

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

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

34

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

37

38

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

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

65 Although higher incidence of FHB events were found in maize-wheat cropping systems, especially
66 under minimum tillage practices (Dill-Macky and Jones 2000; Schöneberg *et al.* 2016; Cromey *et al.*,
67 2002; Edwards & Jennings, 2018; Vogelgsang *et al.* 2011), there is some evidences of *Fusarium* spp.
68 survival reduction due to the effect of maize residues microbiota. For instance, Bateman *et al.* (2007)
69 found that chopped maize tops appeared to suppress stem-base disease in wheats, while increasing the
70 presence of *F. graminearum* in the crop, suggesting some microbial activity for disease suppression was
71 occurring in maize residues. This was supported by previous finding of efficient BCAs, in both in vitro
72 and greenhouse trials, isolated from maize tissues (Mousa *et al.* 2015), root rhizosphere (Abiala *et al.*
73 2015) and residues (Luongo *et al.*, 2005; Singh *et al.*, 2009).

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

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

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

103 To address these objectives, soil and maize residue samples were collected from 8 fields in Brittany,
104 France at 2 time-points: in November 2016 just after maize harvest and again, five months later in
105 April 2017. Metabarcoding sequencing of *16S rRNA* gene, internal transcribed spacer (ITS) and *EF1 α*
106 gene were then used to determine the bacterial, fungal and *Fusarium* communities on those samples. In
107 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:

134 control soil (CS), control soil with maize residues (CM), soil with *F. graminearum* (FS) and soil with
135 maize residues and *F. graminearum* (FM). The experiments were made within a month after sampling
136 soils, using three replications (blocks of 4 x 4 x 6 cm) per treatment and soil, with a total of 72 blocks
137 (4 treatments x 6 soils x 3 replicates) with 20 g of soil, or soil plus maize residues, per block. Pots
138 were incubated in controlled conditions (day/night cycle: 16/8h, 22/18°C and 80% relative humidity)
139 and watered each two days with sterile distilled water.

140

141 **DNA extraction.**

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

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

149

150 **PCR amplification and sequencing.**

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

155 Innovation Centre, Montréal, Canada. Primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R
156 (5'-GACTACHVGGGTATCTAATCC-3') (Herlemann *et al.*, 2011) were used to amplify the variable
157 regions V3 and V4 of 16S rRNA gene; primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3')
158 and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) to amplify the internal
159 transcribed spacer; and primers Fa_150 (5'-CCGGTCACTTGATCTACCAG-3') and Ra-2 (5'-
160 ATGACGGTGACATAGTAGCG-3') (Cobo-Díaz *et al.*, 2019) to amplify the tubulin elongation factor
161 (*ef1 α*) gene of *Fusarium* species.

162

163 **Read filtering.**

164 Sequencing data were processed using QIIME (Quantitative Insights Into Microbial Ecology, version
165 1.9.1) (Caporaso *et al.*, 2010). For 16S rDNA V3-V4 amplicons, the forward (R1) and reverse (R2)
166 paired-end sequences were joined using *multiple_join_paired_ends.py*, followed by
167 *multiple_split_libraries_fastq.py* for demultiplexing. Chimera sequences were removed using
168 UCHIME algorithm (Edgar *et al.*, 2011) implemented in *vsearch* v1.1.3
169 (<https://github.com/torognes/vsearch>) against the ChimeraSlayer database (Haas *et al.*, 2011). Pick
170 open strategy was used to cluster the sequences into Operational Taxonomic Units (OTUs) at 97%
171 similarity cut-off using *pick_open_reference_otus.py*. The taxonomic assignment was performed using
172 UCLUST algorithm (Edgar, 2010) against GreenGenes v13_8 database preclustered at 97% similarity
173 cutoff (McDonald *et al.*, 2012). Chloroplast, mitochondria and “No assigned” OTUs were discarded for
174 further analysis.

175 The R1 and R2 paired-end sequencing reads of ITS amplicons were processed independently using
176 *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 *efl α* 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

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

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

232 **Accession numbers.**

233 Demultiplexed raw sequence data were deposited in the Sequence Read Archive
234 (<http://www.ncbi.nlm.nih.gov/sra>) under BioProject accession number PRJNA497210, while 16S
235 rRNA and ITS sequences from samples taken in 2015 were deposited under BioProject PRJNA429425,
236 with Experiment numbers SRR6475734, SRR6475732, SRR6475727, SRR6475718, SRR6457721,
237 SRR6475715, SRR6457742 and SRR6475744 for 16S rRNA; and SRR6457737, SRR6457735,
238 SRR6457728, SRR6475742, SRR6457747, SRR6457739, SRR6475721 and SRR6457719 for ITS
239 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**

309 Four correlation-based networks of bacterial OTUs were constructed by soil sample per year and maize
310 residue sample. Co-occurrence network of soils from 2016 (soil_2016) showed lower connectivity (less
311 links, average degree and connectedness) than other networks (Table 2). The lowest value for
312 centralization of degree found in soil_2016 indicates more similarity in connectivity values within the
313 nodes of this network, compared to others. Similar observation was found for centralization of stress
314 centrality, which was lower in soil_2015 and soil_2016, meaning that similar values of stress centrality
315 for the nodes within this networks (Table 2). This differences were clearly observed in the sub-network
316 constructed with the nodes with highest value for each topological indexes (degree, stress centrality,
317 betweenness and eigenvector centrality) and the linked nodes, where a higher proportion of nodes
318 belonging to phylum *Gemmatimonadetes* was found for maize network, and *Actinobacteria* and
319 *Acidobacteria* for soil networks (Fig. S2). Moreover, nodes in soil_2016 network presented a decrease
320 in degree value (number of correlations), while some nodes presented higher eigenvalue centrality
321 values compared to other networks (Fig. 5a). Similar observations were found for bacteria-fungi
322 networks, with soil_2016 as the network whose nodes presented lower degree values, followed by
323 maize_2016 and soil_2015 (Fig. 5c). This nodes with highest eigenvalue centrality were mainly
324 *Actinobacteria* belong to *Gailellaceae* family, and no fungal node was found between them (Table S1).
325 Some OTUs negatively correlated with nodes of *Fusarium* spp. were found, including 2 nodes in
326 soil_2016 network (both belonging to Gammaproteobacteria), 4 nodes in soil_2017 network (belonging
327 to Acidobacteria and Ascomycota) and 4 nodes in maize network (including one belonging to
328 Bacteroidetes, two to Ascomycota and one to Gammaproteobacteria) (Table 3). Moreover, up to 7
329 nodes were positively correlated to *Fusarium* nodes in the maize network, among which 3 belonged to
330 *Flavobacterium*, two to *Vishniacozyma*, one to *Sarocladium* and the other to class Sordariomycetes
331 (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).

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

361

362 ***Fungal communities***

363 A total of 2,432,998 sequences of ITS were clustered into 264 OTUs after removing rare OTUs
364 (relative abundance < 0.01%) from 72 mesocosm soil samples. Richness was significantly higher in
365 MC treatment, with 40 ± 16 OTUs in SF, 144 ± 27 in SC, 79 ± 20 in MF and 136 ± 21 in MC (Fig.
366 S5a). No significant differences were found for evenness values, which ranged from 0.60 ± 0.11 to 0.71
367 ± 0.06 (Fig. S5b).

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

370 A significant decrease in *Fusarium* relative abundance was found in MF treatment compared to SF,
371 with significant higher relative abundance of *Epicoccum*, *Podospora*, *Lasiosphaeris* and *Sarocladium*
372 genera, among others (rank test, $p < 0.05$), in MF compared to SF (Fig. S4c). In non-inoculated soils, the
373 addition of maize (MC treatment) induced an increase in the *Podospora*, *Sarocladium*, *Ramularia*,
374 *Cryptococcus*, *Phaeoacremonium* and *Apodus* genera (rank test, $p < 0.05$) and a decrease in the
375 *Acromonium*, *Fusicolla*, *Humicola* and *Ilyonectria* genera, among others, compared to SC treatment
376 (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

401 or suppression is scarce. These findings could be important for the selection of appropriate control
402 strategies in order to determine the predominant *Fusarium* spp. that should be targeted and the field
403 components and wheat stages during which treatments are more likely to affect pathogen populations
404 or their impact on the plant. In the present study, *F. graminearum*, *F. avenaceum*, *F. poae* and *F.*
405 *temperatum* were the most abundant *Fusarium* species found on maize residues, as has already been
406 reported on maize stalks after a 6-month field exposure (Köhl et al, 2015), maize residues collected
407 after harvest (Cobo-Diaz *et al.* 2019, Dill-Macky and Jones 2000), maize stalks and kernels (Basler
408 2016), wheat kernels (Xu *et al.* 2005; Karlsson *et al.* 2016, 2017; Nicolaisen *et al.* 2014) and barley
409 kernels (Schöneberg *et al.* 2016). In contrast, *F. oxysporum* was found as the main species in soil
410 samples, as has been found previously on wheat crops (Edel-Hermann et al, 2015; Silvestro *et al.*,
411 2013; Leblanc et al, 2015). Moreover, an important input of *F. graminearum* and *F. avenaceum* from
412 maize residues to soils was found, which confirmed maize residues as an important source of these
413 *Fusarium* species associated to FHB. Furthermore, the low abundance of this species found in soils
414 sampled before wheat flowering, after maize inter-crops and conventional tillage (except P11 field,
415 which was under minimum tillage, and P23, with not maize residues canopy), support the idea of use
416 conventional tillage approaches to reduce the availability of *Fusarium* spp. pathogens for following
417 crops, as has been suggested before (Schöneberg *et al.* 2016).

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

424 Some strains of *Cryptococcus* were described as effective biocontrol agent (BCA) against *Fusarium*
425 spp. in wheat (Wachowska *et al.* 2013b; Schisler *et al.* 2011) and those genera belong to *Tremellales*
426 could be considered within this group of putative BCA against *Fusarium* spp. because they contain
427 strains previously grouped within *Cryptococcus* genus (Liu *et al.* 2015). This field results were
428 supported with the results obtained in mesocosm experiments, which reported also a increase in relative
429 abundance due to maize residues of genera that harbor strains reported previously as BCA or organism
430 associated to healthy soils in *Fusarium* spp. diseases studies, such as *Cryptococcus* (Wachowska *et al.*
431 2013b; Schisler *et al.* 2011), *Articulospora* (Sugahara *et al.* 2018) and *Sarocladium* (Comby *et al.*
432 2017). Furthermore, maize residues microbial co-occurrence networks presented some OTUs assigned
433 to *Sarocladium*, *Epicoccum* and *Vishniacozyma* genera with significant correlation with OTUs
434 assigned *Fusarium* spp. which could be an evidence of antagonism effect of this genera versus
435 *Fusarium* spp. The same results, except for *Vishniacozyma*, were found for another maize residues
436 sampling in Brittany, France (Cobo-Díaz *et al.* 2019), and their relative abundance increase in soils
437 could have a antagonistic effect against *Fusarium* spp. *Flavobacterium*, *Sphingomonas*, *Pseudomonas*,
438 *Pedobacter* and *Janthinobacterium* were found as the most abundant in our maize samples, as has been
439 reported previously for maize residues (Cobo-Díaz *et al.* 2019), maize rhizospheric soils (Yang *et al.*
440 2017a; García-Salamanca *et al.* 2013; Li *et al.* 2014; Correa-Galeote *et al.* 2016) or even in wheat
441 rhizospheric soils (Yin *et al.* 2013). This bacterial genera were reported as bacterial genera associated
442 with reduced colonization of *Fusarium* spp. in maize stalks (Köhl *et al.* 2015) and/or contains strains
443 characterized as BCA against *Fusarium* spp. (Wachowska *et al.* 2013a,b; Ito *et al.* 2013; Chen *et al.*
444 2018A; De Boer *et al.* 2007; Haack *et al.* 2016). The predominance in maize residues of such genera
445 make them a potential source of bacterial species for plant pathogen control, but no increase of their
446 relative abundance in the corresponding soil samples were observed. Maybe it could be due to the short

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

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

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

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

485

486 **CONCLUSIONS**

487 Maize residues have a stronger influence in fungal communities than bacterial communities
488 composition, although reduction on connectivity indexes for bacterial co-occurrence networks was
489 found due to maize addition. Bacterial communities composition were conserved along the time, with a
490 clear differentiation due to the field instead of time of sampling. Maize residues harbour both bacterial
491 and fungal genera previously reported as biocontrol agents against *Fusarium* spp. or diseases caused by
492 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.

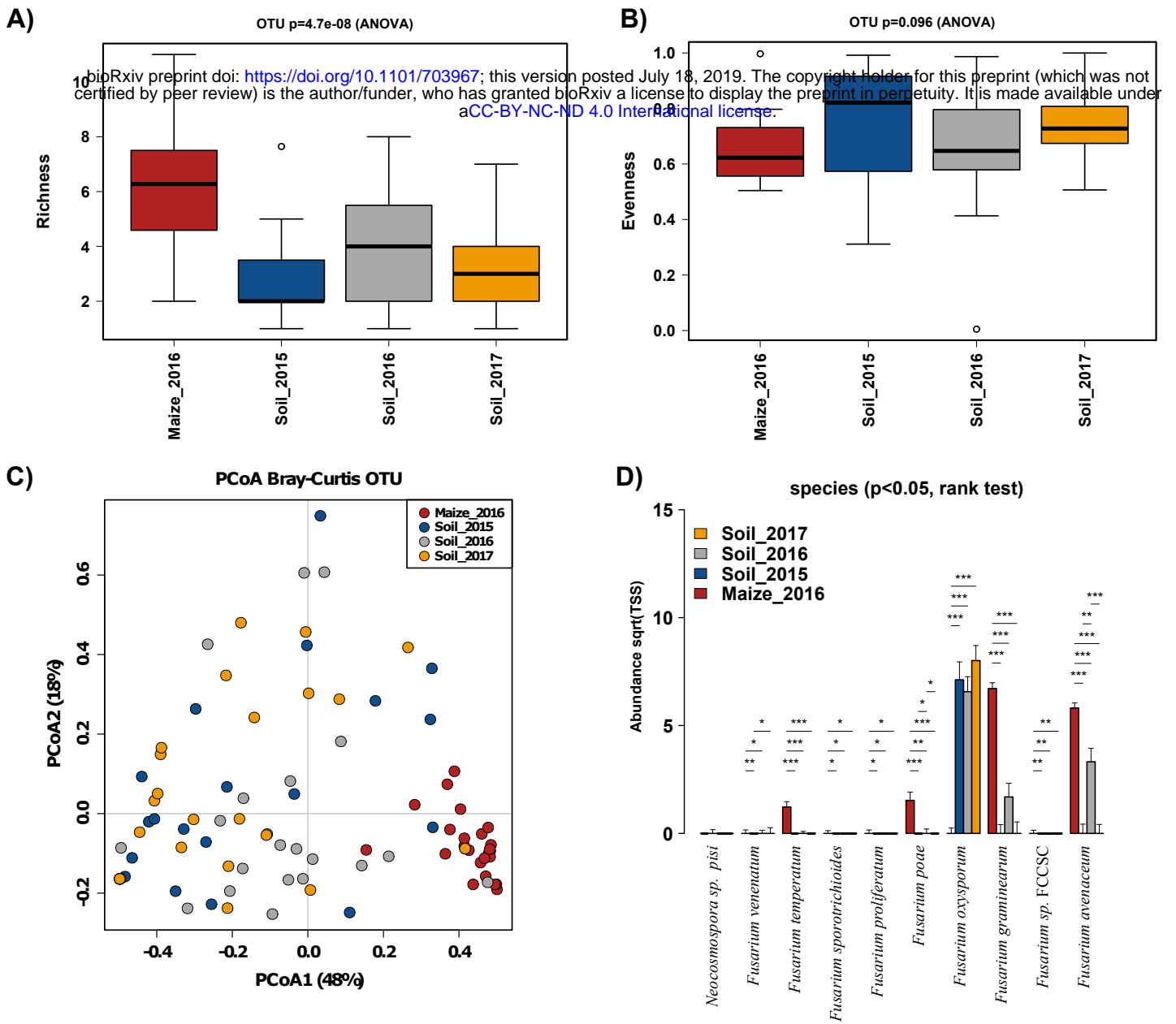


Figure 1. Field *Fusarium* communities. a) Richness index, b) evenness index, c) PCoA plot and d) Rank test analysis.

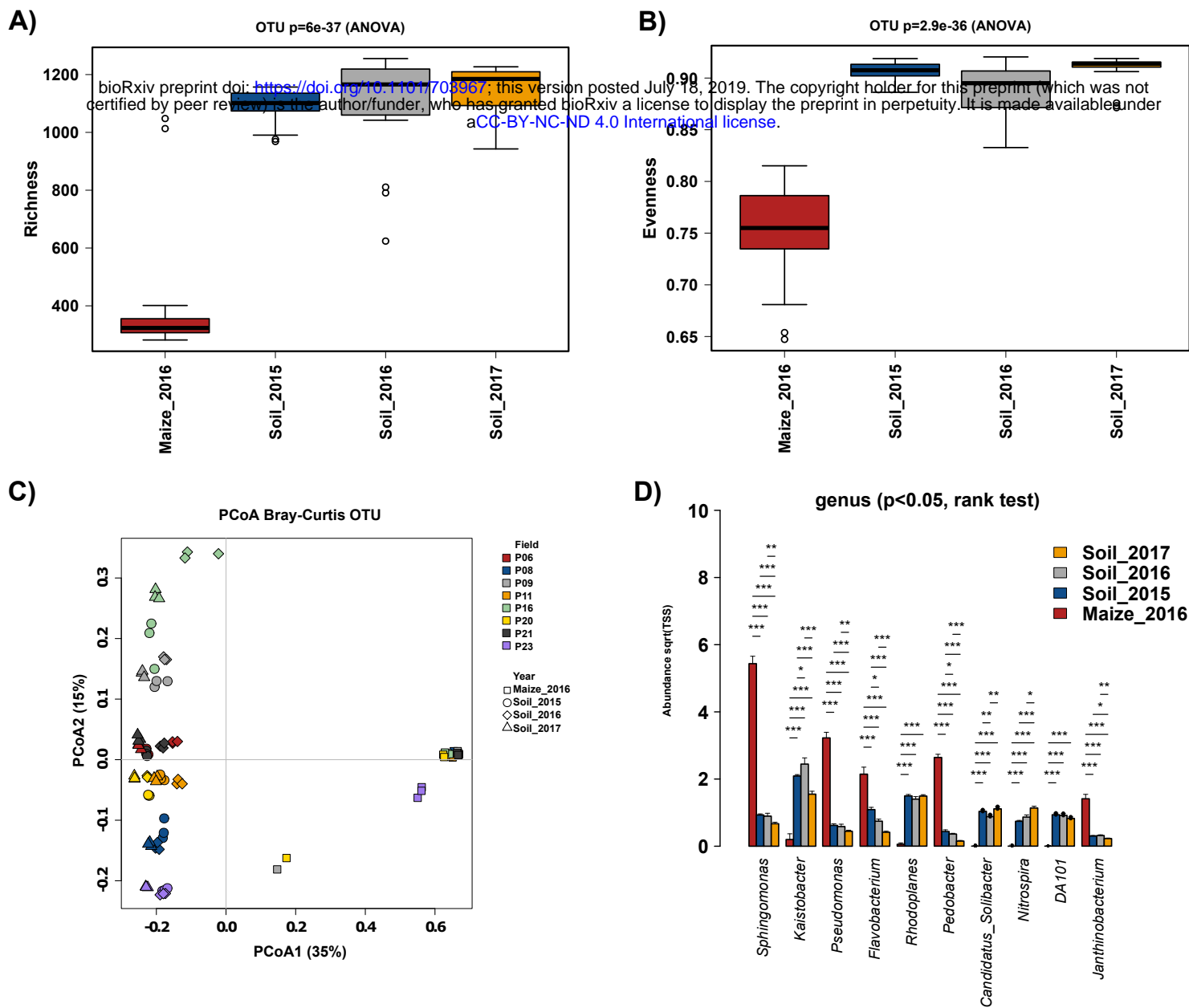
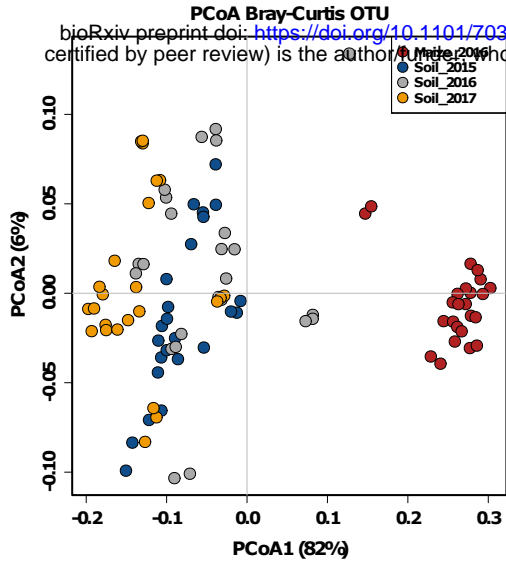


Figure 2. Field bacterial communities. a) Richness index, b) evenness index, c) PCoA plot and d) Rank test analysis.

A)



B)

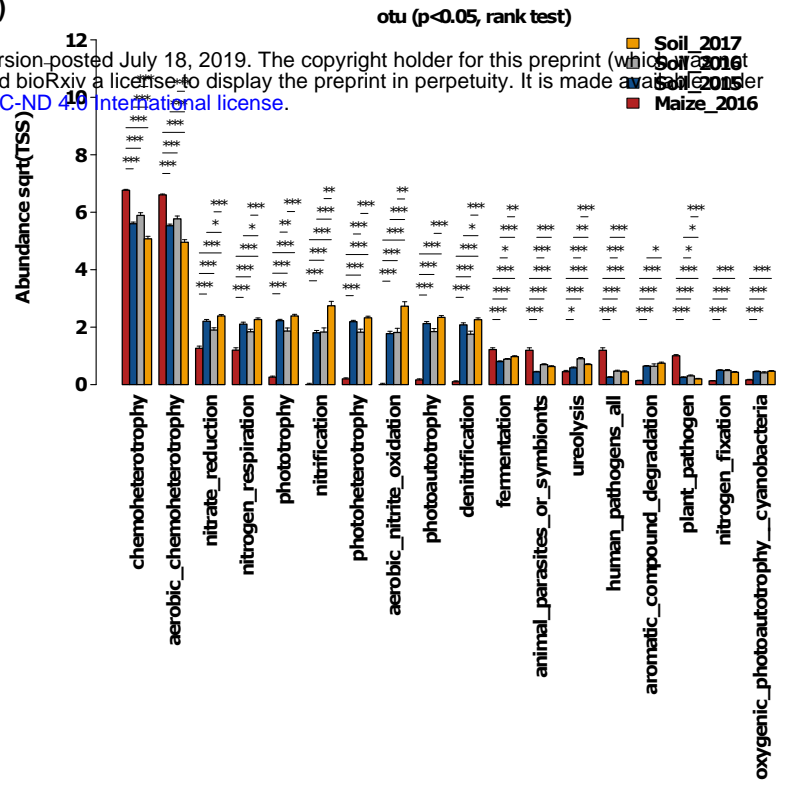


Figure 3. Field bacterial functionality. a) PCoA and b) Rank test analysis using the functional groups obtained by FAPROTAX pipeline.

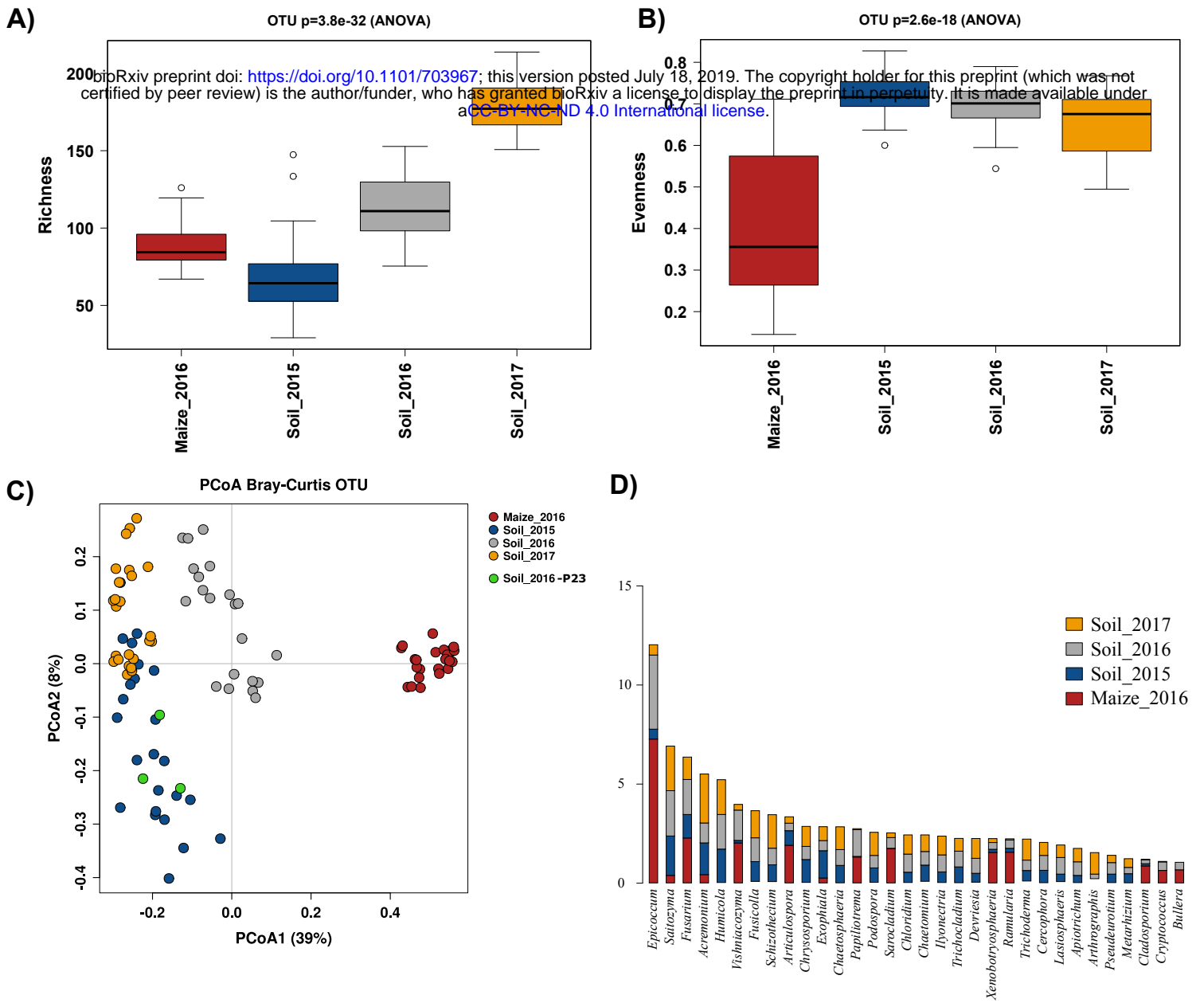


Figure 4. Field fungal communities. a) Richness index, b) evenness index, c) PCoA plot, d) Rank test analysis, and e) core microbiome analysis.

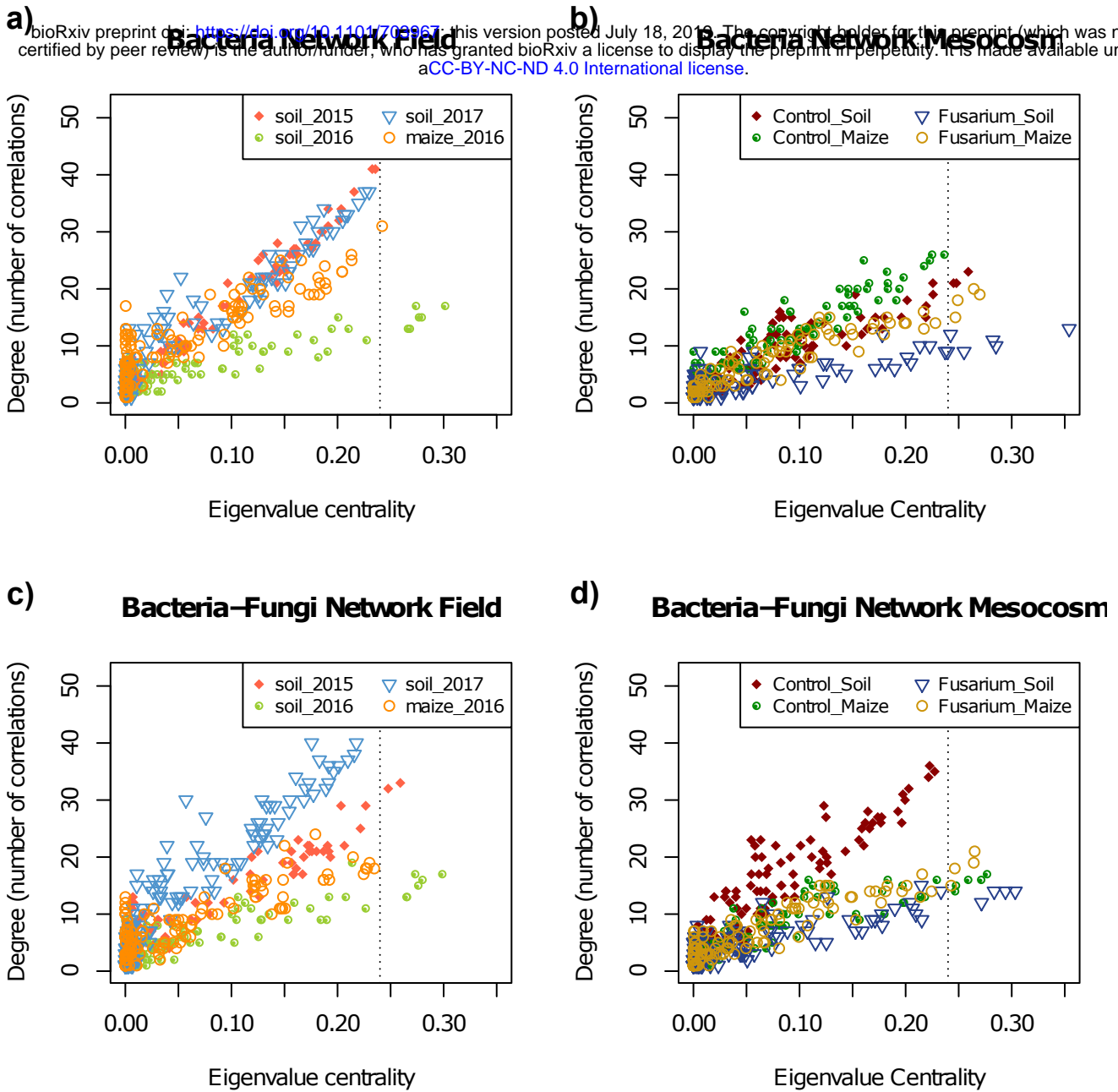


Figure 5. Co-occurrence networks. Degree-Eigenvalue Centrality plot for nodes in a) field bacteria networks, b) mesocosm bacteria networks, c) field bacteria -fungi networks and d) bacteria -fungi networks. Vertical line indicate Eigenvalue centrality equal to 0.24.

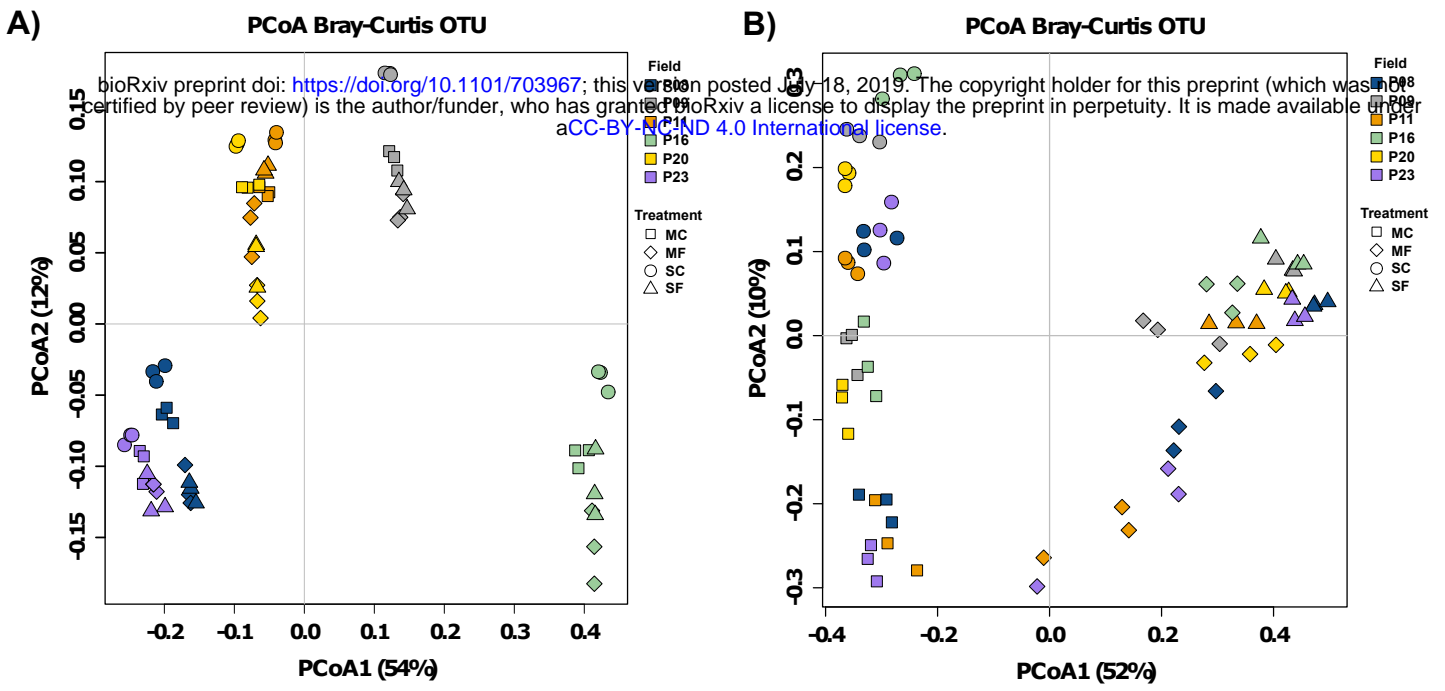


Figure 6. Mesocosm betadiversity analysis. PCoA plot of a) bacterial and b) fungal communities obtained in mesocosm experiment at d15.

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