggest that synergies between the two crops are likely hindered, underscoring the importance of carefully considering partner varieties and technical itineraries in the revival of traditional agricultural systems.

Keywords: agroecology, bacterial communities, cereal-legume mixture, competition, plant nutrient uptake, RNAseq.

Societal Impact Statement

Cereal-legume intercropping is a promising strategy for sustainable agroecosystems that leverages the biological complementarities between species to reduce the need for chemical inputs while enhancing field biodiversity. A traditional method that originated in Central America, where both crops were domesticated, involves intercropping maize with climbing beans, using the maize stalk as support. This ancient practice is experiencing a revival in some contemporary conventional agricultural settings; however, much of the traditional knowledge associated with it, including variety selection and management techniques, has been lost. As a result, modern implementations do not fully harness the beneficial interactions between the species.

1 Introduction

To deliver high-yielding crops, conventional agriculture relies mainly on simplified cropping systems, with a single crop species grown per field (sole cropping) and environmental standardization through the use of chemical inputs. 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 cropping (intercropping), where several crops are grown simultaneously with no distinct row arrangements, relies on biological complementarities and positive species interactions. This approach can improve space, water, and nutrient use, reduce input requirements, enhance pathogen and pest suppression, and even increase crop productivity under low-nitrogen availability (Bedoussac et al. 2015). The benefits of intercropping stem from two key processes: complementarity of resource use and facilitation (Brooker et al. 2015).

The success of cereal-legume intercrops relies on their biological complementarity, which results primarily from the capacity of legumes (*Fabaceae*) to assimilate nitrogen fixed by symbiotic rhizobia. By associating 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 association (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) associations. 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 interconnected species through arbuscular mycorrhizal fungi (Johansen and Jensen 1996; Zhang et al. 2019). Fifth, the recruitment of Plant Growth-Promoting Rhizobacteria (PGPR), such as phloroglucinol-producing *Pseudomonas* on cereal roots, can promote plant growth and provide control over various root pathogens (Mazzola 2004).

While synergies have been documented between cereals and legumes, little is known about the maize-common bean intercropping, especially outside of Central America. Yet this association is of major interest, both 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 – and historically – maize and common bean 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). In this cropping system, known as milpa (that also associates squash), the maize stalk supports the climbing bean, favoring its exposure to light. It is noteworthy that the practice of intercropping maize and bean is commonly observed beyond the geographical region where these crops originated, such as on small farms in sub-Saharan Africa (Nassary et al.

2020) and in various traditional European farming systems. Hence, the intercropping of maize and bean seems to have been introduced and sustained as these crops spread beyond their center of origin.

Despite being abandoned in favor of sole cropping in modern agriculture, this practice was recently revived in some areas. 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 decreased due to the intensification of agriculture and the introduction of maize hybrids. As beans were not suitable for mechanization, their cultivation around Tarbes (Hautes-Pyrénées area) decreased from 12,000 hectares at the beginning of the 20th century to 55 hectares in 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. Two varieties with an identical genetic background (except for a resistance gene), Alaric and Lapujole (hereafter Tarbais bean), were derived from local landraces. Tarbais bean obtained a quality label and a protected geographic indication in 2000, which makes it an emblematic crop with high added value in the region. 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 (Fig. 1).

To gain a deeper understanding of the functioning and potential benefits of intercropping maize and common beans, we focused on the Tarbais-maize system which represents a revival of a traditional farming practice using modern varieties and, most often, conventional agronomic practices. Although these conditions may be suboptimal, we hypothesized that maize-bean intercropping could leverage synergies between the two crops. Due to the lack of research on this particular intercropping, we adopted a descriptive multidisciplinary approach combining agronomy, microbial ecology, and molecular signatures. We recorded technical itineraries, 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. We also investigated the transcriptomic impact of intercropping. Because the detection of molecular signatures of intercropping requires a controlled environment with replicates, we established a controlled field assay in which we compared both agronomic traits and gene expression between sole crop and intercropping of Tarbais bean and two maize hybrids. Our study aimed 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 the transcriptomic level?

2 Materials and Methods

2.1 On-farm analyses

In order to compare sole and intercropping, we identified multiple farms that practiced at least one of the two sole cropping (maize or bean) in addition to the intercropping, seven of which agreed to participate to this study.

2.1.1 Plant and soil sampling for agronomic analyses

We collected plant and soil samples in seven farms located in southwestern France (Tarbes region; Fig. S1). In this region, most farmers use the recent "Label Rouge" bean variety Alaric, an inbred line derived from crosses among local landraces in 1990. The Lapujole variety was derived from Alaric by introgression of an anthracnose resistance gene. Both varieties are therefore genetically similar. Among the seven farmers included in our study, five used the Tarbais bean. Among these, three farmers utilized the Alaric variety, while two used the Lapujole variety. The remaining two farmers used local bean landraces of their own. Several maize varieties were grown, most of which were modern hybrids (Table S1). A single farmer (F4) used a local maize landrace. Only two farms (F6 and F7) practiced the three cropping systems (sole cropped bean, sole cropped maize or maize-bean intercropping).

All plant and soil samples were collected before harvest in October 2017, from 16 fields (one to three fields per farm, Table S1). In each field, we randomly sampled three plots (located > 5 m apart) that served as replicates in the analyses. Each plot consisted of 4 to 5 (sole cropping) or 7 to 10 (intercropping) plants each. Among the 16 fields, seven fields corresponded to intercropping (for a total 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 and pooled them per field to determine soil properties. For the 16 fields, technical itineraries were recorded through semi-directed interviews of the seven farmers.

2.1.2 Soil properties determination

We measured 13 variables on the samples of bulk soil taken at 5-15 cm depth (Table S2). Analyses were performed at the Laboratoire des sols d'Arras (INRAE). 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). From this first sample, a second representative sample was obtained and further grinded at 250 µm (ISO 11464) for analyses requiring finer grinding. Samples from the same field, albeit different plots, were pooled for the subsequent analyses.

Sampling and sieving were carried out after destruction of the organic matter by hydrogen peroxide (H_2O_2). 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 CO_2 release after acidification of the sample by HCl according to NF EN ISO 10693. Finally, the quantification of ammoniacal (Am, NH_4^+) and nitric (Ni, NO_3^-) nitrogen was carried out on all fresh samples (i.e., without pooling samples; Table S3).

2.1.3 Plant phenotyping

All phenotypic and nutrient measures on plants were performed by pooling plants of the same plot together. In addition to the number of plants per sample, we measured 20 traits on the 69 samples (Table 1). Note that we separated vegetative (including maize cobs and bean pod shells) from reproductive (grains for maize or seeds for beans, referred to as propagules) parts. To measure plant nutrient parameters related to nitrogen and carbon content or concentration, we ground the samples into a fine and homogeneous powder. A subsample of each powder sample (0.800–1.200 mg) 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). 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. The ^{15}N abundance was calculated as atom% (percentage of total N) and defined as Asample% $= 100 \times (^{15}N)/(^{15}N + ^{14}N)$ (Marmagne et al. 2022). Note that we also computed N and C content per plant both for vegetative parts (NV_p, CV_p) and propagules (NP_p, CP_p), which generated four additional variables (25 variables in total, Table 1). Collected plant phenotypic data are given in Table S4.

2.1.4 Plant nutrient simulations

To assess the effect of the intercropping on nitrogen cycle, we used the N mineralization as a proxy during presence of the intercropping in fields. Accordingly, we simulated soil nitrogen mineralization 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 accounts for soil water content and daily temperatures recorded in the Tarbais region in 2017 to calculate the real N mineralization. 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 (Table 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 accounted for 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*).

2.1.5 Metabarcoding of bacterial assemblages

Metabarcoding analysis of the soil bacterial community was carried out using 39 composite samples from the three plots in each of the 16 fields. Composite samples of non-rhizosphere (bulk) soil and rhizosphere soil (for each crop) were taken at 5-15 cm depth. For rhizosphere soil, 2 or 3 plants of each species were studied per area

198 (6-9 plants of each species per field) and soil in contact with roots was taken from 2 to 3 different roots per plant.
199 Therefore, soil from roughly 18 roots of each species was sampled per field (~1 g per composite sample) with a
200 sterile scalpel. Likewise, bulk soil was taken from 2 to 3 root-free soil in each of the 3 plots (~1 g non-rhizosphere
201 soil per composite sample).

202 Each composite sample was placed in a 2 ml ZR BashingBead Lysis tube containing beads and ZR
203 stabilization reagent (Zymo Research, Irvine, CA), and was mixed immediately in the field using a bead-beating
204 TerraLyzer lysis system (Zymo Research) for 30 s. DNA extraction was carried out in the laboratory using the
205 FastDNA kit (MP Biomedicals, Santa Ana, CA), following the manufacturer's instructions.

206 We sequenced 16S rRNA genes using Illumina MiSeq technology (2 × 300 bp). Sequencing, sequence
207 trimming and quality control (removal of sequences < 150 bp or with ambiguous base calls) were performed by
208 MR DNA (Shallowater, TX). The remaining sequences were denoised, operational taxonomic units (OTUs;
209 arbitrarily defined with a threshold of 3% divergence) identified, and chimeras removed. Final OTUs were
210 taxonomically classified using BLASTn against RDPII (http://rdp.cme.msu.edu). OTU quantification and
211 taxonomic assignation are given in Tables S5 and S6. We used datasets without singletons (i.e., sequences found
212 a single time among the 39 sequenced samples) to generate rarefaction curves. Data analysis was primarily
213 conducted using the phyloseq R package (McMurdie and Holmes 2013). At each taxonomic level, we visualized
214 the 10 most abundant taxa in the entire dataset using percentage normalization and assessed the proportion of these
215 OTU sequences for each condition. For other analyses, we utilized the rarefy_even_depth function to normalize
216 sample sizes based on the smallest sample size. We computed alpha diversity indices (Observed diversity, Chao1)
217 that represent measures of biodiversity within individual samples. The Observed diversity index simply counts the
218 number of unique taxa observed in a sample, while the Chao1 index estimates the true richness of the sample,
219 taking into account the presence of rare taxa. These indices were calculated using the plot_richness function on
220 the normalized data. Additionally, Unifrac distances, a measure of dissimilarity between microbial communities,
221 were computed between samples and then visualized using Non-metric MultiDimensional Scaling (NMDS) plots.
222 Permutational Multivariate Analysis of Variance (PERMANOVA) was employed to test differences among
223 conditions using the adonis2 function from the vegan R package (https://github.com/vegandevs/vegan), with
224 sample distances as input. The unique and common OTUs between conditions were determined on non-normalized
225 data using Venn diagrams created with the limma package (Ritchie et al. 2015). Furthermore, variations among
226 conditions were assessed using Between Class Analysis (BCA).

227 We relied on differential analysis based on OTU count using negative binomial (a.k.a. Gamma-Poisson)
228 distribution and Wald significance tests to identify discriminant OTUs (with FDR adjusted P-value) between sole
229 cropped and intercropped conditions for maize and bean taken separately. This analysis was performed using the
230 DESeq2 R package (https://github.com/thelovelab/DESeq2).

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

2.1.6 Isotope analysis

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:

$$d15N = 1000[(-\%)(\%)]$$

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:

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

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 propagule (*d15NPdfa*) separately, using the following equation (Shearer et al. 1980):

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

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 = $d15N_{fix}$ when plant N only comes from the atmospheric N (when plants are grown in N-free soil). Here, we took the B value of common bean equal to -1.988. This value was proposed by Farid and Navabi (2015) as the average of *d15N* measurements of a total of 20 randomly selected bean genotypes (Peoples et al. 2002).

2.1.7 Statistical analysis on soil characteristics and plant phenotypic traits

We employed ANOVA to examine whether the 13 soil property variables, nitrogen mineralization (Nsim), irrigation and N fertilization (Nin) varied according to farm and cropping systems (sole cropped bean, sole cropped maize or maize-bean intercropping). Since cropping conditions were not replicated within farms, we were unable to test the interaction between those factors. Therefore, we utilized a linear model including farm and cropping system as fixed effects. Note that the farm effect incorporates differences in the plant varieties, in technical itineraries and in soil characteristics.

We assessed the plant phenotypes of the maize and bean separately, initially employing PCA for graphical analysis. Subsequently, we examined the farm and cropping system effects on plant phenotypes through ANOVA. To assess the interaction between farm and cropping system for each crop, we restricted our analysis to farms practicing both sole cropping and intercropping (F3, F5, F6, F7 for beans, and F1, F2, F4, F6, F7 for maize), and used the three plots per field as replicates to test the farm by cropping system interaction.

To investigate the type of interaction between maize and bean plants, we examined correlations (tested with FDR q-values) between phenotypic traits of both species using intercropping data. Negative correlations are expected in case of competition between species, while positive correlations suggest synergies.

We did not compute the Land Equivalent Ratio, often used to compare production in intercropping versus sole cropping (Mead and Willey, 1980), because the technical itineraries differed between cropping conditions.

2.2 Experimental assays of maize-bean intercropping in Saclay

To assess the effect of maize-bean intercropping at the transcriptomic level, we set up a controlled field experiment in Saclay (France).

2.2.1 Experimental design and technical itinerary

A single bean genotype (the Tarbais variety Alaric, B) 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, INRAE hybrid used 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 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/m² 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 technical itineraries recommended by the Tarbais bean cooperative (residual nitrogen of 26 kg N/ha after winter and application after sowing of 75 kg N/ha of nitrogen in the form of 27% ammonium nitrate). Such nitrogen supply corresponded to a yield target of 30% 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 were inoculated with a mix of symbiotic rhizobia (LEGUMEFIX Phaseolus Inoculant, Legume Technology LTD, United Kingdom). 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. Manual irrigation was applied once during the course of the experiment.

2.2.1 Sampling and phenotyping

Leaf sampling was performed on July 11th 2022 which corresponded 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; Table S7). For maize, we sampled leaf 11, and for beans, we chosen the terminal leaflet of the last fully developed leaf on each plant. We retained only the central portion of the blade from the 10 leaves. Samples were flash-frozen and stored at -80°C. Traits measured on maize and bean plants are given in Table S8.

2.2.2 Quantification of gene expression with QuantSeq 3’RNA-seq

Total RNAs were extracted from 100 mg of tissue with the Nucleospin RNA plant (Macherey-Nagel) kit following the manufacturer instructions. Extracted RNAs were checked for purity and quantity with a bioanalyzer (Agilent). Barcoded libraries were constructed using QuantSeq 3'RNA-Seq Library Prep Kit FWD for Illumina (Lexogen) 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 (Table 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. 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.

2.2.3 Differential gene expression in sole 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 block 2 and 3 (Fig. 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) and 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 False Discovery Rate (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

343 bean expression variance into a cropping system (sole vs intercropping) effect and a block effect. As for maize,
344 we decomposed the expression variance into a cropping system effect, a genotype effect, a block effect and a
345 (Cropping.System × genotype) interaction. Just like for beans, DE genes and adjusted P-values were identified
346 from the sole vs intercropping contrast.

3 Results

3.1 Farms exhibit contrasted soil properties and technical itineraries

Technical itineraries in each farm were recorded (Table S1). The sowing dates for the different farms ranged from April 15th to June 10th. Since manual harvest of beans requires wide space between rows, as well as within rows, sole cropped maize was cultivated at a much higher density (8 plants per m^2) than either intercropped maize and beans (2.5 plants per m^2 for each of the species) or sole cropped beans (2.5 plants per m^2). N-fertilizer input was on average of 189 N unit/ha for sole cropped maize, of 130 N unit/ha for sole cropped beans, and 116 N unit/ha for intercropped maize and beans. Irrigation along the growing season was, on average, 145 mm for sole cropped maize, 150 mm for sole cropped beans, and 100 mm for intercropped maize and beans. We found differences among farms for six out of the 13 soil variables (N, CN, OM, pH, CC, CEC), and for Nsim, Nin and irrigation (Table S9). Differences among cropping systems was significant only for Nin.

Overall, the soil of the 16 fields were all loamy and similar among farms, with content in clay varying from 17.1% to 30.0%, in silt from 36.1% to 62.0% and in sand from 10.3% to 44.8% (Fig. S3). Variability among 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 PCA axis (Fig. 2a). Interestingly, the only organic farm in our panel (F4) clearly appeared as an outlier along this axis (Fig. 2b) and displayed better soil fertility. At a finer scale, soils differed in content of fine silts (Fsi) and coarse sands (Csa) (Dim2, Fig. 2a).

Simulation of soil nitrogen mineralization (Nsim) was positively correlated with CEC, N and OM ($r > 0.63$) while it was negatively correlated with irrigation ($r = -0.79$, Table S10), and shows a negative trend with N fertilization (Fig. 2a).

3.2 Soil bacterial communities associated with plants are shaped by agronomic practices and cropping system.

A total of 4,632,917 16S rRNA sequences were obtained. Sequences abundance varied from 80,394 to 160,393 sequences. Sequence assignation resulted in 11,586 bacterial OTUs from 24 phyla, belonging to 7 phylum levels. When combining data from all farms, the most prevalent phyla were *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi* and *Firmicutes* (Fig. S4).

We observed a closer proximity among samples originating from the same farm, regardless of soil conditions (rhizosphere vs non-rhizosphere), plant species (maize vs bean), or cropping system (intercropping vs sole cropping) (PERMANOVA analyses; Farm effect P-value < 0.001; and see Fig. S5).

Co-inertia analysis of PCA data unveiled a significant association between OTU composition and the 13 soil variables, irrigation, N input, and Nsim (RV = 0.58; P-value = 0.025). The first dimension of soil PCA exhibited a negative contribution to the first dimension of co-inertia (-0.93) while the second dimension of soil PCA contributed positively to the second dimension of co-inertia (0.93). These first two axes of co-inertia together accounted for 61.2% of the total inertia. Notably, Irrigation and Nsim presented the highest values of canonical

weights that defined the second axis of co-inertia (positive for Irrigation: 0.45 and negative for Nsim: -0.43), indicating that these two variables highly impacted OTU composition. F4 and F6 emerged as the most distinct farms on the co-inertia plot (Fig. S6), with the highest mean values of Nsim (158.5 and 107.7 for F4 and F6, respectively). Interestingly, F4 was the only organic farm in our sample and was not irrigated, and F6 also applied low irrigation (Table S1).

The BCA revealed that samples were distinguished by OTUs along a first axis that separated rhizospheric from non-rhizospheric samples. This distinction is clearer for beans than for maize. The second axis differentiated samples by cropping system for both crops (Fig. 3).

Considering all seven farms, we observed no significant difference between alpha diversity of soil microorganisms between maize and beans. However, intercropping conditions displayed a trend toward higher alpha diversity (Observed OTUs and Chao1 indexes) irrespective of the rhizosphere/non-rhizosphere status for both maize and bean (Fig. S7). This increase in diversity under intercropping conditions was significant for maize soils (P adjusted = 0.014). The observed diversity of non-rhizosphere soil in intercropping conditions appeared to be intermediate between the non-rhizosphere soils of intercropped maize and intercropped bean.

Finally, Wald multiple comparison tests carried out with individual OTU indicated that *Vibrio*, *Acinetobacter*, *Pseudonocardia alaniniphila*, *Hydrogenophaga palleronii* and *Pseudoxanthomona koreensis* were more abundant in the sole cropped than the intercropped bean rhizosphere, while *Janthinobacterium lividum* was more abundant in the intercropped bean rhizosphere (Fig. S8). In the maize rhizosphere, *Actinospica*, *Sphingomonas mali* and *Ktedonobacter robiniae* sosp1_30 were more abundant in sole cropped samples while *Thermoactinomyces* was more abundant in intercropped samples (Fig. S9). These 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

Maize kernel yield-related variables (Ple, Psu and Pw, NbP, Sw, Pw, TPw, *d13CP* and *d15NP,* Table 1) were highly positively correlated (Dim1, Fig. 4a). CV_p, NV_p as well as Vw correlated together, and were independent from yield variables (Dim2 in Fig. 4a). The supplementary variables Nin and irrigation correlated positively to Dim1 (r = 0.77, P-value < 0.01 and r = 0.68, P-value < 0.01 respectively), while Nsim correlated negatively (r = -0.78, P-value < 0.01). As expected, there was therefore a positive correlation between nitrogen and water inputs (significant for maize with R = 0.54, P-value < 0.01) and, nutrients and yield (for instance R = 0.57 and P-value < 0.001 for Pw and NP_p). The different fields of a given farm tended to cluster together along the first axis, which suggests that agronomic practices and local soil properties primarily determined maize yield (Fig. 4b).

Sole (M) and intercropped maize (IC) displayed a differentiation as shown by the ellipse plot (Fig. 4b). However, the effect of cropping varied among farms for 11 out of 22 traits (Vw, Pw, Tpw, Ple, Psu, Sw, CV_p, NV_p, CP_p, NP_p, CV), indicating that the cropping effect was modulated by farm-level properties (Fig. S10; Table S11). A single trait (CV) exhibited a consistent direction across farms with smaller values in intercropping than in sole cropping. Four traits were significant for the cropping effect without farm*cropping interaction (Table

420 S11), revealing that maize displayed higher NP, *d15NP, d15NV* but lower PS in intercropping compared with sole
421 cropping (Fig. 5).
422
423 **3.4 Cropping system impacts bean agronomic traits**
424 Sole cropped (B) and intercropped (IC) bean were clearly separated on the first axis of the PCA (Fig. 4d).
425 Many bean traits correlated together. Over 59% of the variation was explained by the two first PC axes (Fig. 4c),
426 with PC1 relating to yield and nutrient uptake traits (Pw, NbS, NbP, PS, CP_p, CV_p, NP_p, Vw, Table 1) while
427 PC2 relates to propagule size (Pwi, Ple and Psu, Table 1). As compared to sole cropping, intercropped beans
428 displayed higher values for propagule size (Ple, Pwi, Psu), TPw, NP, NV, and CP. Lower values were observed in
429 intercropped beans for yield (Vw, Pw, NbP, NbS, PS), propagule (CP_p, NP_p) and vegatative (CV_p) nutrients.
430 For other traits, the effect of the cropping systems varied among farms, as for *d15NV*, *d13CP*, *d15NVdfa*, and
431 NV_p (Fig. S11; Table S11).
432
433 **3.5 Competition prevails in maize-bean intercropping**
434 Correlations between maize and bean traits were computed to delineate the interplay between the two
435 crops, positive correlations being expected under facilitation, while negative correlations being expected under
436 competition. Out of 483 correlations (21 traits for maize and 23 traits for bean), 90 were significant among which
437 56 were negative and 34 were positive, indicating a prevailing trend of competitive rather than synergistic
438 interactions. Notably, *d15NV*, *d15NP*, Vw and CV_p measured in maize were negatively correlated to Vw, Pw,
439 TPw, NbP, Ple, Pwi, Psu, NbS, Sw, NP_p, CP_p and CV_p that were measured on bean indicating inter-species
440 competition for ressources to assimilate N, and C through photosynthesis, leading to a decrease in yield in bean.
441 On the other hand, NV measured on bean positively correlated with Vw, Pw, Tpw, NbP, SW, *d13CP*, NP_p, CP_P
442 and CV_p measured on maize and, *d15NPdfa* and *d15NVdfa* were positively correlated to *d13CV* and *d13CP* (Fig.
443 S12).
444
445 **3.6 Controlled field assay shows that species competition affects bean transcriptomes**
446 We conducted a controlled field assay in Saclay (France) to further assess the impact of intercropping on
447 plant gene expression. Agronomic measures in this experiment revealed that six out of 13 yield and development
448 traits exhibited higher values in sole cropped beans compared to intercropped beans: total seed mass per plant
449 (reduced by 87% from sole cropping to intercropping), number of seeds per plant (reduced by 89%), number of
450 pods per plant (reduced by 89%), width of the main leaflets (reduced by 19%), foliage density (reduced by 56%),
451 and plant height (reduced by 59%; Fig. 6a, b and Fig. S13). Although the two maize varieties strongly differed for
452 all yield and development traits, except for the number of ears per plant and the number of lodged plants, they
453 were both unaffected by the cropping condition (Fig. 6c, d and Fig. S14).
454 We investigated transcriptomic changes in sole cropping versus intercropping stands. First, we tested
455 differences in maize gene expression between genotypes M1 and M2. We found clear distinctions between the two
456 genotypes on the first axis of the MDS plot, that accounted for 66% of the total variation; Fig. 7a). We further

457 detected DE genes predicting that the number of differentially expressed (DE) genes would be higher in beans
458 than in maize, since bean phenotypes were severely affected by the cropping condition as opposed to maize
459 phenotypes. Following our prediction, we detected no DE genes between sole cropped and inter cropped maize
460 plants. In beans, we identified 11 DE genes between M1B and M2B and therefore considered them as biological
461 replicates for the detection of DE genes. Out of 14,993 bean genes, nearly a third (5,070 genes) were DE between
462 cropping conditions (1st MDS axis, Fig. 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 the on-farm fields. It introduces numerous confounding factors that are challenging to control (maize variety, inputs, abiotic and biotic environmental factors) and capture in our analyses as a "farm" effect.

Soil analyzes revealed differences in soil fertility and pH (Table S9) rather than soil texture (Fig. S3, Table S9) across farms. Interestingly, farm F4 which was the only organic farm in our sample displayed elevated values for fertility soil variables such as cation exchange capacity (CEC), total organic carbon (C), total N (N), C:N ratio (CN), organic matter (OM) as well as simulated soil nitrogen mineralization (Nsim; Fig. 1). Whether soil fertility was better because of organic practices or whether the local environment was favorable to the switch to organic farming needs to be assessed. Conversely, farm F6 that used the highest quantity of nitrogen fertilizers (Table S1) displayed low values for fertility soil variables (Fig. 1). More generally, there was a positive correlation between simulated soil nitrogen mineralization and soil fertility variable N ($r = 0.64$; P-value < 0.01) and OM ($r = 0.64$; P-value < 0.01). This suggests that increasing soil organic matter content enhances mineralization by microorganisms, fostering higher CEC and facilitating greater release of nutrient elements into the soil. Overall, these observations are in accordance with the fact that farmers adjust their practices to soil fertility by optimizing nitrogen mineralization in poor soils.

Soil properties may impact indirectly plant nutrient via microbial partners. On one hand, bacterial communities are influenced by soil variables, including soil texture (Girvan et al. 2003), nitrogen content (Zeng 2016), soil pH (Lauber et al. 2008) and water (Li 2021), and locally, by the compounds released in the immediate vicinity of the plants via root exudates (Ulbrich et al., 2022). Here, we found that bacterial communities are primarily driven by technical itineraries (irrigation and N input) and soil fertility, and to a lesser extent by rhizosphere status and cropping system (Fig. S5).

Extensive research has explored the influence of the environment and crop genotype on bacterial assemblages (Bouffaud et al. 2016; de Albuquerque et al. 2022). However, the impact of intercropping on bacterial composition remains understudied (Schlatter et al. 2015). Here, we observed a trend towards higher diversity in soil samples obtained from intercropping than from sole cropping (significant in maize; Fig. S7), a pattern observed in sugarcane–soybean intercropping (Malviya et al. 2021). Interestingly, alpha diversity of between 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, Nature). The combination of root exudates released by both crops may indeed enhance the diversity of the associated bacterial communities. In addition, we found in beans that two of the OTUs that discriminated the cropping systems include PGPR strains

498 (Fig. S8). The *Acinetobacter* spp., which were predominant in sole cropped bean rhizosphere, comprise strains
499 that can produce IAA, siderophores, gibberellin, antibiotics, biosurfactants/bioemulsifiers and solubilize
500 phosphate, potassium, and zinc (Rokhbakhsh-Zamin et al. 2011). Also, *Janthinobacterium lividum* was more
501 abundant in the bean rhizosphere when intercropped with maize. Interestingly, strains of this species can inhibit a
502 number of soil-borne pathogens, including *Pythium ultimum*, *Rhizoctonia solani* and *Rhizoctonia oryzae*, and
503 display biocontrol activity in the wheat rhizosphere (Yin et al., 2021).

504 In cereal-legume associations, cereal roots develop more quickly, utilizing nitrogen before legumes
505 thereby stimulating nodule formation and nitrogen fixation in beans (Hauggaard-Nielsen et al. 2008). Although
506 we did not conduct a detailed examination of root nodules, initial observations revealed no nodules on the beans,
507 suggesting that symbiotic nitrogen fixation was limited, despite the nodulation capability of Tarbais beans
508 (Laguerre et al. 2006). This could be due to two factors: the non-restrictive nitrogen fertilization conditions, which
509 can inhibit nodulation (Voisin et al. 2002), or the absence of the specific rhizobial strains needed for nodulation in
510 the soil (Yan et al. 2014). Because we found presence of the symbiotic strain R*hizobium leguminosarum* (Table
511 S6), our findings suggest that years of conventional farming practices may have diminished the potential benefits
512 of plant-microbial interactions in these fields.

513 In order to assess the potential synergies between the two crops, we examined the impact of intercropping
514 for both maize and beans on plant nutrient and development. Some traits were positively impacted by intercropping
515 such as *d15NV* and NP that increased for both species, NV on bean and *d15NP* on maize, suggesting benefits of
516 the intercropping for nitrogen assimilation (Fig. 5, Table S11). This result was in line with previous ones showing
517 complementarity in N assimilation in maize-legume intercropping (Xing et al., 2023; Hupe et al. 2021).
518 Surprisingly, in our results, N derived from symbiotic interactions in vegetative parts of intercropped beans was
519 lower compared to sole cropped beans, suggesting that the higher N acquisition in the intercropped maize and
520 beans was due to complementarity mechanisms rather than facilitation. Notably, maize had the highest nitrogen
521 acquisition in intercropping, indicating that it benefits from reduced intraspecific competition through greater
522 complementarity. In this setup, bean functions more like a non-fixing crop albeit with a lower capacity than maize
523 to absorb soil nitrogen. Therefore, intercropping effectively manages interspecific competition by reducing
524 intraspecific competition and optimizing spatial complementarity between the above-ground and root systems.

525 We computed pairwise correlations between all maize and bean traits. The majority of significant
526 correlations were negative (56 out of 90), suggesting that competition between the crops outweighed synergies.
527 Yield components in particular, were negatively impacted by intercropping with Pw and PS lower for both species
528 and NbP and NbS lower for bean, as compared to sole cropping situations. Interestingly, TPw and seed size were
529 greater for intercropped than sole cropped beans. Hence, one might speculate that a potential outcome of maize
530 competition, is a response of bean producing fewer seeds but with elevated levels of nutrients (C and N), alongside
531 increased overall weight (TPw). This phenomenon may be influenced by nitrogen uptake, which can affect biomass
532 partitioning and nitrogen investment in vegetative structures through remobilization processes (Voisin et al. 2002).

533 Finally, we designed a randomized-block design field assay in Saclay to provide a first glimpse on the
534 characterization of the molecular signatures of maize-bean intercropping (Montazeaud et al. 2022). We used
535 similar sowing density as practiced on-farm for intercropping and sole cropping of beans and applied low-input
536 conditions. However, environmental conditions (soil, climate) differ between northern and southwestern France,
537 and we also experienced drought during the year of the experimentation. Our results show that competition in
538 intercropping was much stronger than what we observed on-farm. It is interesting to note that under increased
539 competition, the burden falls on the bean and resulted into a strong decline in yield and vegetative development as
540 compared to sole cropping beans. Notably, despite plant density in the intercropping set-up being twice that of sole
541 cropping, bean yield was reduced on average by ~ 87% in the intercropping conditions. Note that early growth of
542 bean remained unaffected. In contrast, maize showed no difference between cropping conditions across all
543 measured traits.
544 Interestingly, gene expression analysis concurred with phenotypic data, revealing no differentially
545 expressed genes in maize but 5,070 DE genes in beans, which indicated a complete rewiring of bean gene
546 expression when suffering drastic competition. Although the experiment was limited in terms of sample size and
547 sampling design—only one stage, one organ—preventing a more detailed analysis of our results in terms of
548 function enrichment or co-expression analyses, these findings open up very interesting avenues for research on the
549 molecular mechanisms implemented in response to species intercropping.

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Author contribution

Conceptualization, M.C., T.F., J.E., Y.M-L, Do.M., M.I.T.; Methodology, N.V-B, M.C., C.B., T.F., J.E., V.P., E.L.C., Da.M., Y.M-L, Do.M., M.I.T.; Software, N.V-B, T.T., C P-L, T.F., Da.M., Y.M-L, Do.M., M.I.T.; Validation N.V-B, L.M., T.T; Formal analysis: N.V-B, L.M., T.T, V.P.; Investigation: N.V-B, B.L., C.P., M.C., M.L.G., J.C., A.M., Y.M-L, Do.M., M.I.T.; Resources: C.P., M.C., C.B., J.C., C. P-L, T.F., M.L.G, V.P., A.M., Y.M-L.; Writing – Original Draft, N.V-B, L.M., Do.M., M.I.T; Writing –Review & Editing, N.V-B, L.M., T.F., J.E., E.L.C, V.P., Da.M., Y.M-L., Do.M. M.I.T.; Vizualization: N.V-B, L.M., T.T.; Supervision, Y.M-L, Do.M. M.I.T; Project administration: Y.M-L, M.I.T.; Funding Acquisition, N. V-B., C. P-L, T.F., Y.M-L, Do.M. M.I.T

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.

Conflict of Interest Statement

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

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785 **TABLES**

786

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)
d15NP	Percent of ^{15}N among total N of the Propagules
d13CP	Percent of ^{13}C among total C of the Propagules
d15NV	Percent of ^{15}N among total N of the Vegetative parts
d13CV	Percent of ^{13}C among total C of the Vegetative parts
NP	Percent Nitrogen (measured in dry matter) in Propagules
CP	Percent Carbon (measured in dry matter) in Propagules
NV	Percent Nitrogen (measured in dry matter) in Vegetative parts
CV	Percent Carbon (measured in dry matter) in Vegetative parts
NbS	Number of Set (ear or pod) of propagules per plant
PS (=NbP/NbS)	Average number of Propagules per Set – ear or pod
Sw (=Pw/NbS)	Average propagule weight per Set – ear or pod (g)
TPw (=1000xPw/NbP)	Thousand Propagules dry weight (g)
d15NVdfa[a]	N in the vegetative parts derived from symbiotic fixation
d15NPdfA[a]	N in the propagules derived from symbiotic fixation

787

FIGURE LEGENDS

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

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

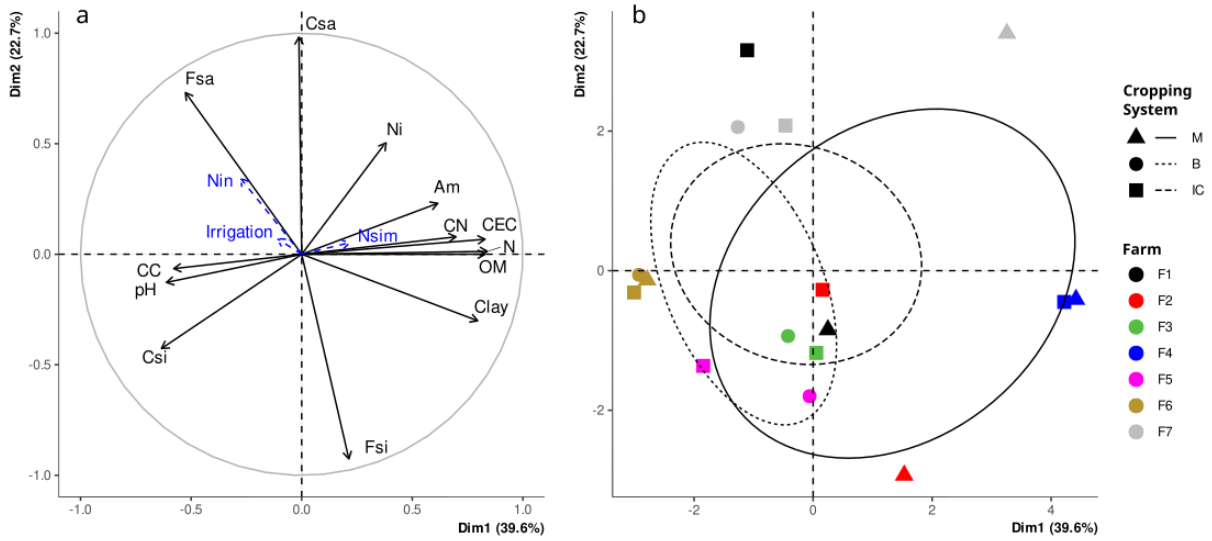
Fig. 3 Between Class Analysis (BCA) computed on 11,586 OTUs. Samples are named according to rhizosphere (R) or non-rhizosphere (NR) soil_maize (M) or bean (B)_ sole cropping (SC) or intercropping (IC). Projection on the two fisrt dimensions are presented with corresponding percentages of total inertia explained by those axes.

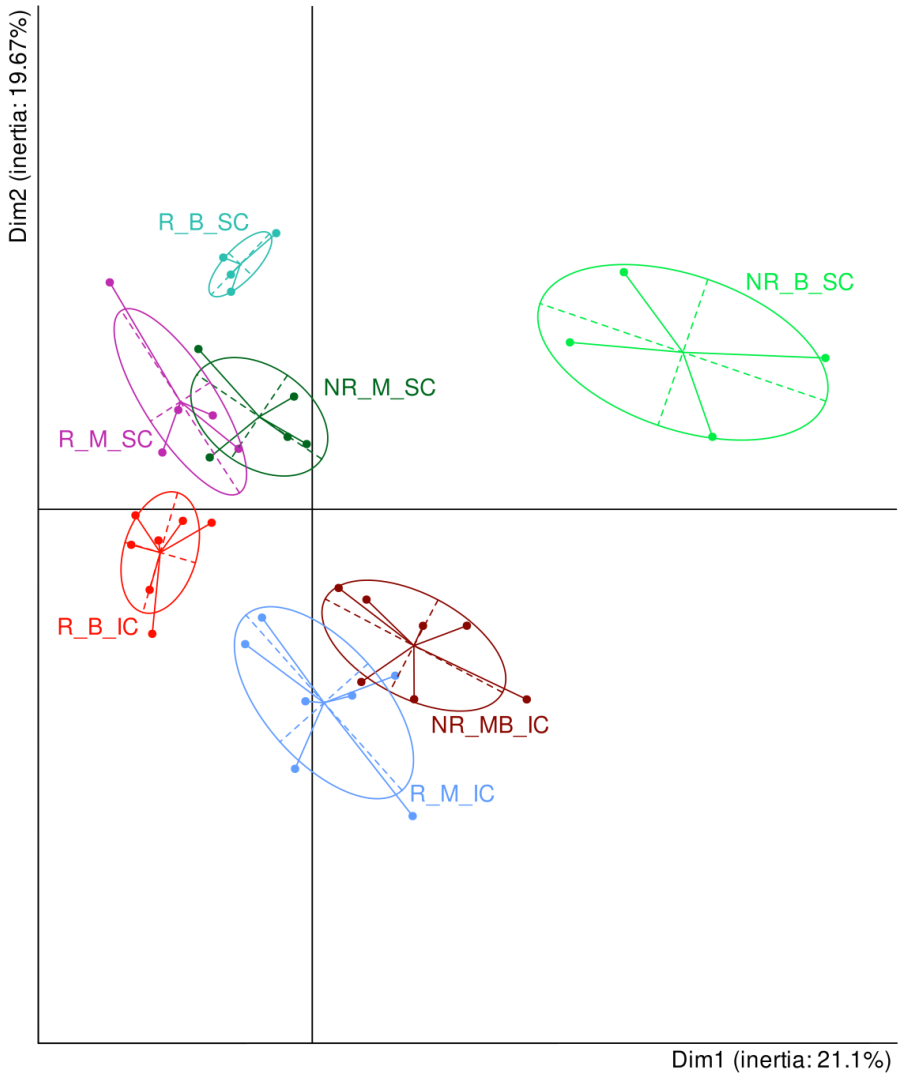
Fig. 4 Principal Component Analysis (PCA) on maize and bean phenotypic and nutrition variables. The two first PCs computed from 22 maize variables (Table 1) are shown with corresponding correlation circle (a) and data projection (b). Similarily, for beans, the two first PCs computed on 24 variables (Table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

Fig. 5 Effect of the cropping system on maize and bean phenotypic and nutrition variables. A two-way ANOVA model with interaction was used to test the effects of the Farm and the cropping system (sole cropping versus intercropping) on phenotypic and nutrition variables (Table 1). Significant P-values for the cropping system effect are indicated by *, ** and *** for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Pink asterisks indicate significance of tests performed on maize traits, while green asterisks on bean traits. Asterisks are underlined when the value is higher in intercropping than in sole cropping system.

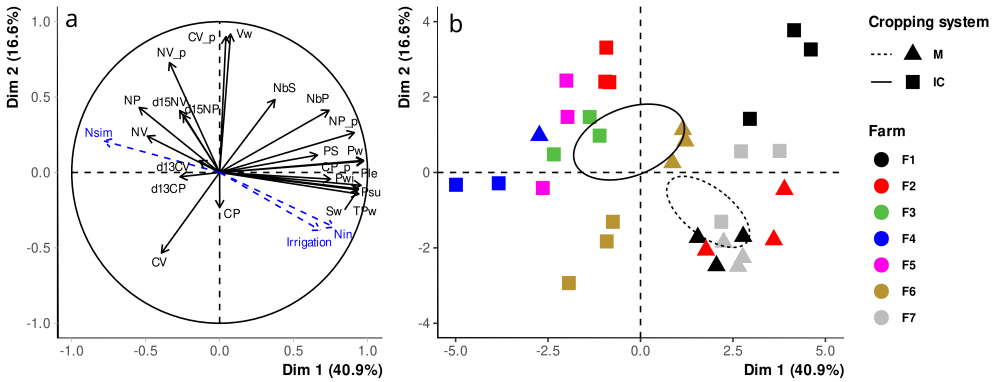
Fig. 6 Development and yield of maize and bean plants under sole and intercropping. Stripcharts display the total seed weight per bean plant (a) and the mean height of bean plants (b), the total kernel weight per maize plant (c) and the mean height of maize plants (d). Blue dots represent the average value per block (1, 2, 3) and horizontal lines indicate the mean for the corresponding cropping system: in a and b, sole cropped maize 1 (M1), sole cropped maize 2 (M2), maize-bean intercropping (M1B and M2B); and in c and d, sole cropped bean (B), and maize-bean intercropping (M1B and M2B). Pictures illustrate sole cropped beans (e) and intercropped beans (f).

825 **Fig. 7 MDS plots of distances between gene expression profiles in sole cropped and intercropped maize and**
826 **bean.** The distances observed between samples in the two MDS plots approximate the typical log2 fold changes
827 between the samples. MDS plots were computed from 16,021 genes for maize samples (a), and from 14,993 genes
828 for bean samples (b). Colors and labels indicate the cropping system of the sample: sole cropped maize of the two
829 different genotypes (M1 or M2), sole cropped bean (B), maize-bean intercropping (M1B or M2B). Labels also
830 indicate the corresponding block (1, 2, 3). The percentage of variation explained by each dimension is shown.

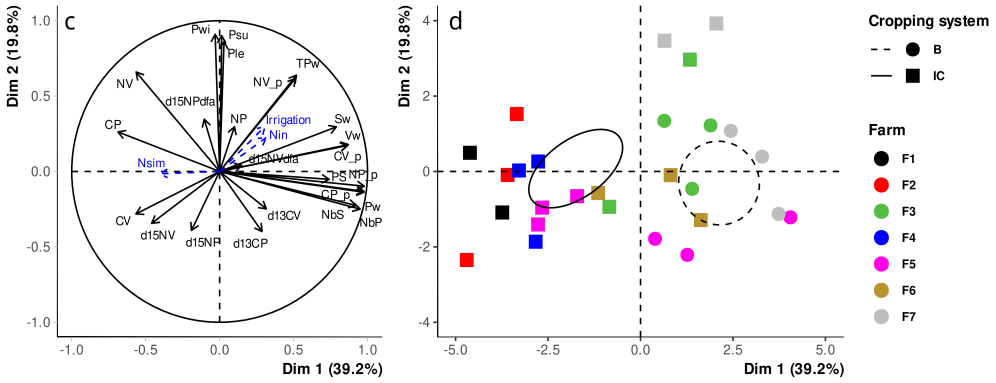

a
Csa
Fsa
Ni
Nin
Am
Irrigation
Nsim
CN
CEC
N
OM
CC
pH
Clay
Csi
Fsi
Dim2 (22.7%)
Dim1 (39.6%)
b
Cropping System
M
B
IC
Farm
F1
F2
F3
F4
F5
F6
F7

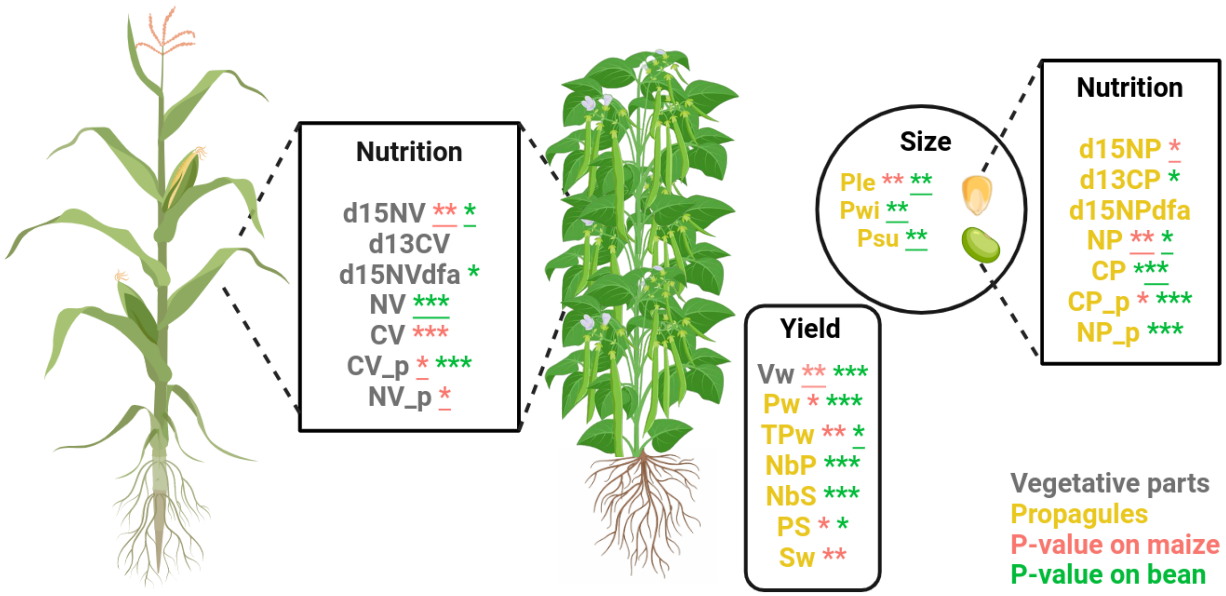

Dim2 (inertia: 19.67%)
R_B_SC
NR_B_SC
NR_M_SC
R_M_SC
R_B_IC
NR_MB_IC
R_M_IC
Dim1 (inertia: 21.1%)

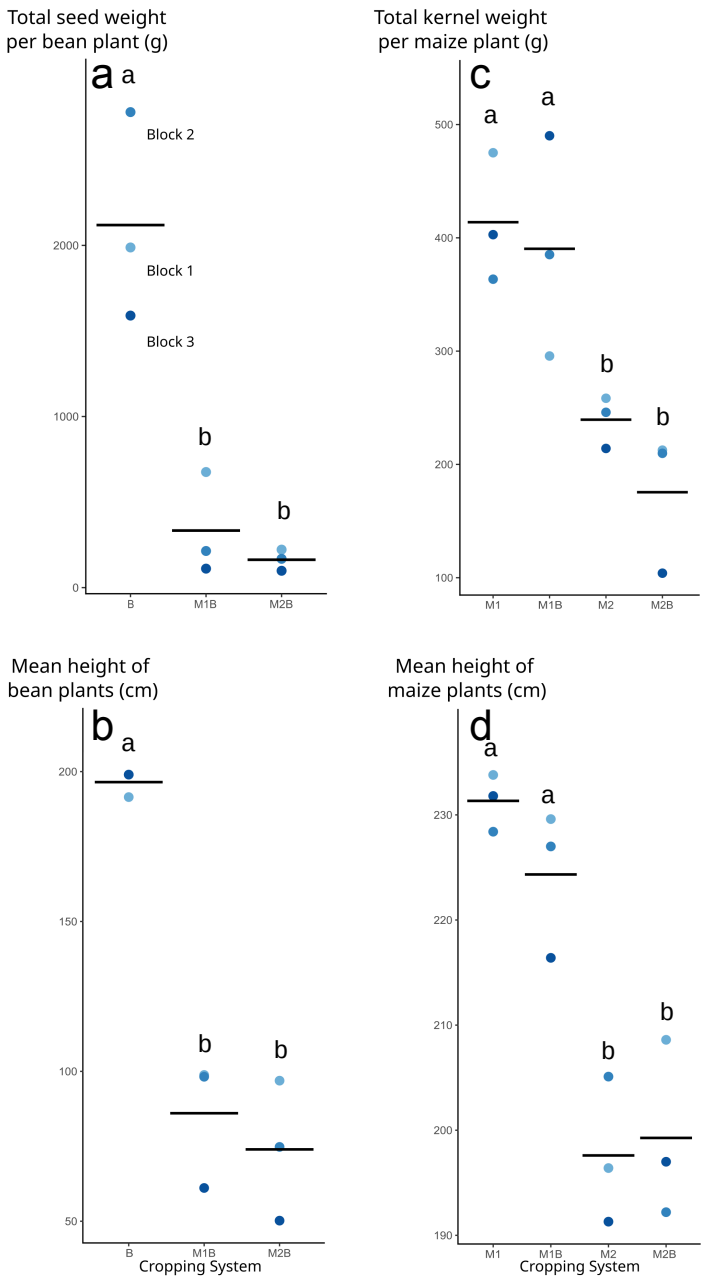
Maize


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

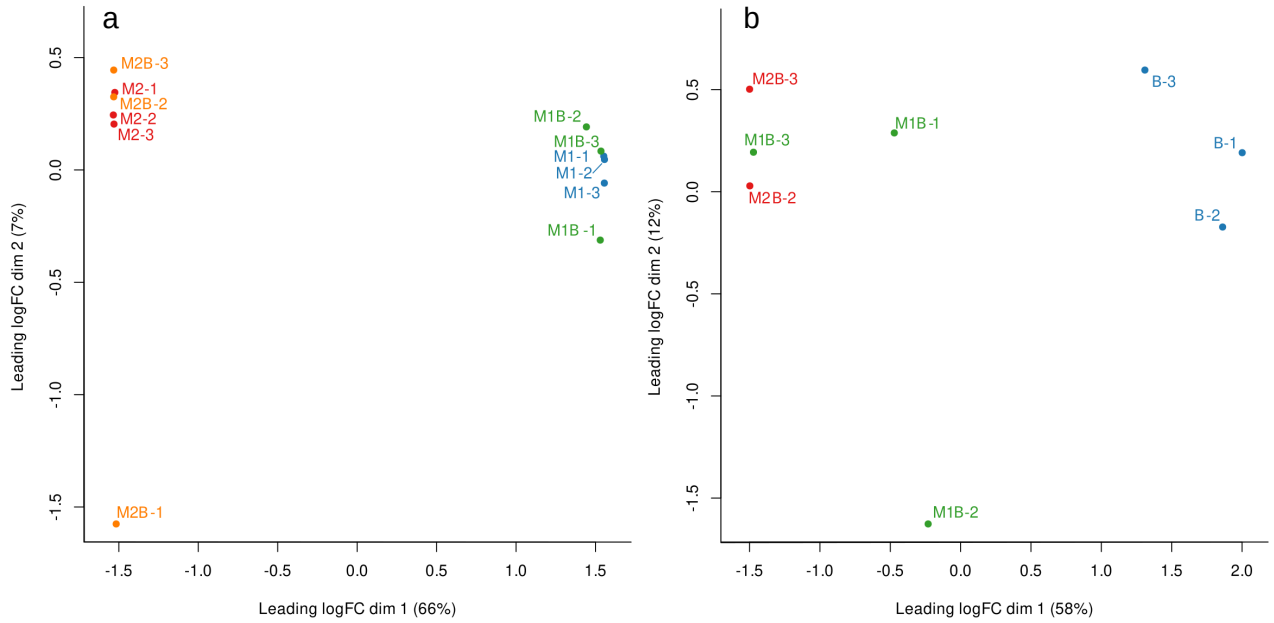

c
d
Dim 1 (39.2%)
Dim 2 (19.8%)
Pwi
Psu
Ple
TPw
NV
NV_p
d15NPdfa
CP
NP
Irrigation
Nin
Sw
Vw
Nsim
d15NVdfa
CV_p
PS
NP_p
CP_p
Pw
NbP
CV
d15NV
d15NP
d13CV
d13CP
NbS
Cropping system
B
IC
Farm
F1
F2
F3
F4
F5
F6
F7


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
P-value on maize
P-value on bean


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
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

e

f


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%)