Additional files

Tables

Table.S1: Description of the analyses conducted to characterize the physicochemical properties of the soils

Measured parameter	Method employed
Soil grinding to 2 mm *	NF ISO 11464
Soil grinding to 250 µm *	NF ISO 11464
Residual humidity *	NF ISO 11465
5-fraction granulometry flag *	NFX 31-107 No decarbonation
Clay (5-fraction) X < 2 µm *	NFX 31-107 No decarbonation
Fine silt (5-fraction) 2 µm < X < 20 µm *	NFX 31-107 No decarbonation
Coarse silt (5-fraction) 20 µm < X < 50 µm *	NFX 31-107 No decarbonation
Fine sand (5-fraction) 50 µm < X < 200 µm *	NFX 31-107 No decarbonation
Coarse sand (5-fraction) 200 µm < X < 2 mm *	NFX 31-107 No decarbonation
Water pH *	NF ISO 10 390
Total organic carbon (Organic matter) *	
Total nitrogen *	NF ISO 13 878 (Method Dumas)
CEC Metson *	NFX 31-130 - (Method SADEF IF07-10D)
BE : solubilization *	NFX31-108
Exchangeable K2O dosage *	NFX 31-108 Quantification ICP AES
Exchangeable MgO dosage *	NFX 31-108 Quantification ICP AES
Exchangeable CaO dosage *	NFX 31-108 Quantification ICP AES
Exchangeable Na2O dosage *	NFX 31-108 Quantification ICP AES
OLIGOS DTPA: solubilization *	NFX 31-121
Iron DTPA dosage *	NFX 31-121 Quantification ICP AES
Manganese DTPA dosage *	NFX 31-121 Quantification ICP AES
Copper DTPA dosage *	NFX 31-121 Quantification ICP AES
Zinc DTPA dosage *	NFX 31-121 Quantification ICP AES
Soluble Boron Boiling Water	NFX 31-122
Sum of cations (acetate)	Calculation

* COFRAC-accredited parameters are indicated by the * symbol in the line header.

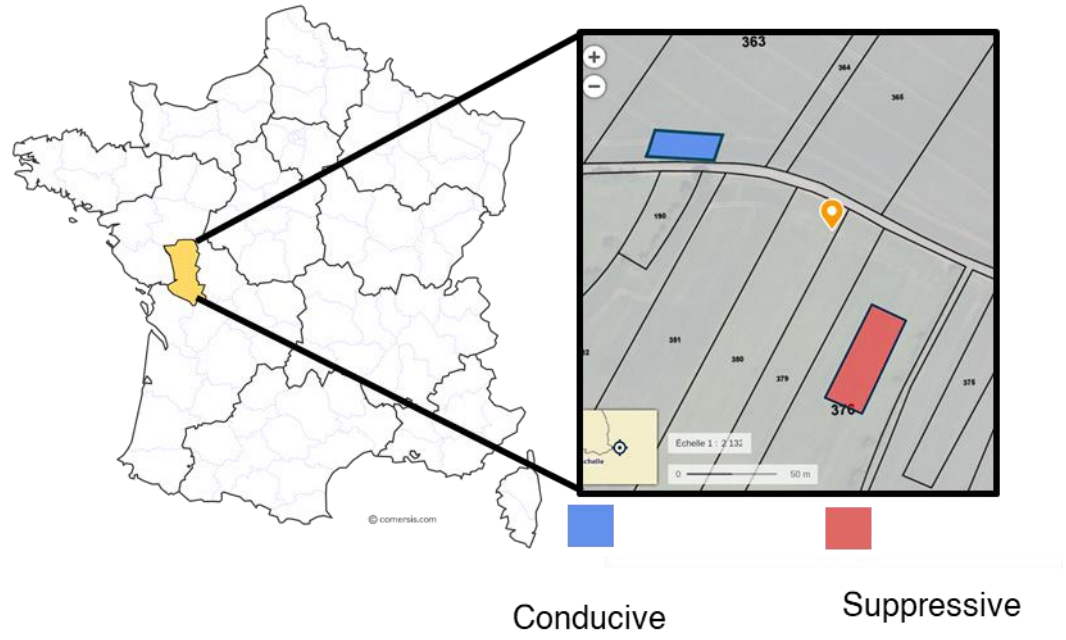
Table.S4: Description of bacteria correlated to parasitic traits but not to plant parasitism suppression

ASV differentially abundant in initial SN soil[a]	Soil	Taxonomic assignation	Parasitic attachment	
			Correlation	Ajdusted P-value
ASV_27058	CN	*Pseudonocardia* sp.	0.273	0.046
			Necrotic symptom ratio	
		Species	Correlation	Ajdusted P-value
ASV_20486	SN	*Luteitalea* sp.	-0.435	0.001
ASV_25744	SN	*Vicinamibacterales* family	-0.338	0.012
ASV_26654	CN	MB-A2-108 (*Actinobacteria)*	-0.316	0.020

[a] ASVs differentially abundant in SN soil correspond to ASVs found with DEseq2, which have significant log2foldchange value after comparisons between initial SN and CN soils
[b] Clusters Blue, Pink and Turquoise correspond to the three cluster of co-abundant ASVs significantly correlated with suppressive parasitic traits, *i.e.* total attachments and relative proportion of necrotic symptoms.

Figures

A



B

Year	Suppressive soil	Conducive soil
2007	Winter wheat	Winter wheat
2008	Winter rapeseed	Temporary pasture
2009	Winter wheat	Winter wheat
2010	Sunflower	Fodder
2011	Winter wheat	Temporary pasture
2012	Winter rapeseed	Temporary pasture
2013	Winter wheat	Temporary pasture
2014	Winter rapeseed	Temporary pasture
2015	Winter wheat	Winter wheat
2016	Winter rapeseed	Winter barley
2017	Winter wheat	Sunflower
2018	Winter barley	Winter wheat
2019	Winter rapeseed	Winter rapeseed

Fig. S1: Summary of the two conducive and suppressive study soils.
A Soils were collected in July 2019 in les Deux-Sèvres (France) in adjacent fields with the numerous broomrape emergences in the conducive soil vs no or few emergences, some presenting necrosis in the suppressive soil. **B** Crop rotations employed by farmers between 2007 until 2017 in the two suppressive and conducive soils.

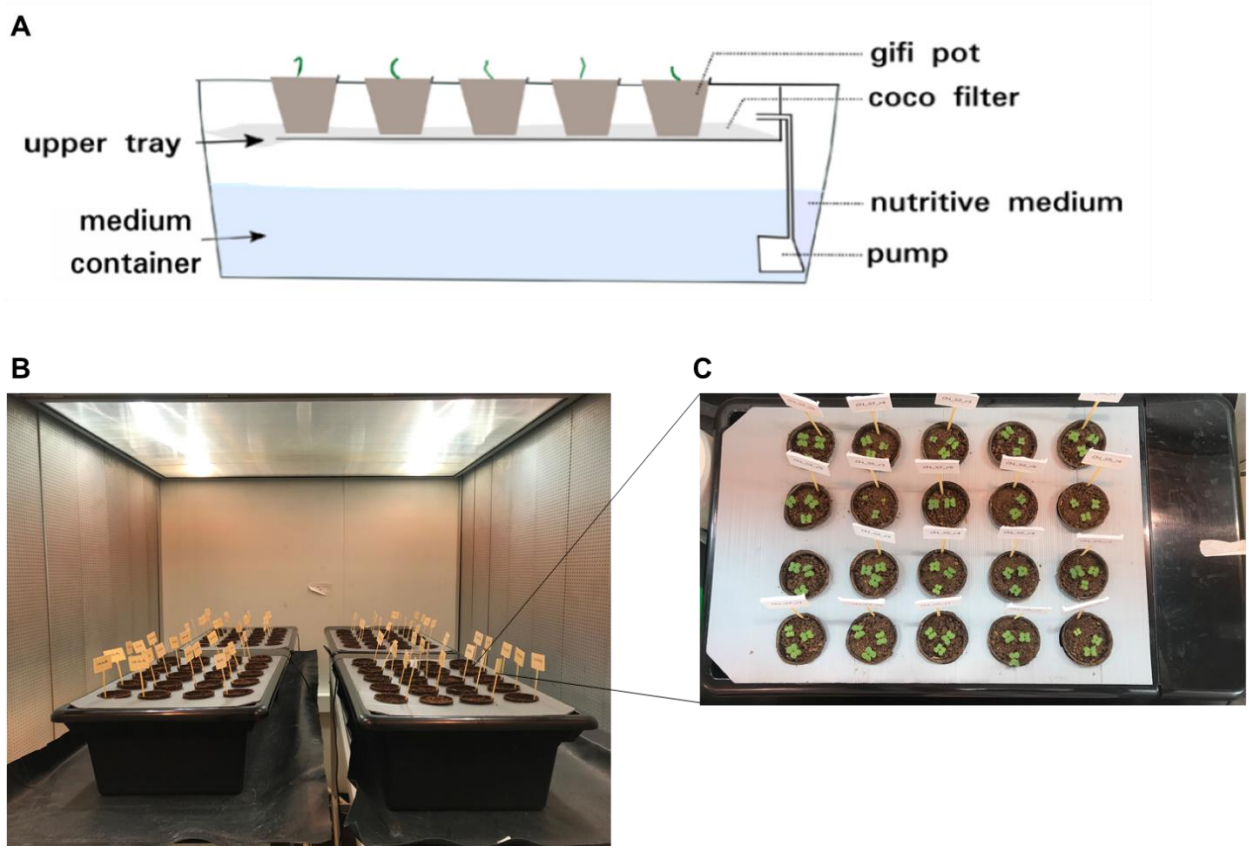


Fig. S2: Co-cultivation design used to simultaneously conduct sampling of root exudates, root parasitic phenotyping and microbial analyses

A Scheme of the nutrient film technique (NFT) tanks used for the hydroponic co-cultivation of rapeseed and broomrape **B** Photo of the NFT tanks in the growth chamber **C** Detail of rapeseed plantlets growing in the conducive native soil in the NFT tank.

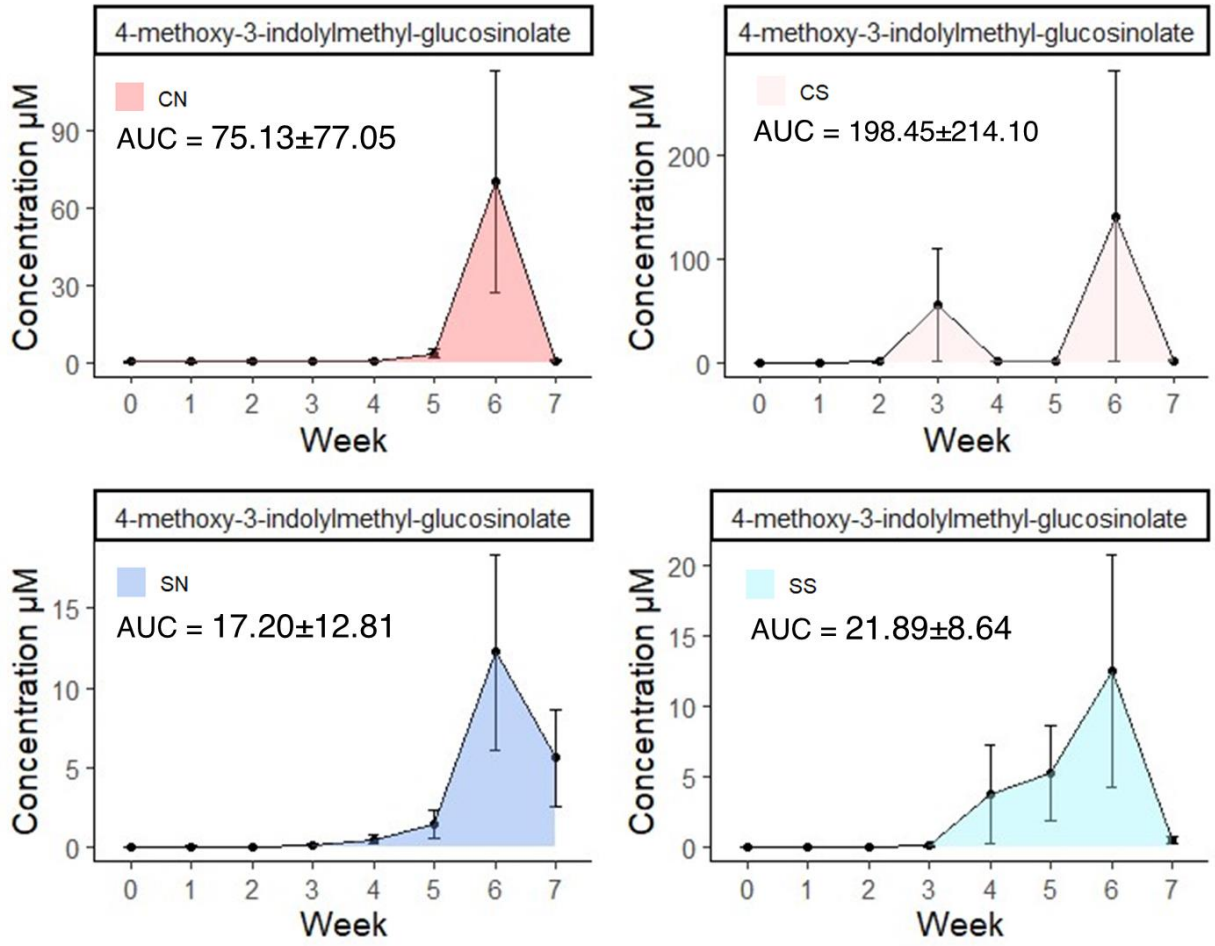


Fig. S3: UPLC-TQD bio-guided quantification of 4-methoxy-3-indolylmethyl-glucosinolate in the eluted fractions from co-cultivation rhizosphere exudates

Analyses were conducted on the four eluted fractions from rhizosphere exudates sampled throughout the three co-cultivations (week 0 to week 7 post sowing) in native conducive (CN) and suppressive (SN) soils or sterilized conducive (CS) and suppressive (SS) soils. 4-methoxy-3-indolylmethyl-glucosinolate was the only glucosinolate detected above threshold (> 1µM). AUC is given for each soil and represents the total concentration of 4-methoxy-3-indolylmethyl-glucosinolate for 8 weeks. Values displayed are means with standard errors as error bars.

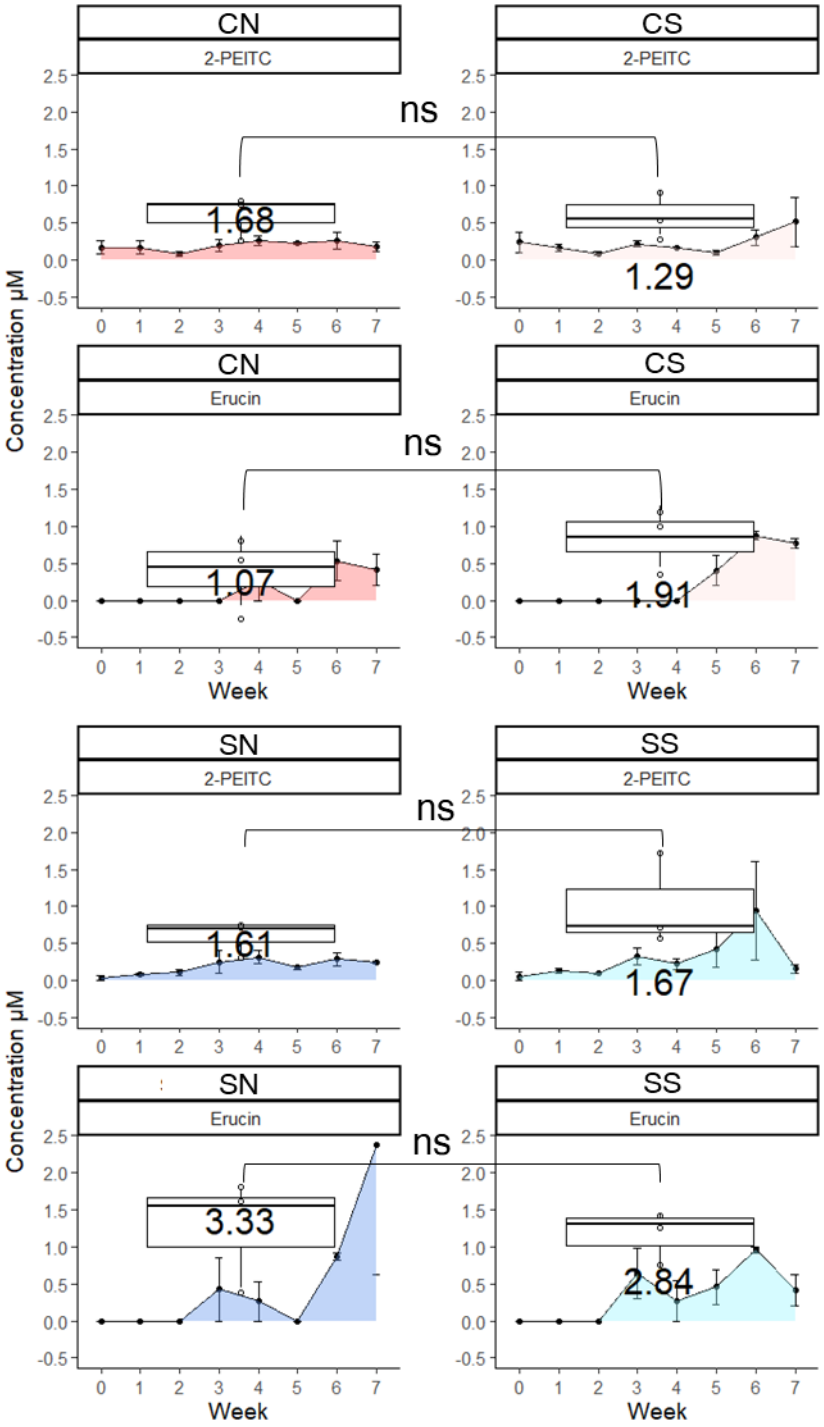


Fig. S4: GC-MS bio-guided quantification of the two isothiocyanates detected in the eluted fractions from rhizosphere exudates of the four native and sterilized soils

Global stimulatory effects were compared thanks the area under the curve (AUC), measured from the 7-week kinetic (Relative absorbance.week^{-1} or Concentration.week^{-1}). Significance between AUC was determined thanks to a non-parametric Kruskall-Wallis rank test and Dunn's Kruskal-Wallis Multiple Comparisons (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001). Values displayed are means with standard errors as error bars.

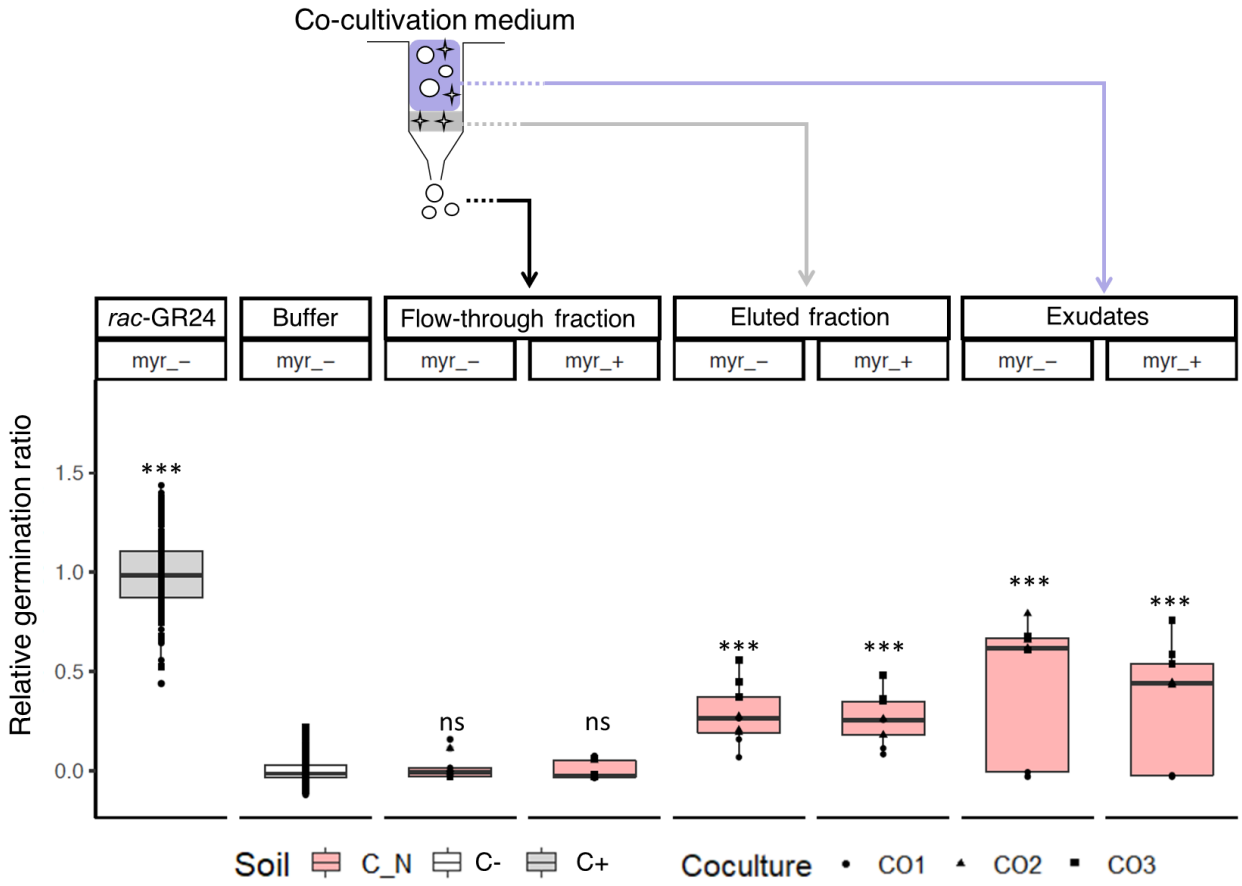


Fig.S5: Germination activity contained in rhizosphere exudates collected in week 2 from the conducive soil

Rhizosphere exudates were concentrated via a solid phase extraction process. As an example, exudates (2-fold dilution), flow-through (2-fold dilution) and eluted (10^2-fold dilution) fractions from week 2 of co-cultivation in the conducive soil (CN), were applied on broomrape seeds. Detection of glucosinolates in exudates is enabled by the addition of myrosinase enzymes (5 $U.L^{-1}$) which cleave them into isothiocyanates. Germination ratios were approached by measuring relative absorbance ($A_{570-690nm}$) on MTT-colored seeds. Significance between AUC was determined thanks to a non-parametric Kruskall-Wallis rank test and Dunn's Kruskal-Wallis Multiple Comparisons (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001)

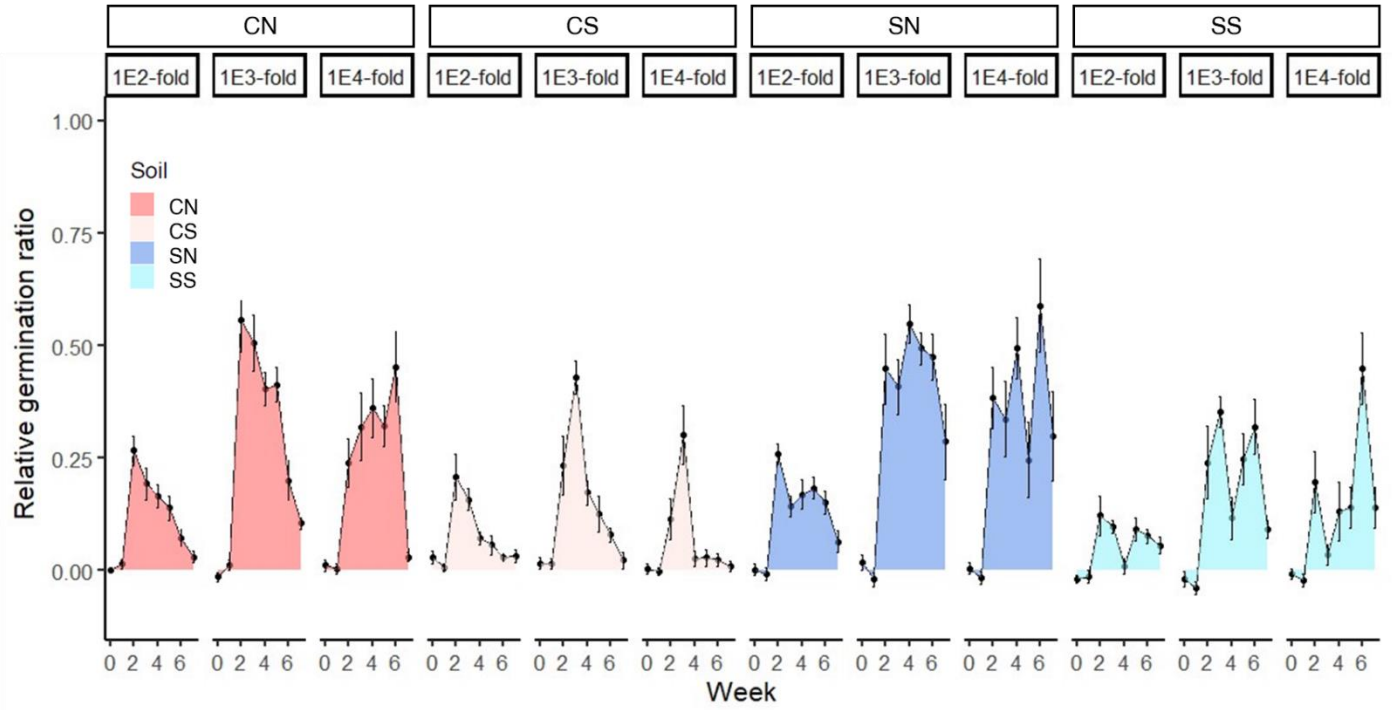


Fig. S6: Germination activity contained in co-cultivation rhizosphere exudates

Germination kinetics of broomrape seeds treated with SPE-eluted fraction (10^2, 10^3 and 10^4-fold dilution) of rhizosphere exudates from native conducive (CN) and suppressive (SN) or sterilized conducive (CS) and suppressive (SS) during 7 weeks of co-cultivation with rapeseed. Germination activities are approached by relative absorbance (A570-690nm) measured on MTT-colored seeds well. Values displayed are means with standard errors as error bars.

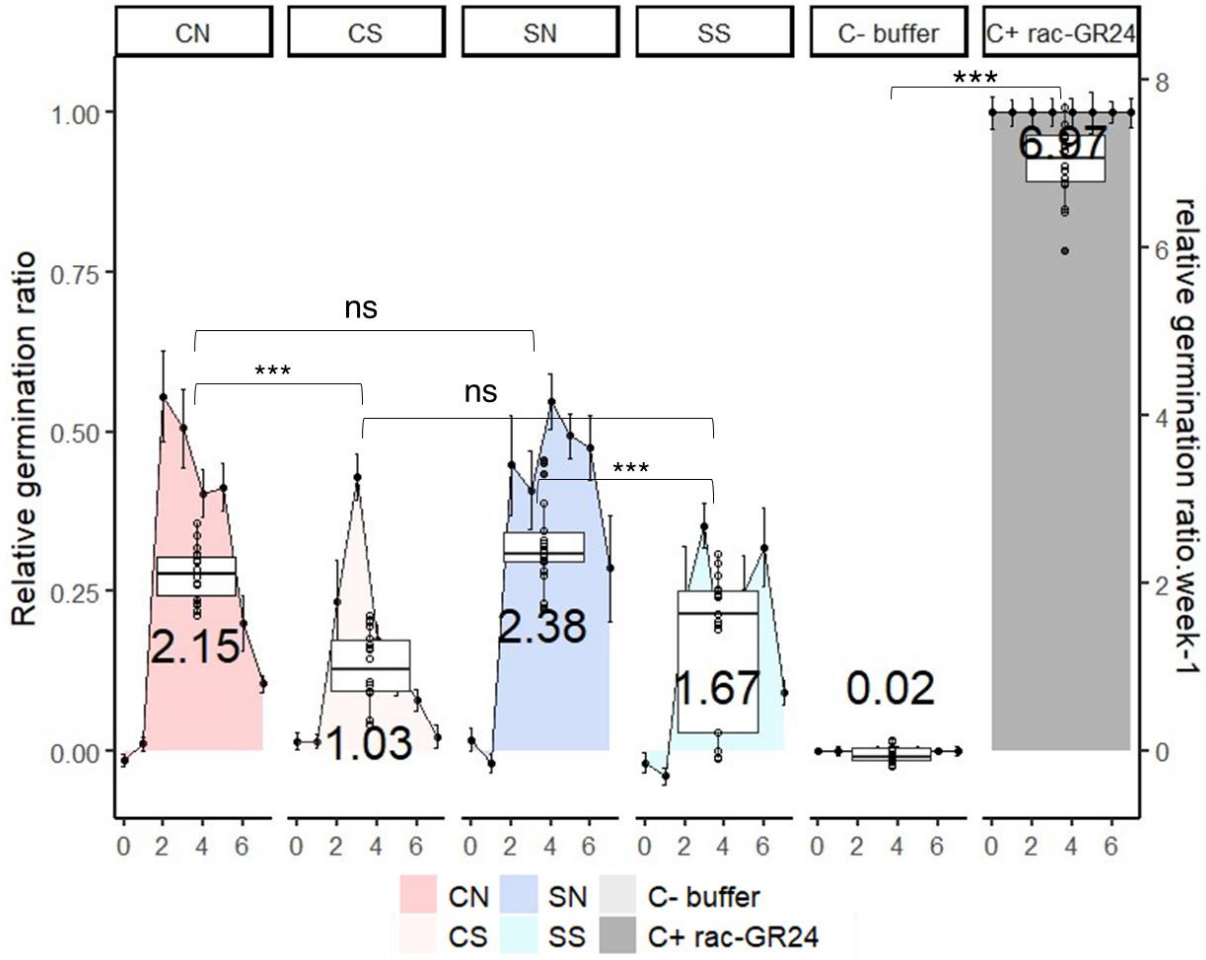


Fig. S7: Effect of soil type and treatment on germination activity of rapeseed rhizosphere exudates

A Germination kinetics of broomrape seeds treated with positive control rac-GR24 (10^{-7} M), negative control buffer (HEPES 1 mM; Plant preservative mixture 0.1% pH 7.5) and SPE-eluted fraction (10^{3}-fold dilution) of rhizosphere exudates from native conducive (CN) and suppressive (SN) soils or sterilized conducive (CS) or suppressive (SS) soils during 7 weeks of co-cultivation with rapeseed. Germination ratios are approached by relative absorbance ($A_{570\text{-}690nm}$) measured on MTT-colored seeds well. Global stimulatory effects were compared through the area under the curve (AUC), measured from the 7-week kinetic (Relative germination ratio.week^{-1}). Significance between AUC was determined thanks to a non-parametric Kruskall-Wallis rank test and Dunn's Kruskal-Wallis Multiple Comparisons (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001). Values displayed are means with standard errors as error bars.

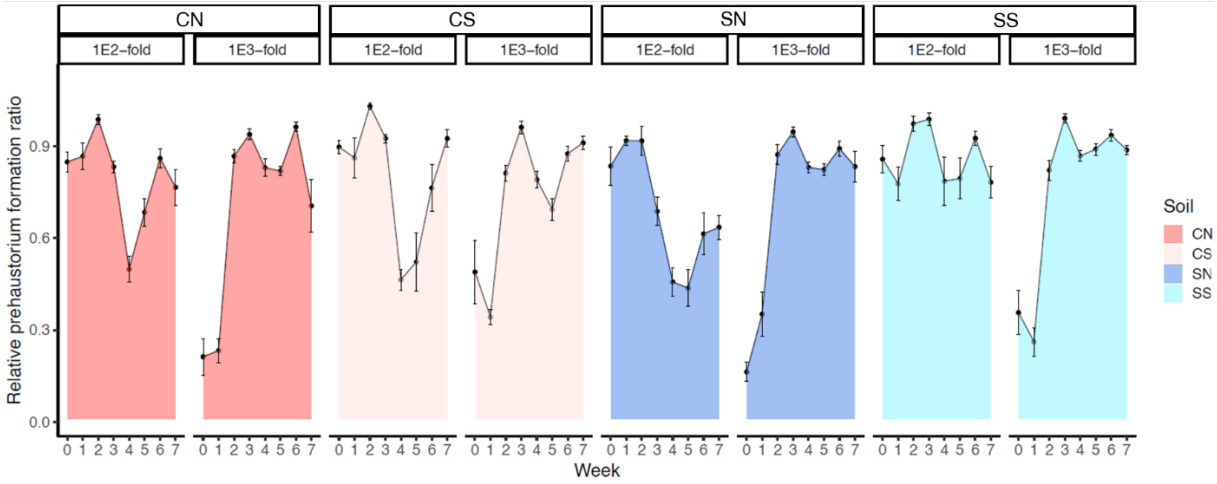


Fig. S8 Prehaustorium activity contained in co-culture rhizosphere exudates

Prehaustorium formation kinetics of germinated broomrape seeds treated with SPE-eluted fraction (10^2, 10^3 and 10^4-fold dilution) of rhizoshere exudates from native conducive (CN) and suppressive (SN) or sterilized conducive (CS) and suppressive (SS) during 7 weeks of co-cultivation with rapeseed. Prehaustorium formation ratios are approached by counting MTT colored germinated seeds presenting papillae at the apex, and reported as a relative ratio with minimum and maximum ratios obtained with positive (TDZ 10^{-8} M) and negative buffer control. Values displayed are means with standard errors as error bars.

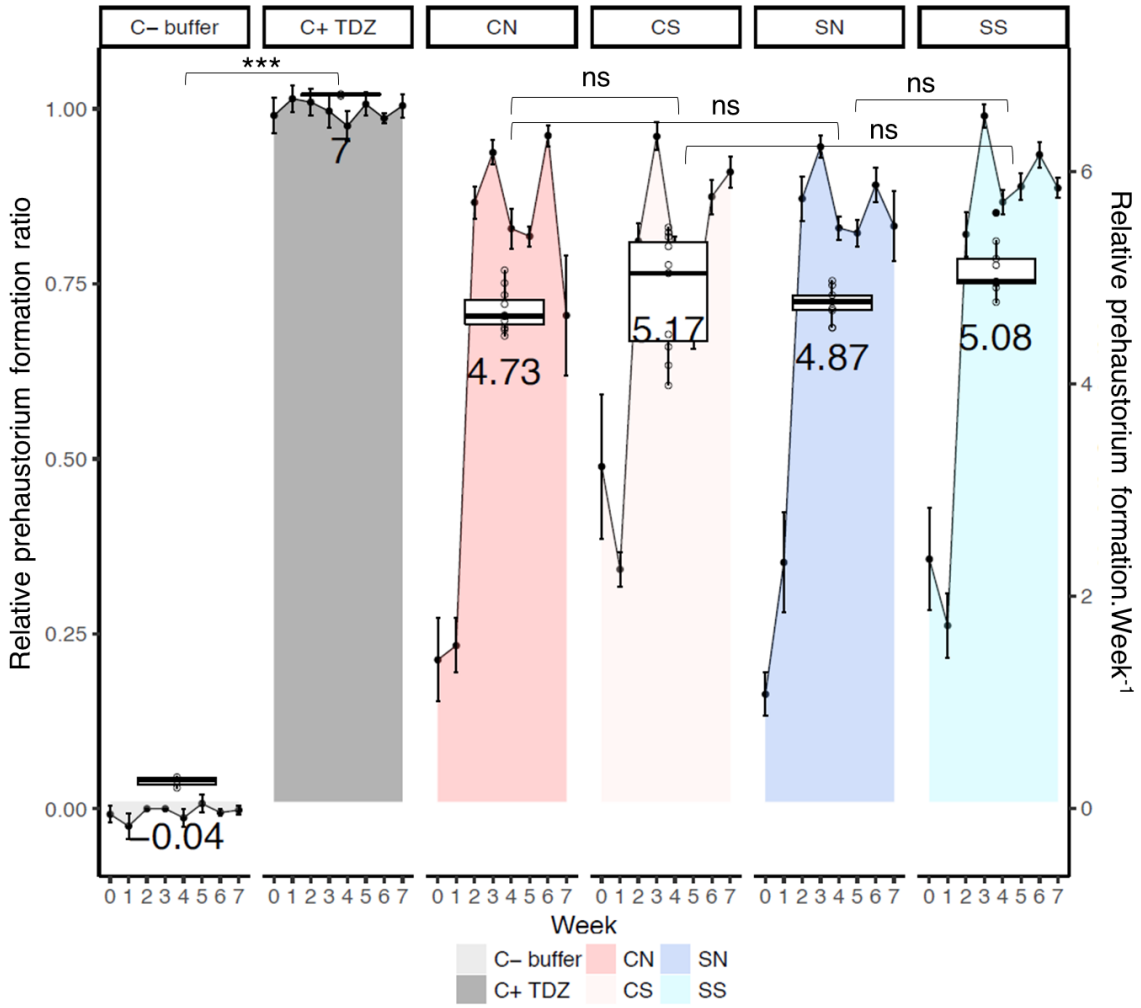
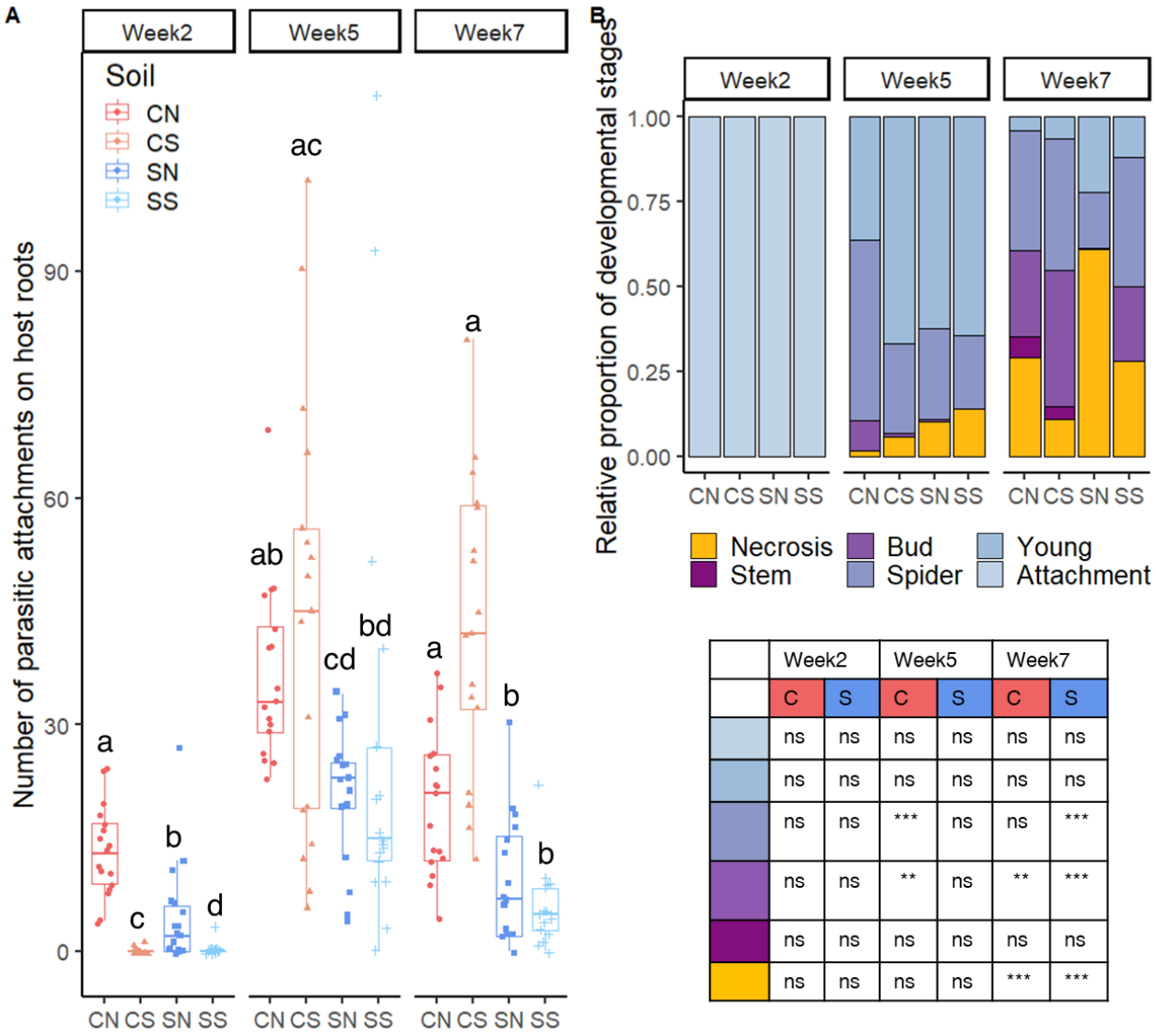


Fig. S9 Stimulatory effect of soil type on the HIF activity of rapeseed rhizosphere exudates

Prehaustorium formation kinetics of germinated broomrape seeds treated with positive control TDZ (10^{-8} M), negative control buffer (HEPES 1 mM; Plant preservative mixure 0.1% pH 7.5) and SPE-eluted fraction (10^{3}-fold dilution) of rhizosphere exudates from native conducive (CN) and suppressive (SN) or sterilized conducive (CS) and suppressive (SS) soils during 7 weeks of co-cultivation with rapeseed. Prehaustorium formation ratios were measured by counting MTT-colored seeds presenting papillae, and reported as relative ratios according to the minimum and maximum ratios obtained with the controls.

Global stimulatory effects were compared thanks the area under the curve (AUC), measured from the 7-week kinetic (Relative prehaustorium formation ratio.week^{-1}). Significance between AUC was determined thanks to a non-parametric Kruskall-Wallis rank test and Dunn's Kruskal-Wallis Multiple Comparisons (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001). Values displayed are means with standard errors as error bars.



	Week2		Week5		Week7	
	C	S	C	S	C	S
	ns	ns	ns	ns	ns	ns
	ns	ns	ns	ns	ns	ns
	ns	ns	***	ns	ns	***
	ns	ns	**	ns	**	***
	ns	ns	ns	ns	ns	ns
	ns	ns	ns	ns	***	***

Fig. S10: Assessment of soil effect on parasitic plant attachment and development

A Total number of *Phelipanche ramosa* attachments on roots of *Brassica napus* growing in co-cultures conducted in the native conducive (CN) or suppressive (SN) or gamma-sterilized conducive (CS) or suppressive (SS) soils on week 2, 5 or 7 after rapeseed sowing. Significance was determined using a pairwise comparisons on a generalized linear mixed model according to a Poisson distribution (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001). **B** Relative proportion of *P. ramosa* developmental stages for the counted attachments. Significance was determined using a pairwise comparisons on generalized linear mixed model via quasi-likelihood for each stage, and are reported in the table law (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001).

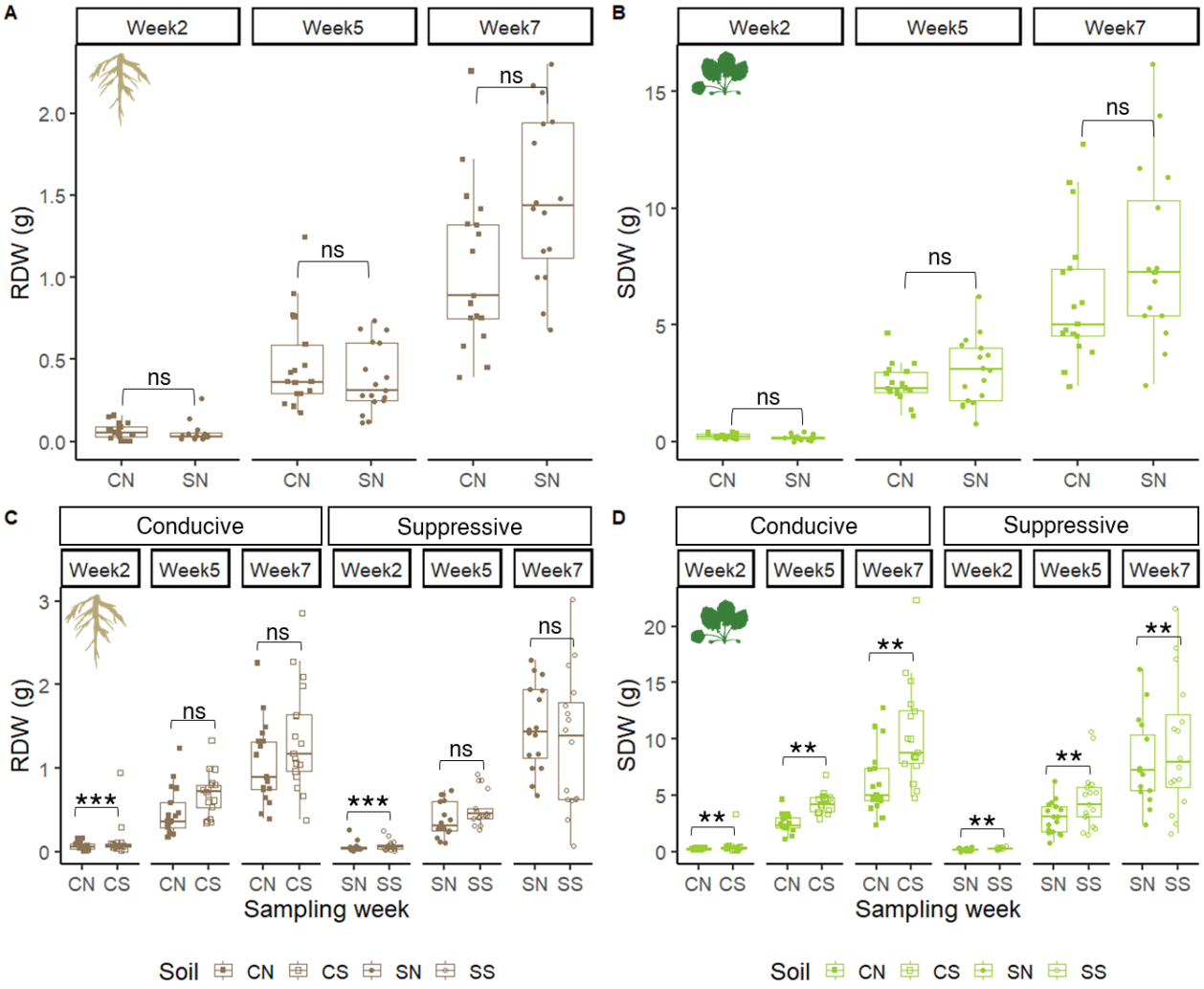


Fig. S11: Assessment of soil effect on host plant growth.

A Comparisons of *B. napus* root dry weight between native conducive (CN) and suppressive (SN) soils during co-cultures, after removal of soil particles as well as broomrape attachments, and incubation at 60°C for 72 h. **B** Comparisons of *B. napus* shoot dry weight between CN and SN soils during co-cultures, after removal of soil particles as well as broomrape attachments, and incubation at 60°C for 72 h. **C** Comparisons of root dry weight between native (CN, SN) and sterilized (CS, SS) soils within a single modality (conducive or suppressive) **D** Comparisons of shoot dry weight between native and sterilized soils within a single modality. Significance was determined using a pairwise comparisons on a generalized linear mixed model with a normal law and logarithmic transformation (ns: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001).

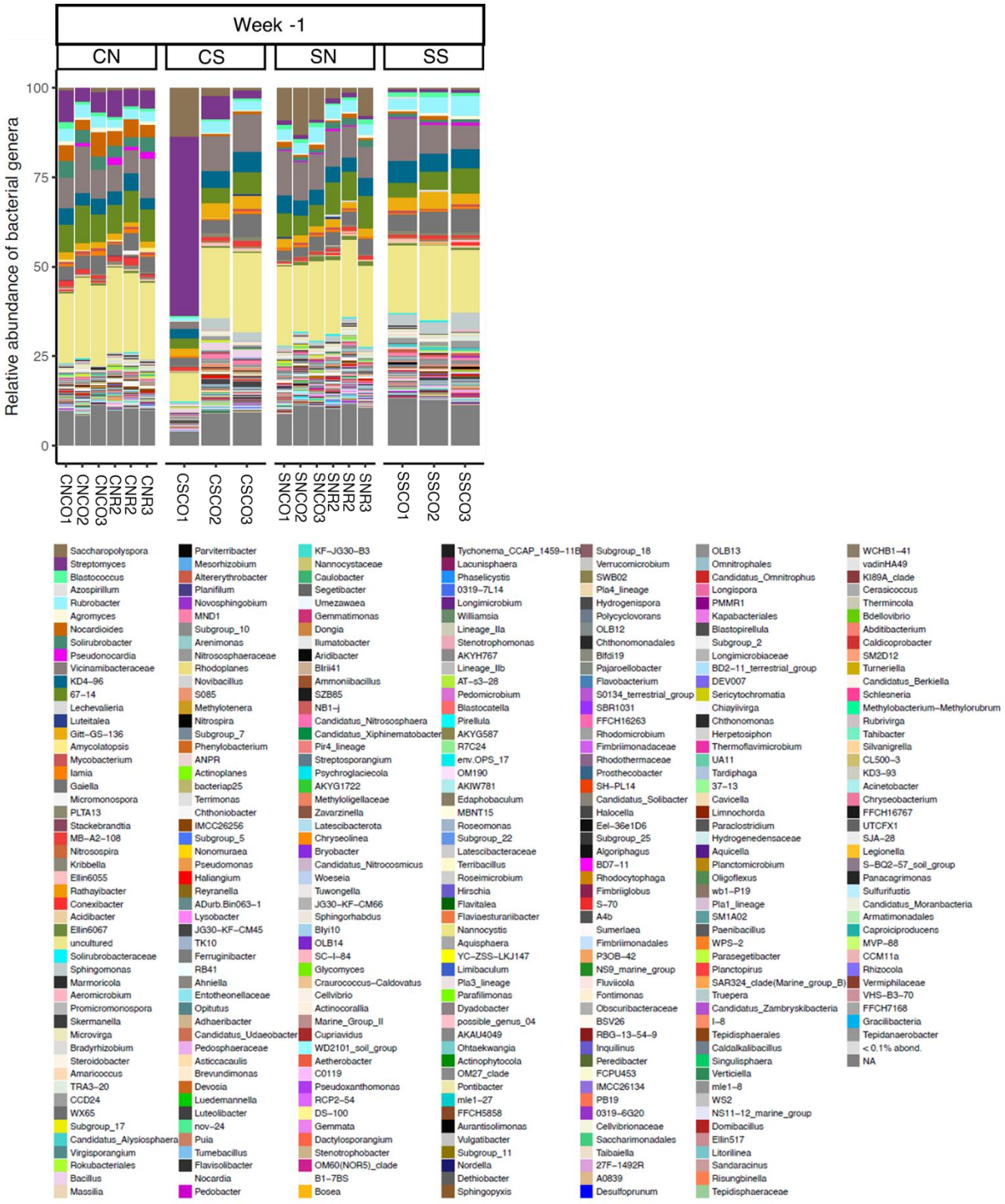


Fig. S12: Comparative description of bacterial communities between the two initial native and sterilized soils

Relative abundance of bacterial genera in the conducive native or sterilized soils (CN, CS) and suppressive native or sterilized soils (SN, SS) at the initial time (R1, R2, R3) and before every co-cultivation (CO1, CO2, CO3). Taxonomic assignations of 16S amplicon was conducted on the UNITE database. Taxa less than 0.1% abundant were grouped into a common class named “<0.1% abond.”, and taxa with no genus attribution were grouped into “NA”, not available

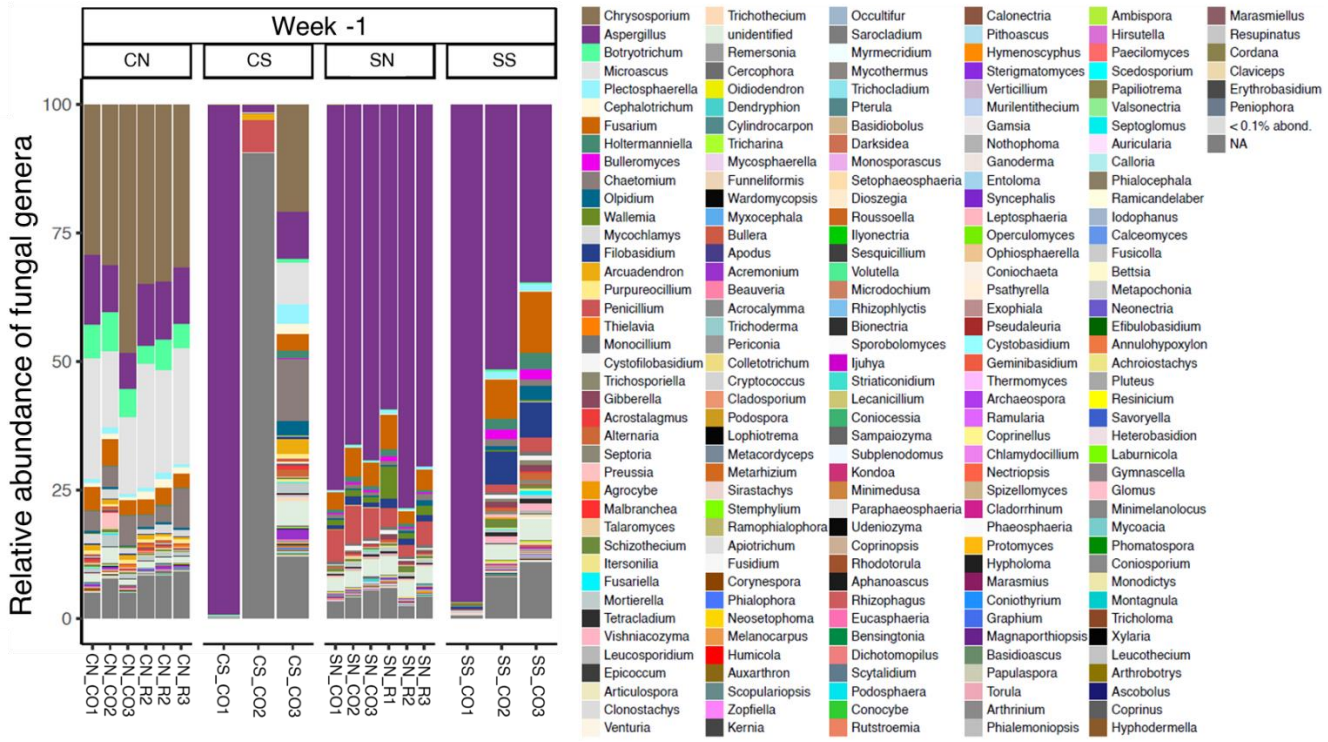


Fig. S13: Comparative description of fungal communities between the two initial native and sterilized soils

Relative abundance of bacterial genera in the conducive native or sterilized soils (CN, CS) and suppressive native or sterilized soils (SN, SS) at the initial time (R1,R2,R3) and before every co-cultivation (CO1, CO2, CO3). Taxonomic assignations of ITS amplicon was conducted on the SILVA database. Taxa less than 0.1% abundant were grouped into a common class named “<0.1% abond.”, and taxa with no genus attribution were grouped into “NA”, not available.

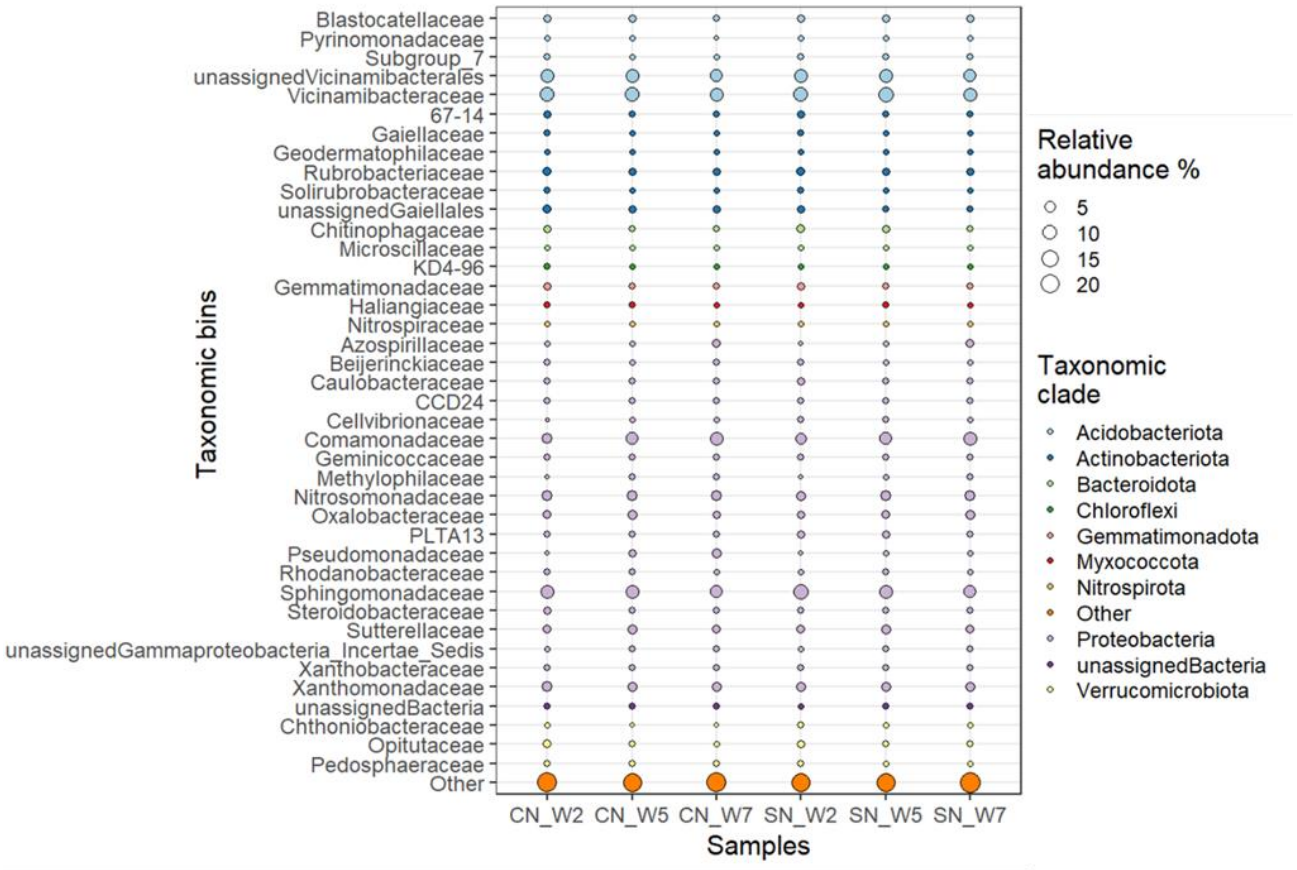
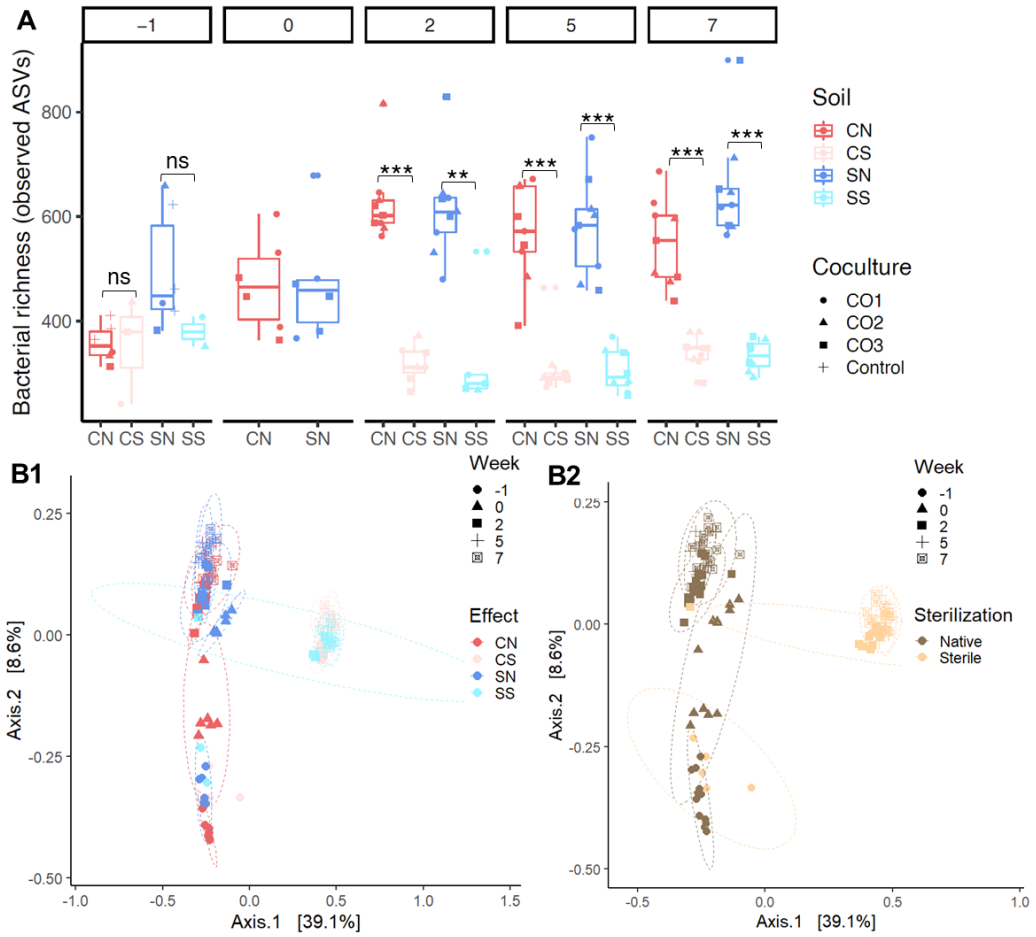


Fig. S14: Comparative description of rhizosphere bacterial communities during parasitism of branched broomrape on rapeseed in conducive or suppressive soils

Bubble plot showing the relative abundance of the different bacterial families and their corresponding taxonomic phylum present on the rhizosphere samples at weeks 2, 5 and 7 of co-cultures in the conducive (CN) and suppressive (SN) native soils. Only the 40 most abundant families are displayed, the rest are grouped in “Other” category. ASVs are coloured according to phylum assignation.



C

Permanova analysis on PCoA for 16S

Resultats	Df	SumOfSqs	R2	F	Pr..F.
Week	**4**	**6.6007784**	**0.1798736**	**12.422967**	**0.001**
Cocultivation	3	0.9150190	0.0249346	2.296143	0.007
Sterilization	**1**	**10.8395921**	**0.2953828**	**81.602433**	**0.001**
Sterilization:Cocultivation	7	1.0755477	0.0293091	1.156703	0.217
Week:Sterilization	3	2.1840331	0.0595157	5.480600	0.001
Cocultivation:Sterilization	2	0.7226593	0.0196927	2.720156	0.005
Week:Cocultivation:Sterilization	6	0.8100481	0.0220741	1.016365	0.402
Residuals	102	13.5490862	0.3692175	NA	NA
Total	128	36.6967640	1.0000000	NA	NA

Fig. S15: Comparative description of bacterial diversity between the four conducive and suppressive, native and sterilized soils during plant parasitism

Alpha and beta diversity were measured on rarefied samples (16,487 reads) and diversity metrics are presented at initial time (week -1: after sampling), after one week in the growth chamber with recirculation of watering medium (week 0) and during co-cultivation (week 2 to 7; host seed was sown in week 0) for native and sterilized conducive (CN, CS) or suppressive soils (SN, SS). **A** Estimation of bacterial richness approached by the Observed indice. Significance is estimated using two-sided Wilcoxon Rank Sum tests (*p < 0.05; ** p < 0.01; *** p < 0.001; ns non-significant). **B** Bacterial composition dissimilarities between soils measured with the Bray-Curtis indice and analyzed on principal coordinate analysis (PCoA). Dissimilarities are projected on axes 1 and 2 with total of 47.7% of inertia. **B1** Ellipses calculated on soil effect **B2** or on sterilization effect. **C** Factors analyzed thanks to Permutational Multivariate Analysis of Variance. High values of R2 (R-square) are highlighted in yellow and indicate the proportion of variation which is explained by the factor.

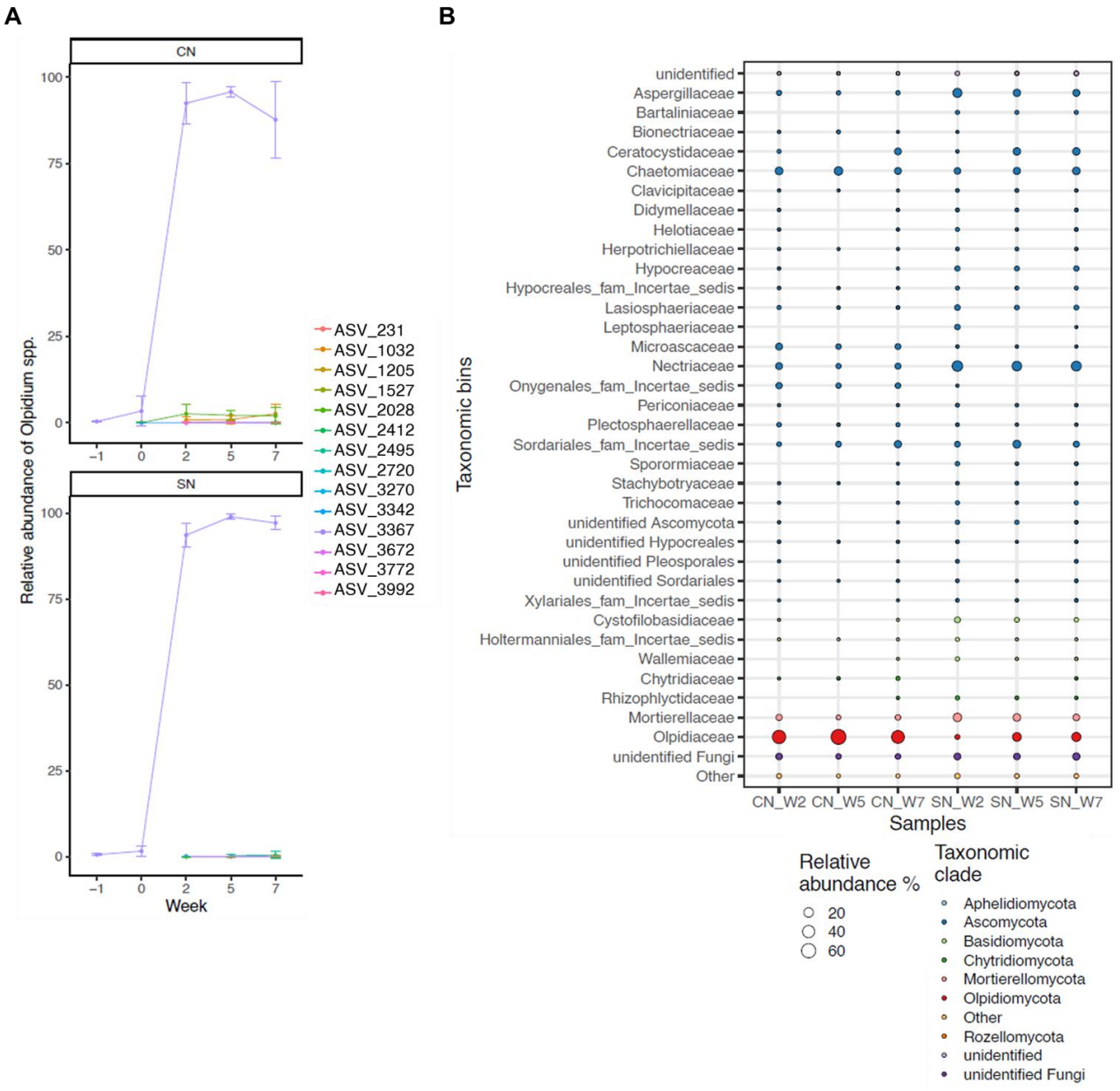
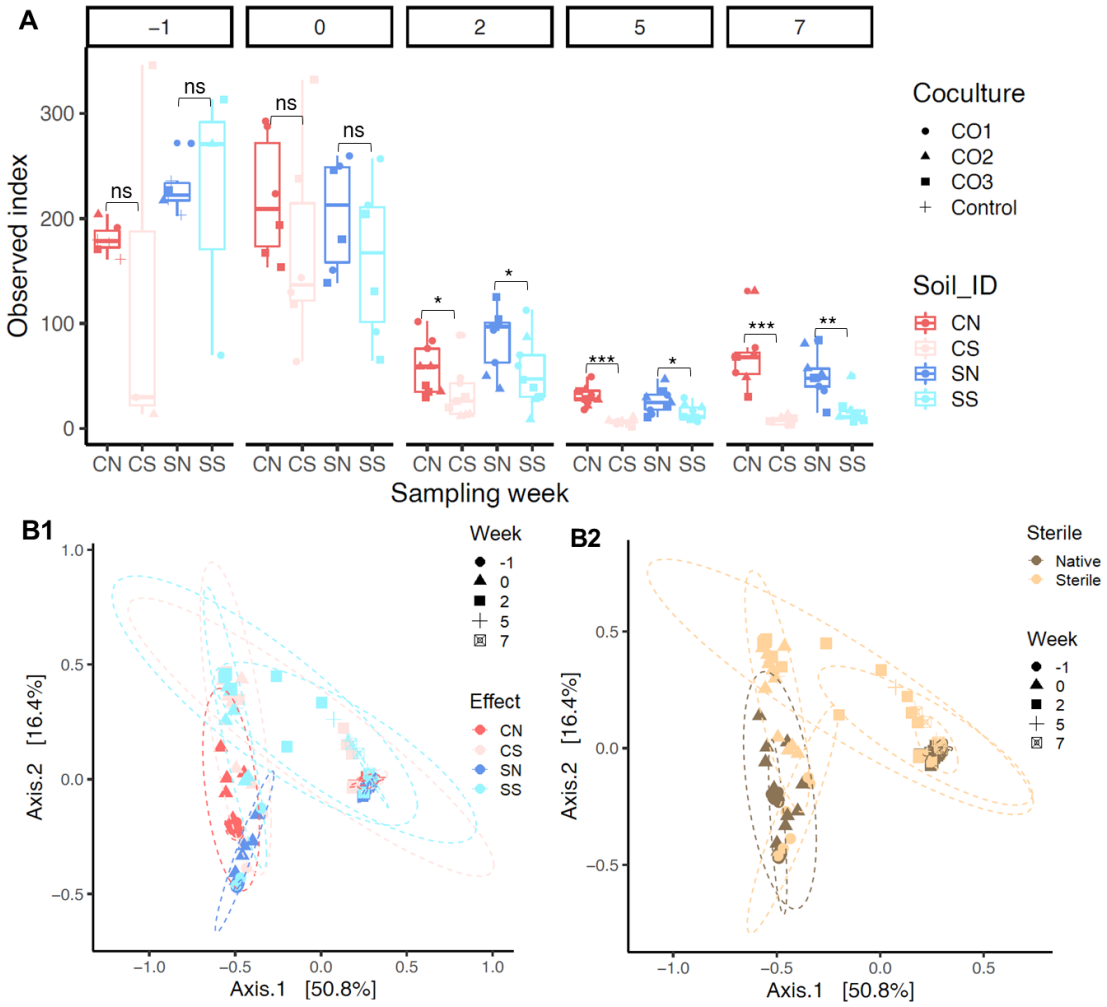


Fig.S16: Evolution of soil fungal communities in the conducive and suppressive soils during co-cultures without *Olpidium brassicae*

A Bubble plot showing the relative abundance of the different fungal families and their corresponding taxonomic phylum present on the rhizosphere samples at weeks 2, 5 and 7 of co-cultures in the conducive (CN) and suppressive (SN) native soils. ASV 3,771 corresponding to *Olpidium brassicae* was removed as it was > 90 % abundant. Only the 40 most abundant families are displayed, the rest are grouped in “Other” category. ASVs are coloured according to phylum assignation. **B** Evolution of the *Olpidium brassicae* ASVs in the whole samples (from soil sampling to week 7 of co-culture).



C

Permanova analysis on PCoA for ITS

Resultats	Df	SumOfSqs	R2	F	Pr..F.
Week	**4**	**18.2601594**	**0.4705749**	**43.753656**	**0.001**
Cocultivation	3	0.5592015	0.0144109	1.786557	0.072
Sterilization	1	1.9731123	0.0508483	18.911309	0.001
Week:Cocultivation	7	0.9325058	0.0240312	1.276801	0.155
Week:Sterilization	4	3.0557670	0.0787489	7.322005	0.001
Cocultivation:Sterilization	2	0.4324487	0.0111445	2.072404	0.064
Week:Cocultivation:Sterilization	7	1.0705343	0.0275883	1.465792	0.080
Residuals	120	12.5202060	0.3226530	NA	NA
Total	148	38.8039351	1.0000000	NA	NA

Fig.S17 Comparative description of fungal diversity between the four conducive and suppressive, native and sterilized soils during plant parasitism

Alpha and beta diversity were measured on rarefied samples (22,865 reads) and diversity metrics are presented at initial time (week -1: after sampling), after one week in the growth chamber with recirculation of watering medium (week 0) and during co-cultivation (week 2 to 7; host seed was sown in week 0) for native and sterilized conducive (CN, CS) or suppressive soils (SN, SS). **A** Estimation of bacterial richness approached by the Observed indice. Significance is estimated using two-sided Wilcoxon Rank Sum tests (*p < 0.05; ** p < 0.01; *** p < 0.001; ns non-significant). **B** Bacterial composition dissimilarities between soils measured with the Bray-Curtis indce and analyzed on principal coordinate analysis (PCoA). Dissimilarities are projected on axes 1 and 2 with total of 65.2% of inertia. **B1** Ellipses calculated on soil effect **B2** or on sterilization effect. **C** Factors analyzed thanks to Permutational Multivariate Analysis of Variance. High values of R2 (R-square) are highlighted in yellow and indicate the proportion of variation which is explained by the factor.

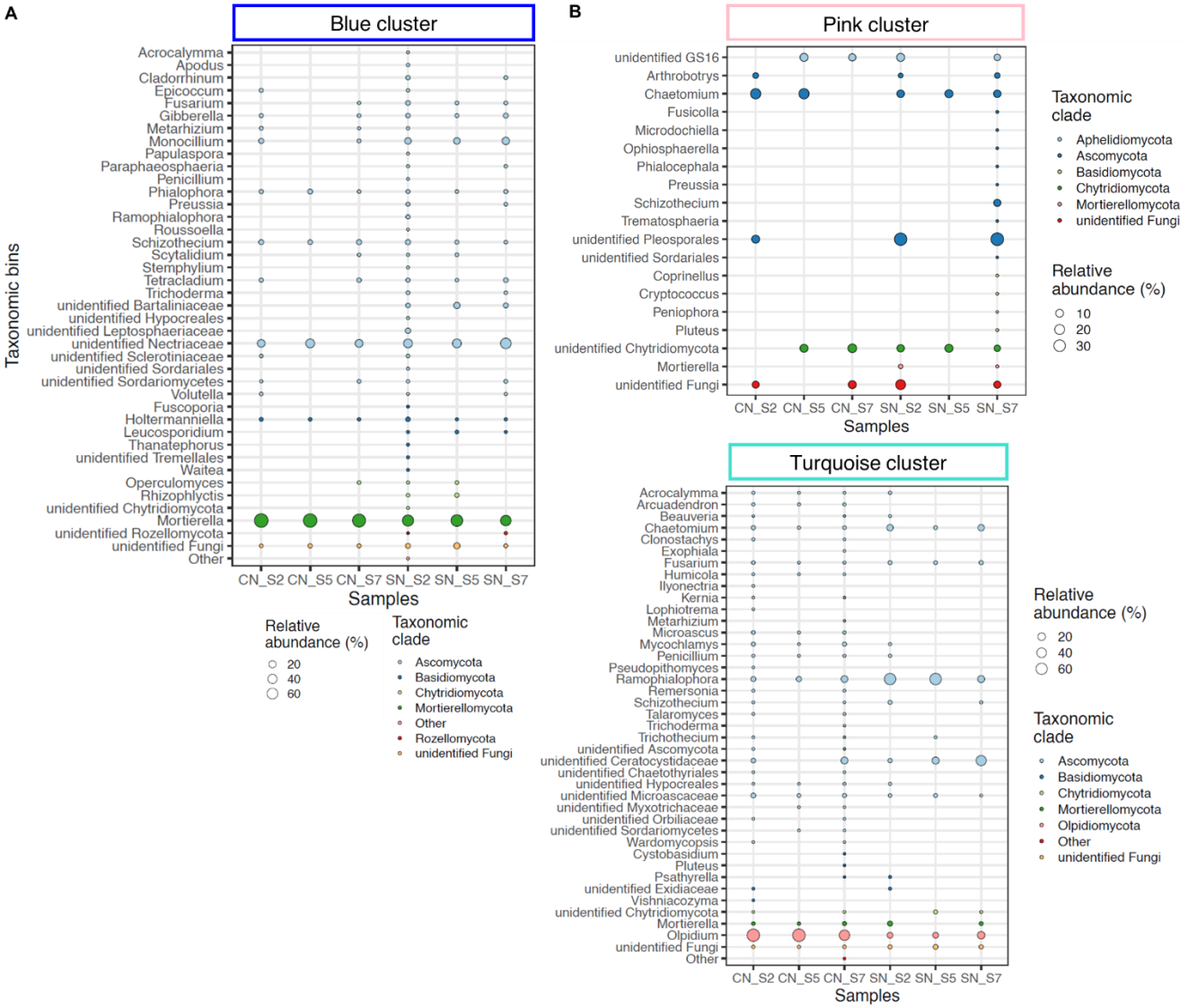


Fig.S18 Abundance evolution of fungal genera from WGCNA clusters during co-cultivation experiment in CN or SN soils

A, B and **C** are bubble plots which display the mean relative abundance (over six replicates) of genera constituting the ASV found in the Blue (81 ASVs), Pink (38 ASVs) and Turquoise (83 ASVs) clusters. These three clusters are correlated to suppressive parasitic traits, *i.e.* reduction of broomrape attachment for Blue and development of necrosis for Turquoise and Pink.