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## Soil microbiota influences clubroot disease by modulating *Plasmodiophora brassicae* and *Brassica napus* transcriptomes

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1 **Soil microbiota influences clubroot disease by modulating *Plasmodiophora***  
2 ***brassicae* and *Brassica napus* transcriptomes**

3  
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14  
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17  
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20  
21 Short title: Soil microbiota and clubroot

22

## 23 Abstract

24

25 The contribution of surrounding plant microbiota to disease development has led to the  
26 postulation of the ‘pathobiome’ concept, which represents the interaction between the  
27 pathogen, the host-plant, and the associated biotic microbial community, resulting or  
28 not in plant disease. The structure, composition and assembly of different plant-  
29 associated microbial communities (soil, rhizosphere, leaf, root) are more and more  
30 described, both in healthy and infected plants. A major goal is now to shift from  
31 descriptive to functional studies of the interaction, in order to gain a mechanistic  
32 understanding of how microbes act on plant growth and defense, and/or on pathogen  
33 development and pathogenicity. The aim herein is to understand how the soil microbial  
34 environment may influence the functions of a pathogen and its pathogenesis, as well  
35 as the molecular response of the plant to the infection, with a dual-RNAseq  
36 transcriptomics approach. We address this question using *Brassica napus* and  
37 *Plasmodiophora brassicae*, the pathogen responsible for clubroot. A time-course  
38 experiment was conducted to study interactions between *P. brassicae*, two *B. napus*  
39 genotypes, and three soils harboring High (H), Medium (M) or Low (L) microbiota  
40 diversities and displaying different levels of richness and diversity. The soil microbial  
41 diversity levels had an impact on disease development (symptom levels and pathogen  
42 quantity). The *P. brassicae* and *B. napus* transcriptional patterns were modulated by  
43 these microbial diversities, and the modulations were dependent of the host genotype  
44 plant and the kinetic time. The functional analysis of gene expressions allowed the  
45 identification of pathogen and plant-host functions potentially involved in the change of  
46 plant disease level, such as pathogenicity-related genes (NUDIX effector) in *P.*  
47 *brassicae* and plant defense-related genes (glucosinolate metabolism) in *B. napus*.

48

## 49 **Author summary**

50

51 The untapped soil microbiota diversity can influence plant tolerance and resistance to  
52 several pests. A better understanding of the mechanisms underlying the plant / pests  
53 / microbiota interaction is required to contribute to the improvement of new plant  
54 protection methods taking into account sustainability, respect for the environment, and  
55 low input utilization. Our work showed that in the *Plasmodiophora brassicae* / *Brassica*  
56 *napus* pathosystem, the soil microbiota diversity modulated the disease symptom level  
57 and the pathogen development. We discovered that soil microbial composition  
58 modulated both the pathogen and the plant expression genes profiles. On one hand,  
59 the pathogen transcriptome was mainly modulated by the microbial communities at the  
60 end of infection, when the pathogen infects a susceptible plant genotype, and the  
61 expression of genes potentially involved in growth and pathogenicity was affected. On  
62 the other hand, the plant transcriptome was more modulated by the microbial  
63 communities at the early step of infection, in the most resistant genotype and the  
64 expression of genes potentially involved in defense was affected. This study provides  
65 new insights into the molecular basis of soil microbiota-mediated modulation of plant  
66 pest diseases.

67

## 68 Introduction

69

70 Plants are constantly interacting with a wide variety of potential pathogens within their  
71 environment that can cause serious diseases affecting agriculture. The development  
72 of biotic plant diseases depends also on the interaction of both plant and pathogen  
73 with the environment. All plant tissues, including leaves [1, 2], seeds [3], and roots [4]  
74 are indeed associated with a multitude of microorganisms (viruses, bacteria, archae,  
75 fungi, protists, oomycetes, nematodes, protozoa, algae,...) assembled in microbial  
76 communities or microbiota. The complex plant-associated microbial community  
77 structure and composition, as well as the complex network of interactions between  
78 microbial species, are crucial in stress tolerance [5], plant development dynamics [6],  
79 yield, nutrition and health [7-10]. This recognition that the plant microbiota may  
80 modulate substantially the disease severity and development led to the 'pathobiome'  
81 postulation, which refers to the pathogenic agent, its surrounding biotic microbial  
82 community and their interactions leading to plant disease [11, 12].

83 In plants, three root-associated microbiota compartments can have a role in the  
84 modulation of disease development: the soil microbiota, which represents a great  
85 reservoir of biological diversity [13], the rhizosphere corresponding to the narrow zone  
86 surrounding and influenced by plant roots [14, 15], and the endosphere (root interior)  
87 in which the microbiota diversity is lower than that estimated outside the root [16-19].  
88 Several studies have established close relationships between the rhizosphere  
89 microbiome composition and the plant immune system [20-23], the host genotype  
90 resistant or susceptible to a pathogen [24], and the life history traits of bioaggressors  
91 [25], but the mechanisms underlying these relationships have still to be deciphered. It  
92 is also known that plants select microbial communities around their roots by specific  
93 root exudates [26], that can also function as an additional layer of defense [8]. The

94 defense barrier constituted by recruited microorganisms can be of different types:  
95 stimulation of defense-related compounds' production by the plant, direct antagonism  
96 against pathogen (production of antibiotics or antifungal compounds), competition with  
97 pathogen for resources [13]. The invasion by a soilborne pathogen led to changes in  
98 indigenous plant-associated microbial communities [27, 28] and then in the defense  
99 barrier.

100 Among biotic stress factors, the soilborne plant pathogens cause major yield or quality  
101 loss in agricultural crops. This is the case of the protist *Plasmodiophora brassicae*, an  
102 obligate biotrophe responsible for clubroot, one of the economically most important  
103 diseases of Brassica crops in the world [29]. The life cycle of this soil-borne pathogen  
104 can be divided into several phases: survival in soil as spores, root hair infection, and  
105 cortical infection [30]. Briefly, during the primary phase of infection, the resting spores  
106 germinate in the soil leading to biflagellate primary zoospores that infect the root hairs.  
107 In these cells, zoospores multiply to form the primary plasmodia. Secondary zoospores  
108 are then released and produce the secondary phase of infection that occurs in the  
109 cortex of the roots of the infected plants. During the second phase, multinucleate  
110 plasmodia cause the hypertrophy (abnormal cell enlargement) and hyperplasia  
111 (uncontrolled cell division) of infected roots into characteristic clubs [31]. These  
112 symptoms obstruct nutrient and water transport, stunt the growth of the plant, and  
113 consequently reduce crop yield and quality. In root galls, different life cycle stages of  
114 *P. brassicae* occur simultaneously.

115 Transcriptomics studies deciphered in part the mechanisms of the host - *P. brassicae*  
116 interaction in simplified experimental conditions, but not in complex soil. During both  
117 the spore germination and the primary zoospore stages, the pathogen showed high  
118 active metabolisms of chitinous cell wall digestion, starch, citrate cycle, pentose  
119 phosphate pathway, pyruvate, trehalose, carbohydrates and lipids [32-34]. During the

120 second phase of infection, genes involved in basal and lipid metabolism were highly  
121 expressed [34], as well as the *G-protein-coupled receptors pathway-related* genes  
122 [35]. These active metabolic pathways allow *P. brassicae* to take up nutrients from the  
123 host cells [30, 36]. During the formation of primary and secondary plasmodia, it is  
124 expected that *P. brassicae* secretes an array of effector proteins triggering growth,  
125 expansion and differentiation of infected host cells. Nevertheless, few RxLR effectors  
126 have been found in *P. brassicae* [32, 37], and no LysM-effectors, known to interfere  
127 with chitin detection in fungal-plant interactions [38], were detected. Some candidate  
128 potential effectors have however been identified from *P. brassicae* [32, 37, 39], such  
129 as Crinkler (CRN) related proteins [40], but their roles in infection and disease  
130 development have still to be identified [36]. Only one effector has been characterized  
131 in detail: a predicted secreted methyltransferase that can mediate methylation of  
132 salicylic, benzoic and anthranilic acids, thereby interfering in the plant salicylic acid-  
133 induced defense [41].

134 Concerning the plant, *P. brassicae* infection altered likewise primary and secondary  
135 metabolism, as pathways involved in lipid, carbohydrate, cell wall synthesis,  
136 lignification-related genes, arginine and proline metabolism [42-46], producing a sink  
137 of plant metabolites assimilated by the pathogen and corresponding to a metabolic  
138 cost for the infested plant. Clubroot infection also modified plant hormone homeostasis  
139 and defense responses, such as cytokinin biosynthesis, auxin homeostasis, salicylic  
140 acid and jasmonic acid metabolism [44-51].

141 During its life cycle, *P. brassicae* can establish potential relationships with microbiota  
142 from soil, rhizospheric soil and roots. Beneficial effect of various specific biocontrol  
143 microorganisms in suppressing clubroot has been demonstrated, such as *Trichoderma*  
144 spp. [52], *Streptomyces* sp. [53, 54], *Heteroconium chaetospora* [55], *Streptomyces*  
145 *platensis* [56], *Bacillus subtilis* [57, 58], *Zhihengliuella aestuarii* B18 [59], *Paenibacillus*

146 *kribbensis* [60], and *Lysobacter antibioticus* [61]. Most of these organisms were  
147 isolated from rhizosphere soil or root endosphere. Mechanisms by which these  
148 microorganisms protect against clubroot are not yet elucidated but could imply  
149 antifungal compounds or molecules up-regulating host plant defense genes. In  
150 addition, the microbe abundance in *B. napus* clubroot infected endosphere roots was  
151 found higher in asymptomatic roots than in symptomatic roots, and the asymptomatic  
152 roots contained many microorganisms with biological control properties and plant  
153 growth promotion functions [62]. In Chinese cabbage, invasion by *P. brassicae*  
154 modified the rhizosphere and root-associated community assembly during the  
155 secondary cortical infection stage of clubroot disease [28]. This shows that the plant  
156 microbiota diversity can modulate the plant response to *P. brassicae* and can be  
157 considered as a potential reservoir of biocontrol microbe for clubroot prevention.  
158 Moreover, in *B. napus*, the plant - microbiota interaction has a role in plant defense  
159 against a phytophagous insect (*Delia radicum*) [25, 63].  
160 In order to gain a mechanistic understanding of how soil microbes boost plant growth  
161 and defense and/or modulate the pathogen development and pathogenicity, a major  
162 challenge is then now to shift from descriptive to functional studies. The aim of this  
163 study is to understand how a single root pathogen, *P. brassicae*, interacts with its host,  
164 the oilseed rape (*B. napus*), considering the role of the soil microbial diversity as a  
165 reservoir of microbial functions related to plant resistance phenotype. To explore how  
166 the soil microbial environment may influence the functions of a pathogen and its  
167 pathogenesis, and the molecular response of the plant to the infection, we evaluated  
168 the effect of different soil microbial diversities obtained by an experimental approach  
169 of dilution to extinction on (i) the phenotype of two plant genotypes harboring different  
170 levels of susceptibility to the clubroot pathogen, and (ii) the transcriptomes of pathogen  
171 and host-plant in interaction.



172

## 173 Results

174

### 175 Characterization of the microbial communities in the initial three soil conditions

176

177 The microbiological composition after recolonization of the three soils manipulated for  
178 having different microbial diversities (High diversity level [H], Medium diversity level  
179 [M] or Low diversity level [L]) was analyzed. As expected, the three soils displayed  
180 optimal fungal and bacterial densities and similar abundances at the end of  
181 recolonization (S1 Fig). Not significant differences for the main soil physicochemical  
182 characteristics were observed between the three soils used (S1 Table). The only  
183 difference concerned the nitrogen form, that was found mainly in the nitrate form in  
184 both H and M and as nitrate and ammonium in L; however, the total nitrogen amount  
185 was similar among the three soils, (0.74 to 0.77 g.kg<sup>-1</sup>).

186 We investigated the effect of the experimental dilution / recolonization on microbiota  
187 diversity. Alpha-diversity (within each modality of soil) was analyzed based on the  
188 OTUs richness and the Shannon diversity index. For bacterial kingdom (Fig 1A), we  
189 observed a statistically significant reduction in richness and specific diversity from H/M  
190 to L microbial modalities. For fungal kingdom (Fig 1B), the fungal richness, and to a  
191 lesser extent the fungal diversity, decreased also from H to L. Beta-diversity (between  
192 soil modalities) was measured for the bacterial and fungal communities (Fig 1C). The  
193 soil microbial diversities differed significantly for bacterial and fungal communities.  
194 Frequencies of bacterial and fungal phyla, genera and OTUs for each microbial  
195 modality are shown in S2 Fig. At the level of phyla, both bacteria and fungi displayed  
196 similar frequencies whatever the soil modality, with *Proteobacteria* and *Ascomycota*  
197 the dominant phyla, respectively. *Bacillus* and *Pseudomonas* on one hand, and

198 *Schizosaccharomyces* and *Fusarium* on the other hand, were major genera  
199 concerning bacteria and fungi, respectively, for the three soils.

200 In conclusion, the soils obtained by microbial diversity manipulation through serial  
201 dilutions displayed different decreasing microbe richness and diversity, validating thus  
202 their use for evaluating their effect on *B. napus* infection by *P. brassicae*.

203

### 204 **Modulation of the plant susceptibility to clubroot according to the soil** 205 **microbiota composition**

206

207 The dry aerial parts were weighted in all experimental conditions (Fig 2A). At Ti  
208 (intermediary time), no significant differences were measured between healthy and  
209 inoculated plants, whatever both the soil microbiota modality and the plant genotype  
210 (except a small difference in H between healthy and inoculated Yudal). On the contrary,  
211 at the final time of the experiment (Tf), the inoculated plants displayed significant  
212 reduced aerial dry weight than healthy plants, whatever both the soil microbiota  
213 modality and the host plant genotype. At this time-point, the weight of aerial parts of  
214 both healthy and inoculated Tenor plants was weaker than in Yudal plants.

215 Concerning the roots (Fig 2B), the Tenor inoculated roots showed heavier dry mass (5  
216 to 6 times more) at Ti and Tf than healthy roots, for each soil microbiota modality. The  
217 Tenor healthy roots had weak growth between Ti and Tf, whatever the soil, whereas  
218 inoculated Tenor had roots 6 times heavier at Tf than at Ti. This is the result of a strong  
219 development of galls in this genotype during this period. Concerning the Yudal root dry  
220 weights, no differences between healthy and infected plants were measured whatever  
221 the microbiota soil dilution and whatever the sampling date, probably because of the  
222 small size of galls clearly visible in Yudal genotype. At Tf, Yudal healthy roots were

223 heavier than Tenor ones because of different root developmental patterns between the  
224 two genotypes.

225 At each sampling time, the soil microbiota modality had overall no effect on both aerial  
226 and root dry weights of healthy and inoculated plants.

227 At Ti and Tf, disease severity of inoculated plants was scored by determining the  
228 disease index (DI) and the DNA pathogen content (Fig 3). For each plant genotype,  
229 the DI showed the progression of disease along time-points: DI is about 50% at Ti and  
230 80 % at Tf for Tenor, and less than 20% at Ti and 50% at Tf for Yudal. Whatever the  
231 soil modality and the sampling date, Yudal displayed lower DI than Tenor. This  
232 expected difference is consistent with the known level of clubroot  
233 resistance/susceptibility already described for these genotypes [64]. The soil  
234 microbiota modality had an effect on DI. For Tenor, at Ti and Tf, the DI was statistically  
235 significantly lower in L compared to H and M, and the highest DI was obtained in M.  
236 The DNA pathogen content followed the same pattern. At Ti, the *P. brassicae* DNA  
237 content was low, making difficult to compare the values between samples. At Tf, the  
238 DNA pathogen content was lower in L than in H and M, and higher in M, providing a  
239 bell-curve. Concerning the Yudal genotype, very low DI and DNA *P. brassicae* content  
240 were observed at Ti, making difficult the interpretation of the results. At Tf, decreasing  
241 gradients of DI and pathogen DNA content were measured through soil dilutions from  
242 H to L: the less rich and diverse soil, the less plant disease and DNA pathogen content.

243

#### 244 **Overview, mapping and validation of RNAseq data**

245

246 Approximately 80 to 100 million (M) reads by sample were obtained, and from 86 to  
247 93% of the reads were mapped to the reference genome that we constructed,  
248 corresponding to the *B. napus* and the *P. brassicae* concatenated genomes.

249 Pathogen gene expression's profiles were clearly clustered by the host plant genotype  
250 at Ti, and both by the soil microbiota modality and the host plant genotype at Tf (S3A  
251 Fig). No similar heatmap was performed with the *B. napus* gene expression profiles  
252 because of a huge number of expressed genes making the figure unreadable.  
253 Hierarchical Cluster Analysis (HCA) (S3B Fig) on the filtered and normalized counts  
254 values concerning *P. brassicae* for each sample at Ti showed no true cluster structure  
255 in function of replicate, soil microbiota diversity or host plant genotype. On the contrary,  
256 at Tf for both host genotypes, the HCA analysis identified separated groups for the  
257 three replicates in H, in a lesser extent in M, and a less good grouping in L. This  
258 indicated that the experimental variation was higher in the more diluted soil microbial  
259 modality (L). Concerning the *B. napus* reads, in healthy (S4A Fig) and inoculated (S4B  
260 Fig) plants, the analysis showed that data clustered first by the host genotype, and  
261 then by the time factor, the soil modality and the replicate.

262

### 263 **Modulation of the *P. brassicae* transcriptome by the soil microbiota composition**

264

265 Table 1 shows the number of DEGs in *P. brassicae* and *B. napus* according to H  
266 compared to M or L, for each inoculated host genotype. The comparisons are focused  
267 on differences between modalities considered closest to the initial state of the soil (*i.e.*  
268 H) and the diluted conditions (M and L).

269

270 Table 1. Number of DEGs in *P. brassicae* and in *B. napus* depending on the soil microbiota diversity levels.

Organism in which DEGs are counted	Infection stage	Host plant genotype	H vs M		H vs L	
			Healthy plants	Infected plants	Healthy plants	Infected plants
<i>P. brassicae</i>	Ti	Yudal	nd	0	nd	0
		Tenor	nd	0	nd	1
	Tf	Yudal	nd	296	nd	0
		Tenor	nd	1827	nd	770
<i>B. napus</i>	Ti	Yudal	0	0	8	64
		Tenor	53	0	814	0
	Tf	Yudal	1852	0	3744	23
		Tenor	883	3	3945	0

271 DEGs, Differentially Expressed Genes; Ti, Intermediary Time; Tf, Final Time; H, High diversity modality; M, Medium diversity modality;  
 272 L, Low diversity modality; nd, not detected.

273

274 Concerning the *P. brassicae* transcriptome, no DEGs between the soil microbiota  
275 modalities were detected at Ti (except only one gene between H and L in infected  
276 Tenor). On the contrary, at Tf, when galls were developed, the transcriptome of *P.*  
277 *brassicae* was different between soils. Interestingly, *P. brassicae* displayed a higher  
278 number of DEGs when infecting Tenor (2597 DEGs between H and both M and L) than  
279 when infecting Yudal (296 DEGs).

280

281 *Modulation of the P. brassicae transcriptome by the soil microbiota composition when*  
282 *infecting Yudal*

283

284 In the interaction with Yudal, only the M condition had an effect on the *P. brassicae*  
285 gene expression compared to H at Tf (Table 1). The complete list of the DEGs is  
286 presented in the S2 Table. Only nine genes among the 296 DEGs were overexpressed  
287 at M compared to H, with a small fold-change between conditions (1.2 to 1.6). No  
288 particular function of these genes can be easily associated with the DI between M and  
289 H (general pathways, such as signalization and chromosome condensation). On the  
290 contrary, a higher number of *P. brassicae* genes (287) were significantly  
291 underexpressed at M compared to H, in the same way than level of disease was lower  
292 at M compared to H. We selected the top 30 most significant down-regulated genes in  
293 M compared to H, with a fold-change greater than 2 (Table 2). Some of these top genes  
294 are potentially involved into the transport of molecules (e.g. *FMN-binding glutamate*  
295 *synthase family*, *MFS transporter Major Facilitator Superfamily*), and in development,  
296 growth and cell differentiation (e.g. *Chitin Synthase\_2*, *Phosphoenolpyruvate*  
297 *carboxykinase*, *Glycosyltransferase*). Other genes were related to pathogenicity,  
298 including *Carbohydrate-binding module family\_18*, *Glycoside hydrolase family\_16*,  
299 and *NUDIX\_hydrolase*.

300

301 Table 2. Selection of top 30 ranking *P. brassicae* highly down-regulated genes (fold-change > 2) in M compared to H at Tf when infecting  
 302 Yudal (Y).

<i>P. brassicae</i> gene	<i>P. brassicae</i> gene expression level		Fold-change	Description	Enzyme Codes
	in Y / H / Tf	in Y / M / Tf			
Pldbri_eH_r1s003g01588	0.43	0.02	11.42	sugar_ABC_transporter_substrate-binding <sup>1</sup>	NA
Pldbri_eH_r1s004g02573	0.69	0.06	10.33	ADP-ribosylation_factor_6 <sup>2</sup>	NA
Pldbri_eH_r1s003g01442	0.58	0.04	8.56	calcium_calmodulin-dependent_kinase_type_IV-like <sup>2</sup>	ec:2.7.11.10
Pldbri_eH_r1s003g01889	1.85	0.34	4.76	NUDIX_hydrolase <sup>3</sup>	ec:3.6.1.65
Pldbri_eH_r1s008g04734	2.32	0.46	4.72	Serine_threonine-_kinase_Sgk3 <sup>2</sup>	ec:3.1.4.4
Pldbri_eH_r1s011g06165	4.32	0.89	4.53	UDP-D-xylose:L-fucose_alpha-1;3-D-xylosyltransferase_1-like <sup>2</sup>	ec:2.4.1.37
Pldbri_eH_r1s015g07579	6.72	1.51	4.21	MFS_transporter <sup>1</sup>	NA
Pldbri_eH_r1s001g00152	1.67	0.40	4.06	carbohydrate-binding_module_family_18 <sup>3</sup>	ec:3.2.1.14
Pldbri_eH_r1s001g00029	7.63	1.84	4.02	WD40_repeat	NA
Pldbri_eH_r1s008g04750	5.45	1.33	3.91	methyltransferase_domain-containing	ec:2.1.1.300
Pldbri_eH_r1s002g01072	7.08	1.78	3.89	FMN-binding_glutamate_synthase_family <sup>1</sup>	ec:1.4.1.14
Pldbri_eH_r1s001g00617	8.55	2.17	3.86	glutamate_NAD(P)+ <sup>2</sup>	ec:1.4.1.23
Pldbri_eH_r1s042g12180	3.67	0.94	3.75	calcium/calmodulin-dependent_protein_kinase_type_IV-like <sup>2</sup>	ec:2.7.11.10
Pldbri_eH_r1s007g04295	21.71	6.20	3.47	Mps1_binder <sup>2</sup>	NA
Pldbri_eH_r1s001g00511	40.71	11.71	3.46	serine_threonine-_kinase_HT1 <sup>2</sup>	ec:2.7.11.10
Pldbri_eH_r1s016g07781	26.37	7.58	3.45	chitin_synthase_2 <sup>2</sup>	ec:2.4.1.16
Pldbri_eH_r1s034g11599	8.64	2.45	3.41	WD-40_repeat_domain-containing	NA
Pldbri_eH_r1s025g10321	8.55	2.46	3.38	maltose_maltodextrin_ABC_substrate_binding_periplasmic <sup>1</sup>	ec:2.5.1.2
Pldbri_eH_r1s014g07095	3.27	0.92	3.35	glucosamine_6-phosphate_N-acetyltransferase <sup>2</sup>	ec:2.3.1.193
Pldbri_eH_r1s001g00671	17.87	5.47	3.23	glutathione-disulfide_reductase	ec:1.8.1.7;ec:1.8.2.3;ec:1.8.1.5
Pldbri_eH_r1s003g01550	8.87	2.75	3.15	glycosyltransferase <sup>2</sup>	ec:2.4.2.38
Pldbri_eH_r1s003g01890	49.53	15.74	3.13	glycoside_hydrolase_family_16 <sup>3</sup>	ec:3.2.1.151
Pldbri_eH_r1s033g11505	14.72	4.86	3.00	glycosyltransferase <sup>2</sup>	ec:2.4.2.38
Pldbri_eH_r1s028g10813	7.61	2.63	2.80	ABC_transporter_G_family <sup>1</sup>	ec:3.6.1.15;ec:3.6.3.43
Pldbri_eH_r1s022g09622	47.16	17.11	2.74	phosphoenolpyruvate_carboxykinase <sup>2</sup>	ec:4.1.1
Pldbri_eH_r1s024g09958	32.88	12.19	2.68	phosphate_ABC_transporter_substrate-binding <sup>1</sup>	ec:3.1.3.1
Pldbri_eH_r1s002g00819	6.83	2.63	2.52	cytochrome_P450	ec:1.6.2.4;ec:1.14.14.1;ec:1.14.21.7;ec:1.16.1.5;ec:1.18.1.7
Pldbri_eH_r1s028g10814	6.19	2.49	2.41	ABC_transporter <sup>1</sup>	ec:3.6.1.3;ec:3.6.1.15;ec:3.6.3.43
Pldbri_eH_r1s009g05056	9.63	4.23	2.28	probable_phospholipid-transporting_ATPase_IA_isoform_X1 <sup>1</sup>	ec:3.6.1
Pldbri_eH_r1s003g01928	56.23	27.59	2.03	chitin_synthase_2 <sup>2</sup>	ec:2.4.1.16

303 Genes potentially involved in transport of molecules<sup>1</sup>, development and growth<sup>2</sup>, or pathogenicity<sup>3</sup>.

304

305 *Modulation of the P. brassicae transcriptome by the soil microbiota composition when*  
306 *infecting Tenor*

307

308 In the interaction with Tenor, 1827 genes of *P. brassicae* were differentially expressed  
309 at Tf between M and H (Table 1), most of them (1360 genes *i.e.* 75%) being  
310 overexpressed in M, and a smaller part (467 genes) underexpressed in M (S3A Table).  
311 Between L and H, there were 770 DEGs (S3B Table), with 532 (*i.e.* 70%) genes  
312 overexpressed in L compared to H and 238 underexpressed. In total, compared to the  
313 normal H level diversity, 621 *P. brassicae* genes were modulated both by M (out of  
314 1827 genes, *ie* 34%) and L (out of 770 genes, *ie* 81%) conditions (S3C Table). Most  
315 of the genes regulated in L were also regulated in M. Moreover, these 621 genes  
316 displayed similar expression profiles: 450 genes were overexpressed at both M and L  
317 compared to H, and conversely for 171 genes. For these 171 genes, the fold-change  
318 was very small (< 1.5 for 169 genes whatever the comparison between soil microbiota  
319 diversities), but the gene expression levels were elevated. On the contrary, among the  
320 450 genes overexpressed in M or L compared to H, 346 displayed a fold-change  
321 sharply higher than 2. The Table 3 shows the top 50 ranking by fold-change genes  
322 among these 346 *P. brassicae* genes overexpressed in M and L compared to H. Many  
323 of them were related to functions of transport (*phospholipid-transporting ATPase*,  
324 *FMN-binding glutamate synthase*, *Ammonium transporter*, *Phosphate*  
325 *ABC\_transporter* or *Potassium transporter*), growth (*Chitin synthase\_2*), detoxification  
326 (*Glutathione\_S transferase*, *Zinc\_C2H2\_type\_family*), or potential pathogenicity (*E3-*  
327 *Ubiquitin ligase*, *alkaline ceramidase*, *cytosolic carboxypeptidase\_4*, *serine*  
328 *carboxypeptidase\_CPVL*).

329



330 Table 3. Selection of top 50 *P. brassicae* genes significantly differentially overexpressed in both M and L compared to H at Tf when  
 331 infecting Tenor (T).

<i>P. brassicae</i> gene	<i>P. brassicae</i> gene expression level			Fold change T / H versus T / M	Fold change T / H versus T / L	Description	Enzyme Codes
	in T / H / Tf	in T / M / Tf	in T / L / Tf				
PIdbra_eH_r1s023g09907	0.05	0.98	0.54	15.53	8.59	E3_ubiquitin_ligase_NRDP1 <sup>3</sup>	NA
PIdbra_eH_r1s007g03979	0.10	0.60	0.66	5.30	5.80	Dynein_light_chain_Tctex-type	NA
PIdbra_eH_r1s028g10892	0.28	1.31	1.46	4.69	5.31	Glucokinase <sup>2</sup>	ec:2.7.1.2, ec:2.7.1.1
PIdbra_eH_r1s035g11711	3.74	14.19	11.63	3.80	3.12	Probable_phospholipid-transporting_ATPase_7_isoform_X1 <sup>1</sup>	ec:3.6.1, ec:3.6.3.1
PIdbra_eH_r1s014g07222	4.08	15.48	12.70	3.75	3.08	Serine_threonine_kinase <sup>2</sup>	ec:2.7.11.10
PIdbra_eH_r1s001g00753	9.55	33.81	25.43	3.53	2.66	Gamma-glutamylcyclotransferase	ec:4.3.2.6
PIdbra_eH_r1s032g11432	2.17	7.53	6.17	3.51	2.87	Glutathione_S-transferase	ec:1.8.1.8, ec:1.5.4.1
PIdbra_eH_r1s008g04734	0.86	3.02	2.83	3.47	3.26	Serine_threonine_kinase_Sgk3 <sup>2</sup>	ec:3.1.4.4
PIdbra_eH_r1s002g01071	4.07	13.84	14.55	3.40	3.57	FMN-binding_glutamate_synthase_family <sup>1</sup>	ec:1.4.1.14
PIdbra_eH_r1s002g01072	2.67	9.15	11.02	3.39	4.08	FMN-binding_glutamate_synthase_family <sup>1</sup>	ec:1.4.1.14
PIdbra_eH_r1s008g04744	0.88	3.02	3.82	3.38	4.27	Alkaline_ceramidase <sup>3</sup>	ec:3.5.1.23
PIdbra_eH_r1s007g04295	10.89	36.75	38.11	3.37	3.50	Mps1_binder <sup>2</sup>	NA
PIdbra_eH_r1s002g00819	3.16	10.48	9.05	3.30	2.86	Cytochrome_P450	ec:1.14.14, ec:1.16.1.5
PIdbra_eH_r1s015g07621	9.57	31.38	28.26	3.28	2.95	Ammonium_transporter <sup>1</sup>	NA
PIdbra_eH_r1s008g04794	1.44	4.67	4.89	3.19	3.35	Zinc_C2H2_type_family	NA
PIdbra_eH_r1s004g02345	15.37	48.78	39.96	3.17	2.60	Cytosolic_carboxypeptidase_4 <sup>3</sup>	ec:3.4.17, ec:3.4.19.11
PIdbra_eH_r1s027g10543	1.92	6.02	5.78	3.13	3.00	Probable_serine_carboxypeptidase_CPVL <sup>3</sup>	ec:3.4, ec:2.3.1.92
PIdbra_eH_r1s017g08171	3.41	10.66	11.56	3.12	3.39	E3_ubiquitin_ligase_UNKL_isoform_X1 <sup>3</sup>	NA
PIdbra_eH_r1s001g00671	7.57	23.37	22.03	3.08	2.91	Glutathione-disulfide_reductase	ec:1.8.1, ec:1.8.2.3
PIdbra_eH_r1s001g00511	19.71	59.05	56.06	3.00	2.85	Serine_threonine_kinase_HT1 <sup>2</sup>	ec:2.7.11.10
PIdbra_eH_r1s003g01889	1.25	3.76	4.62	2.95	3.63	NUDIX_hydrolase <sup>3</sup>	ec:3.6.1.65
PIdbra_eH_r1s024g09958	15.89	46.79	42.00	2.94	2.64	Phosphate_ABC_transporter_substrate-binding <sup>1</sup>	ec:3.1.3.1
PIdbra_eH_r1s006g03794	6.54	19.26	18.82	2.92	2.85	Chitin_synthase_D <sup>2</sup>	ec:2.4.1.12
PIdbra_eH_r1s056g12619	3.23	9.42	9.34	2.92	2.90	Putative_WD_repeat-containing_protein	NA
PIdbra_eH_r1s026g10483	79.60	232.56	209.79	2.92	2.63	Lysosomal_aspartic_protease	ec:3.4.23, ec:3.4.23.2
PIdbra_eH_r1s022g09656	11.68	34.05	39.09	2.91	3.34	Potassium_transporter <sup>1</sup>	NA
PIdbra_eH_r1s002g00884	1.35	3.87	4.21	2.89	3.15	Glutathione_S-transferase_kappa_1	ec:2.5.1.18, ec:1.8.1.8
PIdbra_eH_r1s015g07579	3.80	10.98	10.71	2.88	2.81	MFS_transporter <sup>1</sup>	NA
PIdbra_eH_r1s016g07943	1.28	3.70	4.41	2.88	3.44	Dynein_light_chain	NA
PIdbra_eH_r1s010g05501	5.21	15.04	17.05	2.87	3.26	WD_repeat-containing_54_isoform_X1	NA
PIdbra_eH_r1s006g03824	3.52	10.19	11.22	2.87	3.16	Zinc_C2H2_type_family_(macronuclear)	NA
PIdbra_eH_r1s009g05121	4.39	12.28	11.05	2.78	2.51	Phosphatidylserine_decarboxylase_subunit_beta	ec:4.1.1.65
PIdbra_eH_r1s008g04760	0.66	1.79	1.84	2.76	2.83	Receptor-interacting_serine-threonine_kinase <sup>2</sup>	ec:2.7.1.107
PIdbra_eH_r1s037g11906	1.28	3.57	4.15	2.73	3.17	Phosphate_ABC_transporter_substrate-binding_protein_PstS <sup>1</sup>	ec:3.1.3.1
PIdbra_eH_r1s003g01729	26.32	70.09	69.36	2.66	2.63	Chitin_synthase_(Chitin-UDP-_ac-transferase) <sup>2</sup>	ec:2.4.1.16, ec:2.4.1
PIdbra_eH_r1s007g04126	43.01	113.52	121.98	2.64	2.84	P-type_atpase	ec:3.6.3.7, ec:3.1.3.96
PIdbra_eH_r1s004g02678	9.23	22.88	23.24	2.48	2.52	MFS_general_substrate_transporter <sup>1</sup>	NA
PIdbra_eH_r1s003g01750	4.15	9.82	8.35	2.37	2.01	Phosphatidylinositol_4-kinase_alpha <sup>2</sup>	ec:2.7.11.1
PIdbra_eH_r1s006g03626	7.07	16.68	20.06	2.36	2.83	Mitogen-activated_kinase_kinase_6_isoform_X2 <sup>3</sup>	ec:2.7.11.10
PIdbra_eH_r1s002g01126	14.32	33.48	33.58	2.34	2.34	Serine_threonine_kinase <sup>2</sup>	ec:2.7.11.10, ec:2.7.10.2
PIdbra_eH_r1s003g01487	3.87	9.07	10.00	2.33	2.57	Calcium_calmodulin-dependent_kinase_type_1D-like <sup>2</sup>	ec:2.7.11.10
PIdbra_eH_r1s007g04189	40.96	88.40	101.24	2.16	2.47	Phospholipid-transporting_ATPase_3_isoform_X1 <sup>1</sup>	ec:3.6.1
PIdbra_eH_r1s029g11029	3.14	1.60	1.31	1.99	2.42	TKL_kinase	NA

Pldbra_eH_r1s009g05056	8.47	16.53	19.40	1.94	2.28	Probable_phospholipid-transporting_ATPase_IA_isoform_X1 <sup>1</sup>	ec:3.6.1
Pldbra_eH_r1s010g05586	8.16	15.82	15.52	1.94	1.91	WD_repeat-containing_17	NA
Pldbra_eH_r1s024g09957	26.00	50.22	48.97	1.93	1.88	Phosphate_ABC_transporter_substrate-binding <sup>1</sup>	ec:3.1.3.1
Pldbra_eH_r1s003g01928	44.45	84.65	77.20	1.90	1.74	Chitin_synthase_2 <sup>2</sup>	ec:2.4.1.16, ec:2.4.1
Pldbra_eH_r1s009g05057	20.38	38.26	48.44	1.88	2.37	Probable_phospholipid-transporting_ATPase <sup>1</sup>	ec:3.6.1, ec:3.1.3.96
Pldbra_eH_r1s027g10545	25.49	46.93	45.97	1.84	1.80	Probable_serine_carboxypeptidase_CPVL <sup>3</sup>	ec:3.4.21, ec:3.4.16
Pldbra_eH_r1s001g00135	29.17	51.86	56.72	1.78	1.95	Phospholipid_transporter <sup>1</sup>	ec:3.6.1

332 Genes potentially involved in transport of molecules<sup>1</sup>, development and growth<sup>2</sup>, or pathogenicity<sup>3</sup>.

333

334 *Focus on modulation of the P. brassicae transcriptome by the soil microbiota*  
335 *composition between H and M*

336

337 We focused on the analyses of the *P. brassicae* gene expression between M and H at  
338 Tf because in these two soil microbiota modalities, we observed (i) the most important  
339 differences in pathogen gene expression for both plant genotypes, and (ii) a contrasted  
340 disease phenotype in function of the host plant genotype (Fig 3): lower disease level  
341 in M versus H in Yudal and higher disease level in M versus H in Tenor.

342 The sense of over- or under-expression profiles depending on the soil condition (H or  
343 M) was studied in detail in function of the host genotype. As shown in the Venn diagram  
344 (Fig 4), 1360 *P. brassicae* genes (out of 1827, *i.e.* 74%) when infecting Tenor, and only  
345 9 *P. brassicae* genes (out of 296, *i.e.* 3%) when infecting Yudal were overexpressed  
346 in M compared to H. On the contrary, almost all the genes that were regulated by the  
347 soil microbiota diversity when Yudal was infected (260 out of 296) were  
348 underexpressed in M compared to H, although they were overexpressed in M versus  
349 H when infecting Tenor. The complete list of these 260 genes with the particular  
350 expression profile depending on the H / M levels and the host plant genotypes is  
351 indicated in the S4 Table. Among these 260 genes, a selection of the top 40 genes  
352 ranked according to the fold-change (Table 4) showed that the main functions encoded  
353 by these genes were related to the transport of molecules, the growth and  
354 development, the detoxification process and the pathogenicity. Concerning the 1100  
355 genes specifically overexpressed in the Tenor genotype in L compared to H, most of  
356 them were related to transport of molecules (data not shown).

357

358 Table 4. Selection of top 40 *P. brassicae* differentially expressed genes between H and M at Tf in an opposite sense when infecting  
 359 Yudal (Y) or Tenor (T).

<i>P. brassicae</i> gene	<i>P. brassicae</i> gene expression level		Fold change Y / H versus Y / M	<i>P. brassicae</i> gene expression level		Fold change T / H versus T / M	Description
	in Y / H / Tf	in Y / M / Tf		in T / H / Tf	in T / M / Tf		
Pldbra_eH_r1s001g00029	7.63	1.84	4.02	4.46	12.03	2.70	WD40_repeat
Pldbra_eH_r1s001g00152	1.67	0.40	4.06	1.16	2.79	2.38	carbohydrate-binding_module_family_18 <sup>3</sup>
Pldbra_eH_r1s001g00179	6.45	2.15	2.90	2.86	9.67	3.35	adenylate_guanylate_cyclase <sup>3</sup>
Pldbra_eH_r1s001g00511	40.71	11.71	3.46	19.71	59.05	3.00	Serine_threonine_kinase_HT1 <sup>2</sup>
Pldbra_eH_r1s001g00617	8.55	2.17	3.86	4.59	11.96	2.60	glutamate_NAD(P)+ <sup>2</sup>
Pldbra_eH_r1s001g00671	17.87	5.47	3.23	7.57	23.37	3.08	glutathione-disulfide_reductase <sup>4</sup>
Pldbra_eH_r1s001g00753	42.72	16.10	2.64	9.55	33.81	3.53	gamma-glutamylcyclotransferase <sup>4</sup>
Pldbra_eH_r1s002g00819	6.83	2.63	2.52	3.16	10.48	3.30	cytochrome_P450 <sup>4</sup>
Pldbra_eH_r1s002g00884	1.82	0.48	3.42	1.35	3.87	2.89	glutathione_S-transferase_kappa_1_[Rhodotorula_toruloides_NP11] <sup>4</sup>
Pldbra_eH_r1s002g01072	7.08	1.78	3.89	2.67	9.15	3.39	FMN-binding_glutamate_synthase_family <sup>1</sup>
Pldbra_eH_r1s003g01442	0.58	0.04	8.56	0.29	1.19	4.01	calcium_calmodulin-dependent_kinase_type_IV-like <sup>2</sup>
Pldbra_eH_r1s003g01550	8.87	2.75	3.15	6.10	16.04	2.62	Glycosyltransferase_uncharacterized <sup>2</sup>
Pldbra_eH_r1s003g01889	1.85	0.34	4.76	1.25	3.76	2.95	NUDIX_hydrolase <sup>3</sup>
Pldbra_eH_r1s003g01890	49.53	15.74	3.13	26.18	68.19	2.60	glycoside_hydrolase_family_16 <sup>3</sup>
Pldbra_eH_r1s003g01928	56.23	27.59	2.03	44.45	84.65	1.90	chitin_synthase_2 <sup>2</sup>
Pldbra_eH_r1s006g03824	6.92	2.18	3.10	3.52	10.19	2.87	Zinc_C2H2_type_family_(macronuclear) <sup>4</sup>
Pldbra_eH_r1s007g04126	75.85	26.51	2.85	43.01	113.52	2.64	p-type_atpase
Pldbra_eH_r1s007g04295	21.71	6.20	3.47	10.89	36.75	3.37	Mps1_binder <sup>2</sup>
Pldbra_eH_r1s008g04734	2.32	0.46	4.72	0.86	3.02	3.47	Serine_threonine_kinase_Sgk3 <sup>2</sup>
Pldbra_eH_r1s008g04750	5.45	1.33	3.91	3.65	10.15	2.78	methyltransferase_domain-containing
Pldbra_eH_r1s008g04794	3.37	0.84	3.69	1.44	4.67	3.19	zinc_C2H2_type_family <sup>4</sup>
Pldbra_eH_r1s009g05056	9.63	4.23	2.28	8.47	16.53	1.94	probable_phospholipid-transporting_ATPase_IA_isoform_X1 <sup>1</sup>
Pldbra_eH_r1s009g05121	8.82	2.71	3.20	4.39	12.28	2.78	phosphatidylserine_decarboxylase_subunit_beta
Pldbra_eH_r1s011g06165	4.32	0.89	4.53	2.28	7.67	3.34	UDP-D-xylose:L-fucose_alpha-1, 3-D-xylosyltransferase_1-like <sup>2</sup>
Pldbra_eH_r1s014g07095	3.27	0.92	3.35	1.72	5.57	3.17	glucosamine_6-phosphate_N-acetyltransferase <sup>2</sup>
Pldbra_eH_r1s015g07579	6.72	1.51	4.21	3.80	10.98	2.88	MFS_transporter <sup>1</sup>
Pldbra_eH_r1s016g07781	26.37	7.58	3.45	13.19	38.91	2.95	chitin_synthase_2 <sup>2</sup>
Pldbra_eH_r1s022g09622	47.16	17.11	2.74	22.80	62.09	2.72	phosphoenolpyruvate_carboxykinase <sup>2</sup>
Pldbra_eH_r1s022g09656	23.28	7.19	3.21	11.68	34.05	2.91	potassium_transporter <sup>1</sup>
Pldbra_eH_r1s024g09958	32.88	12.19	2.68	15.89	46.79	2.94	phosphate_ABC_transporter_substrate-binding <sup>1</sup>
Pldbra_eH_r1s025g10321	8.55	2.46	3.38	4.50	10.67	2.38	Maltose_maltodextrin_ABC_substrate_binding_periplasmic <sup>1</sup>
Pldbra_eH_r1s034g10543	2.67	0.79	2.96	1.92	6.02	3.13	probable_serine_carboxypeptidase_CPVL <sup>3</sup>
Pldbra_eH_r1s028g10813	7.61	2.63	2.80	2.22	7.21	3.23	ABC_transporter_G_family <sup>1</sup>
Pldbra_eH_r1s028g10814	6.19	2.49	2.41	1.74	6.07	3.42	ABC_transporter <sup>1</sup>
Pldbra_eH_r1s033g11505	14.72	4.86	3.00	10.02	24.24	2.42	Glycosyltransferase_uncharacterized <sup>2</sup>
Pldbra_eH_r1s034g11599	8.64	2.45	3.41	3.73	11.80	3.15	WD-40_repeat_domain-containing
Pldbra_eH_r1s035g11711	7.18	2.74	2.57	3.74	14.19	3.80	probable_phospholipid-transporting_ATPase_7_isoform_X1 <sup>1</sup>
Pldbra_eH_r1s042g12180	3.67	0.94	3.75	1.55	5.79	3.66	calcium/calmodulin-dependent_protein_kinase_type_IV-like <sup>2</sup>
Pldbra_eH_r1s056g12619	5.28	1.46	3.59	3.23	9.42	2.92	putative_WD_repeat-containing_protein
Pldbra_eH_r1s058g12634	7.62	2.00	3.67	4.64	12.69	2.74	peptidase_M14

360 Genes potentially involved in transport of molecules<sup>1</sup>, development and growth<sup>2</sup>, pathogenicity<sup>3</sup>, or detoxification<sup>4</sup>.

361

362 *Modulation of the P. brassicae transcriptome by the host plant genotype in each*  
363 *condition of soil microbiota composition*

364

365 The number of DEGs in *P. brassicae* according to the plant host genotype for each  
366 microbial diversity is presented in the Fig 5. At Ti, the effect of the host plant genotype  
367 on *P. brassicae* transcriptome was more important in H (445 DEGs) than M (2 DEGs)  
368 or L (60 DEGs), and most of the DEGs in L (78%) were also DEGs in H. Only one gene  
369 (with no known annotation) was differentially expressed according to the host genotype  
370 whatever the soil microbiota diversity. At Tf, a higher number of DEGs was found  
371 between host genotypes for each diversity than at Ti. The effect of the plant genotype  
372 was around 6 times more important in M (3896 DEGs) than in H (604 DEGs) or L (560  
373 DEGs). This is coherent with the observation that the M condition led to a contrasted  
374 disease phenotype in function of the host plant genotype (Figure 3: higher disease  
375 level in H versus M for the infected Yudal and lower disease level in H versus M for the  
376 infected Tenor). There were only 31 common DEGs between H and L and 154 between  
377 H and M, showing a particular *P. brassicae* transcriptome in function of the plant  
378 genotype in H. On the contrary, most of the DEGs in L were also DEGs in M. Finally,  
379 84% (3262 out of 3896) of the *P. brassicae* DEGs between host genotypes in M were  
380 specific of this soil microbiota diversity. A core of 28 DEGs was common to the three  
381 soil modalities; among them, whatever the soil microbiota diversity, 11 and 17 were  
382 under- or over-expressed in Tenor compared to Yudal, respectively. These genes  
383 displayed either unknown functions or functions of the general metabolism (data not  
384 shown).

385

386 **Modulation of the *B. napus* transcriptome by the soil microbiota composition**

387

388 The results of soil diversity manipulation (M versus H and L versus H) at Ti and Tf on  
389 the *B. napus* transcriptome for each genotype, both in healthy and infected plants, are  
390 shown in the Table 1.

391

392 *Modulation of the Yudal transcriptome by the soil microbiota composition*

393

394 In healthy Yudal, a very moderate soil condition's effect on DEGs number at Ti (0 to 8  
395 genes), and a higher effect at Tf (1852 to 3744 genes) were measured.

396 In infected Yudal, the M condition did not modify the gene expression compared to H,  
397 although 64 genes at Ti (S5A Table) and 23 genes at Tf (S5B Table) were differentially  
398 expressed between L and H. Interestingly, the Yudal transcriptome was modified by L  
399 at Ti, although no effect of the diversity on plant disease phenotype was significantly  
400 detectable at this stage (Fig 3). At Tf, the number of the genes that were down/up-  
401 regulated was less than at Ti despite a more pronounced difference in disease  
402 phenotype between L and H. In Table 5 is shown a selection of *B. napus* genes for  
403 which the expression was greatly different in Yudal between L and H. The DEGs  
404 included a large number of genes encoding various proteins involved in plant defense,  
405 and particularly in hormonal pathways.

406

407  
408  
409Table 5. Selection of top Yudal differentially expressed genes between H and L at Ti (A) and Tf (B) when infected by *P. brassicae*.

## A. At Ti.

<i>B. napus</i> gene	<i>B. napus</i> gene expression level		Fold change	Description
	in Y / H / Ti	in Y / L / Ti		
BnaC03g17080D	30.98	0.69	38.50	CYP71A13 <sup>1</sup>
BnaA03g14120D	38.61	2.07	17.82	CYP71A13 = cytochrome P450, family 71, subfamily A, polypeptide 13 <sup>1</sup>
BnaA09g41170D	32.90	2.73	11.44	Tyrosine aminotransferase 3 <sup>3</sup>
BnaA01g28810D	70.33	7.29	9.42	Legume lectin family protein <sup>1</sup>
BnaC09g43040D	9.48	1.04	8.69	GHMP kinase family protein <sup>2</sup>
BnaA01g12970D	13.16	1.37	8.52	CysteineNArich RLK (RECEPTORNAlike protein kinase) 21 <sup>2</sup>
BnaC04g45990D	182.19	22.53	8.05	Serine protease inhibitor, potato inhibitor INAtype family protein <sup>1</sup>
BnaC01g41330D	21.95	3.05	6.75	NucleotideNAdiphosphoNAsugar transferase <sup>1</sup>
BnaA09g00870D	497.35	75.10	6.61	Glutathione SNATransferase F3 <sup>1</sup>
BnaA04g27530D	30.36	5.01	6.05	NA
BnaC04g28910D	17.89	3.04	5.53	FAD/NAD(P)NAbinding oxidoreductase family protein <sup>1</sup>
BnaC02g43390D	26.72	4.70	5.51	0
BnaA04g03320D	70.82	13.00	5.46	JasmonateNAreregulated gene 21 <sup>3</sup>
BnaC01g36670D	117.35	22.88	5.09	CYP72A9 <sup>1</sup>
BnaA05g25490D	18.82	3.61	5.04	Unknown protein
BnaA05g03980D	67.18	13.27	4.98	Beta glucosidase 27 <sup>1</sup>
BnaC09g16910D	1658.97	355.44	4.67	GDSLNAlike Lipase/Acylhydrolase superfamily protein <sup>1</sup>
BnaA05g03390D	21.90	4.89	4.42	Trypsin inhibitor protein 1
BnaC03g17010D	14.16	3.13	4.35	Thioredoxin superfamily protein <sup>1</sup>
BnaA03g60240D	42.64	10.71	3.84	Seven transmembrane MLO family protein <sup>2</sup>
BnaA09g53990D	52.91	13.49	3.84	Pinorensinol reductase 1 <sup>1</sup>
BnaA06g03570D	1.41	8.55	5.60	AuxinNAresponsive GH3 family protein <sup>3</sup>
BnaA03g07790D	2.43	14.87	5.58	ChaperoninNAlike RbcX protein

410  
411  
412Genes potentially involved in plant defense and stress response<sup>1</sup>, signalization pathway<sup>2</sup>, or hormonal and jasmonic acid pathways<sup>3</sup>.

## B. At Tf.

<i>B. napus</i> gene	<i>B. napus</i> gene expression level		Fold change	Description
	in Y / H / Tf	in Y / L / Tf		
BnaA03g55570D	14.21	0.00	112.52	Sulfotransferase 2A <sup>2</sup>
BnaC01g29150D	18.11	0.11	78.16	DefensinNAlike (DEFL) family protein <sup>1</sup>
BnaAnng01940D	63.38	11.11	5.52	Sulfotransferase 2A <sup>2</sup>
BnaA09g50540D	29.06	6.90	4.21	2NAoxoglutarate (2OG) and Fe(II)NAdependent oxygenase superfamily protein <sup>1</sup>
BnaA05g07580D	67.14	16.48	4.04	DonNAGlucosyltransferase 1 <sup>2</sup>
BnaAnng38720D	23.76	7.16	3.39	MATE efflux family protein <sup>2</sup>
BnaC02g22290D	6.75	28.87	3.75	NA
BnaC09g18860D	429.43	1547.24	3.59	Cytochrome P450, family 707, subfamily A, polypeptide 3 <sup>2</sup>

413

Genes potentially involved in plant defense and stress response<sup>1</sup>, or hormonal and jasmonic acid pathways<sup>2</sup>.

414

415 *Modulation of the Tenor transcriptome by the soil microbiota composition*

416

417 In healthy Tenor, similar expression profiles to those of healthy Yudal were found, with  
418 a moderate number of DEGs at Ti between M and H (53 genes), and higher number  
419 between L and H (814 corresponding nearly to only 8 ‰ of the total number of  
420 expressed genes in *B. napus*). At Tf, 883 DEGs between M and H, and 3945 between  
421 L and H were found. In infected Tenor, no genes were differentially expressed between  
422 the soil conditions, except only 3 genes between M and H at Tf.

423

424 *Host plant genotype's effect on the B. napus transcriptome in each modality of soil*  
425 *microbiota composition*

426

427 The global view of DEGs in healthy and infected plants of the two host genotypes,  
428 according to the soil microbiota modality and the interaction time is illustrated in Venn  
429 diagrams (S5 Fig). The number of *B. napus* DEGs between genotypes was huge in  
430 healthy and infected plants, and largely the same whatever the soil microbiota (14,789  
431 to 27,537). In all the studied conditions, the effect of the genotype on plant  
432 transcriptome was very marked since about one third of the genes was differentially  
433 expressed between genotypes whatever the diversity, the time of interaction and the  
434 presence or not of the pathogen.

435

436 *Modulation of the B. napus transcriptome by the infection stage in each modality of*  
437 *soil microbiota composition*

438



439 The number of *B. napus* DEGs in each soil microbiota condition according to the  
440 infection stage showed high changes in transcript levels (S6 Fig). The high number of  
441 DEGs was retrieved for both host plant genotypes, infected or not, and for the three  
442 soil conditions. Whatever the diversity of the soil microbial community, the number of  
443 DEGs was quite similar for both genotypes in healthy plants. In infected plants, the  
444 number of DEGs in Yudal was slightly higher than in Tenor, particularly in H (19230  
445 and 13771 DEGs in Yudal and Tenor, respectively) and L (15560 and 10547 DEGs in  
446 Yudal and Tenor, respectively). Depending on the soil condition, both genotypes  
447 displayed 25 to 50% of common DEGs set between Ti and Tf. A moderate number of  
448 DEGs was shared between plant genotypes and soil microbiota diversities (1388 and  
449 2192 in healthy and infected plants, respectively).

450 By focusing more specifically on the *B. napus* genes that were differentially expressed  
451 between Ti and Tf for both infected genotypes and for the three soil's conditions, 2192  
452 genes were recovered (S6 Fig). Most of them were regulated in the same sense for  
453 Yudal and Tenor according to the time-point (S7A Fig). A slight part of genes had  
454 opposite sense of expression between plant genotypes: 6 genes were underexpressed  
455 in Yudal at Ti compared to Tf but over-expressed in Tenor at Ti compared to Tf, and  
456 34 genes were over-expressed in Yudal at Ti compared to Tf but underexpressed in  
457 tenor at Ti compared to Tf (S7A Fig). The annotation of 33 genes out of the 40 was  
458 retrieved (S7B Fig). Concerning the genes overexpressed in Yudal and  
459 underexpressed in Tenor at Ti compared to Tf, they were mainly related to growth and  
460 plant development. Other genes were related to the response to disease, or involved  
461 in hormonal signalization. Two genes (*WRKY DNA binding protein 11* and *Basic*  
462 *region/leucine zipper motif 53*) encoding for transcription factors were also differentially  
463 expressed between Ti and Tf in a different way according to the plant genotype.

464

## 465 **Discussion**

466

467 The plant-associated microbiota is more and more recognized as important  
468 determinant of plant health and pathogen suppression. As main ways to control  
469 clubroot such as crop rotations and cultivation of varieties carrying major resistance  
470 genes [29, 65] have shown their limits, there is a need to design alternative and durable  
471 methods based on ecological concepts. Exploring and understanding the mechanisms  
472 of disease regulation by microbiota could contribute to the emergence of innovative  
473 plant protection strategies.

474 Our research provides an extensive study of molecular mechanisms involved in  
475 complex host-pathogen interactions modulated by soil microbiota composition, using  
476 dual RNA-Seq to simultaneously capture the transcriptome of the two interacting  
477 partners. This approach has been applied to investigate a variety of host-pathogen  
478 relationships in major plant diseases in simplified in vitro experiments [66-68]. Our  
479 study upgraded the dual RNA-seq approach in more complex and realistic interaction's  
480 conditions.

481

## 482 **Soil microbiota composition and clubroot phenotypes**

483

484 The soil microbial diversity manipulation through serial dilutions ('dilution to extinction'  
485 experiment) led to a decreasing gradient of bacterial and fungal richness and a  
486 modification community' structure, as previously described [25], allowing controlled  
487 experiments using different microbial diversity reservoirs with common soil properties.  
488 We found that the microbial diversity modulated the clubroot development, in different  
489 patterns according to the host plant genotype. Interestingly, when Yudal was infected,  
490 the decrease in microbial diversity led to a proportional decrease in disease level, and

491 in infected Tenor, a bell curve of disease level according to microbial diversity was  
492 found. The invasion of pathogens is often described as linked to the level of microbial  
493 community's diversity and connectedness [69, 70]. It is also known that rhizosphere  
494 and endophytic microbial communities, that play key roles in controlling pathogens [18,  
495 27, 71, 72], are recruited from the communities of microorganisms in the soil in part in  
496 a plant-specific controlled way. It is indeed proved that different genotypes of the same  
497 plant species may have significant impacts on selecting rhizospheric partners through  
498 production of diverse root exudates [16, 73]. For instance, root-associated microbiota  
499 displaying reproducible plant genotype associations was recently identified in maize  
500 [74]. Genotype effects of the plant hosts can be also more important for individual  
501 microbial species [75]. The difference in modulation of clubroot by the soil microbial  
502 diversity between Yudal and Tenor, as well as the higher changes in *P. brassicae*  
503 transcript levels in function of soil microbiota composition when Tenor was infected  
504 compared to Yudal, could be due to a plant genotype's effect on the process of  
505 microbial recruitment. More particularly, missing microbes, or prevalence of 'helper'  
506 microbes, or changes in the strength and connection of the microbes' network between  
507 H, M or L conditions can support the disease's outbreak [76]. Moreover, we previously  
508 showed that not only the structure of microbial communities associated with the  
509 rhizosphere and roots of healthy Brassica plants (*B. rapa*) evolved over time, but also  
510 that the invasion by *P. brassicae* changed root and rhizosphere microbial communities  
511 already assembled from the soil [28]. All these results highlighted the complexity of the  
512 microbial interactions in soil, including interactions between microorganisms, between  
513 microbes and plant, and between microbes and pathogen.

514

515 **Soil microbiota composition and *P. brassicae* transcriptome**

516

517 The global view of distribution of DEGs according to the soil microbiota composition,  
518 in each plant genotype and time-point, showed that the *P. brassicae* transcriptome was  
519 not only more modulated when infected Tenor than Yudal, but also most strongly  
520 activated at Tf than Ti. During its life cycle, *P. brassicae* survives in soil in the form of  
521 resting spores. Sensing signal molecules, such as host root exudate production or  
522 specific soil environment, is essential to exit dormancy, trigger germination and begin  
523 the initial step of the life cycle inside the root: at this stage, suitable conditions in  
524 environment, such as the soil microbial diversity and composition, are necessary. Bi et  
525 al. [35] showed that *P. brassicae* is able to have perception of external signals thanks  
526 to specific signaling pathway and to adapt to its environment. In our study, the very  
527 early step of interaction between *P. brassicae* spores and soil microbiota was not  
528 measured. But the higher *P. brassicae* transcriptome modulation at Tf than at Ti  
529 highlighted the secondary cortical infection stage of clubroot disease as crucial for  
530 interaction between *P. brassicae* and the microbiota. In the same way, the root and  
531 rhizosphere-associated community assemblies in *B. rapa*, particularly the endophytic  
532 bacterial communities, were also strongly modified by *P. brassicae* infection during this  
533 stage [28]. Thus, the disturbance consequences of the interactions between *P.*  
534 *brassicae* and the endophytic communities inside the roots occurred at the tardive date  
535 of sampling, and the effect of soil environment on *P. brassicae* transcriptome was  
536 thereby measurable at the stages where the pathogen was in a close interaction with  
537 its host.

538

539 *The soil microbiota composition affects the expression of P. brassicae genes*  
540 *potentially involved in the transport of molecules*

541

542 At Tf, higher *P. brassicae* amount (and DI) were found in H compared to M in infected  
543 Yudal, whereas lower in H compared to M when infected Tenor. The DEGs in this same  
544 sense as *P. brassicae* amount between H and M were particularly analyzed for both  
545 infected host plant genotypes (Tables 2, 3, 4), and studied in function of their potential  
546 involvement in different functions. This is for example the case for several genes,  
547 overexpressed in conditions where DNA *P. brassicae* content was higher, that were  
548 related to functions of molecule transport. The loss of key biosynthetic pathways is  
549 indeed a common feature of parasitic protists, making them heavily dependent on  
550 scavenging nutrients from their hosts. Salvage of nutrients by parasitic protists is often  
551 mediated by specialized transporter proteins that ensure the nutritional requirements.  
552 This is the case of genes coding for a FMN-binding glutamate synthase, a complex  
553 iron-sulfur flavoprotein that plays a key role in the ammonia assimilation pathways also  
554 found in bacteria, fungi and plants [77, 78], and for a phospholipid transporting ATPase,  
555 a Phosphate\_ABC\_transporter or a Potassium transporter. Some transporters, such  
556 as the Ammonium\_transporters are also expressed during host colonization and  
557 pathogenicity in fungus because of the importance of ammonia in host alkalization  
558 [79, 80]. The soil microbiota composition and then the subsequent recruitment of  
559 endophyte microbes by the plant could affect the *P. brassicae* ability to recruit  
560 nutriment from the host because of potential competition for resource [81].

561

562 *The soil microbiota composition affects the expression of P. brassicae genes*  
563 *potentially involved in growth and development*

564

565 Other examples of DEGs between soil microbial diversities with expression profiles  
566 correlated to clubroot development were related to functions of growth, development  
567 and cell differentiation. For instance, the gene coding for a Chitin synthase, essential

568 for the cell wall chitin depositions during resting spore maturation, was overexpressed  
569 in conditions where clubroot symptoms were more pronounced. The chitin-related  
570 enzymes are enriched in *P. brassicae* genome [32, 37, 39]. Deletion of *chitin synthase*  
571 genes in fungi most often results in developmental defects, which include defective  
572 infection structure development or defunct invasive growth [82, 83]. Concerning the  
573 gene coding for a Phosphoenolpyruvate\_carboxykinase, its differential expression  
574 could make possible to *P. brassicae* a glucose-independent growth [84]. The  
575 differential expression of a gene coding for a Glycosyltransferase could facilitate the  
576 growth as shown in filamentous pathogenic fungi [85].

577

578 *The soil microbiota composition affects the expression of P. brassicae genes*  
579 *potentially involved in pathogenicity*

580

581 Some *P. brassicae* genes coding for potential pathogenicity factors, that were  
582 overexpressed in M compared to H in Tenor and/or underexpressed in M compared to  
583 H in Yudal, may explain in part the different disease phenotype observed in function of  
584 the soil microbial diversities' conditions.

585 This was the case for the gene encoding a Glutathione transferase that was  
586 overexpressed in conditions of important clubroot development symptoms. Glutathione  
587 transferases represent an extended family of multifunctional proteins involved in  
588 detoxification processes and tolerance to oxidative stress. In *Alternaria brassicicola*,  
589 Glutathione transferases participate in cell tolerance to isothiocyanates, allowing the  
590 development of symptoms on host plant tissues [86]. The pathogenicity of *P. brassicae*  
591 could be partly related to its ability to protect itself against such plant defenses  
592 compounds.

593 For other genes putatively related to pathogenicity, we found the same trend of  
594 overexpression in conditions of important clubroot development. The E3-Ubiquitin  
595 ligase is described as a microbial effector protein that evolved the ability to interfere  
596 with the host E3-Ub-ligase proteins to promote disease [87]. The alkaline ceramidase  
597 is involved in the virulence of microbes like *Pseudomonas aeruginosa* [88]. The  
598 cytosolic carboxypeptidase\_4 and the serine carboxypeptidase\_CPVL are also  
599 described as potential factors of virulence with a role in adherence process,  
600 penetration of tissues, and interactions with the immune system of the infected host  
601 [89, 90]. The genes coding for the Carbohydrate-binding module\_family\_18 or the  
602 Glycoside\_hydrolase family\_16 can protect some fungi against plant defense  
603 mechanisms [91, 92]. For instance, CBM18-domain proteins protect from breakdown  
604 by chitinase in some fungi [83]. In Plasmodiophorids, proteins containing a CBM18  
605 domain, could bind to the chitin in order to promote modification into chitosan, a weaker  
606 inducer of immune responses than chitin in many plants [32].  
607 Finally, a conserved effector gene in the genomes of a broad range of phytopathogenic  
608 organisms across kingdoms (bacteria, oomycetes, fungi) [93, 94], the  
609 *NUDIX\_hydrolase*, was found overexpressed in conditions where clubroot symptoms  
610 were highest, according to the soil microbial diversity. In *Arabidopsis thaliana* infected  
611 by *P. brassicae*, proteomics studies had already detected an upregulation of the  
612 NUDIX protein [95]. NUDIX effectors have been validated as pathogenesis players in  
613 a few host–pathogen systems, but their biological functions remain unclear [93].  
614 Further studies are necessary to decipher if *P. brassicae* might share strategy involving  
615 NUDIX effectors described in other plant pathogens. The *NUDIX* gene is a good  
616 pathogenicity candidate gene, potentially responsible for *P. brassicae* infection and  
617 subsequent disease progression and that needs to be functionally assessed.

618

## 619 **Soil microbiota composition and *B. napus* transcriptome**

620

621 *The host plant genotype and the infection's kinetic strongly affect the plant*  
622 *transcriptome whatever the soil microbiota composition*

623

624 In both healthy and infected plants, the number of *B. napus* DEGs between genotypes  
625 was huge and largely shared between soil microbiota, and the number of plants DEGs  
626 between Ti and Tf was also high for each genotype whatever the soil microbiota  
627 composition. This demonstrates that the genetic control of the developmental process  
628 is highly dynamic and complex, and remains largely unknown.

629 The list of common DEGs between Ti and Tf in both genotypes and the three H, M, L  
630 conditions (S7 Fig) was studied more in detail, and particularly the genes  
631 overexpressed in Yudal but underexpressed in Tenor at Ti compared to Tf. These  
632 genes were mainly related to growth and plant development: *Sterol methyltransferase*  
633 *3* [96], *C2H2like zinc finger protein* [97], *BES1/BZR1 homolog 2* [98], *WUSCHEL*  
634 *related homeobox 4* [99], *Expansin A1* [100], *Arabinogalactan protein 22* [101],  
635 *Trichome BireFringence 27* [102], *SKU5 similar 17* [103], *Transcription elongation*  
636 *factor (TFIIS) family protein* [104], *Endoxyloglucan transferase A3* [105], *KIPrelated*  
637 *protein 2* [106], and *Ras related small GTPNAbinding family protein* [107]. Other genes  
638 of the list were related the response to disease, like the *RING/box superfamily protein*  
639 *(family E3 ligase)* [108], the *Eukaryotic aspartyl protease family protein* or the  
640 *Eukaryotic aspartyl protease family protein* [109], the *TRAFlike family protein* [110].  
641 Finally, some other genes were involved in hormonal signalization (*Auxin responsive*  
642 *GH3 family protein*, *Heptahelical transmembrane protein2*), in primary metabolism  
643 (*Glucose-6-phosphate dehydrogenase* playing a key role in regulating carbon flow  
644 through the pentose phosphate pathway), and in stress response (*Galactose*



645 *oxidase/kelch repeat superfamily protein* [111]). Two genes encoding for transcription  
646 factors were also differentially expressed between Ti and Tf in a different way  
647 according to the plant genotype (*WRKY DNA binding protein 11* and *Basic*  
648 *region/leucine zipper motif 53*). The sense of expression of these genes can be  
649 correlated to the level of *P. brassicae* susceptibility of both genotypes: Yudal, known  
650 to be more resistant to clubroot than Tenor, displayed an increase of gene's expression  
651 related to growth and disease response as potential mechanisms of resistance,  
652 whatever the microbial diversity and composition in the soil.

653

654 *The soil microbiota composition affects the plant transcriptome*

655

656 In healthy plants, the soil microbiota composition effect on plant transcriptome was  
657 similar for both genotypes: no effect at Ti and close number of DEGs at Tf. In contrast,  
658 in infected plants, only Yudal transcriptome was affected by the soil microbiota  
659 diversity, and interestingly mainly at Ti. The Yudal DEGs between L and H included a  
660 large number of genes encoding various proteins involved in plant defense, such as  
661 the CYP71A13 (phytoalexin biosynthesis), the  $\beta$ -glucosidase and the Nucleotide  
662 diphospho-sugar transferase (glucosinolates' metabolism), the Pinoresinol reductase  
663 (synthesis of lignane), the oxidoreductase family protein (terpenes' metabolism), the  
664 lectin family protein (plant defense proteins), the serine protease inhibitor and the  
665 inhibitor INAtype family protein (antimicrobial activity), the glutathione transferase F3  
666 (transport of defense compounds), and the Lipase/Acylhydrolase superfamily protein  
667 (growth and plant defense). These proteins may represent critical early molecules in  
668 the plant defense response before disease progression.

669

670 **Complex interactions between plant/pathogen and soil microbiota**

671

672 Our study aimed to decipher the interactions between plant, pathogen and the soil  
673 microbial community to better understand the mechanisms and the host/pathogen  
674 functions involved in disease modulation. We highlighted *P. brassicae* and *B. napus*  
675 DEGs between microbial environment conditions with potential functions involved in  
676 growth and pathogenicity in the pathogen, and defense in the plant. Further studies  
677 (e.g. gene inactivation) are necessary to explore if these proteins have expected  
678 functions in the Plasmodiophorids on one hand, and in *B. napus* on the other hand.

679 In infected plants, even the number of DEGs remained low in *B. napus*, the expression  
680 profile was pretty opposite to that of *P. brassicae* in response to soil microbiota diversity  
681 levels:

682 (i) The plant transcriptome was more modified between H and diluted conditions for  
683 Yudal, a resistant genotype, while the pathogen transcriptome was more modified  
684 between soil microbial modalities when the host plant was Tenor, a clubroot  
685 susceptible genotype.

686 (ii) The plant transcriptome was more modified at Ti than Tf by the soil microbial  
687 diversity, while the pathogen transcriptome was modulated later at Tf.

688 This host plant genotype-dependent and time-lagged response to the soil microbial  
689 composition between the plant and the pathogen transcriptomes suggest a complex  
690 regulatory scheme. The soil microbiome would modulate precociously the plant  
691 defense mechanisms in the partially resistant genotype but would have moderate or  
692 no effect in the susceptible plant, perhaps because of a too high disease level. In  
693 parallel, a direct effect of the soil microbiota composition (key-species for instance) on  
694 the pathogen could also occur in the early stages of infection, with a late visible effect  
695 on the transcriptome of the pathogen. This highlights the importance to perform studies  
696 on very early steps of infection by *P. brassicae*. Moreover, a specific microbial

697 recruitment from the soil diversity in function of the plant genotype could also occur  
698 with subsequent consequences on pathogen metabolism in later step of its  
699 development inside the roots in interaction with endophyte microbes. These latter,  
700 differentially recruited in function of the host plant genotype, could have different effect  
701 on pathogen gene expression during its development inside the roots. In turn, the plant  
702 would affect the pathogen transcriptome by modulating or not some genes involved in  
703 growth and pathogenicity. Mutant approaches (plant and pathogen) could validate  
704 these hypotheses.

705 The mechanisms within the microbial functions present in soils rather than just the  
706 species need also to be studied. The difference in clubroot observed according to both  
707 plant genotypes and soil diversity could be in part explained by the concept of  
708 functional redundancy (defined as the overlapping and equivalent contribution of  
709 multiple species to a particular function) on the one hand, and the non-redundancy of  
710 rare soil microbes playing a key-role in ecosystem on the other hand [112]. Further  
711 thorough studies on microbial endophyte and rhizosphere species and functions  
712 present in both plant genotypes depending on microbial community composition are  
713 necessary to describe if some keystone microbial species/stains of specific bacteria  
714 and/or fungi could explain the clubroot phenotypes. This would require: (i) a more  
715 accurate taxonomic resolution and a more complete description (e.g. protist  
716 community) of the microbial soil compositions; (ii) a study of the functions expressed  
717 by microbial species, as described in some examples of molecular mechanisms  
718 leading to pathogen growth suppression on plant tissues found in the literature [113-  
719 116]. For this, metatranscriptomics approach to analyze the microbial functions  
720 expressed in roots are in progress to better understand the complex interaction plant /  
721 pathogen / microbial environment.

722

## 723 **Materials and methods**

724

### 725 **Preparation of soils harboring different microbial diversity levels**

726

727 The soil preparation to obtain different microbial diversity levels was performed as  
728 described in [25]. The soil was collected at the INRA experimental site La Gruche,  
729 Pacé, France, from the layer -10 to -30 cm. After homogenization, grinding, sieving  
730 and mixing with silica sand (2/3 soil, 1/3 sand), a part of the soil was gamma rays  
731 sterilized at 35 kGy and stabilized for 2 months. The unsterilized soil (100 g of dry soil)  
732 was suspended in 1 L of deionized water and used for serial dilution: undiluted ( $10^0$ ,  
733 High diversity level [H], considered as the reference), diluted at  $10^{-3}$  (Medium diversity  
734 level [M]) or  $10^{-6}$  (Low diversity level [L]). Three dilution processes were performed  
735 corresponding to 3 biological replicates. The sterilized soil (2.5 kg per bag) was  
736 inoculated with 300 mL of each dilution (H, M, L) and incubated in the dark at 18°C  
737 and 50% humidity for 49 days. Every week, microbial respiration and recolonization  
738 were facilitated when opening the bags under hood. The recolonization was followed  
739 by a microbiological count of formed cultivable colonies during the incubation period  
740 (S1 Fig).

741

### 742 **Molecular characterization of soil bacterial and fungal communities**

743

744 After recolonization and before sowing, the three microbial modalities were analyzed  
745 for their physicochemical composition at the Arras soil analysis laboratory (LAS, INRA,  
746 Arras, France) (S1 Table) and for their microbial diversity. The GnS-GII protocol was  
747 used for extraction of DNA from soil samples [117]. Briefly, DNA was extracted from 2  
748 g of dry soil, and then purified by PVPP column and GeneClean [28]. PCR amplification

749 and sequencing were performed at the GenoScreen (Lille, France) using the Illumina  
750 MiSeq 'paired-end' 2x250 bases (16S) for bacteria and Illumina MiSeq 'paired-end'  
751 2x300 bases (18S) for fungi as described previously [25, 28]. The protist diversity was  
752 not included in the analysis. After read assembly, sequences were processed with the  
753 GnS-PIPE bioinformatics developed by Genosol platform [118, 119]. By performing  
754 high-quality sequence clustering, Operational Taxonomic Units (OTUs) were retrieved  
755 and taxonomic assignments were performed comparing OTUs representative  
756 sequences against dedicated reference databases from SILVA [120]. The cleaned  
757 data set is available on the European Nucleotide Archive database system under the  
758 project accession number PRJEB36457. Soil samples accession numbers range from  
759 ERR3842608 to ERR3842625 for 16S and 18S rDNA.

760 The alpha diversity of the communities was analyzed. To compare bacterial or fungal  
761 composition among three soil preparations, the richness of these communities was  
762 characterized by the number of OTUs found in each soil. As metric of taxonomy  
763 diversity, the Shannon diversity index was also determined (package 'vegan' [121]).  
764 Since values were conformed to normality assumptions, linear models LMM function  
765 'lmer', package 'lme4' [122]) were used to examine differences between soil  
766 preparation for these measures. When needed, pairwise comparisons of least squares  
767 means (package 'lsmeans' [123]) and a false discovery rate correction of 0.05 for P-  
768 values [124] were performed.

769 In order to analyse the bacterial and fungal community structure (beta diversity),  
770 principal coordinate analysis (PCoA) was performed on a Bray-Curtis dissimilarity  
771 matrix, obtained from OTUs data, which were normalized using a 1‰ threshold and  
772 log<sub>2</sub>-transformed (package 'vegan' [121]). A type II permutation test was performed on  
773 the PCoA coordinates to compare the community structure of the H, M and L soils  
774 (package 'RVAideMemoire' [125]).

775

## 776 **Plant material and pathogen inoculation**

777

778 The oilseed rape genotypes Tenor and Yudal and the eH isolate of *P. brassicae*  
779 belonging to pathotype P1 [39, 126, 127] were used in this study. Yudal and Tenor  
780 genotypes were chosen because previous assay in our lab showed they display  
781 different responses to clubroot infection: Tenor was more susceptible than Yudal to  
782 eH. Both *B. napus* genotypes were grown in each of the three soils (harboring H, M or  
783 L microbial diversities). For this, seeds of oilseed rape were sown in pots filled with 400  
784 g of experimental soils. Pots were placed in a climatic chamber, in a randomized block  
785 design with the three modalities (H, M, L) and three replicates by dilution factor. For  
786 each oilseed rape genotype, eight plants per soil microbial modality and per replicate  
787 were used. Plants were either not inoculated (healthy plants) or inoculated with a  
788 resting spore suspension of the *P. brassicae* eH isolate. For inoculum production, clubs  
789 propagated on the universal susceptible host Chinese cabbage (*B. rapa* ssp  
790 *pekinensis* cv. Granaat) were collected, homogenized in a blender with sterile water  
791 and separated by filtration through layers of cheesecloth. The resting spores were then  
792 separated by filtration through 500, 100 and 55  $\mu\text{M}$  sieves to remove plant cell debris.  
793 The spore concentration was determined with a Malassez cell and adjusted to  $1.10^7$   
794 spores.mL<sup>-1</sup>. Plant inoculation was done as described in [128]: seven-day-old  
795 seedlings were inoculated by pipetting 1 mL of the spore suspension at  $1.10^7$   
796 spores.mL<sup>-1</sup> to the bottom of the stem of each seedling. The plants were maintained at  
797 22°C (day) and 19°C (night) with a 16h photoperiod, and watered periodically from the  
798 top with a Hoagland nutritive solution to provide nutrients and to maintain a water  
799 retention capacity of 70 to 100%.

800

## 801 **Phenotyping: plant characterization and disease assessment**

802

803 Roots and aerial parts were sampled at two times: 28 days after inoculation (dai)  
804 (intermediary time, Ti) for both genotypes, and 36 dai and 48 dai for Tenor and Yudal  
805 (final time, Tf), respectively. The final time was chosen to have clearly visible galls on  
806 the primary and lateral roots.

807 At each sampling date and for each replicate, the aerial parts of 8 plants were cut,  
808 dried and weighted. As one of the three infected replicates at the final time for Tenor  
809 in L soil displayed no clubroot symptoms in any of the 8 plants, indicating that the  
810 inoculation of these plants was not successful, this sample was removed for all the  
811 analyses. The roots were cut below the collar (in the soil depth from -1 to -6 cm),  
812 separated from soil, and washed twice in sterile water by vortexing 10 sec. Then the  
813 roots were transferred in a petri dish, cut into small pieces, and frozen in liquid nitrogen  
814 then stored at -80°C. After lyophilization, the dry root biomass was measured and the  
815 powder was kept until nucleic acid extraction (DNA for pathogen quantification and  
816 RNA for RNAseq analyses).

817 Disease was assessed at each sampling date after inoculation with *P. brassicae*. First,  
818 clubroot symptoms were evaluated by a disease index calculated with the scale  
819 previously described by Manzanares-Dauleux et al. [128]. Secondly, 1 µL of DNA  
820 extracted from root samples (see 2.5.) was used for quantitative PCR on the  
821 LightCycler® 480 Real-Time PCR System (Roche) to quantify *P. brassicae* amount.  
822 For this, a portion (164 bp) of the target 18S gene was amplified with the following  
823 primers: 5'-ttgggtaattgCGCGCCTG-3' (forward) and 5'-cagcggcaggTCATTCAACA-3'  
824 (reverse). Each reaction was performed in 20 µL qPCR reaction with 10 µL of SYBR  
825 Green Master Mix (Roche), 0.08 µL of each primer (100 µM) and 1 µL of total DNA as  
826 template. The PCR conditions consisted of an initial denaturation at 95°C for 5 min,

827 followed by 45 cycles at 95°C for 10 s and 64°C for 40 s. Standard curves were  
828 constructed using serial dilutions of *P. brassicae* DNA extracted from resting spores.  
829 Quantitative results were then expressed and normalized as the part of the *P.*  
830 *brassicae* mean DNA content in the total root-extracted DNA.

831 To compare the aerial and root biomasses between modalities, linear models were  
832 used (LMM function 'lmer', package 'lme4' [122]). A Wald test ( $\alpha = 5\%$ ) was applied for  
833 evaluating the soil effect in the LMM model. Least Square Means (LSMeans) were  
834 calculated using the 'lsmeans' function of the 'lsmeans' package [123], and the false  
835 discovery rate correction for P-values [124]. Pairwise comparisons of LSMeans were  
836 performed with the Tukey test ( $\alpha = 5\%$ ), using the 'cld' function of the 'lsmeans'  
837 package.

838 Disease data were analyzed using a likelihood ratio test on a cumulative link model  
839 (CLMM; 'clmm' function, 'ordinal' package). LSMeans and pairwise comparisons of  
840 LSMeans were performed as described for biomasses' analyses.

841

## 842 **Nucleic acids isolation from roots**

843

844 At each time-point, the lyophilized roots from the 8 pooled plants of each genotype and  
845 each treatment (with and without *P. brassicae*) were used for nucleic acid extraction.

846 DNA was extracted from 30 mg of lyophilized powder root samples with the NucleoSpin  
847 Plant II Kit (Masherey-Nagel) following the manufacturer's instructions. After  
848 verification of the DNA quality on agarose gel and estimation of the quantity with a  
849 Nanodrop 2000 (Thermoscientific), it was used for *P. brassicae* quantification.

850 Total RNA was extracted from 20 mg of lyophilized powder with the Trizol protocol  
851 (Invitrogen). RNA purity and quality were assessed with a Bioanalyser 2100 (Agilent)  
852 and quantified with a Nanodrop (Agilent).



853

## 854 **Library construction and Illumina sequencing**

855

856 RNA-seq analysis was performed on RNA extracted from roots tissues of two *B. napus*  
857 genotypes infected or not with resting spores of *P. brassicae* (eH isolate) grown in the  
858 three different soils (H, M, L), for three biological replicates, at Ti and Tf.

859 The TruSeq Stranded mRNA Library Sample Prep Kit (Illumina) was used for library  
860 construction. Library pair-end sequencing was conducted on an Illumina HiSeq4000  
861 (Genoscreen, Lille, France) using 2x150 bp and resulting in 2861 paired-end millions  
862 of reads. Briefly, the purified mRNA was fragmented and converted into double-  
863 stranded cDNA withy random priming. Following end-repair, indexed adapters were  
864 ligated. The cDNA fragments of ~350 pb were purified with AMPure beads XP and  
865 amplified by PCR to obtain the libraries for sequencing. The libraries were multiplexed  
866 (six libraries per lane) and sequenced. The cleaned data set is available on the  
867 European Nucleotide Archive database system under the project accession  
868 number PRJEB36458. Samples accession numbers range from ERR3850126 to  
869 ERR3850197.

870

## 871 **Mapping of sequenced reads, assessment of gene expression and identification** 872 **of differentially expressed genes**

873

874 The read quality was undertaken for the quality scores of Q28 and for the read length  
875 of 50 nucleotides using PrinSeq. In order to use a combined host-pathogen genome  
876 as reference for alignment, the genomes of eH *P. brassicae* [39] and *B. napus* [129]  
877 were concatenated, as well as the corresponding annotation files. The high-quality  
878 reads were aligned to the concatenated files using STAR 2.5.2a\_modified. Non-default

879 parameters were minimum intron length 10, maximum intron length 50 000 and mean  
880 distance between paired ends-reads 50 000. For the reads which can align to multiple  
881 locations (parameters set for a maximum of 6 locations), a fraction count for multi  
882 mapping reads was generated. Thanks to genome annotation files, the mapped  
883 sequencing reads were assigned to genomic features using featureCounts v1.5.0-p1,  
884 and counted. After filtering of the read counts below the threshold value (at least 0.5  
885 counts per million in 3 samples), the count reads were then normalized with the  
886 Trimmed Mean of M values (TMM method). Concerning the *P. brassicae* reads, as the  
887 number of reads in the libraries at Ti was much smaller than at the final time (due to  
888 the differences in the infection rate and progression of the pathogen between the  
889 sampling times), the normalization was performed for Ti separately from Tf. So,  
890 analyses of *P. brassicae* were specific of each sampling time, preventing the data  
891 comparison between the time-points. On the contrary, for *B. napus* reads, the  
892 normalization was performed on total libraries, allowing a kinetic analysis of plant  
893 transcriptome.

894 Differential expression analysis was performed using the EdgeR package in R. The  
895 Differentially Expressed Genes (DEGs) with  $FDR \leq 0.05$  from specific comparison lists  
896 were selected for analysis. The functional annotation of DEGs was performed with  
897 Blast2GO 4.1.9 software. Heat maps were generated using the 'heatmap3' package  
898 and Venn Diagrams using the 'VennDiagram' packages in R.

899

## 900 **Figures Captions**

901

902 Fig 1. Bacterial (A) and fungal (B) richness and diversity, and communities' structures  
903 (C) in the three soils used in this study. Mean richness (number of observed OTUs)  
904 and alpha-diversity (Shannon index) for the 3 soil microbial modalities (H, High in black;

905 M, Medium in medium grey; L, Low in white) are presented in bacterial (A) and fungal  
906 (B) communities. Different letters indicate statistically significant differences among  
907 communities at  $P < 0.05$ . Principal coordinates analysis (PCoA) projection of the  
908 communities' structure is shown for bacteria and fungi for the H, M and L diversities  
909 (C).

910

911 Fig 2. Aerial and root biomasses. The dry aerial parts (A) and roots (B) were weighted  
912 for both genotypes (Tenor and Yudal) at different days after inoculation (Ti, 28 dai; Tf  
913 36 or 48 dai). For soil diversity, black, medium grey and white bars correspond to High  
914 (H), Medium (M) and Low (L) diversities, respectively. Error bars represent standard  
915 errors from the means of 8 plants. \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; NS, Non Significant.

916

917 Fig 3. Influence of soil microbiota diversity on clubroot development. Plants were  
918 exposed to High (black), Medium (grey) or Low (white) soil microbial modalities during  
919 28 (Ti), 36 or 48 (Tf) days after inoculation with the eH isolate of *P. brassicae*. The  
920 clubroot symptoms were estimated according to the disease index and the  
921 quantification of *P. brassicae* DNA by qPCR, expressed as a ratio of the 18S DNA  
922 quantity relative to the total DNA. Data are means of 3 biological replicates (12 plants  
923 per replicate) and error bars represent standard errors of the means. Means with  
924 different letters are statistically significantly different according to the analysis of  
925 variance test ( $P < 0.05$ ).

926

927 Fig 4. Number of *P. brassicae* differentially expressed genes (DEGs) at Tf between  
928 High (H) and Medium (M) soil microbial diversity levels when infected Yudal or Tenor.  
929 The Venn diagram shows the number of significantly *P. brassicae* DEGs ( $P < 0.05$ )

930 that are overexpressed ( $M > H$ ) or underexpressed ( $M < H$ ) in M compared to H  
931 according to the host *B. napus* genotypes (Yudal, Y; Tenor, T) at the sampling date Tf.

932

933 Fig 5. Number of *P. brassicae* differentially expressed genes (DEGs) in function of the  
934 host plant genotype for each soil microbial diversity level. The Venn diagram shows  
935 the number of significantly DEGs ( $P < 0.05$ ) according to the host *B. napus* genotypes  
936 (T, Tenor; Y, Yudal) for each soil microbial diversity level (H, High; M, Medium; L, Low)  
937 at the sampling dates Ti and Tf.

938

### 939 **Supporting information captions**

940

941 S1 Fig. Microbiological follow up based on the Colony Forming Units (CFU) method  
942 during the incubation period for bacteria (A) and fungi (B). H, High diversity modality;  
943 M, Medium diversity modality; L, Low diversity modality.

944

945 S2 Fig. Description of the main bacterial and fungal composition in the three soils.  
946 Average relative abundance ( $RA \pm SEM$ ) of the most abundant bacterial phyla (A),  
947 genera (B), OTUs (C), and fungal phyla (D), genera (E), OTUs (F) are shown in High  
948 (H), Medium (M) and Low (L) soil microbial diversities. For each soil, the number of  
949 replicates is  $n=3$ .

950

951 S3 Fig. Overview of all *P. brassicae* transcriptome samples. A. Heatmaps of *P.*  
952 *brassicae* gene expression based on normalized data of expression values. The  
953 heatmaps are based on total reads counts for *P. brassicae* at Ti and Tf for the 3  
954 microbial soil diversities (H, High; M, Medium, L, Low), the two plant genotypes (T,  
955 Tenor; Y, Yudal) and correspond to the mean of the three replicates. B. Hierarchical

956 Cluster Analysis (HCA) of the filtered and normalized counts in the dual-RNAseq  
957 analysis. The analyses are shown for *P. brassicae* reads at Ti and Tf for the 3 soil  
958 microbial diversities (H, High; M, Medium; L, Low), the two plant genotypes (T, Tenor;  
959 Y, Yudal), and the three replicates (a, b, c).

960

961 S4 Fig. Overview of all *B. napus* transcriptome samples. Hierarchical Cluster Analysis  
962 (HCA) of the filtered and normalized counts in the dual-RNAseq analysis in healthy  
963 plants (A) and infected plants (B). The analyses are shown for *B. napus* reads at Ti  
964 and Tf, for the 3 soil microbial diversities (H, High; M, Medium; L, Low), the two plant  
965 genotypes (T, Tenor; Y, Yudal), and the three replicates (a, b, c).

966

967 S5 Fig. Number of *B. napus* differentially expressed genes (DEGs) in function of the  
968 host plant genotype for each soil microbial diversity level when not infected (A) or  
969 infected by *P. brassicae* (B). The Venn diagram shows the number of significantly  
970 DEGs ( $P < 0.05$ ) according to the host *B. napus* genotypes (T, Tenor; Y, Yudal)  
971 infected or not, for each soil microbial diversity level (H, High; M, Medium; L, Low) at  
972 the sampling dates Ti and Tf.

973

974 S6 Fig. Number of *B. napus* differentially expressed genes (DEGs) in function of the  
975 interaction stage for each soil microbial diversity level. The Venn diagrams show the  
976 total number of significantly DEGs ( $P < 0.05$ ) in the *B. napus* genotypes (T, Tenor; Y,  
977 Yudal), healthy (A) or infected by *P. brassicae* (B), at each soil microbial diversity level  
978 (H, High; M, Medium; L, Low), between Ti and Tf.

979

980 S7 Fig. Differentially expressed genes (DEGs) in both infected *B. napus* genotypes  
981 according to the infection's stage whatever the soil microbial diversity. A. The Venn

982 diagram shows the number of significantly DEGs ( $P < 0.05$ ) common in both *B. napus*  
983 genotypes (T, Tenor; Y, Yudal), and common in the three soil microbial diversity levels  
984 (H, High; M, Medium; L, Low), which are down (<) or up (>) regulated at Tf compared  
985 to Tf. B. Heatmaps of the 40 genes surrounded by a grey circles in the figure A. The  
986 expression is based on normalized data of expression values (T, Tenor; Y, Yudal; H,  
987 M, L, High, Medium, Low soil microbial diversity levels).

988

989 S1 Table. Main physicochemical characteristics of the three soils used in this study.

990

991 S2 Table. Description of the *P. brassicae* genes differentially expressed between H  
992 and M at Tf when infecting Yudal (-1: genes underexpressed at H compared to M; 1:  
993 genes overexpressed at H compared to M).

994

995 S3 Table. Description of the *P. brassicae* genes differentially expressed between the  
996 different soil microbiota diversity levels at Tf when infecting Tenor. A. Description of  
997 the *P. brassicae* genes differentially expressed between H and M at Tf when infecting  
998 Tenor (-1: genes underexpressed at H compared to M; 1: genes overexpressed at H  
999 compared to M). B. Description of the *P. brassicae* genes differentially expressed  
1000 between H and L at Tf when infecting Tenor (-1: genes underexpressed at H compared  
1001 to L; 1: genes overexpressed at H compared to L). C. Description of the *P. brassicae*  
1002 genes differentially expressed between H and M and between H and L at Tf when  
1003 infecting Tenor (-1: genes underexpressed at H compared to M or L; 1: genes  
1004 overexpressed at H compared to M or L).

1005

1006 S4 Table. Description of the *P. brassicae* genes differentially expressed between H  
1007 and M at Tf in an opposite sense when infecting Yudal or Tenor (-1: genes  
1008 underexpressed at H compared to M; 1: genes overexpressed at H compared to M).

1009

1010 S5 Table. Effect of soil microbiota diversity levels on infected Yudal gene expression.

1011 A. Description of the 64 *B. napus* Yudal genes differentially expressed between H and  
1012 L at Ti when infected by *P. brassicae* (-1: genes underexpressed at H compared to L;  
1013 1: genes overexpressed at H compared to L). B. Description of the 23 *B. napus* Yudal  
1014 genes differentially expressed between H and L at Tf when infected by *P. brassicae* (-  
1015 1: genes underexpressed at H compared to L; 1: genes overexpressed at H compared  
1016 to L).

1017

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1019

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1024

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1026

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1043

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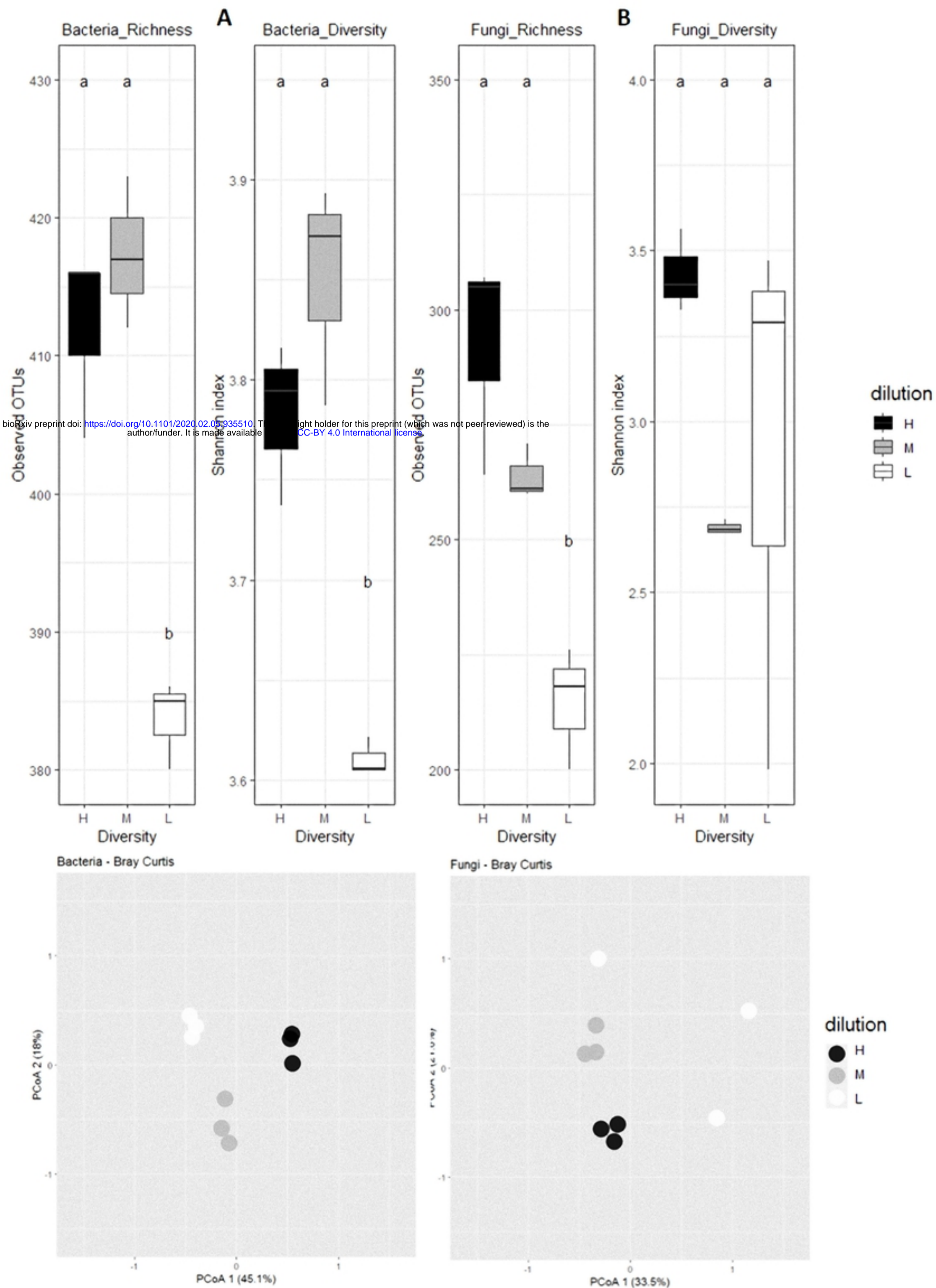


Fig1



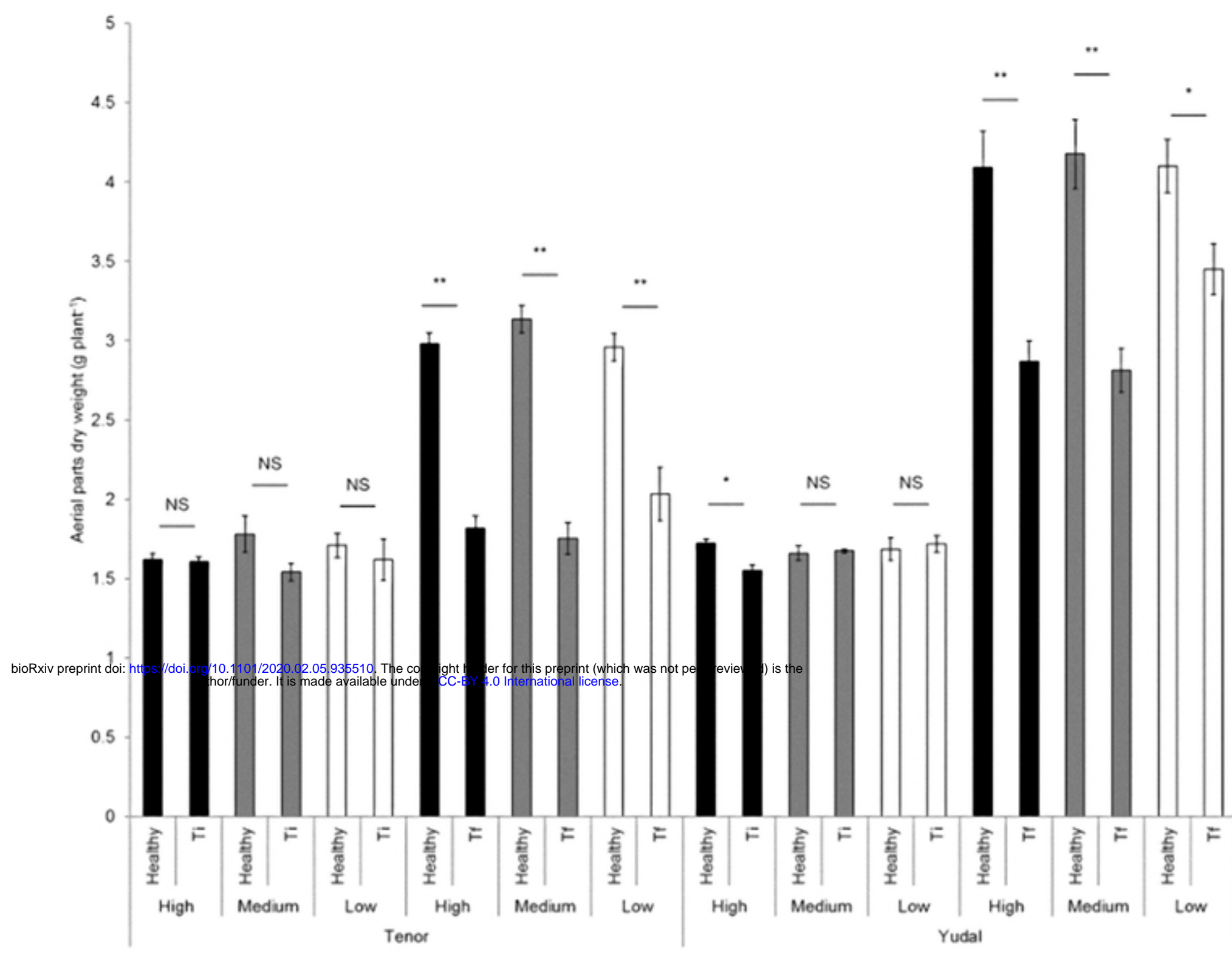
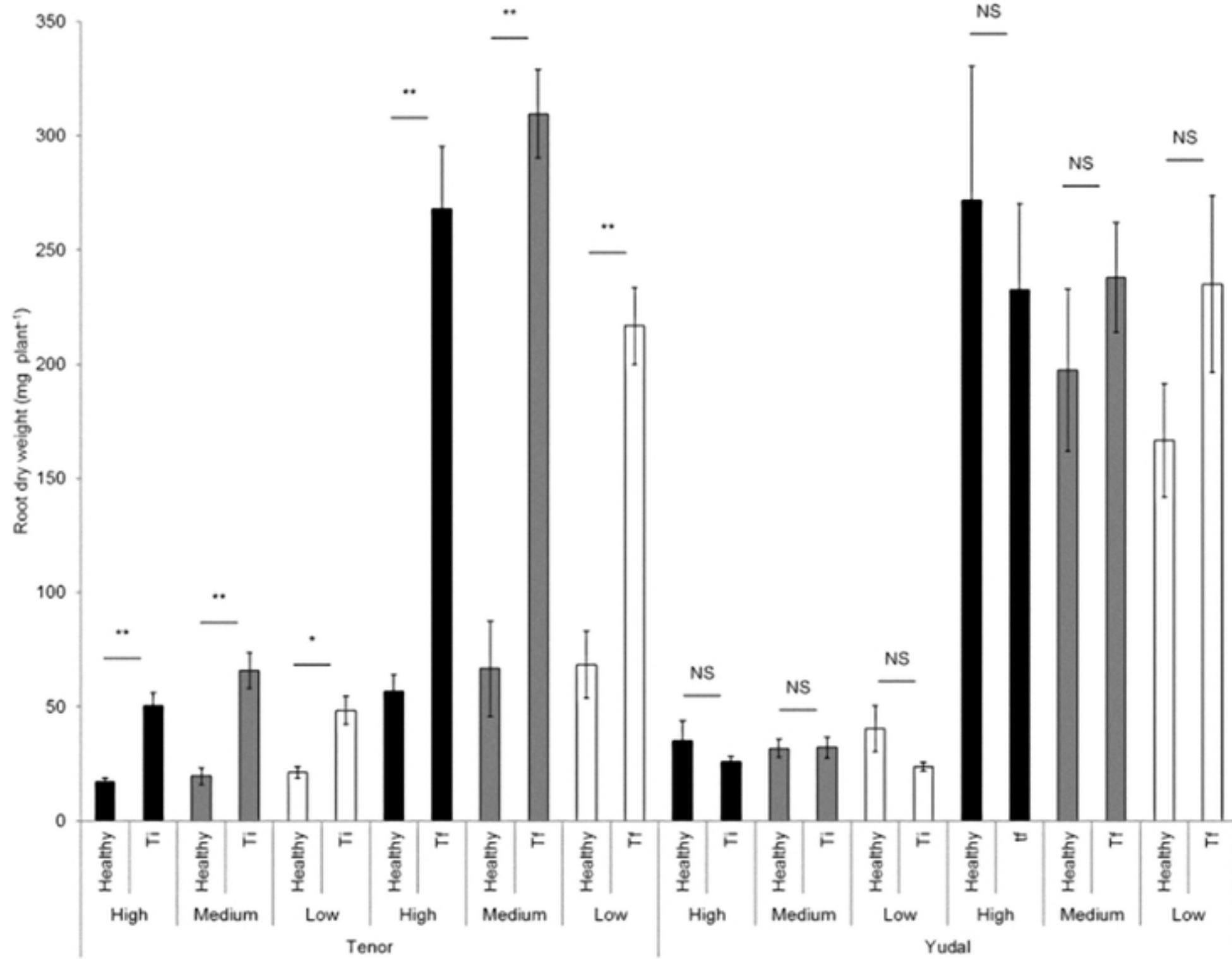
**A****B**

Fig2

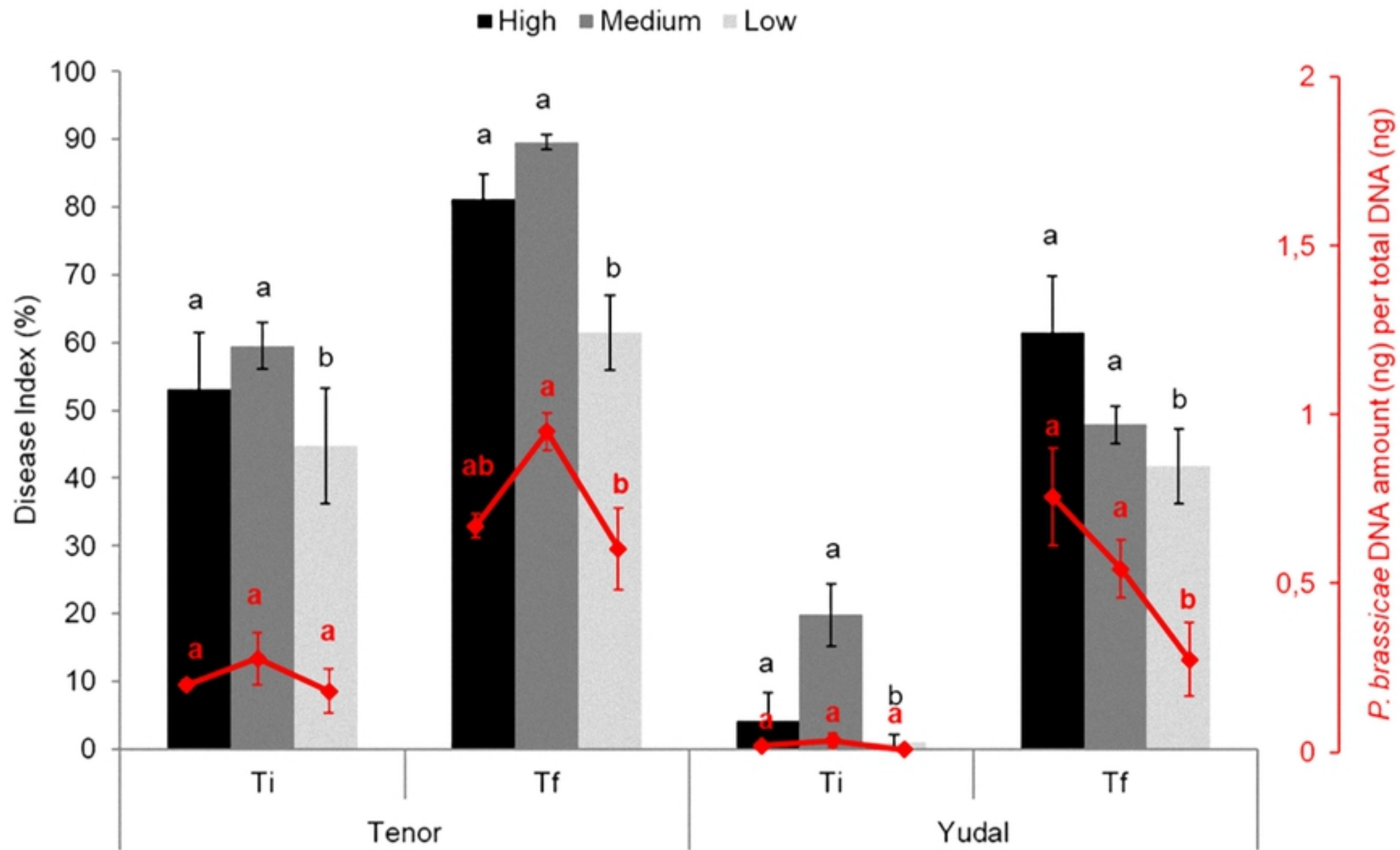


Fig3

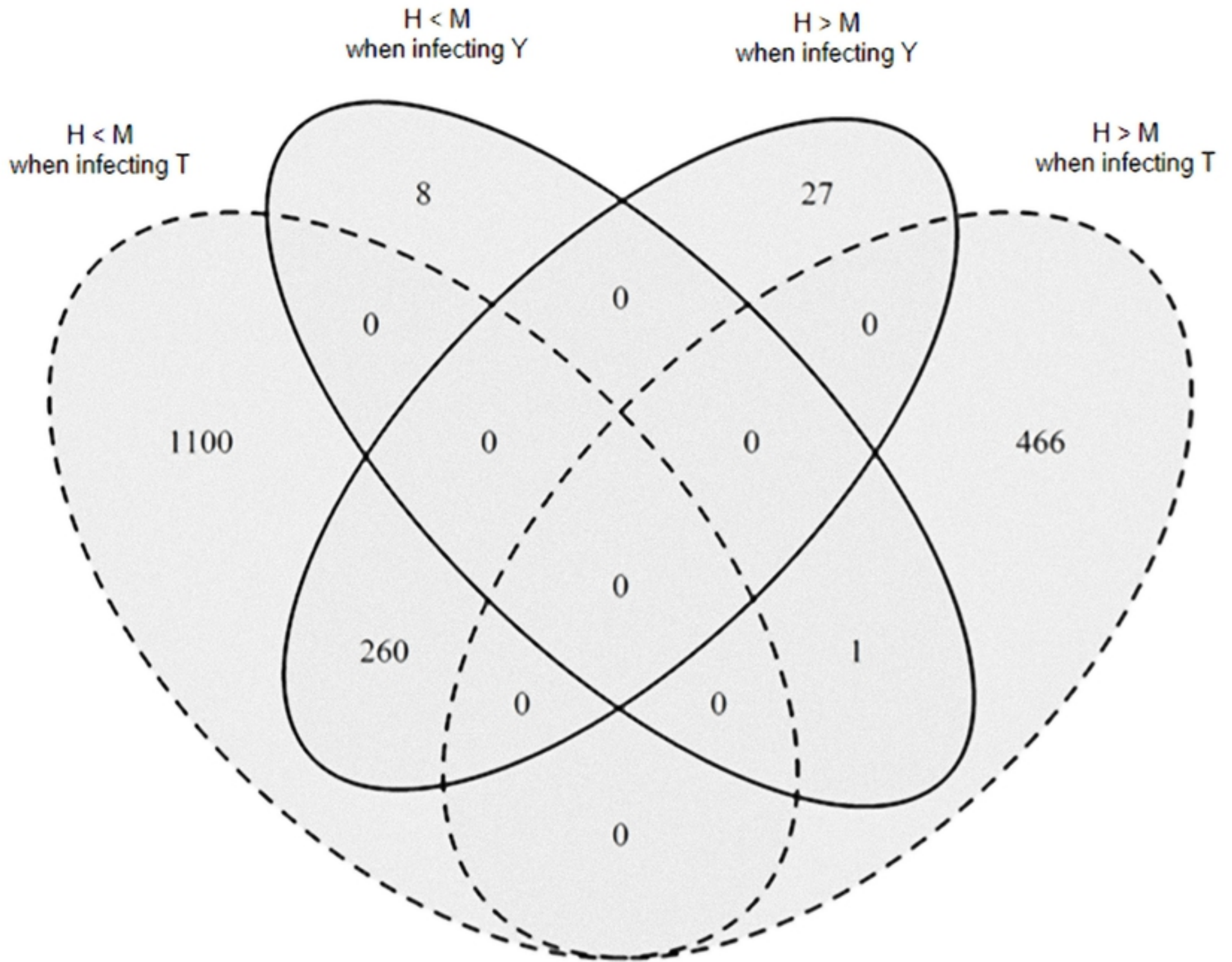
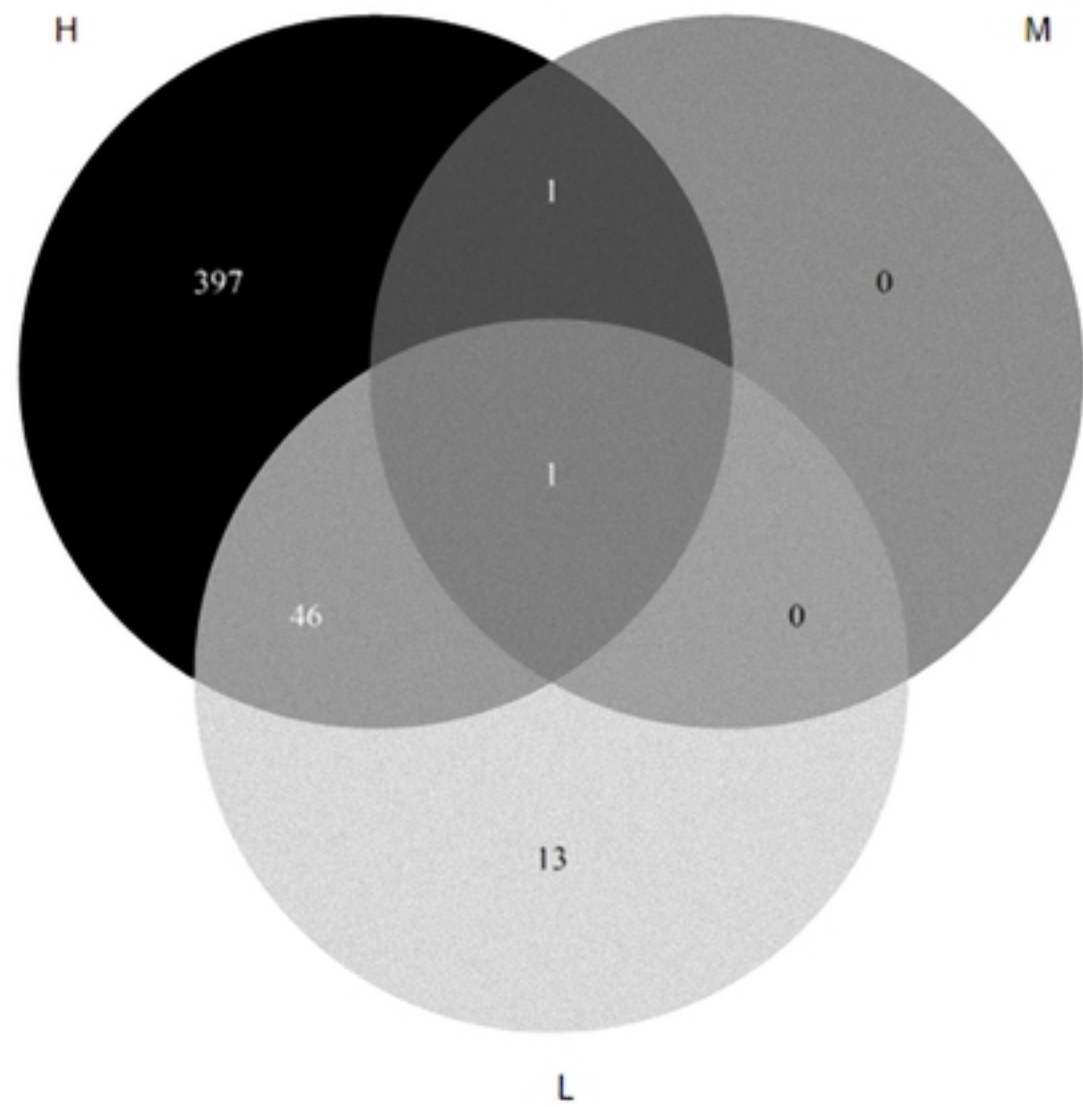


Fig4

*P. brassicae* DEGs in  
Y versus T  
Ti



*P. brassicae* DEGs in  
Y versus T  
Tf

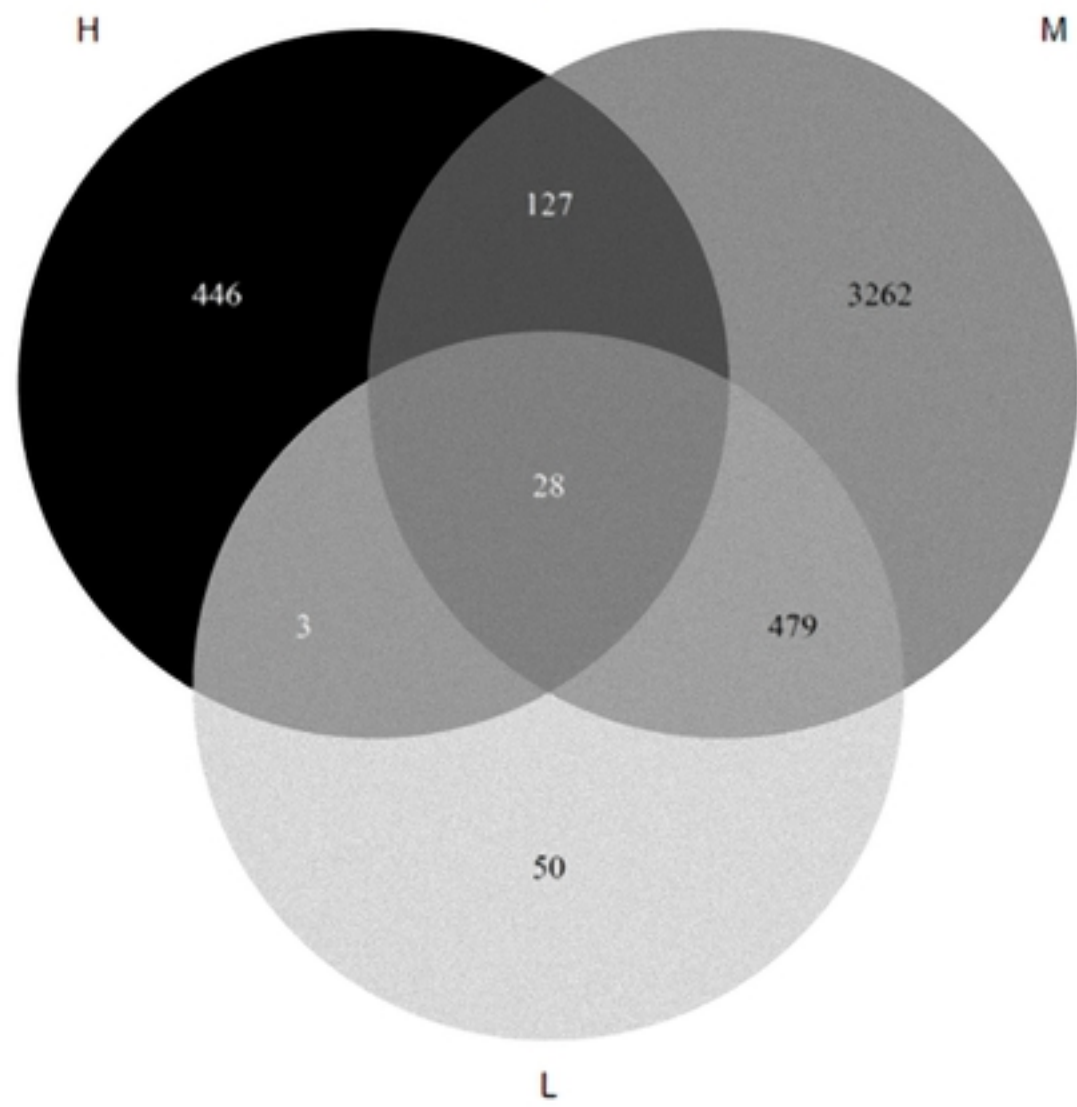


Fig5