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3 **Soil microbiota promotes early developmental stages of *Phelipanche*** 4 ***ramosa* L. Pomel during plant parasitism on *Brassica napus* L.**

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10 **Keywords:** Branched broomrape, soil microbiota, rapeseed, parasitic plant-plant interactions

11 **Abstract**

12 Purpose

13 The root holoparasitic plant *Phelipanche ramosa* has become a major constraint for rapeseed cultivation
14 in western France for the last decades and its control remains challenging. To date, few studies have
15 considered soil microbiota as a third partner of the parasitic plant-plant interaction. Therefore, we here
16 addressed the question of how soil microbiota interferes with host-derived signal metabolites required
17 for host plant recognition by the parasitic plant.

18 Methods

19 Using a branched broomrape infested soil (genetic group 1) from a rapeseed field, we first provided soil
20 physicochemical and microbiological descriptions by metabarcoding, followed by *P. ramosa* seed
21 germination and prehaustorium formation bioassays, and by *in vitro* co-cultivation with *Brassica napus*.

22 Results

23 Co-cultivation in presence of soil microorganisms promoted parasitic plant seed germination and
24 attachments to host's roots. Seed germination assays showed that only the combination of
25 gluconasturtiin (main rapeseed glucosinolate) with soil extracts stimulated broomrape germination.
26 This suggests a microbial conversion of gluconasturtiin into germination stimulants via soil microbial
27 myrosinase enzymes. Furthermore, soil bacteria *Arthrobacter*, *Ralstonia*, *Actinobacterium*,
28 *Proteobacterium* spp. and fungus *Penicillium* spp. were isolated and screened for myrosinase activity.
29 Pre-germinated seeds treated with soil extracts or differentially filtrated soil extracts also promoted
30 the formation of *P. ramosa* prehaustorium and led to more parasitic attachments on rapeseed roots in
31 co-cultivation assays. This thus suggests that this enhancement of parasitic attachments could also be
32 partly attributed to soil microbial production of haustorium inducing factors.

33 Conclusion

34 Soil microbiota influences *B. napus* - *P. ramosa* interaction by altering direct and indirect recognition
35 signals.

36

37 **Abbreviations**

38 **AMF:** Arbuscular mycorrhizal fungi

39 **ASV:** Amplicon sequence variant

40 **GNT:** Gluconasturtiin

41 **GS:** Germination stimulants

42 **GH:** Glycoside hydrolase

43 **SNG:** Sinigrin

44 **TDZ:** Thidiazuron (synthetic cytokinin)

45 **tZ:** *trans*-Zeatin

46

47 **INTRODUCTION**

48 Rhizosecretion of plant-derived compounds (*i.e.* root exudates) improves plant nutrient-seeking and
49 defense abilities, partly by allowing beneficial co-associations with neighboring organisms (Bais et al.
50 2006). Nevertheless, this carbon exchange can also be used as a signal to initiate host invasion (Trivedi
51 et al. 2020; Vives-Peris et al. 2020). Such a mechanism is illustrated in *Orobanche* and *Phelipanche* spp.
52 (broomrapes) which are obligate root holoparasites from the *Orobanchaceae* family that retrieve all of
53 their water and nutrients from a host plant (Heide-Jørgensen 2013). Among broomrapes, *Phelipanche*
54 *ramosa* L. Pomel is a major constraint, worldwide and more specifically in Mediterranean regions, to a
55 wide range of economically important crops such as *Solanaceae* (*Nicotiana tabacum* L., *Solanum*
56 *tuberosum* L., *S. melongena* L.), *Cucurbitaceae* (*Cucumis melo* L., *C. sativus* L.) and *Brassicaceae* (*Brassica*
57 *napus* L., *Sinapis* spp. L.), as well as several weed species (Gibot-Leclerc et al. 2012; Parker 2013).

58 As an adaptive strategy for host detection, broomrape seeds only germinate under suitable
59 abiotic conditions and in response to the fine perception of germination stimulants (GSs) found in the
60 root exudates of many host species (Bouwmeester et al. 2021). Historically, the first class of GSs
61 characterized were the strigolactones, plant hormones derived from the carotenoids (Cook et al. 1972;
62 Yoneyama et al. 2010) further identified as regulating several plant developmental and signaling
63 processes in the rhizosphere such as initiation of symbiosis with arbuscular mycorrhizal fungi
64 (Akiyama et al. 2005; Ćavar et al. 2015). GSs also act as chemotropic signals for *Orobanchaceae* root
65 parasitic plants to direct their radicle towards host roots (Krupp et al. 2021; Ogawa et al. 2022). Yet

66 another adaptive trait is the formation of a specialized intrusive organ at the apex of the parasite radicle,
67 called haustorium. This unique development process occurs upon perception by the parasite of
68 haustorium inducing factors (HIFs) released by the host plants (Yoshida et al. 2016). HIF perception
69 first triggers prehaustorium formation which includes morphological changes such as radicle growth
70 arrest, radial cellular extension, and formation of secretory papillae with adhesive functions (Joel and
71 Losner-Goshen 1994; Goyet et al. 2017). After the prehaustorium development, the haustorial cells then
72 penetrate host tissues to form a vascular bridge, which channels the nutrient flow towards a parasitic
73 tubercle (storage organ). This organ then grows at the expense of the crop, develops adventitious roots
74 and finally produces an above-ground flowering stem which will produce seeds viable for decades in
75 the soil (Joel et al. 2007). Although HIFs triggering prehaustorium formation in hemiparasitic plants
76 have been studied for a long time and shown to belong to the quinones and phenolic compounds family
77 (Goyet et al., 2019), effective HIFs in the holoparasite *P. ramosa* have been recently detected in rapeseed
78 rhizosphere and shown to belong mainly to the cytokinin class of compounds (Goyet et al. 2017).

79 Although described as a generalist parasitic plant, *P. ramosa* also exhibits suboptimal
80 development on some host crops, resulting for instance in France, in its structuration in three genetic
81 populations according to their host preference and geographical distribution (Brault et al. 2007;
82 Stojanova et al. 2019; Huet et al. 2020). *P. ramosa* genetic group 1 is a major drawback for farmers of
83 western France who are facing severe yield losses. To date, no mean to limit its spreading on rapeseed,
84 nor suitable control solutions exist. The occurrence of genetic groups was shown to be due to abiotic
85 factors such as pedo-climatic and agronomic context (Gibot-Leclerc et al. 2003; 2012) but also to
86 differences in sensitivity to host cues during early developmental stages (Stojanova et al. 2019; Huet et
87 al. 2020). Indeed, rapeseed, which is a non-mycorrhizal species, exudes none or only few strigolactones
88 (Auger et al. 2012; Yoneyama et al. 2018; de Saint Germain et al. 2021). This has presumably led *P.*
89 *ramosa* to adapt and perceive other GSs such as isothiocyanates (Auger et al. 2012; Miura et al. 2022).
90 These volatile compounds are the main breakdown products of glucosinolate hydrolysis. They are
91 nitrogen- and sulphur-containing secondary metabolites found almost exclusively in the *Brassicaceae*
92 plant family (Fahey et al. 2001; Wittstock et al. 2016).

93 Because most *P. ramosa* biological cycle takes place in the rhizosphere, which is a complex and
94 rich niche for microbial interactions, plant-associated microbiota has recently been considered as a
95 third partner, contributing to the parasite's life history traits. Recently, microbial communities have
96 been described as a potential tool for broomrape control (Cartry et al. 2021), but also as drivers of
97 broomrape development on its hosts in agrosystems (Hristeva and Denev 2017; Iasur Kruh et al. 2017;
98 Fitzpatrick and Schneider 2020; Huet et al. 2020; Durlík et al. 2021). Durlík et al. (2021) indeed

99 suggested that both *P. ramosa* seed endophytic bacteria *Brevibacterium frigoritolerans* and *Bacillus*
100 *simplex* could promote the germination and longevity in the soil of their parasitic host. Additionally,
101 *Shingobacterium* spp. and *Leptosphaeria maculans* were mostly found in *P. ramosa* seeds of genetic
102 group 1 and potentially facilitate seed germination by improving the production of isothiocyanates as
103 GSs in the rhizosphere of rapeseed (Huet et al. 2020). Indeed, release of isothiocyanates usually occurs
104 upon plant injury, when glucosinolates come in contact with spatially separated endogenous plant
105 myrosinase enzymes (β -thioglucosidase glucohydrolase - EC 3.2.1.147; Wittstock et al. 2016) or
106 alternatively, *via* exogenous myrosinases detected in several microorganisms found in glucosinolate-
107 containing plants and soils (Al-Turki and Dick 2003; Gimsing et al. 2006). Hence, plant-associated
108 microbes gain competitive advantages for carbon acquisition when they can tolerate isothiocyanates
109 (Rumberger and Marschner 2003; Bressan et al. 2009; Szűcs et al. 2018). Plant microbiomes can also
110 alter hormone levels, such as cytokinins, which can be produced in the rhizosphere by a large range of
111 microbial species (Müller et al. 1989; García de Salamone et al. 2001; Spallek et al. 2018) resulting in
112 possible alterations of HIF signaling in broomrape seeds. Occurrence of such microbes in the soil could
113 contribute to the parasitic behavior of *P. ramosa* on *B. napus*, but still needs to be functionally
114 demonstrated.

115 We here aimed to demonstrate that soil microorganisms are able to interfere with host-derived
116 signaling metabolites that control the parasitic plant cycle, and that they ultimately impact the success
117 of the parasitic plant. To do so, we used a soil from a rapeseed field of western France heavily infested
118 with broomrape (*P. ramosa* genetic group 1). The main objectives were i/ to describe the soil-associated
119 bacterial and fungal communities, ii/ to assess the activity of the soil microbiome on *P. ramosa* key pre-
120 parasitic stages including germination and induction of prehaustorium formation, through its
121 interaction with the host-parasite plant allelochemical signaling and iii/ to functionally validate
122 microbial contribution to broomrape germination using fungal and bacterial candidates. This latest part
123 should provide original and useful findings for the management of microbiota in the soil, in relation to
124 the control of *P. ramosa*.

126 MATERIALS AND METHODS

127 The experimental design displaying the link between the different experiments is described in Supp. Fig.S1.

129 Plant materials

130 To preserve seed quantity throughout the study, two batches of parasite seeds were used, Pram120 and
131 Pram123. These seed batches both display similar specific phenotypic characteristics of genetic group
132 1 (Auger et al. 2012; Stojanova et al. 2019; de Saint Germain et al. 2019, 2021; Huet et al. 2020).

133 Pram120 and Pram123 seeds were collected in rapeseed fields at Saint-Jean-d'Angély (France,
134 45°56'40.545" N, 0°31'10.472" O; Charente Maritime, France) in June 2015, and at Benet (46° 22'6" N,
135 0° 35' 45.999" O; Vendée, France) in July 2019 respectively, from dry mature spikes of *P. ramosa*. Seed
136 batches were sieved through a 180-200 µm mesh, and kept in the dark at 25°C until use.

137 *Brassica napus* L. seeds from the highly susceptible to *P. ramosa* variety Veritas CL (Terres
138 Inovia) were provided by DSV France SARL (Terminiers, France) in October 2019, and kept in a tinted
139 glass jar at 4°C.

140 *P. ramosa* and *B. napus* seeds were similarly surface sterilized according to Lechat et al. (2012).
141 Seeds were immersed in a 2.5% (v/v) sodium hypochlorite solution for 3-times 30 s under vigorous
142 shaking and then rinsed 5 min in sterile distilled water. *P. ramosa* seeds were then suspended in an
143 incubation solution (HEPES 1 mM; pH 7.5 adjusted with KOH; PPM 0.1% v/v) at a final seed
144 concentration of 10 mg.mL⁻¹, and incubated 7 days in the dark at 21 °C as a conditioning period (Lechat
145 et al. 2015).

146 **Soil sampling and characterization**

147 Description of soil sampling site, crop itinerary and physicochemical analyses are available on
148 supplementary materials (Supp. Fig. S2).

149 Thirty liters of soil were collected from multiple random sampling point at a 0-20 cm depth in a
150 rapeseed field infested by *P. ramosa* after harvest at Faye-sur-Ardin (46°25'57.72" N, 0°29'19.68" O;
151 Deux-Sèvres, France) in July 2019. This field was under a wheat – barley – rapeseed triennial crop
152 rotation since 2017. Prior to use, the soil was homogenized, hand sieved to remove large debris, and
153 kept in a dark room in a closed container, under low temperature and humidity fluctuations.
154 Physicochemical analyses were performed at the SADEF laboratory (Aspach le bas, France). Texture
155 was loamy with 12.4% clay, 73.2% silt and 14.3% sand. Soil was slightly alkaline (pH of 7.9) with a high
156 organic matter content (67 g.kg⁻¹) and a low C/N ratio (8.6 for values between 8 to 12). To measure the
157 effect of soil microorganisms on plant parasitism, a portion of the sampled soil underwent gamma-
158 sterilization at 35-60 kGy (IONISOS, Pouzauges, France), to eradicate most microorganisms while
159 limiting damages to the soil's integrity (McNamara et al. 2003). Effect of sterilizing treatment was
160 controlled by plating out soil suspensions on a solid rich medium and assessing colony forming units
161 (CFUs). Soil suspensions were prepared as 0.5 g of soil per mL of sterile distilled water macerated for
162 two-hour and then diluted from 1 to 10⁻³. Two hundred µL of these suspensions were then plated on LB
163 agar for bacterial assessment, and on potato dextrose agar (39 g.L⁻¹ PDA, Sigma-Aldrich, in distilled
164 water; pH 5.6) for fungal assessment. LB and PDA plates were then incubated seven days at 28°C and
165 21°C respectively (Balestra and Misaghi 1997; Obire and Anyanwu 2009).

Soil DNA extraction and high-throughput sequencing

Soil DNA was extracted in triplicates from 200 mg of solid materials (bulk soil; after harvest). Sterile distilled water was added as a negative control. Samples were processed using the NucleoSpin® Soil Kit (Macherey-Nagel) according to the recommended standard instructions. Amplicon library were constructed as described by Huet et al. (2020). Two-step PCRs were required for Illumina MiSeq sequencing, with PCR1 used for amplification of the specific V4 regions of bacterial 16S rRNA genes and fungal internal transcribed spacer (ITS1) genes, and PCR2 used to add barcodes and adapters to these amplicons. For bacteria, the paired primers of taxonomic markers used for PCR1 were 16S_515f (GTGCCAGCMGCCGCGGTAA) and 16S_806r (GTGCCAGCMGCCGCGGTAA) (Caporaso et al. 2011). For fungi, paired primers used were ITS1_f (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) (Buée et al. 2009). PCR1 program was 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 45 s, 68 °C for 1min 30 s, final extension at 68 °C for 10 min and final cooling at 10 °C. PCR2 program consisted in 94 °C for 60 s followed by 12 cycles of 94 °C for 60s, 55 °C for 60 s and 72 °C for 60 s and a final extension step of 72 °C for 10 min. Library preparation and sequencing on Illumina MiSeq were performed in the ANAN platform (nucleic acid analysis; SFR QUASAV, Beaucouzé, France).

Bioinformatics analyses

To ensure data reproducibility, sequences were processed through an automated bioinformatics workflow microSysMics (<https://bio.tools/microSysMics>), as reported by Huet et al. (2020). Quality of reads was controlled with Fastqc and Multiqc (Ewels et al. 2016) on demultiplexed Fast. To preserve sample quality (Phred-score >30), 16S reads were trimmed at 225 bp and 220 bp while ITS reads were trimmed at 175 bp and 170 bp for the forward and reverse reads respectively. Prior to the denoising procedure, PCR adapters were removed using Cutadapt (Martin 2011) and reads were truncated to a uniform length. Then, Dada2 processed unique sequences into ASV (Amplicon Sequence Variant), thanks to an error rate algorithm distinguishing biological sequence variations from amplification or sequencing errors. Denoised reads were merged and chimera were omitted from the dataset. Taxonomic assignments were performed using SILVA database for bacteria (Quast et al. 2012; Yilmaz et al. 2014) and UNITE database for fungi (Abarenkov et al. 2020). For further investigations, specific unidentified ASVs were computed using the BLASTn algorithm through the NCBI database. ASVs were numbered by alphabetical order according to the SILVA or UNITE code respectively for bacteria and fungi. Their associated codes and corresponding DNA sequences are available in supplementary data tables (Supp.Table1_16S and Supp.Table1_ITS). Chloroplast and mitochondria ASVs were removed from the abundance table *via* the Phyloseq package (1.34.0). Potential external contaminants were

199 discarded using the package Decontam (1.10.0) with the “either” method (Davis et al. 2018), and a 0.5
200 decision threshold.

201 **Soil extract procedure for biological activity analysis**

202 Soil extracts were obtained by simultaneous extraction of soil living microorganisms and metabolites,
203 and carried out according to an adaptation of several extraction methods using only water and mild
204 centrifugations to avoid death of microorganisms (Morra and Kirkegaard 2002; Gimsing et al. 2005;
205 Choi et al. 2020) The same procedure was used for both native and gamma-sterilized soils. Two
206 replicates of five grams of 1 mm-sieved soil were suspended in 5 mL of sterile distilled water and kept
207 in the dark at 20°C for 1 h under 400 rpm of constant shaking. After centrifugation (500 rpm for 5 min),
208 both supernatants were pooled and pre-filtered on a 0.7 µm glass fiber filters (Merck Millipore) to
209 remove most of soil particles. Half of the pre-filtered extracts were filtered at 0.22 µm with a syringe
210 filter (PES Membrane Millex-GP) to remove microorganisms and extracting soil metabolites. Resulting
211 soil extracts were kept at 4 °C for up to one day before use.

212 **Germination and prehaustorium formation bioassays**

213 Soil extracts were tested on conditioned and pre-germinated *P. ramosa* seeds for germination and
214 prehaustorium formation bioassays, respectively. Sterilizing filtration treatments were carried out on
215 native soil extracts using 0.45 µm (Nylon filter Membrane, Phenomonex) then 0.22 µm (PES Membrane
216 syringe Filter, Millex-GP) to remove part and then all microorganisms (mostly fungi and bacteria)
217 respectively. Additionally, a treatment with a large-spectral biocide (PPM 0.1% v/v) was used to
218 chemically remove microorganisms from the seed suspensions. Germination and prehaustorium-
219 formation bioassays were performed using these different soil extracts, with at least 3 technical and 3
220 biological independent replicates.

221 Germination bioassays were carried out according to Pouvreau et al. (2013) using 50 µL of
222 conditioned seeds (10 mg.mL⁻¹) in 96-well plates (Cell Culture Multiwell Plate Cellstar; Greiner Bio-
223 One). The different soil extracts were tested at 10-fold and 100-fold dilutions in triplicates in a final
224 volume set to 100 µL in HEPES buffer (1 mM; pH 7.5) together with or without 10⁻⁶ M gluconasturtiin
225 (GNT, Phenylethylglucosinolate K-salt from *Nasturtium officinale* L., Phytoplan). GNT was chosen as its
226 main degradation products, 2-PEITC, is primarily known to induce the germination of *P. ramosa* seeds
227 (Auger et al. 2012). Plates were sealed with gas-impermeable filters to prevent inter-well
228 contamination due to volatile degradation products. For positive controls, seed germination was
229 induced with 10⁻⁷ M *rac*-GR24 (synthetic strigolactone kindly provided by F-D Boyer (Centre National
230 de la Recherche Scientifique, Gif-sur-Yvette, France)). Seeds were treated with (1 mM; pH 7.5) HEPES
231 buffer solution as negative controls. Direct induction of germination or presence of residual

232 glucosinolates was assessed by using control wells without GNT, or in presence of exogenous
233 myrosinase (thioglucosidase from *Sinapis alba*, Sigma-Aldrich; 5 mU.mL⁻¹ final concentration)
234 respectively (Auger et al. 2012). Plates were incubated at 21 °C in the dark for 4 days. Seeds were
235 stained by adding 5 µL of thiazolyl methylthiazolyldiphenyl-tetrazolium bromide (MTT; Sigma-Aldrich;
236 5 g.L⁻¹) per well, and the number of germinated seeds was then determined (purple seeds) the following
237 day under binocular (Olympus SZX10; Olympus Europa GmbH). Germination ratio was then expressed
238 as a ratio relative to the average ratio of germination with *rac*-GR24-treated seeds within the same
239 plate.

240 Prehaustorium formation bioassays were conducted according to Goyet et al. (2017). Five mL of
241 conditioned seeds (10 mg.mL⁻¹) were rinsed 3 times in sterile distilled water and suspended in 10 mL
242 of germination medium (HEPES 1 mM, pH 7.5, *rac*-GR24 10⁻⁷ M) to reach 5 mg.mL⁻¹ seed concentration.
243 After 5 min, 50 µL of treated seeds (around 50 seeds) were distributed in 96 well plates, sealed with
244 parafilm, and incubated at 21 °C in the dark. After 4 days, germination was controlled and should have
245 reached a minimum of 70% germination ratio. The incubation medium was then carefully removed and
246 replaced with 90 µL of HEPES buffer solution (1 mM; pH 7.5). Ten microliters of soil extracts were then
247 added at 10-fold and 100-fold dilutions in six replicates. In addition, seeds were treated with the
248 synthetic cytokinin thidiazuron (TDZ; PESTANAL[®] analytical standard, Sigma-Aldrich; 10⁻⁸ M) as
249 positive control (Goyet et al. 2019) and with buffer solution as negative control. Plates were incubated
250 at 21 °C in the dark. After 3 days, seeds were stained by adding 5 µL of MTT per well. Ratio of germinated
251 seeds developing prehaustorium (occurrence of papillae and root tip swelling) was evaluated the
252 following day under binocular (Olympus SZX10; Olympus Europa GmbH), and was reported as a ratio
253 relative to the average ratio of prehaustorium formation on TDZ-treated germinated seeds on the same
254 plate.

255 **Mini-rhizotron co-cultivation for germination and aggressiveness assays**

256 Germination of *B. napus* seeds was induced by placing surface-sterilized seeds between two sterile glass
257 microfiber filters (150 mm diameter, Macherey-Nagel) moistened with 15 mL of sterile distilled water
258 in a round Petri-dish (150 mm diameter, 15 mm height, Dutscher). Petri-dishes were kept 7 days in a
259 growth chamber (21 °C, 16 h photoperiod with 110 µmol m⁻² s⁻¹ photosynthetically active radiation,
260 PAR).

261 To investigate the interacting effect of soil microbiota and the host plant on broomrape seed
262 germination and aggressiveness (*i.e.* number of broomrapes attached to host roots), two distinct co-
263 cultivation assays were set using *P. ramosa* conditioned seeds or seeds with pre-induced prehaustorium
264 formation for germination and aggressiveness assays respectively. These assays were conducted

265 according to Gauthier et al. (2012) and Billard et al. (2020) with some minor modifications: one-week
266 old rapeseed plantlets were transferred onto glass fiber filters in mini-rhizotron systems, with either
267 one plantlet for germination assays, or two plantlets for aggressiveness assays to ensure sufficient root
268 surface for attachment of germinated *P. ramosa* seeds. Mini-rhizotrons consisted of a square Petri-dish
269 (120 mm diameter, 17 mm height, Greiner) uniformly filled with sterile rock wool moistened with 50
270 mL of 50% (v/v) autoclaved Tadano et Tanaka medium pH 7.5 (Tadano and Tanaka, 1976) or 50%
271 (v/v) of Coïc medium pH 6.8 (Coic and Lesaint 1975), for germination or aggressiveness assays
272 respectively. Mini-rhizotrons were sealed on three sides with parafilm, covered with aluminum and
273 incubated in the growth chamber under the same conditions. Plants were watered with 10 to 40 mL of
274 medium every 2 days, depending on plant phenology requirements.

275 For inoculation, sterile conditioned broomrape seeds were treated with either unfiltered or 0.22
276 μm -filtered soil extracts (1/6-fold dilution) or buffer solution (HEPES 1 mM, pH 7.5) as a negative
277 control for germination test, and spun before spreading on roots. Seeds with pre-induced
278 prehaustorium formation were prepared 6 days earlier as described in Goyet et al. (2017). Sterile
279 conditioned *P. ramosa* seeds were rinsed 3-time and were then placed in germination medium (HEPES
280 1mM, pH 7.5, *rac*-GR24 10^{-7} M) in 50 mL tissue culture flasks (Becton Dickinson, Franklin Lakes, NJ,
281 Etats-Unis) at 21 °C in the dark. After 4 days, seeds were rinsed 3-time and suspended in a medium
282 containing filtered or unfiltered soil extracts (1/6-fold dilution), or buffer solution (HEPES 1 mM, pH
283 7.5) as a negative control, or *trans*-zeatin (10^{-7} M, *tZ* dissolved in 50% acetonitrile, Olchemin, Czech
284 Republic) as a positive control (Billard et al. 2020), 2 days before rapeseed root inoculation. The quality
285 of broomrape seed sets was assessed beforehand regarding germination and prehaustorium formation.
286 Two-week old rapeseed roots were then inoculated by spreading either 10 mg of conditioned
287 broomrape seeds for germination assays onto the one root, or 12.5 mg of seeds pre-induced for
288 prehaustorium formation onto the two roots after 4 weeks for aggressiveness assays.

289 During co-cultivation assays dedicated to germination assessment, broomrape germination
290 ratios were determined in mini-rhizotrons on weeks 1 and 2 post inoculation by evaluating the number
291 of germinated seeds and total seeds in 4 squares of 1 cm² nearby the roots, and total broomrape
292 attachments on weeks 2 to 4 post root inoculation. Broomrape attachments were also characterized
293 according to Gibot-Leclerc et al. (2012) as either young tubercles (no adventitious roots), spider-like
294 tubercles (numerous adventitious roots) tubercles or older tubercles already bearing a flowering bud.
295 Each mini-rhizotron was repeated 4 times for each treatment modality as 4 independent biological
296 replicates. During co-cultivation assays dedicated to aggressiveness assessment, broomrape

297 attachments were determined daily from day 7 to day 20 post root inoculation. Each treatment was
298 conducted as nine mini-rhizotron replicates.

299 **Isolation of myrosinase producing microorganisms by glucosinolate enrichment in minimum** 300 **media**

301 Myrosinase producing bacteria and fungi were isolated following an enrichment isolation method using
302 selective media with glucosinolate as the sole source of carbon (based on Albaser et al. 2016). Five
303 hundred mg of 1 mm - sieved soil were suspended in 50 mL tissue culture flasks (Becton Dickinson)
304 containing either 10 mL of M9-sinigrin medium (SNG; Sinigrin hydrate, Sigma-Aldrich) for bacteria and
305 fungi, or PDB-sinigrin medium for fungi only. M9-SNG medium was prepared with 2 mL of M9 solution
306 5X (64 g.L⁻¹ Na₂HPO₄, 15 g.L⁻¹ KH₂PO₄, 2.5 g.L⁻¹ NaCl and 5 g.L⁻¹ NH₄Cl), 2 mL of 24.65 g.L⁻¹ MgSO₄ and
307 10 µL of 14.7 g.L⁻¹ CaCl₂, and 2 mL of 10 g.L⁻¹ SNG filtrated at 0.22 µm with syringe filter in sterile distilled
308 water (pH 7.2). PDB-SNG medium was prepared with 8 mL of ¼ PDB solution (6 g.L⁻¹) with 2 mL of 10
309 g.L⁻¹ SNG filtrated at 0.22 µm with syringe filter in sterile distilled water (pH 5.6). Flasks were incubated
310 in the dark for 2 days under 100 rpm shaking at 30 °C or 21 °C for bacteria and fungi respectively. One
311 milliliter of culture medium was then collected and subcultured in 4 ml of fresh M9-SNG or PDB-SNG
312 medium and incubated at 30 °C or 21 °C respectively for another two days. This step was repeated two
313 times. One milliliter was then collected, and serial dilutions were plated onto LB agar at 30 °C or PDA at
314 21 °C for 4 days respectively.

315 In addition, to increase fungal collection, serial dilutions of soil extracts were plated onto the
316 non-selective medium PDA and incubated at 21 °C for 7 days. Finally, distinct colonies were isolated
317 and purified on new solid LB or PDA medium for 5 days and grown in liquid LB or PDB medium for 2
318 days. Pure cultures were then stored at -80 °C in 40 % glycerol (v/v) until further molecular and
319 biological characterizations.

320 **Validation of myrosinase producing microorganisms using germination assays**

321 Validation of glucosinolate-degrading abilities of microbial isolates was assessed in vitro using
322 broomrape seeds as a proxy for isothiocyanate detection. Beforehand, pure microbial isolates were
323 suspended in buffer solution (HEPES 1 mM; pH 7.5) to maintain germination conditions for *P. ramosa*.
324 Bacterial suspensions were set to OD_{600nm} = 0.01 in plate using an Absorbance Microplate Reader
325 (POLARstar Omega, BMG Labtech GmbH), and fungi solutions were set to 10⁵ spores mL⁻¹ in plate using
326 a hemocytometer. Germination bioassays were carried out in 96-well plates as described above. Similar
327 controls were used. Germination ratios were expressed as mentioned for germination assays, relatively
328 to the positive control with rac-GR24.

329 **Taxonomic identification of myrosinase producing isolated strains**

330 Molecular analyses were conducted on either single-pure bacterial colonies or mycelia, grown on solid
331 medium. Microbial material was suspended in 100 μ L of sterile ultra-pure water and incubated for 5
332 min at 94 $^{\circ}$ C in order to allow cell lysis cells and release DNA. DNA amplifications were then performed
333 using GoTaq $^{\circledR}$ (G2 Flexi DNA Polymerase, Promega France) according to manufacturer's
334 recommendations. In a final volume of 50 μ L, 2 μ L of heated DNA suspensions were mixed with 1 μ L of
335 dNTP mix (10 mM, Promega France), 1 μ L of upstream and downstream primers and GoTaq $^{\circledR}$ reagents
336 (10 μ L of 5X Green Buffer, 5 μ L of MgCl₂ Solution (25 mM) and 0.25 μ L of GoTaq $^{\circledR}$). The paired primers
337 16S_515f (GTGCCAGCMGCCGCGGTAA) and 16S_806r (GTGCCAGCMGCCGCGGTAA) were used to
338 amplify bacterial DNA (Caporaso et al. 2011). The paired primers ITS1_f
339 (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) were used to amplify fungal
340 DNA (Buée et al. 2009). PCR amplification were carried out using a MyCyclerTM thermal Cycler system
341 (Bio-Rad) according to Barret et al. (2015) with some modifications, 95 $^{\circ}$ C for 2 min followed by 35
342 cycles of 95 $^{\circ}$ C for 45 s, 50 $^{\circ}$ C or 52 $^{\circ}$ C for 45 s for bacteria and fungi respectively, 72 $^{\circ}$ C for 1 min, then
343 final elongation at 72 $^{\circ}$ C for 5 min and final cooling at 10 $^{\circ}$ C. Sequencing was performed at Eurofins
344 Genomics (Ebersberg, Germany). Taxonomic assignments were computed using the BLASTn algorithm
345 on the NCBI database (Altschul et al. 1990). Species was assigned when it belonged to the top hit, *i.e.*
346 single species with identity percentage above 95%.

347 **Statistical analyses**

348 All results were computed on RStudio v1.4.1103 (R Core v4.0.3), and analyzed with different statistical
349 approaches according to data distribution and experimental design.

350 For co-culture experiments, effects of soil extracts on broomrape germination and attachment
351 were compared across treatments (3 modalities) and weeks (from 1 to 4) with ANOVAs (analysis of
352 variance) on Mixed-Effect Models using the Car package v3.0-10, (Fox and Weisberg 2019) and the lme4
353 package v1.1-26 (Bates et al. 2015). Fixed effects were compared with multi comparisons of estimated
354 means with the emmeans package 1.5.5-1 (Lenth 2021). For germination assays, a logit transformation
355 was required for the percentage data, and were modeled with a linear mixed model (lmer), assuming
356 normal distribution of germinated seeds. Square position (1 to 4), biological replicate, experimenters,
357 and mini-rhizotron identification were used as random factors. For broomrape attachments, Poisson
358 law was selected as a large part of the dataset are small counts, and data were modeled with a
359 generalized mixed model (glmer), with a square-root link function. For relative abundance of the
360 different development stages, each stage was used as explanatory variable (Young, Spider, Bud and
361 Necrosis tubercle) using a glmmPQL, a glmer based on a *quasi* law for logistic regression. GlmmPQL

362 were employed in this case, as it is usually required when explanatory variable is only available as a
363 proportion but follows binomial law. For both total attachment and development stage models,
364 biological replicate, and mini-rhizotron individuals were used as random factors.

365 In both germination and prehaustorium-formation bioassays, integration of positive and
366 negative controls resulted in an unbalanced design; therefore, soil treatment was used as a unique
367 factor to cover all modalities including soil dilution, soil sterilization and co-treatments. On the one
368 hand, the large proportion of zeros in the germination dataset limited the identification of a known
369 statistical law and therefore the use of parametric tests. Data were analyzed with a Kruskal rank sum
370 test (stats package v4.0.3) and comparisons of effects were done with multiple pairwise comparison
371 procedures (asbio package v1.6-7). On the other hand, prehaustorium-formation data were analyzed
372 with a parametric mixed-model, using a glmmPQL based on a *quasi* law for logistic regression. Fixed
373 effects were compared with multi comparisons of estimated, and biological and technical replicates
374 were included as random factors.

375 RESULTS

376 Bacterial and fungal profiles of broomrape infested soil

377 Bacterial and fungal communities present in the rapeseed soil were described on three independent
378 soil samples by sequencing the 16S and ITS marker genes respectively. After bioinformatics processing,
379 the number of reads ranged from 12,519 to 14,892 for bacteria, and from 35,005 to 45,425 for fungi.
380 Bacterial communities were composed of 877 distinct amplicon sequence variants (ASVs), clustered
381 into 29 phyla, 163 families and 189 genera, while fungal communities were composed of 545 distinct
382 ASVs clustered into 9 phyla, 95 families and 136 genera. For abundance description, taxa with
383 abundance less than 0.1% were grouped into a common class named “< 0.1%”. ASVs from SILVA and
384 UNITE identifiers with corresponding DNA sequences for 16S and ITS can be found in supplementary
385 data (Supp.TableS1; Supp.TableS2 respectively).

386 At the phylum level, the four most abundant bacterial phyla were *Actinobacteriota* (mean=
387 $34.45 \pm \text{sd} = 1.11\%$), *Proteobacteria* ($23.31 \pm 3.04\%$), *Acidobacteriota* ($21.91 \pm 2.71\%$) and *Chloroflexi*
388 ($5.29 \pm 0.90\%$) (Supp. Fig.S3A). Bacterial families were homogeneously distributed among the samples,
389 and characterized by a high number of low abundant taxa, with $68.36 \pm 7\%$ of them being less than 1%
390 abundant (Fig.1). Nevertheless, the most abundant families were the *Pseudonocardiaceae*
391 ($11.97 \pm 3.99\%$), the *Vicinamibacteraceae* ($10.72 \pm 0.86\%$) and families less than 0.1% abundant
392 ($6.71 \pm 0.45\%$). In addition, 4 *Streptomyces* ASVs ($2.1 \pm 0.54\%$), one *Bacillus* ASV ($0.08 \pm 0.13\%$), and one
393 *Pseudomonas* ASV ($0.02 \pm 0.04\%$) were detected. Species assignments were not available from either the
394 SILVA or the NCBI data base.

395 The majority of fungal ASVs corresponded to the phyla *Ascomycota* (74.52±8.56%), followed by
396 a smaller proportion of *Olpidiomycota* (9.86±7.58%) and *Basidiomycota* (6.15±0.42%) (Supp. Fig.S3B).
397 A very small proportion of the phylum *Glomeromycota* (0.05±0.03%) was present, mainly composed of
398 the genera *Funneliformis* (33.33±57.74%), *Paraglomus* (26.09±45.19%) and *Archaeospora*
399 (28.49±38.60%), namely arbuscular mycorrhizal fungi (AMF). Fungal families were less
400 homogeneously distributed than bacterial families and had smaller proportions of few abundant taxa
401 (24.97±2.55%) (Fig.2). On average, *Aspergillaceae* members were dominant in the samples
402 (29.89±14.83), followed by *Olpidiaceae* (9.86±7.58%), *Plectosphaerellaceae* (8.81±7.89%), *Nectriaceae*
403 (6.08±1.93%), and families less than 0.1% abundant (6.42±0.72%). The *Aspergillaceae* family was
404 mostly composed of *Aspergillus* (65.12±6.16%), *Penicillium* (34.69±6.13%) genera, and one
405 unidentified genus (0.19±0.03%). The different *Aspergillus* species were presented in supplementary
406 Table S3, with prevalence of ASV 853 (76.07±1.14%). After a BLASTn analysis, ASV 853 shared 100%
407 identity with *A. europaeus* and *A. fumigatus* but no exact assignation could be determined.

408 No metabarcoding analyses were conducted on gamma on sterilized soil to search for microbial
409 communities. Nevertheless, the average values of colony forming bacteria and fungi were respectively
410 $4.80 \cdot 10^3 \pm 1.11 \cdot 10^3$ (mean±sd) and 11.55 ± 4.88 CFU g⁻¹ dry native soil, whereas average values were of
411 8.67 ± 6.46 bacterial and 1.01 ± 0.98 fungal CFU.g⁻¹ dry sterilized soil thus confirming sterilizing
412 treatment effectiveness.

413 **Effect of soil microorganisms on germination signal in mini-rhizotron systems**

414 Germination of broomrape seeds was monitored in mini-rhizotron systems at one- and two- weeks post
415 rapeseed root inoculation. Inoculum consisted of broomrape seeds suspended either in control buffer,
416 buffered unfiltered soil extracts or 0.22 µm-filtered soil extracts (Fig.3A). Seed viability was assessed
417 separately *via* addition of synthetic strigolactone *rac*-GR24, and led to identical and high seed
418 germination ratio for all treatments after 4 days (mean= 94.64±sd= 2.27%, p-value >0.05).

419 Control treatment with buffer and treatment with 0.22 µm-filtered soil extracts containing
420 water-soluble soil metabolites resulted in similar germination ratios after two weeks of co-cultivations.
421 Germination ratios were close to 0 during the first week but increased considerably during the second
422 week to reach $47.72 \pm 27.51\%$ (median±sd) and $41.97 \pm 34.73\%$, respectively (p-value >0.05). This thus
423 suggests the release of GSs from rapeseed roots after 2 weeks of co-cultivation without any effect of
424 water-soluble soil metabolites. Furthermore, treatment with unfiltered soil extracts significantly
425 increased broomrape germination ratios over the two weeks compared to the control, as germination
426 ratios reached $41.55 \pm 25.21\%$ (p-value <0.0001) on the first week and $76.84 \pm 26.15\%$ (p-value <0.0001)
427 on the second week. Besides, in the presence of unfiltered soil extracts, germination ratio on week 1

428 was similar to the control buffer treatment on week 2 (p-value = 0.99), indicating a one-week delay of
429 germination in the absence of microorganisms. Altogether, these results highlight the amplification of
430 broomrape seed germination signal in the presence of microorganisms from soil extracts.

431 Subsequent effects of soil on broomrape attachment to rapeseed roots and subsequent tubercle
432 development was assessed at two, three, and four weeks post inoculation (Fig.3B). Treatment with
433 unfiltered soil extracts only impacted the number of attached parasites at three weeks post inoculation
434 with over 6.8 times more broomrape tubercles compared to the 0.22 μ m filtered extract (p-value
435 =0.004) and buffer treatment (p-value <0.0001). However, differences in number of attached parasites
436 at four weeks post inoculation were no longer significant. There were 56.63 \pm 51.81 (mean \pm sd) attached
437 tubercles for unfiltered soil extract treatment, and 42.47 \pm 46.22 for 0.22 μ m filtered soil extract
438 treatment compared to 42.32 \pm 33.35 attached tubercles for buffer control treatment (p-values =0.596
439 and =0.376 respectively). Nevertheless, unfiltered soil extract treatments resulted in higher
440 proportions of advanced spider tubercles at four weeks post inoculation with 59.69 \pm 19.93% compared
441 to buffer control with 21.79 \pm 24.30% (p-values<0.0001) while treatments with buffer solution and 0.22
442 μ m filtered soil extract displayed higher proportions of young tubercles with respectively
443 76.42 \pm 27.37% (p-values <0.0001) and 59.46 \pm 24.77% (p-value =0.042) compared to unfiltered extract
444 treatment with 39.39 \pm 19.67% (Fig.3C). These results suggested that the microbial-mediated signal
445 enabled faster kinetics of broomrape seed germination, and thus faster kinetics of attachment to host
446 roots and subsequent post attachment development.

447 **Effect of soil microorganisms and metabolites on broomrape seed germination in presence of** 448 **glucosinolates in *in vitro* assays**

449 Four days after induction treatment in 96-well plates, no *P. ramosa* seed germinated in the presence of
450 either the negative control (HEPES buffer) or the soil extracts alone for any dilutions (0%, p-value
451 >0.05; Fig.4). This suggests that both metabolites and microorganisms from soil extracts had no direct
452 impact on the germination of broomrape seeds. Furthermore, no inhibitory effect was observed on seed
453 germination after co-treatment with germination stimulant *rac*-GR24 (10⁻⁸ M) associated with either
454 0.22 μ m filtered or unfiltered soil at 2 dilutions. At the 10-fold dilution, seeds germinated at relative
455 ratios of 1.00 \pm 0.05 (median \pm sd) and 0.92 \pm 0.05 respectively for filtered and unfiltered soil extracts (p-
456 value =1), similarly to the control (*rac*-GR24 only) with 1.01 \pm 0.05 (p-value >0.05; Supp. Fig.S4A).

457 In contrast, broomrape seed co-treatment with soil extracts and exogenous gluconasturtiin
458 (GNT; 10⁻⁶ M) significantly enhanced seed germination ratios ranging from 0 to 0.90 \pm 0.08 (median \pm sd)
459 to 0.97 \pm 0.13 for the 10-fold and 100-fold soil extract dilutions respectively (p-value <0.0001 for both
460 dilutions), compared to seeds treated with positive control *rac*-GR24 (10⁻⁷ M). As GNT alone does not

461 induce any seed germination (p-value =1), this result suggests the release of GSs from GNT hydrolysis
462 by soil microorganisms. Control co-treatments with exogenous myrosinase (5 mU.mL⁻¹) and 0.22 µm-
463 filtered soil extracts confirmed the absence of residual glucosinolate among the water-soluble soil
464 metabolites, whereas co-treatments with GNT confirmed the absence of residual extra-cellular
465 myrosinase (null germination ratio, Supp. Fig.S4B).

466 All co-treatments of any of the sterilized extracts (soil extract filtrations, biocidal treatment,
467 gamma sterilization) with GNT (10⁻⁶ M) were similarly inactive on *P. ramosa* seed germination (null
468 germination) compared to the buffer control for all soil extract dilutions (p-values >0.05; Fig.4). In
469 addition, both 0.45 µm and 0.22 µm filtrations of native soil extracts prevented GNT-mediated
470 germination, suggesting that the soil activity was concentrated in unfiltered extracts, with microbes
471 larger than 0.45 µm.

472 **Bacterial and fungal candidates responsible for glucosinolate degradation in rapeseed soil**

473 Isolation procedures from enrichment cultures revealed nine bacterial isolates on minimum medium
474 M9 (8 growing on LB agar and 1 on PDA), as well as nine fungal isolates (2 on M9 and 7 on ¼ strength
475 PDB). Bacteria were identified via sequencing of 16S rRNA amplicons, and fungi via sequencing of ITS1.
476 Nucleotide sequences were compared in NCBI database (Table 1) and assigned to one uncultured
477 *Actinobacterium* sp., one uncultured *Proteobacterium*, four *Arthrobacter* spp., one *Ralstonia picketti*, and
478 one *Chryseobacterium* sp. for bacteria. As for fungi, all isolates belonged to *Penicillium* genus. Four
479 additional strains of *Aspergillus* spp. were isolated from the soil extracts on PDA plates (non-selective
480 medium for *Aspergillus* spp.).

481 All isolates were screened for their ability to degrade glucosinolates and to induce *P. ramosa*
482 seed germination in 96-well plate germination assays using the seeds as bioindicators (Table 1). No
483 germination activity was observed for any strains in the absence of GNT, meaning that there was no
484 direct microbial production of effective GSs. The tested strains were reported as myrosinase-producing
485 microorganisms when they were able to induce seed germination in presence of GNT (10⁻⁶ M). Out of
486 the 13 tested fungi, isolates previously grown with SNG and within the *Penicillium* genus showed
487 myrosinase activity. None of the *Aspergillus* strains showed any myrosinase activity. In total, 2 strains
488 Pen_M9_1 and Pen_M9_2 isolated from M9 and SNG medium, as well as 1 strain Pen_PDB_5 from PDB
489 and SNG were able to degrade GNT into broomrape GSs. For bacteria, myrosinase activity was detected
490 for all *Arthrobacter* strains and for *Ralstonia*, *Actinobacterium* and *Proteobacterium* strains. Out of 9, all
491 8 isolates but Chry_M9_9 were able to induce GNT-mediated germination.

Effect of soil microorganisms and metabolites on broomrape aggressiveness

Broomrape aggressiveness was monitored using mini-rhizotron systems, by inoculating rapeseed seedlings with pre-germinated *P. ramosa* seeds (at mean = $94.78 \pm \text{sd} = 1.48\%$). Prehaustorium induction on an aliquot of *P. ramosa* seeds was also assessed separately in 96-well plates the day after, with a maximum formation ratio of $79.32 \pm 9.70\%$ for the positive control (trans-zeatin; tZ 10^{-7} M) and $2.09 \pm 3.70\%$ for the negative control (HEPES buffer).

In mini-rhizotrons, attachments to rapeseed roots of pre-germinated and treated seeds were evaluated from day seven post inoculation. Attachments were first observed on the 10th day for each treatment (Fig.5A). Filtered and unfiltered soil extracts, and *tZ* treatments increased broomrape aggressiveness with respectively 3.89 ± 5.62 (mean \pm sd), 3.22 ± 2.05 and 5.40 ± 5.50 attachments, compared to the control buffer with 0.63 ± 0.74 attachments (p -values ≤ 0.01) from day 10 and throughout the assay. At the end of the experiment, the attachment number increased significantly by $21.31 \pm 4.54\%$ in conditions treated with unfiltered soil extracts, compared to the negative control. No significant differences (p -values > 0.05) in tubercle development were nevertheless observed, all stages considered (Fig.5B). No increase of the parasitic kinetic was triggered by soil extract treatments, as all conditions had similar daily attachment ratios (number of new attachments reported to total final attachments; p -values > 0.05 ; Supp. Fig.S5). Altogether, these results show a combined effect of soil metabolites and microorganisms on broomrape aggressiveness, through improvements of the attachment ratio of germinated seeds.

Characterization of soil activities on induction of prehaustorium formation using *in vitro* assays

After three days of treatment with unfiltered soil extracts, pre-germinated seeds exhibited shorter radicles and developed prehaustoria with ratios of 0.71 ± 0.17 (median \pm sd) at the 10-fold and 0.08 ± 0.11 at the 100-fold dilutions, relatively to seeds treated with the positive control (TDZ 10^{-8} M; ratio of $1.00 \pm 0.05 \approx 95.15 \pm 5.53\%$; Fig.6). The 10-fold dilution strongly differed from the control buffer treatment which did not induced prehaustoria (0 ± 0.004 ; p -value < 0.0001), thus showing that soil may be involved in the release of HIFs. Prehaustorium formation ratios were lower for the 100-fold dilution for all treatment, suggesting a dilution effect of HIFs in the microbial suspension.

Prehaustorium formation ratios were also assessed using mechanic (filtrations) and chemical (PPM addition) sterilization procedures as well as soil gamma-sterilization treatment. This resulted in two divergent patterns according to the chosen method. Indeed, mechanical sterilization induced decreased activities compared to the 10-fold diluted unfiltered extracts, with relative prehaustorium formation ratios of 0.42 ± 0.22 for soil extracts filtrated at $0.45 \mu\text{m}$ (p -value = 0.0006) and 0.25 ± 0.13 for soil extracts filtrated at $0.22 \mu\text{m}$ (p -value < 0.0001). Similarly, chemical sterilization with PPM reduced

525 relative Prehaustorium formation ratios to 0.40 ± 0.17 compared to 10-fold diluted unfiltered soil
526 extracts (p-value < 0.0001). These results suggest that both soil extract metabolites and microorganisms
527 contribute to the induction of prehaustorium formation. In contrast, gamma-sterilized soil extracts
528 considerably increased prehaustorium formation ratios up to levels similar to the positive control,
529 without any effect of the $0.22 \mu\text{m}$ filtration for both extract dilutions (p-value $= 0.104$). Indeed, relative
530 prehaustorium formation ratios of the 10-fold diluted gamma-sterilized soil extracts were of 0.98 ± 0.05
531 and 0.97 ± 0.07 for the $0.22 \mu\text{m}$ filtered and unfiltered extracts respectively (p-value $= 0.989$). Thus, this
532 suggests an artifact effect of soil sterilization by gamma-irradiation causing the release of metabolites
533 with HIF activity.

535 DISCUSSION

536 The confirmation of microbial contribution in the *B. napus* - *P. ramosa* interaction is of major
537 interest in the integrated and global understanding of the parasitic plant biological cycle in its agro-
538 ecological environment. This study shows that a rapeseed soil contains a microbiota that promotes both
539 the parasitic plant germination and aggressiveness through the release of glucosinolate-breakdown
540 products and HIFs.

541 **Rapeseed soil microorganisms hydrolyze host plant glucosinolates into isothiocyanates in the** 542 **rhizosphere**

543 In the present study, using *B. napus* - *P. ramosa* co-cultivation experiments, we have assessed the effect
544 of the soil activity on the rhizosphere signaling leading to broomrape seed germination. We first
545 observed that, in absence of soil microorganisms, *B. napus* alone was able to induce more than 45% of
546 broomrape seed germination after two weeks of co-cultivations, thus suggesting the occurrence of GSs
547 in the vicinity of roots. Most likely, these compounds include 2-phenylethyl isothiocyanate (2-PEITC),
548 as it is the main GS exuded by rapeseed (Auger et al. 2012). Indeed, rapeseed roots may release
549 isothiocyanates, through the degradation of tissues associated with root growth, during which
550 glucosinolates come in contact with endogenous myrosinase (Rumberger and Marschner 2003). Non-
551 enzymatic degradation of glucosinolates in the roots have also been reported through induction by
552 strong thermal and chemical stresses (*e.g.* pH >10 ; Blažević et al. 2015). Although glucosinolate stability
553 is still under exploration, it has been shown to vary according to the side chain structure of the
554 compound (aromatic, aliphatic, indolic) and plant species (Blažević et al. 2015; Dekker et al. 2009). In
555 the present study, glucosinolates are thus unlikely to be easily degraded due to their structure under
556 the used conditions (21°C in the dark in buffered medium).

557 Interestingly, transfer of soil microorganisms to the mini-rhizotron co-cultivation systems
558 favored parasitic seed germination, in turn accelerating the whole parasitic cycle. This indicates an
559 amplification of the host pre-existing germination signal by the hydrolysis of the host-exuded
560 glucosinolates thanks to soil microbial myrosinase activities (Fahey et al. 2001; Rumberger and
561 Marschner 2003; Bressan et al. 2009; Auger et al. 2012). This hypothesis is further supported by *in vitro*
562 germination assays which showed that soil microorganisms stimulated broomrape seed germination,
563 only when supplied with the main glucosinolate of rapeseed roots GNT (Kirkegaard and Sarwar 1998).
564 This result suggests GNT hydrolysis into 2-PEITC in the incubation medium (Auger et al. 2012).
565 Moreover, in the absence of soil microorganisms, the transformation of GNT into GSs required addition
566 of exogenous myrosinase, which is the only enzyme able to click the S-glycosidic bond present in GNT
567 (Naumoff 2011; Bhat and Vyas 2019). Thus, the present study provides strong evidence of microbial
568 myrosinase-like activity in the studied rapeseed soil. The complete loss of activity observed after
569 sterilizing treatments (0.22 μm filtrations, PPM and gamma-sterilization) further confirmed the
570 microbial origin of the germination-promoting activity. This activity was also shown to originate from
571 microorganisms larger than 0.45 μm , as filtration at 0.45 μm prevents the germination. Further analyses
572 using intermediate filters or including specific fungicide or bactericide treatments are nevertheless
573 required to establish the bacterial and/or fungal origin of the observed activity.

574 Interestingly, while myrosinase activity has already been reported in soils (Al-Turki and Dick
575 2003; Gimsing et al. 2006), this report is the first to emphasize its role as a factor contributing to the
576 whole parasitic plant - plant interaction.

577 **Rapeseed soil microorganisms alone do not produce germination stimulants**

578 Direct release of GSs by soil microorganisms was not observed in the present study, as no broomrape
579 seed germination occurred *in vitro* upon treatment with unfiltered or filtered soil extracts alone.
580 Moreover, as no germination was observed with or without exogenous myrosinase treatment, it
581 appears that no residual glucosinolate nor GS were detected in the soil extracts. Nevertheless, we
582 cannot out rule that the parasitic seeds were not able to detect putative isothiocyanates, as they could
583 be too diluted or rapidly degraded by soil microorganisms, or also because of apolar interactions may
584 prevent them to be properly solubilized in water (Sarwar et al. 1998; Rumberger and Marschner 2003).
585 Indeed, Auger et al. (2012) successfully extracted glucosinolate-breakdown products from rapeseed
586 soil using dichloromethane solvent, showing that GSs can be retained by soil particles.

587 To date, only few studies have described the production of GSs by microorganisms. For instance,
588 fusicoccins (carbocyclic diterpenoids) and their derivatives isolated from the fungus *Fusicoccum*
589 *amygdali*, the causal agent of peach and almond canker, as well as Ophiobolins (sesquiterpenes) from

590 pathogenic *Bipolaris* fungal species were shown to induce germination of *P. ramosa* and other
591 broomrape species (Yoneyama et al. 1998; Evidente et al. 2006; Fernández-Aparicio et al. 2008;
592 Okazawa et al. 2021). However most of these studies focused on non-crop specific or plant pathogenic
593 microorganisms as tools for suicidal germination approaches. Yet, no study has considered the role of
594 microorganisms naturally occurring in the soil during the parasitic plant cycle. Altogether, these results
595 emphasize the tri-partite nature of the signaling mechanisms leading to *P. ramosa* germination in
596 rapeseed fields.

597 **Rapeseed microbiome is structured by the rapeseed metabolome**

598 The prevalence of isothiocyanates in the rhizosphere of rapeseed and other *Brassicaceae* species is
599 known to induce a strong biocidal activity against numerous soilborne bacteria and fungi (Smith and
600 Kirkegaard 2002; Aires et al. 2009; Sotelo et al. 2015). It was also shown that, under different pH and
601 cellular conditions (*e.g.* presence of Fe³⁺, epithiospecifier-like proteins, glucosinolate side-chain
602 structure), glucosinolates can rearrange into other breakdown compounds (thiocyanates, nitriles,
603 oxazolidine-2-thiones and epithionitriles) which also bear biocidal properties (Wittstock et al. 2016).
604 Overall, this toxicity shapes the microbiota structure in the rhizosphere of *Brassicaceae* (Bressan et al.
605 2009). Fungal communities are especially impacted and undergo severe reductions in diversity and
606 richness, as they are usually more sensitive to these toxic compounds (Smith and Kirkegaard 2002;
607 Hollister et al. 2013). This explains the absence of AMF (arbuscular mycorrhizal fungi) in rhizosphere
608 of isothiocyanate-producing plant species (Wang et Qiu 2006; Vierheilig and Ocampo 1990; Schreiner
609 and Koide 1993), which is also demonstrated by the very low proportion of AMF phylum
610 *Glomeromycota* recovered in the rapeseed soil of the present study (<0.1%).

611 Microbial community rearrangements have also been observed after brassicaceous plant
612 material or isothiocyanate amendment as indirect effects of isothiocyanate toxicity. They are generally
613 associated with an increase in the proportion of *Pseudomonas* spp., *Bacillus* spp. and taxa from
614 *Actinomycetales* including *Streptomyces* spp. (Hollister et al. 2013; Ren et al. 2018; Hu et al. 2015), which
615 are well-known to suppress bacterial and fungal soilborne pathogens of rapeseed (Cohen et al. 2005;
616 Sun et al. 2017; Jelušić et al. 2021). Although no soil microbiota analysis was performed prior to
617 rapeseed cropping in our study, glucosinolate-degradation products possibly induced the selection of a
618 high proportion of the *Pseudonocardiaceae* (order *Actinomycetales*). Among this family, genus
619 *Saccharopolyspora*, which contains numerous species producing a wide variety of biologically active
620 secondary metabolites, is mainly represented (Sayed et al. 2020). Global effects of glucosinolates on
621 microbial communities also result in combining toxicity effects of glucosinolate degradation products
622 together with beneficial effects of allelopathy and nutrient release. Indeed, at the small scale, glucose

623 molecules resulting from the glucosinolate hydrolysis can be used as a carbon substrate, thus conferring
624 competitive advantages for myrosinase-producing and isothiocyanate-tolerant microorganisms
625 (Rumberger and Marschner 2003; Bressan et al. 2009; Szűcs et al. 2018). We thus hypothesize a
626 distribution of these specific traits among several groups of microorganisms found in the rapeseed
627 rhizosphere.

628 **Distribution of myrosinase activity among microorganisms isolated from rapeseed soil**

629 We demonstrated that a rapeseed cultivated soil contains microorganisms able to degrade
630 glucosinolates thanks to their myrosinase activity and thus able to induce broomrape seed germination.
631 Indeed, we isolated both bacterial and fungal candidates harboring myrosinase activities. Notably, this
632 study reveals a myrosinase-like activity of *Penicillium* strains with three out of nine isolates inducing
633 about 45% of broomrape seed germination in presence of glucosinolates. However, the myrosinase
634 activity was not evenly distributed among the genera. Only one out of the seven PDB-isolated
635 *Penicillium* exhibited myrosinase activities in a more complex medium (SNG and potato dextrose). This
636 suggests the ability of *Penicillium* spp. to tolerate toxicity of glucosinolate-breakdown products and
637 develop on different available sources of carbon, either from the medium itself or from co-metabolism
638 with co-occurring myrosinase-containing organisms. To date, most of the identified glucosinolate-
639 degrading fungi belong to *Aspergillus* or *Fusarium* species, such as *A. sydowii*, *A. clavatus*, *A. flavus*, *A.*
640 *niger*, *A. terreus* and *F. oxysporum* (Ohtsuru et al. 1973; Smits et al. 1993; Sakorn et al. 1999;
641 Rakariyatham and Sakorn 2002; Rakariyatham et al. 2005; Galletti et al. 2008; Wang et al. 2012; Szűcs
642 et al. 2018). Interestingly, both *Aspergillus* and *Penicillium* genera belong to the *Aspergillaceae* family,
643 which was the most abundant family in the studied rapeseed soil (20%). However, among all the species
644 identified in this study, only *A. flavus*, which was present in low quantities, has previously been
645 described to degrade glucosinolates. Nevertheless, we tested the ability of the four isolated *Aspergillus*
646 strains to degrade glucosinolates into GSs. In our conditions, none of *Aspergillus* strains at a
647 concentration of around 10^3 – 10^4 spores per mL, were able to induce broomrape germination in
648 presence of GNT (10^{-6} M). However, these strains were isolated on a classic medium (PDA) without
649 glucosinolate, which may be required to select myrosinase active strains and/or to induce their
650 myrosinase activity. Indeed, fungal myrosinases may function similarly as bacterial myrosinases that
651 were characterized as inducible rather than constitutive enzymes (Albaser et al. 2016). Likewise,
652 Gimsing et al. (2006) found that degradation of glucosinolates was higher in soils that had been
653 cultivated with glucosinolate-containing crops compared to soils cultivated with other crops. This
654 suggests a selection and/or stimulation of myrosinase-producing microorganisms. Further
655 experiments may be nevertheless required to properly conclude on the contribution of these isolates

656 to the germination-promoting signaling in rapeseed rhizosphere. No *Fusarium* strain, was isolated from
657 the studied soil. This may be due to their low relative abundance in the studied soil (less than 3%), but
658 also to the competition with other species on non-selective PDA medium. Nevertheless, *Fusarium* spp.
659 have been found to both degrade glucosinolates and tolerate isothiocyanates (Smits et al. 1993;
660 Ishimoto et al. 2000). This thus highlights the need to further investigate the contribution of *Fusarium*
661 spp. in the germination signaling. *F. oxysporum* might be considered of great interest in this case, as this
662 species is pathogenic to many broomrapes and was dominant in our studied soil (Andolfi et al. 2005;
663 Aybeke 2020; Gibot-Leclerc et al. 2022).

664 In addition to the mycobiota, myrosinases are also found in several bacteria mostly found in
665 human or animal gut, such as enterobacteria or lactic acid bacteria (Palop et al. 1995; Cheng et al. 2004;
666 Mullaney et al. 2013). Bacterial myrosinase activities were also recently characterized in *Leclercia*
667 *adecarboxylata* and *Citrobacter* WEY1 strains isolated from the rhizosphere of rapeseed seedlings and
668 adult plants respectively (Albaser et al. 2016; Tie et al. 2021). Gene sequence structures differ between
669 bacterial myrosinases and those of plants and insects suggesting different hydrolysis mechanisms and
670 strategies for glucosinolate-breakdown. Plant myrosinase belong the glycoside hydrolase 1 (GH1)
671 enzyme family (Francis et al. 2002; Naumoff 2011; Mahn et al. 2014), whereas bacterial ones were
672 assigned to multiple GH families. On the one hand, Albaser et al. (2016) described a GH enzyme with
673 myrosinase activity encoded by genes of GH3 family in a *Citrobacter* strain. On the other hand, Cordeiro
674 et al. (2015) found GHs with possible myrosinase-like activities encoded by genes of GH1 and GH4
675 families in strains of SNG-degrading *Escherichia coli*. In the present work, myrosinase activity was
676 detected in eight out of the nine soil bacterial strains isolated from M9 SNG-medium. Most of these
677 strains were characterized as members of the genera *Arthrobacter*, *Ralstonia* and two uncultured
678 *Actinobacterium* and *Proteobacterium*. In the same way as for fungi, incubation of bacteria with SNG did
679 not constitutively result in myrosinase enzymatic activity, since Chry_M9_9 (*Chryseobacterium* sp.)
680 could not degrade GNT into GSs in the tested conditions. Interestingly, *Arthrobacter* genus is known to
681 be prevalent in soils and rhizospheres and is involved in the transformation of several organic carbon
682 substrates, such as aromatic hydrocarbons (Stevenson, 1967). In a recent study, a variety of
683 *Arthrobacter* strains isolated at different depth in a sediment substrate exhibited a high number of
684 carbohydrate-active enzymes from the CAZymes database (carbohydrate-active enzyme database
685 Drula et al. 2022), many of them being glycoside hydrolases (Gushgari-Doyle et al. 2022). They
686 displayed high genomic capacities for utilization of carbohydrates and other carbon substrates, which
687 is somewhat similar to their abilities to degrade glucosinolate in rapeseed soils.

Rapeseed soil microorganisms increase broomrape aggressiveness through HIF release

This study provides an original demonstration of the direct contribution (*i.e.* without requiring a host plant) of soil microorganisms in the induction of prehaustorium formation in root-parasitic plants.

Seeds treated with soil microorganisms and/or soil metabolites indeed displayed more attachments to rapeseed roots, which was also similar to treatments with cytokinins (tZ 10^{-7} M). Cytokinins are known effective HIFs that induce the formation of prehaustorial structures on *P. ramosa* germinated seeds (Goyet et al. 2017) as well as on *P. ramosa* microcalli (Billard et al. 2020). Thus, we suggest that the studied soil contains HIFs that trigger prehaustorium formation and lead to a higher attachment ratio of broomrape seeds. The hypothesis of the HIFs' microbial origin was strengthened by the *in vitro* assays. Indeed, soil microorganisms induced up to 66% of prehaustorium formation in absence of the host plant. This ratio was decreased after biological or mechanical soil sterilization. The contribution of microorganisms present in both the 0.45 μ m-filtered fraction and the 0.22 μ m-filtered fraction was confirmed by the progressive yet significant loss of activity after these filtrations with ratios of 0.49 and 0.22 of prehaustorium formation, respectively. The residual activity in the filtered extracts is suspected to be the outcome from HIFs released in the medium during soil extraction procedure, as it has already been observed with cytokinin-producing microorganisms (Müller et al. 1989; García de Salamone et al. 2001; Spallek et al. 2018). Chemical bio-guided analysis or pharmacological approaches using specific competitive inhibitors could be considered to further characterize these microbial HIFs (Goyet et al. 2017).

This strong HIF activity was also detected in the gamma-sterilized extracts and in both filtered and unfiltered soil extracts. This excludes a direct microbial activity. Unlike biocidal treatment, gamma-irradiation possibly results in the breakdown of microbial DNA structure. This might subsequently allow the release of adenine molecules, used as precursor for (naturally occurring) cytokinin biosynthesis which in turn leads to artefactual HIF activity (Frebort et al., 2011; Sakakibara 2010).

HIF-producers represent a large diversity of microbial species with diverse ecological roles

Host-derived quinones and cytokinins have been identified as HIFs in *Orobanchaceae* (Bandaranayake et al. 2010; Goyet et al. 2019; Aoki et al. 2022). The present study highlights that soil microorganisms can also contribute to cytokinin signaling in the rhizosphere. These phytohormones are highly conserved and widely distributed among living organisms including plants, bacteria and fungi, making them perfect candidates for allelopathic signaling across kingdoms (Tirichine et al. 2007; Spíchal 2012). A large diversity of microbial species releases cytokinins for their own development and nutrient uptake as free-living bacteria and fungi, or as chemical signaling to control the host plant growth and development during beneficial or detrimental interactions (Frebort et al. 2011; Chanclud and Morel

2016). For instance, PGP (plant-growth promoting) bacteria *Azospirillum* spp. (*A. lipoferum*, *A. brasilense*), *Bacillus* sp. (*B. subtilis*) and *Pseudomonas* spp. (*P. fluorescens*, *P. putida*) synthesize cytokinins and increase cytokinin levels *in planta* (Arkhipova et al. 2006; Esquivel-Cote et al. 2010; Pallai et al. 2012). However, in the present study, few of these organisms were detected in the studied soil (one *Pseudomonas* (0.02%) and one *Bacillus* (0.08%) ASVs). Also, phytopathogenic microorganisms produce cytokinins as effectors to dampen the host defenses upon root infection (Chanclud and Morel 2016, Spallek et al., 2018). For instance, in rapeseed, Trdá et al. (2017) showed that *Leptosphaeria maculans*, the fungal agent of Blackleg disease (Howlett et al., 2001), was able to release cytokinins both *in vitro*, without any precursor, and inside its host, by modifying cytokinin levels after tissue colonization. Although many potential HIF producers exist among the microorganisms in this study, no candidate can be proposed to date.

CONCLUSIONS

The present study highlights the importance of integrating the soil microbiota in the allelopathic signaling shaping of host plant- root parasitic plant interactions. We showed here that microbial contribution was characterized as beneficial for *P. ramosa*, facilitating host-recognition mechanisms during interactions for carbon acquisition in rapeseed rhizosphere. More specifically, microbe-related decomposition of glucosinolates efficiently releases germination stimulants, a.k.a. isothiocyanates, while microbe-related production of HIFs promotes broomrape attachments to host roots.

To a larger extent, these microbial driving forces, as well as rapeseed cropping intensification, may eventually contribute to host specialization of *P. ramosa* on *B. napus* in western France. These findings also disclose a dilemma for *Brassicaceae* cropping in an agroecological context. Indeed, although isothiocyanates act as biofumigants and suppress soilborne pathogens, *Brassicaceae* are likely to promote broomrape infestation and suppress mutualist symbiosis for the next crop.

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761 **Competing Interests/Conflicts of interest**

762 The authors declare that the research was conducted in the absence of any commercial or financial
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764 **Author Contributions**

765 Lisa Martinez, Lucie Poulin, Jean-Bernard Pouvreau, Christophe Jestin and Philippe Simier contributed
766 to the study conception and design. Material preparation and data collection were performed by Ph.D
767 student Lisa Martinez. Analyses were conducted by Lisa Martinez with the supervision of Lucie Poulin,
768 Jean-Bernard Pouvreau and Philippe Simier. The first draft of the manuscript was written by Lisa
769 Martinez and all authors commented on previous versions of the manuscript. All authors read and
770 approved the final manuscript.

771 **Data Availability**

772 The datasets generated during the current study can be found in the
773 <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA854370>

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1122 **Tables**
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1124 **Table 1** Screening for presence or absence of myrosinase-like activity in bacteria and fungi isolated
1125 from a rapeseed soil using either selective or non-selective media amended or not with sinigrin (5 mM).
1126 Germination assays were conducted in 96-well plates in presence of GNT (5 10^{-6} M) with broomrape
1127 seeds as bioindicators for perception of glucosinolate-breakdown products.

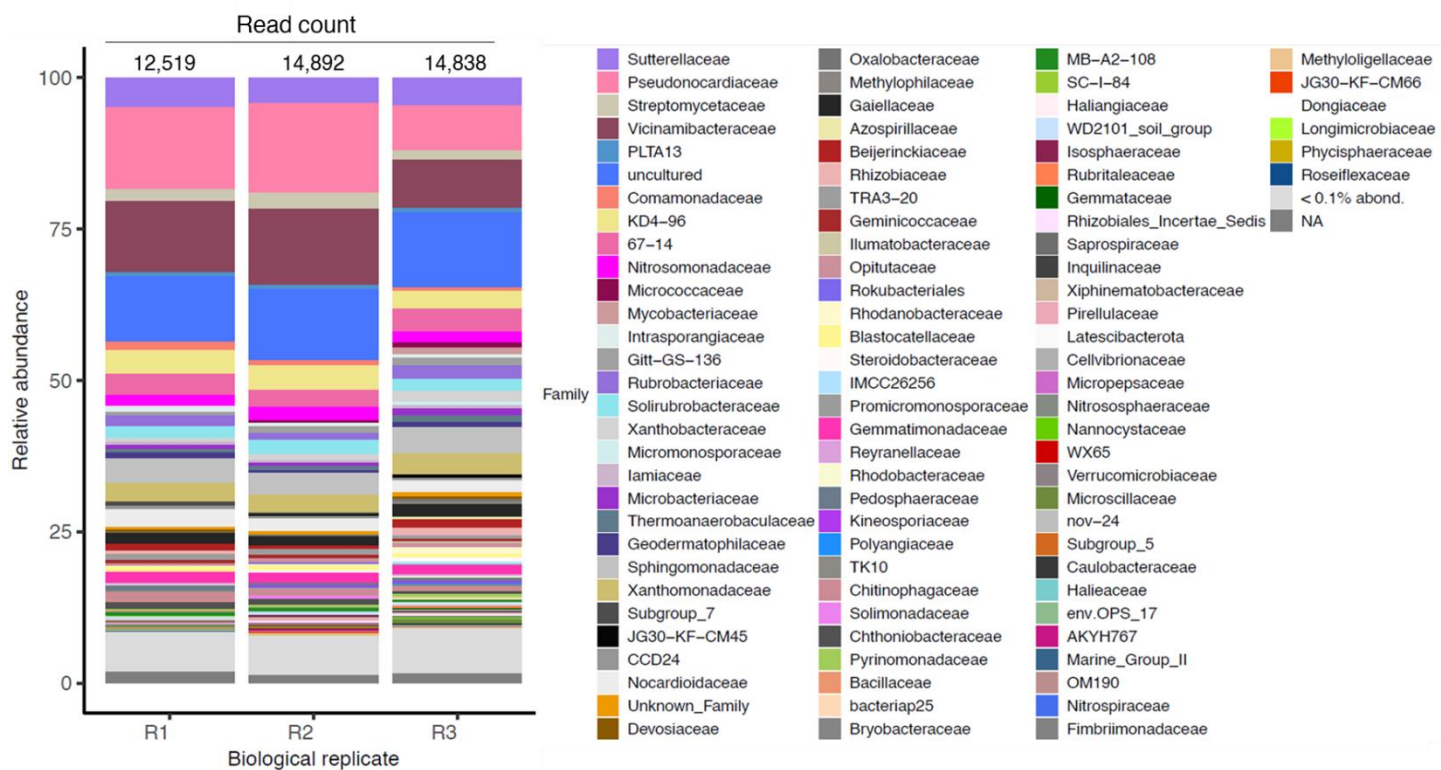
Kingdom	Taxonomic assignment ^a	Strain name	Relative germination % ^{b,c}	Medium
Bacteria	Uncultured <i>Actinobacterium</i>	Act_M9_1	96.68±1.86%	M9+Sinigrin
	<i>Arthrobacter</i> sp.	Art_M9_2	75.31±15.94%	
	<i>Arthrobacter</i> sp.	Art_M9_3	69.08±6.02%	
	<i>Arthrobacter</i> sp.	Art_M9_4	78.42±0.89%	
	<i>Ralstonia picketti</i>	Ral_M9_5	90.12±8.73%	
	Uncultured <i>Proteobacterium</i>	Prot_M9_6	74.72±5.98%	
	<i>Arthrobacter</i> sp.	Art_M9_7	67.94±2.18%	
	<i>Arthrobacter</i> sp.	Art_M9_8	94.68±11.93%	
	<i>Chryseobacterium</i> sp.	Chry_M9_9	0%	
Fungi	<i>Penicillium</i> sp.	Pen_M9_1	46.32±26.00%	M9+Sinigrin
	<i>Penicillium echinalutum</i>	Pen_M9_2	44.50±25.33%	
	<i>Penicillium</i> sp.	Pen_PDB_1	0%	PDB+Sinigrin
	<i>Penicillium</i> sp.	Pen_PDB_2	0%	
	<i>Penicillium</i> sp.	Pen_PDB_3	0%	
	<i>Penicillium</i> sp.	Pen_PDB_4	0%	
	<i>Penicillium</i> sp.	Pen_PDB_5	46.19±21.27%	
	<i>Penicillium</i> sp.	Pen_PDB_6	0%	
	<i>Penicillium</i> sp.	Pen_PDB_7	0%	
	<i>Aspergillus ochraceus</i>	Asp_PDA_1		PDA
<i>Aspergillus</i> sp.	Asp_PDA_2	0%		
<i>Aspergillus versicolor</i>	Asp_PDA_3			
<i>Aspergillus</i> sp.	Asp_PDA_4			

1128 ^a All strains were molecularly characterized using the V4 regions of 16S rRNA (16S) and internal
1129 transcribed spacer (ITS1) phylogenetic markers for bacteria and fungi respectively. Species was
1130 assigned when it belonged to the top hit, i.e. single species with identity percentage above 95%.

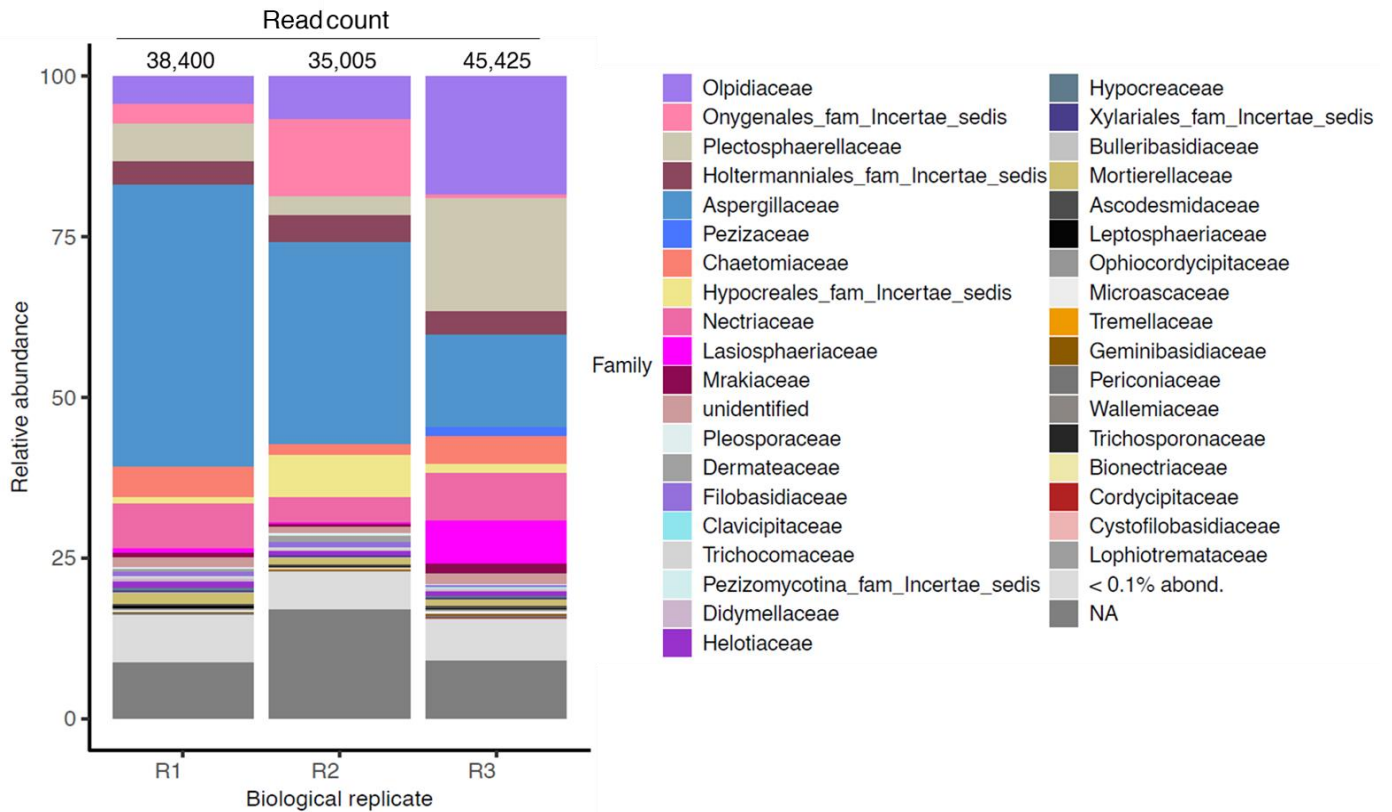
1131 ^b Percentages (mean±sd) are expressed as the number of germinated seeds on total seeds, relatively to
1132 the average ratio obtained with the positive control (rac-GR24 10^{-7} M).

1133 ^c Activity is validated and highlighted in red when relative germination is different than zero at the
1134 highest concentration of 0.01 OD₆₀₀ and 10^{-5} spores mL⁻¹ for bacteria and fungi respectively.

1137 **Figures**



1139 **Fig.1** Relative abundance levels of bacterial families, expressed as mean percentages \pm sd of all amplicon
 1140 sequence variant (ASVs) detected in the rapeseed soil. DNA extraction was performed on three soil
 1141 samples and amplification targeted the V4 region of the 16S rRNA genes. Taxonomic assignments were
 1142 performed on the SILVA database. Taxa less than 0.1% abundant were grouped into the “< 0.1% abund.”
 1143 class, and taxa with no family assignment of family level were grouped into the “NA” class (not
 1144 available).
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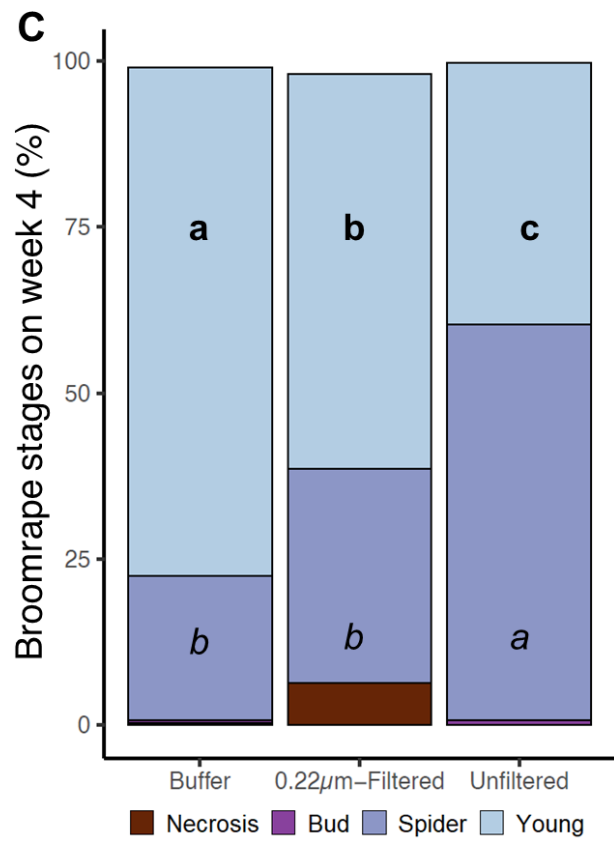
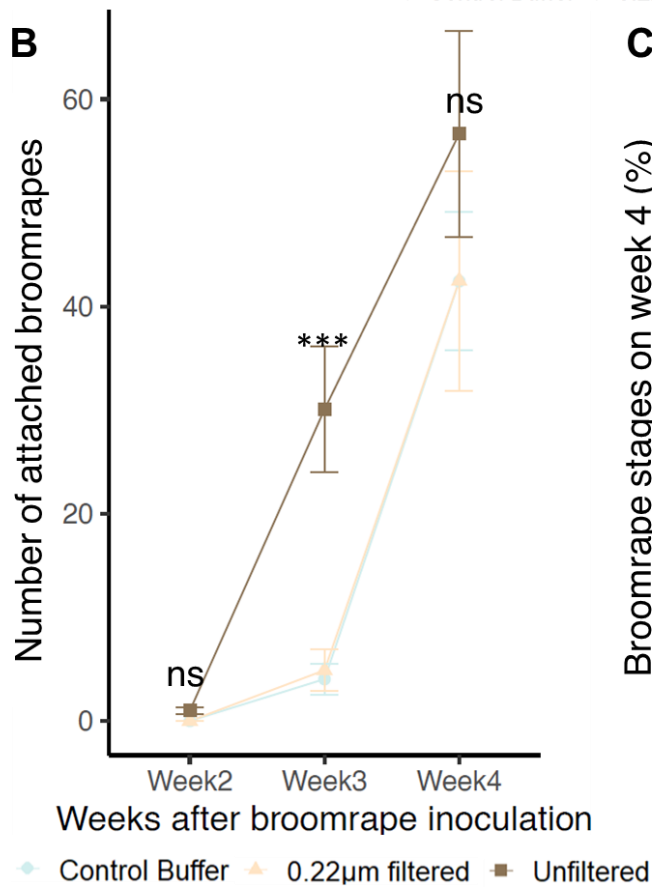
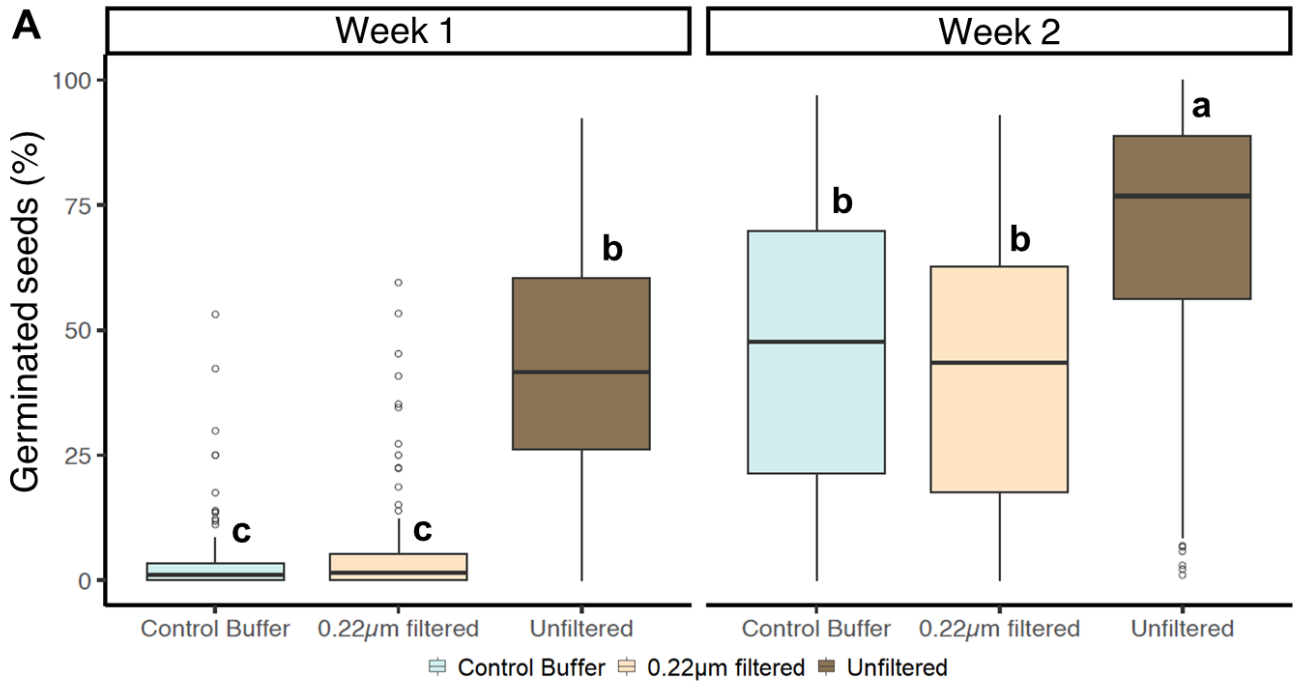
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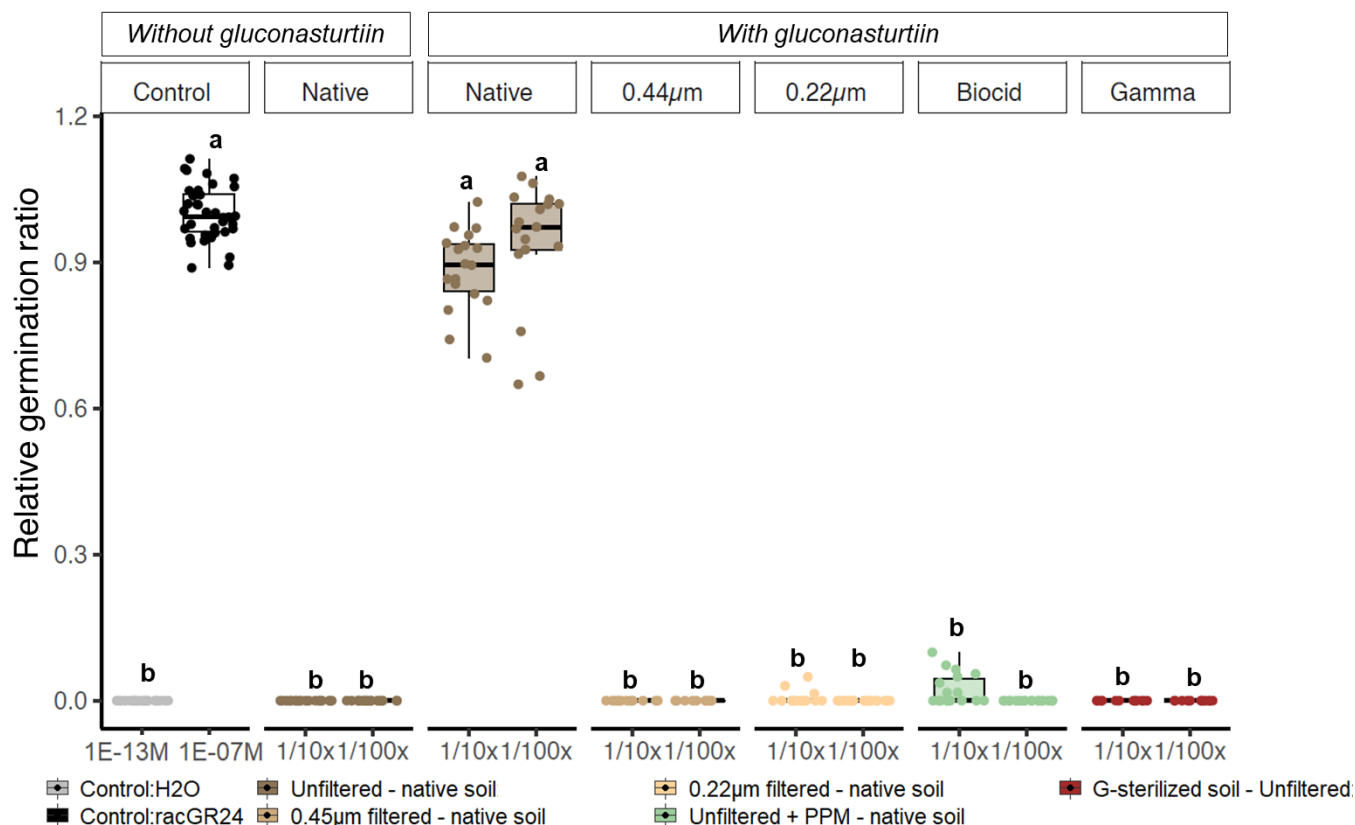
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Fig.2 Relative abundance levels of fungal families, expressed as mean percentages \pm sd of all amplicon sequence variant (ASVs) detected in the rapeseed soil. DNA extraction was performed on three soil samples and amplification targeted the ITS region. Taxonomic assignments were performed on the UNITE database. Taxa less than 0.1% abundant were grouped into the “< 0.1% abund.” class, and taxa with no family assignment of family level were grouped into the “NA” class (not available).



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1154 **Fig.3A** Germination of broomrape seeds in mini-rhizotron systems, 1 and 2 weeks post broomrape
1155 inoculations on rapeseed roots. Percentages were calculated on 4 squares of 1cm². Different
lowercase letters indicate significant differences of treatments (p-value <0.05) determined by
ANOVA. **B** Successful broomrape attachment on rapeseed roots in mini-rhizotron systems, 2, 3 and
4 weeks post broomrape inoculation. Values are displayed as mean with standard deviation as
error bars. Asterisks indicate significant differences 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'not
significant (ns) ', compared to the control modality and determined by ANOVA on a Poisson
distributed model. **C** Average proportion of the different broomrape developmental stages,
reported as a relative ratio to the average number of total attached parasites. Stages correspond
to Young (young tubercle without adventitious root), Spider (old tubercle bearing adventitious
roots) and bud (bud with adventitious roots but without a stem). Different lowercase letters
indicate significant differences of treatments (p-value <0.05) determined by ANOVA on logistic
models for each stage. Bold and italic letters indicate comparisons between young and spiders
respectively. No letter was displayed when there was no significant difference between modalities
(i.e. Bud and Necrosis stages).



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Fig.4 Effect of soil extract and exogenous gluconasturtiin on broomrape germination. Germination of broomrape seeds after a four-day treatment in 96-well plates with unfiltered soil extracts at a 10-fold and 100-fold dilution with and without gluconasturtiin (GNT 10^{-6} M), or after co-treatment with GNT and treated soil extracts: 0.45 μ m and 0.22 μ m syringe filtration, addition of large spectral biocide (Plant-Preservative Mixture 0.1% v/v) with the unfiltered extract, and soil gamma-sterilization. Ratios are expressed as the number of germinated seeds on total seeds, relatively to the average ratio obtained with the positive control (racGR24 10^{-7} M, a synthetic strigolactone, maximum germination ratio = 1 $\approx 70.12 \pm 4.37\%$ of germinated seeds). Different lowercase letters indicate significant differences (p -value < 0.05), and determined by a Kruskal-Wallis rank sum test (non-normal data) and multiple pairwise comparison.

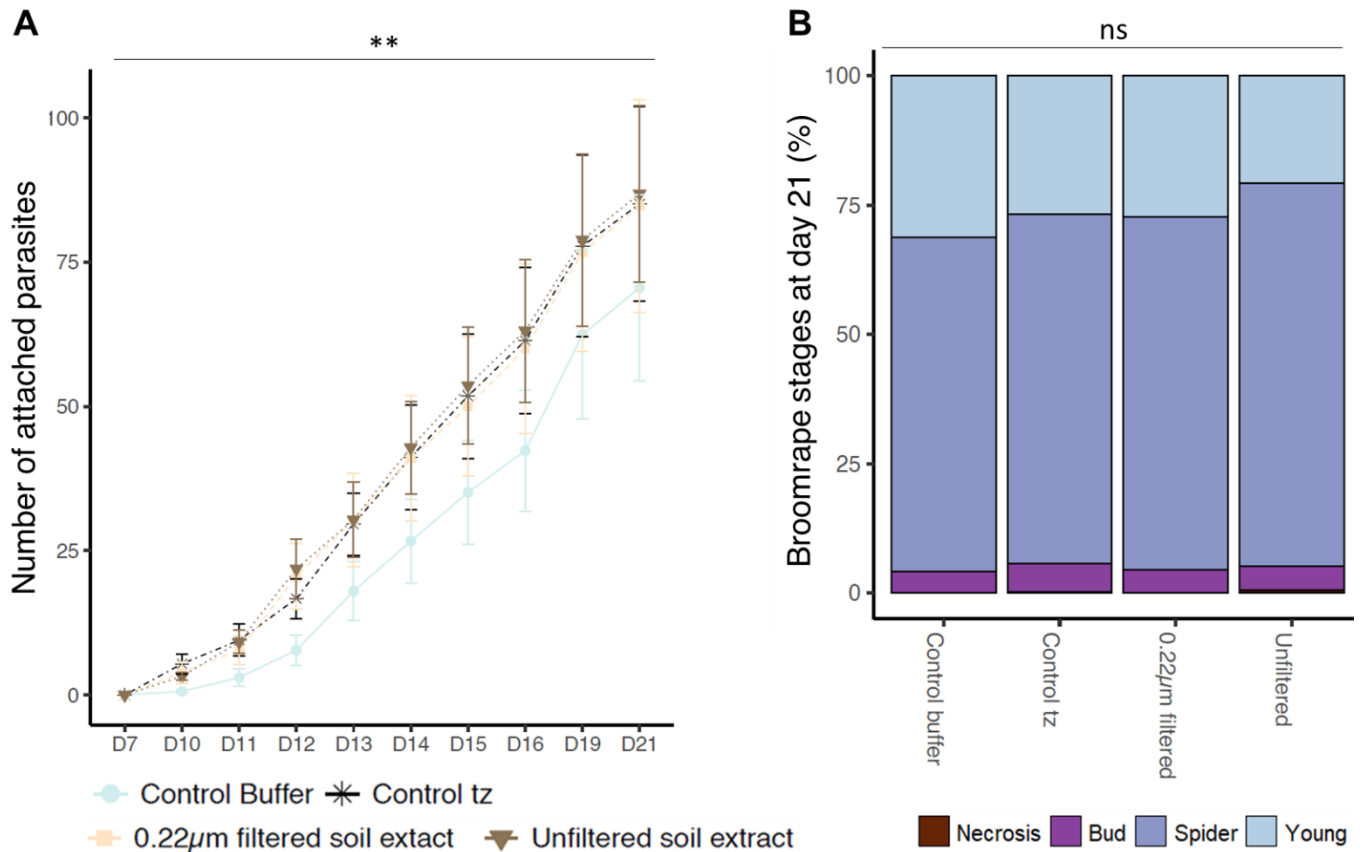
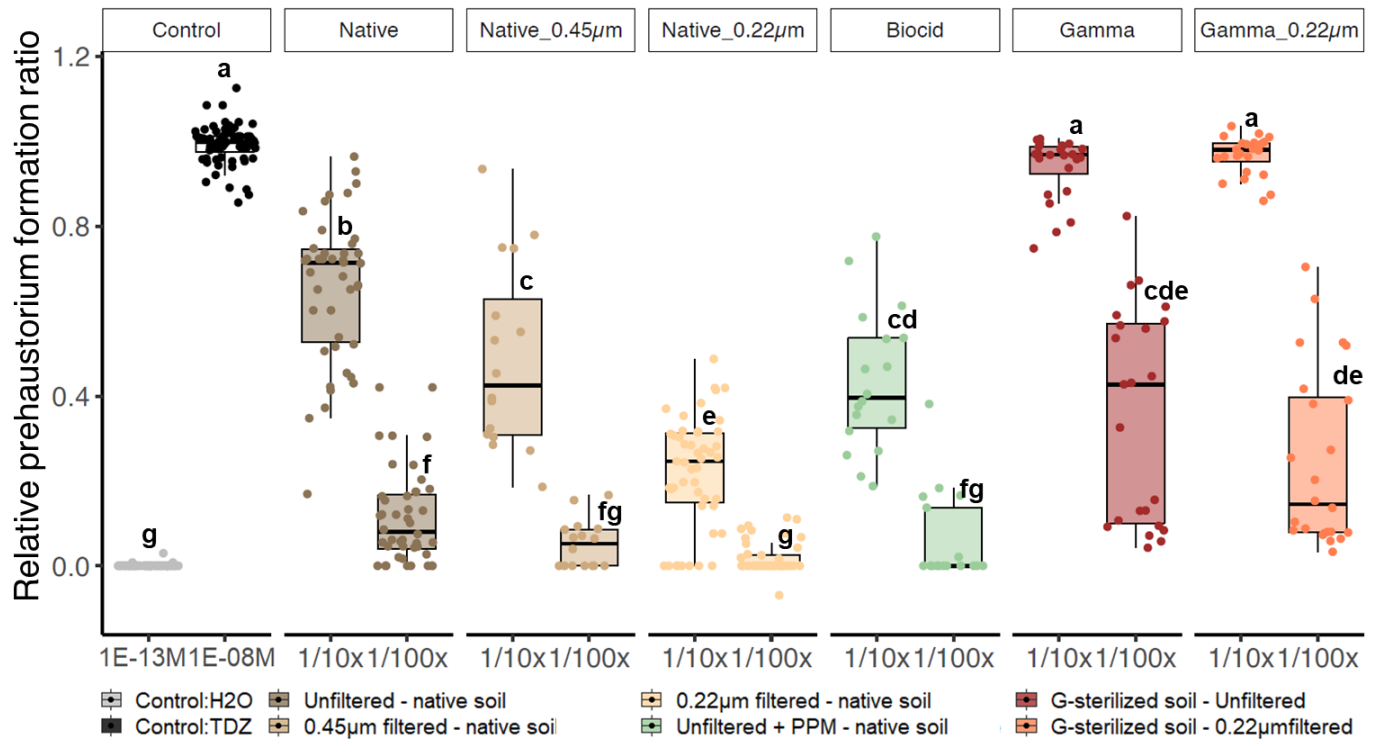


Fig.5A Broomrape attachments to rapeseed roots in mini-rhizotrons, on day 7 to 21 post seed inoculation. Values are displayed as mean with standard deviation as error bars. Asterisks indicate significant p-values compared to the control modality 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns'; determined by an ANOVA on a Poisson distributed model. **B** Corresponding tubercle developmental stages of the broomrape attachments to roots in mini-rhizotrons at day 21. Stages correspond to Young (young tubercle without adventitious root), Spider (old tubercle bearing adventitious roots) and bud (bud with adventitious roots but without a stem). Aggressiveness assays were conducted on germinated and 48 h pre-treated seeds with either control buffer solution, unfiltered soil extract or 0.22µm filtered soil extract or cytokinin (tZ 10⁻⁷ M). Differences of treatments (p-value <0.05) are determined by ANOVA on logistic models for each stage, but are not significant for any stage between modalities (ns).



1190

1191 **Fig.6** Effect of soil extracts on prehaustorium formation in *P. ramosa*. Prehaustorium formed after a
 1192 three-day treatment in 96-well plates with unfiltered soil extracts or treated soil extracts: 0.45 µm and
 1193 0.22 µm syringe filtration, addition of large spectral biocide (Plant-Preservative Mixture 0.1% v/v) with
 1194 the unfiltered extract and soil gamma-sterilization. Ratios are expressed as the number of germinated
 1195 seeds exhibiting prehaustoria (swollen radicles with papillae) on total germinated seeds, relatively to
 1196 the average ratio obtained with the positive control (TDZ 10⁻⁸ M, a cytokinin-like molecule). Different
 1197 lowercase letters indicate significant differences of treatments (p-value < 0.05), determined by an
 1198 ANOVA on a logistic model.
 1199