1 **Maize residues changes soil fungal composition and decrease soil** 2 **microbial co-occurrence networks complexity.**

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14 **ABSTRACT.**

15 *Fusarium graminearum* (*Fg*) can cause different diseases in cereals and maize crops worldwide, and a
16 correct management of previous crop residues could decrease disease incidence and/or severity.
17 Bacterial, fungal and *Fusarium* communities were studied by metabarcoding approach in 8 agricultural
18 fields with wheat-maize rotation system in Brittany, France, during three years. Additionally, shift in
19 microbial communities were evaluated under mesocosm experiments in soils amended or not with
20 maize residues and/or *Fg* isolate. Bacterial communities composition were highly influenced by crop
21 soil origin in both environmental and mesocosm soils, while bacteria co-occurrence network

complexity was decreased by maize residues in environmental samples and *Fg* treatment in mesocosm samples. Maize residues altered slightly bacteria-fungi co-occurrence networks, while all treatments on mesocosm experiments showed lower complexity in bacteria-fungi networks than Control Soil treatment. A clear input of fungal genera *Epicoccum*, *Fusarium*, *Vishniacozyma*, *Articulospora*, *Papiliotrema*, *Sarocladium*, *Xenobotryosphaeria*, *Ramularia*, *Cladosporium*, *Cryptococcus* and *Bullera* from maize residues to soil were observed for both environmental and mesocosm samples. Moreover, an increase of *F. graminearum* and *F. avenaceum* was observed in soils where maize residues were presented. Finally, microbial co-occurrence networks reported some OTUs significantly correlated to *Fusarium* spp. OTUs, such as those assigned to *Epicoccum*, *Vishniacozyma* and *Sarocladium* fungal genera, previously reported as efficient biocontrol agents versus *Fusarium* spp. Moreover, a decrease of complexity was observed for soil bacterial and bacterial-fungal networks due to maize addition in both environmental and mesocosm communities.

Keywords: microbial communities, maize residues, crop soils, *Fusarium* species, co-occurrence networks

39 INTRODUCTION.

40 *Fusarium* Head Blight (FHB) is a devastating fungal disease of small-grain cereals, including wheat,
 41 caused mainly by members of *Fusarium* complexes (Bateman *et al.* 2007; Dean *et al.* 2012). Beyond
 42 significantly reduced yields, the main consequence is grain contamination with mycotoxins, including
 43 type A and B trichothecenes, produced by toxigenic *Fusarium* spp. (Smith *et al.* 2016; Tralamazza *et*
 44 *al.* 2016). Among *Fusarium* spp. responsible for FHB, *F. graminearum* (Fg) is considered the fourth
 45 most economically-important plant fungal pathogen (Dean *et al.* 2012), and is within the most frequent
 46 species associated to FHB in Europe, besides *F. culmorum*, *F. avenaceum* and *F. poae* (Xu *et al.* 2005;
 47 Hellin *et al.* 2016). Moreover, a shift from *F. culmorum* to *F. graminearum* as the main *Fusarium*
 48 species has been recently observed in European cereal crops (Nielsen *et al.* 2011; Scauflaire *et al.*
 49 2011). Climatic change is the principal hypothesis put forward, although the increase in maize-wheat
 50 rotation crops may also contribute to this increase in *F. graminearum* at the expense of *F. culmorum*
 51 (Nielsen *et al.*, 2011). FHB control represents a major scientific challenge given the multiplicity of
 52 causal agents and the complex mechanisms leading to mycotoxin contamination.

53 The life cycle of *Fusarium* spp. and especially *F. graminearum* is relatively well known (Champeil *et*
 54 *al.*, 2004). *F. graminearum* is able to survive for several years saprophytically in soil and especially on
 55 crop residues which provide a carrier and nutrients necessary for its growth (Leplat *et al.*, 2013). Based
 56 on mesocosm experiments, the latter authors demonstrated a higher Fg growth on soil amended with
 57 residues than on bare soil while, among crop residues, higher growth was found on soil amended with
 58 maize residues, which provided the best carrying capacity over wheat or rapeseed residues (Leplat *et*
 59 *al.*, 2016). Similar observations were obtained from field studies (Schaafsma *et al.*, 2005; Blandino *et*
 60 *al.*, 2010). Overall, the risk of FHB is recognized to be higher when crop residues are left on the soil
 61 surface and with direct sowing (Maiorano *et al.*, 2008). Therefore, residues, and soil after residue

decomposition, are considered as the primary source of inoculum responsible for FHB. In spite of this, the composition and diversity of *Fusarium* spp. in these components have received much less attention than in grains.

Although higher incidence of FHB events were found in maize-wheat cropping systems, especially under minimum tillage practices (Dill-Macky and Jones 2000; Schöneberg *et al.* 2016; Cromeey *et al.*, 2002; Edwards & Jennings, 2018; Vogelgsang *et al.* 2011), there is some evidences of *Fusarium* spp. survival reduction due to the effect of maize residues microbiota. For instance, Bateman *et al.* (2007) found that chopped maize tops appeared to suppress stem-base disease in wheats, while increasing the presence of *F. graminearum* in the crop, suggesting some microbial activity for disease suppression was occurring in maize residues. This was supported by previous finding of efficient BCAs, in both in vitro and greenhouse trials, isolated from maize tissues (Mousa *et al.* 2015), root rhizosphere (Abiala *et al.* 2015) and residues (Luongo *et al.*, 2005; Singh *et al.*, 2009).

By colonizing previous crop residues and soil, *Fusarium* spp. can therefore interact with the microbiota associated with these components. Recent advances in next generation sequencing technologies (NGS) has allowed researchers to deepen our knowledge of bacterial and fungal communities in both soils (Chen *et al.* 2015; Zhao *et al.* 2016) and plants (Cobo-Díaz *et al.* 2019, Zhou *et al.* 2016). Beyond the description of compositions and diversities of microbial communities, metabarcoding data can also be applied to predict the functionality of microbial communities (Louca *et al.* 2016; Nguyen *et al.* 2016) and examine network interactions (Vacher *et al.* 2016). The accurate description of the field microbiota, using such state-of-the-art technologies in combination with co-occurrence networks, may thus contribute to better understand the mechanisms underlying *Fusarium* spp./microbiota interactions, which has never been undertaken so far. Such knowledge may thus provide chances to develop innovative biocontrol approaches against FHB, which performance have been disappointing so far.

Indeed, it is now agreed that the soil- or plant-associated microbiota serves as a protective barrier against pathogens. While residues may represent a carrier for pathogenic organisms, including *Fusarium* spp., the removal of previous crop residues could also deprive the soil from taxa with suppressive functionalities towards such pathogens. Some candidate antagonistic organisms against *Fusarium* spp. have been isolated from maize root rhizosphere (Abiala *et al.*, 2015), maize endophytes (Mosua *et al.*, 2015), maize residues (Luongo *et al.*, 2005; Singh *et al.*, 2009) and agricultural soils (He *et al.*, 2009). It was recently shown that the microbiota of maize residues were dominated by genera that contain strains previously reported as biocontrol agents, as well as plant pathogenic genera, such as *Fusarium*, *Acremonium*, *Phoma*, *Pseudomonas* and *Erwinia* (Cobo-Díaz *et al.*, 2019). Therefore, a better understanding of the maize residues effects on *Fusarium* spp/ microbiota interactions may help improve crop management practices under conservation tillage systems. This could either rely on inoculative biocontrol approaches on maize residues to reduce FHB incidence or severity in following crops.

In this context, the objectives of the present work were to: i) study the dynamics of *Fusarium* and microbial communities and their interactions in soil and maize residues on agricultural fields under wheat/maize rotation; ii) determine the influence of maize residues and *F. graminearum* inoculation on soil microbial communities mesocosm conditions; iii) identify putative taxa of interest significant correlated to *Fusarium* spp. in co-occurrence networks analysis.

To address these objectives, soil and maize residue samples were collected from 8 fields in Brittany, France at 2 time-points: in November 2016 just after maize harvest and again, five months later in April 2017. Metabarcoding sequencing of *16S rRNA* gene, internal transcribed spacer (ITS) and *EF1 α* gene were then used to determine the bacterial, fungal and *Fusarium* communities on those samples. In addition, our microbial community profiles were also compared to an additional time-point in April

108 2015 which was included in a previous study from our laboratory (Legrand *et al.*, 2018). The influence
109 of maize residues on soil microbial communities were confirmed under mesocosm conditions with soil
110 samples inoculated or not with *Fg*.

111

112 MATERIALS AND METHODS

113 Soil and maize stalk sampling.

114 Soils were selected from an initial amount of 31 agronomics fields sampled on April 2015 in Brittany,
 115 France (28)(Legrand et al, 2019), and a total of 8 soils were sampled in 2 additional dates, in November
 116 2016 and April 2017. Fields localization and crop practices are described in Table 1. Fields were under
 117 maize/wheat rotation (in winter/spring rotation) for at least the last 4 years, except P16 and P23 that
 118 had no cultivation yet and onion, respectively, in sampling of April 2017. Wheat crop soils were taken
 119 one month before flowering and maize crop soils within 3 days after harvest. In each field, 15
 120 randomly points were selected, and the first 5 cm of soil (with a hand auger of 6 cm Ø); and, for
 121 November 2016 sampling, the above-ground part of one maize stalks with nodal region and leaves
 122 were randomly sampled in each point, at the same date of soil sampling. For soil P23, where not maize
 123 residues canopy was leave on soil, some maize residues were also taken from those not collected
 124 during harvest (Fig S1). Soil samples were stored at 4°C until were sieved with a 2 mm Ø mesh before
 125 DNA extraction, that was done within 24 h after sampling. Stalks were stored at 4°C until DNA
 126 extraction, made within 1 week.

127 Mesocosm experiment.

128 Soils and maize samples from fields P08, P09, P11, P16, P20 and P23 were employed on mesocosm
 129 experiment. Maize residues stems were cut in pieces of around 2 cm and then crushed in a blender
 130 machine, and 5 g of maize residues were added per 100 g of soil in “Maize” treatments. The maize
 131 residues added in each soil were those picked from the same field. A *F. graminearum* inoculum,
 132 prepared according to Legrand *et al.* (2018), was added to “*Fusarium*” treatments at a proportion of 2 g
 133 of maize infected kernel per 100 g of soil. A total of 4 treatments were tested for the 6 selected fields:

control soil (CS), control soil with maize residues (CM), soil with *F. graminearum* (FS) and soil with maize residues and *F. graminearum* (FM). The experiments were made within a month after sampling soils, using three replications (blocks of 4 x 4 x 6 cm) per treatment and soil, with a total of 72 blocks (4 treatments x 6 soils x 3 replicates) with 20 g of soil, or soil plus maize residues, per block. Pots were incubated in controlled conditions (day/night cycle: 16/8h, 22/18°C and 80% relative humidity) and watered each two days with sterile distilled water.

DNA extraction.

DNA was extracted from 200 mg of crushed maize stalks and leaves using FastDNA[®] SPIN kit (MP Biomedicals, Santa Ana, CA) following the manufacturer's instructions. For soil samples, DNA was extracted from 1 g of soil using NucleoSpin[®] Kit for Soil (Machery-Nagel, Dueren, De) according to the manufacturer's instructions. Quality and concentration of purified DNA were determined using a UV spectrophotometer (NanoDrop1000, Thermo Scientific, USA).

Aliquots from environmental and mesocosm DNA extracted samples were diluted in at least 10 ng/μl before sending to the sequencing company for metabarcoding approaches.

PCR amplification and sequencing.

Soil DNA extracted at day 15 for the 4 treatments (and three replicates) in mesocosm experiment for soils P08, P09, P11, P16, P20 and P23 (72 mesocosm samples); 72 field soil samples and 24 maize samples were used for amplicon sequencing by Illumina Miseq PE300. PCR amplification, Miseq libraries preparation and sequencing was performed at the McGill University and Génome Québec

Innovation Centre, Montréal, Canada. Primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') (Herlemann *et al.*, 2011) were used to amplify the variable regions V3 and V4 of 16S rRNA gene; primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) to amplify the internal transcribed spacer; and primers Fa_150 (5'-CCGGTCACTTGATCTACCAG-3') and Ra-2 (5'-ATGACGGTGACATAGTAGCG-3') (Cobo-Díaz *et al.*, 2019) to amplify the tubulin elongation factor (*ef1α*) gene of *Fusarium* species.

Read filtering.

Sequencing data were processed using QIIME (Quantitative Insights Into Microbial Ecology, version 1.9.1) (Caporaso *et al.*, 2010). For 16S rDNA V3-V4 amplicons, the forward (R1) and reverse (R2) paired-end sequences were joined using *multiple_join_paired_ends.py*, followed by *multiple_split_libraries_fastq.py* for demultiplexing. Chimera sequences were removed using UCHIME algorithm (Edgar *et al.*, 2011) implemented in vsearch v1.1.3 (<https://github.com/torognes/vsearch>) against the ChimeraSlayer database (Haas *et al.*, 2011). Pick open strategy was used to cluster the sequences into Operational Taxonomic Units (OTUs) at 97% similarity cut-off using *pick_open_reference_otus.py*. The taxonomic assignment was performed using UCLUST algorithm (Edgar, 2010) against GreenGenes v13_8 database preclustered at 97% similarity cutoff (McDonald *et al.*, 2012). Chloroplast, mitochondria and “No assigned” OTUs were discarded for further analysis.

The R1 and R2 paired-end sequencing reads of ITS amplicons were processed independently using *multiple_split_libraries_fastq.py*. ITS1 and ITS2 regions were first extracted separately from forward

177 and reverse fasta files respectively, using ITSx v1.0.11 (Bengtsson-Palme *et al.*, 2013) before being
 178 concatenated in a new file. A chimera filtering was made on concatenated file using the UCHIME
 179 algorithm (Edgar *et al.*, 2011) with VSEARCH v1.1.3 (<https://github.com/torognes/vsearch>) and a
 180 modified version of the UNITE/INSDC representative/reference sequences version 7.2 (UNITE
 181 Community 2017) as reference database. The modification consisted in extracting ITS1 and ITS2
 182 regions by ITSx software and concatenated them in the modified version of the database. The ITS1-
 183 ITS2 concatenated file of non-chimeric sequences was used for OTU picking running the QIIME script
 184 *pick_open_reference_otus.py*, with BLAST (Altschul *et al.*, 1990) as taxonomic assignment method
 185 and a modified version of UNITE plus INSD non-redundant ITS database version 7.1 (Kõljalg *et al.*
 186 2013). Again, the modified version consisted in concatenating ITS1 and ITS2 regions after extracting
 187 them using ITSx software. Those OTUs assigned to genus *Didymella* were checked manually, by
 188 BLAST on the web service (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) versus nt database to improve the
 189 taxonomic assignation, and in some cases were reassigned to genus *Epicoccum*, which was not
 190 presented in the last versions of UNITE database. Only OTUs assigned to kingdom Fungi were used
 191 for further analysis. The taxonomy for fungi known to have both sexual and asexual stages was
 192 replaced by accepted names according to Chen *et al.* (2018).

193 The library DADA2 (Callahan *et al.*, 2016) was used in R version 3.5.0 (r Development Core Team,
 194 2017) for *eflα* sequences filtering. Forward and reverse read pairs were trimmed and filtered, with
 195 forward reads truncated at 270 nt and reverse reads at 210 nt, no ambiguous bases allowed, and each
 196 read required to have less than two expected errors based on their quality scores. Amplicon Sequence
 197 Variants (ASVs) were independently inferred from the forward and reverse of each sample using the
 198 run-specific error rates, and then read pairs were merged requiring at least 15 bp overlap. The ASV

199 sequences were grouped in OTUs by *pick_otus.py* QIIME script, using a 98% of similarity cutoff.
 200 OTUs representative sequences along with references were used for phylogenetic tree taxonomic
 201 assignation, according to Cobo-Díaz et al (2019).

202 **Statistical analysis.**

203 To minimize the inflation of rare OTUs in the community analysis, samples with less than 1,000
 204 sequences and taxa with less than 0.01 percent relative abundance across all samples were removed,
 205 using the corresponding options in Calypso web tool (Zakrzewski *et al.*, 2017). Total sum
 206 normalization and square root transformation (Hellinger transformation) were done for data
 207 normalization. Principal Coordinates analysis (PCoA) was computed with the normalized data using
 208 Bray-Curtis distance metric in Calypso web tool (Zakrzewski *et al.*, 2017) while adonis test were done
 209 by *vegan* R-package (Oksanen). Richness and evenness indexes were calculated with normalized data
 210 and a previous samples rarefaction to the number of reads for the smallest sample. Wilcoxon-rank test
 211 or ANOVA test were used to compare taxa relative abundance on normalized data, and Core
 212 Microbiome analysis were computed at genus level using 0.70 as core relation samples in group. All
 213 the statistical analysis listed were made in Calypso web tool (Zakrzewski *et al.*, 2017).

214 Metabolic and ecologically relevant functions were annotated by FAPROTAX (Louca *et al.* 2016) for
 215 the 16S rRNA gene OTU, and Wilcoxon-rank test were used to compare functions relative abundance
 216 on hellinger transformed data in Calypso web tool (Zakrzewski *et al.*, 2017).

217 **Network analysis.**

218 The 200 most abundant OTUs were extracted from both bacterial and fungal otu-tables, and
 219 joined before were uploaded in the Molecular Ecological Network analysis Pipeline (MENAP) (Deng
 220 *et al.* 2012) in order to construct the corresponding Molecular Ecological Network (MEN) using two

major steps. First, the pairwise similarity of OTU abundance across the samples was used to create a Pearson correlation matrix. Then, an adjacency matrix was determined by Random Matrix Theory (RMT)-based approach using a regressed Poisson distribution for the prediction of the nearest neighbor spacing distribution of eigenvalues.

Those nodes with the highest value of degree, betweenness, stress centrality and/or eigenvector centrality were extracted beside the nodes with a significant correlation value (edge) to plot the corresponding sub-network. Ruby homemade scripts were used to select the nodes and edges used for sub-network plots, and to add taxonomic information from OTU table to nodes files. Network graphs were plotted by Gephi 0.9.2 (Bastian *et al.* 2009) using Fruchterman Reingold spatialisation (Fruchterman *et al.* 1991). Nodes and network topological indices were calculated within the MENAP webtool (Deng *et al.* 2012).

Accession numbers.

Demultiplexed raw sequence data were deposited in the Sequence Read Archive (<http://www.ncbi.nlm.nih.gov/sra>) under BioProject accession number PRJNA497210, while 16S rRNA and ITS sequences from samples taken in 2015 were deposited under BioProject PRJNA429425, with Experiment numbers SRR6475734, SRR6475732, SRR6475727, SRR6475718, SRR6457721, SRR6475715, SRR6457742 and SRR6475744 for 16S rRNA; and SRR6457737, SRR6457735, SRR6457728, SRR6475742, SRR6457747, SRR6457739, SRR6475721 and SRR6457719 for ITS region.

240 **RESULTS.**

241 **Shift in soil microbial communities along rotation**

242 *Fusarium* communities

243 A total of 2,914,818 *eflα* sequences were clustered into 31 OTUs (6 of them with abundance lower
244 than 0.01 % of total *eflα* sequences) assigned to *Fusarium* or *Neocosmospora* species, after filtering
245 raw reads from 24 maize residues samples and 72 soil samples. Replicates 5SP09A and 7SP06C were
246 removed for further analysis due to a low number of sequences obtained. Maize samples showed
247 significant ($p < 0.05$) higher richness (6.3 ± 2.1 OTUs) than soil samples (from 2.7 ± 1.6 to 4.1 ± 2.2),
248 while no significant differences were found for evenness (Fig. 1a,b).

249 The *Fusarium* spp. composition of maize samples was significantly different to that of soil samples
250 (adonis test, $R^2 = 0.29$, p -value = 0.001) and there was no significant differences between soil samples
251 (adonis test, $R^2 = 0.04$, p -value = 0.084), while parcels showed significant differences for soil samples
252 (adonis test, $R^2 = 0.32$, p -value = 0.001). PCoA of *Fusarium* OTUs illustrated such differentiation
253 among soil samples (Fig. 1c).

254 *F. oxysporum* was the most abundant species in soil samples, with significant higher values (rank test,
255 $p < 0.05$) than in maize samples while maize samples were significantly dominated by *F. graminearum*
256 and *F. avenaceum*. In addition, *F. avenaceum* was significantly more abundant in soil samples from
257 2016 than the other years (Fig. 1d). Other species with significant higher abundances in maize samples
258 than in soil samples included *F. poae*, *F. temperatum* and *Fusarium* sp. FCCSC (*Fusarium citricola*
259 species complex), and to a lesser extent, *F. venenatum*, *F. sporotrichioides* and *F. proliferatum* (Fig.
260 1d).

261

262 *Bacterial* communities

263 A total of 2,030,793 sequences of *16S rRNA* gene were clustered into 1,753 OTUs after removing rare
264 OTUs (relative abundance < 0.01%) from 24 maize residue and 72 soil samples. Maize samples had
265 significant (ANOVA, p-value < 0.001) lower richness (385 ± 201 OTUs) than soil samples (from 1090
266 ± 57 to 1444 ± 88 OTUs) while no significant differences were observed depending on year in soil
267 samples (Fig. 2a). Similar pattern was observed for evenness, with significant (ANOVA, p-value <
268 0.001) lower levels in maize samples (0.75 ± 0.05) than in soil samples (from 0.88 ± 0.03 to $0.91 \pm$
269 0.01) (Fig. 2b).

270 Soil samples were mainly grouped by field (adonis test, $R^2 = 0.38$, p-value = 0.001) in PCoA analysis,
271 and were significant differentiated from maize samples (adonis test, $R^2 = 0.52$, p-value = 0.001) (Fig.
272 2c).

273 The *Sphingomonas*, *Pseudomonas*, *Flavobacterium*, *Pedobacter*, and *Janthinobacterium* genera were
274 significantly more abundant (rank test, $p < 0.05$) in maize samples than in soil samples (Fig. 2d), but no
275 increase of these genera was found on soil samples from 2016 compared to other years. The
276 *Kaistobacter*, *Rhodoplanes*, *Nitrospira* and *DA101* genera were significantly more abundant in soil
277 samples than in maize samples (rank test, $p < 0.05$) (Fig. 2d).

278 At functional level, estimated by FAPROTAX, there were significant differences between maize and
279 soil samples (adonis test, $R^2 = 0.76$, $p = 0.001$) and also within soil samples (adonis test, $R^2 = 0.18$,
280 $p = 0.001$) (Fig. 3a). Some functional groups were highly represented in maize samples, including those
281 related to chemoheterotrophy, plant pathogen and fermentation; while soil samples were enriched in
282 functions related to nitrogen cycle and phototrophy, among others (Fig. 3b). Similar pattern was
283 observed when comparing soil samples from 2016 to the 2 other years, which had significant higher
284 values for chemoheterotrophy and plant pathogen in 2016, and lower values for functions related to
285 nitrogen cycle and phototrophy (Fig. 3b).

286

287 ***Fungal communities***

288 A total of 2,121,490 sequences of ITS were clustered into 440 OTUs after removing rare OTUs
 289 (relative abundance < 0.01%) from 24 maize residues and 72 soil samples. There was a significant
 290 increase in soil richness throughout years, with values of 69 ± 29 , 113 ± 22 and 179 ± 16 OTUs for
 291 2015, 2016 and 2017, respectively, while maize had lower richness (88 ± 15 OTUs) than soil from
 292 2016 (Fig. 4a). Evenness values were also significantly lower in maize samples (0.40 ± 0.18) compared
 293 to soil samples (from 0.65 ± 0.08 to 0.72 ± 0.06) (Fig. 4b).

294 There was a significant effect of field (adonis test, $R^2 = 0.38$, p-value = 0.001) and year (adonis test, R^2
 295 = 0.20 p-value = 0.001) in the composition of soil fungal communities, while soil samples were
 296 significantly differentiate from maize residues samples (adonis test, $R^2 = 0.41$, p-value = 0.001) (Fig.
 297 4c). PCoA, at OTU level, showed that soil samples from 2016 were clustered away from the other 2
 298 years, except for field P23 (Fig. 4c). Interestingly, this field had no maize residues left on the surface
 299 during 2016 harvest.

300 The *Epicoccum*, *Fusarium*, *Vishniacozyma*, *Articulospora*, *Papiliotrema*, *Sarocladium*,
 301 *Xenobotryosphaeria*, *Ramularia*, *Cladosporium*, *Cryptococcus* and *Bullera* genera were significantly
 302 more abundant (rank test, $p < 0.05$) in maize samples than in soil samples, and, except for *Articulospora*,
 303 they were also significantly more abundant (rank test, $p < 0.05$) in soil samples from 2016 than the other
 304 two years (Fig. 4d). Soil samples were dominated by *Saitozyma*, *Acremonium*, *Humicola*, *Fusicolla*,
 305 *Schizothecium*, *Chrysosporium* and *Exophiala* genera, with significant higher abundance in soil
 306 samples than in maize samples (Fig. 4d).

307

308 **Molecular Ecological Network analysis in field samples**

Four correlation-based networks of bacterial OTUs were constructed by soil sample per year and maize residue sample. Co-occurrence network of soils from 2016 (soil_2016) showed lower connectivity (less links, average degree and connectedness) than other networks (Table 2). The lowest value for centralization of degree found in soil_2016 indicates more similarity in connectivity values within the nodes of this network, compared to others. Similar observation was found for centralization of stress centrality, which was lower in soil_2015 and soil_2016, meaning that similar values of stress centrality for the nodes within this networks (Table 2). This differences were clearly observed in the sub-network constructed with the nodes with highest value for each topological indexes (degree, stress centrality, betweenness and eigenvector centrality) and the linked nodes, where a higher proportion of nodes belonging to phylum *Gemmatimonadetes* was found for maize network, and *Actinobacteria* and *Acidobacteria* for soil networks (Fig. S2). Moreover, nodes in soil_2016 network presented a decrease in degree value (number of correlations), while some nodes presented higher eigenvalue centrality values compared to other networks (Fig. 5a). Similar observations were found for bacteria-fungi networks, with soil_2016 as the network whose nodes presented lower degree values, followed by maize_2016 and soil_2015 (Fig. 5c). This nodes with highest eigenvalue centrality were mainly *Actinobacteria* belong to *Gailellaceae* family, and no fungal node was found between them (Table S1). Some OTUs negatively correlated with nodes of *Fusarium* spp. were found, including 2 nodes in soil_2016 network (both belonging to Gammaproteobacteria), 4 nodes in soil_2017 network (belonging to Acidobacteria and Ascomycota) and 4 nodes in maize network (including one belonging to Bacteroidetes, two to Ascomycota and one to Gammaproteobacteria) (Table 3). Moreover, up to 7 nodes were positively correlated to *Fusarium* nodes in the maize network, among which 3 belonged to *Flavobacterium*, two to *Vishniacozyma*, one to *Sarocladium* and the other to class Sordariomycetes (Table 3).

332

333 **Influence of maize residues and *F. graminearum* inoculation in soil microbial communities under**
 334 **mesocosm conditions.**

335 ***Bacterial communities.***

336 A total of 1,689,356 sequences of *16S rRNA* gene were clustered into 1,822 OTUs after removing rare
 337 OTUs (relative abundance < 0.01%) from 72 mesocosm soil samples. Excluding samples from field
 338 P16, which presented lowest values for alpha diversity indices, *Fg* inoculation or amendment with
 339 maize residues induced higher richness than in control samples (1289 ± 61 OTUs in SC compared to
 340 1367 ± 54 OTUs, 1341 ± 55 OTUs, in MC and SF respectively)(Fig. S3a). When comparing samples
 341 amended with maize residues, *Fg* inoculation induced significant lower evenness values, (0.85 ± 0.06
 342 versus 0.90 ± 0.01 , in MF and MC, respectively)(Fig. S3b).

343 In terms of bacterial compositional structure, differences due to treatment (adonis test, $R^2 = 0.09$, p-
 344 value = 0.007) were much less than the variation between fields (adonis test, $R^2 = 0.70$, p-value =
 345 0.001), as was shown in PCoA plot (Fig. 6a).

346 The addition of maize residues induced a significant increase in *Sphingomonas*, *Opitutus*,
 347 *Chthoniobacter* and *Fimbriimonas* relative abundance and a decrease in *Methylibium*, *Nitrospira* and
 348 *Terracoccus* whether or not soils were inoculated with *Fg* (Fig. S3c,d). In non-inoculated soil, the
 349 addition of maize induced a significant higher levels of other genera such as *Cellvibrio*, *Devosia*,
 350 *Phenylobacterium*, *Luteolibacter*, *Caulobacter*, *Luteibacter* and *Prostheco bacter* and lower levels for
 351 *Kaistobacter*, *Pseudomonas* and *Nitrospira* genera (Fig. S3d). Whether or not soil were amended with
 352 maize residues, *Fg* inoculation induced an increase in relative abundance for *Sphingomonas*,
 353 *Burkholderia*, *Pseudomonas*, *Rhodanobacter*, *Cellvibrio*, *Opitutus* and *Rhizobium*, while *Kaistobacter*
 354 and *Rhodoplanes* were decreased (Fig. S3e).

At functional level, estimated by FAPROTAX, the addition of maize residues increased the relative abundance of OTUs assigned to nitrogen fixation, fermentation and cellulolysis, among other functions (Fig. S4a). Fg inoculation induced an increase in nitrogen fixation and plant pathogen functions, among others, and a decrease in others functions associated to nitrogen metabolism, such as nitrogen respiration in soil treatments (Fig. S4b) and nitrogen respiration, nitrate reduction and denitrification in maize residues treatments (Fig. S4c).

361

362 ***Fungal communities***

A total of 2,432,998 sequences of ITS were clustered into 264 OTUs after removing rare OTUs (relative abundance < 0.01%) from 72 mesocosm soil samples. Richness was significantly higher in MC treatment, with 40 ± 16 OTUs in SF, 144 ± 27 in SC, 79 ± 20 in MF and 136 ± 21 in MC (Fig. S5a). No significant differences were found for evenness values, which ranged from 0.60 ± 0.11 to 0.71 ± 0.06 (Fig. S5b).

PCoA showed a significant clustering of samples by treatment (adonis test, $R^2 = 0.62$, p-value = 0.001), while slight differences were found by field (adonis test, $R^2 = 0.12$, p-value = 0.04) (Fig. 6b).

A significant decrease in *Fusarium* relative abundance was found in MF treatment compared to SF, with significant higher relative abundance of *Epicoccum*, *Podospora*, *Lasiosphaeris* and *Sarocladium* genera, among others (rank test, $p < 0.05$), in MF compared to SF (Fig. S4c). In non-inoculated soils, the addition of maize (MC treatment) induced an increase in the *Podospora*, *Sarocladium*, *Ramularia*, *Cryptococcus*, *Phaeoacremonium* and *Apodus* genera (rank test, $p < 0.05$) and a decrease in the *Acremonium*, *Fusicolla*, *Humicola* and *Ilyonectria* genera, among others, compared to SC treatment (Fig. S4d).

377

378 **Molecular Ecological Network analysis in mesocosm experiment.**

379 After removing samples for soil P16, which presented an important increase of *Rhodanobacter* genus
 380 for all the treatments (data not shown), co-occurrence network analysis were constructed for both
 381 bacterial and fungal data pooled together, and with bacterial data only. In bacteria networks, CM
 382 treatment showed the highest values for total links and average degree, with a progressive decrease for
 383 CS, FM and FS networks for both parameters (Table 4). Moreover, FS treatment presented the lowest
 384 values for average clustering coefficient, centralization of degree and density, while had the highest
 385 values for average path distance, harmonic geodesic distance, centralization betweenness and
 386 centralization of eigenvector centrality (Table 4). Lower values for the degree eigenvalue centrality
 387 proportion was observed in nodes from FS in bacteria networks (Fig. 5b) and in nodes from all
 388 treatments except CS for bacteria-fungi network (Fig. 5d). Nodes with highest eigenvalue centrality
 389 were mainly assigned to Kaistobacter genus and/or Proteobacteria phylum, and no fungal node was
 390 detected within them (Table S1).

391 Finally, fungi-fungi intra-kingdom and fungi-bacteria inter-kingdom connections were higher in
 392 mesocosm treatments, except for FS treatment for f-f connections, compared to environmental
 393 networks,. (Table 5). Moreover, presence of maize residues decrease the levels of the 3 kind of
 394 connections (bacteria-bacteria, fungi-fungi, and fungi-bacteria) for soil environmental and mesocosm
 395 networks, except for ff connection in mesocosm (Table 5).

396

397 **DISCUSSION.**

398 Basic but yet unanswered questions regarding the ecology of *Fusarium* spp. still remain. First,
 399 although soil and residues constitute the main FHB inoculum sources (Bateman *et al.* 2007; Fernandez
 400 *et al.* 2008), the knowledge on microbial ecology in residues and its influence on pathogen dispersion

or suppression is scarce. These findings could be important for the selection of appropriate control strategies in order to determine the predominant *Fusarium* spp. that should be targeted and the field components and wheat stages during which treatments are more likely to affect pathogen populations or their impact on the plant. In the present study, *F. graminearum*, *F. avenaceum*, *F. poae* and *F. temperatum* were the most abundant *Fusarium* species found on maize residues, as has already been reported on maize stalks after a 6-month field exposure (Köhl et al, 2015), maize residues collected after harvest (Cobo-Diaz *et al.* 2019, Dill-Macky and Jones 2000), maize stalks and kernels (Basler 2016), wheat kernels (Xu *et al.* 2005; Karlsson *et al.* 2016, 2017; Nicolaisen *et al.* 2014) and barley kernels (Schöneberg *et al.* 2016). In contrast, *F. oxysporum* was found as the main species in soil samples, as has been found previously on wheat crops (Edel-Hermann et al, 2015; Silvestro *et al.*, 2013; Leblanc et al, 2015). Moreover, an important input of *F. graminearum* and *F. avenaceum* from maize residues to soils was found, which confirmed maize residues as an important source of these *Fusarium* species associated to FHB. Furthermore, the low abundance of this species found in soils sampled before wheat flowering, after maize inter-crops and conventional tillage (except P11 field, which was under minimum tillage, and P23, with not maize residues canopy), support the idea of use conventional tillage approaches to reduce the availability of *Fusarium* spp. pathogens for following crops, as has been suggested before (Schöneberg *et al.* 2016).

Those fungal genera promoted by maize residues addition harbour maize or/and wheat pathogens species, such as *Cladosporium*, *Fusarium* and *Epicoccum*; and other plant pathogens, such as *Sarocladium* and *Ramularia*. Conversely, strains with antagonism effect against *F. graminearum* in wheat were found for *Epicoccum* (Luongo *et al.* 2005; Jensen *et al.*, 2016), *Cladosporium* (Luongo *et al.* 2005) and *Sarocladium* (Comby *et al.* 2017). Another genera deposited in soil from maize residues were *Vishniacozyma*, *Papiliotrema*, *Cryptococcus* and *Bullera*, which belong to order *Tremellales*.

Some strains of *Cryptococcus* were described as effective biocontrol agent (BCA) against *Fusarium* spp. in wheat (Wachowska *et al.* 2013b; Schisler *et al.* 2011) and those genera belong to *Tremellales* could be considered within this group of putative BCA against *Fusarium* spp. because they contain strains previously grouped within *Cryptococcus* genus (Liu *et al.* 2015). This field results were supported with the results obtained in mesocosm experiments, which reported also a increase in relative abundance due to maize residues of genera that harbor strains reported previously as BCA or organism associated to healthy soils in *Fusarium* spp. diseases studies, such as *Cryptococcus* (Wachowska *et al.* 2013b; Schisler *et al.* 2011), *Articulospora* (Sugahara *et al.* 2018) and *Sarocladium* (Comby *et al.* 2017). Furthermore, maize residues microbial co-occurrence networks presented some OTUs assigned to *Sarocladium*, *Epicoccum* and *Vishniacozyma* genera with significant correlation with OTUs assigned *Fusarium* spp. which could be an evidence of antagonism effect of this genera versus *Fusarium* spp. The same results, except for *Vishniacozyma*, were found for another maize residues sampling in Brittany, France (Cobo-Díaz *et al.* 2019), and their relative abundance increase in soils could have a antagonistic effect against *Fusarium* spp. *Flavobacterium*, *Sphingomonas*, *Pseudomonas*, *Pedobacter* and *Janthinobacterium* were found as the most abundant in our maize samples, as has been reported previously for maize residues (Cobo-Díaz *et al.* 2019), maize rhizospheric soils (Yang *et al.* 2017a; García-Salamanca *et al.* 2013; Li *et al.* 2014; Correa-Galeote *et al.* 2016) or even in wheat rhizospheric soils (Yin *et al.* 2013). This bacterial genera were reported as bacterial genera associated with reduced colonization of *Fusarium* spp. in maize stalks (Köhl *et al.* 2015) and/or contains strains characterized as BCA against *Fusarium* spp. (Wachowska *et al.* 2013a,b; Ito *et al.* 2013; Chen *et al.* 2018A; De Boer *et al.* 2007; Haack *et al.* 2016). The predominance in maize residues of such genera make them a potential source of bacterial species for plant pathogen control, but no increase of their relative abundance in the corresponding soil samples were observed. Maybe it could be due to the short

time lapsed between harvest and sampling, which was no more than 3 days. Furthermore, maize residues generate changes in the soil nitrogen transformations (Li *et al.* 2019) and increase soil nitrogen content (Maresma *et al.* 2018), and could generate the decrease, in relative abundance, of some bacterial functions related to nitrogen metabolism observed in our field samples.

Differences in the soil bacterial composition structure among years was lower than variation between fields, while fungal communities composition were significant different between soil samples from 2016 and the other 2 years, except for P23, whose samples from 2016 were within the 2015-2017 group. This field was the only one not amended with maize residues, although the base of the stalks were leave on crop (see Fig. S1). This maize residues influence on fungal communities observed in PCoA analysis was highlighted with the increase of relative abundance in soils after maize harvest of many fungal genera that clearly comes from those residues left on the field, while not increase was found on bacterial genera due to maize residues. Moreover, mesocosm experiment results corroborated this important influence of maize residues (and also *Fg* inoculation) on fungal communities structure and composition, while not influence was found for bacteria.

This stronger influence in fungal than bacterial communities had been observed in soil transplant experiments along 6 years (Zhao *et al.*, 2019). Moreover, it have been found that microbial diversity or taxonomical information are not sensitive enough as indicator of ecosystem perturbations (Karimi *et al.* 2016) and in some cases significant changes in bacterial co-occurrence patterns were found while no differences were observed in diversity indexes (He *et al.* 2017). The use of ecological networks as indicators of environmental quality had reported that higher levels of perturbation correlated to lower complexity in microbial networks (Karimi *et al.* 2016, Lupatini *et al.* 2014, Zappellini *et al.* 2015, Sauvadet *et al.* 2016), including disease incidence as perturbation factor (Yang *et al.*, 2017b). In our study, although bacterial communities did not present strong differences due to

maize presence, or even along years of sampling, their co-occurrence networks presented a significant decrease of connectivity and nodes degree values when maize were added to soil samples. The main factor could be changes in interaction between species due to the increase of nutrients and also the differences in fungal communities composition observed by maize residues addition. Moreover, this changes on bacterial networks was accentuated with the increase of importance for nodes belonging to *Actinobacteria* phylum, which although were not abundant in the communities, had an important role within co-occurrence networks. Phylum *Actinobacteria* was found previously as a key taxon on bacterial soil networks, where it could reduce the chance of soil plant pathogen invasion for tobacco bacterial wilt disease (Yang *et al.* 2017b). The decrease of connectivity values has also been observed on soil bacterial communities due to land use, where higher density of links were found in natural forest soils than pasture or field and plantations soils (Lupatini *et al.* 2014), so that maize addition could be considered as a strong perturbation of soil microbial co-occurrence networks. Furthermore, the not clearly existence of keystone taxa (data not shown) could be advantageous to the ecosystem functionality, as the lost or decrease on any microbial taxa is not going to weaken the inter correlation network of the ecosystem (Toju *et al.* 2018).

485

486 CONCLUSIONS

Maize residues have a stronger influence in fungal communities than bacterial communities composition, although reduction on connectivity indexes for bacterial co-occurrence networks was found due to maize addition. Bacterial communities composition were conserved along the time, with a clear differentiation due to the field instead of time of sampling. Maize residues harbour both bacterial and fungal genera previously reported as biocontrol agents against *Fusarium* spp. or diseases caused by this genera, but only an increase in relative abundance of those genera belonged to fungi were observed

493 in both field and mesocosm soils amended with maize residues. Some OTUs belonged to those BCA
 494 genera, such as the bacteria *Flavobacterium* and the fungal *Epicoccum*, *Vishniacozyma* and
 495 *Sarocladium*, presented significant co-occurrence with *Fusarium* spp. OTUs, mainly in maize
 496 networks. Further experiments and studies has to be done to clarify their effect in *Fusarium* disease
 497 suppression in following crops, and to found which agricultural practices can increase the presence of
 498 those BCA genera in soil, and reduce of FHB events or severity.

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757 **Table 1.** Characteristics of sampled fields.

758 **Table 2.** Topological properties of field bacteria networks.

759 **Table 3.** Nodes in field networks with significant co-occurrence with *Fusarium* spp. nodes

760 **Table 4.** Topological properties of mesocosm bacteria networks.

761 **Table 5.** Analysis of the proportion of intra-kingdom interactions in the co-occurrence networks.

762

763 **Figure 1. Field *Fusarium* communities.** a) Richness index, b) evenness index, c) PCoA plot and d)
764 Rank test analysis.

765 **Figure 2. Field bacterial communities.** a) Richness index, b) evenness index, c) PCoA plot and d)
766 Rank test analysis.

767 **Figure 3. Field bacterial functionality.** a) PCoA and b) Rank test analysis using the functional groups
768 obtained by FAPROTAX pipeline.

769 **Figure 4. Field fungal communities.** a) Richness index, b) evenness index, c) PCoA plot, d) Rank test
770 analysis, and e) core microbiome analysis.

771 **Figure 5. Co-occurrence networks.** Degree-Eigenvalue Centrality plot for nodes in a) field bacteria
772 networks, b) mesocosm bacteria networks, c) field bacteria-fungi networks and d) bacteria-fungi
773 networks. Vertical line indicate Eigenvalue centrality equal to 0.24.

774 **Figure 6. Mesocosm betadiversity analysis.** PCoA plot of a) bacterial and b) fungal communities
775 obtained in mesocosm experiment at d15.

776

777

778 **Table S1. Nodes topological characteristics and taxonomic assignment.** Only nodes with highest
779 eigenvalue centrality (> 0.24) were indicated. “Network” column indicates to which networks belong
780 (year number for field soil networks, “Maize” for maize network, treatment for mesocosm networks).

781 **Figure S1. Sampled fields in November 2016.** Photos of sampled fields a) P08, b) P09, c) P20 and d)
782 P23. Maize residues from P23 were used for silage and not left in crop.

783 **Figure S2. Bacteria co-occurrence sub-network.** Nodes with highest topological characteristics
784 (plotted in yellow) and those correlated to them were plotted. Nodes were colored by phylum and edges
785 according to positive (green) or negative (red) correlation between nodes linked.

786 **Figure S3. Mesocosm bacterial communities.** a) Richness index, b) evenness index, c) Rank test
787 analysis for *Fusarium* treatments, d) Rank test analysis for Control treatments and e) Rank test analysis
788 compared *Fusarium* treatments versus control treatments.

789 **Figure S4. Mesocosm bacterial functionality.** a) Rank test analysis for control treatments, b) soil
790 treatments and c) maize treatments, using the functional groups obtained by FAPROTAX pipeline.

791 **Figure S5. Field fungal communities.** a) Richness index, b) evenness index, c) Rank test analysis for
792 *Fusarium* treatments, d) Rank test analysis for Control treatments.

Figure 1. Field *Fusarium* communities. a) Richness index, b) evenness index, c) PCoA plot and d) Rank test analysis.

Figure 2. Field bacterial communities. a) Richness index, b) evenness index, c) PCoA plot and d) Rank test analysis.

Figure 3. Field bacterial functionality. a) PCoA and b) Rank test analysis using the functional groups obtained by FAPROTAX pipeline.

Figure 4. Field fungal communities. a) Richness index, b) evenness index, c) PCoA plot, d) Rank test analysis, and e) core microbiome analysis.

Figure 5. Co-occurrence networks. Degree-Eigenvalue Centrality plot for nodes in a) field bacteria networks, b) mesocosm bacteria networks, c) field bacteria -fungi networks and d) bacteria -fungi networks. Vertical line indicate Eigenvalue centrality equal to 0.24.

Figure 6. Mesocosm betadiversity analysis. PCoA plot of a) bacterial and b) fungal communities obtained in mesocosm experiment at d15.

Table 1

Code	Location (City)	GPS Coordenates	April 2015	November 2016	April 2017	Tillage	Fertilizers
P06	Gouesnou	48.447893, -4.435354	Wheat	Maize	Wheat	Conventional	Chemical + Manure
P08	Plouvenez Lochrist	48.598551, -4.240471	Wheat	Maize	Wheat	Conventional	Chemical + Manure
P09	Porspoder	48.451933, -4.628142	Wheat	Maize	Wheat	Conventional	Chemical + Manure
P11	Loudéac	48.218545, -2.806619	Wheat	Maize	Wheat	Minimum	Chemical + Manure
P16	Bannalec	47.932287, -3.712028	Wheat	Maize	Nothing	Conventional	Unknown
P20	Plabennec	48.500746, -4.440550	Wheat	Maize	Wheat	Conventional	Chemical + Manure
P21	Kergoz Bannalec	47.958804, -3.705690	Wheat	Maize	Wheat	Conventional	Chemical
P23	Plouider	48.606743, -4.281656	Wheat	Maize *	Onion	Conventional	Chemical + Manure

* Maize used for silage

Table 2

Network Indexes	2015 (0.690)	2016 (0.820)	2017 (0.760)	maize (0.710)
Total nodes	110	127	108	145
Total links	661	304	698	763
R square of power-law	0.496	0.909	0.39	0.305
Average degree (avgK)	12.018	4.787	12.926	10.524
Average clustering coefficient (avgCC)	0.449	0.346	0.473	0.598
Average path distance (GD)	2.699	3.747	3.044	3.935
Geodesic efficiency (E)	0.465	0.34	0.421	0.333
Harmonic geodesic distance (HD)	2.149	2.939	2.373	3.006
Maximal degree	41	17	37	31
Centralization of degree (CD)	0.271	0.098	0.229	0.144
Maximal betweenness	453.174	1312.223	1300.332	1717.985
Centralization of betweenness (CB)	0.066	0.156	0.213	0.147
Maximal stress centrality	3426	4244	12784	39741
Centralization of stress centrality (CS)	0.48	0.487	2.038	3.5
Maximal eigenvector centrality	0.236	0.301	0.23	0.242
Centralization of eigenvector centrality (CE)	0.174	0.257	0.168	0.193
Density (D)	0.11	0.038	0.121	0.073
Transitivity (Trans)	0.568	0.416	0.604	0.569
Connectedness (Con)	0.748	0.56	0.963	1
Efficiency	0.863	0.945	0.883	0.933

Table 3

Network	Node name	Phylum *	Taxonomy	Fusarium node	Fusarium species **
Negative correlations					
2016	b_4360511	c__Gammaproteobacteria	f__Enterobacteriaceae	f_SH020374.07FU_GQ505688	s__Gibberella_intricans (FIESC)
2016	b_1566691	c__Gammaproteobacteria	g__Pseudomonas	f_SH020374.07FU_GQ505688	s__Gibberella_intricans (FIESC)
2017	b_1108199	p__Acidobacteria	f__Koribacteraceae	f_SH031935.07FU_AB586992_refs	s__Gibberella_zeae
2017	b_1108199	p__Acidobacteria	f__Koribacteraceae	f_SH022239.07FU_KU901536_reps	s__Gibberella_tricineta
2017	f_New.ReferenceOTU154	p__Ascomycota	g__Chaetosphaeria	f_SH031935.07FU_AB586992_refs	s__Gibberella_zeae
2017	f_New.ReferenceOTU154	p__Ascomycota	g__Chaetosphaeria	f_SH495279.07FU_KT268914_reps_singleton	s__Gibberella_tricineta
Maize	b_New.ReferenceOTU191	p__Bacteroidetes	g__Hymenobacter	f_New.ReferenceOTU284	s__Fusarium_venenatum
Maize	f_SH019454.07FU_KP859013_refs	p__Ascomycota	g__Microdochium	f_New.ReferenceOTU284	s__Fusarium_venenatum
Maize	f_New.ReferenceOTU246	p__Ascomycota	g__Epicoccum	f_SH026899.07FU_AB587010_refs	s__Gibberella_fujikuroi
Maize	b_New.ReferenceOTU445	c__Gammaproteobacteria	f__Enterobacteriaceae	f_SH026899.07FU_AB587010_refs	s__Gibberella_fujikuroi
Positive correlations					
Maize	b_264229	p__Bacteroidetes	g__Flavobacterium	f_SH022239.07FU_KU901536_reps	s__Gibberella_tricineta
Maize	b_264229	p__Bacteroidetes	g__Flavobacterium	f_SH495279.07FU_KT268914_reps_singleton	s__Gibberella_tricineta
Maize	f_SH004916.07FU_HQ875391_refs	p__Basidiomycota	g__Vishniacozyma	f_SH489173.07FU_KR909450_reps	s__Gibberella_tricineta
Maize	f_SH004916.07FU_HQ875391_refs	p__Basidiomycota	g__Vishniacozyma	f_SH495279.07FU_KT268914_reps_singleton	s__Gibberella_tricineta
Maize	f_SH479820.07FU_KJ160145_reps_singleton	p__Ascomycota	c__Sordariomycetes	f_SH026899.07FU_AB587010_refs	s__Gibberella_fujikuroi
Maize	b_509372	p__Bacteroidetes	g__Flavobacterium	f_SH026899.07FU_AB587010_refs	s__Gibberella_fujikuroi
Maize	f_SH024466.07FU_HG965034_refs	p__Ascomycota	g__Sarocladium	f_SH026899.07FU_AB587010_refs	s__Gibberella_fujikuroi

* Class for Proteobacteria

** Taxonomical assignment by UNITE database

Table 4

Network Indexes	CM (0.860)	CS (0.880)	FM (0.860)	FS (0.900)
Total nodes	131	111	112	93
Total links	557	410	351	196
R square of power-law	0.642	0.598	0.695	0.758
Average degree (avgK)	8.504	7.387	6.268	4.215
Average clustering coefficient (avgCC)	0.447	0.458	0.425	0.346
Average path distance (GD)	3.664	3.147	3.596	4.234
Geodesic efficiency (E)	0.364	0.394	0.363	0.304
Harmonic geodesic distance (HD)	2.748	2.539	2.758	3.286
Maximal degree	26	23	20	13
Centralization of degree (CD)	0.137	0.145	0.126	0.098
Maximal betweenness	1396.241	716.186	743.481	1320.59
Centralization of betweenness (CB)	0.152	0.106	0.106	0.289
Maximal stress centrality	20169	8982	3948	4020
Centralization of stress centrality (CS)	2.201	1.344	0.546	0.878
Maximal eigenvector centrality	0.237	0.259	0.27	0.354
Centralization of eigenvector centrality (CE)	0.181	0.194	0.211	0.298
Density (D)	0.065	0.067	0.056	0.046
Transitivity (Trans)	0.527	0.442	0.439	0.409
Connectedness (Con)	0.759	0.752	0.721	0.836
Efficiency	0.923	0.921	0.932	0.957

Table 5

Sample	Number of nodes		Number of interactions			Theoretical Max. interactions			% of interactions ⁴		
	bact	fung	bb	ff	fb	bb ¹	ff ²	fb ³	bb	ff	fb
2015	95	112	440	3	74	4465	6216	10640	9.85	0.05	0.70
2016	132	160	304	4	50	8646	12720	21120	3.52	0.03	0.24
2017	112	181	698	58	269	6216	16290	20272	11.23	0.36	1.33
Maize	138	166	500	27	68	9453	13695	22908	5.29	0.20	0.30
FM	114	22	351	10	48	6441	231	2508	5.45	4.33	1.91
FS	112	9	267	0	20	6216	36	1008	4.30	0.00	1.98
CS	130	44	680	17	137	8385	946	5720	8.11	1.80	2.40
CM	113	40	317	23	49	6328	780	4520	5.01	2.95	1.08

¹ estimated by $x = n! / (2x(n-1)!)$, where n is the number of bacteria nodes

² estimated by $x = n! / (2x(n-1)!)$, where n is the number of fungi nodes

³ estimated by $x = n_f \times n_b$, where n_f and n_b are the number of fungi and bacteria nodes, respectively

⁴ percentage of interactions found from the theoretical maximum interactions