Title: Differential dynamics of microbial community networks help identify microorganisms interacting with residue-borne pathogens: the case of *Zymoseptoria tritici* in wheat

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Abstract

Background - Wheat residues are a crucial determinant of the epidemiology of Septoria tritici blotch, as they support the sexual reproduction of the causal agent *Zymoseptoria tritici*. We aimed to characterize the effect of infection with this fungal pathogen on the microbial communities present on wheat residues, and to identify microorganisms interacting with it. We used metabarcoding to compare the microbiome associated with wheat residues placed outdoors, with and without preliminary *Z. tritici* inoculation, comparing a first set of residues in contact with the soil and a second set without contact with the soil, on four sampling dates in two consecutive years.

Results - The diversity of the tested conditions, leading to the establishment of different microbial communities according to the origins of the constitutive taxa (plant only, or plant and soil), highlighted the effect of *Z. tritici* on the wheat residue microbiome. Several microorganisms were affected by *Z. tritici* infection, even after the disappearance of the pathogen. Linear discriminant analyses and ecological network analyses were combined to

describe the communities affected by infection. The number of fungi and bacteria promoted or inhibited by inoculation with *Z. tritici* decreased over time, and was smaller for residues in contact with the soil. The interactions between the pathogen and other microorganisms appeared to be mostly indirect, despite the strong position of the pathogen as a keystone taxon in networks. Direct interactions with other members of the communities mostly involved fungi, including other wheat pathogens.

Conclusions - Our results provide essential information about the alterations to the microbial community in wheat residues induced by the mere presence of a fungal pathogen, and vice versa. Species already described as beneficial or biocontrol agents were found to be affected by pathogen inoculation. The strategy developed here can be viewed as a proof-of-concept for crop residues serving as a particularly rich ecological compartment, with a high diversity of taxa originating from both the plant and soil compartments, and for *Z. tritici*-wheat as a model pathosystem. By revealing putative antagonistic interactions, we demonstrate that this strategy can be used to improve the biological control of residue-borne diseases.

Keywords

Ecological interaction networks, metabarcoding, microbial communities, microbiome, pathobiome, Septoria tritici blotch, wheat residues.

Background

Septoria tritici blotch (STB) is one of the most important disease of wheat (*Triticum aestivum*), causing yield losses averaging 20% on susceptible wheat varieties and 5–10% on wheat varieties selected for disease resistance and sprayed with fungicide in Northwestern Europe [1]. It is caused by the hemibiotrophic, heterothallic, ascomycete fungus *Zymoseptoria tritici* [2], which initiates its sexual reproduction on senescent tissues [3]. STB is clonally propagated between wheat plants during the cropping season by pycnidiospores (asexual spores), which are splash-dispersed upwards over short distances. Wind-dispersed ascospores (sexual spores), mostly produced on wheat residues, initiate subsequent epidemics. Thus, wheat residues are a crucial, but often neglected determinant of the epidemiology of STB during the interepidemic period, as they support the sexual reproduction of the pathogen, maintaining diversity within populations and influencing adaptive dynamics in response to selection pressures [4], through the rapid evolution of fungicide resistance [5–8] or the breakdown of wheat resistance genes [9], for example.

The identification of microorganisms interacting with pathogens is an increasingly important issue for both academic and operational research on the development of biological control solutions [10,11]. In plant, animal and human epidemiology, increasing numbers of studies are trying to characterize variant microbial populations associated with specific disease stages, or temporal changes in the microbial populations during disease progression [12–14]. The pathogen and its cohort of associated microorganisms, which may influence its persistence, transmission and evolution, are together known as the “pathobiome” [15]. Pathobiome research has advanced significantly with the advent of high-throughput sequencing technologies, which have made it possible to describe and follow the diversity of

68 the microbial communities associated with the pathogen during its life cycle, during both the
69 epidemic and interepidemic periods.

70 The dynamics of microbial communities have been studied in detail during the
71 vegetative and reproductive stages of the plant life cycle, but very few studies during and after
72 plant senescence (e.g. [16,17]). The specific, central position of crop residues in agrosystems
73 was long neglected, but these residues should be seen as both a fully-fledged matrix and a
74 transient compartment: a compartment originating from the plant (temporal link), but in close
75 contact with the soil (spatial link), with variable rates of degradation over the following
76 cropping season, according to the plant species, the cropping practices used, and the climatic
77 conditions in the year concerned [16,19–21]. In addition, the rare studies focusing on the
78 evolution of microbial communities in crop residues performed to date were conducted in
79 microcosms, with sterilized residues (e.g. [22]), in which this compartment is much less
80 complex than under natural conditions.

81 Several studies have investigated the potential beneficial effects of microorganisms for
82 limiting the development of a plant pathogen during its saprophytic stage on natural crop
83 residues (e.g. *Aureobasidium pullulans* and *Clonostachys rosea* inhibiting the sexual stage of
84 *Didymella rabiei* on chickpea residues [23]; *Trichoderma harzianum* [24,25],
85 *Microsphaerelopsis* sp. [26], *C. rosea* [27,28] and Streptomyces sp. [29] reducing *Fusarium*
86 *graminearum* inoculum (perithecia, the sexual fruiting bodies) on wheat or maize residues, as
87 exhaustively summarized in [30]). Other studies have focused on the general impact of
88 cropping practices, such as the increase in microbial soil antagonists induced by the addition
89 of green manure to the soil (e.g. [19,31]). Some phyllosphere microorganisms selected for
90 their antifungal activity against *Z. tritici* (*Bacillus megaterium* [32]; *Pseudomonas fluorescens*
91 [33]; *Cryptococcus* sp., *Rhodotorula rubra* and *Penicillium lilacinum* [34]; *T. harzianum* [35];
92 *Trichoderma koningii* [36]) have been tested *in planta* against the asexual, pathogenic stage of

the pathogen (typically on wheat seedlings), but not against the pathogen during its sexual, saprophytic stage. Moreover, no microbial antagonists of *Z. tritici* have been isolated from wheat residues, despite the dense population of this habitat with a high diversity of microbial taxa [16].

The taxonomic structure of microbial communities associated with maize [17] and wheat [16] residues has recently been described under natural conditions. In addition to *Z. tritici*, the microbial communities associated with wheat include *Clonostachys* sp., *Aureobasidium* sp., *Chaetomium* sp. and *Cryptococcus* sp. [16], all of which are potential competitors. However, the presence of microorganisms in the same ecological niche, as highlighted in such descriptive approaches, does not necessarily mean that interactions actually occur between them. Many other non-interacting microorganisms (pathogens, endophytes) are also present on the residues. Moreover, microbial communities change during the physical degradation of the residues, probably modifying interactions between microorganisms over time [16]. Ecological network analysis has made it possible to detect putative interactions between microorganisms. For instance, Jakuschkin *et al*. [13] detected significant changes in foliar fungal and bacterial communities following the infection of pedunculate oak with *Ersysiphe alphitoides* (the causal agent of oak powdery mildew), and Cobo-Diaz *et al*. [17] identified candidate antagonists of toxigenic *Fusarium* spp. among the species present in maize residues. The use of co-occurrence networks in these two studies highlighted a set of bacteria and fungi that might be useful for managing plant pathogens.

In this study, our goal was to identify fungi and bacteria potentially interacting with *Z. tritici* during its sexual reproduction on wheat residues. To this end, we compared the structure of microbial communities associated with wheat residues with and without *Z. tritici* inoculation, by metabarcoding, combining linear discriminant analyses (LDA) and ecological network analyses. The response of microbial communities to *Z. tritici* infection was assessed

during the interepidemic period between two successive crops, for two sets of wheat residues, one left outdoors in contact with the soil, and the other left outside but not in contact with the soil, at different sampling dates during two consecutive years. The diversity of experimental conditions was expected to lead to the establishment of different microbial communities according to the origin of the constitutive taxa (plant or soil), thereby increasing the probability of detecting effects of *Z. tritici* on the residue microbiome, and of the residue microbiome on *Z. tritici*.

Results

Overall diversity of the bacterial and fungal communities on residues

The response of the residue microbiome to *Z. tritici* inoculation was assessed by analyzing the composition of the fungal and bacterial communities of wheat residues, after inoculation with *Z. tritici* (*n*=240) or in the absence of inoculation (*n*=240). We also investigated the impact of cropping season (*n*=2), season (*n*=4), and soil contact (*n*=2) on the dynamics of these communities (see materials and methods for a detailed explanation of the experimental design; **Figure 1**).

We investigated the structure of the residue microbiome by analyzing the v4 region of the 16S rRNA gene and ITS1. Overall, 996 bacterial amplicon sequence variants (ASVs) and 520 fungal ASVs were obtained from 390 and 420 samples, respectively. Some samples (July 2016) were removed from the analysis due to the co-amplification of chloroplasts.

The high relative abundance (RA) of ASVs affiliated to *Z. tritici* in samples collected in July 2016 (21.5±9.8%) and 2017 (30.3±7.1%) highlights successful colonization of the wheat tissues by this pathogen following inoculation (**Figure 2**). However, the RA of *Z. tritici* rapidly decreased to 2±1.64% and 1.4±0.9% on residues not in contact with the soil ("above

143 ground" residues) collected in October 2016 and 2017, respectively, and this species was
144 below the limit of detection in December and February. For residues in contact with soil, this
145 decrease occurred more rapidly, with *Z. tritici* already undetectable in samples collected in
146 October.

147 Alpha diversity, estimated with the Shannon index, was low in July for both bacterial
148 (2.70±0.75) and fungal communities (1.82±0.19; **Suppl. Figure 1**). A gradual increase was
149 then observed during residue degradation. *Z. tritici* inoculation had no impact on bacterial
150 alpha-diversity, but decreased fungal diversity (Kruskal-Wallis: $p = 0.008$). More specifically,
151 bacterial diversity was higher in inoculated residue samples in July 2017 (2.92±0.80 for
152 inoculated samples versus 2.47±0.6 for non-inoculated samples; Wilcoxon: $p = 0.022$), but no
153 such difference was detected for the other sampling dates. Conversely, for fungal
154 communities, inoculation had no effect in July, but led to a significant decrease in diversity in
155 subsequent months during the second cropping season (October and December 2017, for the
156 two soil contact conditions).

157 Beta diversity analysis (Bray-Curtis index) showed large dissimilarities between
158 bacterial community composition in July and at the other sampling dates, as illustrated in the
159 hierarchical clustering of the samples, justifying separate analyses and MDS representations
160 (**Figure 3**). Inoculation with *Z. tritici* had a minor effect on bacterial communities, with only
161 5% of the variance explained for samples collected in July (PERMANOVA: $p = 0.004$). By
162 contrast, in the same month, inoculation was the structuring factor for fungal communities,
163 accounting for 33% of the variance (PERMANOVA: $p = 0.001$). For subsequent samplings
164 (October, December and February), temporal conditions (seasonality and cropping season)
165 were the main factors influencing fungal communities. Soil contact was the main structuring
166 factor for bacterial communities, with a stronger effect than seasonality or cropping season
167 (**Table 1**).

168

169 **Impact of contact with the soil on microbial communities**

170 The significant impact of soil contact on microbial communities highlighted differences
171 in the process of wheat residue colonization. MDS analysis suggested that the communities of
172 "above ground" residue samples collected in October were less different from those collected
173 in July than from the communities of "soil contact" samples also collected in October **(Figure**
174 **4)**. Contact with the soil, therefore, caused a greater change in communities, suggesting
175 competition between plant-associated taxa and soil-borne taxa. Taxonomic differences
176 between the communities present on residues in contact with the soil and those present in
177 above ground residues were highlighted in linear discriminant analysis (LDA).

178 Some classes of taxa (e.g. *Bacilli*, *Sphingobacteria*, *Betaproteobacteria*,
179 *Dothideomycetes*, *Pezizomycetes*) were particularly abundant only in above ground residues,
180 suggesting that they were mostly derived from the plant. By contrast, other classes (e.g.
181 *Alphaproteobacteria*, *Agaricomycetes*, *Cytophagia*, *Gammaproteobacteria*) were more
182 prevalent in residues in contact with soil, suggesting that they originated from the soil (**Suppl.**
183 **Figure 2**). The abundance of some classes varied with cropping season (e.g. *Flavobacteria*).
184 Soil contact had a large impact for *Dothideomycetes* and *Bacilli*, which were highly abundant
185 in July, but rapidly decreased in frequency when the residues were in contact with the soil.
186 *Pezizomycetes*, absent in July, colonized only the above ground residues. Conversely, the
187 percentage of reads associated with *Alphaproteobacteria*, which was quite high in July, and
188 *Cytophagia*, which was low in July, increased over time, particularly in residues in contact
189 with the soil. Similarly, *Agarycomycetes*, which was completely absent in July, colonized
190 only residues in contact with the soil.

191 At the genus level, 87 (excluding "unclassified") of the 273 genera (60/190 for bacteria;
192 27/83 for fungi) identified displayed differences in abundance between above ground residues

and residues in contact with the soil, for at least one date (**Figure 4**). For example, *Bosea*, *Rhizobium*, *Nocardioides*, *Pseudomonas*, and *Sphingomonas* were more abundant in residues in contact with the soil, whereas *Cladosporium*, *Massilia*, *Paracoccus*, *Stagonospora* and *Cryptococcus* were more abundant in above ground residues.

Impact of *Z. tritici* inoculation on microbial communities

The influence of *Z. tritici* on the RA of residue microbiome members was assessed, through LDA scores. In total, the RA of 115 ASVs (74 bacterial ASVs and 41 fungal ASVs) was significantly affected by *Z. tritici* inoculation, for at least one sampling date (listed in **Suppl. Figure 3**). The effect of inoculation on microbial communities persisted throughout the experiment, despite the absence of *Z. tritici* detection from December onwards (**Figure 2**). ASVs with significant differences in RA decreased over time for residues in contact with the soil (**Suppl. Table 1**). By contrast, for above ground residues, the number of differential ASVs increased until December, in both cropping seasons (20 ASVs in December 2016-2017; 31 ASVs in December 2017-2018).

Inoculation with *Z. tritici* decreased the number of fungal ASVs, including those affiliated to *Sarocladium*, *Gibellulopsis* and *Blumeria*, and increased the number of bacterial ASVs affiliated to *Curtobacterium* and *Brachybacterium* (listed in **Suppl. Figure 3**). The ASVs affected by inoculation differed between above ground residues and residues in contact with soil. The pattern of change (i.e. promoted or inhibited by inoculation) was always the same within a given year, regardless of soil contact conditions. For example, *Brachybacterium* and *Curtobacterium* were promoted by inoculation, in both soil contact conditions, whereas *Sarocladium* was inhibited by inoculation, in both soil contact conditions.

Impact of the actual presence of *Z. tritici* on microbial communities

Microbial analysis networks combining bacterial and fungal datasets were used to predict the potential interactions between *Z. tritici* and members of microbial communities associated with wheat residues.

Dynamics of ecological interaction networks **–** The dataset was split according to the effects previously described (cropping season, seasonality, soil contact conditions). Six ecological interaction networks were generated per experimental year, corresponding to residue samples in contact with the soil and above ground residues, collected in October, December, and February (**Figure 5**). The networks for July are presented in **Suppl. Figure 4**. The mean number of nodes in the network (205.3±47.5) increased over the season (**Suppl. Table 1**). Overall, networks were very sparse, with a mean node degree of 2.76±0.43. For each network, the positive/negative edge ratio decreased over time, reaching 1.0-1.5 in February. Most nodes were common to October, December and February. *Z. tritici* was one of the fungi with the largest number of degrees and greatest betweenness (measurement of centrality in a graph based on the shortest paths) for above ground samples in October. By contrast, for samples in contact with soil, it was absent the first year and had low betweenness and degree values for the second year (**Figure 6**).

Subnetworks highlighting direct interactions between Z. tritici and other microorganisms **–** Ecological interaction networks were combined with LDA to investigate the interactions between *Z. tritici* and members of the microbial communities of residues (**Figure 7**). Only 13 of the 115 ASVs affected by inoculation (LDA) were in direct interaction with *Z. tritici*, indicating an indirect effect of *Z. tritici* on the community (no direct connection between the microorganisms).

Microorganisms with the same differential pattern (i.e. “promoted by inoculation” or “promoted in the absence of inoculation”) did not interact negatively with each other in networks. Conversely, microorganisms with opposite differential patterns systematically

243 interacted negatively with each other. These results highlight the consistency of the LDA and
244 ecological interaction network analysis approaches.

245 The subnetworks generated with microorganisms presenting differential relative
246 abundances and their adjacent nodes were strongly connected: each subnetwork consisted of a
247 principal component and, in some cases, smaller components of less than four nodes (**Figure**
248 **7**).

249 Only a few direct interactions between *Z. tritici* and other microorganisms were
250 highlighted in ecological interaction networks. Some ASVs affiliated to the same genus had
251 opposite interaction trends with *Z. tritici*, such as *Fusarium* ASVs in July 2017, or
252 *Cladosporium* ASVs in October 2016, consistent with the findings of LDA analyses. In some
253 cases, the same ASV had different interaction trends at different sampling dates or in different
254 years. This was the case for *Acremonium* ASVs (negative interaction in October 2016,
255 positive interaction in October 2017). Some genera, such as *Blumeria*, *Sarocladium*, and
256 *Penicillium*, interacted only negatively with *Z. tritici*. *Symmetrospora*, *Brachybacterium*, and
257 *Monographella* interacted only positively with *Z. tritici*.

258

259 ## Discussion

260

261 By sequencing the microbial communities of 420 samples of wheat residues, we
262 obtained a total of 996 bacterial ASVs and 520 fungal ASVs. Using this large dataset, we
263 estimated the potential interactions occurring between a plant pathogen (*Z. tritici*) and the
264 members of microbial communities associated with crop residues in field conditions. By
265 combining two approaches — LDA and network analysis — we were able to demonstrate an
266 effect of pathogen infection, even after disappearance of the pathogen, on the structure and
267 composition of the microbial communities during residue degradation.

268

269 **Effect of soil contact on microbial communities**

270 Our aim here was not to characterize the organisms colonizing wheat residues, but our
271 findings nevertheless highlight major changes in the microbial community over time for
272 residues in contact with soil. The taxa favored in above ground residues, such as
273 *Cladosporium*, *Alternaria*, *Pedobacter* and *Massilia*, were already present on the plant. This
274 is consistent with previous findings showing a decrease in the abundance of these plant-
275 associated taxa during the degradation of residues in contact with soil and the colonization of
276 these residues with soil-borne competitors, such as *Chaetomium*, *Torula*, and *Nocardioides*
277 [16]. Some fungal genera not present in July were favored by above ground conditions (e.g.
278 *Cryptococcus*, *Stagonospora*, and *Myrmecridium*). This finding is consistent with our
279 knowledge of fungal dispersal processes, mostly involving aerial spores.

280

281 **Disappearance of *Z. tritici* during residue degradation**

282 *Z. tritici* rapidly disappeared between October and December. This finding is surprising
283 in light of the quantitative epidemiological data acquired for the same plot, which suggested
284 that *Z. tritici* ascospores may be ejected from residues until March [3,37]. The observed
285 disappearance of *Z. tritici* may be due to lower levels of contamination of adult wheat plants
286 in residues than would be achieved in the field after natural infection. Indeed, in field
287 conditions, *Z. tritici* establishes itself on all parts of the plant (leaves, but also sheaths and
288 stems) through multiple secondary infections, driven by the repeated splash dispersal of
289 asexual spores, leading to an accumulation of contaminating raindrops at the points of
290 insertion of the leaf sheaths. The single inoculation event in the greenhouse resulted in
291 contamination principally of the leaves, the organs most exposed to spraying, with relatively
292 little contamination of the stems and sheaths, the parts of the plant most resistant to

293 degradation. Indeed, the results of a previous study [16] support this hypothesis: in the same
294 field, during the same season, *Z. tritici* was detected in wheat residues originating from plants
295 grown in natural conditions until February, and even May, with a similar metabarcoding
296 approach.
297
298 **Effect of *Z. tritici* on microbial communities**
299 Endophytes and pathogens induce changes in plant tissues (e.g. necrosis), which may
300 themselves modify the microbial communities inhabiting the plant (e.g. impact of secondary
301 saprophytes or opportunistic pathogens [38]; selection of microorganisms by secondary
302 metabolites produced by microorganisms or the plant; [39,40]). This general phenomenon
303 may explain the impact of *Z. tritici* on the microbial communities observed in both LDA and
304 network analysis. The impact of *Z. tritici* on residues, even after its disappearance between
305 October and December, persisted until February, particularly for fungal communities. Within
306 microbial networks, *Z. tritici* was one of the keystone taxa, despite its low abundance, in
307 above ground residues in October (**Suppl. Figure 5**). The high levels of *Z. tritici* in July
308 (between 10 and 40% of reads) account for its central position in the network. The number of
309 microorganisms displaying changes in abundance due to *Z. tritici* inoculation decreased
310 during residue degradation. This finding highlights the resilience of the community (i.e. its
311 ability to return to its original composition after a disturbance, in this case, *Z. tritici*
312 inoculation) [41].
313
314 **Specific interactions with *Z. tritici***
315 Most of the predicted interactions with *Z. tritici* involved fungi, such as *Fusarium*,
316 *Blumeria* or *Cladosporium*. *Z. tritici* infection has been shown to be associated with the
317 accumulation of H_2O_2 [42]. This compound is known to inhibit biotroph fungal pathogens

318 [43], such as *Blumeria graminis* [44,45]. This may explain the negative interaction between *Z.*
319 *tritici* and *B. graminis* in July and October 2017-2018. In addition, *Z. tritici* infection induces
320 leaf necrosis, potentially decreasing wheat susceptibility to *B. graminis*, due to a significant
321 physiological interaction during the latent, endophytic period of *Z. tritici* development [45].
322 H_2O_2 is also known to promote necrotrophic agents, such as *Fusarium*. We detected both
323 positive and negative interactions between *Z. tritici* and *Fusarium*, depending on the ASV
324 considered. On adult wheat plants, such differential interactions have been demonstrated in
325 log-linear analyses [46], with both species giving positive results on stem bases and negative
326 results on the upper parts of stems. Positive interactions between *Z. tritici* and *Cladosporium*
327 have also been demonstrated on adult plants [46], consistent with our findings for wheat
328 residues. These results lend a biological meaning to the interactions detected, confirming the
329 relevance of network analyses for highlighting ecological interactions within crop residue
330 communities.

331 *Trichoderma* was more abundant in residues from wheat plants inoculated with *Z. tritici*
332 (July 2016), as shown by LDA (**Suppl. Figure 4**). Conversely, *Epicoccum* and *Cryptococcus*
333 were more abundant in residues from non-inoculated wheat plants (October 2016). The
334 overabundance of those taxa, described as biocontrol agents in previous studies [34–36,47],
335 was influenced by the presence of the pathogen. However, no direct interactions between *Z.*
336 *tritici* and these species could be established. This exemplifies the difficulties highlighting
337 beneficial species within complete microbial communities. These difficulties are not specific
338 to the residue compartment and also apply to the spermosphere [48], phyllosphere [49] and
339 rhizosphere compartments [14,50].

340

341 **Other interactions**

342 Other interactions between ASVs highlighted in the network analysis were examined in
343 light of published results for fungal pathogens of cereals. For instance, it has already been
344 shown that *B. graminis* growth on barley is inhibited by *Trichoderma harzianum* [51] and
345 *Stagonospora norodum* [52], that *Stenetrophomas maltophila* attenuates the seedling blight of
346 wheat caused by *F. graminearum* [53], that *Acremonium zeae* has antibiotic activity against
347 *Fusarium verticillioides* [54], and that *Chaetomium sp.* produces compounds (e.g. chaetomin)
348 active against *Alternaria triticimaculans* [34]. Conversely, certain non-pathogenic bacteria
349 were shown to be associated with significantly more disease on wheat caused by *B. graminis*
350 and *Z. tritici* and to "help" *Phaeosphaeria nodorum* to infect wheat tissues [55]. Newtoon *et*
351 *al*. [38] has proposed the hypothesis of "induced susceptibility" to explain such an interaction
352 between bacteria and biotroph fungal pathogens.

353 The analysis of ecological network also suggested that intra-kingdom interactions were
354 favoured over inter-kingdom interactions in certain conditions (**Suppl. Table 2**). This may
355 reflect differences in ecological niches and dynamics, as illustrated by the temporal changes
356 in microbial communities over a season, with a densification of the networks during residue
357 degradation. Further investigations are required to determine whether inter- or intra-kingdom
358 interactions are more intense, and thus more promising for use in biocontrol engineering.
359 Should we preferentially focus on fungal communities to improve the management of a fungal
360 disease, and on bacterial communities to improve the management of a bacterial disease?
361

362 **Identification of beneficial species, and potential biocontrol agents**

363 Network models provide new opportunities for enhancing disease management and can
364 be helpful for biocontrol. Our study, combining LDA and ecological network analyses based
365 on a metabarcoding approach and differential conditions (plants inoculated with a pathogen or
366 left non-inoculated; plant residues in contact with soil vs. residues not in contact with the

soil), fits into the framework described by Poudel *et al.* [56], which considers several types of network analyses, including pathogen-focused analyses, taking into account diseased and healthy plant hosts, with a view to elucidating direct and indirect pathogen-focused interactions within the pathobiome.

Network analyses revealed no significant direct interactions between *Z. tritici* and microorganisms reported to be useful biocontrol agents. However, pathogen infection had a strong effect on the entire microbial community present in residues during the course of their degradation. Most of the interactions were difficult to interpret. Several interactions appeared to be transient, changing over time with residue degradation, and their presence or absence depended on whether the residues were in contact with the soil. This suggests that interactions between microorganisms are not very stable, and can be modified by changes in the environment, for example, or by the arrival of a new microorganism.

The neglect of complex interactions between biocontrol agents and their biotic environment (the plant, the soil and their microbiomes), the physical and chemical properties of which change over time, may account for lower levels of efficacy in field conditions than in laboratory conditions (concerning the phyllosphere, e.g. [38], but also the residue compartment, e.g. [57]). Indeed, several studies have demonstrated the value of studying the effect of entire communities on biotic and abiotic stresses rather than the effects of single species. For example, resistance to *B. cinerea* in *Arabidopsis thaliana* was shown to be not due to a single species, but to the action of the microbiome as a whole [58]. By comparing the structure of microbial communities associated with *Brassica rapa* plants inoculated with the root pathogen *Plasmodiophora brassicae*, Lebreton *et al*. [14] showed significant shifts in the temporal dynamics of the root and rhizosphere microbiome communities during root infection. Moreover, the rhizospheres of plants infected with *P. brassicae* were significantly

391 more frequently colonized with a *Chytridiomycota* fungus, suggesting interactions between
392 these two microorganisms.

393 The most frequently studied cases of microbial community effects include “suppressive
394 soils”, which provide defense against soil-borne pathogens, rendering them unable to establish
395 themselves or to persist in the soil or the plant [59]. The basis and dynamics of this disease
396 suppression vary, and suppression may be general or specific, under the control of antibiotic-
397 producing *Pseudomonas* or *Streptomyces* populations, for example [60]. Differences in the
398 composition, structure and diversity of microbial communities on crop residues remain poorly
399 understood, and further studies are required to determine the potential for use in biocontrol
400 not of single agents, but of microbial communities, as for these suppressive soils. Despite this
401 ecological reality, the current perception of biocontrol engineering is still too often limited to
402 the action of a single species, even a single strain, with a direct, strong and durable effect
403 against a plant pathogen.

404

405 **Potential utility of the residue microbiome**

406 Improving our understanding of the relationship between biodiversity and ecosystem
407 functioning will require the development of methods integrating microorganisms into the
408 framework of ecological networks. Exhaustive descriptions of microbial diversity combined
409 with network analysis are particularly useful for identifying species within microbial
410 communities of potential benefit for disease management [56]. By revealing antagonistic
411 interactions between pathogen species (e.g. *Z. tritici*) and other microorganisms, our study
412 suggests that this strategy could potentially improve the control of residue-borne diseases, as
413 suggested by another recent study on *Fusarium* [17]. This strategy, which has been developed
414 separately for the plant [61,62] and soil [14,50,63] compartments, would undoubtedly benefit
415 from further development on crop residues. Indeed, decreasing the presence of pathogens on

residues during the interepidemic period can decrease disease development on subsequent crops [21]. The strategy developed here can be viewed as a proof-of-concept for the use of crop residues as a particularly rich ecological compartment containing a high diversity of taxa originating from both the plant and soil compartments, and for the use of *Z. tritici*-wheat as a model pathosystem. Understanding the complex interactions between a pathogen, crop residues and other microbiome components in the shaping of a plant-protective microbiome is essential, to improve the efficacy of biocontrol agents and to preserve existing beneficial equilibria through the adoption of appropriate agricultural practices.

Methods

We investigated the effect of *Z. tritici* on the diversity of the wheat microbiome and the effect of the wheat microbiome on *Z. tritici*, by characterizing the composition of the microbial communities of 420 residue samples (210 per year) from plants with and without preliminary *Z. tritici* inoculation. The residues were placed outdoors, either directly in contact with the soil in a field plot or “above ground”, i.e. not in contact with the soil, to assess the effect of their colonization by microorganisms originating from the soil, the plant and the air on the saprophytic development of *Z. tritici*. We investigated the persistence of interactions between the pathogen and the whole microbial community, and changes in those interactions over time, by sampling the residues before exposure to outdoor conditions (in July), and every two months thereafter (in October, December, and February) (**Figure 1**).

Preparation of wheat residues

The 420 wheat residue samples were obtained from 60 winter wheat cv. Soissons plants grown in a greenhouse in each of the two years of the study, as described in [64]: two weeks

after sowing, seedlings were vernalized for eight weeks in a growth chamber and then transplanted into pots. Three stems per plant were retained. Half the wheat plants were inoculated with a mixture of four *Z. tritici* isolates (two Mat1.1. isolates and two Mat1.2 isolates; [65]) to ensure that sexual reproduction occurred as in natural conditions. This equiproportional conidial suspension was prepared and adjusted to a concentration of 2×10^5 spores.mL^{-1}, as previously described [64]. Thirty plants were inoculated at the late heading stage in early May, by spraying with 10 mL of inoculum suspension. The other thirty plants were sprayed with water, as a control. Inoculated and non-inoculated plants were enclosed in transparent plastic bags for three days to ensure moist conditions favoring pathogen infection. Septoria tritici blotch lesions appeared three to four weeks after inoculation (**Figure 1A**). All plants were kept in the same greenhouse compartment until they reached complete maturity (mid-July).

For each "inoculated" and "non-inoculated" condition, stems and leaves were cut into 2 cm-long pieces and homogenized to generate the "wheat residues", which were then distributed in 105 nylon bags (1.4 g per bag; **Figure 1B**) for each set of inoculation conditions, in each year.

Exposure of residues to natural conditions

Ninety nylon bags were deposited in contact with the soil in a field plot (the "soil contact" treatment) or without contact with the soil ("above ground" residue treatment). Thirty batches of residues (15 inoculated and 15 non-inoculated) were used to characterize the communities present in July before the exposure of the residues in the nylon bags to natural conditions. The field plot ("OWO" in [16]; Grignon experimental station, Yvelines, France; 48°51′N, 1°58′E) was the same in both cropping seasons. It was sown with wheat in 2015-2016, with oilseed rape in 2016-2017, and with wheat in 2017-2018. The 90 bags for the "soil

466 contact" treatment were deposited in the OWO field plot (**Figure 1C**) in late July, at 15
467 sampling points 20 m apart (three "inoculated" and three "non-inoculated" bags at each
468 sampling point). The 90 bags of the "above ground" treatment were placed on plastic grids
469 exposed to outdoor conditions and located about 300 m from the OWO field plot (**Figure**
470 **1D**).

471 We assessed the impact of seasonality on the fungal and bacterial communities on
472 residues by collecting samples of each "inoculated" and "non-inoculated" treatment at three
473 dates (October, December and February): 15 bags from plastic grids ("above ground"
474 treatment) and one bag from each sampling point in the field ("soil contact" treatment) At
475 each date, nylon bags were opened, the residues were rinsed with water and air-dried in
476 laboratory conditions. Residues were then crushed with a Retsch™ Mixer Mill MM 400 for
477 60 seconds at 30Hz with liquid nitrogen in a Zirconium oxide blender.

478

479 **Total DNA extraction**

480 Total DNA was extracted with the DNeasy Plant Mini kit (Qiagen, France), with a
481 slightly modified version of the protocol recommended by the manufacturer. Powdered
482 residues (20 mg), 450 µL of Buffer AP1 preheated to 60°C, RNase A and Reagent DX (450:
483 1: 1) were mixed vigorously for 15 s in a 2 mL Eppendorf tube. Buffer P3 (130 µL) was
484 added to each tube, which was then shaken manually for 15 s, incubated at -20°C, and
485 centrifuged (1 min, 5000 g). The supernatant (450 µL) was transferred to a spin column and
486 centrifuged (2 min, 20000 g). The filtrate (200 µL) was transferred to a new tube, to which
487 sodium acetate (200 µL, 3 M, pH 5) and cold 2-propanol (600 µL) were added. DNA was
488 precipitated by incubation at -20°C for 30 min and recovered by centrifugation (20 min,
489 13000 g). The pellet was washed with cold ethanol (70%), dried, and dissolved in 50 µL of
490 AE buffer.

491

492 **PCR and Illumina sequencing**

493 Fungal and bacterial communitiesprofiles were analyzed by amplifying ITS1 and the v4
494 region of the 16S rRNA gene, respectively. Amplifications were performed with ITS1F/ITS2
495 [66] and 515f/806r [67] primers. All PCRs were run in a total volume of 50 µL, with 1x
496 Qiagen Type-it Multiplex PCR Master Mix (Type-it® Microsatellite PCR kit Cat
497 No./ID: 206243), 0.2 µM of each primer, 1x Q-solution® and 1 µl DNA (approximately 100
498 ng). The PCR mixture was heated at 95°C for 5 minutes and then subjected to 35 cycles of
499 amplification [95°C (1 min), 60°C (1 min 30 s), 72°C (1 min)] and a final extension step at
500 72°C (10 min). PCR products were purified with Agencourt® AMPure® XP (Agencourt
501 Bioscience Corp., Beverly, MA). A second round of amplification was performed with 5 µl of
502 purified amplicons and primers containing Illumina adapters and indices. PCR mixtures were
503 heated at 94°C for 1 min, and then subjected to 12 cycles of amplification [94°C (1 min),
504 55°C (1 min), 68°C (1 min)] and a final extension step at 68°C (10 min). PCR products were
505 purified and quantified with Invitrogen QuantIT™ PicoGreen®. Purified amplicons were
506 pooled in equimolar concentrations, and the final concentration of the library was determined
507 with the qPCR NGS library quantification kit (Agilent). Libraries were sequenced in four
508 independent runs with MiSeq reagent kit v3 (600 cycles).

509

510 **Sequence processing**

511 Runs were analyzed separately. Primer sequences were first cut off in the fastq files
512 with Cutadapt [68]. Files were then processed with DADA2 v.1.8.0 [69] according to the
513 recommendations for the “DADA2 Pipeline Tutorial (1.8)” workflow [70], with quality
514 trimming adapted for each run (**Suppl. Table 3**).

A mock sample consisting of equimolar amounts of DNA from known microorganisms was included in each run (see **Suppl. Figure 6**) to establish a detection threshold for spurious haplotypes. At a threshold of ≤ 0.3 ‰ of the size of the library, amplicon sequence variants (ASVs) were considered spurious and were removed from the sample. We used the naive Bayesian classifier on RDP trainset 14 [71] and the UNITE 7.1 database [72] to assign ASVs. ASVs as assigned to chloroplasts (for bacteria) or unclassified at the phylum level (for bacteria and fungi) were also removed from each sample. Due to large proportion of chloroplast sequences among the 16S rRNA gene products, all samples from July 2017 were removed from the analysis.

Differential community analysis

For microbial community analyses, the total library size of each sample was standardized by normalization by proportion. The experimental conditions taken into account were cropping season (2016-2017 and 2017-2018), seasonality (four sampling dates: July, October, December, and February), inoculation with *Z. tritici* (inoculated and non-inoculated), soil contact (soil contact and above ground treatments). The Shannon diversity index was used to assess the effect of each set of conditions on fungal and bacterial diversity. The divergence of microbial communities between samples was assessed by calculating the Bray-Curtis dissimilarity matrix with the phyloseq package (v 1.24.2 [73]), and then illustrated by MDS and clustering based on the average linkage method (ape package v 5.2. [74]). PERMANOVA was performed with the “margin” option, to test the effect of each factor on communities (adonis2 function, vegan package [75]).

A linear discriminant analysis (LDA) implemented in Galaxy [76] (LefSe, http://huttenhower.org/galaxy) was used to characterize the differential abundances of fungal and bacterial taxa between each soil contact condition and each *Z. tritici* inoculation

condition. In this analysis, differences in the relative abundance of taxa between treatments were evaluated with a Kruskal-Wallis test; a Wilcoxon test was used to check, by pairwise comparisons, whether all subclasses agreed with the trend identified in the Kruskal-Wallis test. The results were used to construct an LDA model, to discriminate between taxa in the different conditions. For the comparison between "soil contact" and "above ground" treatments, inoculation condition was used as a subclass, with the Wilcoxon test alpha value set at 0.05, and the alpha value of the Kruskal-Wallis test set at 0.01. For the comparison between "inoculated" and "non-inoculated" treatments, the alpha value of the Kruskal Wallis test was set at 0.01 (no subclasses). For both analyses, the threshold for the LDA analysis score was set at 2.0.

Ecological interaction network analyses

For characterization of interactions within the different wheat residue microbial communities, we used ecological interactions networks calculated with SPIEC-EASI [77] for combined bacterial and fungal datasets [78]. The same parameters were used for all networks. The non-normalized abundance dataset was split on the basis of sampling date and soil contact condition. Each of the datasets included 30 samples (15 inoculated samples and 15 non-inoculated samples). Infrequent ASVs were filtered out by defining a threshold of a minimum of six occurrences. We used the neighborhood selection as graphical inference model (Meinshausen and Bühlmann MB method) with SPIEC-EASI, as this method has been shown to outperform most of the other available methods (e.g. CCREPE, SPARCC, SPIEC-EASI (glasso)) [77]. The StARS variability threshold was set at 0.05. Networks were then analyzed with the igraph package (version 1.2.2. [79]). Scripts for network construction and analysis are available from GitHub (see Availability of data and materials).

Subnetworks for analysis of the *Z. tritici* pathobiome

We used a dual approach to characterize interactions between *Z. tritici* and the other taxa, based on: (i) the LDA scores obtained in differential analyses between *Z. tritici* inoculation conditions ("inoculated" and "non-inoculated" treatments); (ii) ecological network analysis. LDA identified taxa affected by inoculation conditions (definition of classes for samples) and network analysis identified interactions at the sample scale (without prior assumptions). Subnetworks of differential ASVs and their adjacent nodes were established by combining these two approaches. Subnetworks were visualized with Cytoscape V. 3.6.1 [80]

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Availability of data and materials

The raw sequencing data are available from the European Nucleotide Archive (ENA) under study accession number PRJEB31818. We provide the command-line script for data analysis and all necessary input files via GitHub (https://github.com/LydieKerdraon/MicrobialNetworkAnalysis-WheatResidues).

Authors' contributions

LK, FS, VL, MB conceived the study, participated in its design, and wrote the manuscript. LK conducted the experiments and analyzed the data. FS and VL supervised the project. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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822

Figure and table captions

Figure 1. Preparation of wheat residues.

(A) Adult wheat plants were inoculated with *Zymoseptoria tritici* under greenhouse conditions.

(B) Sealed nylon bags containing wheat residues, consisting of stem and leaf fragments of approximately 2 cm in length (red yarn for residues from wheat plants inoculated with *Z. tritici*; white yarn for those from non-inoculated plants).

(C) "Soil contact" treatment: nylon bags were left on the ground of the field and partially covered with soil (one of the 15 sampling points).

(D) "Above ground" treatment: plastic grids containing nylon bags placed outside the field.

Figure 2. Relative abundance of *Zymoseptoria tritici*. Each box represents the distribution of the relative abundances of genera for the 15 sampling points. Wilcoxon tests were performed for inoculation condition (NS: not significant; * *p*-value<0.05; ** *p*-value <0.01; *** *p*-value <0.001).

Figure 3. Dissimilarities between microbial communities. Beta diversity analyses for fungal (A, B) and bacterial (C, D) communities originating from 420 wheat residue samples. Hierarchical clustering (A, C) and multidimensional scaling (B, D) are based on the compositional distances between samples (Bray Curtis distance matrix).

(A, C) Visualization of compositional distances between samples through hierarchical clustering with the average linkage method. The samples (15 sampling points per treatment) corresponding to the two cropping seasons (year) are represented by the two colored horizontal series (2016-2017, 2017-2018). Effects of seasonality are highlighted by different

848 colours, corresponding to the different sampling dates (July: green; October: red; December:
849 blue; February: gray). The intensity of the colors distinguishes between samples obtained
850 from plants inoculated with *Z. tritici* (I, dark hues) and non-inoculated samples (NI, light
851 hues). “Above ground” and “soil contact” treatments are represented by horizontal lines, with
852 each sample considered separately.
853 (B, D) Visualization of compositional distances between samples through multidimensional
854 scaling (MDS). Each data point corresponds to one sample of wheat residues. The shape of
855 the points (circles: 2016-2017; triangles: 2017-2018) corresponds to the cropping season (year
856 effect); the colors, similar to those used in graphs A and C, correspond to the sampling dates
857 (seasonality effect). For fungal communities, MDS analysis was performed on all samples
858 together, whereas for bacterial communities, the analyses of the July samples and samples
859 from all other sampling dates (October, December, and February) were separated, in
860 accordance with the large differences between the communities of these samples shown in the
861 clustering analysis (C). For a sake of clarity, the MDS are shown according to the soil contact
862 condition.
863
864 **Figure 4.** Changes in the relative abundance of microbial taxa over time.
865 (A, C) Diversity and dominance of the 30 most abundant (30/107) fungal genera (A) and the
866 50 most abundant (50/189) bacterial genera (B) distributed in all samples distinguishing
867 between the different experimental conditions: i.e. cropping season (2016-2017; 2017-2018),
868 contact with soil (“above ground” and “soil contact” treatments), seasonality (July: green;
869 October: red; December: blue; February: gray), and inoculation with *Zymoseptoria tritici*
870 (inoculated: dark hues; non-inoculated: light hues).
871 (B, D) Significant differences in relative abundance of fungal (B) and bacterial (D) genera
872 between the samples in “soil contact” (red) and “above ground” (blue) samples in linear

discriminant analysis (LDA). The *Z. tritici* inoculation condition was used as a subclass to avoid interference in the LDA. Only genera with a *p*-value < 0.05 for the Kruskal-Wallis test and an LDA score > 2 are displayed.

Figure 5. Temporal dynamics of co-occurrence networks.

(A) Networks based on bacterial and fungal ASVs combined. In all networks, circles and squares correspond to bacterial and fungal ASVs, respectively, with colors representing class. Isolated nodes are not shown. Edges represent positive (green) or negative (red) interactions. The Venn diagram highlights the number of non-isolated nodes common and specific to "above ground" (AG) and "soil contact" (SC) treatments for each sampling date (October, December, February).

(B) Percentage of reads associated with fungal and bacterial classes for each network. Isolated nodes are included. Colors are the same as in (A). (C) Upset plot of bacterial and fungal non-isolated nodes common and specific to sampling date for each treatment.

Figure 6. Betweenness, centrality and degree of each ASV in the October networks. Nodes with high betweenness, centrality and high degree values are considered to be keystone taxa in the networks. The genera of the fungal and bacterial ASVs with the highest degree and centrality are shown: *Acrem(onium); Clado(sporium); Devos(ia); Epico(ccum); Frond(ihabitans); Myrme(cridium); Neorh(izobium); Pedob(acter); Rhizo(bium); SphiG(=Sphingomonas); Strep(tomyces); Uncl.(assified); Zymos(eptoria).* The betweenness, centrality and degree of each ASV in the networks for the other sampling dates (July, December, and February) are presented in **Supplementary Figure 5**.

Figure 7. Subnetworks based on the data in Figure 4A and composed of differential bacterial and fungal ASVs identified in residue samples (originating from wheat plants inoculated and non-inoculated with *Zymoseptoria tritici*) and of the first adjacent nodes. Node color corresponds to the results of LefSe differential analysis between inoculated (orange) and non-inoculated (blue) treatments. Only genera with *p*-values < 0.01 for the Kruskal-Wallis tests and LDA scores > 2 were retained for the plot. The first adjacent nodes of each differential ASV are not named, except for ASVs interacting with *Z. tritici*. Edges represent positive (green) or negative (red) interactions. Differential ASVs are plotted with genus name abbreviations: *Acido(vorax); Acrem(onium); Aerom(icrobium); Alkal(ibacterium); Alter(naria); Aquab(acterium); Arthr(obacter); Blume(ria); Botry(osporium); Brach(ybacterium); Brevi(bacterium); Brevu(ndimonas); Chaet(omium); Chrys(eobacterium); Clado(sporium); Crypt(ococcus); Curto(bacterium); Desem(zia); Devos(ia); Epico(ccum); Falsi(rhodobacter); Flavo(bacterium); Frond(ihabitans); Fusar(ium); Gibel(lulopsis); Halom(onas); Massi(lia); MethB(=Methylobacterium); MethP(=Methylophilus); Monog(raphella); Neorh(izobium); Neose(tophoma); Nocar(dioides); Novih(erbaspirillum); Panto(ea); Parac(occus); Param(icrothyrium); Pedob(acter); Penic(illium); Phaeo(sphaeria); PhaeP(sphaeriopsis); Plano(coccus); PlanM(=Planomicrobium); Pseud(omonas); PseuP(=Pseudopithomyces); Rhizo(bium); Rhoda(nobacter); Salin(irepens); Sangu(ibacter); Saroc(ladium); SphiB(=Sphingobium); SphiG(=Sphingomonas); Stago(nospora); Steno(trophomonas); Symme(trospora); Terri(bacillus); Torul(a); Trich(oderma); Uncl.(assified); Vario(vorax); Zymos(eptoria).*

Table 1. Results of PERMANOVAs analyzing the effects of cropping season, sampling date, contact with soil and inoculation on fungal and bacterial communities. Factors were tested

921 with the adonis2 function of the vegan package. PERMANOVAs were performed with all
922 tested factors together, with the “margin” option.
923

Additional files

Supplementary Table 1. Sequence filtering for each run.

Supplementary Table 2. Analysis of the proportion of intra-kingdom interactions (between two fungal ASVs and between two bacterial ASVs) and inter-kingdom interactions (between a fungal ASV and a bacterial ASV) in the ecological networks. The statistical significance of the under- or over-representation of inter-kingdom interactions (when F-B residuals < 1 or > 1, respectively) was established by a χ^2 test of independence performed on the contingency table ($\chi^2 < 0.001$).

Supplementary Table 3. Number of ASVs detected for each analysis performed on the dataset and properties of residue microbial ecological networks.

Supplementary Figure 1. Alpha diversity of microbial communities associated with residues. Observed richness (number of ASVs) and diversity (Shannon index), in four sets of experimental conditions (cropping season, contact with soil, seasonality, *Zymoseptoria tritici* inoculation). Each box represents the distribution of the number of ASVs and Shannon index for 15 sampling points per treatment. Wilcoxon tests were performed for inoculation condition (inoculated, non-inoculated) and sampling date (July, October, December, February; NS: not significant; * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001).

Supplementary Figure 2. Seasonal shift, from October to February, in the relative abundance of a selection of bacterial (A) and fungal (B) classes present on wheat residues (originating from wheat plants inoculated and not inoculated with *Zymoseptoria tritici*) according to cropping season (2016-2017, 2017-2018) and soil contact condition (in contact with the soil

950 or above ground). Each box represents the distribution of class relative abundances for the 15
951 sampling points per treatment. Wilcoxon tests were performed for soil contact condition (NS:
952 not significant; * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001).
953
954 **Supplementary Figure 3.** Significant differences in the dominance of fungal and bacterial
955 genera between wheat residues originating from inoculated (orange) and non-inoculated
956 (blue) wheat plants in linear discriminant analyses (LDA), according to three sets of
957 experimental conditions (cropping season, soil contact, seasonality). Only ASVs with *p*-
958 values < 0.01 for the Kruskal-Wallis test and LDA scores > 2 are displayed.
959
960 **Supplementary Figure 4.** Residue microbial ecological networks based on bacterial and
961 fungal ASVs combined for July (no contact with soil) for each cropping season (2016-2017,
962 2017-2018). Circles and squares correspond to bacterial and fungal ASVs, respectively, with
963 colors represent classes. Isolated nodes are not shown. Edges represent positive (green) or
964 negative (red) interactions.
965
966 **Supplementary Figure 5.** Betweenness, centrality and degree of each ASV in the networks.
967 Nodes with high betweenness, centrality and high degree values are considered to be keystone
968 taxa in the networks. The genera of the fungal and bacterial ASVs with the highest degree and
969 centrality are indicated: *Acrem(onium); Actin(oplanes); Aquab(acterium); Artic(ulospora);*
970 *Brevi(bacterium); Clado(sporium); Devos(ia); Epico(ccum); Frond(ihabitans); Massi(lia);*
971 *Mesor(hizobium); MethP(=Methylophilus); Myrme(cridium); Neorh(izobium);*
972 *Nocar(dioides); Pedob(acter); Phaeo(sphaeria); PlanM(=Planomicrobium);*
973 *Promi(cromonospora); Pteru(la); Rhizo(bium); Schiz(othecium); SphiG(=Sphingomonas);*
974 *SphiP(=Sphingopyxis); Strep(tomyces); Torul(a); Uncl.(assified); Zymos(eptoria).*

Supplementary Figure 6. Mocks analysis for the two fungal sequencing runs (A, C) and the two bacterial sequencing runs (D, F).

(A, D) Composition of the mocks. All microbial DNAs were pooled at equimolar concentrations.

(B, E) Filter on the relative abundance of ASVs. The library size was normalized by proportion before analysis. The red line corresponds to a threshold at 3 ‰ of the size of the library.

(C, F) ASVs detected in each mock. The 40 most abundant fungal ASVs are indicated (C), whereas all bacterial ASVs are indicated (F). The name of the ASVs corresponds to the taxonomic affiliation to the genus. All genera present in fungal mocks were detected (*Gibberella* and *Fusarium* are synonymous), while some bacterial genera were not detected in bacterial mocks, which differed only from one ASV. The red line corresponds to a threshold at 3 ‰ of the size of the library.

Table 1 - Results of the PERMANOVAs analyzing the effects of cropping season, sampling date, contact with soil and inoculation on fungal and bacterial assemblages. Factors were tested with the adonis2 function of the vegan package. PERMANOVAs were performed with all tested factors together, with the "margin" option

		Factors tested	Proportion of the variance explained	*p*-value
Fungi	July	Season	0.197	0.001
		Inoculation	0.333	0.001
	Oct. - Dec. - Feb.	Season	0.217	0.001
		Sampling date	0.136	0.001
		Contact with soil	0.096	0.001
		Inoculation	0.012	0.001
Bacteria	July	Season	0.192	0.001
		Inoculation	0.051	0.004
	Oct. - Dec. - Feb.	Season	0.128	0.001
		Sampling date	0.168	0.001
		Contact with soil	0.195	0.001
		Inoculation	0.006	0.001

Figure 1

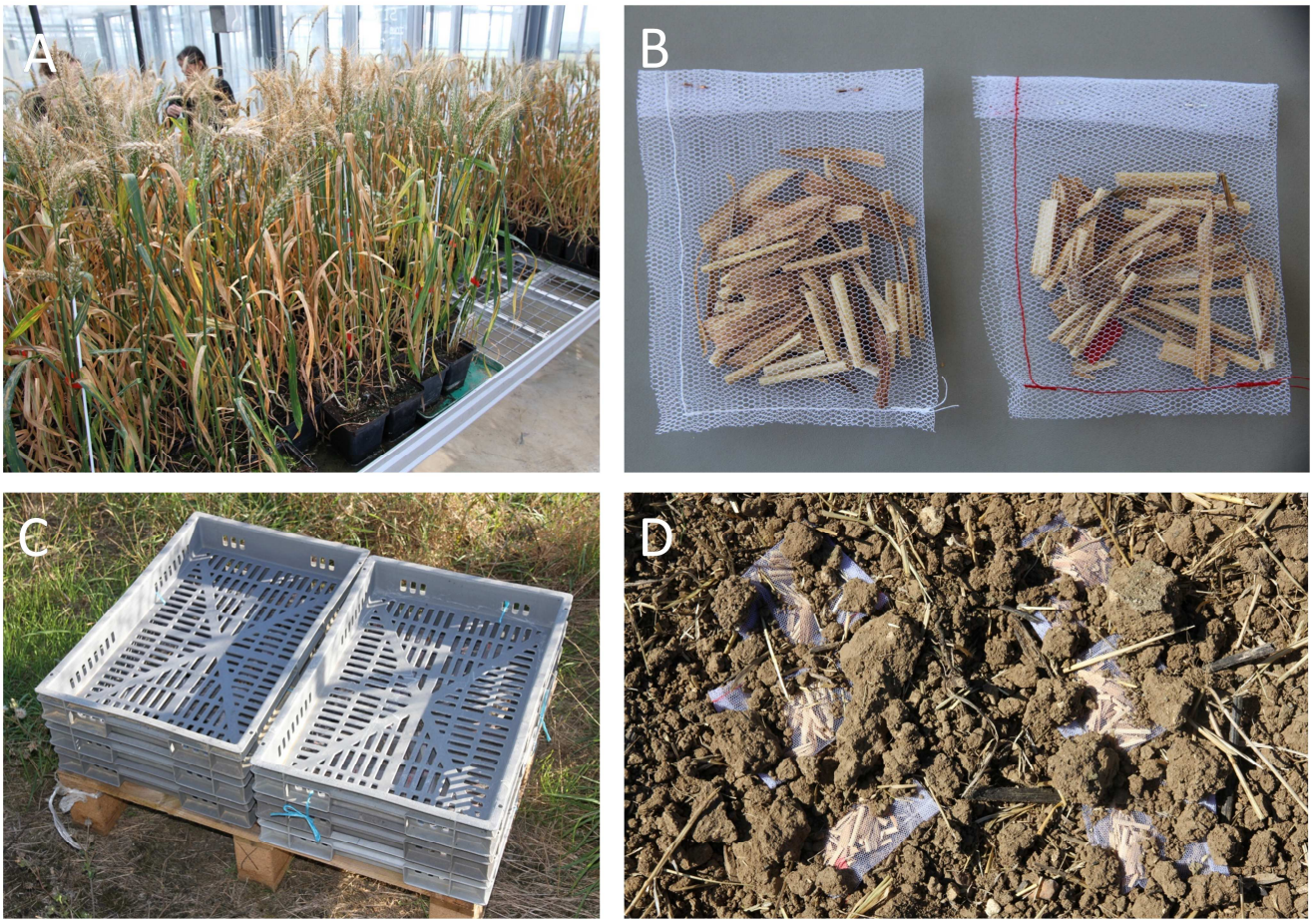


Figure 2

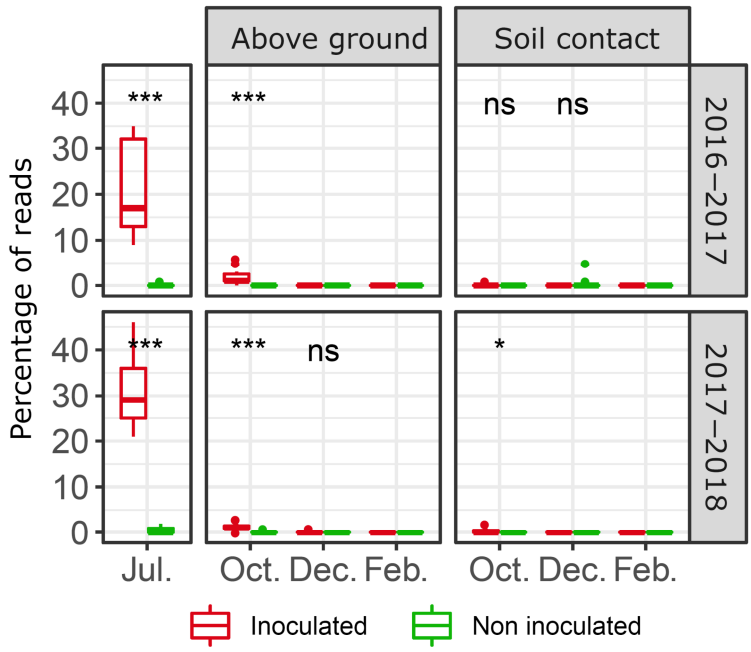


Figure 3

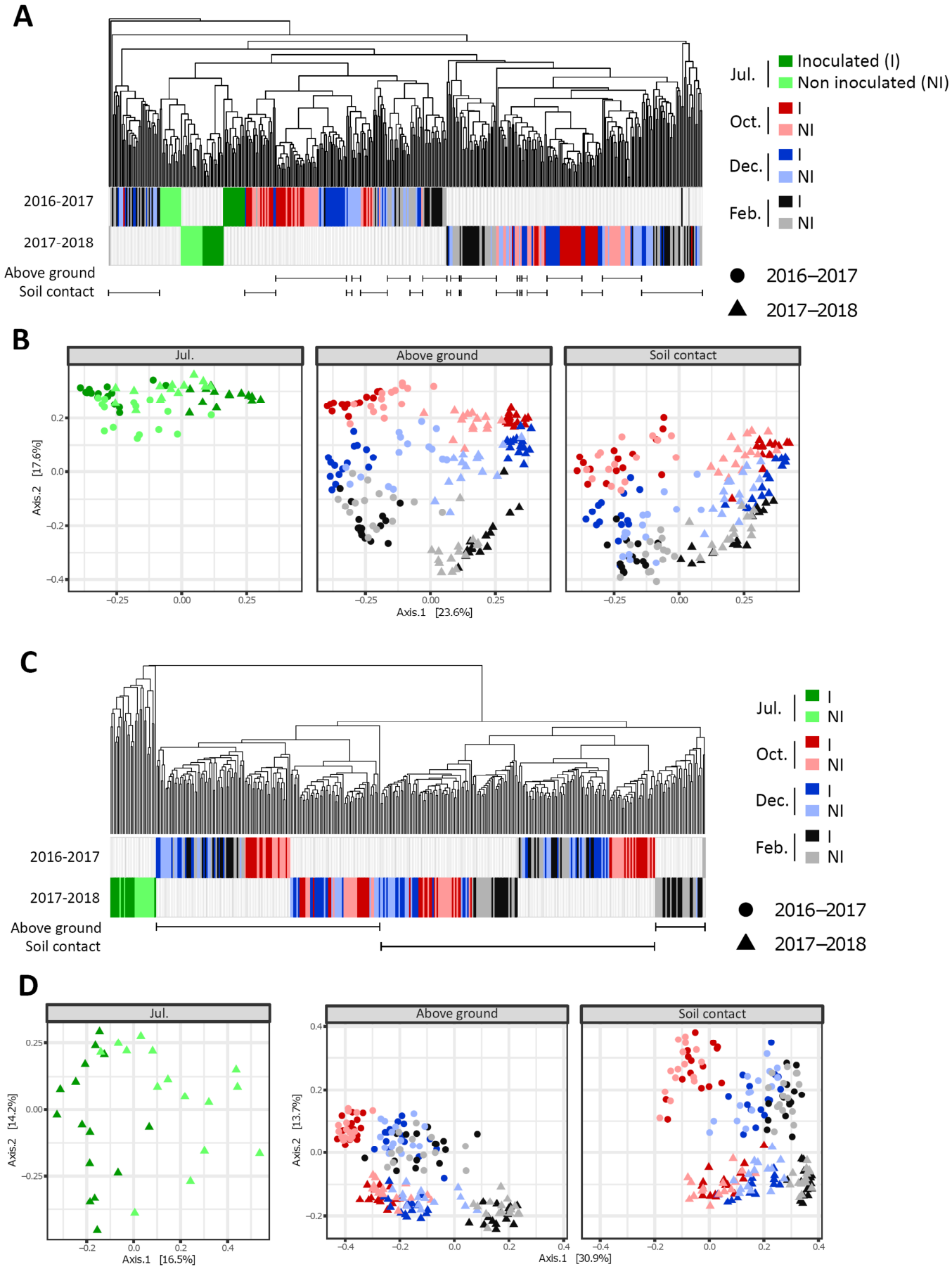


Figure 4

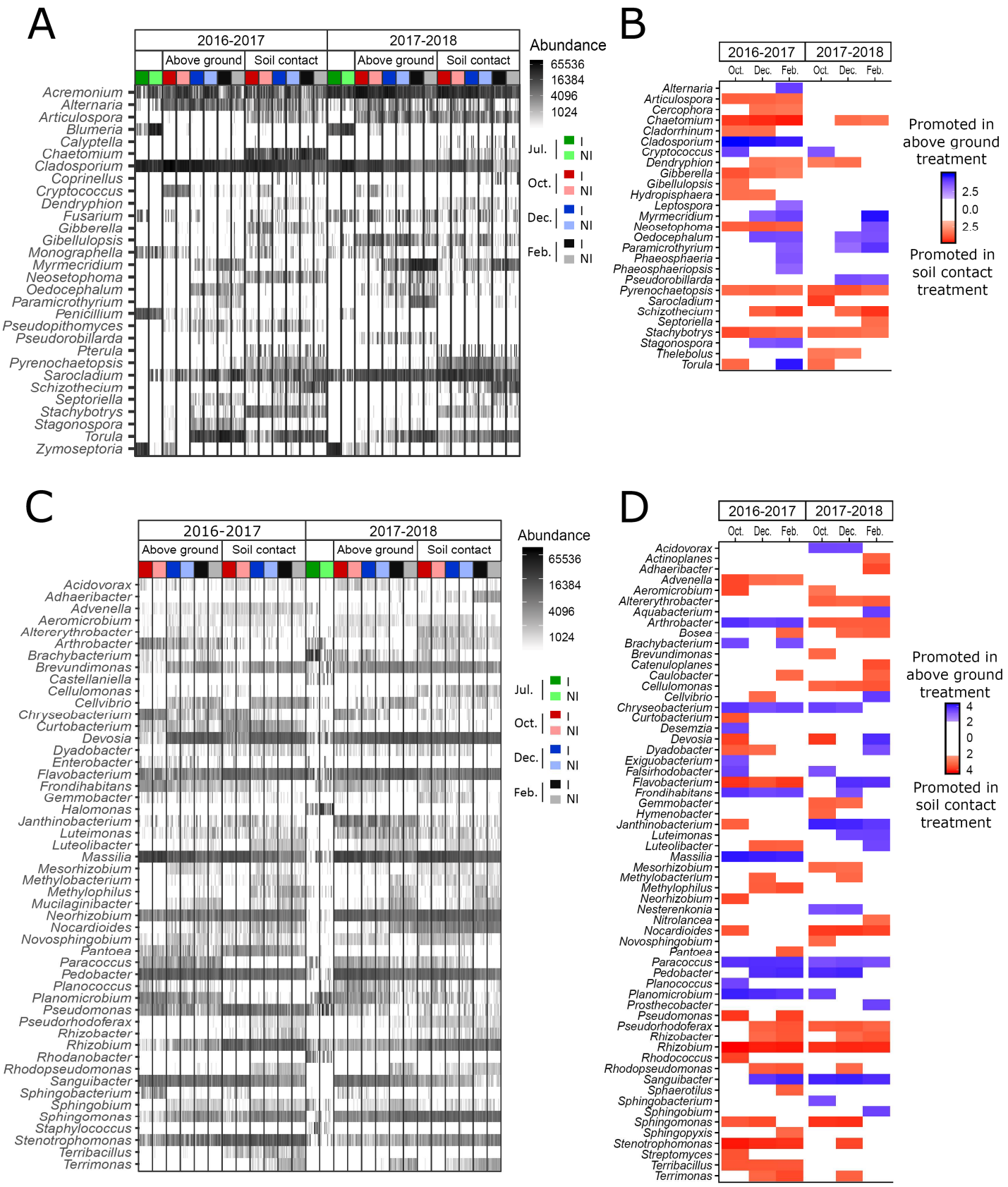


Figure 5

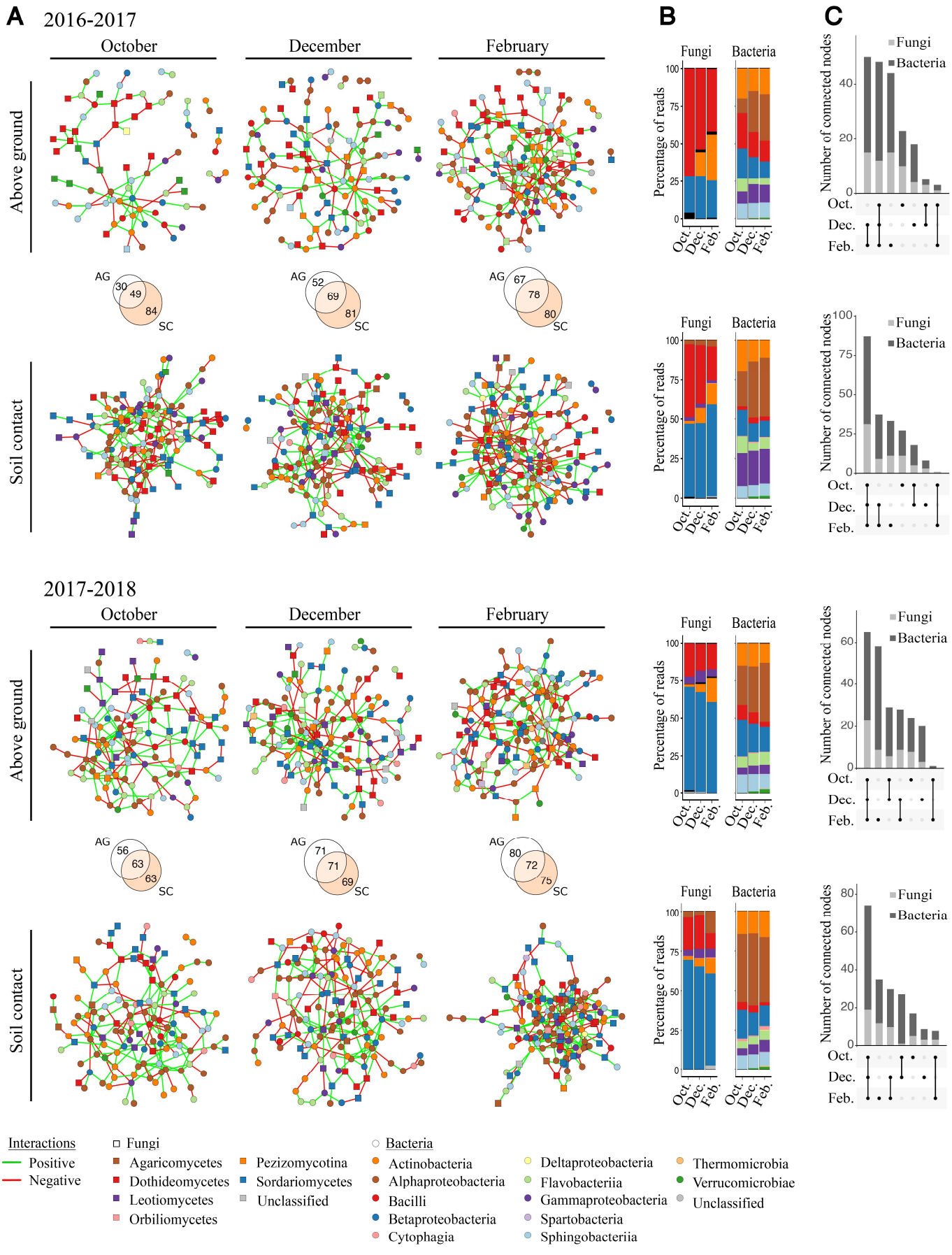


Figure 6

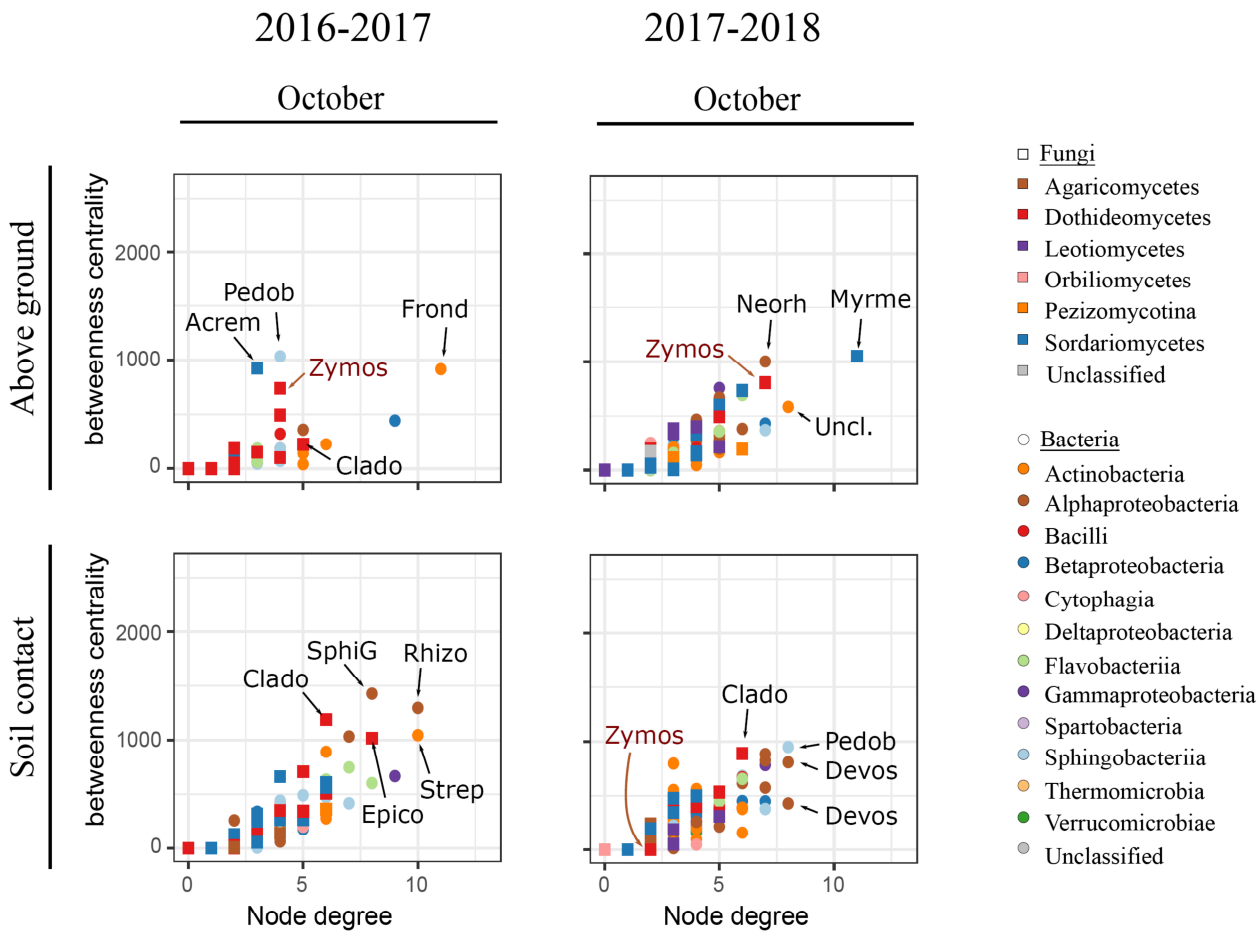
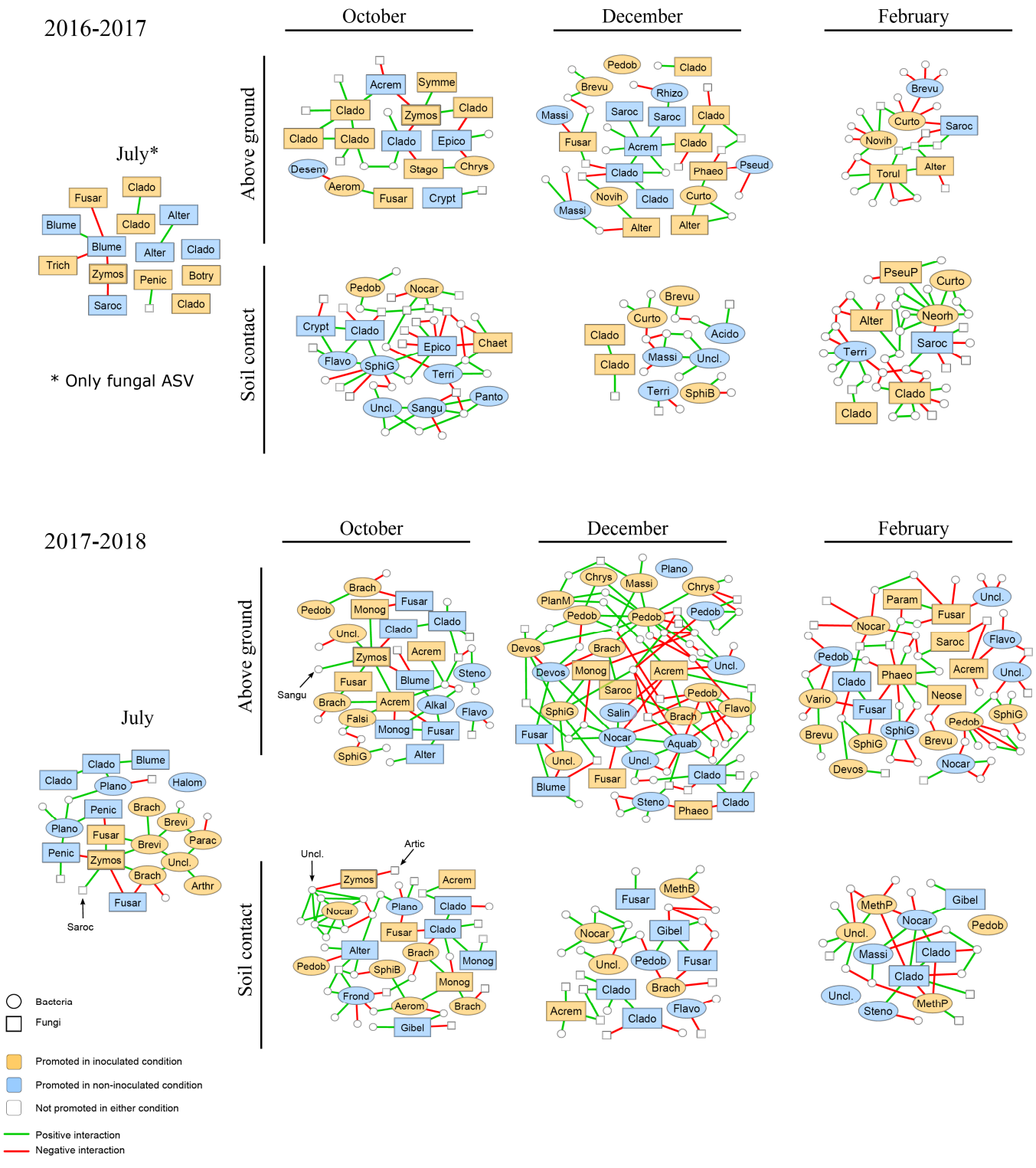


Figure 7



Supplementary Table 1 - Sequence filtering for each run

Run	Primers	Sequence number (paired end)	Sequence quality trimming (F/R)	Selection by sequence length	Quality sequence number after DADA2 analysis
#1	ITS1F / ITS2	10 536 086 (×2)	220 / 210	-	7 164 826
#2	515f / 806r	8 368 872 (×2)	230 / 200	253 bp	5 562 335
#3	ITS1F / ITS2	10 216 508 (×2)	220 / 190	-	6 965 664
#4	515f / 806r	9 975 344 (×2)	220 / 170	253 bp	5 734 825

Supplementary Table 2 - Analysis of the proportion of intra-kingdom interactions (between two fungal ASVs and between two bacterial ASVs) and inter-kingdom interactions (between a fungal ASV and a bacterial ASV) in the ecological networks. The statistical significance of the under- or over-representation of inter-kingdom interactions (when F-B residuals < 1 or > 1, respectively) was established by a χ^2 test of independence performed on the contingency table ($\chi^2 < 0.001$).

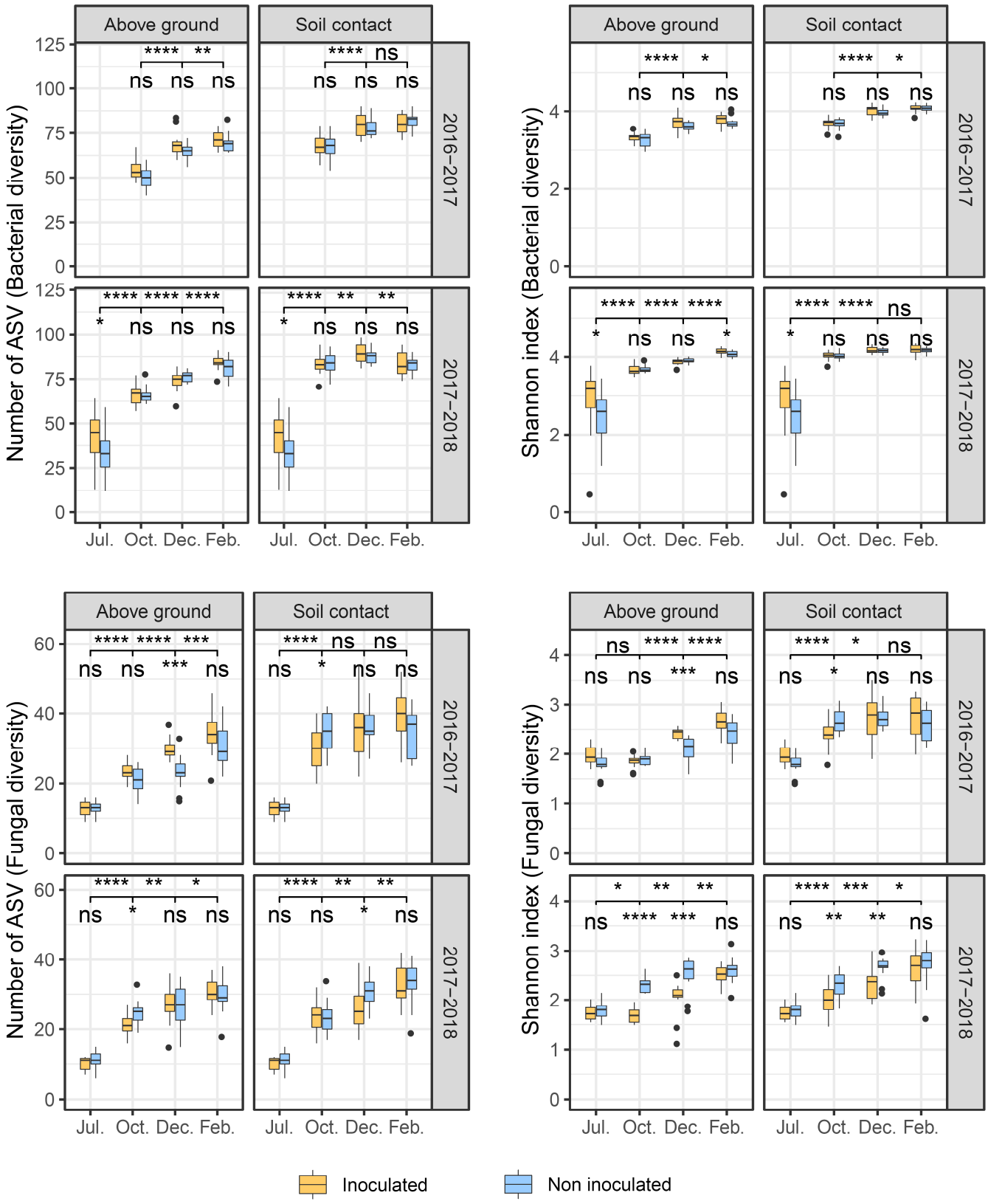
Networks [1]	Number of species		Number of interactions			Theoretical maximum number of interactions			Residuals		
	F [2]	B [3]	F-F	B-B	F-B	F-F [4]	B-B [5]	F-B [6]	F-F	B-B	F-B
Oct. 2016-2017, above ground	32	73	17	57	17	496	2628	2336	1.856	0.545	-1.939
Oct. 2016-2017, contact with soil	52	90	32	121	69	1326	4005	4680	1.060	-0.736	0.358
Dec. 2016-2017, above ground	39	100	19	86	42	741	4950	3900	0.340	0.036	-0.266
Dec. 2016-2017, contact with soil	51	105	25	145	74	1275	5460	5355	-0.772	0.236	0.160
Feb. 2016-2017, above ground	48	107	30	110	65	1128	5671	5136	1.109	-0.866	0.508
Feb. 2016-2017, contact with soil	55	110	32	136	90	1485	5995	6050	0.208	-1.170	1.505
July 2017-2018	16	60	8	45	9	120	1770	960	0.216	1.476	-2.201
Oct. 2017-2018, above ground	39	93	28	93	62	741	4278	3627	1.309	-1.321	1.019
Oct. 2017-2018, contact with soil	31	109	17	140	43	465	5886	3379	-1.413	2.172	-2.144
Dec. 2017-2018, above ground	42	110	22	119	68	861	5995	4620	-0.598	-0.253	0.733
Dec. 2017-2018, contact with soil	35	116	19	149	50	595	6670	4060	-1.384	1.949	-1.849
Feb. 2017-2018, above ground	44	119	27	136	72	946	7021	5236	-0.207	-0.081	0.244
Feb. 2017-2018, contact with soil	46	114	26	135	91	1035	6441	5244	-0.752	-0.978	1.845

[1] according to sampling date, cropping season and contact with soil
[2] fungal species
[3] bacterial species
[4] estimated by $C_2^{n_F} = \frac{n_F!}{2 \times (n_F-2)!}$
[5] estimated by $C_2^{n_B}$
[6] estimated by $n_F \times n_B$

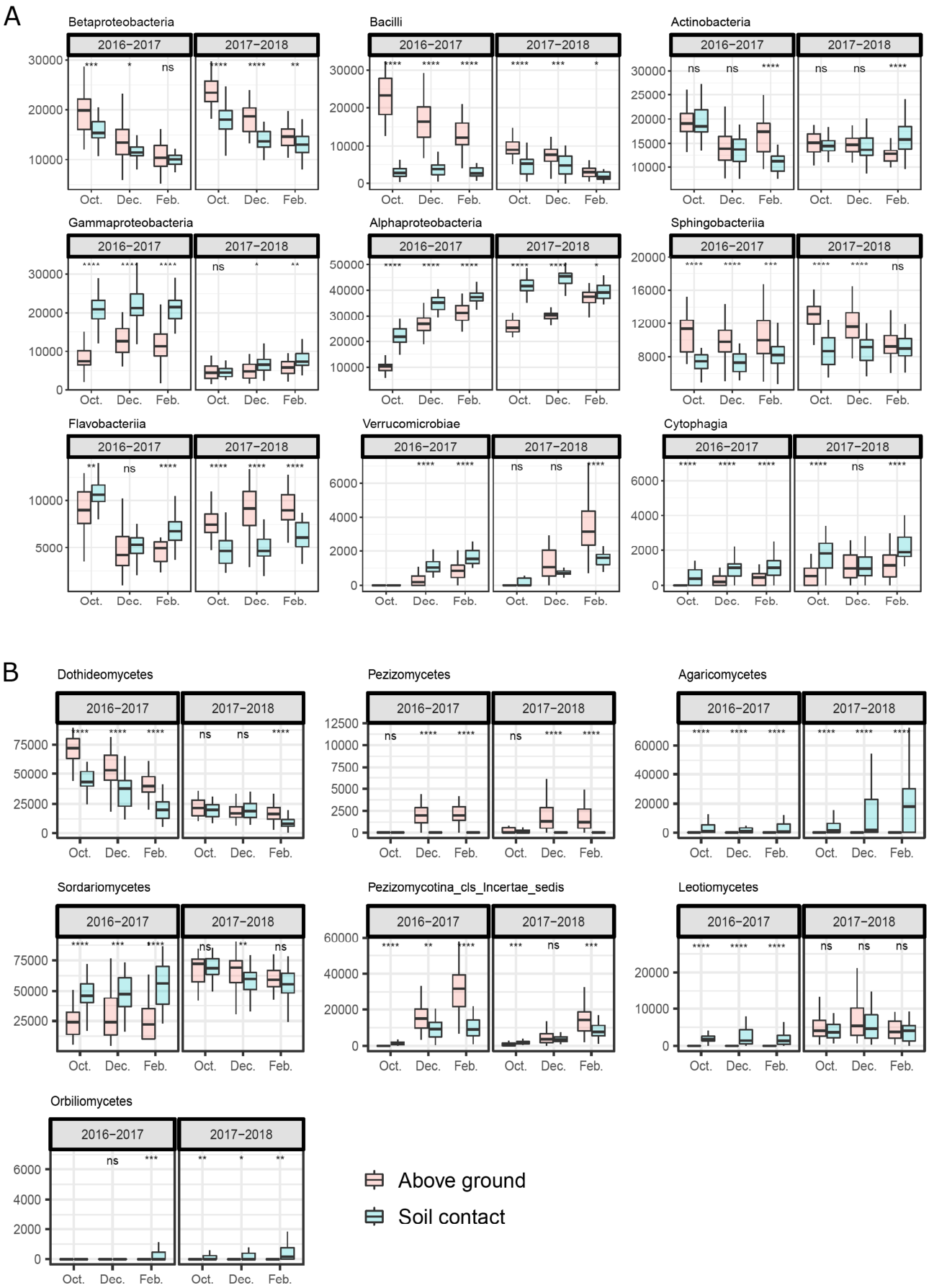
Supplementary Table 3 - Number of ASVs detected for each analysis performed on the dataset and properties of residue microbial ecological networks

			All taxa		Number of taxa promoted in inoculated condition			Number of taxa promoted in non-inoculated condition			Number of taxa promoted in contact with the soil (SC)			Number of taxa promoted without contact with the soil (AG)			Network analysis					
Year	Sampling date	Soil condition	F	B	F	B	Total	F	B	Total	F	B	Total	F	B	Total	F	B	Total	Interacting node	Isolated node	*Z. tritici* interaction
2016-2017	Jul.	-	36	/	8	/	8	6	/	6	-	-	-	-	-	-	20	/	20	12	8	2
2016-2017	Oct.	AG	61	182	8	2	10	4	1	5	13	31	44	9	18	27	32	73	105	79	26	4
2016-2017	Oct.	SC	106	167	1	2	3	3	6	9							52	90	142	133	9	nd
2016-2017	Dec.	AG	101	260	7	4	11	5	4	9	13	36	49	6	16	22	39	100	139	121	18	nd
2016-2017	Dec.	SC	164	227	2	3	5	0	4	4							51	105	156	150	6	nd
2016-2017	Feb.	AG	138	285	2	2	4	1	1	2	13	37	50	14	20	34	48	107	155	145	10	nd
2016-2017	Feb.	SC	179	238	4	2	6	1	1	2							55	110	165	158	7	nd
2017-2018	Jul.	-	36	286	2	7	9	6	3	9	-	-	-	-	-	-	16	60	76	60	16	6
2017-2018	Oct.	AG	87	197	5	6	11	7	3	10	8	36	44	5	20	25	39	93	132	119	13	7
2017-2018	Oct.	SC	117	216	4	6	10	5	2	7							31	109	140	126	14	2
2017-2018	Dec.	AG	104	252	5	13	18	4	9	13	7	43	50	6	22	28	42	110	152	142	10	nd
2017-2018	Dec.	SC	158	230	1	4	5	5	2	7							35	116	151	140	11	nd
2017-2018	Feb.	AG	135	340	6	8	14	2	6	8	8	33	41	10	21	31	44	119	163	152	11	nd
2017-2018	Feb.	SC	193	253	0	4	4	3	4	7							46	114	160	147	13	nd

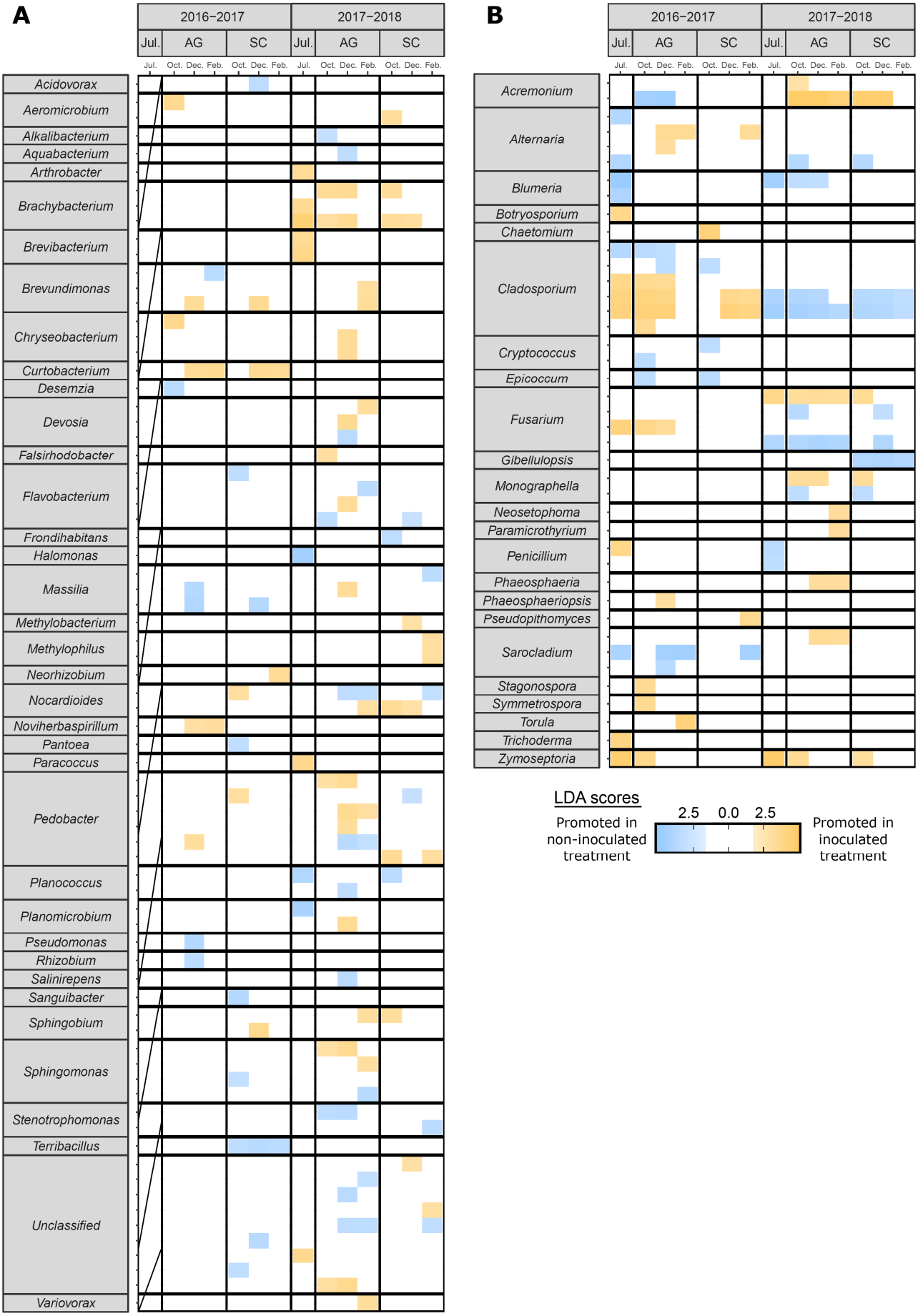
Supplementary Figure 1



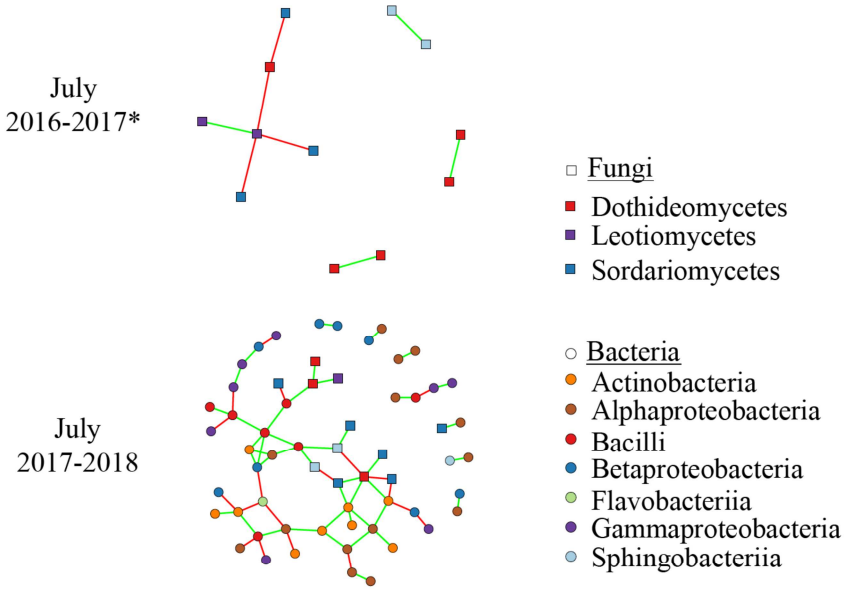
Supplementary Figure 2



Supplementary Figure 3

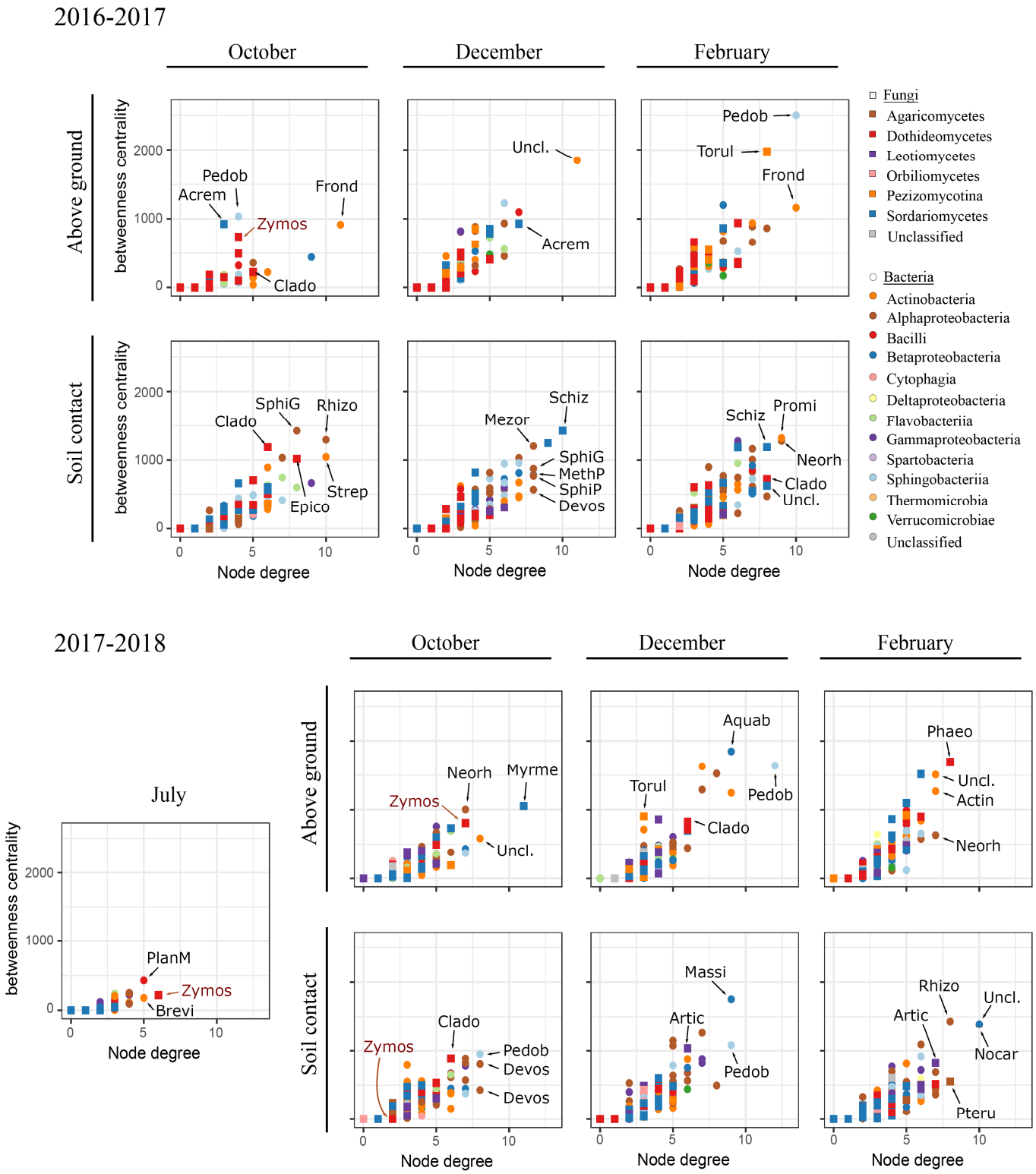


Supplementary Figure 4



* only fungal ASV

Supplementary Figure 5

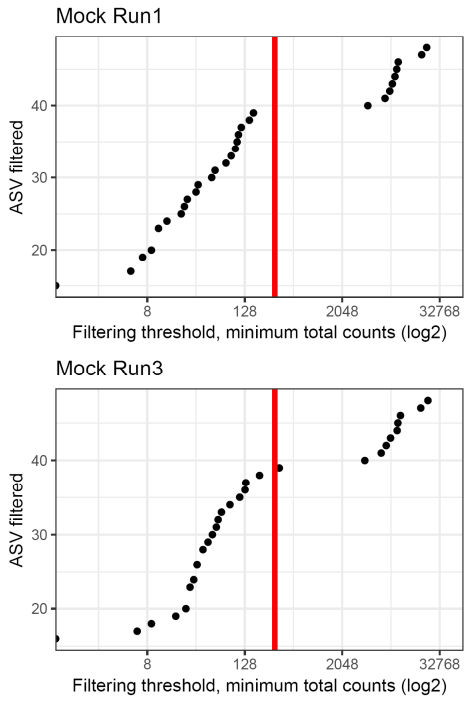


Supplementary Figure 6

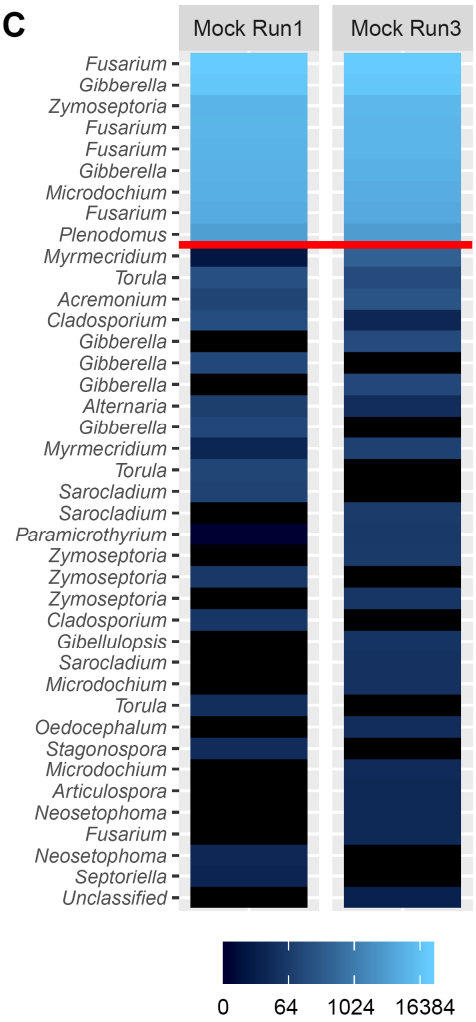
A

Fusarium avenaceum
Fusarium culmorum
Fusarium langsethiae
Fusarium moniliforme
Fusarium sambucinum
Fusarium subglutinans
Fusarium temperatum
Fusarium tricinctum
Plenodomus biglobosus
Microdochium bolleyi
Zymoseptoria tritici

B



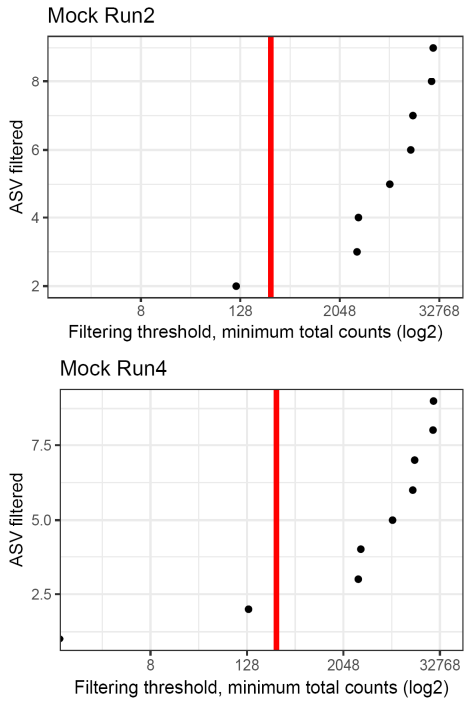
C



D

Agrobacterium sp
Brevibacillus sp
Brevundimonas sp
Erwinia sp
Labedella sp
Morganella sp
Paenibacillus sp
Pantoea agglomerans
Pseudomonas sp
Stenotrophomonas sp
Xanthomonas sp

E



F