Perennial soil characteristics are the main factor driving in vitro inhibition of the wheat fungal pathogen Fusarium graminearum in a french case study

Lena Simon, Thierry Langin, Benjamin Nowak, Gaëlle Marliac

Received: 26 January 2023 | Revised: 22 November 2023 | Accepted: 24 November 2023

DOI: 10.1111/sum.12998

RESEARCH PAPER

Soil Use and Management | BRITISH SOCIETY OF SOIL SCIENCE | WILEY

Perennial soil characteristics are the main factor driving in vitro inhibition of the wheat fungal pathogen *Fusarium graminearum* in a French case study

Lena Simon[1,2] | **Thierry Langin**[1] | **Benjamin Nowak**[3] | **Gaëlle Marliac**[1,2]

[1]INRAE, GDEC, Université Clermont Auvergne, Clermont-Ferrand, France

[2]VetAgro Sup, Université Clermont Auvergne, Lempdes, France

[3]AgroParisTech, INRAE, VetAgro Sup, UMR Territoires, Université Clermont Auvergne, Clermont-Ferrand, France

Correspondence
Lena Simon, UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales, INRAE Site de Crouël, Université Clermont Auvergne, Clermont-Ferrand F-63000, France.
Email: lena.simonbousquet@gmail.com

Funding information
International Research Center on Sustainable AgroEcosystems (IRC-SAE, ISITE CAP20-25)

Abstract

Soils are termed 'suppressive' when they can limit the emergence and propagation of plant diseases. However, little is known regarding what factors determine suppressiveness and whether they could be improved for a given soil. Agricultural practices, such as tillage systems, influence the properties of the soil, and could be a lever to improve soil suppression of pathogens. In this study, we investigated the impact of soil type and crop management practices on soil suppressiveness towards *Fusarium graminearum*, a major winter wheat (*Triticum aestivum*) pathogen. As it is transmitted via crop residues left on soil, *F. graminearum* is susceptible to soil suppression. Soil suppressiveness has been evaluated in more than a 100 sampled fields in the Limagne plain (Puy-de-Dôme, France), to represent a great diversity of soil types (either calcisol, vertisol or fluvisol) and cropping systems (organic, reduced tillage or intensive cropping systems). The physicochemical composition of the sampled soils and crop management practices, identified through farmer surveys, were included in a generalized linear mixed-effect model to explain soil fungistasis. A fungistasis test was performed by putting a plug of PDA-agar inoculated with *Fusarium* in contact with the soil and measuring the area of fungal growth. This test revealed a wide variety of soil fungistatic properties, ranging from conducive soils (22% of soil covered by fungal growth) to very suppressive soils (1% of soil covered). Suppressiveness was related to soil perennial properties (p-value <.001) rather than practices. Overall, these results show that soil type and composition play a key role in fungistasis, and that soil suppressiveness is a complex, multifactorial process.

KEYWORDS

agroecology, agroecosystem, crop diseases, fungistasis, fusarium head blight, soil ecosystem services

1 | INTRODUCTION

More and more attention is given to the opportunities to harness soil ecosystem services that could play a part in pest regulation. In particular, soil microorganisms are critical to ecosystem services: they are involved in biogeochemical cycles that take place in the soil, they promote soil resilience and stability, and they interact directly with plants and plant pathogens (Ahemad & Kibret, 2014; Banerjee & van der Heijden, 2023; Welbaum et al., 2004). In some cases, soils can even prevent or directly limit the diseases caused by soil-borne crop pathogens. These soils, called suppressive, are defined by their capacity to limit the occurrence of disease even in presence of the pathogen (Scher & Baker, 1980). This property is therefore an interesting opportunity to limit the use of pesticides in agriculture.

The origin of soil suppressiveness is thought to be the soil microbiome, as is suggested by the complete or partial loss of the suppressive properties after sterilization (Boer et al., 1998; Scher & Baker, 1980). Despite the efforts to study soil suppression, descriptions of this phenomenon in situ are still lacking. Often, attention is given to particular strain of antagonist or synthetic communities, but the functioning of the microbiome is highly dependent on environmental conditions and interactions within the community (de Boer et al., 2019). Moreover, uncultured microbes could play a part in soil suppressiveness. The overall microbial diversity, abundance and activity that enable competition with pathogens are the result of soil perennial physicochemical properties such as carbon, pH or clay content. The microbiome is also shaped by variable soil properties (nitrogen, phosphorus, potassium and organic matter content) as well as overall land use (Mhete et al., 2020; Weyman-Kaczmarkowa & Pędziwilk, 2000; Xue et al., 2018). Crop management techniques can directly or indirectly impact the soil microbial life and activity (Welbaum et al., 2004). For instance, soil tillage, fertilization or liming modulate soil properties such as structure (Alletto et al., 2010), organic matter, nutrient content, and pH, while it has been shown that these properties influence soil microbiome. For example, higher organic carbon content promote soil microbial diversity (Esmaeilzadeh-Salestani et al., 2021). These various management practices consequently affect specific soil properties involved in pathogen control and soil fungistasis, i.e. the limitation of fungal growth and development by the soil. It has notably been observed by Palojärvi et al. (2020) that reduced tillage and no tillage increased soil suppressiveness towards *Fusarium culmorum* relatively to tilled soil. Additionally, soil variable characteristics could respond differently to agricultural practices (Viaud et al., 2018) and we lack the knowledge on the effects of agricultural practices on various soil types on fungistasis potential.

Part of the life cycle of *Fusarium graminearum* (*Fg*) occurs within the soil and could be affected by soil suppression. This fungus is the main species responsible for Fusarium Head Blight (FHB), one of the most important cereal diseases worldwide. *F. graminearum* produces toxins *in planta*, such as deoxynivalenol, that are harmful for animals and humans (Schmale III & Bergstrom, 2003). FHB decreases yield and causes major losses each year in Europe and the USA (Goswami & Kistler, 2004). To this day, the preventive use of fungicides remains one of the main resorts to fight FHB, along with agronomic practices such as crop rotation and soil tillage to bury crop residues. To better control this pathogen, it is fundamental to understand what determines its suppression in the top layer of soil, in contact with the crop residues. We hypothesized that soil fungistasis could be influenced by (i) soil perennial properties and (ii) agricultural practices through the variation of soil variable properties. We also hypothesized that practices such as organic amendments, residues management and cover crops that promote microbial diversity and activity might stimulate soil suppressiveness. To test these hypotheses, we provided a simple and efficient assessment of soil fungistatic properties towards *F. graminearum*, with samples from farm plots described with a detailed record of crop management practices. We sampled over a hundred plots in Limagne, Puy-de-Dôme (France), an important region with respect to cereal production, and often faced with important losses caused by FHB. To maximize the diversity of the crop management practices, we selected 19 farms conducted in either intensive or agroecological (organic or soil conservation) agriculture. This substantial sampling also allowed us to base this study on three soil types representative of this area. The aim of this study is to determine which physicochemical and agronomic factors could enhance soil fungistasis. This project is based on a large-scale approach to better represent field conditions and include several environmental factors.

2 | MATERIALS AND METHODS

2.1 | Context of the study area

The Limagne plain is a sedimentary formation, near the city of Clermont-Ferrand and crossed by the Allier River (Puy-de-Dôme, France, latitude: 45°53′13.73″ N, longitude: 3°14′20.19″ E) (Figure 1). It covers about 200,000 ha at an average elevation of 350 m. Pedogenesis has produced three main soil types: shallow to deep calcareous clay soils (calcisols), heavy and deep clays (vertisols) and sandy alluvium soils (fluvisols). The three main types of soils represented in the Limagne plain are notably distinguished by clay percentage, calcium carbonate percentage, pH(H_2O),

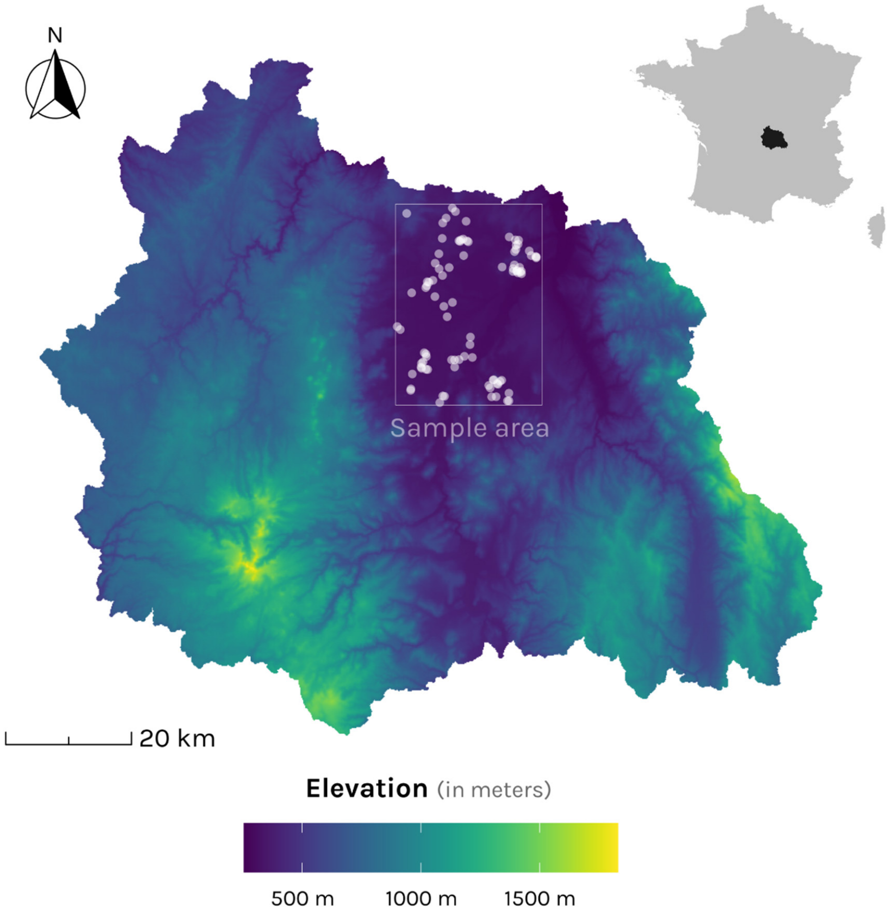

FIGURE 1 Sampling area in the department of Puy de Dôme, France. The samples were taken in the area inside the rectangle, that is approximately 25 km wide and 30 km long.

density and rock fragments (>2 mm) percentage (Nowak & Marliac, 2020; World Reference Base, FAO, n.d.).

The dominant arable land use is production of winter bread wheat (*Triticum aestivum*) and grain maize (*Zea mays*) (Agreste, 2010). Cereal production represents 64% of the agricultural area of the plain. The crops are mainly managed with intensive practices, with maize/wheat rotations. The average yields for wheat crops over the last 30 years range between 6 and 7 t/ha. Plots with maize in the rotation are often irrigated, and some of the plots use cover crops, mainly in conservation and organic farming.

The climate is semi-continental, and a mountainous relief to the West generates a Foehn effect that greatly reduces rainfall: the average annual precipitation was 573 mm between 2000 and 2018 (Météo France, 2019). For the same period, the mean annual temperature was 12.2°C. The average thermal amplitude is moderate (difference of 16.4°C between the average temperature of the hottest and coldest month) but extreme events can occur, especially during the summer when there are periods of several days with temperatures above 40°C. For the year of sampling, annual precipitation was 575.30 mm and average temperature was 13.0°C (Météo France, 2022).

2.2 | Plot characterization

Samples were taken from 103 plots from 19 farms in the plain of Limagne (Figure 1). The diversity of agricultural practices in the sample was maximized by selecting eight farms (24 plots) under organic farming, two (19 plots) under conservation farming, with minimum soil tillage (no ploughing) and use of cover crops and nine (60 plots) managed with intensive practices. Organic farming is defined by the absence of phytosanitary treatments and the use of organic amendments. Conservation farming aims to reduce soil tillage, as well as to diversify and maximize soil cover. Intensive farming can resort to soil tillage and/or phytosanitary treatments and is often less diversified regarding crop rotation.

Since these broad categories may cover a wide diversity of agricultural practices, surveys were conducted to define them more precisely. Agricultural practices during the year of sampling were characterized in detail for each plot with the farmers. The plots were characterized by agricultural practices that were relevant regarding microbial activity and fungistasis, i.e. practices that can influence soil organic matter, structure, and pathogen load.

Variables included in agricultural practices analyses comprised the sensitivity of the previous crop to *Fg*, the previous crop residue management, the type of soil tillage, organic amendments, the amount of nitrogen per hectare, the number of months without soil cover previous to the crop of the sampling year and the Treatment Frequency Index (TFI) (Table 1). The TFI is an indicator used to monitor pesticide use in European agriculture (Butault et al., 2011). This index corresponds to the number of full registration doses of the commercial products applied during the season (Jørgensen, 1999). It is calculated for each plot by adding every treatment performed on that plot with the following formula:

$$TFI = \sum \frac{Applied\ Dose}{Reference\ Dose} \times \frac{Treated\ Surface}{Total\ Surface}$$

The reference doses are provided by the French ministry of agriculture (Ministère de l'agriculture et de l'alimentation, 2022). These practices were differentially represented among the three main systems from which the plots originated. These data reflect the principles of each system with, for instance, no phytosanitary treatments or mineral fertilization on organic plots, and no soil inversion tillage in conservation agriculture (Table 2).

2.3 | Soil sampling

Bulk soil samples were collected in May 2021 during the wheat flowering period. The samples were taken at least 4 m from any border, at a 150 mm-depth. Five sampling points separated by 2 m were chosen for each plot and pooled. All the equipment was disinfected with ethanol between every plot.

2.4 | Physico-chemical analysis

The texture of the soil (clay, silt, and sand fractions, NF X31-107), and its chemical properties i.e. pH(H_2O) (NF ISO 10390), available phosphorus (Olsen method, NF ISO 11263), exchangeable potassium (NF ISO 23470), organic matter concentration (NF ISO 14235), carbonates concentration (NF 10693), Cationic Exchange Capacity (CEC) (NF ISO 23470) and DTPA-extracted iron and copper (NF X31-121) were measured by an agronomic analysis laboratory (SADEF).

TABLE 1 Agricultural practices variables included in the model.

Variable	Type	Encoding	Min	Max
Previous crop sensitivity	Discrete	0 = previous crop is not host to *Fusarium* 1 = previous crop is host to *Fusarium*	0	1
Crops residue management	Discrete	0 = residues were removed 1 = residues were left on the plot	0	1
Organic fertilization	Discrete	0 = no organic fertilization 1 = organic fertilization	0	1
Soil tillage	Discrete	0 = no tillage 1 = reduced tillage 2 = inversion tillage	0	2
Mineral N	Continuous	kg/ha of nitrogen during the year of sampling and before sampling	0	181.9
Soil cover	Discrete	Number of months without crops	0	4
Treatment frequency index (TFI)	Continuous	As calculated according to official dosage of each treatment (1 full dose = 1 unit)	0	6.24

TABLE 2 Agricultural practices representation among the three management systems. Discrete variables are expressed as percentages and continuous variables with their mean in each category.

	Intensive	Conservation	Organic
Residues left on plot	86%	95%	42%
Organic fertilization	6%	0%	37%
No tillage	4%	63%	0%
Reduced tillage	50%	37%	73%
Average mineral N (kg/ha)	167.2	173.5	0
Average months without cover	1.2	0.5	0.5
Average TFI	2	3.5	0

2.5 | Fungistasis test

The method used to assess fungal growth and soil suppressiveness was adapted from the surface method from Boer et al. (1998).

We used the strain *MDC_Fg1* previously isolated in field experiments (Alouane et al., 2021). Each soil was air dried after sampling and stored at room temperature before use. The samples were rehydrated with sterilized water to reach 33% of water content 8 days prior to the experiment. For every sample, 20 g of soil were placed in Petri dishes. The soils were then hydrated to reach water saturation. Discs of potato dextrose agar (PDA) medium were placed on the soil and inoculated with 100 μL of a suspension of *Fg* spores (10^6 spores/mL). Each soil inoculation was replicated three times. The dishes were then sealed with parafilm and incubated for 6 days at 23°C. Controls consisted of the same inoculated discs deposited in empty Petri dishes. Spore germination was measured after 6 days by image analysis with Fiji software from photos of the dishes. The area of hyphae development was used as the indicator for fungal growth, and expressed as a percentage of surface covered.

2.6 | Statistical analysis

The data was analysed with R software (version 4.0.5). The perennial physical (clay, silt and sand portions) and chemical (pH, CEC, iron and carbonates content) characteristics of the soils were used to project all plots in a Principal Components Analysis, and a hierarchical clustering on Principal Components was performed to classify soil in 5 subgroups. Only clay and sand were included to describe soil texture to avoid redundancy. Clustering was performed with the 'FactoMineR' package, and results were plotted with the 'factoextra' package. A generalized linear mixed model was then constructed following a quasi-binomial distribution with the glmmPQL function of the "MASS" package. The model included (i) soil perennial characteristics through the clustering output, (ii) soil variable characteristics (copper, phosphorus, potassium and organic matter), and (iii) agricultural practices (TFI, tillage, previous crop, residues), and (iv) the interaction between previous crop and residues. Pseudoreplication for each plot in the fungistasis test was included as a fixed effect. Collinearity of the model variables was analysed beforehand with the package 'olsrr'. Independence of the residuals was tested with a Durbin-Watson test ('car' package), followed by a Shapiro–Wilk test to assess the normality of the distribution using the 'stats' package. Pairwise comparisons of the clusters were performed on the output of the model with Tukey tests, with the 'emmeans' package.

3 | RESULTS

3.1 | Soils vary in suppressive potential

There was a wide variety in the responses of the soils to the strains. The *MDC_Fg1* strains showed hyphal growth ranging from 1% to 22% of the surface of soil with a mean of 9.00%, with the more permissive soils allowing growth more than 20-fold the growth of the more suppressive ones (Figure 2). All soils diminished fungal growth compared to the control that covered 79% of the surface of the Petri dish. High fungal growth was considered as the expression of a permissive soil, i.e. with low fungistatic potential. Low fungal growth was attributed to suppressiveness and high fungistatic potential. The eight most conducive soils seemed to differ from the rest of the distribution with an average growth exceeding 15%, whereas the rest of the values were equally distributed around the mean. There was no significant difference of fungal growth on soils from organic, conservation and intensive farming (p-value = .8533). All three systems presented soils with high, low and intermediate suppressive properties.

3.2 | Diversity of soils across the Limagne plain

The soils were grouped into five clusters, determined by their texture i.e. clay and sand content, as well as CEC, pH, iron, and carbonates content (Table 3). These variables explained 56.6% of the variance in the first axis and 24.9% in the second. The variables that contributed the most to the first axis of the PCA are the Iron, CEC, and sand content (respectively 20.8%, 20.7% and 19.6%), and the carbonates, clay content, and pH for the second axis (respectively 33.4%, 22.4% and 18.6%).

The first cluster, comprised of 12 soils, is composed of the most sandy soils with highest iron concentration, lowest pH, CEC, and carbonates content (Table 3). The second cluster consists of 16 sandy to silty soils (the average sand fraction of 388 g/kg being above the overall mean but lower than the first cluster) with no difference in pH, iron or carbon, and a significantly lower CEC. The third cluster (17 soils) represents clay soils with a pH slightly more acidic than average, higher iron content and less carbon. The fourth cluster (26 soils) consists of silty alkaline soils with high carbon and low iron concentration. Finally the fifth cluster (32 soils) includes alkaline clay soils, with high CEC and low iron content.

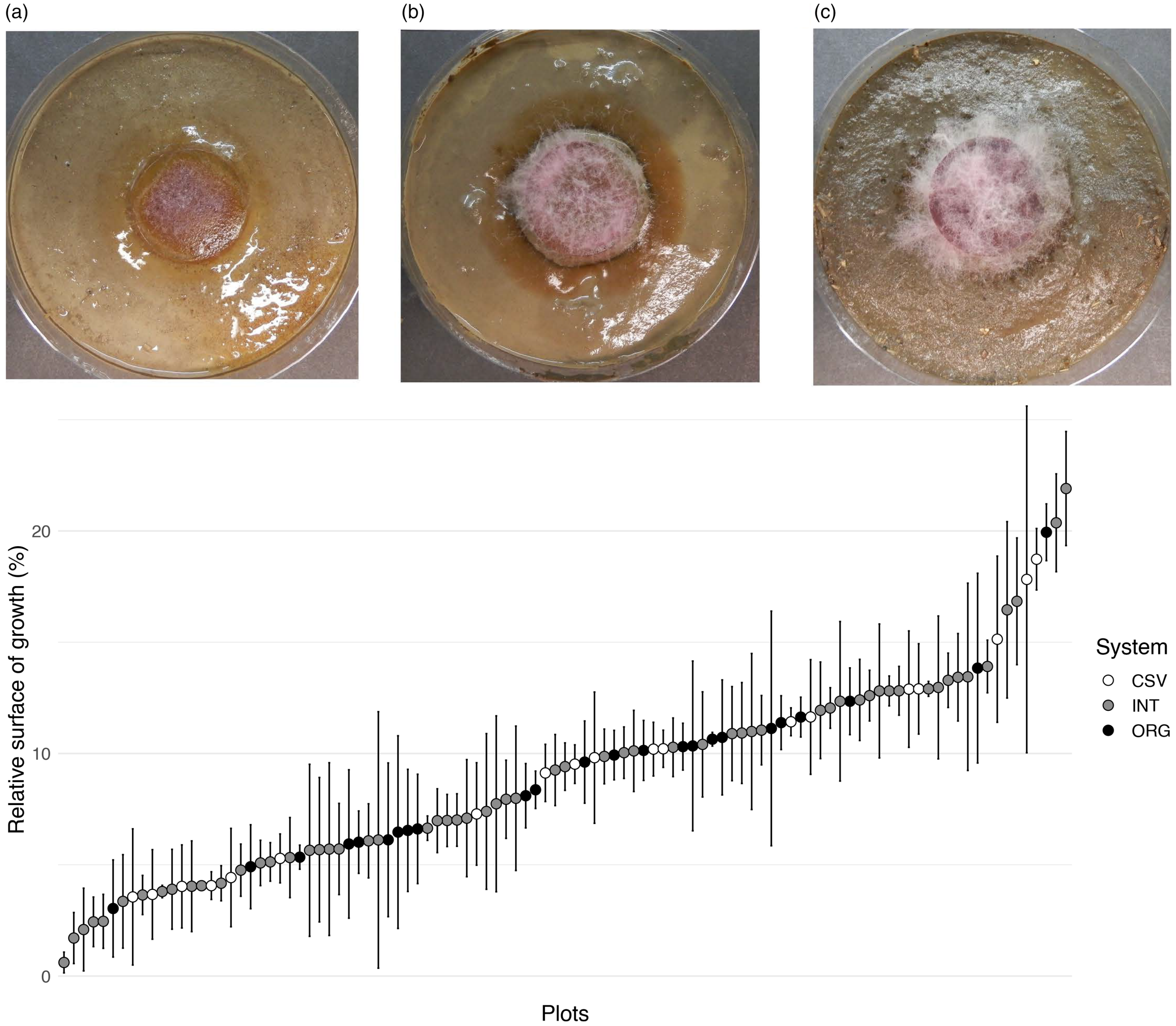

FIGURE 2 Relative growth of *Fusarium graminearum* on soils from 103 plots representing three types of crop management. Soils that exhibit smaller fungal growth are the most suppressive. Black (ORG) = organic farming, tillage, no pesticides; white (INT) = intensive farming, tillage, pesticides; grey (CSV) = soil conservation farming, no tillage, pesticides. Dots show the mean value for each soil sample and the errors bars show the standard deviation between the three replicates. Pictures featured above are examples of (a) suppressive, (b) intermediate and (c) conducive soils.

TABLE 3 Soil physicochemical characteristics of each cluster.

Variable	Mean in cluster 1	Mean in cluster 2	Mean in cluster 3	Mean in cluster 4	Mean in cluster 5	Overall mean
Clay	**205*** **	**348*** **	**588*** **	443	**587*** **	469
Sand	**542*** **	**388*** **	**174** **	216	**151*** **	254
Carbonates	**0.00** **	45.50	**1.59*** **	**215.96*** **	57.72	79.78
Fe	**95.17*** **	18.35	**47.76** **	**10.18*** **	**14.88** **	29.02
pH	**6.65*** **	7.94	**7.04*** **	**8.20*** **	**8.02** **	7.73
CEC	**141.17*** **	**287.38** **	391.8	372.0	**438.91*** **	356.02

Note: Variables whose mean in the cluster significantly differ from the overall mean after a *v* test (Husson, Lê and Pagès, 2016) are in bold. Lighter and darker cells signify significantly lower and higher values, respectively. Carbonates = total CO_3^{2-} (g/kg); CEC = cation-exchange capacity with cobaltihexamine method (mEq/kg); Clay = soil particles <2 µm (g/kg); Fe = Iron measured with DTPA method (mg/kg); pH = pH H_2O; Sand = soil particles >50 µm (g/kg).

Significance codes: *$p < .05$, **$p < .01$ and ***$p < .001$.

3.3 | Effects of soil type and agricultural practices

The generalized mixed model including soil type cluster, soil organic matter content, phosphorus, potassium, copper, TFI, tillage type, previous crop sensitivity to *Fg*, the management of residues, mineral nitrogen fertilization, and soil cover revealed that only soil cluster significantly affected soil fungistasis (p-value = .0005) (Table 4). Variable soil characteristics as well as agricultural practices did not influence fungal growth significantly in this model.

The output of the model was used to compare fungistatic potential between the five soil type clusters (Figure 4). The results were significantly different between soils of cluster 3 and clusters 1 (p-value = .0046), and 5 (p-value = .0094). Cluster 1 presented the lowest average value for fungal growth (5.6%), thus gathering the most suppressive soils. The highest values of fungal growth were found in cluster 3 that also presented the highest mean (10.3%). This cluster represents soils with the highest clay content, a relatively low pH, high iron and low carbon concentration.

TABLE 4 Analysis of deviance table (Type II Wald chi-squared tests) of the linear mixed-effect model including soil cluster and agricultural practices variables.

	Value	Std. error	DF	*t*-value	*p*-value
(Intercept)	−3.49	0.79	120	−4.41	.00
Previous crop	−0.32	0.48	42	−0.67	.51
Residues	−0.05	0.46	42	−0.11	.91
Reduced tillage	0.44	0.24	42	1.88	.07
Inversion tillage	0.32	0.27	42	1.19	.24
Organic matter	0.01	0.01	42	1.05	.3
P	−2.57	2.04	42	−1.26	.21
K	−0.13	0.32	42	−0.41	.68
Cu	0.04	0.03	42	1.68	.1
TFI	0.07	0.08	42	0.94	.35
Cluster 2	0.45	0.27	42	1.68	.1
Cluster 3	1.12	0.3	42	3.75	.00***
Cluster 4	0.46	0.33	42	1.4	.17
Cluster 5	0.18	0.33	42	0.55	.58
Cover	−0.07	0.21	42	−0.34	.73
N fertilization	0	0	42	−0.11	.92
Organic fertilization	0.03	0.31	42	0.11	.91
Previous crop: Residues	0.37	0.52	42	0.7	.49

Note: Formula = fg − previous. crop × residues + tillage + Organic. Matter + P + K + Cu + IFT + cluster + cover + N fertilization + Organic fertilization, random = ~1|plot.

Significance codes: *p < .05, **p < .01 and ***p < .001.

Soils belonging to this cluster tended to be more conducive to *Fg*, i.e. exhibit weaker suppressive properties.

Clusters 2, 4 and 5 presented average fungal growth of respectively 9%, 9.6% and 9.0% (Figure 3). Clusters 2 and 4 did not differ significantly from the other clusters.

4 | DISCUSSION

With an experimental design that reproduces the development of *Fusarium graminearum* in the field, this study showed striking differences in the suppressive power of soils against this fungus, even though the soil samples originated from a restricted area (approximately 750 km^2) (Figure 1). The most suppressive soil almost completely inhibited the growth of *Fusarium graminearum* (Figure 2). Although the absolute difference between the most and least suppressive soil was relatively limited compared to the control, this inhibition of pathogens in their saprotrophic stage at the field level could determine the potential for disease outbreak. These results should be put in perspective by other work regarding the suppression mechanisms that occur at different phases of the fungal

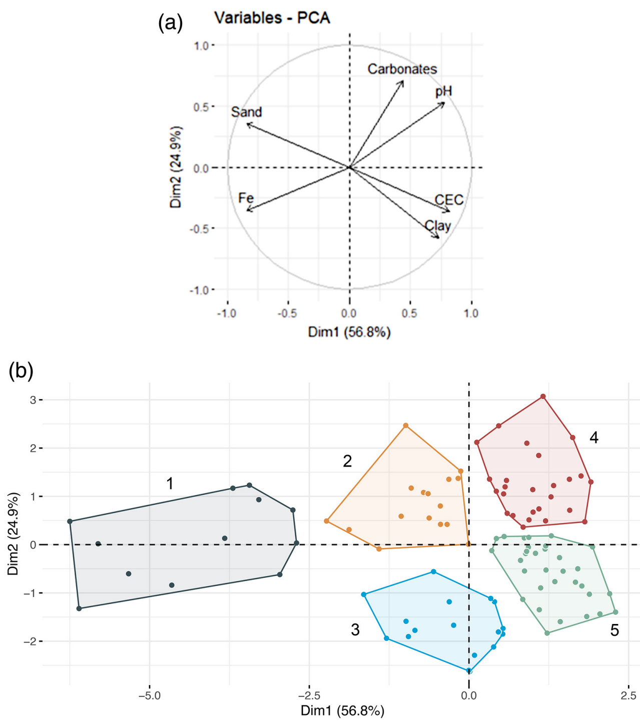

FIGURE 3 Hierarchical Clustering on Principal Components of soil samples based on perennial characteristics. (a) PCA of the perennial soil characteristics. Carbonates = total CO_3^{2-} (g/kg), CEC = Cation-Exchange Capacity with cobaltihexamine method (mEq/kg); Clay = soil particles <2 µm (g/kg); Fe = Iron measured with DTPA method (mg/kg); pH = pH H_2O; Sand = soil particles >50 µm (g/kg). (b) Hierarchical Clustering on Principal Components.

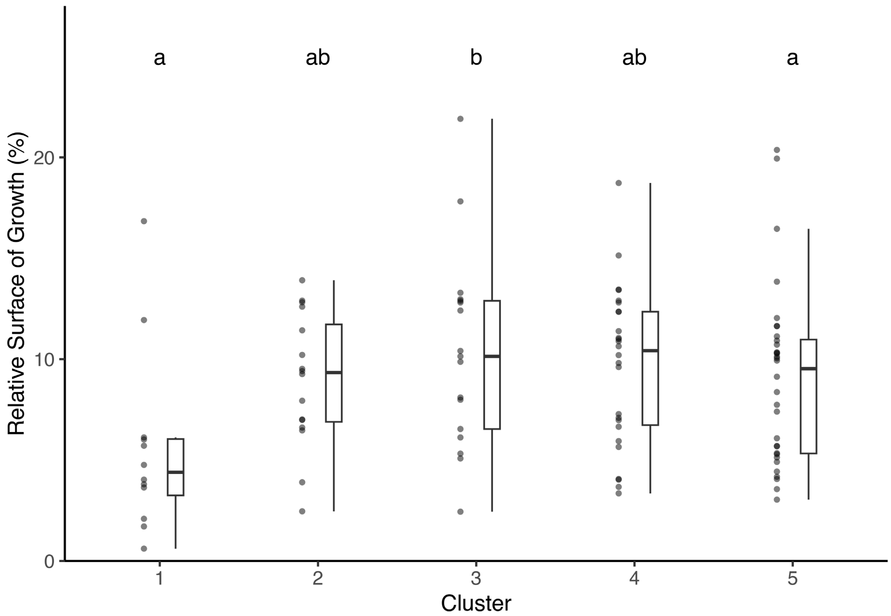

FIGURE 4 Growth of *Fusarium graminearum* on soil per soil cluster. Black dots represent raw data. Means not sharing any letter are significantly different by the Tukey-test at the 5% level of significance. The boxplot represents the first quartile, median and third quartile.

cycle: soils are also considered suppressive if they limit the impact of the pathogen indirectly by increasing plant immunity and overall fitness (Gómez Expósito et al., 2017).

We expected soil fungistasis to vary depending on parameters influenced by both soil type and agricultural practices. Agricultural practices can impact soil properties differentially depending on soil type: for instance, the effect of tillage on soil structure is deeply linked to the clay content of soils (Roger-Estrade et al., 2014). To be able to distinguish these factors, we proposed a generalized linear mixed-effect model including the soil type, determined beforehand with clustering on perennial soil characteristics, crop management data as well as variable soil characteristics that can be affected by those practices (Table 4). Notably, organic matter promotes bacterial abundance, diversity and activity and thus increases the chances for soil suppressiveness (Perez et al., 2008). In our model, only the soil perennial characteristics, in the form of soil cluster, significantly influenced pathogen inhibition. Sandy soils tend to have lower organic matter content but surprisingly, the sandiest soils (cluster 1), that were also slightly more acidic soils, exhibited stronger suppressive properties (Figure 4). Furthermore, organic matter content was not significantly associated with fungistasis in our model (Table 4). We expected soils with lower pH to favour the development of this fungus, as fungi generally thrive in acidic pH (Rousk et al., 2009) but the opposite was observed in cluster 3. However, the difference in pH is relatively moderate and this level of acidity might not be sufficient to favour fungi over bacteria. In general, and particularly in our samples, soil pH and iron content are strongly negatively correlated (results not shown). In this same cluster, iron content was indeed significantly higher than average. In a study comparing one suppressive and one non-suppressive soil, Kloepper et al. (1980) proposed that soil suppressiveness could be associated with the activity of *Pseudomonas* siderophores that limit the availability of iron. In this study, the authors showed that the addition of iron to the suppressive soil made it more conducive, as it was made available to the pathogen. However, in our case, the soils that have highest iron content (cluster 1) are also the most suppressive ones (Figure 4). This could be that iron is not the key component that explains fungistasis in this case.

Several studies have previously shown the influence of farming practices on the development of *F. graminearum* in the field, through their impact on the soil microbiome. The survival of *F. graminearum* on crop residues was investigated by Perez et al. (2008) on three distinct soils. They found green manure to increase the number of putative *Fusarium* antagonists. Sipilä et al. (2012) examined the impact of tillage on *F. culmorum* suppression on six agricultural fields, and found that microbial and fungal biomass were correlated with fungistasis, and that tillage reduced fungal biomass. De Corato et al. (2020) found that tomato-wheat rotation in a plot, compared with tomato monoculture, decreased the abundance of *Fusarium oxysporum* in the tomato rhizosphere and improved the health of the tomato plants cultivated on this soil. In a microcosm experiment with sandy soil, Bonanomi et al. (2017) found the frequent applications of organic amendments increased soil fungistasis towards four fungal species. Legrand et al. (2019) evaluated the effect of tillage and organic amendments on soil fungistasis in an assay including 31 wheat plots. They discovered that soils under minimum tillage practices exhibited stronger *F. graminearum* suppression than soils from plots under intensive tillage. Contrary to what was observed in previous work, in our results, the cropping system and specific agricultural practices were not associated with suppression. Studies that focus on a particular management practice provide precious insight but often lack the complexity of field conditions. Conversely, even with a large number of samples, it remains difficult to unravel the origin of these mechanisms in diversified environments.

In our experimental design, the fungus was able to grow thanks to a plug of PDA medium deposited on the soil. Fungal growth was therefore not limited by nutrients, as would be confirmed by the controls with solely the piece of PDA medium. According to Schlatter et al. (2017), soils can exhibit either general or specific suppressive properties. In the case of general suppression, the main mechanism is likely competition with soil microorganisms. In such conditions, the development of a wide variety of soil-borne pathogens can be limited (Weyman-Kaczmarkowa & Pędziwilk, 2000). Specific suppressive properties on the other hand could originate in antagonism mediated by particular microbial species rather than the entire microbial community. Given these results, the suppression regarding *F. graminearum* in our samples could be due to a specific suppression mechanism at the saprophytic stage, involving antagonistic microorganisms rather than a general suppression mechanism explained by competition. Additionally, the mechanism of suppression could vary among the sampled soils, depending on soil types and physicochemical properties. As fungal growth was inhibited even when the fungus not in direct contact with the soil, this suppression is likely the result of the production of Volatile Organic Compounds (VOCs) by the microbial community. This phenomenon has been observed on pathogens such as *Phytophthora capsica, Rhizoctonia solani, Fusarium oxysporum, and Pythium intermedium*, by isolated bacterial species as well as soil samples (de Boer et al., 2019; Syed-Ab-Rahman et al., 2019; Van Agtmaal et al., 2018). Notably, Van Agtmaal et al. (2018) found that inhibition of *F. oxysporum* by VOCs was increased by reduced till.

Our fungistasis test can be used to assess inhibition of other fungal pathogens. To go further, crop management practices should be studied retrospectively over longer time spans. This way, the cumulated years of specific land use that can affect the soil will be considered to explain specific soil properties at a given time.

5 | CONCLUSION

By taking into account soil characteristics and farming practices in the same study, we were able to identify the perennial soil characteristics as the main driver of soil fungistasis. Our work proposes a simple and efficient method to evaluate soil capacity to limit the development of a pathogen, as well as an insight on the impact of both crop management practices and soil type on this multifactorial phenomenon. This project is the first to undertake the study of over a 100 field samples with this approach.

ACKNOWLEDGEMENTS

The authors acknowledge the support received from the International Research Center on Sustainable AgroEcosystems (IRC-SAE, ISITE CAP20-25).

The authors would like to thank all the farmers that allowed sampling in their plots, Stéphane Sauvat for the collection of the samples, Julie Billom (VetAgro Sup) and Hannah Rey-Yang (Clermont Auvergne University) for proofreading the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Lena Simon https://orcid.org/0000-0003-2512-1178

REFERENCES

Agreste (2010) Recensement agricole 2010. http://agreste.agriculture.gouv.fr/recensement-agricole-2010/resultats-donnees-chiffrees/ (accessed December 13th, 2019).

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King saud University-science*, *26*, 1–20. https://doi.org/10.1016/j.jksus.2013.05.001

Alletto, L., Coquet, Y., & Roger-Estrade, J. (2010). Two-dimensional spatial variation of soil physical properties in two tillage systems. *Soil Use and Management*, *26*, 432–444. https://doi.org/10.1111/j.1475-2743.2010.00295.x

Alouane, T., Rimbert, H., Bormann, J., González-Montiel, G. A., Loesgen, S., Schäfer, W., Freitag, M., Langin, T., & Bonhomme, L. (2021). Comparative genomics of eight *Fusarium graminearum* strains with contrasting aggressiveness reveals an expanded open pangenome and extended effector content signatures. *International Journal of Molecular Sciences*, *22*, 6257. https://doi.org/10.3390/ijms22126257

Banerjee, S., & van der Heijden, M. G. A. (2023). Soil microbiomes and one health. *Nature Reviews. Microbiology*, *21*, 6–20. https://doi.org/10.1038/s41579-022-00779-w

Boer, W. D., Gunnewiek, P. J. A. K., & Woldendorp, J. W. (1998). Suppression of hyphal growth of soil-borne fungi by dune soils from vigorous and declining stands of *Ammophila arenaria*. *The New Phytologist*, *138*(1), 107–116.

Bonanomi, G., Gaglione, S., Cesarano, G., Sarker, T. C., Pascale, M., Scala, F., & Zoina, A. (2017). Frequent applications of organic matter to agricultural soil increase fungistasis. *Pedosphere*, *27*(1), 86–95. https://doi.org/10.1016/S1002-0160(17)60298-4

Butault, J., Delame, N., Jacquet, F., & Zardet, G. (2011). L'utilisation des pesticides en France: état des lieux et perspectives de réduction.

de Boer, W., Li, X., Meisner, A., & Garbeva, P. (2019). Pathogen suppression by microbial volatile organic compounds in soils. *FEMS Microbiology Ecology*, *95*(8), fiz105. https://doi.org/10.1093/femsec/fiz105

De Corato, U., Patruno, L., Avella, N., Salimbeni, R., Lacolla, G., Cucci, G., & Crecchio, C. (2020). Soil management under tomato-wheat rotation increases the suppressive response against *Fusarium* wilt and tomato shoot growth by changing the microbial composition and chemical parameters. *Applied Soil Ecology*, *154*, 103601. https://doi.org/10.1016/j.apsoil.2020.103601

Esmaeilzadeh-Salestani, K., Bahram, M., Ghanbari Moheb Seraj, R., Gohar, D., Tohidfar, M., Eremeev, V., Talgre, L., Khaleghdoust, B., Mirmajlessi, S. M., Luik, A., & Loit, E. (2021). Cropping systems with higher organic carbon promote soil microbial diversity. *Agriculture, Ecosystems and Environment*, *319*, 107521. https://doi.org/10.1016/j.agee.2021.107521

Gómez Expósito, R., de Bruijn, I., Postma, J., & Raaijmakers, J. M. (2017). Current insights into the role of rhizosphere bacteria in disease suppressive soils. *Frontiers in Microbiology*, *8*, Article 2529. https://doi.org/10.3389/fmicb.2017.02529

Goswami, R. S., & Kistler, H. C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*, *5*, 515–525. https://doi.org/10.1111/j.1364-3703.2004.00252.x

Husson, F., Lê, S., & Pagès, J. (2016). *Analyse de données avec R*. Presses Universitaires de Rennes.

Jørgensen, L. N. (1999). Denmarks action plant for pesticides: Status and role of research. *Nordic Agricultural Research.*, *81*, 197–203.

Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. N. (1980). Pseudomonas siderophores: A mechanism explaining disease-suppressive soils. *Current Microbiology*, *4*, 317–320. https://doi.org/10.1007/BF02602840

Legrand, F., Chen, W., Cobo-Díaz, J. F., Picot, A., & Floch, G. L. (2019). Co-occurrence analysis reveal that biotic and abiotic factors influence soil fungistasis against *Fusarium graminearum*. *FEMS Microbiology Ecology*, *95*(5), fiz056. https://doi.org/10.1093/femsec/fiz056

Météo France (2019) Station météorologique de Clermont-Ferrand. https://donneespubliques.meteofrance.fr/?fond=contenu&id_contenu=37 (accessed in 2019).

Météo France (2022) Station météorologique de Clermont-Ferrand https://donneespubliques.meteofrance.fr/?fond=contenu&id_contenu=37 (accessed in 2022).

Mhete, M., Eze, P. N., Rahube, T. O., & Akinyemi, F. O. (2020). Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. *Scientific African*, *7*, e00246. https://doi.org/10.1016/j.sciaf.2019.e00246

Nowak, B., & Marliac, G. (2020). Optimization of carbon stock models to local conditions using farmers' soil tests: A case study with AMGv2 for a cereal plain in Central France. *Soil Use and Management*, *36*(4), 633–645. https://doi.org/10.1111/sum.12608

Palojärvi, A., Kellock, M., Parikka, P., Jauhiainen, L., & Alakukku, L. (2020). Tillage system and crop sequence affect soil disease suppressiveness and carbon status in boreal climate. *Frontiers in Microbiology*, *11*, 534786. https://doi.org/10.3389/fmicb.2020.534786

Perez, C., Dill-Macky, R., & Kinkel, L. L. (2008). Management of soil microbial communities to enhance populations of *Fusarium graminearum*-antagonists in soil. *Plant and Soil*, *302*, 53–69. https://doi.org/10.1007/s11104-007-9455-6

Roger-Estrade, J. J., Labreuche, J., & Boizard, H. H. (2014). *Importance du travail du sol: Typologie des modes de mise en œuvre et effet sur le rendement des cultures*. Savoir Faire (Quae).

Rousk, J., Brookes, P. C., & Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology*, *75*(6), 1589–1596. https://doi.org/10.1128/AEM.02775-08

Scher, F. M., & Baker, R. (1980). Mechanism of biological control in a *Fusarium*-suppressive soil. *Phytopathology*, *70*(5), 412–417. https://doi.org/10.1094/PHYTO-70-412

Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., & Paulitz, T. (2017). Disease suppressive soils: New insights from the soil microbiome. *Phytopathology*, *107*(11), 1284–1297. https://doi.org/10.1094/PHYTO-03-17-0111-RVW

Schmale, D. G., III, Bergstrom, G. C., & Fusarium head blight [WWW document]. (2003). Fusarium Head Blight. Retrieved 17 May 2022 in https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/Fusarium.aspx

Sipilä, T. P., Yrjälä, K., Alakukku, L., & Palojärvi, A. (2012). Cross-site soil microbial communities under tillage regimes: Fungistasis and microbial biomarkers. *Applied and Environmental Microbiology*, *78*(23), 8191–8201. https://doi.org/10.1128/AEM.02005-12

Syed-Ab-Rahman, S. F., Carvalhais, L. C., Chua, E. T., Chung, F. Y., Moyle, P. M., Eltanahy, E. G., & Schenk, P. M. (2019). Soil bacterial diffusible and volatile organic compounds inhibit Phytophthora capsici and promote plant growth. *The Science of the total environment*, *692*, 267–280. https://doi.org/10.1016/j.scitotenv.2019.07.061

Van Agtmaal, M., Straathof, A. L., Termorshuizen, A., Lievens, B., Hoffland, E., & De Boer, W. (2018). Volatile-mediated suppression of plant pathogens is related to soil properties and microbial community composition. *Soil Biology and Biochemistry*, *117*, 164–174. https://doi.org/10.1016/j.soilbio.2017.11.015

Viaud, V., Santillàn-Carvantes, P., Akkal-Corfini, N., Le Guillou, C., Prévost-Bouré, N. C., Ranjard, L., & Menasseri-Aubry, S. (2018). Landscape-scale analysis of cropping system effects on soil quality in a context of crop-livestock farming. *Agriculture, Ecosystems and Environment*, *265*, 166–177. https://doi.org/10.1016/j.agee.2018.06.018

Welbaum, G. E., Sturz, A. V., Dong, Z., & Nowak, J. (2004). Managing soil microorganisms to improve productivity of agro-ecosystems. *Critical Reviews in Plant Sciences*, *23*(2), 175–193. https://doi.org/10.1080/07352680490433295

Weyman-Kaczmarkowa, W., & Pędziwilk, Z. (2000). The development of fungi as affected by pH and type of soil, in relation to the occurrence of bacteria and soil fungistatic activity.

Microbiological Research, *155*(2), 107–112. https://doi.org/10.1016/S0944-5013(00)80045-2

World Reference Base | FAO SOILS PORTAL | Food and Agriculture Organization of the United Nations [WWW Document], (n.d) Retrieved April 28, 2023. https://www.fao.org/soils-portal/data-hub/soil-classification/world-reference-base/en/

Xue, P.-P., Carrillo, Y., Pino, V., Minasny, B., & McBratney, A. B. (2018). Soil properties drive microbial community structure in a large scale transect in South Eastern Australia. *Scientific Reports*, *8*(1), 11725. https://doi.org/10.1038/s41598-018-30005-8

How to cite this article: Simon, L., Langin, T., Nowak, B., & Marliac, G. (2023). Perennial soil characteristics are the main factor driving in vitro inhibition of the wheat fungal pathogen *Fusarium graminearum* in a French case study. *Soil Use and Management*, *00*, 1–11. https://doi.org/10.1111/sum.12998