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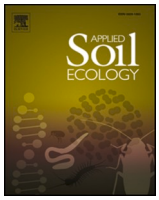

Research paper

Manure amendments and fungistasis, and relation with protection of wheat from *Fusarium graminearum*

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ABSTRACT

Certain soils promote crop health because they are pathogen-suppressive (i.e., fungistatic) or disease-suppressive, but the effect of soil management on these properties is not fully understood. Here, we tested the hypothesis that manure could favor fungistasis by screening 26 manured or non-manured wheat fields from Serbia for their ability to control survival/growth of the fungal plant pathogen *Fusarium graminearum* Fg1. Quantitative PCR showed that the pathogen grew after inoculation in all 26 autoclaved soils. In absence of autoclaving, the pathogen was stable or grew in 16 soils (37 % manured) but declined in the 10 others (70 % manured). For most soils, there was no significant link between soil chemistry and fungistasis, except with Mionica in western/central Serbia. Mionica soils MI2 and MI3, which had received manure, exhibited higher levels of organic matter and potassium compared with soils MI4 and MI5, which had not received manure and were non-fungistatic. Using Mionica soils, we then tested the hypothesis that fungistatic (manured) soils rather than non-fungistatic (non-manured) soils would protect wheat from *F. graminearum* disease. Indeed, fungistatic soils were suppressive to wheat damping-off. Non-fungistatic soil MI4 was conducive, as expected, but non-fungistatic soil MI5 turned out to be suppressive. Metabarcoding showed that the structure of prokaryotic and fungal rhizosphere communities depended mostly on field location, with a significant effect of *F. graminearum* inoculation. In conclusion, our findings show that certain farming practices (here, manure amendments) may promote soil fungistasis towards *F. graminearum*. However, both fungistatic and non-fungistatic soils can be suppressive to *F. graminearum* disease in wheat, and their differences in rhizosphere microbiota suggest different phytoprotection mechanisms.

1. Introduction

Soil hosts a diversified community of microorganisms, which present beneficial, detrimental, or neutral effects on plants (Berendsen et al., 2012; Vacheron et al., 2013). The resulting impact on plant health and performance depends on multiple microbe-microbe and plant-microbe interactions. Within the complex rhizosphere ecosystem, these multiple interactions may lead to effective plant protection, despite the presence of virulent pathogen(s) and environmental conditions favorable for disease development. In soils where this emerging property takes place, plants exhibit limited or no disease symptoms and such soils are termed disease-suppressive soils (Alabouvette, 1986; Hornby, 1983; Raaijmakers et al., 2009; Schlatter et al., 2017; Mitsuboshi et al., 2018).

Practically speaking, disease suppressiveness refers to the inherent ability of certain soils to actively restrict the population size, physiological activity, or negative effects of microbial phytopathogens. While several studies attributed suppressiveness to particular soil physico-chemical properties (Stotzky and Torrence Martin, 1963; Almario et al.,

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2014), the soil microbiome plays a prominent role (Mazurier et al., 2009; Almario et al., 2014; Ossowicki et al., 2020), which may be reflected by differences in soil microbiota composition between disease-suppressive soils and their conducive counterparts (Kyselková et al., 2009; Ossowicki et al., 2020).

In disease-suppressive soils, plant-protecting soil microorganisms inhibit pathogens directly, through competition or antagonism, or indirectly by stimulating other plant-associated microorganisms or inducing plant immune responses (Mazzola, 2002; Raaijmakers et al., 2009). Often, these interactions take place in the rhizosphere, and thus the analysis of disease-suppressiveness has focused on rhizosphere interactions (Almario et al., 2014; Ossowicki et al., 2020). However, specific plant-beneficial interactions can also be driven from the bulk soil, thereby reducing saprophytic survival of pathogens (Leplat et al., 2013; de Boer et al., 2019; Legrand et al., 2019), a property often referred to as fungistasis in the case of fungal pathogens (Garbeva et al., 2011; Sipilä et al., 2012; Legrand et al., 2019). Certain agricultural practices such as organic amendments can impact the soil microbiota, with the potential to influence both soil fungistasis and disease suppressiveness (Cuesta et al., 2012; Mousa and Raizada, 2016; Bonanomi et al., 2017; De Corato, 2020). Indeed, manure application can influence soil structure, brings allochtonous microorganisms into soil, provides a range of organic substrates that may modify survival of both soil-borne pathogens and plant-beneficial microorganisms, and is likely to influence soil microbial diversity (Legrand et al., 2018a, 2019; Tang et al., 2023; Tao et al., 2020; Todorović et al., 2023). Arguably, the effects of both fungistasis and rhizosphere-based disease-suppressiveness can be expected to add up in terms of phytoprotection efficacy, but these two aspects have rarely been considered together.

In this work, we focused on *Fusarium graminearum*, a pathogen causing a range of diseases at different stages of wheat development, including damping-off disease early on, root and collar rot, and later Fusarium Head Blight (Leslie and Summerell, 2006; Goswami and Kistler, 2004). Interestingly, soils suppressive to *Fusarium* diseases have been evidenced in different geographic regions and *Fusarium* pathosystems (Ossowicki et al., 2020; Siegel-Hertz et al., 2018; Todorović et al., 2023), and *F. graminearum* can also be strongly affected by fungistasis (Legrand et al., 2019). Microorganisms antagonistic to this pathogen have been described (Legrand et al., 2017; Besset-Manzoni et al., 2019).

Here, we tested the hypotheses that soil fungistasis is associated with specific soil physico-chemistry properties, can be enhanced by manure amendments, and may promote rhizosphere-based disease-suppressiveness. We screened 26 agricultural soils from five locations in Serbia, which represent different soil types (chernozems, pseudogleys, eutric cambisols, and vertisols), and comprise manured and non-manured soils at each location, for their ability to inhibit the development of *F. graminearum*. After characterizing fungistatic and non-fungistatic soils, contrasted soils from one location were then assessed for suppressiveness of *Fusarium graminearum*-mediated damping-off of wheat and compared based on fungal and bacterial microbiota diversity in the wheat rhizosphere.

2. Material and methods

2.1. Soil sampling

Soil sampling was conducted in 26 agricultural fields from five locations of Serbia, i.e., Sombor (SO) and Novi Karlovci (NK) in northern Serbia, and Valjevo (VA), Mionica (MI), and Čačak (CA) in western/central Serbia (Table 1; Fig. 1A). Some fields received manure amendments regularly but others did not, and all soils were managed with tillage, fertilizers and pesticides (Table 1). In all fields, wheat was predominant within the crop rotation (Table 1). In each field, 6 areas at intervals of 10 m were sampled in October 2020 (no crop present). The top few centimeters of soil were carefully removed, and soil samples were collected at a 5–20 cm depth. These individual subsamples were then combined into one composite sample per field. The composite soils samples were then sieved (0.5 cm), and stones, roots and other organic material were removed. Soil was also collected in June 2021 to perform plant testing using four fields in Mionica, i.e., MI2 (planted with maize), MI3 and MI5 (planted with wheat), and MI4 (meadow) (Table 1).

2.2. Soil physicochemical analysis

Soil physicochemical analysis was carried out at the Fruit Research Institute in Čačak (Serbia), using 500-g samples. Mechanical properties of the soils were determined by dry sieving procedures, disaggregation with 4 % $Na_4P_2O_710H_2O$ and the pipetting method. Cation exchange capacity (CEC; cmol/kg) and CEC saturation (%) were determined using the Kappen method. Agrochemical soil determinations included pH (measured in H_2O) and soil contents in humus (determined using the Kotzmann method; expressed as %), organic matter (determined by combustion; %), total nitrogen (recalculated from humus content; %), readily-available phosphorus (extracted with ammonium lactate; mg/kg), readily-available potassium (extracted with ammonium lactate; mg/kg), and total iron (determined by HCl:HNO_3 extraction at a ratio of 1:3; %).

2.3. Preparation of fungal mycelia and spore suspension

The virulent strain *Fusarium graminearum* MDC_Fg1 (hereafter Fg1), obtained from contaminated cereals in northern France (Alouane et al., 2018), was used in the experiments. To obtain mycelia for the fungistasis experiment, the fungus was grown for eight days at 20–22 °C on Potato Dextrose Agar (PDA; Conda Pronadisa, Madrid, Spain). To prepare the inoculum, a protocol adapted from Legrand et al. (2019) was followed. Maize grains were soaked in water at 22 °C for 72 h. They were then ground to Ø 1–2 mm and put into 2-l Erlenmeyer flasks, which were autoclaved two times for 20 min at 121 °C with a 24-h interval. After autoclaving, inoculation was done with 7-mm-diameter plugs taken from the edge of 8-day-old PDA cultures of *F. graminearum* Fg1. The flasks were incubated 10 days at 22 °C with vigorous shaking for 5 min once a day to promote kernel colonization, and colonized kernels were used as an inoculum.

To obtain spore suspension used in the damping-off experiment, we employed Mung Bean Broth (MBB) (Evans et al., 2000), which was prepared by adding 40 g of organic mung bean seeds in 1 l of boiling water and leaving to infuse and cool down for 10 min. After that, beans were discarded and 50 ml of the resulting medium was poured into 250-ml Erlenmeyer flasks and autoclaved for 20 min at 121 °C. MBB (50 ml) was inoculated with ten 7-mm-diameter plugs taken from the edge of a 8-day-old *F. graminearum* Fg1 PDA plate and incubated for six days at 22 °C and 180 rpm agitation (Incubator Shaker Series I26, New Brunswick Scientific, Edison, NJ, USA). On the sixth day, the preculture was diluted one-tenth with fresh MBB and incubated under the same conditions for 10 additional days. The culture was filtered using sterile Miracloth to discard mycelium and centrifuged for 10 min at 4700 *g* (Avanti J-E Series, Beckman Coulter, Fullerton, CA, USA) at room temperature. The resulting pellet was washed twice with sterile water. Titration of spores in the suspension was performed using a Thoma counting chamber.

2.4. Evaluation of soil fungistasis to Fusarium graminearum Fg1

The fungistatic status of each soil was assessed using a protocol adapted from Legrand et al. (2019). In brief, prior to inoculation, four 1-g autoclaved and four 1-g non-autoclaved samples of each soil, as well as four 1-ml aliquots of *F. graminearum* Fg1 inoculum were collected and stored at −20 °C before quantifying *F. graminearum*. The experiment was done in 20-ml vials containing 15 g soil, which were autoclaved (for 20 min at 121 °C on two consecutive days) or not, and then inoculated (600

Table 1
Location, field ID, type and quantity (t/ha) of manure, recent cropping field history, use of fertilizers and pesticides, postharvest residues management, field symptoms of Fusarium Head Blight of wheat, soil type and GPS coordinates of 26 Serbian soils.

Location	Field ID	Type of animal manure	Manure quantity	Recent cropping history [b]	Use of fertilizers	Use of pesticides	Use of fungicides	Postharvest residues management	Recent wheat fusariosis observations	Soil type [c]	GPS coordinates
Mionica	MI2	Sheep	80 t/ha	**Alfalfa**-wheat-maize-wheat-maize	Yes	Yes	No	Ploughing	No	Vertisol	44.24611 N 20.10431 E
	MI3	Sheep	80 t/ha	**Sunflower**-wheat-maize-wheat-maize	Yes	Yes	No	Ploughing	No	Vertisol	44.24540 N 20.10350 E
	MI4	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Vertisol	44.24745 N 20.10012 E
	MI5	–	–	**Meadow**-wheat-maize-wheat-maize	Yes	Yes	No	Ploughing	No	Vertisol	44.24759 N 20.09931 E
Valjevo	VA1	Sheep, cattle and chicken	70–80 t/ha	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Eutric cambisol	44.33050 N 19.968102 E
	VA2	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Burning	No	Eutric cambisol	44.330491 N 19.966663 E
	VA3	–	–	**Oat**-wheat-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Eutric cambisol	44.330466 N 19.969106 E
	VA4	Sheep, cattle and chicken	70–80 t/ha	**Maize**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Eutric cambisol	44.330110 N 19.968102 E
	VA5	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing and burning	No	Pseudogley	44.351892 N 19.981415 E
	VA6	Cattle	80 t/ha	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing and burning	No	Pseudogley	44.351155 N 19.978144 E
	VA7	Cattle	80 t/ha	**Maize**-wheat-maize-wheat-maize	Yes	Yes	No	Ploughing	No	Pseudogley	44.355395 N 19.977465 E
	VA8	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Pseudogley	44.355012 N 19.977650 E
Novi Karlovci	NK1	Cattle	14.5 t/ha	**Wheat**-maize-sunflower-beetroot	Yes	Yes	Yes	Ploughing	No	Chernozem	45.060182 N 20.215013 E
	NK2	Cattle	14.5 t/ha	**Wheat**-maize-sunflower-beetroot	Yes	Yes	Yes	Ploughing	No	Chernozem	45.060066 N 20.215213 E
	NK3	–	–	**Wheat**- sunflower-beetroot-maize-beetroot	Yes	Yes	Yes	Ploughing	No	Chernozem	45.088806 N 20.102067 E
	NK4	–	–	**Wheat**- sunflower-beetroot-maize-beetroot	Yes	Yes	Yes	Ploughing	No	Chernozem	45.088011 N 20.099312 E
Sombor	SO1	–	–	**Wheat**-soybean-maize	Yes	Yes	Yes	Ploughing	Yes	Chernozem	45.758696 N 19.1840320 E
	SO2	–	–	**Wheat**-soybean-maize	Yes	Yes	Yes	Ploughing	Yes	Chernozem	45.746168 N 19.159358 E
	SO3	a	35 t/ha	**Wheat**-maize-wheat-maize	Yes	Yes	Yes	Ploughing	Yes	Chernozem	45.750012 N 19.170019 E
	SO4	a	35 t/ha	**Wheat**-maize-wheat-maize	Yes	Yes	Yes	Ploughing	Yes	Chernozem	45.750839 N 19.172977 E
Čačak	CA1	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	Yes	Vertisol	43.89897 N 20.54435 E
	CA2	Cattle	30–40 t/ha	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Vertisol	43.89910 N 20.54450 E
	CA3	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	Yes	Vertisol	43.89905 N 20.54312 E
	CA4	Cattle	30–40 t/ha	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	No	Vertisol	43.89930 N 20.54315 E
	CA5	–	–	**Wheat**-maize-wheat-maize-wheat	Yes	Yes	No	Ploughing	Yes	Vertisol	43.8867833 N 20.5462167 E
	CA6	Cattle	30–40 t/ha	**Maize**-wheat-maize-wheat-maize	Yes	Yes	No	Ploughing	No	Vertisol	43.8878667 N 20.5475167 E

[a] Type of animal manure is not known.
[b] Soil for fungistasis tests was taken in October 2020 (no crop present), and soil for plant tests was taken in June 2021 at Mionica (maize in MI2, wheat in MI3 and MI5, meadow in MI4).
[c] Based on Tanasijević et al. (1964) and Nejgebauer et al. (1971).

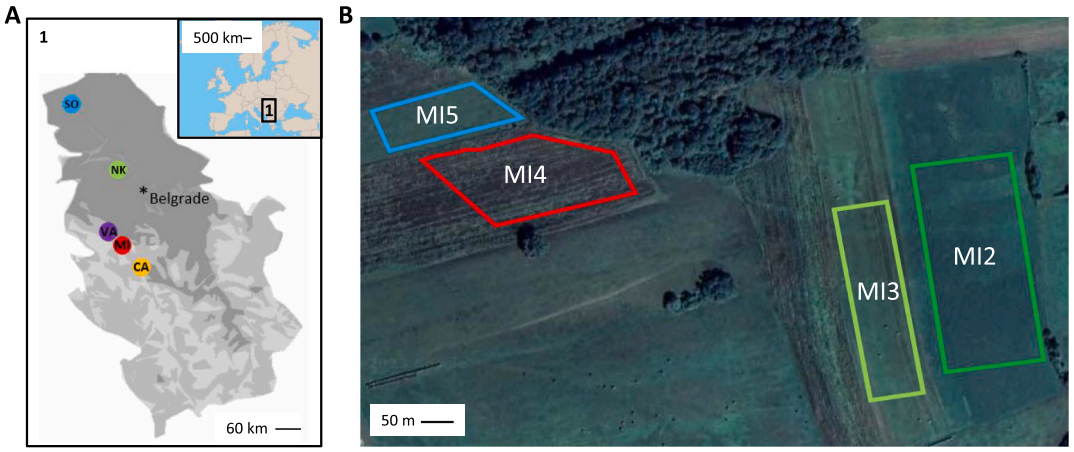

Fig. 1. Locations where soils were sampled in Serbia. A. Five locations in Serbia, i.e., Sombor (SO), Novi Karlovci (NK), Valjevo (VA), Mionica (MI) and Čačak (CA) where the 26 fields were sampled. **B.** Aerial picture of the four sampling fields MI2, MI3, MI4 and MI5 at Mionica, visualized in Google Maps [Map data ©2023, Google].

µl of mycelia inoculum) or not (600 µl of sterile water), giving for each of the 26 soils (i) 4 inoculated, autoclaved vials, (ii) 4 inoculated, non-autoclaved vials, and (ii) 4 non-inoculated, non-autoclaved vials, i.e., $26 \times (4 + 4 + 4) = 312$ vials. The vials were arranged following a randomized block design and incubated in the dark at 60 % air humidity and 20 °C. Every three days, vials were weighted to estimate water loss, and the corresponding amount was added back. At 15 days (Legrand et al., 2019), all soil samples were lyophilized (Lyophilizator, Alpha 1-4LSC, Christ, Germany) for 48 h, 1 g soil was sampled from each vial and stored at −20 °C until DNA extraction.

Total DNA was extracted from 0.5 g soil for each of the 520 samples (208 samples before inoculation and 312 samples at 15 days) and the Fg1 inoculum added, using FastDNA Spin Kit for Soil (MP Biomedicals, Illkirch-Graffenstaden, France), according to manufacturer's instructions. Using primers Fg16N-F/Fg16N-R, *F. graminearum* DNA was quantified by qPCR using a CFX-96TM Real-Time PCR System (Bio-Rad, Hercules, CA, USA), as previously described (Legrand et al., 2018b; Nicholson et al., 1998 and Supplementary Data). Obtained number of copies.µl^{-1} were transformed into number of copies.g^{-1} soil and normalized to the total DNA quantity extracted from 0.5 g of soil and expressed as a number of copies.g^{-1} dry soil, as previously done (Bouffaud et al., 2016). The amount in the Fg1 inoculum was calculated for 1 ml (same calculation as for 1 g of soil), extrapolated to the 600 µl used to inoculate 15 g of soil, and expressed per g of soil. This amount was subtracted from the DNA quantity found in each sample of 1 g of soil. All results were $\log_{10}$-transformed for subsequent analysis. Mean values and standard deviation were calculated. The fungistasis level was calculated according to the formula: $\Delta day15 = \log_{10}$(Fg1 DNA in soil at 15 days after inoculation) - $\log_{10}$(Fg1 DNA in the inoculum).

2.5. Wheat damping-off suppressiveness assay

The wheat damping-off suppressiveness assay with *F. graminearum* Fg1 was conducted with the four MI soils (Fig. 1B), in a plant growth chamber (FitoClima, 10.000 EH, ARALAB, Rio de Mouro, Portugal) with 16 h of day at 20 °C, 8 h dark period at 18 °C and a relative humidity of 80 %. Soils had been collected in June 2021. For each soil, 100 seeds of winter wheat (*Triticum aestivum*) variety Récital were distributed into 20 pots (height 12 × 10 × 10 cm; 5 seeds per pot) filled with 250 g of soil mixed with sterile siliceous sand (granulometry 0.6–1.6 mm, Gedimat, Dagneux, France; autoclaved twice, at 24 h interval) in a 50:50 ratio. In 10 pots, the seeds were inoculated with 100 µl of spore suspension (10^6 spores per seed), while seeds in the other 10 pots received 100 µl of water (control). The experiment followed a randomized block design with 10 blocks ($n = 10$). The plants were watered every 3 days by adding water under each pot, to maintain water content close to 21 % *w*/w.

The number of germinated seeds was recorded at 14 days, and (i) the number of plants alive, (ii) shoot length (cm), (iii) dry shoot biomass (mg), and (iv) dry shoot density (i.e., shoot length divided by dry shoot biomass; mg/cm) were measured at 28 days.

At 28 days, six blocks were used to sample the root system of one plant per pot. Loosely-adhering soil was discarded by shaking. Roots and tightly-adhering rhizosphere soils were frozen in liquid nitrogen, lyophilized for 48 h and then stored at −20 °C. Root-adhering soil was mechanically separated (using sterile tweezers) and 0.5 g of soil was used for DNA extraction with the FastDNA SPIN kit for Soil and the FastPrep instrument (MP Biomedicals), following manufacturer's instructions. DNA was eluted in 80 µl DNase-free water and quantified using Qubit dsDNA High sensitivity Assay Kit with an Invitrogen Qubit 4.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) for low DNA concentrations. DNA quality was assessed using a UV spectrophotometer (NanoPhotometer NP80, Implen, Munich, Germany).

2.6. 16S rRNA gene and ITS sequencing from rhizospheric DNA

16S rRNA gene and ITS sequencing using an Illumina platform was performed on rhizospheric DNA from MI2, MI4 and MI5 inoculated and non-inoculated samples from the disease-suppressiveness experiment. Bacterial diversity in rhizosphere ($n = 6$ samples) was assessed by Illumina MiSeq sequencing (2 × 250 bp) of V3-V4 region of the 16S rRNA gene using primers Uni341F and Uni806R (Yu et al., 2005; Caporaso et al., 2011; Sundberg et al., 2013). The purification of PCR products, the construction of amplicon libraries and Illumina MiSeq sequencing with 2 × 250 bp paired-end reads were carried out by Novogene (Cambridge, UK; see Table S1 and Supplementary Data for further detail).

The fungal ITS2 region was amplified using the primers fITS7/ITS4 (Gardes and Bruns, 1993; Ihrmark et al., 2012). Primers were equipped with Illumina adaptors (Nextera XT Index Kit, Illumina). To obtain high-fidelity amplification, PCR was performed using Kapa Hifi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA). Construction of amplicon libraries and paired-end Illumina MiSeq sequencing (2 × 300 bp) were performed at the Department of Soil Ecology, UFZ-Helmholtz Centre for Environmental Research in Halle (Saale, Germany) (for further detail, see Table S1 and Supplementary Data).

2.7. Sequence data processing

Amplicon sequencing datasets from 16S rRNA gene and ITS were handled independently. Sequences from the 16S rRNA gene dataset were processed and classified using the R package DADA2 (Divisive Amplicon

Denoising Algorithm) v.1.12.1 pipeline (Callahan et al., 2016). Using the "FilterAndTrimmed" function, quality filtering and trimming stages were executed. Reads shorter than 100 bp were removed, allowing two errors per read. ITS sequences were processed using dadasnake v.10 (Weißbecker et al., 2020; https://github.com/a-h-b/dadasnake), with the DADA2 package in R (v.3.6.1; Callahan et al., 2016). Only reads with the expected amplification primers were kept, and primer sequences were cut using cutadapt v.1.18 (Martin, 2011). The amplicon reads were truncated to a minimum base quality of 7, with a minimum length of 70 nucleotides for the forward and reverse reads. For both datasets, read pairs were merged with zero mismatches, and exact sequence variants were determined as ASVs (Amplicon Sequence Variants). Chimeric reads were removed using the DADA2 "consensus" algorithm. For the 16S rRNA gene dataset, the ASVs were assigned taxonomically using the SILVA database v.138 (Quast et al., 2013), while the UNITE database v.9 (Abarenkov et al., 2022) was used to assign the ITS2 gene amplicon sequences taxonomically using the mothur implementation of the Bayesian Classifier (Schloss et al., 2009). During this process, any unclassified ASVs and those identified as chloroplasts, mitochondria, or eukaryotes in the 16S rRNA gene sequences were excluded from the analysis. The phyla nomenclature was maintained as suggested by the Silva database v.138 (Quast et al., 2013). The 16S rRNA gene primers have been designed to target both the archaeal and bacterial domains; hereafter, we refer to this subset of the microbiota as the prokaryotic community. For ITS, all ASVs assigned to Fungi were kept. In both datasets, the rarefaction curves tended to reach a plateau, indicating that the sequencing method supplied sufficient sequences to cover most of the diversity (Fig. S1). Prokaryotic and fungal taxa were identified at the genus level when possible, otherwise at family or order level.

2.8. Statistical analyses

All the data were analyzed at $P < 0.05$, using the R v.4.2.1. software (https://www.r-project.org). The relationships among soil samples based on their physicochemical composition were assessed with Non-metric MultiDimensional Scaling (NMDS) using vegan package (Oksanen et al., 2022). The data were centered and scaled, Euclidean distances were used as distance metric and two dimensions were kept for ordination with NMDS. The stress value was <0.1. Fitting variables into NMDS plot and testing their significance were done with the envfit function.

For qPCR data, outliers were detected using the Grubbs' test (Grubbs, 1969; Burns et al., 2005) and discarded. This comprised one replicate from soil CA1, one from soil CA4, one from soil VA4, one from soil VA6 and one from soil MI2 for inoculated, non-autoclaved soils at 15 days, and one replicate from soil CA4 for inoculated, autoclaved soils at 15 days. At 15 days, in some samples, the amount of *F. graminearum* Fg1 DNA was at the quantification limit (lowest DNA concentration at which the quantification can be achieved), equal to 4.95×10^5 gene copies.g^{-1} dry soil, or below. This comprised all replicates (4 in total) of soils MI3, VA5, VA2 and CA6, three replicates of soils MI2, VA4, VA7, VA1 and CA4, two replicates of CA5 and CA3, and one replicate of soils MI4, SO4 and NK4. qPCR data are presented as means ± standard errors. Firstly, these data were processed using an ANOVA, followed by Fisher's LSD tests from the *agricolae* package (de Mendiburu, 2023), to assess differences in fungistasis levels for 26 fields. Secondly, a two-way ANOVA was performed to assess the effects of field location × manure amendment. Thirdly, differences between manured vs. non-manured fields at all 5 locations were tested with ANOVA and LSD tests.

The plant growth chamber experiment followed a randomized block design with 10 replicates (i.e., 10 pots). The data for the number of germinated seeds at 2 and 4 weeks did not display normal distribution and homogeneity of variance, based on Shapiro and Levene tests, respectively, so Kruskal-Wallis and post-hoc Dunn's tests were used to compare treatments. For shoot length, shoot biomass and shoot density, the data displayed normal distribution and homogeneity of variance, so an ANOVA followed by Tukey's HSD tests was used. Additionally, for plant shoot length, shoot biomass and shoot density, *t*-tests were performed to compare plants grown in manured vs. non-manured soils. For shoot length, biomass and density, the plants that did not germinate were regarded as missing data (NA). In the case of shoot biomass measurements, one plant was discarded from MI2 inoculated soil and one from MI3 inoculated soil, and in the case of shoot density, two plants were discarded from the MI2 inoculated soil and one from MI3 inoculated soil because of extreme values.

For the microbial communities, samples with low number of reads or ASVs were discarded. Specialized R package functions were used to determine taxa relative abundances, alpha and beta diversities and to perform statistical tests. Alpha diversity was computed, and sequences were rarefied based on the lowest number of sequences identified among samples, with a minimum of 41,961 sequences for 16S rRNA gene and 34,482 sequences for ITS. Alpha diversity indices were computed for each rarefied sample using the phyloseq (McMurdie and Holmes, 2013), microbiome (Lahti and Shetty, 2018), or vegan (Oksanen et al., 2022) packages. Kruskal–Wallis tests were used to assess changes in alpha diversity with 10,000 permutations. If the Kruskal–Wallis test led to rejecting the null hypothesis ($P < 0.05$), LSD tests with Bonferroni correction were conducted to compare categories using *agricolae* package (de Mendiburu, 2023). Kruskal–Wallis tests were also used to assess the effect of inoculation on the relative abundance of phyla.

Beta diversity analysis was carried out using the rarefied datasets and the ASVs for both 16S rRNA gene and ITS. The dissimilarity among samples was determined by calculating the Bray-Curtis distance. The statistical significance of the comparisons was assessed using a permutation analysis of variance (PERMANOVA) with 10,000 permutations using the adonis2 function of the vegan package (Oksanen et al., 2022). NMDS was employed to visually represent the microbial communities with the ggplot2 (Wickham, 2011) package. Analysis of similarities (ANOSIM) (with 10,000 permutations) was used to compare microbial communities of the three soils (MI2, MI4 and MI5), while pairwise comparisons were used for pairwise comparisons of microbial communities (for MI5 vs. MI4, MI5 vs. MI2, and MI4 vs. MI2).

We employed a negative binomial Wald test implemented in DESeq2 v.1.18.1 within the phyloseq R package to identify taxa with significant differences to test for differential abundance (DA) on unrarefied reads (Love et al., 2014). After the Benjamini-Hochberg correction method, the taxa were considered differentially abundant when the adjusted *P* value was below 0.05. We tested the control against *Fusarium* inoculation (Fg1 samples) for each soil.

For the analysis of *Fusarium* diversity, all ASVs affiliated with the genus *Fusarium* were kept. When possible, the taxonomic identification at the species level was used, based on the UNITE database (Nilsson et al., 2019). In each soil and inoculation condition, the proportion of *Fusarium* reads among the total number of fungal reads was computed, as well as the proportion of reads for each identified *Fusarium* species among the total number of *Fusarium* reads. To assess the impact of Fg1 inoculation on the abundance of each identified *Fusarium* species, ASV data from the eleven retrieved *Fusarium* species were treated by Kruskal-Wallis tests, followed by post-hoc LSD tests with Benjamini-Hochberg correction ($P < 0.05$).

3. Results

3.1. Soil fungistasis of Serbian fields against Fusarium graminearum

Before soil inoculation (day 0), *F. graminearum* was not found in any of the 26 soils analyzed, implying that any observed Fg DNA increase or decrease over the 15 days of experiment was specifically due to Fg1 growth or decline after inoculation. When autoclaved soils were used, growth of *F. graminearum* Fg1 took place in all soils during the 15 days of soil incubation, to a magnitude of 2 $\log_{10}$ units or more (Fig. 2A). In non-autoclaved soils, levels of Fg1 DNA at day 15 were always lower than in

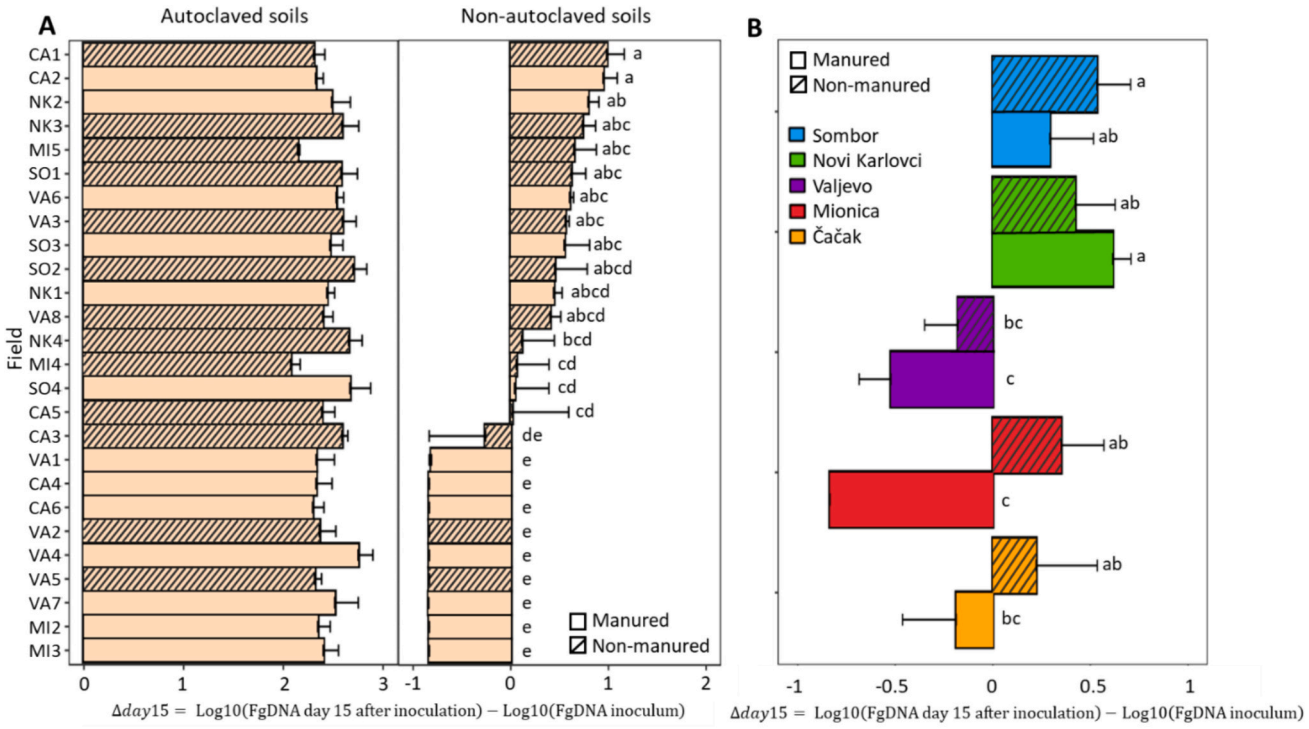

Fig. 2. Difference in the quantities of *F. graminearum* Fg1 DNA in soil between day 0 and 15 days after inoculation. A. Differences in DNA quantity of *F. graminearum* Fg1 in 26 inoculated Serbian soils, from Valjevo (VA), Sombor (SO), Novi Karlovci (NK), Mionica (MI) and Čačak (CA), which were autoclaved or not autoclaved. Results are presented as means with standard errors. Striped bars indicate soils without manure amendments and non-striped bars indicate soils with manure amendments. Letters a-e are used to show statistical differences between individual soils (ANOVA and LSD tests, $P < 0.05$). **B.** Comparison of manured vs. non manured soils at each location. Results are presented as means with standard errors. Striped bars are used for soils without manure amendments and non-striped bars for soils with manure amendments. Letters a-c are used to show statistical differences between soil conditions (ANOVA and LSD tests, $P < 0.05$).

autoclaved soils. The pathogen quantities remained stable or even increased in 16 of 26 non-autoclaved soils as compared with the initial inoculum, while the amount of Fg1 DNA decreased in the 10 others (i.e., 38 %; all from western/central Serbia), indicating a strong fungistasis potential attributable to soil microbiota in these 10 soils. Subsequently, soils in which the amount of Fg1 DNA decreased were thus defined as displaying fungistasis.

When considering the effect of manuring on fungistasis, 7 out of 10 non-autoclaved soils (70 %) displaying fungistasis originated from manured fields, whereas only 6 of 16 non-autoclaved non-fungistatic soils (37 %) had received manure (Fig. 2A). When locations were considered, fungistasis was found for the three western/central Serbia locations (Valjevo, Mionica and Čačak) for manure-amended soils, and only for Valjevo and Čačak for non-manured soils. Two-way ANOVA ($P < 0.05$) showed that field location and manure amendments were significant factors, but the interaction between them was not significant. Fg1 growth was significantly lower in manured soils than in non-manured soils from Mionica (LSD test, $P < 0.01$), with a similar trend (although not significant) in soils from Čačak (Fig. 2B).

3.2. Relation between soil composition and fungistasis

Non-metric MultiDimensional Scaling (NMDS) of soil physicochemical data showed that soils mainly clustered according to their geographical location (permutation test, $P < 0.001$), which coincided also with particular soil types, whereas manure amendment or fungistasis level did not have an over-riding effect overall (Fig. 3, non-significant in permutation test). However, higher organic matter content (OM) was found in fungistatic (and manured) Mionica soils MI2 (7.66 %) and MI3 (6.96 %), compared with non-fungistatic (and non-manured) soils MI4 (5.87 %) and MI5 (5.88 %) (Table S2 and Fig. 3). Higher potassium (K) content was also evidenced in fungistatic soils MI2

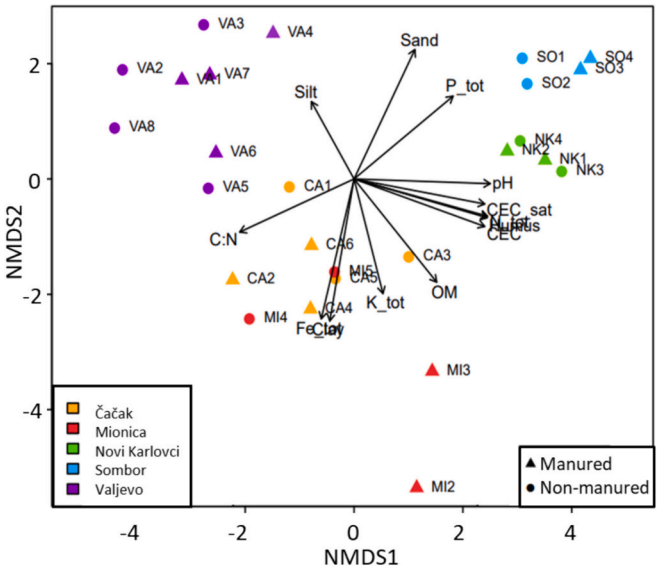

Fig. 3. Non-metric MultiDimensional Scaling analysis of the physico-chemical composition (see Table S2 for details) of the 26 Serbian fields.

(370 mg/kg) and MI3 (293 mg/kg), than in non-fungistatic soils MI4 (218 mg/kg) and MI5 (184 mg/kg). When comparing fungistatic and non-fungistatic soils at other locations (Valjevo or Čačak), fungistatic soils did not display higher contents in organic matter or potassium compared with non-fungistatic soils, and they did not exhibit any other chemical particularity. In summary, soils exhibited particularities according to their location of origin (and soil type), and in Mionica

according to fungistasis status (confounded with manure usage; with higher OM and K contents). Therefore, there was no global relation between soil composition and fungistasis.

3.3. Suppressiveness of Mionica soils against F. graminearum-induced wheat damping-off

Based on the contrasted fungistasis results and the link with manure amendments found at Mionica, we selected these soils for a wheat damping-off suppressiveness assay with *F. graminearum* Fg1. At 14 days after sowing, the number of germinated seeds was statistically lower upon pathogen inoculation in soil MI4, whereas the difference was not significant in soils MI2, MI3, and MI5 (Fig. 4A). Similarly, at 4 weeks, the number of plants alive was statistically lower in *F. graminearum* Fg1-inoculated vs. non-inoculated MI4 soils, while the difference was not significant in the three other soils (Fig. 4B). Inoculation with *F. graminearum* Fg1 did not significantly impact wheat shoot length (Fig. S2A), but it resulted in lower dry shoot biomass (Fig. S2B) and shoot density (Fig. S2C) in soil MI2. In addition, dry shoot biomass, shoot length and shoot density were higher overall (*t*-tests, all $P < 10^{-12}$) in manured soils (MI2 and MI3) than in non-manured soils (MI4 and MI5). In summary, non-fungistatic (non-manured) soil MI4 was conducive to wheat damping-off caused by *F. graminearum* Fg1 but non-fungistatic (non-manured) soil MI5 turned out to be suppressive, whereas both fungistatic (manured) soils MI2 and MI3 were suppressive based on wheat germination and survival (even though plant growth in MI2 was affected by the pathogen).

3.4. Diversity and genetic structure of prokaryotic and fungal rhizospheric communities

When assessing the link between rhizosphere microbial diversity and disease-suppressiveness status of Mionica soils, metabarcoding data for the 16S rRNA gene (prokaryotic community) pointed to similar diversity levels for the three soils. Indeed, the only difference was that Pielou index (evenness) in the fungistatic, suppressive soil MI2 (also the only manured soil) was significantly higher than in non-fungistatic soils MI4 (conducive) and MI5 (suppressive) when inoculated with Fg1 (Fig. 5C). The effect of Fg1 inoculation on bacterial alpha diversity was not significant, regardless of the soil and the diversity index. With ITS metabarcoding data (fungal community) from the rhizosphere, the Shannon (diversity; Fig. 5D) and Pielou (evenness; Fig. 5F) indices but not the Chao1 index (richness; Fig. 5E) were statistically higher (i) in soils MI4 (non-fungistatic, non-suppressive) and MI2 (fungistatic, suppressive) than in MI5 (non-fungistatic, suppressive) in the absence of inoculation, and (ii) in soil MI4 than in MI5 when Fg1 had been inoculated. Inoculation itself resulted only in a lower Pielou index in soil MI4 (Fig. 5F).

NMDS plots based on Bray-Curtis distances showed that microbial communities clustered largely according to the field of origin, for the prokaryotic (Fig. 6A) and especially the fungal communities (Fig. 6B). Indeed, individual soils accounted for 42.6 % (for prokaryotes) and 60.0 % (for fungi) of the variations in community structure (PERMANOVA, both at $P < 0.001$), whereas merely 3.7 % (prokaryotes; $P = 0.048$) and 4.0 % (fungi; $P = 0.023$) of the differences were attributed to inoculation (Table S3). When considering each soil separately, the effect of Fg1 inoculation was significant in most cases, i.e., for MI2 ($P = 0.009$ for prokaryotes and $P = 0.004$ for fungi), MI5 ($P = 0.004$ for prokaryotes and $P = 0.048$ for fungi), and MI4 ($P = 0.004$ for prokaryotes but $P > 0.05$ for fungi).

In summary, most differences in prokaryotic alpha diversity were not significant, whereas fungi in soil MI5 (non-fungistatic, suppressive) displayed lower Shannon and Pielou indices. In addition, microbial community structure depended mostly on the field of origin, with a modest significant effect of inoculation.

3.5. Composition of the prokaryotic rhizosphere community

The most abundant rhizosphere phyla in soils MI2, MI4 and MI5 were the same, i.e., *Proteobacteria*, *Actinobacteriota, Firmicutes, Chloroflexi, Verrucomicrobiota* and *Crenarchaeota.* The 20 most abundant taxa (the lowest likely taxonomic information available for an ASV, often at the genus level) in the prokaryotic community represented an average of 56.2 % (MI2 = 54.2 %, MI4 = 55.3 %, MI5 = 58.6 %) of the sequences (Fig. 7A, B and C). Some of these most abundant taxa were evidenced in all three soils, e.g., the *Actinobacteriota Gaiella* (average 4.6 % of the reads) and a taxon affiliated to the order *Gaiellales* (average 13.0 %). Some were found in specific soil(s), as for (i) an *Acidobacteriota* taxon from the order *Vicinamibacterales* (1.1 %) and various *Actinobacteriota*, i.e., the genera *Microlunatus* (2.1 %) and *Rubrobacter* (1.6 %), a

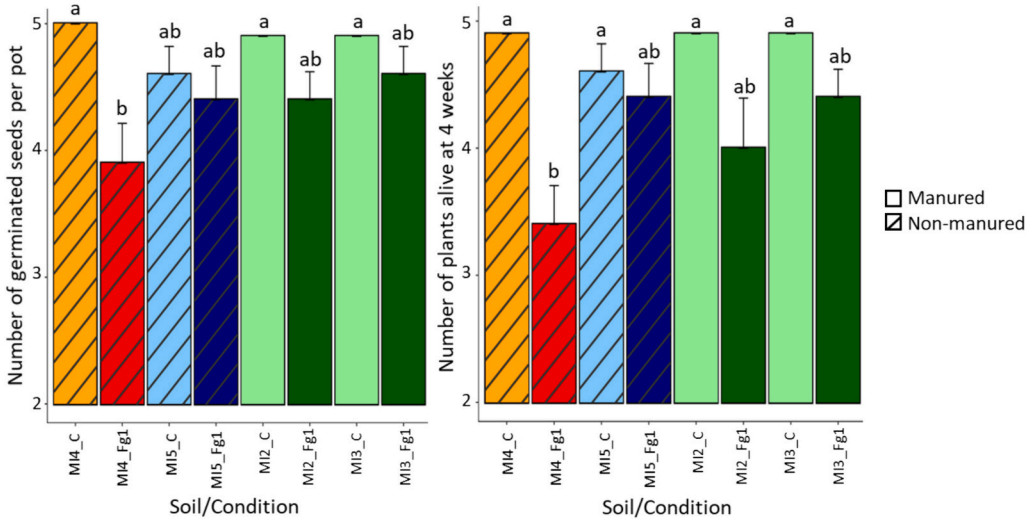

Fig. 4. Wheat suppressiveness assay with soils from Mionica (MI4, MI5, MI2 and MI3), non-inoculated (shown as MIi_C) or inoculated with *F. graminearum* Fg1 (shown as MIi_Fg1). Non-manured soils are represented with stripes. Soil MI4 (orange-red) is non-fungistatic and non-suppressive to wheat damping-off caused by *F. graminearum* Fg1, soil MI5 (light blue-navy blue) is non-fungistatic and suppressive, while soils MI2 and MI3 (light green-forest green) are both fungistatic and suppressive. All results are presented as means and standard errors (n = 10). Statistical differences between soil conditions are shown with letters a and b (Kruskal-Wallis and Dunn's tests, $P < 0.05$). **A.** Number of germinated wheat seeds per pot (out of 5) at 2 weeks. **B.** Number of wheat plants alive per pot at 4 weeks.

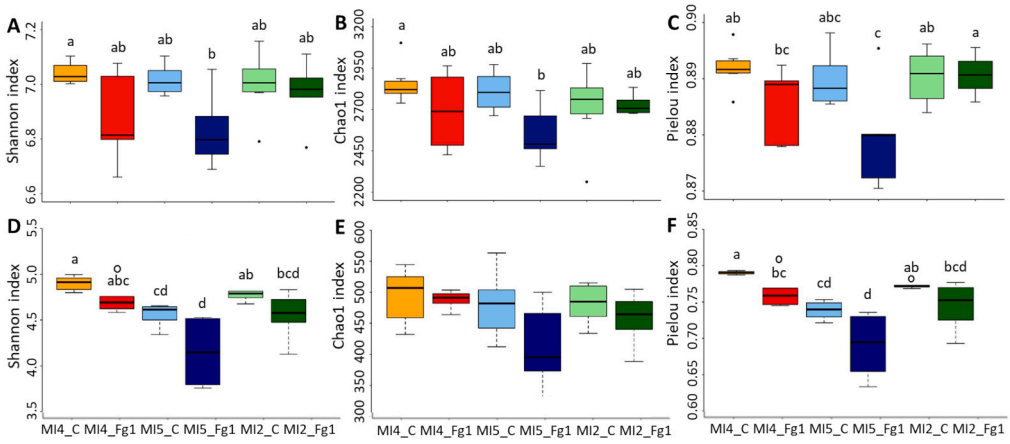

Fig. 5. Alpha diversity of prokaryotic (A, B, C) and fungal (D, E, F) rhizosphere communities in soils from Mionica (MI4, MI5, and MI2) inoculated with *F. graminearum* Fg1 (shown as MI_Fg1) or non-inoculated (shown as MI_C). Letters a-d indicate statistical relations between soils × inoculation (Fg1 or not) combinations (Kruskal–Wallis tests, followed by Fisher's Least Significant Difference (LSD) tests with Bonferroni correction, $P < 0.05$).

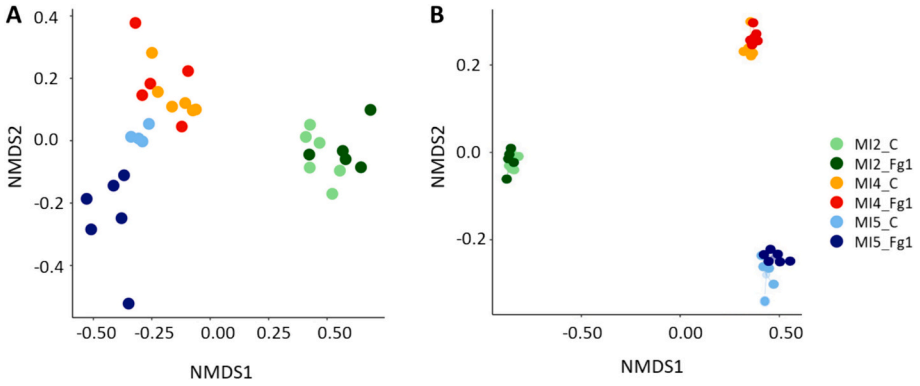

Fig. 6. Non-metric MultiDimensional Scaling (NMDS) of soils from Mionica MI2, MI4, and MI5 inoculated with *F. graminearum* Fg1 (shown as MIi_Fg1) or non-inoculated (shown as MIi_C) based on rhizosphere metabarcoding of prokaryotic (A) and fungal (B) communities. ANOSIM (10,000 permutations) indicated that the between-groups difference was larger than the within-groups difference ($P = 10^{-4}$ for prokaryotes and 10^{-4} for fungi). All pairwise comparisons (for MI5 vs. MI4, MI5 vs. MI2, and MI4 vs. MI2) for prokaryotes were $P = 10^{-3}$ and $P = 10^{-3}$ for fungi.

Microtrichales genus (1.3 %) and a *Ilumatobacteraceae* genus (1.1 %), which were evidenced only in soil MI2, (ii) the *Proteobacteria* genus *Sphingomonas* in soil MI4 (1.4 %), (iii) an *Elsterales* (*Proteobacteria*) genus in soil MI5 (1.0 %), and (iv) the *Actinobacteriota* genera *Conexibacter* (2.0 %), *Marmoricola* (1.0 %), *Intransporangium* (1.3–1.1 %) and *Acidothermus* (2.0–1.9 %) in soils MI4 and MI5.

Inoculation with *F. graminearum* Fg1 resulted in a significant increase ($P < 0.05$) in the rhizosphere relative abundance of the phylum *Firmicutes* in the non-fungistatic soils MI4 (from 10.8 % to 15.8 %) and MI5 (from 10.1 % to 14.4 %) (Fig. 8). In the fungistatic MI2 soil, pathogen inoculation caused a modest but significant increase ($P < 0.05$) in the relative abundance of *Actinobacteriota* (from 48.0 % to 50.9 %) and *Proteobacteria* (from 13.7 % to 17.1 %), but led to somewhat lower levels of *Crenarchaeota* (from 5.4 % to 1.5 %) and *Chloroflexi* (from 5.4 % to 4.9 %) ($P < 0.05$).

Differential analysis was also used to identify individual taxa that differed significantly ($P < 0.05$) in relative abundance between *Fusarium*-inoculated and non-inoculated samples, at the scale of the whole rhizosphere community. Among the 1493 identified prokaryotic taxa (Fig. 9), this concerned 17 taxa in soil MI4 (non-fungistatic, non-suppressive), 45 taxa in soil MI5 (non-fungistatic, suppressive), and 17 taxa in soil MI2 (fungistatic, suppressive). Most of the taxa were found exclusively in one of the three soils, but *Gemmatimonas* (*Gemmatimonadota*) was evidenced in all three soils, with a lower abundance in inoculated than in non-inoculated samples (by 0.8, 0.5 and 1.2 $\log_2$ units for soils MI2, MI4 and MI5, respectively). In both soils MI4 and MI5, a taxon belonging to the candidate group SC-I-84 (*Proteobacteria*) was found more in non-inoculated than in inoculated samples (by 0.8 and 1.2 $\log_2$ units, respectively), as for a *Myxococcota* taxon from the candidate group Blrii41 (by 0.6 and 1.2 $\log_2$ units, respectively), whereas the opposite was found for *Bacillus* (by 0.7 and 0.9 $\log_2$ units, respectively), *Paenibacillus* (by 0.8 and 0.9 $\log_2$ units, respectively) and *Pelosinus* (*Firmicutes*) (by 1.2 and 2.0 $\log_2$ units, respectively). *Sphingobium* (*Proteobacteria*) was more abundant in inoculated than in non-inoculated samples of soils MI2 and MI5 (by 7.0 and 8.0 $\log_2$ units, respectively). These inoculation effects concerned also some of the 20 most abundant prokaryotic taxa, i.e., for *Solirubrobacter* in soil MI4, *Candidatus Udaeobacter* and *Bacillus* for soil MI5 and for *Nitrososphaeraceae* in soil MI2.

In summary, the wheat rhizosphere of the three soils shared the main phyla and the majority of the most abundant taxa, yet several taxa were soil specific. Additionally, soil inoculation with *F. graminearum* Fg1 impacted the rhizosphere microbial community, but often with soil-specific effects.

3.6. Composition of the fungal rhizosphere community

In each soil, *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* were the phyla harboring the most abundant taxa. However, differences were found between soils, as in soils MI4 and MI5 the phylum *Chytridiomycota* was also present. The 20 most abundant fungal taxa (considered at the

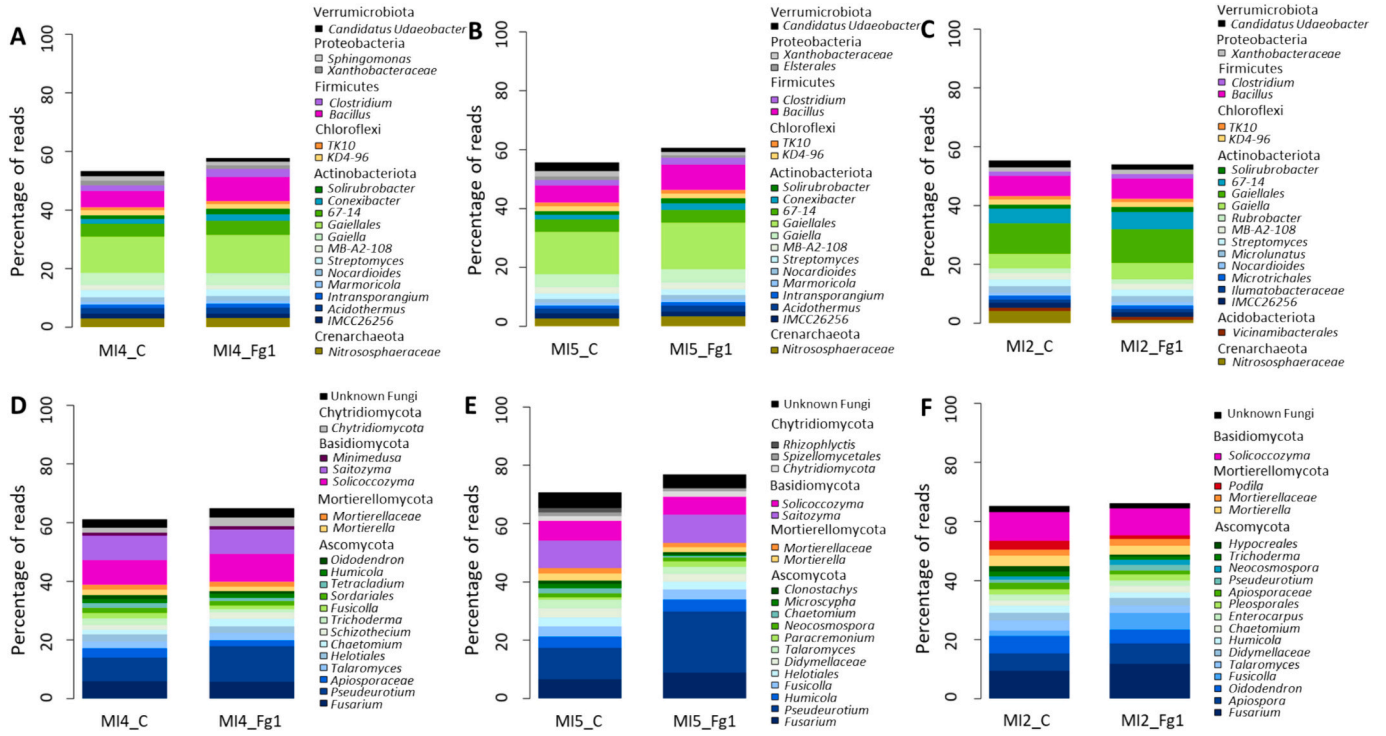

Fig. 7. Top 20 most abundant prokaryotic (A, B, C) and fungal taxa (D, E, F) in the wheat rhizosphere of soils from Mionica MI4, MI5, and MI2. MIi_C, control (non-inoculated soils); MIi_Fg1, *F. graminearum*-inoculated soils. The 20 most abundant taxa (the lowest taxonomic information available for an ASV; often at genus level) in the prokaryotic community represented 55.3 % (non-inoculated MI2 soil), 53.9 % (Fg1-inoculated MI2 soil), 53.2 % (non-inoculated MI4 soil), 57.7 % (Fg1-inoculated MI4 soil), 55.7 % (non-inoculated MI5 soil) and 60.5 % (Fg1-inoculated MI5 soil) of the sequences, whereas the 20 most abundant fungal taxa represented 65.2 % (non-inoculated MI2 soil), 66.1 % (Fg1-inoculated MI2 soil), 61.1 % (non-inoculated MI4 soil), 64.8 % (Fg1-inoculated MI4 soil), 70.7 % (non-inoculated MI5 soil) and 76.9 % (Fg1-inoculated MI5 soil) of the sequences.

genus level or higher rank if genus information was not available) represented 61 % (in non-inoculated MI4 soil) to 77 % (in Fg1-inoculated MI5 soil) of the reads in rhizosphere samples (Fig. 7D, E and F). Distinctive features were evidenced in particular soil(s), as (i) *Podila* (a *Mortierellaceae* genus; representing 10 % of the reads), *Hypocreales*, *Apiospora*, *Pleosporales, Enterocarpus* were found only in soil MI2, (ii) *Schizothecium*, *Sordariales*, *Tetracladium*, *Minimedusa* only in soil MI4, (iii) *Clonostachys*, *Microscypha*, *Paracremonium* only in soil MI5, (iv) *Pseudeurotium, Helotiales, Humicola*, *Saitozyma* only in soils MI4 and MI5, (v) *Apiosporaceae*, *Chaetomium*, *Trichoderma*, *Oidodendron* only in soils MI4 and MI2, and (vi) *Neocosmospora*, *Didymellaceae* only in soils MI5 and MI2.

At phylum level, inoculation with *F. graminearum* Fg1 resulted into a significant increase ($P < 0.05$) in the rhizosphere relative abundance of the *Chytridiomycota* in the non-fungistatic soil MI4 (from 5.4 % to 7.8 %), and a decrease of the *Mortierellomycota* (from 8.7 % to 6.6 %) in the fungistatic soil MI2 (Fig. 8).

When differential analysis was performed to assess inoculation effects on the whole fungal community (Fig. 10), decreased levels were found for the *Ascomycota* genera *Beauvaria* (by 5.8 $\log_2$ units) and *Collarina* (by 4.9 $\log_2$ units) in soil MI4 (non-fungistatic, non-suppressive), for four genera (of distinct phyla) including *Waitea* (*Basidiomycota*; by 24 $\log_2$ units), *Microscypha* (*Ascomycota*; by 2 $\log_2$ units) in soil MI5 (non-fungistatic, suppressive), and the four *Ascomycota* genera *Septoria* (by 22 $\log_2$ units) and *Purpureocillium* (by 2 $\log_2$ units) in soil MI2 (fungistatic, suppressive), whereas higher levels were found for *Atractium* (by 2.5 $\log_2$ units) and *Scutellinia* (by 3.0 $\log_2$ units) in soil MI2. Inoculation effects were also observed (Kruskal-Wallis tests and Fisher's tests with Bonferroni correction) for some of the 20 most abundant fungal taxa, but these effects were not significant anymore with the more stringent differential analysis.

In summary, the three soils harbored representatives from the phyla *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* in rhizosphere samples, while taxa from the order *Chytridiomycota* were found only in soils MI4 and MI5. Similarly to the prokaryotic community, soil inoculation with *F. graminearum* Fg1 impacted fungal rhizosphere community.

3.7. Composition of the Fusarium community

In the absence of *F. graminearum* Fg1 inoculation, the *Fusarium* genus represented 9.4 % of all rhizosphere fungi in non-suppressive soil MI4 (also non-fungistatic), vs. only 5.9 % and 6.6 % in suppressive soils MI5 (non-fungistatic) and MI2 (fungistatic), respectively (Table S4). The *Fusarium* genus was more prevalent in Fg1-inoculated vs. non-inoculated rhizosphere for soil MI4 (up to 11.8 %) and MI2 (up to 8.8 %), but not for soil MI5. Within the genus, inoculation increased ($P < 0.05$) rhizosphere levels of *F. graminearum* from 2.6 % to 25.0 % of all *Fusarium* sequences for MI4, 0.9 % to 29.0 % for MI5, and 0.5 % to 20.0 % for MI2 (Fig. 11). In addition, a small decrease in levels of *F. equiseti* was found in soil MI4 after inoculation (Table S4).

4. Discussion

F. graminearum is an important crop pathogen (Valverde-Bogantes et al., 2020) and its infectious cycle includes a phase where the fungus must survive in the soil before infecting new seedlings (Pereyra et al., 2004; Cobo Díaz et al., 2019). Certain soils can negatively affect survival and growth of fungal pathogens, including *F. graminearum*, a property referred to as fungistasis (Lockwood, 1977; Garbeva et al., 2011; Legrand et al., 2019). Though fungistasis is an important soil trait in the context of sustainable agriculture and may belong to mechanisms contributing to disease suppressiveness, it remains insufficiently understood.

As in the case of disease suppressiveness, fungistasis is mostly

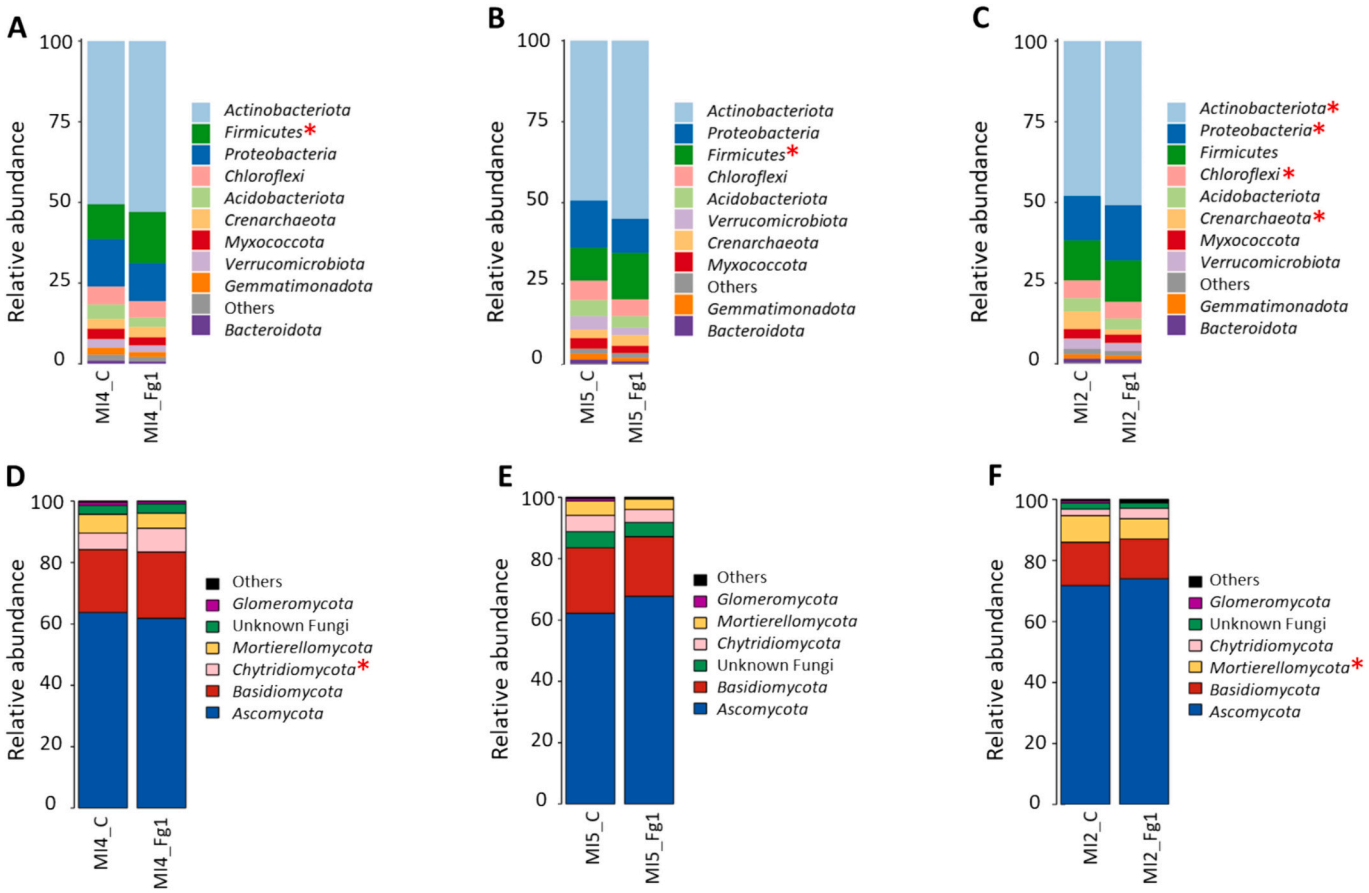

Fig. 8. Relative abundance of prokaryotic (A, B, C) and fungal phyla (D, E, F) in the rhizosphere of Mionica soils MI4, MI5 and MI2. MIi_C, control; MIi_Fg1, *F. graminearum*-inoculated soils. Asterisks indicate significant differences between each inoculated vs. non-inoculated soil based on Kruskal-Wallis tests followed by Fisher's Least Significant Difference (LSD) tests with Bonferroni correction ($P < 0.05$).

provided by living microorganisms in soil (Lockwood, 1977) and may be influenced by soil physicochemical properties and soil management practices (Zhang et al., 2020; Bellini et al., 2023). In this context, we screened 26 fields from five localities in Serbia (northern and western/central regions) for their fungistatic properties and tested the relationship between fungistasis, previous soil manuring and soil physicochemical composition. The screening evidenced 10 soils in which the pathogen *F. graminearum* declined significantly and the observed fungistasis was obviously due to antifungal properties of native soil microbiota, as *F. graminearum* grew readily when the 10 soils were sterilized, to the same extent as in the non-fungistatic soils. Autoclaving may change certain properties of soil organic matter (Berns et al., 2008), but importantly this did not prevent pathogen growth.

In this study, fungistasis was found with soils from Valjevo (eutric cambisols and pseudogleys), Čačak (vertisols) and Mionica (vertisols), located in a hilly region in western/central Serbia, where agriculture is less intensive and follows more traditional practices without use of fungicides than in northern plains of Serbia, where soils also differ (chernozems). Here, a majority of fungistatic soils and a minority of non-fungistatic soils were manured soils. Particularly in Mionica location, only manured soils were fungistatic, and they differed statistically from non-manured soils based on fungistasis levels. Animal manure amendments introduces new microorganisms to the soils, supplies nutrients and impacts the resident microbiota (Mousa and Raizada, 2016; Su et al., 2022), which may in turn have negative effects on pathogen growth and survival, but this was not sufficient to develop fungistasis in the chernozems of northern Serbia. Our study showed that manuring was a significant factor influencing fungistasis, but that the positive relationship between manuring and fungistasis cannot be generalized as it is also affected by (probably mixed) effects of location, soil type and prevailing management practices.

The positive effect of manure on fungistasis may also be related to different soil physicochemical properties, as they can influence microbiota functioning including phytoprotection properties (Sipilä et al., 2012; Almario et al., 2014). For example, soil fungistasis to *F. graminearum* was related to manganese and nitrogen contents in Brittany soils (Legrand et al., 2019), but here soil types were more diverse and physicochemical properties varied primarily with geographic location (and soil type, Fig. 3). In Mionica, where the relation between manure and fungistasis was significant, the manured, fungistatic soils displayed higher organic matter and potassium contents, which may be due to manuring itself (Aziz et al., 2010). In another study, the sole amendment of soil with potassium phosphite enriched the soil community with antagonistic bacteria and affected survival of the tomato pathogen *Ralstonia solanacearum* (Su et al., 2022). Based on the current results, it seems that particular soil composition (e.g., higher organic matter and potassium content) may favor fungistasis.

Using soils from Mionica, we further tested whether fungistatic soils would also be disease-suppressive, as this possibility has been mentioned in earlier works (Lockwood, 1977; Garbeva et al., 2011). Here, the two Mionica fungistatic (manured) soils MI2 and MI3 were suppressive to *F. graminearum* disease of wheat, and it could be that either fungistasis promoted rhizosphere-based disease-suppressiveness or was sufficient to prevent pathogen attack on plant. Interestingly, one non-fungistatic soil (MI5) was disease-suppressive as well, raising the possibility that suppressiveness in this case entailed (at least in part) induced resistance triggered by the soil microbiota. In addition, this shows that manuring was not necessarily the primary determinant for

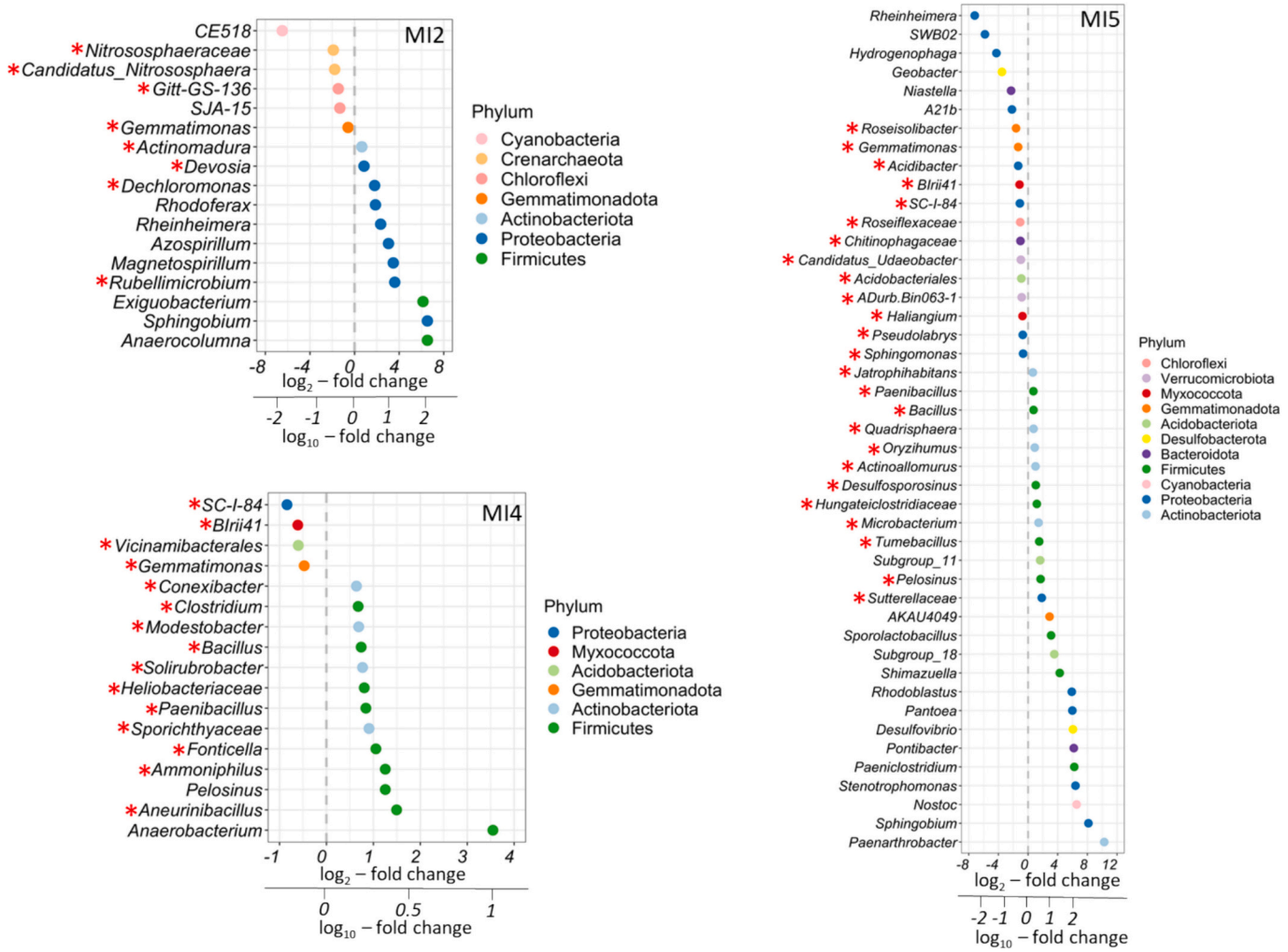

Fig. 9. Differential abundance analysis of prokaryotic taxa in the wheat rhizosphere of soils from Mionica MI2, MI4 and MI5 following inoculation with *F. graminearum* Fg1. The X axes are shown with $\log_2$- and $\log_{10}$-fold changes. All taxa shown were affected by inoculation ($P < 0.05$), and those representing >0.1 % of all sequences are indicated with an asterisk.

biocontrol microorganisms in the rhizosphere of soils from Mionica. Finally, the other non-manured non-fungistatic soil (MI4) was disease-conducive. However, during the soil sampling for suppressiveness assay (June 2021), fields in Mionica were grown with maize (MI2), wheat (MI3 and MI5) or a meadow (MI4) (Table 1), whereas soil for the fungistasis assay was sampled in October 2020, when fields in Mionica were (or had been) grown with alfalfa (MI2), sunflower (MI3), wheat (MI4) or a meadow (MI5). Perhaps this contributed to the difference between the non-manured soils MI4 and MI5. More generally, the current approach based on the comparison of soil from farmers' fields (rather than using an experimental field site with controlled conditions) is interesting because it connects with the reality of true farming conditions, but a key limitation is the lack of control on crop rotation and crop genotypes. Both can have a strong influence on soil-borne pathogens and plant-beneficial microorganisms (Gruet et al., 2024; Renoud et al., 2020; Nannipieri et al., 2023; Todorović et al., 2023; Yuan et al., 2021).

A wide range of bacterial and fungal taxa may be involved in disease suppression (Cha et al., 2016; Kloepper et al., 1980; Tamietti and Alabouvette, 1986; Weller et al., 2002; Kyselková and Moënne-Loccoz, 2012; Ossowicki et al., 2020) and phytoprotection may often result from a joint contribution of various microbial taxa (Alabouvette et al., 1985; Rouxel and Sedra, 1989; Rasmussen et al., 2002; Kyselková et al., 2009; Ossowicki et al., 2020). Complex analyses of microbial communities from disease-suppressive soils against conducive soils may be thus helpful to reveal microbial taxa potentially involved in disease suppressiveness (Borneman et al., 2004; Kyselková et al., 2009). In the present study, *Sphingobium* (*Alphaproteobacteria*) increased its abundance after pathogen inoculation by two orders of magnitude in the disease suppressive soils MI2 and MI5, but not in the conducive soil MI4 (Fig. 9), making it a candidate for further examination of its role in protecting wheat from *F. graminearum* disease. Indeed, *Sphingobium* is known for its biocontrol properties (Boss et al., 2022; Van Bruggen et al., 2014) and its increased abundance in conditions suppressive to *Fusarium oxysporum* banana wilt was reported on several occasions (Fu et al., 2017; Hong et al., 2020). In general, however, the microbial (both prokaryotic and fungal) communities were mostly soil-specific (Fig. 6), and it is likely that different mechanisms, provided by different microbial taxa, were involved in disease suppressiveness in MI2 and MI5.

For example, soil MI2 maintained its original prokaryotic diversity in rhizosphere upon *F. graminearum* inoculation, in contrast to MI5 (and MI4, both non-fungistatic), where the prokaryotic diversity decreased with pathogen introduction (significant for Pielou index and a trend for Shannon and Chao1 indices, Fig. 5A, B and C). High microbial diversity is an important barrier against invasion of pathogens in soil in general (Van Elsas et al., 2012) and might be promoted by manure addition to soil (Zhong et al., 2010). It is thus likely that manure amendments in MI2 sustained high prokaryotic diversity that could contribute to

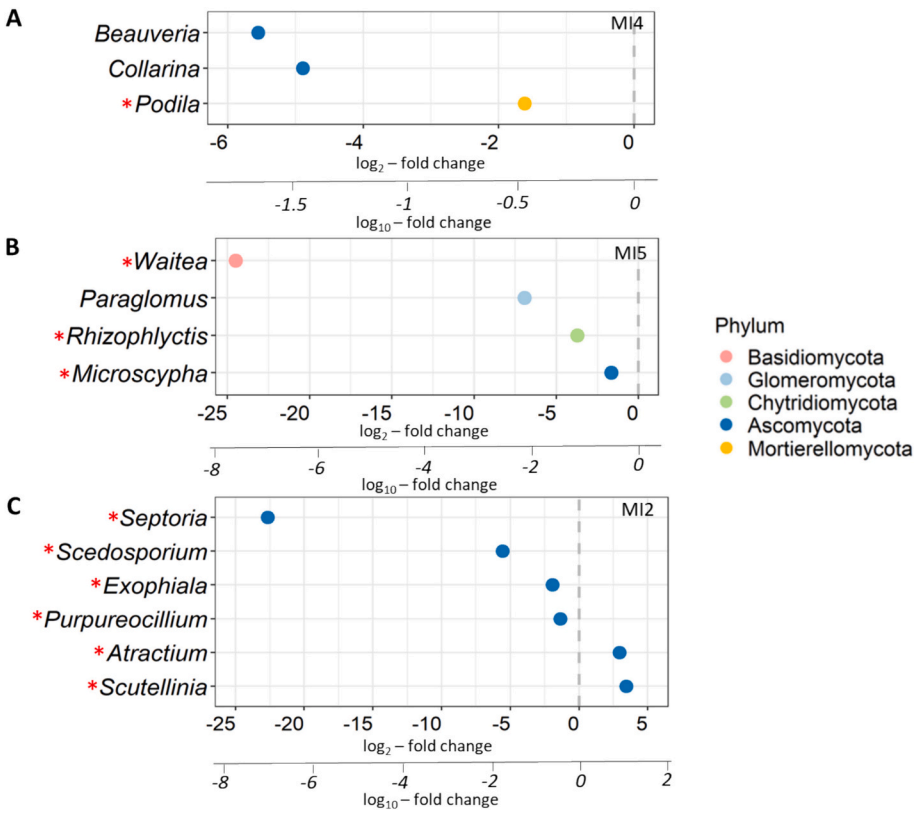

Fig. 10. Differential abundance analysis of fungal taxa in the wheat rhizosphere of soils from Mionica MI4 (A), MI5 (B) and MI2 (C) following inoculation with *F. graminearum* Fg1. The X axes are shown with log_2- and log_{10}-fold changes. All taxa shown were affected by inoculation ($P < 0.05$), and those representing > 0.1 % of all sequences are indicated with an asterisk.

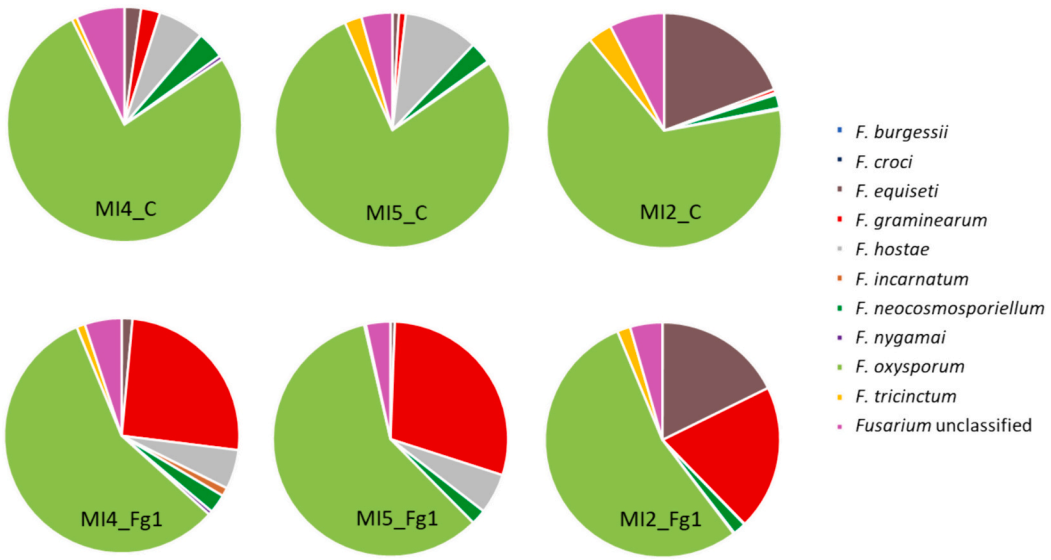

Fig. 11. Proportion of the different *Fusarium* species in the wheat rhizosphere of soils from Mionica MI4, MI5 and MI2 inoculated (MIi_Fg1) or not (MIi_C) with *Fusarium graminearum* Fg1.

fungistasis and to some extent also to disease suppression. In addition, a closer look at *Fusarium* composition in wheat rhizosphere (Fig. 11) indicated that the share of *F. graminearum* remained at lower levels in wheat rhizosphere of MI2, as compared to MI4 and MI5 (both for inoculated and non-inoculated samples). There was a strikingly high proportion of *F. equiseti* in the MI2 rhizosphere, which remained approximately in a 1:1 ratio with *F. graminearum* in inoculated MI2 samples. *F. equiseti* is known for biocontrol of *F. oxysporum* by reducing growth of the Fusarium wilt pathogen in spinach (Horinouchi et al., 2010), so it might be also considered as an agent contributing to fungistasis and disease-suppressiveness in the case of *F. graminearum* in MI2. In contrast, in the other suppressive (but non-fungistatic) soil MI5, the proportion of *F. graminearum* remained at higher levels comparable to that of the conducive soil MI4, pointing to the importance of rhizosphere

interactions for wheat protection in soil MI5. Stimulation of other plant-beneficial taxa or plant defenses is one possible explanation, as showed in other cases (Almario et al., 2014; Van Peer et al., 1991; Tamietti et al., 1993; Leeman et al., 1995).

In conclusion, we identified manure as an important farming practice for achieving soil fungistasis towards the wheat pathogen *F. graminearum*. On the example of Mionica, where manure is of particular importance for fungistasis, we showed that the two fungistatic soils were also suppressive to *F. graminearum* disease in wheat. The fungistasis, however, may not be the key factor determining disease-suppressiveness, as one non-fungistatic soil from Mionica was also disease-suppressive, which was reflected by particularities in rhizosphere microbial diversity of the individual soils.

CRediT authorship contribution statement

Irena Todorović: Investigation, Writing – original draft, Data curation, Formal analysis. **Danis Abrouk:** Investigation, Data curation, Formal analysis. **Nicolas Fierling:** Investigation. **Martina Kyselková:** Investigation. **Marie-Lara Bouffaud:** Investigation, Data curation, Formal analysis. **François Buscot:** Investigation. **Adriana Giongo:** Formal analysis, Investigation, Data curation. **Kornelia Smalla:** Investigation, Resources, Funding acquisition. **Adeline Picot:** Resources. **Vera Raičević:** Visualization, Funding acquisition. **Jelena Jovičić-Petrović:** Funding acquisition, Investigation, Resources, Supervision. **Yvan Moënne-Loccoz:** Conceptualization, Funding acquisition, Validation, Writing – review & editing. **Daniel Muller:** Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

For 16S rRNA gene and ITS, the raw amplicon data were deposited at the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA1010537.

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Ethical statement

The experiments did not involve human participants and/or animals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105506.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2022. UNITE mothur release for Fungi. Version 16.10.2022. In: UNITE community. https://doi.org/10.15156/BIO/2483919.

Alabouvette, C., 1986. Fusarium-wilt suppressive soils from the Châteaurenard region: review of a 10-year study. Agronomie 6, 273–284. https://doi.org/10.1051/agro:19860307.

Alabouvette, C., Couteaudier, Y., Louvet, J., Bremeersch, P., Richard, P., Soulas, M.-L., 1985. Recherches sur la résistance des sols aux maladies. XI. Etude comparative du comportement des *Fusarium* spp. dans un sol résistant et un sol sensible aux fusarioses vasculaires enrichis en glucose. Agronomie 5, 63–68. https://doi.org/10.1051/agro:19850109.

Almario, J., Muller, D., Défago, G., Moënne-Loccoz, Y., 2014. Rhizosphere ecology and phytoprotection in soils naturally suppressive to Thielaviopsis black root rot of tobacco. Environ. Microbiol. 16, 1949–1960. https://doi.org/10.1111/1462-2920.12459.

Alouane, T., Rimbert, H., Fabre, F., Cambon, F., Langin, T., Bonhomme, L., 2018. Genome sequence of *Fusarium graminearum* strain MDC_Fg1, isolated from bread wheat grown in France. Microbiol. Resour. Announc. 7, e01260–18 https://doi.org/10.1128/MRA.01260-18.

Aziz, T., Ullah, S., Sattar, A., Nasim, M., Farooq, M., Khan, M., 2010. Nutrient availability and maize (*Zea mays*) growth in soil amended with organic manures. Int. J. Agric. Biol. 12, 621–624. doi:10–070/RAS/2010/12–4–621–624.

Bellini, A., Gilardi, G., Idbella, M., Zotti, M., Pugliese, M., Bonanomi, G., Gullino, M.L., 2023. *Trichoderma* enriched compost, BCAs and potassium phosphite control fusarium wilt of lettuce without affecting soil microbiome at genus level. Appl. Soil Ecol. 182, 104678 https://doi.org/10.1016/j.apsoil.2022.104678.

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486. https://doi.org/10.1016/j.tplants.2012.04.001.

Berns, A.E., Philipp, H., Narres, H.D., Burauel, P., Vereecken, H., Tappe, W., 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur. J. Soil Sci. 59, 540–550. https://doi.org/10.1111/j.1365-2389.2008.01016.x.

Besset-Manzoni, Y., Joly, P., Brutel, A., Gerin, F., Soudiere, O., Langin, T., Prigent-Combaret, C., 2019. Does *in vitro* selection of biocontrol agents guarantee success *in planta*? A study case of wheat protection against *Fusarium* seedling blight by soil bacteria. PLoS One 14, e0225655. https://doi.org/10.1371/journal.pone.0225655.

de Boer, W., Li, X., Meisner, A., Garbeva, P., 2019. Pathogen suppression by microbial volatile organic compounds in soils. FEMS Microbiol. Ecol. 95, fiz105. https://doi.org/10.1093/femsec/fiz105.

Bonanomi, G., Gaglione, S.A., Cesarano, G., Sarker, T.C., Pascale, M., Scala, F., Zoina, A., 2017. Frequent applications of organic matter to agricultural soil increase fungistasis. Pedosphere 27, 86–95. https://doi.org/10.1016/S1002-0160(17)60298-4.

Borneman, J., Olatinwo, R., Yin, B., Beckers, J.O., 2004. An experimental approach for identifying microorganisms involved in specified functions: utilisation for understanding a nematode suppressive soil. Australas. Plant Pathol. 33, 151–155. https://doi.org/10.1071/AP04007.

Boss, B.L., Wanees, A.E., Zaslow, S.J., Normile, T.G., Izquierdo, J.A., 2022. Comparative genomics of the plant-growth promoting bacterium *Sphingobium* sp. strain AEW4 isolated from the rhizosphere of the beachgrass *Ammophila breviligulata*. BMC Genomics 23, 1–14. https://doi.org/10.1186/s12864-022-08738-8.

Bouffaud, M.-L., Renoud, S., Moënne-Loccoz, Y., Muller, D., 2016. Is plant evolutionary history impacting recruitment of diazotrophs and *nifH* expression in the rhizosphere? Sci. Rep. 6, 21690. https://doi.org/10.1038/srep21690.

Burns, M.J., Nixon, G.J., Foy, C.A., Harris, N., 2005. Standardisation of data from real-time quantitative PCR methods – evaluation of outliers and comparison of calibration curves. BMC Biotechnol. 5, 1–13. https://doi.org/10.1186/1472-6750-5-31.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107.

Cha, J.-Y., Han, S., Hong, H.-J., Cho, H., Kim, D., Kwon, Y., Kwon, S.-K., Crüsemann, M., Bok Lee, Y., Kim, J.F., Giaever, G., Nislow, C., Moore, B.S., Thomashow, L.S.,

Weller, D.M., Kwak, Y.-S., 2016. Microbial and biochemical basis of a Fusarium wilt-suppressive soil. ISME J. 10, 119–129. https://doi.org/10.1038/ismej.2015.95.
Cobo Díaz, J., Baroncelli, R., Le Floch, G., Picot, A., 2019. Combined metabarcoding and co-occurrence network analysis to profile the bacterial, fungal and *Fusarium* communities and their interactions in maize stalks. Front. Microbiol. 10, 261. https://doi.org/10.3389/fmicb.2019.00261.
Cuesta, G., García-de-la-Fuente, R., Abad, M., Fornes, F., 2012. Isolation and identification of actinomycetes from a compost-amended soil with potential as biocontrol agents. J. Environ. Manag. 95, S280–S284. https://doi.org/10.1016/j.jenvman.2010.11.023.
De Corato, U., 2020. Disease-suppressive compost enhances natural soil suppressiveness against soil-borne plant pathogens: A critical review. Rhizosphere 13, 100192. https://doi.org/10.1016/j.rhisph.2020.100192.
de Mendiburu, F., 2023. agricolae: Statistical Procedures for Agricultural Research. https://doi.org/10.32614/CRAN.package.agricolae.
Evans, C.K., Xie, W., Dill-Macky, R., Mirocha, C.J., 2000. Biosynthesis of deoxynivalenol in spikelets of barley inoculated with macroconidia of *Fusarium graminearum*. Plant Dis. 84, 654–660. https://doi.org/10.1094/PDIS.2000.84.6.654.
Fu, L., Penton, C.R., Ruan, Y., Shen, Z., Xue, C., Li, R., Shen, Q., 2017. Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil Biol. Biochem. 104, 39–48. https://doi.org/10.1016/j.soilbio.2016.10.008.
Garbeva, P., Hol, W.H.G., Termorshuizen, A.J., Kowalchuk, G.A., de Boer, W., 2011. Fungistasis and general soil biostasis – A new synthesis. Soil Biol. Biochem. 43, 469–477. https://doi.org/10.1016/j.soilbio.2010.11.020.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.
Goswami, R.S., Kistler, H.C., 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5, 515–525. https://doi.org/10.1111/j.1364-3703.2004.00252.x.
Grubbs, F.E., 1969. Procedures for detecting outlying observations in samples. Technometrics 11, 1–21. https://doi.org/10.1080/00401706.1969.10490657.
Gruet, C., Abrouk, D., Börner, A., Muller, D., Moënne-Loccoz, Y., 2024. D genome acquisition and breeding have had a significant impact on interaction of wheat with ACC deaminase producers in soil or ACC deaminase potential activity in the rhizosphere. Soil Biol. Biochem. 198, 109392. https://doi.org/10.1016/j.soilbio.2024.109392.
Hong, S., Jv, H., Lu, M., Wang, B., Zhao, Y., Ruan, Y., 2020. Significant decline in banana Fusarium wilt disease is associated with soil microbiome reconstruction under chilli pepper-banana rotation. Eur. J. Soil Biol. 97, 103154. https://doi.org/10.1016/j.ejsobi.2020.103154.
Horinouchi, H., Muslim, A., Hyakumachi, M., 2010. Biocontrol of Fusarium wilt of spinach by the plant growth promoting fungus *Fusarium equiseti* GF183. J. Plant Pathol. 92, 249–254.
Hornby, D., 1983. Suppressive soils. Annu. Rev. Phytopathol. 21, 65–85. https://doi.org/10.1146/annurev.py.21.090183.000433.
Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x.
Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N., 1980. *Pseudomonas* siderophores: A mechanism explaining disease-suppressive soils. Curr. Microbiol. 4, 317–320. https://doi.org/10.1007/BF02602840.
Kyselková, M., Kopecký, J., Frapolli, M., Défago, G., Ságová-Marečková, M., Grundmann, G., Moënne-Loccoz, Y., 2009. Comparison of rhizobacterial community composition in soil suppressive or conducive to tobacco black root rot disease. ISME J. 3, 1127–1138. https://doi.org/10.1038/ismej.2009.61.
Kyselková, M., Moënne-Loccoz, Y., 2012. Pseudomonas and other microbes in disease-suppressive soils. In: Lichtfouse, E. (Ed.), Organic Fertilisation, Soil Quality and Human Health. Sustainable Agriculture Reviews, 9. https://doi.org/10.1007/978-94-007-4113-3_5.
Lahti, L., Shetty, S., et al., 2018. Introduction to the microbiome R package. http://microbiome.github.com/microbiome.
Leeman, M., van Pelt, J.A., Den Ouden, F.M., Heinsbroek, M., Bakker, P.A.H.M., Schippers, B., 1995. Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85, 1021–1027. https://doi.org/10.1094/Phyto-85-1021.
Legrand, F., Picot, A., Cobo-Díaz, J.F., Chen, W., Le Floch, G., 2017. Challenges facing the biological control strategies for the management of Fusarium head blight of cereals caused by *F. graminearum*. Biol. Control 113, 26–38. https://doi.org/10.1016/j.biocontrol.2017.06.011.
Legrand, F., Picot, A., Cobo-Díaz, J.F., Carof, M., Chen, W., Le Floch, G., 2018a. Effect of tillage and static abiotic soil properties on microbial diversity. Appl. Soil Ecol. 132, 135–145. https://doi.org/10.1016/j.apsoil.2018.08.016.
Legrand, F., Picot, A., Cobo-Díaz, J.F., Cor, O., Barbier, G., Le Floch, G., 2018b. Development of qPCR assays to monitor the ability of *Gliocladium catenulatum* J1446 to reduce the cereal pathogen *Fusarium graminearum* inoculum in soils. Eur. J. Plant Pathol. 152, 285–295. https://doi.org/10.1007/s10658-018-1473-0.
Legrand, F., Chen, W., Cobo-Díaz, J.F., Picot, A., Le Floch, G., 2019. Co-occurrence analysis reveal that biotic and abiotic factors influence soil fungistasis against *Fusarium graminearum*. FEMS Microbiol. Ecol. 95, fiz056. https://doi.org/10.1093/femsec/fiz056.
Leplat, J., Friberg, H., Abid, M., Steinberg, C., 2013. Survival of *fusarium graminearum*, the causal agent of fusarium head blight. A review. Agron. Sustain. Dev. 33, 97–111. https://doi.org/10.1007/s13593-012-0098-5.
Leslie, J.F., Summerell, B.A., 2006. The *Fusarium* Laboratory Manual. John Wiley and Sons, Ames. https://doi.org/10.1002/9780470278376.
Lockwood, J.L., 1977. Fungistasis in soils. Biol. Rev. 52, 1–43. https://doi.org/10.1111/j.1469-185X.1977.tb01344.x.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 1–21. https://doi.org/10.1186/s13059-014-0550-8.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17, 10–12. https://doi.org/10.14806/ej.17.1.200.
Mazurier, S., Corberand, T., Lemanceau, P., Raaijmakers, J., 2009. Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to Fusarium wilt. ISME J. 3, 977–991. https://doi.org/10.1038/ismej.2009.33.
Mazzola, M., 2002. Mechanisms of natural soil suppressiveness to soilborne diseases. Antonie Van Leeuwenhoek 81, 557–564. https://doi.org/10.1023/A:1020557523557.
McMurdie, P.J., Holmes, S.P., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.
Mitsuboshi, M., Kioka, Y., Noguchi, K., Asakawa, S., 2018. Evaluation of suppressiveness of soils exhibiting soil-borne disease suppression after long-term application of organic amendments by the co-cultivation method of pathogenic *Fusarium oxysporum* and indigenous soil microorganisms. Microbes Environ. 33, 58–65. https://doi.org/10.1264/jsme2.ME17072.
Mousa, W.K., Raizada, M.N., 2016. Natural disease control in cereal grains. Encycl. Food Grains 4, 257–263. https://doi.org/10.1016/B978-0-12-394437-5.00206-0.
Nannipieri, P., Hannula, S.E., Pietramellara, G., Schloter, M., Sizmur, T., Pathan, S.I., 2023. Legacy effects of rhizodeposits on soil microbiomes: a perspective. Soil Biol. Biochem. 184, 109107 https://doi.org/10.1016/j.soilbio.2023.109107.
Nejgebauer, V., Živković, B., Tanasijević, Đ., Miljković, N., 1971. Pedological map of Vojvodina, scale 1: 50000. Institute for Agricultural Research, Novi Sad.
Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., Joyce, D., 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol. Mol. Plant Pathol. 53, 17–37. https://doi.org/10.1006/pmpp.1998.0170.
Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Köljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47, D259–D264. https://doi.org/10.1093/nar/gky1022.
Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B, Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2022. Vegan: community ecology package. https://doi.org/10.32614/CRAN.package.vegan.
Ossowicki, A., Tracanna, V., Petrus, M.L.C., van Wezel, G., Raaijmakers, J.M., Medema, M.H., Garbeva, P., 2020. Microbial and volatile profiling of soils suppressive to *Fusarium culmorum* of wheat. Proc. Biol. Sci. 287, 20192527. https://doi.org/10.1098/rspb.2019.2527.
Pereyra, S.A., Dill-Macky, R., Sims, A.L., 2004. Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis. 88, 724–730. https://doi.org/10.1094/PDIS.2004.88.7.724.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., Moënne-Loccoz, Y., 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321, 341–361. https://doi.org/10.1007/s11104-008-9568-6.
Rasmussen, P.H., Knudsen, I.M.B., Elmholt, S., Jensen, D.F., 2002. Relationship between soil cellulolytic activity and suppression of seedling blight of barley in arable soils. Appl. Soil Ecol. 19, 91–96. https://doi.org/10.1016/S0929-1393(01)00177-9.
Renoud, S., Bouffaud, M.L., Dubost, A., Prigent-Combaret, C., Legendre, L., Moënne-Loccoz, Y., Muller, D., 2020. Co-occurrence of rhizobacteria with nitrogen fixation and/or 1-aminocyclopropane-1-carboxylate deamination abilities in the maize rhizosphere. FEMS Microbiol. Ecol. 96, fiaa062. https://doi.org/10.1093/femsec/fiaa062.
Rouxel, F., Sedra, H., 1989. Résistance des sols aux maladies (mise en évidence de la résistance d'un sol de la palmeraie de Marrakech aux fusarioses vasculaires). Al Awamia 66, 35–54.
Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., Paulitz, T., 2017. Disease suppressive soils: New insights from the soil microbiome. Phytopathology 107, 1284–1297. https://doi.org/10.1094/PHYTO-03-17-0111-RVW.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Siegel-Hertz, K., Edel-Hermann, V., Chapelle, E., Terrat, S., Raaijmakers, J.M., Steinberg, C., 2018. Comparative microbiome analysis of a Fusarium wilt

suppressive soil and a Fusarium wilt conducive soil from the Châteaurenard region. Front. Microbiol. 9, 568.
Sipilä, T., Yrjälä, K., Alakukku, L., Palojärvi, A., 2012. Cross-site soil microbial communities under tillage regimes: Fungistasis and microbial biomarkers. Appl. Environ. Microbiol. 78, 8191–8201. https://doi.org/10.1128/AEM.02005-12.
Stotzky, G., Torrence Martin, R., 1963. Soil mineralogy in relation to the spread of fusarium wilt of banana in Central America. Plant Soil 18, 317–337. https://doi.org/10.1007/BF01347232.
Su, L., Feng, H., Mo, X., Sun, J., Qiu, P., Liu, Y., Zhang, R., Kuramae, E.E., Shen, B., Shen, Q., 2022. Potassium phosphite enhanced the suppressive capacity of the soil microbiome against the tomato pathogen *Ralstonia solanacearum*. Biol. Fertil. Soils 58, 553–563. https://doi.org/10.1007/s00374-022-01634-z.
Sundberg, C., Al-Soud, W.A., Larsson, M., Alm, E., Yekta, S.S., Svensson, B.H., Sørensen, S.J., Karlsson, A., 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol. Ecol. 85, 612–626. https://doi.org/10.1111/1574-6941.12148.
Tamietti, G., Alabouvette, C., 1986. Résistance des sols aux maladies : XIII - Rôle des *Fusarium oxysporum* non pathogènes dans les mécanismes de résistance d'un sol de Noirmoutier aux fusarioses vasculaires. Agronomie 6, 541–548. https://doi.org/10.1051/agro:19860606.
Tamietti, G., Ferraris, L., Matta, A., Abbattista Gentile, I., 1993. Physiological responses of tomato plants grown in *Fusarium* suppressive soil. J. Phytopathol. 138, 66–76. https://doi.org/10.1111/j.1439-0434.1993.tb01361.x.
Tanasijević, Đ.M., Jeremić, M.R., Filipović, Đ.M., Aleksić, Ž.B., Nikodijević, V.Č., Antonović, G.M., Spasojević, M.S., 1964. Pedological Map of Western and Northwestern Serbia with the Surroundings of Belgrade. In: Scale 1: 50,000. Institute for Land Studies, Belgrade -Topčider.
Tang, T., Sun, X., Liu, Q., Dong, Y., Zha, M., 2023. Treatment with organic manure inoculated with a biocontrol agent induces soil bacterial communities to inhibit tomato Fusarium wilt disease. Front. Microbiol. 13, 1006878 https://doi.org/10.3389/fmicb.2022.1006878.
Tao, C., Li, R., Xiong, W., Shen, Z., Liu, S., Wang, B., Ruan, Y., Geisen, S., Shen, Q., Kowalchuk, G.A., 2020. Bio-organic fertilizers stimulate indigenous soil *Pseudomonas* populations to enhance plant disease suppression. Microbiome 8, 137. https://doi.org/10.1186/s40168-020-00892-z.
Todorović, I., Moënne-Loccoz, Y., Raičević, V., Jovičić-Petrović, J., Muller, D., 2023. Microbial diversity in soils suppressive to *Fusarium* diseases. Front. Plant Sci. 14, 1228749. https://doi.org/10.3389/fpls.2023.1228749.
Vacheron, J., Desbrosses, G., Bouffaud, M.-L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F., Prigent-Combaret, C., 2013. Plant growth-promoting rhizobacteria and root system functioning. Front. Plant Sci. 4, 356. https://doi.org/10.3389/fpls.2013.00356.
Valverde-Bogantes, E., Bianchini, A., Herr, J.R., Rose, D.J., Wegulo, S.N., Hallen-Adams, H.E., 2020. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can. J. Plant Pathol. 4, 315–329.
Van Bruggen, A.H.C., Francis, I.M., Jochimsen, K.N., 2014. Non-pathogenic rhizosphere bacteria belonging to the genera *Rhizorhapis* and *Sphingobium* provide specific control of lettuce corky root disease caused by species of the same bacterial genera. Plant Pathol. 63, 1384–1394.
Van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V., Salles, J., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc. Natl. Acad. Sci. USA 109 (4), 1159–1164.
Van Peer, R., Niemann, G.J., Schippers, B., 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81, 728–734. https://doi.org/10.1094/Phyto-81-728.
Weißbecker, C., Schnabel, B., Heintz-Buschart, A., 2020. Dadasnake, a snakemake implementation of DADA2 to process amplicon sequencing data for microbial ecology. GigaScience 9, giaa135. https://doi.org/10.1093/gigascience/giaa135.
Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40, 309–348. https://doi.org/10.1146/annurev.phyto.40.030402.110010.
Wickham, H., 2011. ggplot2. Wiley interdisciplinary reviews: computational statistics 3, 180–185.
Yu, Y., Lee, C., Kim, J., Hwang, S., 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 89, 670–679. https://doi.org/10.1002/bit.20347.
Yuan, X., Wang, B., Hong, S., Xiong, W., Shen, Z., Ruan, Y., Dini-Andreote, F., 2021. Promoting soil microbial-mediated suppressiveness against Fusarium wilt disease by the enrichment of specific fungal taxa via crop rotation. Biol. Fertil. Soils 57, 1137–1153. https://doi.org/10.1007/s00374-021-01594-w.
Zhang, H., Chen, W., Zhao, B., Phillips, L.A., Zhou, Y., Lapen, D.R., Liu, J., 2020. Sandy soils amended with bentonite induced changes in soil microbiota and fungistasis in maize fields. Appl. Soil Ecol. 146, 103378 https://doi.org/10.1016/j.apsoil.2019.103378.
Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., Shen, W., 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil 326, 511–522.