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

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Keywords: traditional farming, cereal-legume association, agronomy, competition, plant nutrition, bacterial communities, RNAseq.

Abstract

Cereal-legume intercropping is emerging as a promising strategy for fostering more sustainable agroecosystems. The traditional farming system of maize-bean intercropping, originating from Central America, where both crops were domesticated, is experiencing a revival in modern agricultural settings. This is exemplified in southwestern France, where maize hybrids are intercropped with the recently commercialized Tarbais bean variety. Here, we combined on-farm investigations of agronomic practices, plant yield and nutrition measurements, characterization of soil and bacterial assemblages by metabarcoding, along with transcriptomic assays to compare the performance of sole cropped maize and beans to intercropped plants. Agronomic practices and soil-related variables differed among farms, with results suggesting that farmers adjust the former according to soil mineralization level. Despite this farm-specific effect, we detected a notable increase in bacterial diversity in intercropping, both in bulk and rhizosphere soil. Multivariate analyses unveiled a significant impact of cropping systems on agronomic traits in maize and beans, with frequent farm-by-cropping system interactions (11 of 22 traits in maize and 12 of 24 traits in bean). Competition dominated maize-bean intercropping, with 54 of 89 significant correlations between maize and bean traits being negative. Beans were notably impacted by this competition, yielding fewer seeds in intercropped conditions, albeit exhibiting higher seed size and weight, as well as nitrogen and carbon percentages. We set up a controlled field assay to quantify differentially expressed (DE) genes between cropping conditions for the two species. While competition between the two crops was much more severe than on-farm, we found that it primarily affected the bean and its transcriptome, with nearly 30% of DE genes vs none in maize. Overall, our findings suggest that synergies between the two crops are likely hindered, underscoring the importance of carefully considering partner varieties and agronomic practices in the revival of traditional agricultural systems.

1 Introduction

To deliver high-yielding crops, conventional agriculture relies mainly on simplified cropping systems, with a single crop species grown on a field in a given growing season (sole croppring) and environment standardization through the use of chemical inputs. With the current environmental challenges, there is a growing concern for more sustainable agroecosystems based on ecological regulations defined as nature-based solutions (Beillouin 2021; Fréville et al. 2022; Gaba et al. 2015; Keichinger et al. 2021; Li et al. 2023). Such remobilization can be implemented, for example, through mixed cropping (intercropping), where several crops are grown simultaneously with no distinct row arrangement. This approach can improve space, water, and nutrient use efficiency, 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), which have both been exploited in traditional intercropping systems.

Recent studies recognize the potential of intercropping as a more sustainable approach compared with sole cropping in modern agriculture (Beillouin 2021). 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. 2016). 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 leads to increased 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), maize-alfafa (Ma 2023), wheat-faba bean (Li et al. 2016), wheat-chickpea (Betencourt et al. 2012) 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 after wheat (Erenstein et al. 2022) and

common bean is the most widely used legume for direct human consumption (Uebersax et al. 2023) – 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 garbure soup and 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, different 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 potential synergies between the two crops. Due to the lack of research on this particular intercropping association we adopted a multidisciplinary approach that we defined as a convergence of agronomy, microbial ecology, and molecular signatures. We conducted surveys with farmers about their technical itineraries, performed agronomic and nutrition 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 field experiment 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: Firstly, how do inter- and sole-cropping differ in terms of agronomic practices, plant nutrition, yield and rhizosphere bacterial assemblages? Secondly, what abiotic factors influence plant nutrition, yield, and the interactions between maize and Tarbais beans in intercropping? Lastly, can we identify either positive or negative interactions between Tarbais bean and maize partners in intercropping, both at the phenotypic and transcriptomic levels?

2 Material and methods

2.1 On-farm analyses

2.1.1 Plant and soil sampling for agronomic analyses

We collected plant and soil samples in seven farms located in southwerstern 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.

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) of 4 to 5 (sole cropping) or 7 to 10 (intercropping) plants each. Seven fields corresponded to intercropping (21 plots), five to maize sole cropping (15 plots) and four to bean sole cropping (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 retrieved through semi-directed interviews of the 7 farmers.

2.1.2 Soil properties determination

We measured 13 variables on the samples of bulk soil (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 (Ciesielski et al. 1997; 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 nutrition measures on plants were performed by pooling plants of the same plot together. In addition to the number of plants per sample, we measured 21 traits (Table 1) on the 69 samples. We first separated vegetative (including maize cobs and bean pod shells) from reproductive (grains or seeds) parts for maize and beans, respectively. We determined dry weight of vegetative and reproductive parts. We counted ears/pods and propagules (grains/seeds), and measured propagule size. We further measured plant nutrition parameters related to nitrogen and carbon content or concentration. To do so, the vegetative and reproductive organ samples were ground 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 DW. The ^{15}N abundance was calculated as atom% (percentage of total N) and defined as Asample% = 100 × (^{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 nutrition 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 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 (6-9 plants of each species per field) and soil in contact with roots was taken from 2 to 3 different roots per plant. Therefore, soil from roughly 18 roots of each species was sampled per field (~1 g per composite sample) with a sterile scalpel. Likewise, bulk soil was taken from 2 to 3 root-free soil in each of the 3 plots (~1 g non-rhizosphere soil per composite sample).

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

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

We relied on differential analysis based on OTU count using negative binomial (a.k.a. Gamma-Poisson) distribution and Wald significance tests to identify discriminant OTUs (with FDR adjusted P-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 technical itineraries 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/).

2.1.6 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:

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

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:

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

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 (Amarger et al. 1977; 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.

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 agronomic conditions differed between cropping conditions.

2.2 Experimental assays of maize-bean intercropping in Saclay

2.2.1 Sampling and phenotyping

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). Bean and maize plants were sown at a density of 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 corresponding to the technical itineraries for Tarbais bean in southwestern France (residual nitrogen of 26 units after winter and application after sowing of 75 units 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.

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 inter-cropping. 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. Beans were sown after maize emergence 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.

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 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 × genotype) interaction. Just like for beans, DE genes and adjusted P-values were identified from the sole vs intercropping contrast.

3 Results

3.1 Farms exhibit contrasted soil properties and agronomic practices

Agronomic management in each farm was documented through interviews (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). This finding suggests that farmers adjust their practices according to soil mineralization level.

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 without irrigation in our sample, and F6 also practices low irrigation (none in sole cropped bean, the lowest level among the other farms irrigating in sole cropped maize, and 105 mm in intercropping (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 7 farms, 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 rhizosphere soils of maize (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).

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 and, nutrition and yield. 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 S11), revealing that maize displayed higher NP, *d15NP, d15NV* but lower PS in intercropping compared with sole cropping (Fig. 5).

3.4 Cropping system impacts bean agronomic traits

Nodules were absent from bean roots in all conditions (NS reported as 0; Table S4); therefore, the NS variable was not included in further analyses. Sole cropped (B) and intercropped (IC) bean were clearly separated on the first axis of the PCA (Fig. 4d). Many bean traits correlated together. Over 59% of the variation was explained by the two first PC axes (Fig. 4c), with PC1 relating to yield and nutrition traits (Pw, NbS, NbP, PS, CP_p, CV_p, NP_p, Vw, Table 1) while PC2 relates to propagule size (Pwi, Ple and Psu, Table 1). As compared to sole cropping, intercropped beans displayed higher values for propagule size (Ple, Pwi, Psu), TPw, NP, NV, and CP. Lower values were observed in intercropped beans for yield (Vw, Pw, NbP, NbS, PS), propagule (CP_p, NP_p) and vegatative (CV_p) nutrition. For other traits, the effect of the cropping systems varied among farms, as for *d15NV*, *d13CP*, *d15NVdfa*, and NV_p (Fig. S11; Table S11).

3.5 Correlations of agronomic traits between maize and bean intercropped plants

Correlations between maize and bean traits were computed to delineate the interplay between the two crops, positive correlations being expected under facilitation, while negative correlations being expected under competition. Out of 528 correlations (22 traits for maize and 24 traits for bean), 89 were significant among which 54 were negative and 35 were positive, indicating a prevailing trend of competitive rather than synergistic interactions. Notably, *d15NV*, *d15NP*, Vw and CV_p measured in maize were negatively correlated to Vw, Pw, TPw, NbP, Ple, Pwi, Psu, NbS, Sw, NP_p, CP_p and CV_p that were measured on bean indicating inter-species competition for carbon and nitrate leading to a decrease in yield in bean. On the other hand, NV measured on bean positively correlated with Vw, Pw, Tpw, NbP, SW, *d13CP*, NP_p, CP_P and CV_p measured on maize and, *d15NPdfa* and *d15NVdfa* were positively correlated to *d13CV* and *d13CP* (Fig. S12).

3.6 Experimental validation shows that species competition affects bean transcriptomes

To better assess the effects of maize-bean intercropping on plant development, phenology, and yield-related traits while controlling environmental effects – including soil, crop management practices and environmental conditions – we set up an experiment with three replicates (blocks) in Saclay (France). We employed equivalent plant density as the one observed in Tarbais for intercropping (5 plants per square meter). For sole cropping, in order to facilitate comparisons, we used half the number of plants per square meter for beans as observed in Tarbais but also for maize (cultivated at much higher densities in Tarbais - see Table S1). In addition, we adopted low-input conditions as recommended by the Tarbais bean cooperative, namely minimal nitrogen (<60 N units, we added no herbicides, no pesticides, nor did we apply any P and K inputs) and limited irrigation (manual irrigation once during the season). Note that we encountered a drought with only 94.5 mm of cumulated rainfall for the three first months of the growing season.

Agronomic measures revealed that six out of 13 yield and development traits exhibited higher values in sole cropped beans compared to intercropped beans: total seed mass per plant (reduced by 87% from sole cropping to intercropping), number of seeds per plant (reduced by 89%), number of pods per plant (reduced by 89%), width of the main leaflets (reduced by 19%), foliage density (reduced by 56%), and plant height (reduced by 59%; Fig. 6a, b and Fig. S13). Although the two maize varieties strongly differed for all yield and development traits, except for the number of ears per plant and the number of lodged plants, they were both unaffected by the cropping condition (Fig. 6c, d and Fig. S14).

We investigated transcriptomic changes in sole cropping versus intercropping stands. First, we tested differences in maize gene expression between genotypes M1 and M2. We found clear distinctions between the two genotypes on the first axis of the MDS plot, that accounted for 66% of the total variation; Fig. 7a). We further detected DE genes predicting that the number of differentially expressed (DE) genes would be higher in beans than in maize, since bean phenotypes were severely affected by the cropping condition as opposed to maize phenotypes. Following our prediction, we detected no DE genes between sole cropped and inter-cropped maize plants. In beans, we identified 11 DE genes between M1B and M2B and therefore considered them as biological replicates for the detection of DE genes. Out of 14,993 bean genes, nearly a third (5,070 genes) were DE between 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 (Maitra et al. 2021). 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.

Farmers interviews uncovered a surprising diversity in farming technical itineraries. First, the maize variety is not reasoned as a partner for the bean but rather as an opportunity to avoid the complex and costly use of artificial support for beans (nets). The variety of maize used depends on the one cultivated as a sole crop on the farm or in a nearby farm; it is not selected based on its compatibility with beans. It therefore varied across farms. Second, conventional farms (F4 excluded) applied different types and quantities of inputs varying from 18 to 220 N units per hectare and from 0 to 270 mm of water (Table S1). In addition to differences in technical itineraries, soil analyses revealed significant differences across farms (Table S9). Main differences were associated with soil fertility and pH rather than soil texture. 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). Conversely, farm F6 that used the highest quantity of nitrogen fertilizers and water in intercropping (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 nutrition via microbial partners. On one hand, bacterial communities are influenced by soil variables, including soil texture (Girvan et al. 2003), nitrogen content (Zeng 2016), phosphorus content (DeForest and Scott 2010), and soil pH (Lauber et al. 2008) and, locally, by the compounds released in the immediate vicinity of the plants via root exudates (Badri and Vivanco 2009; Ulbrich 2022). Here, we found that bacterial communities are primarily driven by agronomic practices and soil fertility, and to a lesser extent by rhizosphere status and cropping system (Fig. S5). Noteworthy, F4 which was the only organic farm in our survey displayed the highest soil fertility (Fig. 2). Those two farms also exhibited the lowest water irrigation and highest mean values of Nsim (158.5 and 107.7 for F4 and F6, respectively) across their plots. Depletion of soil water may affect the abundance and diversity of soil microorganisms (Li 2021), a process that can occur through the reduction of soil organic carbon (Bastida 2017).

Soil microorganisms are widely acknowledged to play a significant role in soil nitrogen transformation and mineralization (Paśmionka et al. 2021). Indeed, bacterial communities play an essential role in plant nutrition (Bai et al. 2022). For example, PGPR are known to modulate plant development and productivity (Das 2022), in part because they produce indoleacetic acid (Olanrewaju 2017), ammonia (Pérez-Montaño 2014), and other compounds of interest. The rhizosphere microbiota can also interfere with plant pathogens by producing antimicrobial (Rezzonico et al. 2007) or siderophores (Kloepper et al. 1980). 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 plant species association on bacterial composition remains understudied (Schlatter et al. 2015). To address this gap, we investigated the influence of cropping systems on bacterial assemblages. Overall, we observed a higher diversity in rhizosphere samples obtained from intercropping than from sole cropping (Fig. S7), a pattern observed in sugarcane–soybean intercropping (Malviya et al. 2021). One possible explanation is that the combination of root exudates released by both crops has the potential to generate a more diverse range of rhizosphere micro-environments, thereby enhancing 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 (Fig. S8). The *Acinetobacter* spp., which were predominant in sole cropped bean rhizosphere, comprise strains that can produce IAA, siderophores, gibberellin, antibiotics, biosurfactants/bioemulsifiers and solubilize phosphate, potassium, and zinc (Sachdev 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 2021).

Previous results have reported that in cereal-legume association, cereal roots develop faster and use soil resources including available nitrogen prior to legumes, thereby stimulating atmospheric nitrogen fixation and nodule formation by beans (Hauggaard-Nielsen et al. 2008; Hauggaard-Nielsen et al. 2009). We observed no root nodules on beans in any of the farms, despite the fact that Tarbais bean is capable of nodulation (Laguerre et al. 2006). There are two plausible albeit non-exclusive explanations for this observation: the non-restrictive nitrogen fertilization condition inhibited nodulation in beans, previous research has shown this possibility in beans (Hardarson 1993) and pea (Voisin et al. 2002), or the specific rhizobial strains necessary for nodulation of bean roots were not present in the soil (Yan et al. 2014). Altogether, these results suggest that years of conventional agronomic practices in these fields have reduced the potential benefit of plant-microbial interactions.

In order to assess the potential synergies between the two crops, we examined the impact of intercropping for both maize and beans on plant nutrition and development. On one hand, some traits were positively impacted by intercropping such as *d15NV* and NP that increased for both species, NV on bean and *d15NP* on maize, suggesting benefits of the intercropping for nitrogen assimilation (Fig. 5, Table S11). This

result was in line with previous results showing complementarity in N assimilation in maize-legume intercropping (Xing 2023; Berruti et al. 2016; Hupe et al. 2021), cereals-legume (Moyer-Henry et al. 2006). Surprisingly, N derived from symbiotic interactions in vegetative parts of intercropped beans was lower compared to sole cropped beans, suggesting that the higher N acquisition in the intercropped maize and beans was due to complementarity mechanisms rather than facilitation (Singh et al. 2023). On the other hand, the yield components were negatively impacted by intercropping, with Pw and PS lower for both species and NbP and NbS lower for bean, as compared to sole cropping situations. Interestingly, TPw and seed size were higher for intercropped than sole cropped beans. Hence, one might speculate that a potential outcome of maize competition, is a response of bean producing fewer seeds but with elevated levels of nutrients (NP, CP), alongside increased overall weight (TPw). This phenomenon may be influenced by nitrogen nutrition, which can affect biomass partitioning and nitrogen investment in vegetative structures through remobilization processes (Voisin et al. 2002). We computed pairwise correlations between all maize and bean traits. The majority of significant correlations were negative (56 out of 90), suggesting that competition between the crops outweighed synergies.

The primary strength, yet limitation, of this study lies in the direct sampling of plants from the fields. While this approach enables us to draw conclusions about current technical itineraries, it also introduces numerous confounding factors that are challenging to control (maize variety, field management, abiotic and biotic environmental factors). We therefore designed a randomized-block design field experiment in Saclay (northern France) to confirm our observations, and to provide a first glimpse on the characterization of the molecular signatures of maize-bean intercropping (Montazeaud et al. 2022). We used similar sowing density as practiced on-farm for intercropping and sole cropping of beans and applied low-input conditions. However, environmental conditions (soil, climate) differ between northern and southwestern France, and we also experienced drought during the year of the experimentation. Our results show that competition in intercropping was ultimately stronger than we observed on-farm. It is interesting to note that under increased competition, the burden falls on the bean and resulted into a strong decline in yield and vegetative development as compared to sole cropping beans. Notably, despite plant density in the intercropping set-up being twice that of sole cropping, bean yield was reduced on average by ~ 87% in the intercropping conditions. 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 5,070 DE genes in beans, which indicated a complete rewiring of bean gene expression when suffering drastic competition. Although the experiment was limited in terms of sample size and sampling design—only one stage, one organ—preventing a more detailed analysis of our results in terms of function enrichment or co-expression analyses, these findings open up very interesting avenues for research on the molecular mechanisms implemented in response to species intercropping.

5 Conclusion

We adopted a multidisciplinary approach to investigate the potential benefits of maize-bean intercropping in the context of the revival of traditional farming systems in modern agriculture settings in southwestern France. Our results highlight a diversity of technical itineraries lacking a consensus, yet predominantly reliant on high-input conventional agronomic practices. Additionally, we found that while bacteria are chiefly influenced by these practices and soil composition, intercropping increases microbial diversity, both in rhizosphere and non-rhizosphere soil compartments. Some bacterial OTUs may have a positive impact on plants such as PGPRs. Overall, intercropping exerts a detrimental effect on development- and yield-related traits for both species, attributable to interspecies competition. This competition disproportionately affects bean, despite its higher commercial value. These findings underscore the necessity of reevaluating both the crop varieties and associated agronomic practices to fully harness the potential synergies between species in sustainable agro-ecosystems.

DECLARATIONS

- **Acknowledgements**

We are extremely grateful to Hélène Corti, a retired technician who helped us collecting the data. In addition, we would like to thank Marie-Hélène Jeuffroy for insightful discussion at the start of the project. This work would have not been possible without the contribution of farmers and the Coopérative du Haricot Tarbais.

- **Funding**

This work was supported by the French National Research Agency (ANR) as part of the Investissements d'Avenir Programme (LabEx BASC; ANR-11-LABX-0034) and the Biosphera Graduate School of the Université Paris-Saclay (BSE-2022-03). GQE-Le Moulon and POPS platform both benefit from the support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007). GQE-Le Moulon also benefits from the support of the Institut Diversité, Ecologie et Evolution du Vivant (IDEEV). N.V-B was financed for three months by the European Union's Horizon 2020 research and innovation programme (grant agreement No. 862862) and, subsequently, by a doctoral contract from SPS through the Doctoral school "Sciences du Végétal ED567".

- **Disclaimer**

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- **Conflicts of interest/Competing interests**

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

- **Consent to participate**

Farmers verbal informed consents were obtained prior to the interviews and data collection. We anonymized the farmers by providing approximate locations for farms, and only report technical itineraries from the interviews avoiding any personal information.

- **Availability of data and material**

Raw reads from metabarcoding data have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number XXX. Raw reads from QuantSeq 3'RNA-seq have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number XXX. 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.

- **Code availability**

Codes for RNAseq and phenotypic data analyses are available at recherche.data.gouv.fr with the DOI https://doi.org/10.57745/BZMPT9.

- **Authors' contributions**

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

REFERENCES

Amarger, N., Mariotti, A., & Mariotti, F. (1977). Essai d'estimation du taux d'azote fixé symbiotiquement chez le lupin par le traçage isotopique naturel (^{15}N). Comptes Rendus de l'Académie des Sciences, Série D, 284, 2179-2182.

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. Plant, Cell & Environment, 32(6), 666-681. https://doi.org/10.1111/j.1365-3040.2009.01926.x

Bai, B., Liu, W., Qiu, X., Zhang, J., Zhang, J., & Bai, Y. (2022). The root microbiome : Community assembly and its contributions to plant fitness. Journal of Integrative Plant Biology, 64(2), 230-243. https://doi.org/10.1111/jipb.13226

Bastida, F., Torres, I. F., Hernández, T., & García, C. (2017). The impacts of organic amendments : Do they confer stability against drought on the soil microbial community? Soil Biology & Biochemistry, 113, 173-183. https://doi.org/10.1016/j.soilbio.2017.06.012

Bedoussac, L., Journet, E.-P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E. S., Prieur, L., & Justes, E. (2015). Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agronomy for Sustainable Development, 35(3), 911-935. https://doi.org/10.1007/s13593-014-0277-7

Beillouin, D., Ben-Ari, T., Malézieux, E., Seufert, V., & Makowski, D. (2021). Positive but variable effects of crop diversification on biodiversity and ecosystem services. Global Change Biology, 27(19), 4697-4710. https://doi.org/10.1111/gcb.15747

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate : A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57(1), 289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Betencourt, E., Duputel, M., Colomb, B., Desclaux, D., & Hinsinger, P. (2012). Intercropping promotes the ability of durum wheat and chickpea to increase rhizosphere phosphorus availability in a low P soil. Soil Biology & Biochemistry, 46, 181-190. https://doi.org/10.1016/j.soilbio.2011.11.015

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., Santilocchi, R., Zeuli, P. S., Gioia, T., Logozzo, G., Attene, G., Nanni, L., & Papa, R. (2013). Molecular analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. New Phytologist, 197(1), 300-313. https://doi.org/10.1111/j.1469-8137.2012.04377.x

Bonnain-Dulon, R., & Brochot, A. (2004). De l'authenticité des produits alimentaires. Ruralia, 14, 969. http://journals.openedition.org/ruralia/969

Bouffaud, M.-L., Renoud, S., Moënne-Loccoz, Y., & Muller, D. (2016). Is plant evolutionary history impacting recruitment of diazotrophs and *nifH* expression in the rhizosphere? Scientific Reports, 6(1), 21690. https://doi.org/10.1038/srep21690

Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., McKenzie, B. M., Pakeman, R. J., Paterson, E., Schob, C., Shen, J. B., Squire, G., Watson, C. A., Zhang, C. C., … White, P. J. (2015). Improving intercropping : A synthesis of research in agronomy, plant physiology and ecology. New Phytologist, 206(1), 107-117. https://doi.org/10.1111/nph.13132

Ciesielski, H., Sterckeman, T., Santerne, M., & Willery, J. P. (1997). A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. Agronomie, 17(1), 9-16. https://doi.org/10.1051/agro:19970102

Clivot, H., Mouny, J.-C., Duparque, A., Dinh, J.-L., Denoroy, P., Houot, S., Vertès, F., Trochard, R., Bouthier, A., Sagot, S., & Mary, B. (2019). Modeling soil organic carbon evolution in long-term arable experiments with AMG model. Environmental Modelling & Software, 118, 99-113. https://doi.org/10.1016/j.envsoft.2019.04.004

Das, P. P., Singh, K. R., Nagpure, G., Mansoori, A., Singh, R. P., Ghazi, I. A., Kumar, A., & Singh, J. (2022). Plant-soil-microbes : A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. Environmental Research, 214, 113821. https://doi.org/10.1016/j.envres.2022.113821

de Albuquerque, T. M., Mendes, L. W., Rocha, S. M. B., Antunes, J. E. L., de Sousa Oliveira, L. M., Melo, V. M. M., Oliveira, F. A. S., de Araujo Pereira, A. P., da Silva, V. B., Gomes, R. L. F., de Alcantara Neto, F., de Almeida Lopes, A. C., de Moura Rocha, M., & Araujo, A. S. F. (2022). Genetically related genotypes of cowpea present similar bacterial community in the rhizosphere. Scientific Reports, 12(1), 3472. https://doi.org/10.1038/s41598-022-06860-x

DeForest, J. L., & Scott, L. G. (2010). Available organic soil phosphorus has an important influence on microbial community composition. Soil Science Society of America Journal, 74(6), 2059-2066. https://doi.org/10.2136/sssaj2009.0426

Di Tommaso, P., Chatzou, M., Floden, E. W., Barja, P. P., Palumbo, E., & Notredame, C. (2017). Nextflow enables reproducible computational workflows. Nature Biotechnology, 35(4), 316-319. https://doi.org/10.1038/nbt.3820

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR : Ultrafast universal RNA-seq aligner. Bioinformatics (Oxford, England), 29(1), 15-21. https://doi.org/10.1093/bioinformatics/bts635

Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., & Prasanna, B. M. (2022). Global maize production, consumption and trade : Trends and R&D implications. Food Security, 14(5), 1295-1319. https://doi.org/10.1007/s12571-022-01288-7

Ewels, P. A., Pelzer, A., Fillinger, S., Patel, H., Alneberg, J., Wilm, A., Garcia, M. U., Di Tommaso, P., & Nahsen, S. (2020). The nf-core framework for community-curated bioinformatics pipelines. Nature Biotechnology, 38(3), 271-271. https://doi.org/10.1038/s41587-020-0435-1

Farid, M., & Navabi, A. (2015). N_2 fixation ability of different dry bean genotypes. Canadian Journal of Plant Science, 95(6), 1243-1257. https://doi.org/10.4141/cjps-2015-084

Fréville, H., Montazeaud, G., Forst, E., David, J., Papa, R., & Tenaillon, M. I. (2022). Shift in beneficial interactions during crop evolution. Evolutionary Applications, 15(6), 905-918. https://doi.org/10.1111/eva.13390

Gaba, S., Lescourret, F., Boudsocq, S., Enjalbert, J., Hinsinger, P., Journet, E. P., Navas, M. L., Wery, J., Louarn, G., Malezieux, E., Pelzer, E., Prudent, M., & Ozier-Lafontaine, H. (2015). Multiple cropping systems as drivers for providing multiple ecosystem services : From concepts to design. Agronomy for Sustainable Development, 35(2), 607-623. https://doi.org/10.1007/s13593-014-0272-z

Girvan, M. S., Bullimore, J., Pretty, J. N., Osborn, A. M., & Ball, A. S. (2003). Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied and Environmental Microbiology, 69(3), 1800-1809. https://doi.org/10.1128/AEM.69.3.1800-1809.2003

Hardarson, G. (1993). Methods for enhancing symbiotic nitrogen fixation. Plant and Soil, 152(1), 1-17. https://doi.org/10.1007/BF00016329

Hauggaard-Nielsen, H., Jørnsgaard, B., Kinane, J., & Jensen, E. S. (2008). Grain legume–cereal intercropping : The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renewable Agriculture and Food Systems, 23(1), 3-12. https://doi.org/10.1017/S1742170507002025

Hauggaard-Nielsen, H., Mundus, S., & Jensen, E. S. (2009). Nitrogen dynamics following grain legumes and subsequent catch crops and the effects on succeeding cereal crops. Nutrient Cycling in Agroecosystems, 84(3), 281-291. https://doi.org/10.1007/s10705-008-9242-7

Hupe, A., Naether, F., Haase, T., Bruns, C., Heß, J., Dyckmans, J., Joergensen, R. G., & Wichern, F. (2021). Evidence of considerable C and N transfer from peas to cereals via direct root contact but not via mycorrhiza. Scientific Reports, 11(1), 11424. https://doi.org/10.1038/s41598-021-90436-8

Johansen, A., & Jensen, E. S. (1996). Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. Soil Biology & Biochemistry, 28(1), 73-81. https://doi.org/10.1016/0038-0717(95)00117-4

Keichinger, O., Viguier, L., Corre-Hellou, G., Messéan, A., Angevin, F., & Bockstaller, C. (2021). Un indicateur évaluant la diversité globale des rotations : De la diversité des cultures aux services écosystémiques. Agronomie, Environnement & Sociétés, 11(1), 183-201. https://doi.org/10.54800/dgr543

Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature, 286(5776), 885-886. https://doi.org/10.1038/286885a0

Lamy, I., Revellin, C., Breuil, M. C., & Chaussod, R. (2006). Response of rhizobial populations to moderate copper stress applied to an agricultural soil. Microbial Ecology, 52, 426-435. https://doi.org/10.1007/s00248-006-9081-5

Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology & Biochemistry, 40(9), 2407-2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Li, B., Li, Y.-Y., Wu, H.-M., Zhang, F.-F., Li, C.-J., Li, X.-X., Lambers, H., & Li, L. (2016). Root exudates drive interspecific facilitation by enhancing nodulation and N_2 fixation. Proceedings of the National Academy of Sciences of the United States of America, 113(23), 6496-6501. https://doi.org/10.1073/pnas.1523580113

Li, C., Dong, Y., Li, H., Shen, J., & Zhang, F. (2016). Shift from complementarity to facilitation on P uptake by intercropped wheat neighboring with faba bean when available soil P is depleted. Scientific Reports, 66(1), 18663. https://doi.org/10.1038/srep18663

Li, C., Stomph, T.-J., Makowski, D., Li, H., Zhang, C., & Zhang, F. (2023). The productive performance of intercropping. Proceedings of the National Academy of Sciences of the United States of America, 120(2), e2201886120. https://doi.org/10.1073/pnas.2201886120

Li, H. (2021). Irrigation has a higher impact on soil bacterial abundance, diversity and composition than nitrogen fertilization. Scientific Reports, 11(1), 16901. https://doi.org/10.1038/s41598-021-96234-6

Li, H., Shen, J., Zhang, F., Clairotte, M., Drevon, J.-J., Le Cadre, E., & Hinsinger, P. (2008). Dynamics of phosphorus fractions in the rhizosphere of common bean (*Phaseolus vulgaris* L.) and durum wheat (*Triticum turgidum durum* L.) grown in monocropping and intercropping systems. Plant and Soil, 312(1), 139-150. https://doi.org/10.1007/s11104-007-9512-1

Li, L., Li, S. M., Sun, J. H., Zhou, L. L., Bao, X. G., Zhang, H. G., & Zhang, F. S. (2007). Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proceedings of the National Academy of Sciences of the United States of America, 104(27), 11192-11196. https://doi.org/10.1073/pnas.0704591104

Li, S. M., Li, L., Zhang, F. S., & Tang, C. (2004). Acid phosphatase role in chickpea/maize intercropping. Annals of Botany, 94(2), 297-303. https://doi.org/10.1093/aob/mch140

Lun, A. T. L., Chen, Y., & Smyth, G. K. (2016). It's DE-licious : a recipe for differential expression analyses of RNA-seq experiments using quasi-likelihood methods in edgeR. In E. Mathé & S. Davis (Éds.), Statistical Genomics (Vol. 1418, p. 391-416). Springer New York. https://doi.org/10.1007/978-1-4939-3578-9_19

Ma, H. (2023). Maize/alfalfa intercropping enhances yield and phosphorus acquisition. Field Crops Research, 303, 109136. https://doi.org/10.1016/j.fcr.2023.109136

Maitra, S., Hossain, A., Brestic, M., Skalicky, M., Ondrisik, P., Gitari, H., Brahmachari, K., Shankar, T., Bhadra, P., Palai, J. B., Jena, J., Bhattacharya, U., Duvvada, S. K., Lalichetti, S., & Sairam, M. (2021). Intercropping—A low input agricultural strategy for food and environmental security. Agronomy, 11(2), 343. https://doi.org/ 10.3390/agronomy11020343

Malviya, M. K., Solanki, M. K., Li, C.-N., Wang, Z., Zeng, Y., Verma, K. K., Singh, R. K., Singh, P., Huang, H.-R., Yang, L.-T., Song, X.-P., & Li, Y.-R. (2021). Sugarcane-legume intercropping can enrich the soil microbiome and plant growth. Frontiers in Sustainable Food Systems, 5, 606595. https://doi.org/10.3389/fsufs.2021.606595

Marmagne, A., Masclaux-Daubresse, C., & Chardon, F. (2022). Modulation of plant nitrogen remobilization and postflowering nitrogen uptake under environmental stresses. Journal of Plant Physiology, 277, 153781. https://doi.org/10.1016/j.jplph.2022.153781

Matsuoka, Y., Mitchell, S. E., Kresovich, S., Goodman, M., & Doebley, J. (2002). Microsatellites in *Zea* – variability, patterns of mutations, and use for evolutionary studies. Theoretical and Applied Genetics, 104(2), 436-450. https://doi.org/10.1007/s001220100694

Mazzola, M. (2004). Assessment and management of soil microbial community structure for disease suppression. Annual Review of Phytopathology, 42, 35-59. https://doi.org/10.1146/annurev.phyto.42.040803.140408

McMurdie, P. J., & Holmes, S. (2013). phyloseq : an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE, 8(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mead, R., & Willey, R. W. (1980). The concept of a 'land equivalent ratio' and advantages in yields from intercropping. Experimental Agriculture, 16(3), 217-228. https://doi.org/10.1017/S0014479700010978

Meng, L., Zhang, A., Wang, F., Han, X., Wang, D., & Li, S. (2015). Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Frontiers in Plant Science, 6, 135939. https://doi.org/10.3389/fpls.2015.00339

Metson, A. J. (1957). Methods of chemical analysis for soil survey samples. Soil Science, 83(3), 245. https://doi.org/10.7931.dl1-sbb-012

Montazeaud, G., Flutre, T., Ballini, E., Morel, J.-B., David, J., Girodolle, J., Rocher, A., Ducasse, A., Violle, C., Fort, F., & Fréville, H. (2022). From cultivar mixtures to allelic mixtures : Opposite effects of allelic richness between genotypes and genotype richness in wheat. New Phytologist, 233(6), 2573-2584. https://doi.org/10.1111/nph.17915

Moyer-Henry, K. A., Burton, J. W., Israel, D. W., & Rufty, T. W. (2006). Nitrogen transfer between plants : A ^{15}N natural abundance study with crop and weed species. Plant and Soil, 282(1), 7-20. https://doi.org/10.1007/s11104-005-3081-y

Mugi-Ngenga, E., Bastiaans, L., Zingore, S., Anten, N. P. R., & Giller, K. E. (2022). The role of nitrogen fixation and crop N dynamics on performance and legacy effects of maize-grain legumes intercrops on smallholder farms in Tanzania. European Journal of Agronomy, 141, 126617. https://doi.org/10.1016/j.eja.2022.126617

Nassary, E. K., Baijukya, F., & Ndakidemi, P. A. (2020). Productivity of intercropping with maize and common bean over five cropping seasons on smallholder farms of Tanzania. European Journal of Agronomy, 113, 125964. https://doi.org/10.1016/j.eja.2019.125964

Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology, 33(11), 197. https://doi.org/10.1007/s11274-017-2364-9

Parnaudeau, V. V., Reau, R. R., & Dubrulle, P. P. (2012). Un outil d'évaluation des fuites d'azote vers l'environnement à l'échelle du système de culture : Le logiciel Syst'N. Innovations Agronomiques, 21, 59-70. http://www7.inra.fr/ciag/revue/volume_21_septembre_2012

Paśmionka, I. B., Bulski, K., & Boligłowa, E. (2021). The participation of microbiota in the transformation of nitrogen compounds in the soil—A review. Agronomy, 11(5), 977. https://doi.org/10.3390/agronomy11050977

Patel, Y., Zhu, C., Yamaguchi, T. N., Bugh, Y. Z., Tian, M., Holmes, A., Fitz-Gibbon, S. T., & Boutros, P. C. (2024). NFTest : Automated testing of Nextflow pipelines. Bioinformatics, 40(2), btae081. https://doi.org/10.1093/bioinformatics/btae081

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods, 14(4), 417-419. https://doi.org/10.1038/nmeth.4197

Peña-Cabriales, J. J., & Castellanos, J. Z. (1993). Effects of water stress on N_2 fixation and grain yield of *Phaseolus vulgaris* L. Plant and Soil, 152(1), 151-155. https://doi.org/10.1007/BF00016345

Peoples, M. B., Boddey, R. M., & Herridge, D. F. (2002). Quantification of Nitrogen Fixation (Chapter 13). In G. J. Leigh (Éd.), Nitrogen Fixation at the Millennium (p. 357-389). Elsevier Science. https://doi.org/10.1016/B978-044450965-9/50013-6

Pérez-Montaño, F., Alías-Villegas, C., Bellogín, R. A., del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., López-Baena, F. J., Ollero, F. J., & Cubo, T. (2014). Plant growth promotion in cereal and leguminous agricultural important plants : From microorganism capacities to crop production. Microbiological Research, 169(5), 325-336. https://doi.org/10.1016/j.micres.2013.09.011

Poggio, S. L. (2005). Structure of weed communities occurring in monoculture and intercropping of field pea and barley. Agriculture, Ecosystems & Environment, 109(1), 48-58. https://doi.org/10.1016/j.agee.2005.02.019

Rezzonico, F., Zala, M., Keel, C., Duffy, B., Moënne-Loccoz, Y., & Défago, G. (2007). Is the ability of biocontrol fluorescent pseudomonads to produce the antifungal metabolite 2,4-diacetylphloroglucinol really synonymous with higher plant protection? New Phytologist, 173(4), 861-872. https://doi.org/10.1111/j.1469-8137.2006.01955.x

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research, 43(7), e47. https://doi.org/10.1093/nar/gkv007

Robinson, M. D., & Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biology, 11(3), R25. https://doi.org/10.1186/gb-2010-11-3-r25

Rodriguez, C., Carlsson, G., Englund, J.-E., Flöhr, A., Pelzer, E., Jeuffroy, M.-H., Makowski, D., & Jensen, E. S. (2020). Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. European Journal of Agronomy, 118, 126077. https://doi.org/10.1016/j.eja.2020.126077

Rokhbakhsh-Zamin, F., Sachdev, D., Kazemi-Pour, N., Engineer, A., Pardesi, K. R., Zinjarde, S. S., Dhakephalkar, P. K., & Chopade, B. A. (2011). Characterization of plant-growth-promoting traits of *Acinetobacter* species isolated from rhizosphere of *Pennisetum glaucum*. Journal of Microbiology and Biotechnology, 21(6), 556-566. https://doi.org/10.4014/jmb.1012.12006

Schlatter, D. C., Bakker, M. G., Bradeen, J. M., & Kinkel, L. L. (2015). Plant community richness and microbial interactions structure bacterial communities in soil. Ecology, 96(1), 134-142. https://doi.org/10.1890/13-1648.1

Shearer, G., & Kohl, D. H. (1988). Natural ^{15}N abundance as a method of estimating the contribution of biologically fixed nitrogen to N_2-fixing systems : Potential for non-legumes. Plant and Soil, 110(2), 317-327. https://doi.org/10.1007/BF02226812

Shearer, G., Kohl, D. H., & Harper, J. E. (1980). Distribution of ^{15}N among plant parts of nodulating and nonnodulating isolines of soybeans. Plant Physiology, 66(1), 57-60. https://doi.org/10.1104/pp.66.1.57

Singh, A., Schöb, C., & Iannetta, P. P. M. (2023). Nitrogen fixation by common beans in crop mixtures is influenced by growth rate of associated species. BMC Plant Biology, 23(1), 253. https://doi.org/10.1186/s12870-023-04204-z

Smith, T., Heger, A., & Sudbery, I. (2017). UMI-tools : Modeling sequencing errors in Unique Molecular Identifiers to improve quantification accuracy. Genome Research, 27(3), 491-499. https://doi.org/10.1101/gr.209601.116

Uebersax, M. A., Cichy, K. A., Gomez, F. E., Porch, T. G., Heitholt, J., Osorno, J. M., Kamfwa, K., Snapp, S. S., & Bales, S. (2023). Dry beans (*Phaseolus vulgaris* L.) as a vital component of sustainable agriculture and food security—A review. Legume Science, 5(1), e155. https://doi.org/10.1002/leg3.155

Ulbrich, T. C., Rivas-Ubach, A., Tiemann, L. K., Friesen, M. L., & Evans, S. E. (2022). Plant root exudates and rhizosphere bacterial communities shift with neighbor context. Soil Biology & Biochemistry, 172, 108753. https://doi.org/10.1016/j.soilbio.2022.108753

Voisin, A.-S., Salon, C., Munier-Jolain, N. G., & Ney, B. (2002). Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). Plant and Soil, 242(2), 251-262. https://doi.org/10.1023/A:1016214223900

Xing, Y., Yu, R.-P., An, R., Yang, N., Wu, J.-P., Ma, H.-Y., Zhang, J.-D., Bao, X.-G., Lambers, H., & Li, L. (2023). Two pathways drive enhanced nitrogen acquisition via a complementarity effect in long-term intercropping. Field Crops Research, 293, 108854. https://doi.org/10.1016/j.fcr.2023.108854

Yan, J., Han, X. Z., Ji, Z. J., Li, Y., Wang, E. T., Xie, Z. H., & Chen, W. F. (2014). Abundance and diversity of soybean-nodulating rhizobia in black soil are impacted by land use and crop management. Applied and Environmental Microbiology, 80(17), 5394-5402. https://doi.org/10.1128/AEM.01135-14

Yin, C., Casa Vargas, J. M., Schlatter, D. C., Hagerty, C. H., Hulbert, S. H., & Paulitz, T. C. (2021). Rhizosphere community selection reveals bacteria associated with reduced root disease. Microbiome, 9(1), 86. https://doi.org/10.1186/s40168-020-00997-5

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., & Chu, H. (2016). Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biology & Biochemistry, 92, 41-49. https://doi.org/10.1016/j.soilbio.2015.09.018

Zhang, C., Postma, J. A., York, L. M., & Lynch, J. P. (2014). Root foraging elicits niche complementarity-dependent yield advantage in the ancient 'three sisters' (maize/bean/squash) polyculture. Annals of Botany, 114(8), 1719-1733. https://doi.org/10.1093/aob/mcu191

Zhang, D., Zhang, C., Tang, X., Li, H., Zhang, F., Rengel, Z., Whalley, W. R., Davies, W. J., & Shen, J. (2016). Increased soil phosphorus availability induced by faba bean root exudation stimulates root growth and phosphorus uptake in neighbouring maize. New Phytologist, 209(2), 823-831. https://doi.org/10.1111/nph.13613

Zhang, S., Lehmann, A., Zheng, W., You, Z., & Rillig, M. C. (2019). Arbuscular mycorrhizal fungi increase grain yields : A meta-analysis. New Phytologist, 222(1), 543-555. https://doi.org/10.1111/nph.15570

Zizumbo-Villarreal, D., & Colunga-GarcíaMarín, P. (2010). Origin of agriculture and plant domestication in West Mesoamerica. Genetic Resources and Crop Evolution, 57(6), 813-825. https://doi.org/10.1007/s10722-009-9521-4

TABLES

Table 1: Description of phenotypes and nutrition 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
NS[a]	Nodulation score, 0 = no nodule

FIGURES

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

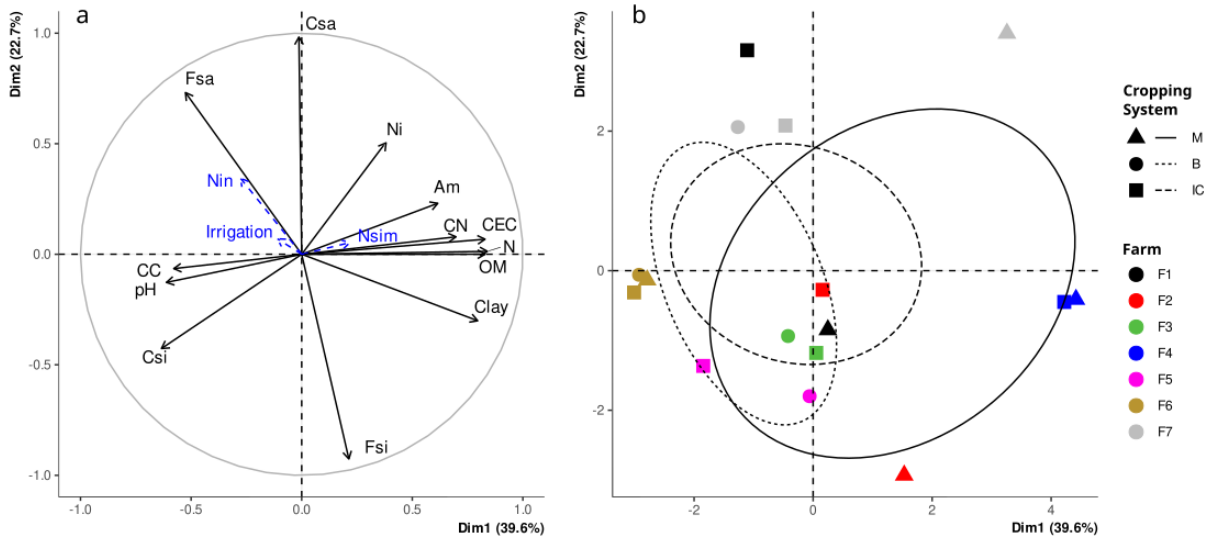

Fig. 2 Principal Component Analysis (PCA) computed on 13 soil variables per field. The two first axes are shown. A: Correlation circle of the 13 soil variables with three supplementary variables added to the PCA (in blue): Nsim which estimates the soil nitrogen mineralization, the Irrigation and nitrogen 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 Csa: Coarse sands – 200-2000 µm (g.kg-1 soil), Fsa: Fine sands – 50-200 µm (g.kg-1 soil), CC: Calcium Carbonate (CaCO3, g.kg-1 soil), pH, Csi: Coarse silts – 20-50 µm (g.kg-1 soil), Fsi: Fine silts – 2-20 µm (g.kg-1 soil), Clay: Clay – <2 µm (g.kg-1 soil), OM: Organic Matter, N: Total N (g.kg-1 soil), CEC: Cation Exchange Capacity (Metson, cmol+.kg-1), CN: C:N ratio, Am: 0.5M KCl-extractable NH4+ N content (mg.kg-1 soil), Ni: 0.5M KCl-extractable NO3- N content (mg.kg-1 soil).

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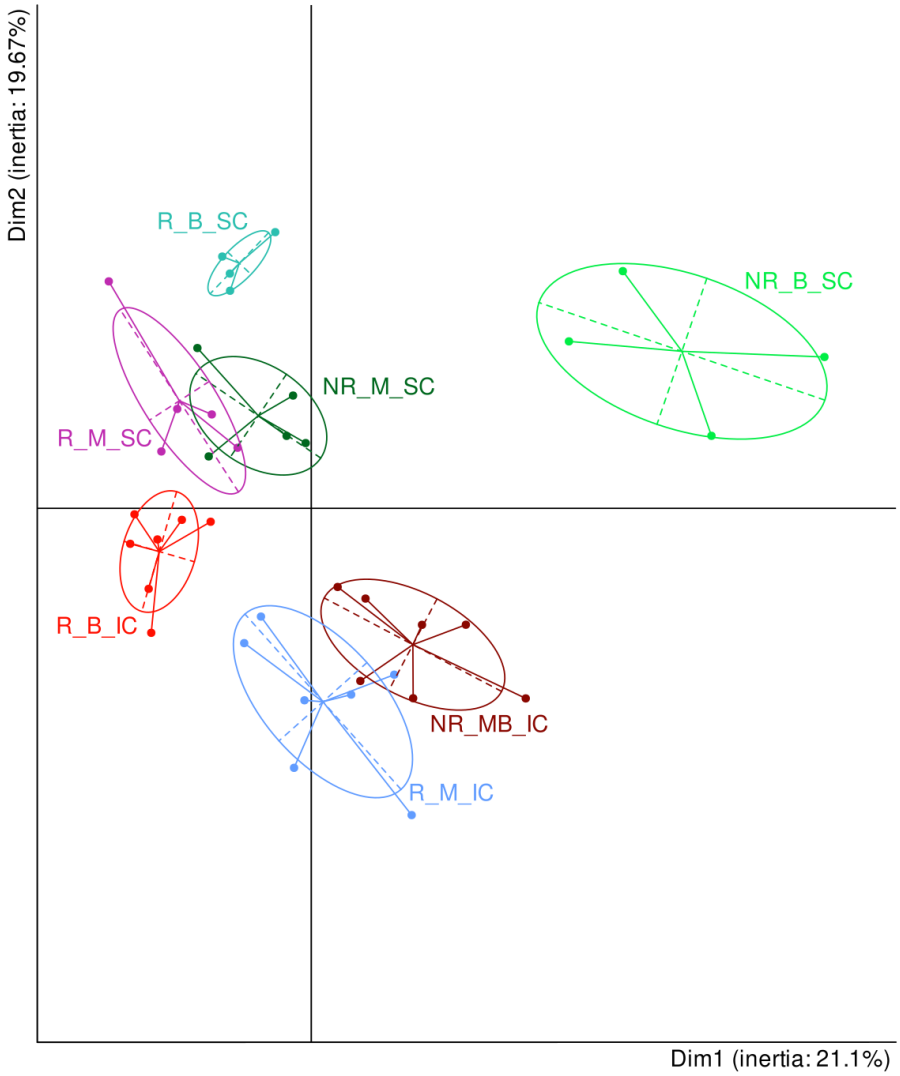

Fig. 3 Between Class Analysis (BCA) computed on 11,586 OTUs. Samples are named according to rhizosphere (R) or non-rhizosphere (NR) soil_maize (M) or bean (B)_ sole cropping (SC) or intercropping (IC). Projection on the two fisrt dimensions are presented with corresponding percentages of total inertia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

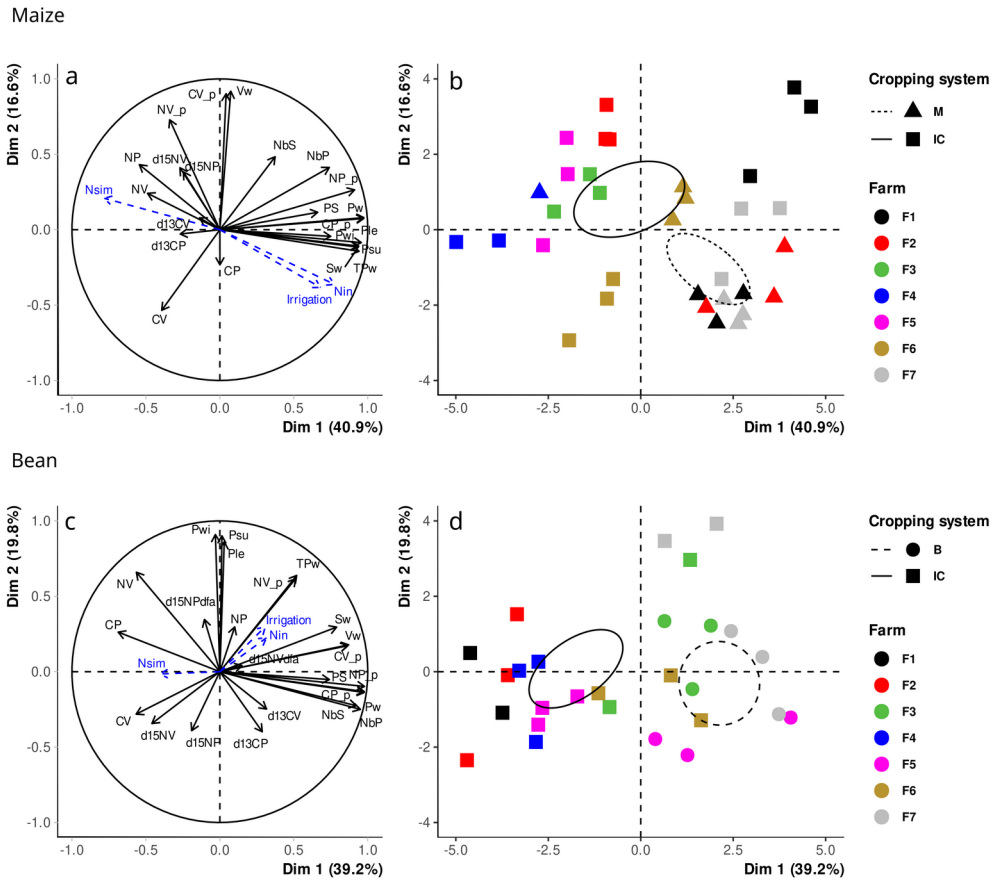

Fig. 4 Principal Component Analysis (PCA) on maize and bean phenotypic and nutrition variables. The two first PCs computed from 22 maize variables are shown with corresponding correlation circle (a) and data projection (b). Similarily, for beans, the two first PCs computed on 24 variables are shown with 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). Variable names are 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: Average number of Propagules per Set – ear or pod, Sw: Average propagule weight per Set – ear or pod (g), TPw: Thousand Propagules dry weight (g), d15NVdfa: N in the vegetative parts derived from symbiotic fixation, d15NPdfa: N in the propagules derived from symbiotic fixation.

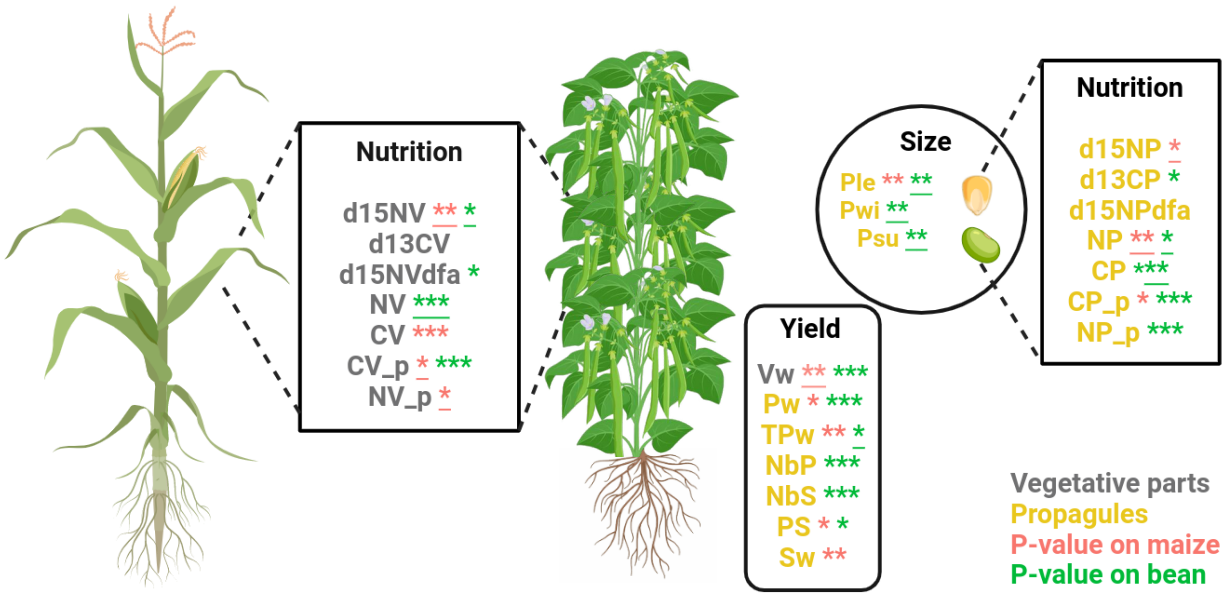

Fig. 5 Effect of the cropping system on maize and bean phenotypic and nutrition variables. A two-way ANOVA model with interaction was used to test the effects of the Farm and the cropping system (sole cropping versus intercropping) on phenotypic and nutrition variables (Table 1). Significant P-values 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. Variable names are 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: Average number of Propagules per Set – ear or pod, Sw: Average propagule weight per Set – ear or pod (g), TPw: Thousand Propagules dry weight (g), d15NVdfa: N in the vegetative parts derived from symbiotic fixation, d15NPdfa: N in the propagules derived from symbiotic fixation.

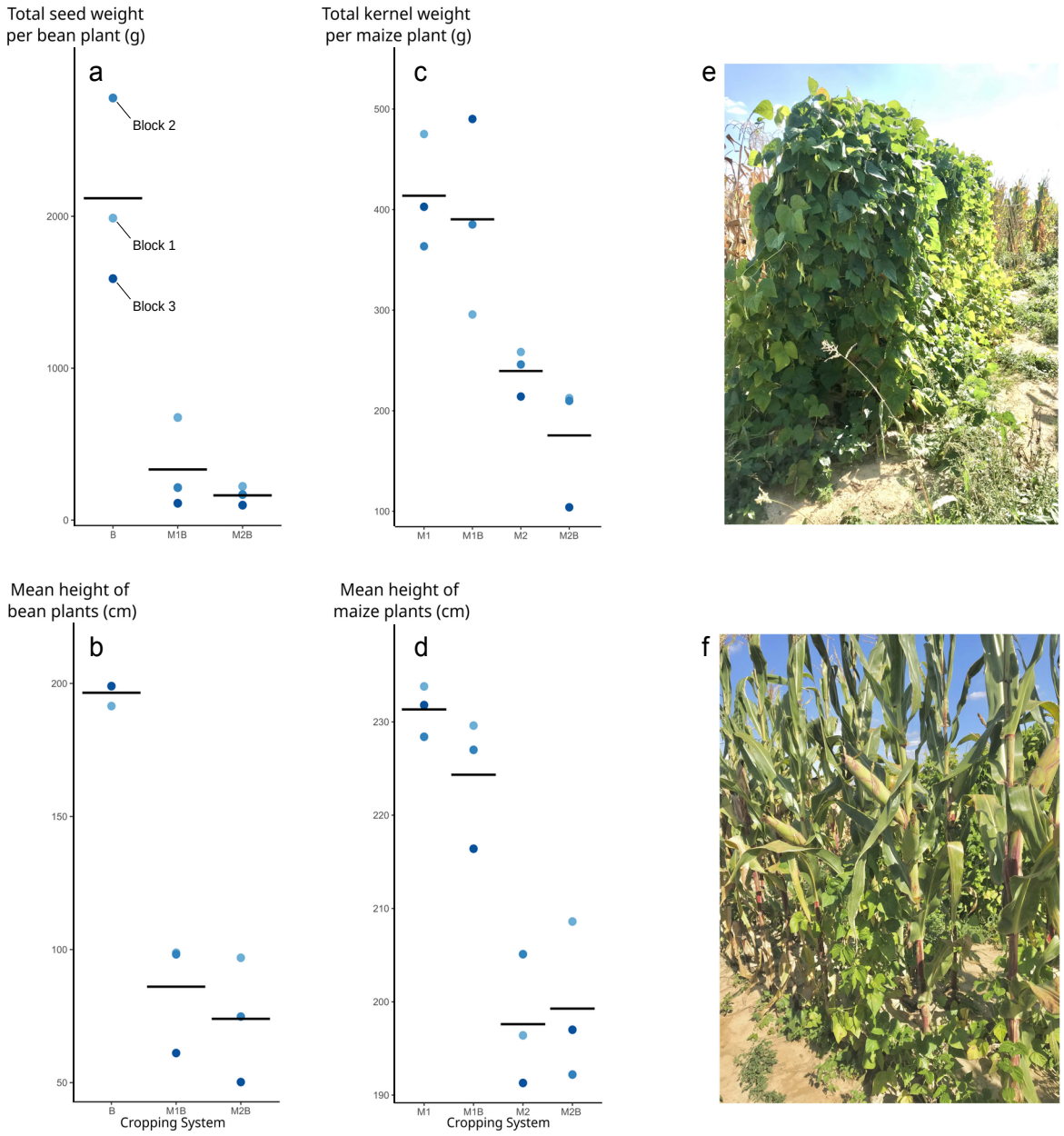

Fig. 6 Development and yield of maize and bean plants under sole and intercropping. Stripcharts display the total seed weight per bean plant (a) and the mean height of bean plants (b), the total kernel weight per maize plant (c) and the mean height of maize plants (d). Blue dots represent the average 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).

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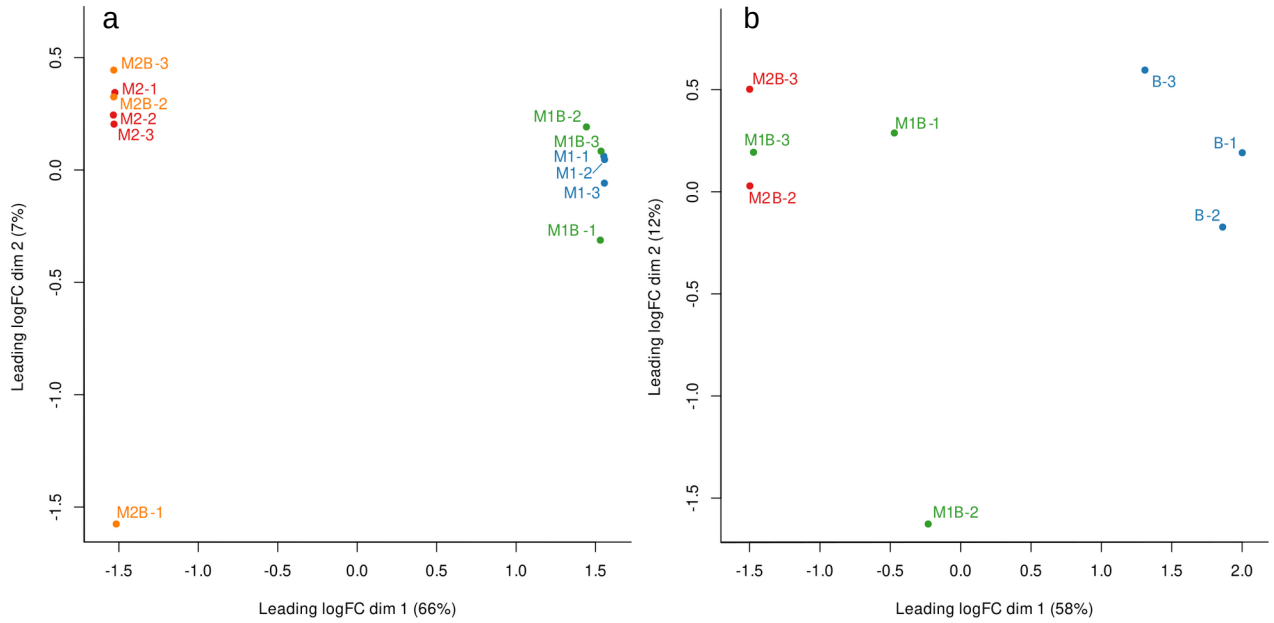

Fig. 7 MDS plots of distances between gene expression profiles in sole cropped and intercropped maize and bean. The distances observed between samples in the two MDS plots approximate the typical log2 fold changes between the samples. MDS plots were computed from 16,021 genes for maize samples (a), and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