

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

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16 NUDIX effector

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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⁻¹).

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 ¹	NA
Pldbri_eH_r1s004g02573	0.69	0.06	10.33	ADP-ribosylation_factor_6 ²	NA
Pldbri_eH_r1s003g01442	0.58	0.04	8.56	calcium_calmodulin-dependent_kinase_type_IV-like ²	ec:2.7.11.10
Pldbri_eH_r1s003g01889	1.85	0.34	4.76	NUDIX_hydrolase ³	ec:3.6.1.65
Pldbri_eH_r1s008g04734	2.32	0.46	4.72	Serine_threonine-_kinase_Sgk3 ²	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 ²	ec:2.4.1.37
Pldbri_eH_r1s015g07579	6.72	1.51	4.21	MFS_transporter ¹	NA
Pldbri_eH_r1s001g00152	1.67	0.40	4.06	carbohydrate-binding_module_family_18 ³	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 ¹	ec:1.4.1.14
Pldbri_eH_r1s001g00617	8.55	2.17	3.86	glutamate_NAD(P)+ ²	ec:1.4.1.23
Pldbri_eH_r1s042g12180	3.67	0.94	3.75	calcium/calmodulin-dependent_protein_kinase_type_IV-like ²	ec:2.7.11.10
Pldbri_eH_r1s007g04295	21.71	6.20	3.47	Mps1_binder ²	NA
Pldbri_eH_r1s001g00511	40.71	11.71	3.46	serine_threonine-_kinase_HT1 ²	ec:2.7.11.10
Pldbri_eH_r1s016g07781	26.37	7.58	3.45	chitin_synthase_2 ²	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 ¹	ec:2.5.1.2
Pldbri_eH_r1s014g07095	3.27	0.92	3.35	glucosamine_6-phosphate_N-acetyltransferase ²	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 ²	ec:2.4.2.38
Pldbri_eH_r1s003g01890	49.53	15.74	3.13	glycoside_hydrolase_family_16 ³	ec:3.2.1.151
Pldbri_eH_r1s033g11505	14.72	4.86	3.00	glycosyltransferase ²	ec:2.4.2.38
Pldbri_eH_r1s028g10813	7.61	2.63	2.80	ABC_transporter_G_family ¹	ec:3.6.1.15;ec:3.6.3.43
Pldbri_eH_r1s022g09622	47.16	17.11	2.74	phosphoenolpyruvate_carboxykinase ²	ec:4.1.1
Pldbri_eH_r1s024g09958	32.88	12.19	2.68	phosphate_ABC_transporter_substrate-binding ¹	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 ¹	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 ¹	ec:3.6.1
Pldbri_eH_r1s003g01928	56.23	27.59	2.03	chitin_synthase_2 ²	ec:2.4.1.16

303 Genes potentially involved in transport of molecules¹, development and growth², or pathogenicity³.

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				
Pldbra_eH_r1s023g09907	0.05	0.98	0.54	15.53	8.59	E3_ubiquitin_ligase_NRDP1 ³	NA
Pldbra_eH_r1s007g03979	0.10	0.60	0.66	5.30	5.80	Dynein_light_chain_Tctex-type	NA
Pldbra_eH_r1s028g10892	0.28	1.31	1.46	4.69	5.31	Glucokinase ²	ec:2.7.1.2, ec:2.7.1.1
Pldbra_eH_r1s035g11711	3.74	14.19	11.63	3.80	3.12	Probable_phospholipid-transporting_ATPase_7_isoform_X1 ¹	ec:3.6.1, ec:3.6.3.1
Pldbra_eH_r1s014g07222	4.08	15.48	12.70	3.75	3.08	Serine_threonine_kinase ²	ec:2.7.11.10
Pldbra_eH_r1s001g00753	9.55	33.81	25.43	3.53	2.66	Gamma-glutamylcyclotransferase	ec:4.3.2.6
Pldbra_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
Pldbra_eH_r1s008g04734	0.86	3.02	2.83	3.47	3.26	Serine_threonine_kinase_Sgk3 ²	ec:3.1.4.4
Pldbra_eH_r1s002g01071	4.07	13.84	14.55	3.40	3.57	FMN-binding_glutamate_synthase_family ¹	ec:1.4.1.14
Pldbra_eH_r1s002g01072	2.67	9.15	11.02	3.39	4.08	FMN-binding_glutamate_synthase_family ¹	ec:1.4.1.14
Pldbra_eH_r1s008g04744	0.88	3.02	3.82	3.38	4.27	Alkaline_ceramidase ³	ec:3.5.1.23
Pldbra_eH_r1s007g04295	10.89	36.75	38.11	3.37	3.50	Mps1_binder ²	NA
Pldbra_eH_r1s002g00819	3.16	10.48	9.05	3.30	2.86	Cytochrome_P450	ec:1.14.14, ec:1.16.1.5
Pldbra_eH_r1s015g07621	9.57	31.38	28.26	3.28	2.95	Ammonium_transporter ¹	NA
Pldbra_eH_r1s008g04794	1.44	4.67	4.89	3.19	3.35	Zinc_C2H2_type_family	NA
Pldbra_eH_r1s004g02345	15.37	48.78	39.96	3.17	2.60	Cytosolic_carboxypeptidase_4 ³	ec:3.4.17, ec:3.4.19.11
Pldbra_eH_r1s027g10543	1.92	6.02	5.78	3.13	3.00	Probable_serine_carboxypeptidase_CPVL ³	ec:3.4, ec:2.3.1.92
Pldbra_eH_r1s017g08171	3.41	10.66	11.56	3.12	3.39	E3_ubiquitin_ligase_UNKL_isoform_X1 ³	NA
Pldbra_eH_r1s001g00671	7.57	23.37	22.03	3.08	2.91	Glutathione-disulfide_reductase	ec:1.8.1, ec:1.8.2.3
Pldbra_eH_r1s001g00511	19.71	59.05	56.06	3.00	2.85	Serine_threonine_kinase_HT1 ²	ec:2.7.11.10
Pldbra_eH_r1s003g01889	1.25	3.76	4.62	2.95	3.63	NUDIX_hydrolase ³	ec:3.6.1.65
Pldbra_eH_r1s024g09958	15.89	46.79	42.00	2.94	2.64	Phosphate_ABC_transporter_substrate-binding ¹	ec:3.1.3.1
Pldbra_eH_r1s006g03794	6.54	19.26	18.82	2.92	2.85	Chitin_synthase_D ²	ec:2.4.1.12
Pldbra_eH_r1s056g12619	3.23	9.42	9.34	2.92	2.90	Putative_WD_repeat-containing_protein	NA
Pldbra_eH_r1s026g10483	79.60	232.56	209.79	2.92	2.63	Lysosomal_aspartic_protease	ec:3.4.23, ec:3.4.23.2
Pldbra_eH_r1s022g09656	11.68	34.05	39.09	2.91	3.34	Potassium_transporter ¹	NA
Pldbra_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
Pldbra_eH_r1s015g07579	3.80	10.98	10.71	2.88	2.81	MFS_transporter ¹	NA
Pldbra_eH_r1s016g07943	1.28	3.70	4.41	2.88	3.44	Dynein_light_chain	NA
Pldbra_eH_r1s010g05501	5.21	15.04	17.05	2.87	3.26	WD_repeat-containing_54_isoform_X1	NA
Pldbra_eH_r1s006g03824	3.52	10.19	11.22	2.87	3.16	Zinc_C2H2_type_family_(macronuclear)	NA
Pldbra_eH_r1s009g05121	4.39	12.28	11.05	2.78	2.51	Phosphatidylserine_decarboxylase_subunit_beta	ec:4.1.1.65
Pldbra_eH_r1s008g04760	0.66	1.79	1.84	2.76	2.83	Receptor-interacting_serine-threonine_kinase ²	ec:2.7.1.107
Pldbra_eH_r1s037g11906	1.28	3.57	4.15	2.73	3.17	Phosphate_ABC_transporter_substrate-binding_protein_PstS ¹	ec:3.1.3.1
Pldbra_eH_r1s003g01729	26.32	70.09	69.36	2.66	2.63	Chitin_synthase_(Chitin-UDP-_ac-transferase) ²	ec:2.4.1.16, ec:2.4.1
Pldbra_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
Pldbra_eH_r1s004g02678	9.23	22.88	23.24	2.48	2.52	MFS_general_substrate_transporter ¹	NA
Pldbra_eH_r1s003g01750	4.15	9.82	8.35	2.37	2.01	Phosphatidylinositol_4-kinase_alpha ²	ec:2.7.11.1
Pldbra_eH_r1s006g03626	7.07	16.68	20.06	2.36	2.83	Mitogen-activated_kinase_kinase_6_isoform_X2 ³	ec:2.7.11.10
Pldbra_eH_r1s002g01126	14.32	33.48	33.58	2.34	2.34	Serine_threonine_kinase ²	ec:2.7.11.10, ec:2.7.10.2
Pldbra_eH_r1s003g01487	3.87	9.07	10.00	2.33	2.57	Calcium_calmodulin-dependent_kinase_type_1D-like ²	ec:2.7.11.10
Pldbra_eH_r1s007g04189	40.96	88.40	101.24	2.16	2.47	Phospholipid-transporting_ATPase_3_isoform_X1 ¹	ec:3.6.1
Pldbra_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 ¹	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 ¹	ec:3.1.3.1
Pldbra_eH_r1s003g01928	44.45	84.65	77.20	1.90	1.74	Chitin_synthase_2 ²	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 ¹	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 ³	ec:3.4.21, ec:3.4.16
Pldbra_eH_r1s001g00135	29.17	51.86	56.72	1.78	1.95	Phospholipid_transporter ¹	ec:3.6.1

332 Genes potentially involved in transport of molecules¹, development and growth², or pathogenicity³.

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 ³
Pldbra_eH_r1s001g00179	6.45	2.15	2.90	2.86	9.67	3.35	adenylate_guanylate_cyclase ³
Pldbra_eH_r1s001g00511	40.71	11.71	3.46	19.71	59.05	3.00	Serine_threonine_kinase_HT1 ²
Pldbra_eH_r1s001g00617	8.55	2.17	3.86	4.59	11.96	2.60	glutamate_NAD(P)+ ²
Pldbra_eH_r1s001g00671	17.87	5.47	3.23	7.57	23.37	3.08	glutathione-disulfide_reductase ⁴
Pldbra_eH_r1s001g00753	42.72	16.10	2.64	9.55	33.81	3.53	gamma-glutamylcyclotransferase ⁴
Pldbra_eH_r1s002g00819	6.83	2.63	2.52	3.16	10.48	3.30	cytochrome_P450 ⁴
Pldbra_eH_r1s002g00884	1.82	0.48	3.42	1.35	3.87	2.89	glutathione_S-transferase_kappa_1_[Rhodotorula_toruloides_NP11] ⁴
Pldbra_eH_r1s002g01072	7.08	1.78	3.89	2.67	9.15	3.39	FMN-binding_glutamate_synthase_family ¹
Pldbra_eH_r1s003g01442	0.58	0.04	8.56	0.29	1.19	4.01	calcium_calmodulin-dependent_kinase_type_IV-like ²
Pldbra_eH_r1s003g01550	8.87	2.75	3.15	6.10	16.04	2.62	Glycosyltransferase_uncharacterized ²
Pldbra_eH_r1s003g01889	1.85	0.34	4.76	1.25	3.76	2.95	NUDIX_hydrolase ³
Pldbra_eH_r1s003g01890	49.53	15.74	3.13	26.18	68.19	2.60	glycoside_hydrolase_family_16 ³
Pldbra_eH_r1s003g01928	56.23	27.59	2.03	44.45	84.65	1.90	chitin_synthase_2 ²
Pldbra_eH_r1s006g03824	6.92	2.18	3.10	3.52	10.19	2.87	Zinc_C2H2_type_family_(macronuclear) ⁴
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 ²
Pldbra_eH_r1s008g04734	2.32	0.46	4.72	0.86	3.02	3.47	Serine_threonine_kinase_Sgk3 ²
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 ⁴
Pldbra_eH_r1s009g05056	9.63	4.23	2.28	8.47	16.53	1.94	probable_phospholipid-transporting_ATPase_IA_isoform_X1 ¹
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 ²
Pldbra_eH_r1s014g07095	3.27	0.92	3.35	1.72	5.57	3.17	glucosamine_6-phosphate_N-acetyltransferase ²
Pldbra_eH_r1s015g07579	6.72	1.51	4.21	3.80	10.98	2.88	MFS_transporter ¹
Pldbra_eH_r1s016g07781	26.37	7.58	3.45	13.19	38.91	2.95	chitin_synthase_2 ²
Pldbra_eH_r1s022g09622	47.16	17.11	2.74	22.80	62.09	2.72	phosphoenolpyruvate_carboxykinase ²
Pldbra_eH_r1s022g09656	23.28	7.19	3.21	11.68	34.05	2.91	potassium_transporter ¹
Pldbra_eH_r1s024g09958	32.88	12.19	2.68	15.89	46.79	2.94	phosphate_ABC_transporter_substrate-binding ¹
Pldbra_eH_r1s025g10321	8.55	2.46	3.38	4.50	10.67	2.38	Maltose_maltodextrin_ABC_substrate_binding_periplasmic ¹
Pldbra_eH_r1s027g10543	2.67	0.79	2.96	1.92	6.02	3.13	probable_serine_carboxypeptidase_CPVL ³
Pldbra_eH_r1s028g10813	7.61	2.63	2.80	2.22	7.21	3.23	ABC_transporter_G_family ¹
Pldbra_eH_r1s028g10814	6.19	2.49	2.41	1.74	6.07	3.42	ABC_transporter ¹
Pldbra_eH_r1s033g11505	14.72	4.86	3.00	10.02	24.24	2.42	Glycosyltransferase_uncharacterized ²
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 ¹
Pldbra_eH_r1s042g12180	3.67	0.94	3.75	1.55	5.79	3.66	calcium/calmodulin-dependent_protein_kinase_type_IV-like ²
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 molecules¹, development and growth², pathogenicity³, or detoxification⁴.

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

408

409 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 ¹
BnaA03g14120D	38.61	2.07	17.82	CYP71A13 = cytochrome P450, family 71, subfamily A, polypeptide 13 ¹
BnaA09g41170D	32.90	2.73	11.44	Tyrosine aminotransferase 3 ³
BnaA01g28810D	70.33	7.29	9.42	Legume lectin family protein ¹
BnaC09g43040D	9.48	1.04	8.69	GHMP kinase family protein ²
BnaA01g12970D	13.16	1.37	8.52	CysteineNArich RLK (RECEPTORNAlike protein kinase) 21 ²
BnaC04g45990D	182.19	22.53	8.05	Serine protease inhibitor, potato inhibitor INAtype family protein ¹
BnaC01g41330D	21.95	3.05	6.75	NucleotideNAdiphosphoNAsugar transferase ¹
BnaA09g00870D	497.35	75.10	6.61	Glutathione SNATransferase F3 ¹
BnaA04g27530D	30.36	5.01	6.05	NA
BnaC04g28910D	17.89	3.04	5.53	FAD/NAD(P)NAbinding oxidoreductase family protein ¹
BnaC02g43390D	26.72	4.70	5.51	0
BnaA04g03320D	70.82	13.00	5.46	JasmonateNAreregulated gene 21 ³
BnaC01g36670D	117.35	22.88	5.09	CYP72A9 ¹
BnaA05g25490D	18.82	3.61	5.04	Unknown protein
BnaA05g03980D	67.18	13.27	4.98	Beta glucosidase 27 ¹
BnaC09g16910D	1658.97	355.44	4.67	GDSLNAlike Lipase/Acylhydrolase superfamily protein ¹
BnaA05g03390D	21.90	4.89	4.42	Trypsin inhibitor protein 1
BnaC03g17010D	14.16	3.13	4.35	Thioredoxin superfamily protein ¹
BnaA03g60240D	42.64	10.71	3.84	Seven transmembrane MLO family protein ²
BnaA09g53990D	52.91	13.49	3.84	Pinorensinol reductase 1 ¹
BnaA06g03570D	1.41	8.55	5.60	AuxinNAresponsive GH3 family protein ³
BnaA03g07790D	2.43	14.87	5.58	ChaperoninNAlike RbcX protein

410 Genes potentially involved in plant defense and stress response¹, signalization pathway², or hormonal and jasmonic acid pathways³.

411

412 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 ²
BnaC01g29150D	18.11	0.11	78.16	DefensinNAlike (DEFL) family protein ¹
BnaAnng01940D	63.38	11.11	5.52	Sulfotransferase 2A ²
BnaA09g50540D	29.06	6.90	4.21	2NAoxoglutarate (2OG) and Fe(II)NAdependent oxygenase superfamily protein ¹
BnaA05g07580D	67.14	16.48	4.04	DonNAGlucosyltransferase 1 ²
BnaAnng38720D	23.76	7.16	3.39	MATE efflux family protein ²
BnaC02g22290D	6.75	28.87	3.75	NA
BnaC09g18860D	429.43	1547.24	3.59	Cytochrome P450, family 707, subfamily A, polypeptide 3 ²

413 Genes potentially involved in plant defense and stress response¹, or hormonal and jasmonic acid pathways².

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 alkalinization
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 nutriments 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 β -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 log2-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 μM 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⁻¹. 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⁻¹ 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'-ttgggtaatttgcgcgcctg-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

1044 **References**

1045

- 1046 1. Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. The Phyllosphere: Microbial
1047 Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics*.
1048 2016;47(1):1-24. doi: 10.1146/annurev-ecolsys-121415-032238.
- 1049 2. Ploch S, Rose LE, Bass D, Bonkowski M. High Diversity Revealed in Leaf-Associated Protists
1050 (Rhizaria: Cercozoa) of Brassicaceae. *Journal of Eukaryotic Microbiology*. 2016;63(5):635-41. doi:
1051 10.1111/jeu.12314.
- 1052 3. Barret M, Guimbaud JF, Darrasse A, Jacques MA. Plant microbiota affects seed transmission of
1053 phytopathogenic microorganisms. *Mol Plant Pathol*. 2016;17(6):791-5. doi: 10.1111/mpp.12382.
- 1054 4. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, et al. Defining the core
1055 *Arabidopsis thaliana* root microbiome. *Nature*. 2012;488(7409):86-90. doi: 10.1038/nature11237.
- 1056 5. Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, et al. Improved plant
1057 resistance to drought is promoted by the root-associated microbiome as a water stress-dependent
1058 trait. *Environ Microbiol*. 2015;17(2):316-31. doi: 10.1111/1462-2920.12439.
- 1059 6. Chaparro JM, Badri DV, Vivanco JM. Rhizosphere microbiome assemblage is affected by plant
1060 development. *ISME J*. 2014;8(4):790-803. doi: 10.1038/ismej.2013.196.
- 1061 7. Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM. Potential impact of soil microbiomes on
1062 the leaf metabolome and on herbivore feeding behavior. *New Phytol*. 2013;198(1):264-73. doi:
1063 10.1111/nph.12124.
- 1064 8. Berendsen RL, Pieterse CM, Bakker PA. The rhizosphere microbiome and plant health. *Trends*
1065 *Plant Sci*. 2012;17(8):478-86. doi: 10.1016/j.tplants.2012.04.001.
- 1066 9. van der Heijden MG, Hartmann M. Networking in the Plant Microbiome. *PLoS Biol*.
1067 2016;14(2):e1002378. doi: 10.1371/journal.pbio.1002378.

- 1068 10. Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, et al. Deciphering
1069 the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science*. 2011;332(6033):1097-100.
1070 doi: 10.1126/science.1203980.
- 1071 11. Vayssier-Taussat M, Albina E, Citti C, Cosson JF, Jacques MA, Lebrun MH, et al. Shifting the
1072 paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. *Front Cell Infect*
1073 *Microbiol*. 2014;4:29. doi: 10.3389/fcimb.2014.00029.
- 1074 12. Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma LJ, et al. Ecology and Genomic Insights
1075 into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annu Rev Phytopathol*. 2017;55:61-83.
1076 doi: 10.1146/annurev-phyto-080516-035641.
- 1077 13. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y. The rhizosphere: a
1078 playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*.
1079 2008;321(1-2):341-61. doi: 10.1007/s11104-008-9568-6.
- 1080 14. Muller DB, Vogel C, Bai Y, Vorholt JA. The Plant Microbiota: Systems-Level Insights and
1081 Perspectives. *Annu Rev Genet*. 2016;50:211-34. doi: 10.1146/annurev-genet-120215-034952.
- 1082 15. Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome: significance of plant
1083 beneficial, plant pathogenic, and human pathogenic microorganisms. *Fems Microbiol Rev*.
1084 2013;37(5):634-63. doi: 10.1111/1574-6976.12028.
- 1085 16. Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, et al.
1086 Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature*.
1087 2012;488(7409):91-5. doi: 10.1038/nature11336.
- 1088 17. Vandenkoornhuysen P, Quaiser A, Duhamel M, Le Van A, Dufresne A. The importance of the
1089 microbiome of the plant holobiont. *New Phytol*. 2015;206(4):1196-206. doi: 10.1111/nph.13312.
- 1090 18. Hassani MA, Duran P, Hacquard S. Microbial interactions within the plant holobiont.
1091 *Microbiome*. 2018;6(1):58. doi: 10.1186/s40168-018-0445-0.
- 1092 19. Turner TR, James EK, Poole PS. The plant microbiome. *Genome Biol*. 2013;14(6):10. doi:
1093 10.1186/gb-2013-14-6-209.
- 1094 20. Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P. Interplay Between Innate Immunity
1095 and the Plant Microbiota. *Annu Rev Phytopathol*. 2017;55:565-89. doi: 10.1146/annurev-phyto-
1096 080516-035623.
- 1097 21. Bakker P, Pieterse CMJ, de Jonge R, Berendsen RL. The Soil-Borne Legacy. *Cell*.
1098 2018;172(6):1178-80. doi: 10.1016/j.cell.2018.02.024.
- 1099 22. Vannier N, Agler M, Hacquard S. Microbiota-mediated disease resistance in plants. *PLoS*
1100 *Pathog*. 2019;15(6):e1007740. doi: 10.1371/journal.ppat.1007740.
- 1101 23. Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, et al. Salicylic acid
1102 modulates colonization of the root microbiome by specific bacterial taxa. *Science*.
1103 2015;349(6250):860-4. doi: 10.1126/science.aaa8764.
- 1104 24. Yao H, Wu F. Soil microbial community structure in cucumber rhizosphere of different
1105 resistance cultivars to fusarium wilt. *FEMS Microbiol Ecol*. 2010;72(3):456-63. doi: 10.1111/j.1574-
1106 6941.2010.00859.x.
- 1107 25. Lachaise T, Ourry M, Lebreton L, Guillerm-Erckelboudt AY, Linglin J, Paty C, et al. Can soil
1108 microbial diversity influence plant metabolites and life history traits of a rhizophagous insect? A
1109 demonstration in oilseed rape. *Insect Sci*. 2017;24(6):1045-56. doi: 10.1111/1744-7917.12478.
- 1110 26. Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, et al. Root exudates drive the soil-borne legacy
1111 of aboveground pathogen infection. *Microbiome*. 2018;6(1):156. doi: 10.1186/s40168-018-0537-x.
- 1112 27. Erlacher A, Cardinale M, Grosch R, Grube M, Berg G. The impact of the pathogen *Rhizoctonia*
1113 *solani* and its beneficial counterpart *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome.
1114 *Front Microbiol*. 2014;5:175. doi: 10.3389/fmicb.2014.00175.
- 1115 28. Lebreton L, Guillerm-Erckelboudt AY, Gazengel K, Linglin J, Ourry M, Glory P, et al. Temporal
1116 dynamics of bacterial and fungal communities during the infection of *Brassica rapa* roots by the protist
1117 *Plasmodiophora brassicae*. *PLoS One*. 2019;14(2):e0204195. doi: 10.1371/journal.pone.0204195.
- 1118 29. Dixon GR. The Occurrence and Economic Impact of *Plasmodiophora brassicae* and Clubroot
1119 Disease. *Journal of Plant Growth Regulation*. 2009;28(3):194-202. doi: 10.1007/s00344-009-9090-y.

- 1120 30. Kageyama K, Asano T. Life Cycle of Plasmodiophora brassicae. Journal of Plant Growth
1121 Regulation. 2009;28(3):203-11. doi: 10.1007/s00344-009-9101-z.
- 1122 31. Tommerup IC, Ingram DS. Life-cycle of *Plasmodiophora brassicae* woron. in *brassica* tissue
1123 cultures and in intact roots. New Phytol. 1971;70(2):327-32. doi: 10.1111/j.1469-8137.1971.tb02531.x.
- 1124 32. Schwelm A, Fogelqvist J, Knaust A, Julke S, Lilja T, Bonilla-Rosso G, et al. The Plasmodiophora
1125 brassicae genome reveals insights in its life cycle and ancestry of chitin synthases. Sci Rep.
1126 2015;5:11153. doi: 10.1038/srep11153.
- 1127 33. Schwelm A, Dixelius C, Ludwig-Müller J. New kid on the block – the clubroot pathogen genome
1128 moves the plasmodiophorids into the genomic era. European Journal of Plant Pathology.
1129 2015;145(3):531-42. doi: 10.1007/s10658-015-0839-9.
- 1130 34. Bi K, He Z, Gao Z, Zhao Y, Fu Y, Cheng J, et al. Integrated omics study of lipid droplets from
1131 Plasmodiophora brassicae. Sci Rep. 2016;6:36965. doi: 10.1038/srep36965.
- 1132 35. Bi K, Chen T, He Z, Gao Z, Zhao Y, Liu H, et al. Comparative genomics reveals the unique
1133 evolutionary status of Plasmodiophora brassicae and the essential role of GPCR signaling pathways.
1134 Phytopathology Research. 2019;1(1). doi: 10.1186/s42483-019-0018-6.
- 1135 36. Perez-Lopez E, Waldner M, Hossain M, Kusalik AJ, Wei Y, Bonham-Smith PC, et al. Identification
1136 of Plasmodiophora brassicae effectors - A challenging goal. Virulence. 2018;9(1):1344-53. doi:
1137 10.1080/21505594.2018.1504560.
- 1138 37. Rolfe SA, Strelkov SE, Links MG, Clarke WE, Robinson SJ, Djavaheeri M, et al. The compact
1139 genome of the plant pathogen Plasmodiophora brassicae is adapted to intracellular interactions with
1140 host Brassica spp. BMC Genomics. 2016;17:272. doi: 10.1186/s12864-016-2597-2.
- 1141 38. Kombrink A, Thomma BP. LysM effectors: secreted proteins supporting fungal life. PLoS
1142 Pathog. 2013;9(12):e1003769. doi: 10.1371/journal.ppat.1003769.
- 1143 39. Daval S, Belcour A, Gazengel K, Legrand L, Gouzy J, Cottret L, et al. Computational analysis of
1144 the Plasmodiophora brassicae genome: mitochondrial sequence description and metabolic pathway
1145 database design. Genomics. 2019;111(6):1629-40. doi: 10.1016/j.ygeno.2018.11.013.
- 1146 40. Zhang D, Burroughs AM, Vidal ND, Iyer LM, Aravind L. Transposons to toxins: the provenance,
1147 architecture and diversification of a widespread class of eukaryotic effectors. Nucleic Acids Res.
1148 2016;44(8):3513-33. doi: 10.1093/nar/gkw221.
- 1149 41. Ludwig-Muller J, Julke S, Geiss K, Richter F, Mithofer A, Sola I, et al. A novel methyltransferase
1150 from the intracellular pathogen Plasmodiophora brassicae methylates salicylic acid. Mol Plant Pathol.
1151 2015;16(4):349-64. doi: 10.1111/mpp.12185.
- 1152 42. Gravot A, Grillet L, Wagner G, Jubault M, Lariagon C, Baron C, et al. Genetic and physiological
1153 analysis of the relationship between partial resistance to clubroot and tolerance to trehalose in
1154 Arabidopsis thaliana. New Phytol. 2011;191(4):1083-94. doi: 10.1111/j.1469-8137.2011.03751.x.
- 1155 43. Gravot A, Deleu C, Wagner G, Lariagon C, Lugan R, Todd C, et al. Arginase induction represses
1156 gall development during clubroot infection in Arabidopsis. Plant Cell Physiol. 2012;53(5):901-11. doi:
1157 10.1093/pcp/pcs037.
- 1158 44. Chen J, Pang W, Chen B, Zhang C, Piao Z. Transcriptome Analysis of Brassica rapa Near-Isogenic
1159 Lines Carrying Clubroot-Resistant and -Susceptible Alleles in Response to Plasmodiophora brassicae
1160 during Early Infection. Front Plant Sci. 2015;6:1183. doi: 10.3389/fpls.2015.01183.
- 1161 45. Ludwig-Müller J. Glucosinolates and the clubroot disease: defense compounds or auxin
1162 precursors? Phytochemistry Reviews. 2008;8(1):135-48. doi: 10.1007/s11101-008-9096-2.
- 1163 46. Li L, Long Y, Li H, Wu X. Comparative Transcriptome Analysis Reveals Key Pathways and Hub
1164 Genes in Rapeseed During the Early Stage of Plasmodiophora brassicae Infection. Frontiers in Genetics.
1165 2020;10(1275). doi: 10.3389/fgene.2019.01275.
- 1166 47. Agarwal A, Kaul V, Faggian R, Rookes JE, Ludwig-Müller J, Cahill DM. Analysis of global host
1167 gene expression during the primary phase of the Arabidopsis thaliana–Plasmodiophora brassicae
1168 interaction. Functional Plant Biology. 2011;38(6):462. doi: 10.1071/fp11026.
- 1169 48. Schuller A, Kehr J, Ludwig-Muller J. Laser microdissection coupled to transcriptional profiling
1170 of Arabidopsis roots inoculated by Plasmodiophora brassicae indicates a role for brassinosteroids in
1171 clubroot formation. Plant Cell Physiol. 2014;55(2):392-411. doi: 10.1093/pcp/pct174.

- 1172 49. Lemarie S, Robert-Seilaniantz A, Lariagon C, Lemoine J, Marnet N, Jubault M, et al. Both the
1173 Jasmonic Acid and the Salicylic Acid Pathways Contribute to Resistance to the Biotrophic Clubroot
1174 Agent *Plasmodiophora brassicae* in *Arabidopsis*. *Plant Cell Physiol*. 2015;56(11):2158-68. doi:
1175 10.1093/pcp/pcv127.
- 1176 50. Malinowski R, Novák O, Borhan MH, Spíchal L, Strnad M, Rolfe SA. The role of cytokinins in
1177 clubroot disease. *European Journal of Plant Pathology*. 2016;145(3):543-57. doi: 10.1007/s10658-015-
1178 0845-y.
- 1179 51. Siemens J, Keller I, Sarx J, Kunz S, Schuller A, Nagel W, et al. Transcriptome Analysis of
1180 *Arabidopsis* Clubroots Indicate a Key Role for Cytokinins in Disease Development. *Molecular Plant-
1181 Microbe Interactions*[®]. 2006;19(5):480-94. doi: 10.1094/mpmi-19-0480.
- 1182 52. Cheah LH, Veerakone S, Kent G. Biological control of clubroot on cauliflower with *Trichoderma*
1183 and *Streptomyces* spp. *New Zealand Plant Protection*. 2000;53(0). doi: 10.30843/nzpp.2000.53.3642.
- 1184 53. Cheah LH, Kent G, Gowers S, New Zealand Plant Protection Society INC, New Zealand Plant
1185 Protection Society INC. Brassica crops and a *Streptomyces* sp as potential biocontrol for clubroot of
1186 Brassicas. *New Zealand Plant Protection, Vol 54. New Zealand Plant Protection-Series. 54. Rotorua:
1187 New Zealand Plant Protection Soc; 2001. p. 80-3.*
- 1188 54. Lee SO, Choi GJ, Choi YH, Jang KS, Park DJ, Kim CJ, et al. Isolation and characterization of
1189 endophytic actinomycetes from Chinese cabbage roots as antagonists to *Plasmodiophora brassicae*. *J
1190 Microbiol Biotechnol*. 2008;18(11):1741-6. doi: 10.4014/jmb.0800.108.
- 1191 55. Lahlali R, McGregor L, Song T, Gossen BD, Narisawa K, Peng G. *Heteroconium chaetospora*
1192 induces resistance to clubroot via upregulation of host genes involved in jasmonic acid, ethylene, and
1193 auxin biosynthesis. *PLoS One*. 2014;9(4):e94144. doi: 10.1371/journal.pone.0094144.
- 1194 56. Shakeel Q, Lyu A, Zhang J, Wu M, Chen S, Chen W, et al. Optimization of the cultural medium
1195 and conditions for production of antifungal substances by *Streptomyces platensis* 3-10 and evaluation
1196 of its efficacy in suppression of clubroot disease (*Plasmodiophora brassicae*) of oilseed rape. *Biological
1197 Control*. 2016;101:59-68. doi: 10.1016/j.biocontrol.2016.06.007.
- 1198 57. Guo S, Li X, He P, Ho H, Wu Y, He Y. Whole-genome sequencing of *Bacillus subtilis* XF-1 reveals
1199 mechanisms for biological control and multiple beneficial properties in plants. *J Ind Microbiol
1200 Biotechnol*. 2015;42(6):925-37. doi: 10.1007/s10295-015-1612-y.
- 1201 58. Zhao J, Wu Y-X, Ho H-H, Chen Z-J, Li X-Y, He Y-Q. PBT1, a novel antimicrobial protein from the
1202 biocontrol agent *Bacillus subtilis* XF-1 against *Plasmodiophora brassicae*. *European Journal of Plant
1203 Pathology*. 2016;145(3):583-90. doi: 10.1007/s10658-016-0905-y.
- 1204 59. Luo Y, Dong D, Gou Z, Wang X, Jiang H, Yan Y, et al. Isolation and characterization of
1205 *Zhihengliuella aestuarii* B18 suppressing clubroot on *Brassica juncea* var. *tumida* Tsen. *European
1206 Journal of Plant Pathology*. 2017;150(1):213-22. doi: 10.1007/s10658-017-1269-7.
- 1207 60. Xu SJ, Hong SJ, Choi W, Kim BS. Antifungal Activity of *Paenibacillus kribbensis* Strain T-9 Isolated
1208 from Soils against Several Plant Pathogenic Fungi. *Plant Pathol J*. 2014;30(1):102-8. doi:
1209 10.5423/PPJ.OA.05.2013.0052.
- 1210 61. Zhou L, Li M, Yang J, Wei L, Ji G. Draft Genome Sequence of Antagonistic Agent Lysobacter antibioticus 13-6.
1211 *Genome Announcements*. 2014;2(5):e00566-14. doi: 10.1128/genomeA.00566-14.
- 1212 62. Zhao Y, Gao Z, Tian B, Bi K, Chen T, Liu H, et al. Endosphere microbiome comparison between
1213 symptomatic and asymptomatic roots of *Brassica napus* infected with *Plasmodiophora brassicae*. *PLoS
1214 One*. 2017;12(10):e0185907. doi: 10.1371/journal.pone.0185907.
- 1215 63. Ourry M, Lebreton L, Chaminade V, Guillerm-Erckelboudt A-Y, Hervé M, Linglin J, et al.
1216 Influence of Belowground Herbivory on the Dynamics of Root and Rhizosphere Microbial Communities.
1217 *Frontiers in Ecology and Evolution*. 2018;6. doi: 10.3389/fevo.2018.00091.
- 1218 64. Aigu Y, Laperche A, Mendes J, Lariagon C, Guichard S, Gravot A, et al. Nitrogen supply exerts a
1219 major/minor switch between two QTLs controlling *Plasmodiophora brassicae* spore content in
1220 rapeseed. *Plant Pathol*. 2018;67(7):1574-81. doi: 10.1111/ppa.12867.
- 1221 65. Hwang SF, Strelkov SE, Feng J, Gossen BD, Howard RJ. *Plasmodiophora brassicae*: a review of
1222 an emerging pathogen of the Canadian canola (*Brassica napus*) crop. *Mol Plant Pathol*. 2012;13(2):105-
1223 13. doi: 10.1111/j.1364-3703.2011.00729.x.

- 1225 66. Oh Y, Donofrio N, Pan H, Coughlan S, Brown DE, Meng S, et al. Transcriptome analysis reveals
1226 new insight into appressorium formation and function in the rice blast fungus *Magnaporthe oryzae*.
1227 *Genome Biol.* 2008;9(5):R85. doi: 10.1186/gb-2008-9-5-r85.
- 1228 67. Westermann AJ, Gorski SA, Vogel J. Dual RNA-seq of pathogen and host. *Nat Rev Microbiol.*
1229 2012;10(9):618-30. doi: 10.1038/nrmicro2852.
- 1230 68. Wolf T, Kammer P, Brunke S, Linde J. Two's company: studying interspecies relationships with
1231 dual RNA-seq. *Curr Opin Microbiol.* 2018;42:7-12. doi: 10.1016/j.mib.2017.09.001.
- 1232 69. Mallon CA, Le Roux X, van Doorn GS, Dini-Andreote F, Poly F, Salles JF. The impact of failure:
1233 unsuccessful bacterial invasions steer the soil microbial community away from the invader's niche.
1234 *ISME J.* 2018;12(3):728-41. doi: 10.1038/s41396-017-0003-y.
- 1235 70. Yan Y, Kuramae EE, de Hollander M, Klinkhamer PG, van Veen JA. Functional traits dominate
1236 the diversity-related selection of bacterial communities in the rhizosphere. *ISME J.* 2017;11(1):56-66.
1237 doi: 10.1038/ismej.2016.108.
- 1238 71. Podolich O, Ardanov P, Zaets I, Pirttilä AM, Kozyrovskaya N. Reviving of the endophytic bacterial
1239 community as a putative mechanism of plant resistance. *Plant and Soil.* 2014;388(1-2):367-77. doi:
1240 10.1007/s11104-014-2235-1.
- 1241 72. Lugtenberg BJ, Caradus JR, Johnson LJ. Fungal endophytes for sustainable crop production.
1242 *FEMS Microbiol Ecol.* 2016;92(12). doi: 10.1093/femsec/fiw194.
- 1243 73. Mahoney AK, Yin C, Hulbert SH. Community Structure, Species Variation, and Potential
1244 Functions of Rhizosphere-Associated Bacteria of Different Winter Wheat (*Triticum aestivum*) Cultivars.
1245 *Front Plant Sci.* 2017;8:132. doi: 10.3389/fpls.2017.00132.
- 1246 74. Walters WA, Jin Z, Youngblut N, Wallace JG, Sutter J, Zhang W, et al. Large-scale replicated field
1247 study of maize rhizosphere identifies heritable microbes. *Proceedings of the National Academy of*
1248 *Sciences.* 2018;115(28):7368-73. doi: 10.1073/pnas.1800918115.
- 1249 75. Haney CH, Samuel BS, Bush J, Ausubel FM. Associations with rhizosphere bacteria can confer
1250 an adaptive advantage to plants. *Nat Plants.* 2015;1(6). doi: 10.1038/nplants.2015.51.
- 1251 76. Blaser MJ. The microbiome revolution. *J Clin Invest.* 2014;124(10):4162-5. doi:
1252 10.1172/JCI78366.
- 1253 77. van den Heuvel RH, Curti B, Vanoni MA, Mattevi A. Glutamate synthase: a fascinating pathway
1254 from L-glutamine to L-glutamate. *Cell Mol Life Sci.* 2004;61(6):669-81. doi: 10.1007/s00018-003-3316-
1255 0.
- 1256 78. Gaufichon L, Rothstein SJ, Suzuki A. Asparagine Metabolic Pathways in Arabidopsis. *Plant Cell*
1257 *Physiol.* 2016;57(4):675-89. doi: 10.1093/pcp/pcv184.
- 1258 79. Shnaiderman C, Miyara I, Kobiler I, Sherman A, Prusky D. Differential activation of ammonium
1259 transporters during the accumulation of ammonia by *Colletotrichum gloeosporioides* and its effect on
1260 appressoria formation and pathogenicity. *Mol Plant Microbe Interact.* 2013;26(3):345-55. doi:
1261 10.1094/MPMI-07-12-0170-R.
- 1262 80. Vylkova S. Environmental pH modulation by pathogenic fungi as a strategy to conquer the host.
1263 *PLoS Pathog.* 2017;13(2):e1006149. doi: 10.1371/journal.ppat.1006149.
- 1264 81. Bauer MA, Kainz K, Carmona-Gutierrez D, Madeo F. Microbial wars: Competition in ecological
1265 niches and within the microbiome. *Microb Cell.* 2018;5(5):215-9. doi: 10.15698/mic2018.05.628.
- 1266 82. Kong LA, Yang J, Li GT, Qi LL, Zhang YJ, Wang CF, et al. Different chitin synthase genes are
1267 required for various developmental and plant infection processes in the rice blast fungus *Magnaporthe*
1268 *oryzae*. *PLoS Pathog.* 2012;8(2):e1002526. doi: 10.1371/journal.ppat.1002526.
- 1269 83. Liu Z, Zhang X, Liu X, Fu C, Han X, Yin Y, et al. The chitin synthase FgChs2 and other FgChs co-
1270 regulate vegetative development and virulence in *F. graminearum*. *Sci Rep.* 2016;6:34975. doi:
1271 10.1038/srep34975.
- 1272 84. Nitzsche R, Gunay-Esiyok O, Tischer M, Zagoriy V, Gupta N. A plant/fungal-type
1273 phosphoenolpyruvate carboxykinase located in the parasite mitochondrion ensures glucose-
1274 independent survival of *Toxoplasma gondii*. *J Biol Chem.* 2017;292(37):15225-39. doi:
1275 10.1074/jbc.M117.802702.

- 1276 85. King R, Urban M, Lauder RP, Hawkins N, Evans M, Plummer A, et al. A conserved fungal
1277 glycosyltransferase facilitates pathogenesis of plants by enabling hyphal growth on solid surfaces. *PLoS*
1278 *Pathog.* 2017;13(10):e1006672. doi: 10.1371/journal.ppat.1006672.
- 1279 86. Calmes B, Morel-Rouhier M, Bataille-Simoneau N, Gelhaye E, Guillemette T, Simoneau P.
1280 Characterization of glutathione transferases involved in the pathogenicity of *Alternaria brassicicola*.
1281 *BMC Microbiol.* 2015;15:123. doi: 10.1186/s12866-015-0462-0.
- 1282 87. Duplan V, Rivas S. E3 ubiquitin-ligases and their target proteins during the regulation of plant
1283 innate immunity. *Front Plant Sci.* 2014;5:42. doi: 10.3389/fpls.2014.00042.
- 1284 88. Heung LJ, Luberto C, Del Poeta M. Role of sphingolipids in microbial pathogenesis. *Infect*
1285 *Immun.* 2006;74(1):28-39. doi: 10.1128/IAI.74.1.28-39.2006.
- 1286 89. Monod M, Capoccia S, Lechenne B, Zaugg C, Holdom M, Jousson O. Secreted proteases from
1287 pathogenic fungi. *Int J Med Microbiol.* 2002;292(5-6):405-19. doi: 10.1078/1438-4221-00223.
- 1288 90. Muszewska A, Stepniewska-Dziubinska MM, Steczkiewicz K, Pawlowska J, Dziedzic A, Ginalski
1289 K. Fungal lifestyle reflected in serine protease repertoire. *Sci Rep.* 2017;7(1):9147. doi:
1290 10.1038/s41598-017-09644-w.
- 1291 91. Abramyan J, Stajich JE. Species-specific chitin-binding module 18 expansion in the amphibian
1292 pathogen *Batrachochytrium dendrobatidis*. *MBio.* 2012;3(3):e00150-12. doi: 10.1128/mBio.00150-12.
- 1293 92. Liu P, Stajich JE. Characterization of the Carbohydrate Binding Module 18 gene family in the
1294 amphibian pathogen *Batrachochytrium dendrobatidis*. *Fungal Genet Biol.* 2015;77:31-9. doi:
1295 10.1016/j.fgb.2015.03.003.
- 1296 93. Dong S, Wang Y. Nudix Effectors: A Common Weapon in the Arsenal of Plant Pathogens. *PLoS*
1297 *Pathog.* 2016;12(8):e1005704. doi: 10.1371/journal.ppat.1005704.
- 1298 94. Singh K, Winter M, Zouhar M, Rysanek P. Cyclophilins: Less Studied Proteins with Critical Roles
1299 in Pathogenesis. *Phytopathology.* 2018;108(1):6-14. doi: 10.1094/PHYTO-05-17-0167-RVW.
- 1300 95. Devos S, Laukens K, Deckers P, Van Der Straeten D, Beeckman T, Inze D, et al. A hormone and
1301 proteome approach to picturing the initial metabolic events during *Plasmodiophora brassicae* infection
1302 on *Arabidopsis*. *Mol Plant-Microbe Interact.* 2006;19(12):1431-43. doi: 10.1094/mpmi-19-1431.
- 1303 96. Schaeffer A, Bronner R, Benveniste P, Schaller H. The ratio of campesterol to sitosterol that
1304 modulates growth in *Arabidopsis* is controlled by *STEROL METHYLTRANSFERASE 2;1*. *The Plant Journal.*
1305 2001;25(6):605-15. doi: 10.1046/j.1365-313x.2001.00994.x.
- 1306 97. Kielbowicz-Matuk A. Involvement of plant C(2)H(2)-type zinc finger transcription factors in
1307 stress responses. *Plant Sci.* 2012;185-186:78-85. doi: 10.1016/j.plantsci.2011.11.015.
- 1308 98. Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J. A new class of transcription factors
1309 mediates brassinosteroid-regulated gene expression in *Arabidopsis*. *Cell.* 2005;120(2):249-59. doi:
1310 10.1016/j.cell.2004.11.044.
- 1311 99. Zhao Y, Hu Y, Dai M, Huang L, Zhou DX. The WUSCHEL-related homeobox gene *WOX11* is
1312 required to activate shoot-borne crown root development in rice. *Plant Cell.* 2009;21(3):736-48. doi:
1313 10.1105/tpc.108.061655.
- 1314 100. Marowa P, Ding A, Kong Y. Expansins: roles in plant growth and potential applications in crop
1315 improvement. *Plant Cell Rep.* 2016;35(5):949-65. doi: 10.1007/s00299-016-1948-4.
- 1316 101. Showalter AM. Arabinogalactan-proteins: structure, expression and function. *Cellular and*
1317 *molecular life sciences.* 2001;2001 v.58 no.10(no. 10):pp. 1399-417.
- 1318 102. Bischoff V, Nita S, Neumetzler L, Schindelasch D, Urbain A, Eshed R, et al. *TRICHOME*
1319 *BIREFRINGENCE* and its homolog *AT5G01360* encode plant-specific DUF231 proteins required for
1320 cellulose biosynthesis in *Arabidopsis*. *Plant Physiol.* 2010;153(2):590-602. doi:
1321 10.1104/pp.110.153320.
- 1322 103. Sedbrook JC, Carroll KL, Hung KF, Masson PH, Somerville CR. The *Arabidopsis* *SKU5* gene
1323 encodes an extracellular glycosyl phosphatidylinositol-anchored glycoprotein involved in directional
1324 root growth. *Plant Cell.* 2002;14(7):1635-48. doi: 10.1105/tpc.002360.
- 1325 104. Van Lijsebettens M, Grasser KD. Transcript elongation factors: shaping transcriptomes after
1326 transcript initiation. *Trends Plant Sci.* 2014;19(11):717-26. doi: 10.1016/j.tplants.2014.07.002.

- 1327 105. Akamatsu T, Hanzawa Y, Ohtake Y, Takahashi T, Nishitani K, Komeda Y. Expression of
1328 endoxyloglucan transferase genes in acaulis mutants of Arabidopsis. *Plant Physiol.* 1999;121(3):715-
1329 21. doi: 10.1104/pp.121.3.715.
- 1330 106. Vandepoele K, Raes J, De Veylder L, Rouze P, Rombauts S, Inze D. Genome-wide analysis of
1331 core cell cycle genes in Arabidopsis. *Plant Cell.* 2002;14(4):903-16. doi: 10.1105/tpc.010445.
- 1332 107. Hall A. The cellular functions of small GTP-binding proteins. *Science.* 1990;249(4969):635-40.
1333 doi: 10.1126/science.2116664.
- 1334 108. Zeng LR, Park CH, Venu RC, Gough J, Wang GL. Classification, expression pattern, and E3 ligase
1335 activity assay of rice U-box-containing proteins. *Mol Plant.* 2008;1(5):800-15. doi: 10.1093/mp/ssn044.
- 1336 109. Xia Y, Suzuki H, Borevitz J, Blount J, Guo Z, Patel K, et al. An extracellular aspartic protease
1337 functions in Arabidopsis disease resistance signaling. *EMBO journal.* 2004;2004 v.23 no.4(no. 4):pp.
1338 980-8. doi: 10.1038/sj.emboj.7600086.
- 1339 110. Huang S, Chen X, Zhong X, Li M, Ao K, Huang J, et al. Plant TRAF Proteins Regulate NLR Immune
1340 Receptor Turnover. *Cell Host Microbe.* 2016;19(2):204-15. doi: 10.1016/j.chom.2016.01.005.
- 1341 111. Song JB, Gao S, Sun D, Li H, Shu XX, Yang ZM. miR394 and LCR are involved in Arabidopsis salt
1342 and drought stress responses in an abscisic acid-dependent manner. *BMC Plant Biology.*
1343 2013;13(1):210. doi: 10.1186/1471-2229-13-210.
- 1344 112. Hol WH, de Boer W, de Hollander M, Kuramae EE, Meisner A, van der Putten WH. Context
1345 dependency and saturating effects of loss of rare soil microbes on plant productivity. *Front Plant Sci.*
1346 2015;6:485. doi: 10.3389/fpls.2015.00485.
- 1347 113. Cordovez V, Carrion VJ, Etalo DW, Mumm R, Zhu H, van Wezel GP, et al. Diversity and functions
1348 of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Front*
1349 *Microbiol.* 2015;6:1081. doi: 10.3389/fmicb.2015.01081.
- 1350 114. Santhanam R, Luu VT, Weinhold A, Goldberg J, Oh Y, Baldwin IT. Native root-associated
1351 bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proc Natl*
1352 *Acad Sci U S A.* 2015;112(36):E5013-20. doi: 10.1073/pnas.1505765112.
- 1353 115. Cha JY, Han S, Hong HJ, Cho H, Kim D, Kwon Y, et al. Microbial and biochemical basis of a
1354 *Fusarium* wilt-suppressive soil. *ISME J.* 2016;10(1):119-29. doi: 10.1038/ismej.2015.95.
- 1355 116. Chapelle E, Mendes R, Bakker PA, Raaijmakers JM. Fungal invasion of the rhizosphere
1356 microbiome. *ISME J.* 2016;10(1):265-8. doi: 10.1038/ismej.2015.82.
- 1357 117. Plassart P, Terrat S, Thomson B, Griffiths R, Dequiedt S, Lelievre M, et al. Evaluation of the ISO
1358 standard 11063 DNA extraction procedure for assessing soil microbial abundance and community
1359 structure. *PLoS One.* 2012;7(9):e44279. doi: 10.1371/journal.pone.0044279.
- 1360 118. Terrat S, Christen R, Dequiedt S, Lelievre M, Nowak V, Regnier T, et al. Molecular biomass and
1361 MetaTaxogenomic assessment of soil microbial communities as influenced by soil DNA extraction
1362 procedure. *Microb Biotechnol.* 2012;5(1):135-41. doi: 10.1111/j.1751-7915.2011.00307.x.
- 1363 119. Terrat S, Dequiedt S, Horigue W, Lelievre M, Cruaud C, Saby NP, et al. Improving soil bacterial
1364 taxa-area relationships assessment using DNA meta-barcoding. *Heredity (Edinb).* 2015;114(5):468-75.
1365 doi: 10.1038/hdy.2014.91.
- 1366 120. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene
1367 database project: improved data processing and web-based tools. *Nucleic Acids Res.*
1368 2013;41(Database issue):D590-6. doi: 10.1093/nar/gks1219.
- 1369 121. Oksanen J, Blanchet GF, Friendly M, Kindt R, Legendre P, McGlenn D, et al. Package 'vegan'.
1370 2019.
- 1371 122. Bates DM. *lme4: Mixed-effects modeling with R*: Springer; 2010.
- 1372 123. Lenth RV. *Least-Squares Means: TheRPackageIsmmeans*. *Journal of Statistical Software.*
1373 2016;69(1). doi: 10.18637/jss.v069.i01.
- 1374 124. Benjamini Y. Discovering the false discovery rate. *Journal of the Royal Statistical Society: Series*
1375 *B (Statistical Methodology).* 2010;72(4):405-16. doi: 10.1111/j.1467-9868.2010.00746.x.
- 1376 125. Hervé M. Package 'RVAideMemoire' 2019.
- 1377 126. Some A, Manzanares MJ, Laurens F, Baron F, Thomas G, Rouxel F. Variation for virulence on
1378 *Brassica napus* L amongst *Plasmodiophora brassicae* collections from France and derived single-spore
1379 isolates. *Plant Pathol.* 1996;45(3):432-9. doi: 10.1046/j.1365-3059.1996.d01-155.x.

- 1380 127. Fahling M, Graf H, Siemens J. Pathotype separation of *Plasmodiophora brassicae* by the host
1381 plant. *J Phytopathol-Phytopathol Z.* 2003;151(7-8):425-30. doi: 10.1046/j.1439-0434.2003.00744.x.
1382 128. Manzanares-Dauleux MJ, Divaret I, Baron F, Thomas G. Evaluation of French *Brassica oleracea*
1383 landraces for resistance to *Plasmodiophora brassicae*. *Euphytica.* 2000;113(3):211-8. doi:
1384 10.1023/a:1003997421340.
1385 129. Chalhoub B, Denoeud F, Liu SY, Parkin IAP, Tang HB, Wang XY, et al. Early allopolyploid
1386 evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science.* 2014;345(6199):950-3. doi:
1387 10.1126/science.1253435.

1388

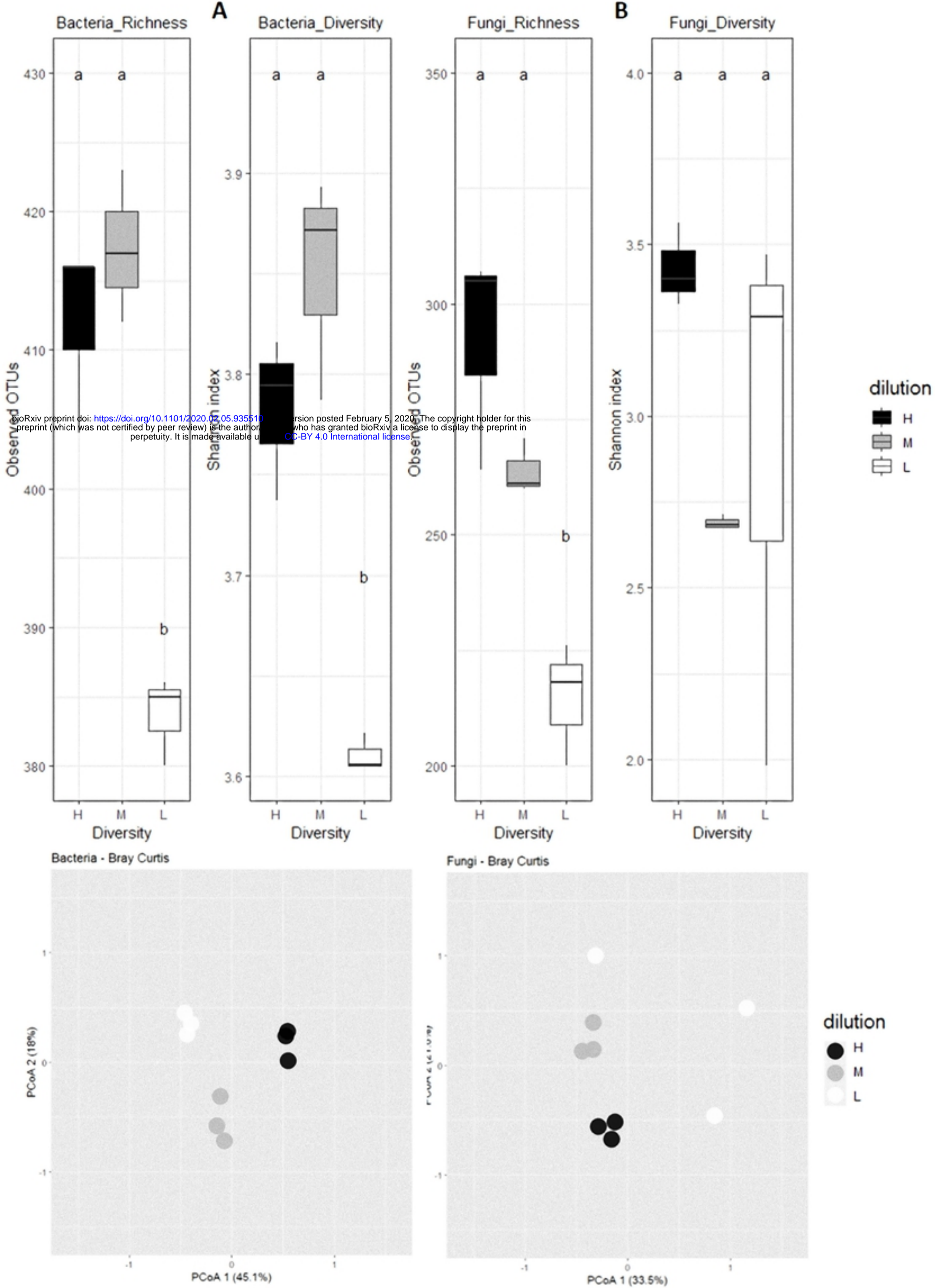


Fig1

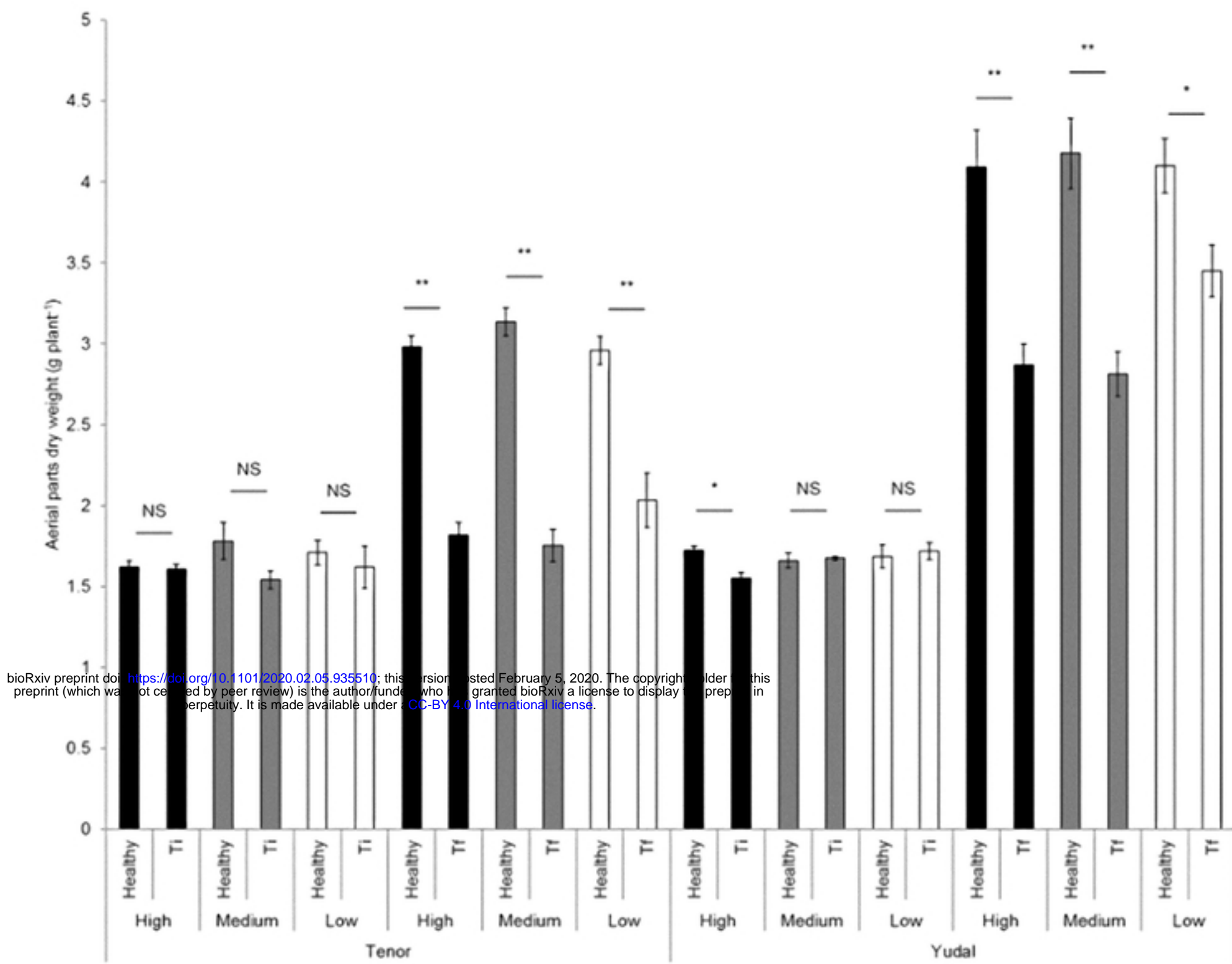
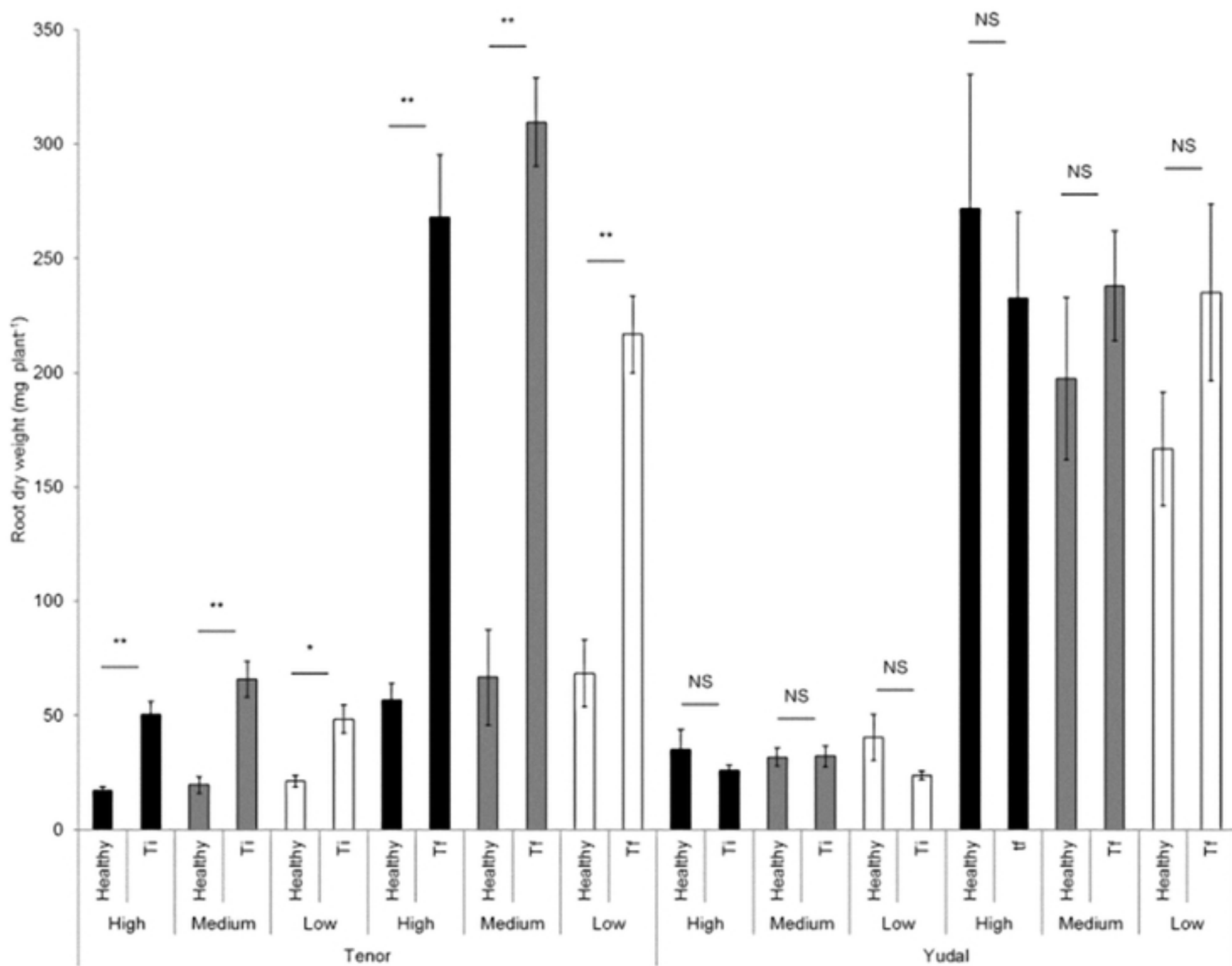
A**B**

Fig2

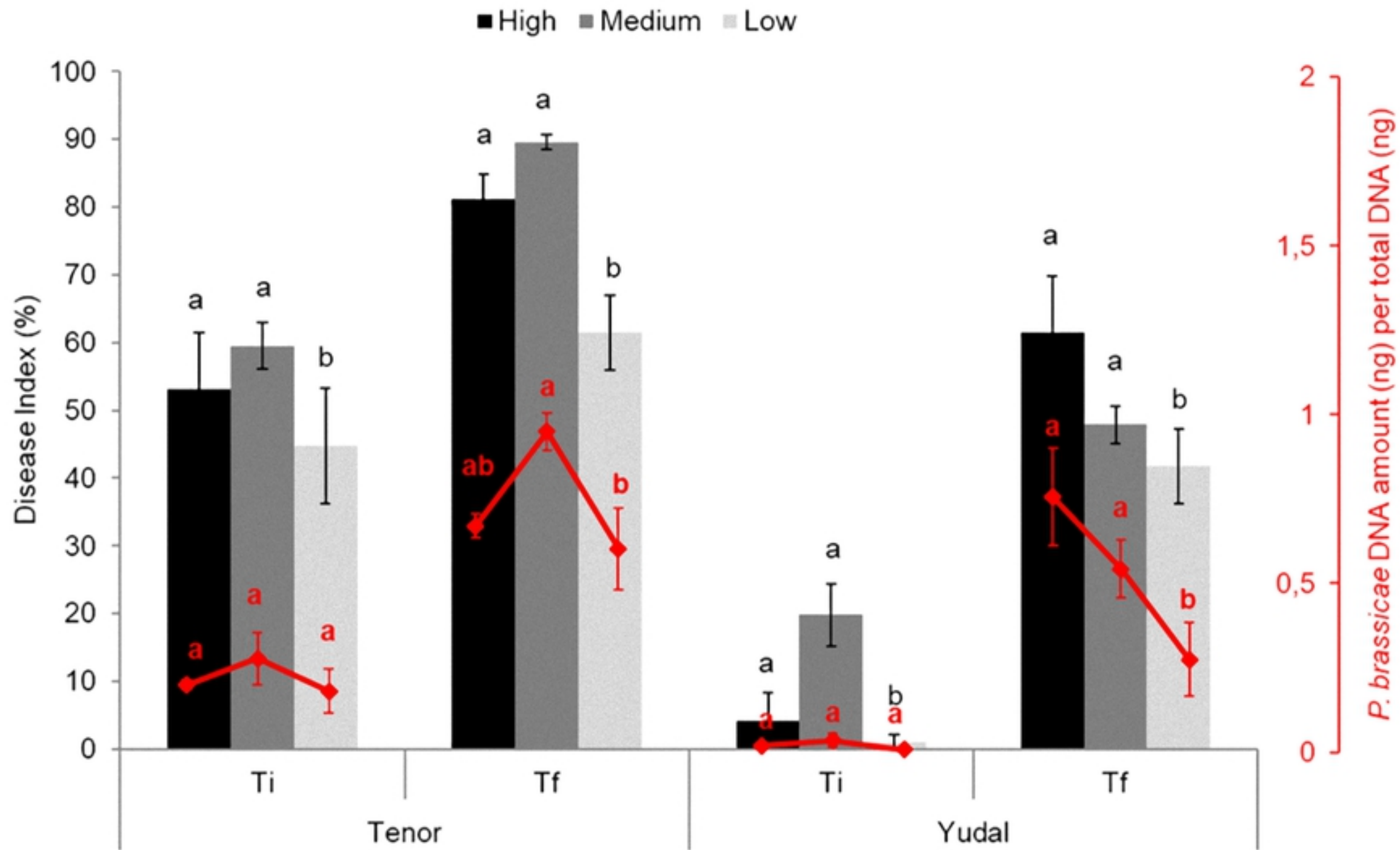


Fig3

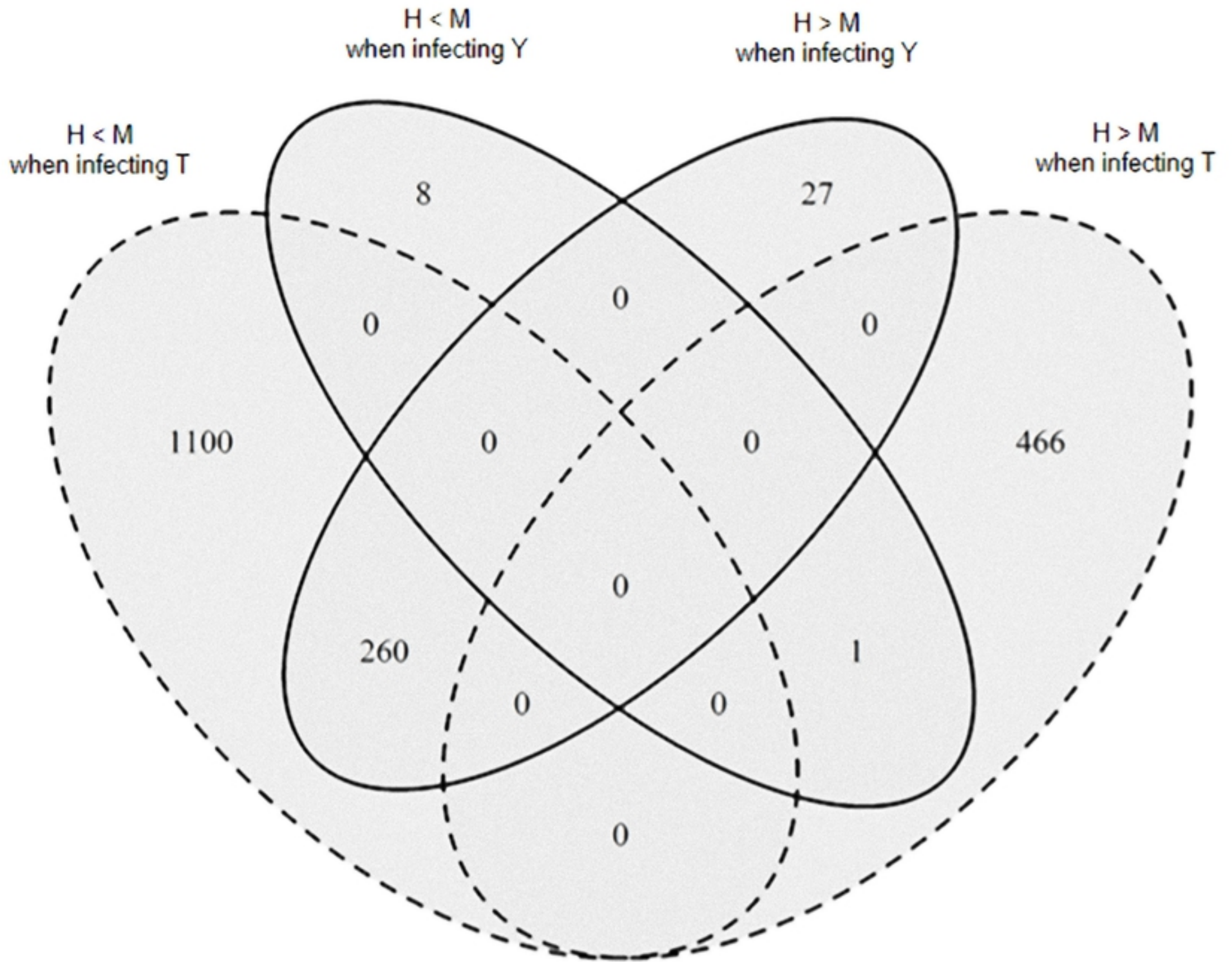
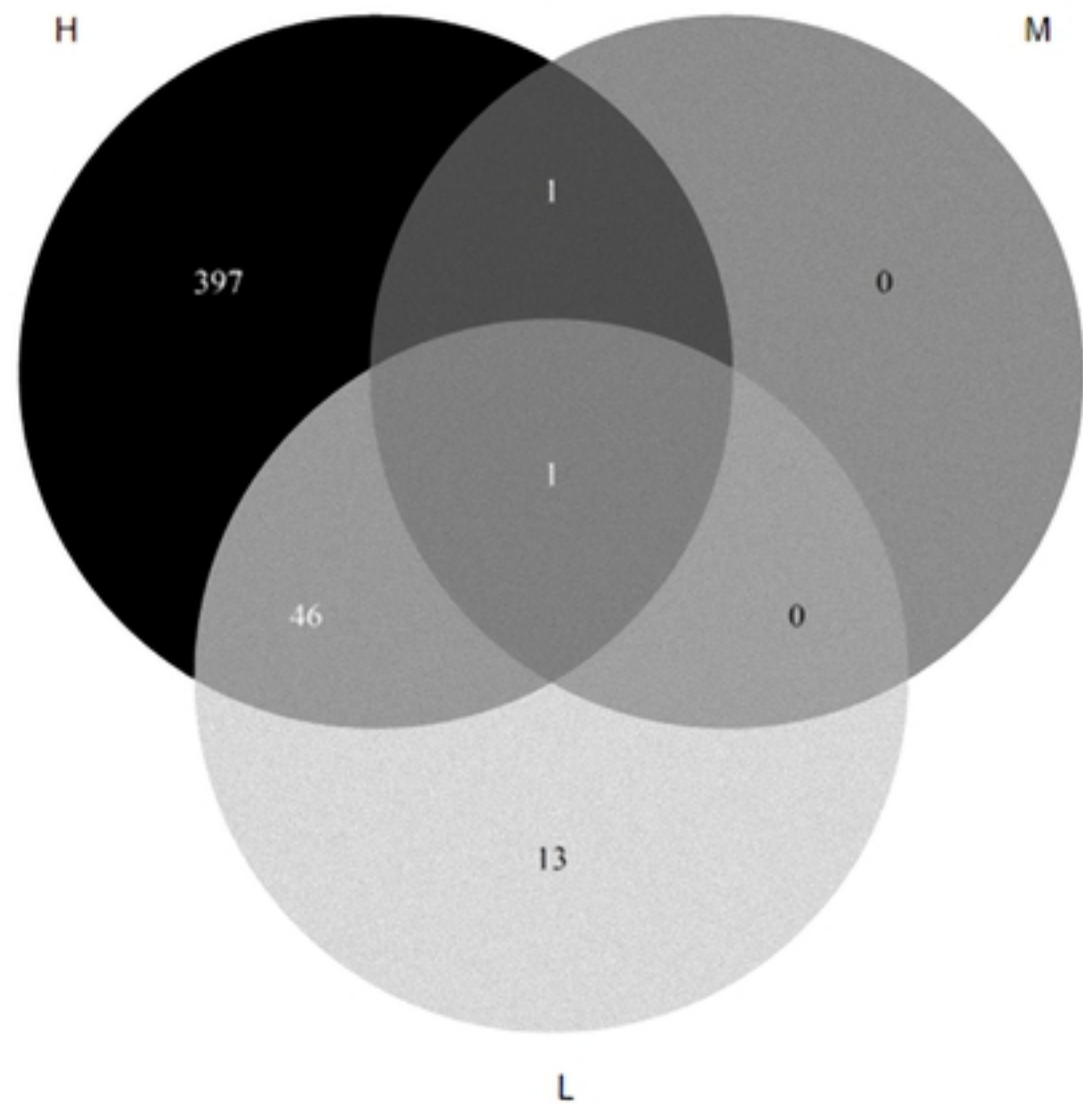


Fig4

P. brassicae DEGs in
Y versus T
Ti



P. brassicae DEGs in
Y versus T
Tf

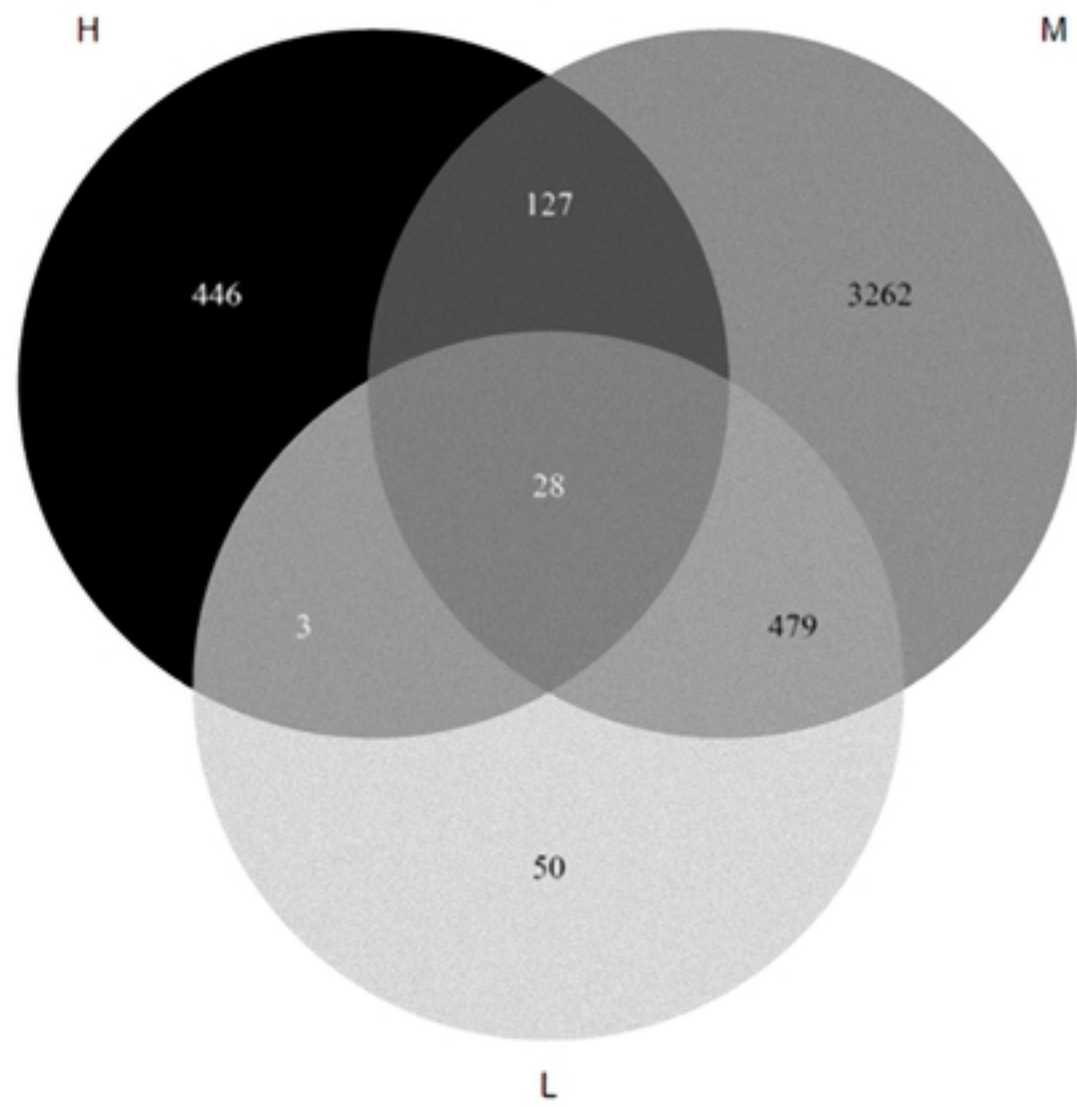


Fig5