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Soil suppressiveness to *Pythium ultimum* in ten European long-term field experiments and its relation with soil parameters

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ABSTRACT

Soil suppressiveness to pathogens is defined as the capacity of soil to regulate soil-borne pathogens. It can be managed by agricultural practices, but the effects reported so far remain inconsistent. Soil suppressiveness is difficult to predict and for this reason different soil properties have been linked to it with the aim to find informative indicators, but these relationships are not conclusive. The objectives of this study were i) to test if soil suppressiveness is affected by long-term agricultural management such as tillage and organic matter (OM) addition; ii) to understand the direct and indirect relationships between soil suppressiveness and labile organic carbon fractions; and iii) to understand the relationship between soil suppressiveness and other chemical, physical and biological soil quality indicators. We measured soil suppressiveness with a bioassay using *Pythium ultimum* - *Lepidium sativum* (cress) as a model system. The bioassay was performed in soils from 10 European long-term field experiments (LTEs) which had as main soil management practices tillage and/or organic matter addition. We found that the site had a stronger influence on soil suppressiveness than agricultural practices. Reduced tillage had a positive effect on the suppressive capacity of the soil across sites using an overall model. Organic farming and mineral fertilization increased soil suppressiveness in some LTEs, but no overall effect of OM was found when aggregating the LTEs. Soil suppressiveness across LTEs was linked mainly to microbial biomass and labile carbon in the soil, but not to total soil organic matter content. From structural equation modelling (SEM) we conclude that labile carbon is important for the maintenance of an abundant and active soil microbial community, which is essential for the expression of soil suppressiveness. However, soil suppressiveness could only partly (25%) be explained by the soil parameters measured, suggesting that other mechanisms contribute to soil suppressiveness such as the presence and the activity of specific bacterial and fungal taxa with high biocontrol activity.

1. Introduction

Diseases caused by soil-borne pathogens are among the most important limiting factors for plant growth and productivity in agriculture (Oerke, 2006). Soils can regulate and suppress soil-borne pathogens to a certain extent, a capacity that is highly desirable when developing robust cropping systems that aim to rely less on chemical inputs. This capacity of the soil is known as soil suppressiveness to pathogens or disease suppressiveness of soils (throughout the manuscript we will refer to it as soil suppressiveness) and has been related to chemical, physical and biological soil parameters (Janvier et al., 2007). The capacity of soils to regulate soil-borne plant pathogens is an essential element of soil quality (Larkin, 2015). Previous investigations have shown evidence that biological, and in particular microbiological, properties play a crucial role in determining soil suppressiveness (Thuerig et al., 2009; Fuchs et al., 2014). General soil suppressiveness to pathogens relates to the activity, biomass and diversity of soil organisms and is based on the collective capacity of non-pathogenic constituents of soil and rhizosphere microbiomes to compete with and be antagonistic to pathogens. Specific soil suppressiveness to pathogens is the result of the presence of specific microbial taxa, such as *Pseudomonas* spp. and *Streptomyces* spp., which act as antagonists through antibiosis, and production of enzymes or siderophores (Schlatter et al., 2017). Specific suppressiveness is considered less persistent than

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general suppressiveness (Mazzola, 2002). Soil suppressiveness mechanisms and expression vary according to the pathogen considered. For some pathogens, soil suppressiveness it has often been detected, mainly as one type (e.g. specific soil suppressiveness for *Gaeumannomyces graminis* and *Fusarium* spp.), or as a combined effect of both suppressiveness types, e.g., *Rhizoctonia solani*, *Pythium* spp. (Postma et al., 2008; Cook, 2014; Yadav et al., 2015), while for others it has less often and more recently been observed, e.g., Meloidogyne spp. (Silva et al., 2018). For most soil pathogens the microorganisms and the mechanisms involved in soil suppressiveness are not know. However, soil suppressiveness probably originates from a combined effect of general and specific soil suppressiveness (Postma et al., 2008; Yadav et al., 2015).

Agricultural management can influence soil suppressiveness in the short as well as in the long term through its effects on soil physical, chemical and biological properties (Bailey and Lazarovits, 2003; Sánchez-Moreno and Ferris, 2007). Many studies have shown that compost addition can have a positive short-term effect on soil suppressiveness (Boehm et al., 1993; van Os and van Ginkel, 2001; Pascual et al., 2002; Bonanomi et al., 2007b, c; Chen and Nelson, 2008; Alfano et al., 2011). Fewer studies have addressed the short-term effects of other types of organic matter input such as manure addition (Aryantha et al., 2000; Darby et al., 2006; Tamm et al., 2010), or addition of other organic amendments (Stone et al., 2003) on soil suppressiveness. Although there is less information available regarding long-term management effects on soil suppressiveness, some studies indicate positive effects of long-term application of practices such as reduction of tillage intensity (Pankhurst et al., 2002; Peters et al., 2003; Campos et al., 2016; van Agtmaal et al., 2018), crop residue retention (Medvecky et al., 2007), crop rotation (Manici et al., 2005) and organic farming (Bonanomi et al., 2018a). Generally, intensive agricultural management (i.e. deep soil cultivation, mineral fertilizers, pesticides, and little organic matter supply) is associated with a decrease in soil biodiversity, including natural enemies and competitors of pathogens, pests and weeds, and consequently a decreased soil suppressiveness is expected (van Elsas et al., 2002; Crowder and Jabbour, 2014). However, the effect of management on soil suppressiveness can be variable, for example the effect of tillage (Yadav et al., 2015) or of organic matter input (Tamm et al., 2010) has been found to be contradictory. Expanding our knowledge on long-term agricultural practices that increase soil suppressiveness could contribute to the development of a more sustainable disease control in agricultural settings.

Soil suppressiveness is difficult to predict due to the interaction of different pathogenic and antagonistic species, heterogeneous distribution of pathogens at field, landscape and regional level, and the incomplete understanding of the mechanisms behind the phenomenon. Since direct measurement of soil suppressiveness using plant-pathogen systems is time-consuming, and requires infrastructure (e.g. growth chambers, clean benches) and trained staff, there is the need of indicators which can help in its assessment. However, the identification of such indicators is one of the main challenges of soil quality assessment in agriculture. Studies that aimed to identify relationships between soil suppressiveness and soil chemical, physical and biological parameters (Höper and Alabouvette, 1996; Darby et al., 2006; Postma et al., 2008) found inconsistent correlations probably depending on the pathogens and antagonists and the system under study (Janvier et al., 2007). Yet, some studies indicate that the quality of the organic matter may play an important role in soil suppressiveness (Hoitink and Boehm, 1999; Bonanomi et al., 2010; Dignam et al., 2018). Specifically, labile carbon fractions and their characteristics have been associated with soil suppressiveness (Darby et al., 2006; Saadi et al., 2010; van Overbeek et al., 2012; Cao et al., 2016). Labile carbon is a part of the total organic carbon which is available as a source of energy to microorganisms, therefore being correlated to microbial abundance and activity (Haynes, 2005). Labile carbon has received growing attention recently as a novel soil quality indicator and, in our previous work, it resulted to be linked with various soil quality indicators that have already been linked to soil suppressiveness (Bongiorno et al., 2019). As such, labile carbon might be important in soil suppressiveness because of its positive impact on general microbial activity and on pathogen antagonists' presence and activity. However, the mechanistic interactions between labile organic carbon, microbial biomass and activity, and soil suppressiveness have not been elucidated yet.

The objectives of the current study were i) to test if soil suppressiveness is affected by long-term agricultural management such as tillage and organic matter (OM) addition; ii) to understand the direct and indirect relationships between soil suppressiveness and labile organic carbon fractions; and iii) to understand the relationship between soil suppressiveness and other soil quality indicators (chemical, physical and biological). To this end, we sampled soils in different long-term field experiments selected from a range of pedoclimatic zones in Europe. We hypothesised that long-term reduced tillage and increased OM addition will result in higher soil suppressiveness, that labile organic carbon, through its positive effect on soil microbial biomass and activity will be an important driver for soil suppressiveness, and that soil suppressiveness will be linked more to soil biological than physical and chemical parameters.

2. Materials and methods

2.1. Experimental sites and management

We selected 10 European long-term field experiments (LTEs) with a minimum duration of 5 years and a mean duration of 19 years to investigate the effects of different intensities of tillage and organic matter management on soil suppressiveness (Fig. 1, Table S1). These LTEs were located in five European pedoclimatic zones: Dfb and Dfc (continental climate with cold winters and warm summers without a dry season or with cold winters and temperate summers without a dry season, respectively), Cfb and Csb (temperate climate with warm summer with or without dry season, respectively) and Bsk (arid cold steppe climate) (Köppen, 1918). In addition, the LTEs covered six different soil types (Vertic Cambisol, Haplic Luvisol, Haplic Fluvisol, Gleyic Podzol, Eutric Gleysol, and Eutric Cambisol (WRB, 2014) (Table S1).

Eight LTEs consisted of arable crops, two LTEs of permanent crops (PT1, ES4). All LTEs had individual tillage and fertilization regimes, which were classified in two main treatment factors: tillage (T) and organic matter addition (OM) (Fig. 1). The contrast in tillage was categorised as conventional tillage (CT, ploughing to 20–25 cm depth) versus reduced tillage (RT, tillage to 0–10 cm), the level of OM addition was categorised as low organic matter input (LOW, no organic matter additions or only mineral fertilization) versus high organic matter input (HIGH, organic matter additions or organic matter additions with mineral fertilizer) as in Bongiorno et al. (2019). LTEs had either a complete randomized block design or a split plot design with 3 or 4 replicates per treatment which was taken into account into the statistical models (Table S1).

2.2. Sampling procedure and sample handling

The soil samples were collected in spring 2016 before any major soil management was applied to the plots. Each sample comprised 20 soil cores, which were randomly collected in the central area of a plot to circumvent border effects. In the trials with tillage included in the management factor (CH1, CH2, NL1, NL2, SL1, HU4, ES4), samples were taken from 0 to 10 cm and 10–20 cm soil depth with the exception of NL1 experiment, where samples were taken from 0 to 15 cm and 15–30 cm (Table S1). For these tillage management trials, only the soil samples from the 0–10 cm (0–15 cm for NL1) were used. In the trials with fertilization as the only management factor (CH3, HU1, PT1), samples were taken from 0 to 20 cm soil depth, and this layer was used for the current study. The total number of samples used in this study

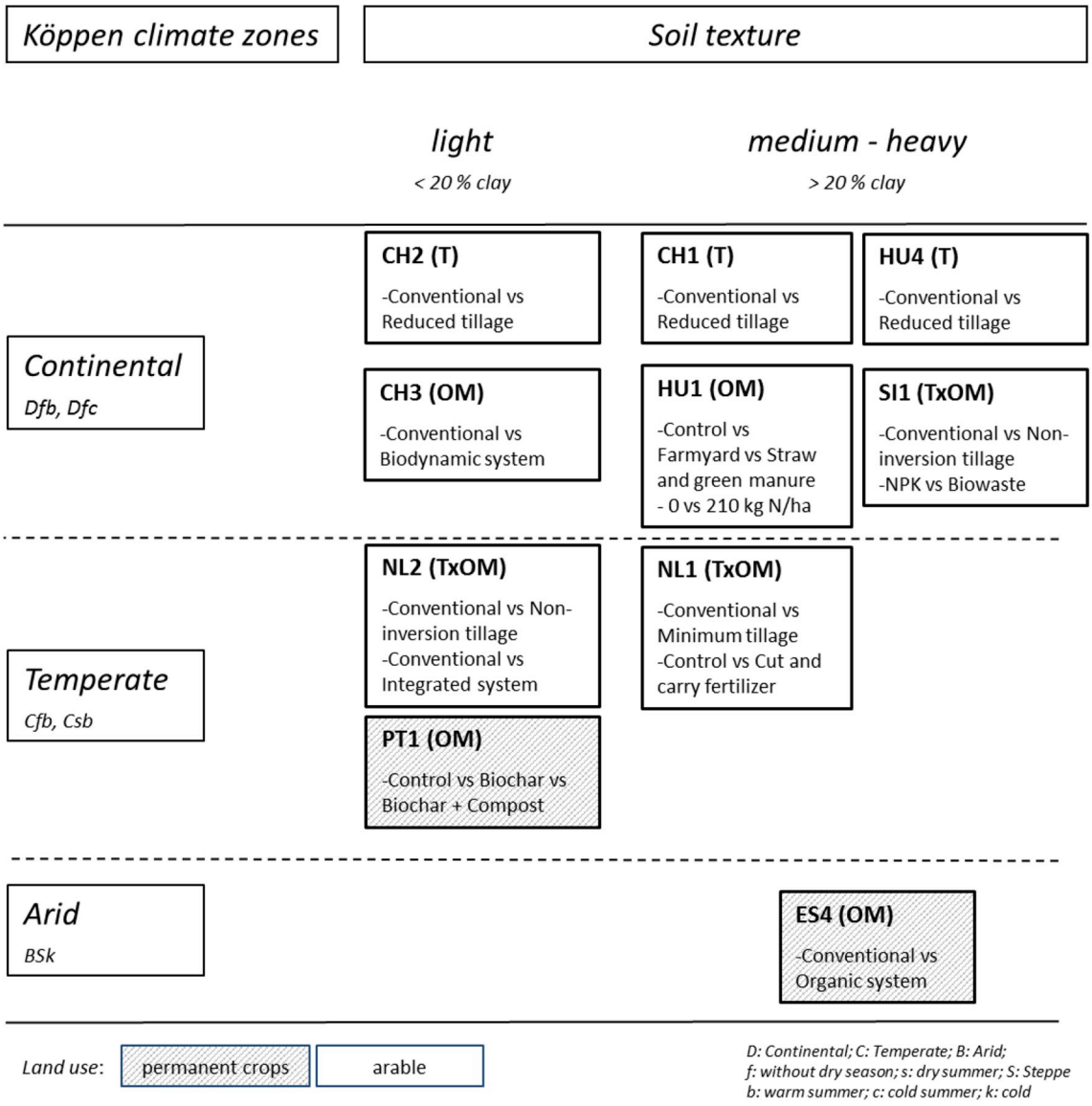

Fig. 1. Main pedoclimatic characteristics and management practices (i.e. tillage or organic matter input, or a combination of the two practices) of ten European long-term field experiments. *T* tillage, *OM* organic matter addition. *CH1* Frick trial, *CH2* Aesch trial, *HU4* Keszthely trial, *CH3* DOK trial, *HU1* Keszthely trial, *SL1* Tillorg trial, *NL2* de Peel trial, *NL1* BASIS trial, *PT1* Vitichar trial, *ES4* Pago trial. For detailed information about the experiments see Table S1 in the supplementary materials.

was 101. Upon collection, a subsample was air-dried (40 °C) and another part was stored field moist at 3 °C. Field-moist samples were sent in cooling boxes to Wageningen University (The Netherlands), the Research Institute of Organic Agriculture (FiBL, Frick, Switzerland), and the University Miguel Hernandez (Alicante, Spain), and dry samples were sent to the University of Trier (Germany) shortly after collection. Soil samples were sieved to 5 mm at the sampling location or immediately after shipping and, if field moist, stored at 3 °C. Biological parameters were assessed within 3 months, while chemical, and physical were assessed within 6 months after sampling. The soil suppressiveness bioassays were performed within one year after sampling. The soil suppressiveness measured with the *Pythium*-cress bioassay have shown in previous studies and trials to yield constant results in same soils for a period of two years (see results of the reference natural soil "REC", and the soil "THE" and "STC" in Thuerig et al. (2009) and Tamm et al. (2010)).

2.3. Chemical, physical and biological parameters

Several chemical, physical and biological soil parameters were measured by various laboratories and details about the methodology used are presented in Table 1.

2.4. Soil suppressiveness bioassays

We used *Pythium ultimum – Lepidium sativum* (cress) as a model pathosystem to test the soil suppressiveness under standardized laboratory conditions. The *P. ultimum* - cress bioassay has been successfully used as a model pathosystem (or indicator) for general disease suppressiveness in previous studies (Thuerig et al., 2009; Tamm et al., 2010).

The bioassay was based on the protocol of Tamm et al. (2010). In short, cress was sown on soils which had or had not been inoculated with *P. ultimum* two days before sowing. A *P. ultimum* concentration usually causing distinct disease symptoms but not complete yield losses was selected. The protocol of Tamm et al. (2010) was modified as

Table 1
Overview on methods used to determine chemical, physical, and biological parameters linked with soil functions as measured in the framework of the iSQAPER project, and the methods used to measure labile carbon fractions (Bongiorno et al., 2019).

Parameters	Methodology	Unit	Laboratory of analysis
Chemical parameters			
Total organic carbon (TOC)	SIST ISO 10694: Soil quality - Determination of organic and total carbon after dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
Total nitrogen (TN)	SIST ISO 13878:1999: Soil quality - Determination of total nitrogen content by dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
pH	$CaCl_2$ determination- SIST ISO 10390:2006: Soil quality - Determination of pH	–	University of Ljubljana (SL)
Cation exchange capacity (CEC)	ISO 13536:1995 - Soil quality - Determination of the potential cation exchange capacity and exchangeable cations using barium chloride solution buffered at pH = 8,1	mmol 100 g^{-1} soil	University of Ljubljana (SL)
Plant available phosphorus (P_2O_5)	ÖNORM L 1087 - modification: ammonium lactate extraction	mg kg^{-1} soil	University of Ljubljana (SL)
Available phosphorus (P-Olsen)	SIST ISO 11263-1996	mg kg^{-1} soil	University of Ljubljana (SL)
Plant available potassium (K_2O)	ÖNORM L 1087 - modification: ammonium lactate extraction	mg kg^{-1} soil	University of Ljubljana (SL)
Exchangeable magnesium, calcium, sodium, and potassium (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+})	ammonium acetate extraction; Soil survey laboratory methods manual, 1992	mg kg^{-1} soil	University of Ljubljana (SL)
Physical parameters			
Water stable aggregates (WSA)	Wet sieving method modified as in Kandeler (1996)	mg kg^{-1} soil	FiBL (CH)
Bulk density (BD)	Volumetric assessment with ring	g cm^{-3}	Field assessment by LTE owners
Silt, Clay and Sand	SIST ISO 11277:2011: Soil quality - Determination of particle size distribution in mineral soil material - Method by sieving and sedimentation	%	University of Ljubljana (SL)
Penetration resistance	Pressure needed to insert penetrometer in the soil	Mpa	Field assessment by LTE owners
Water holding capacity (WHC)	Calculated with a pedotransfer function using the % clay, silt and total organic carbon (Tóth et al., 2015)	%	Wageningen University & Research (NL)
Biological parameters			
Microbial biomass carbon (MBC)	Fumigation extraction method (Vance et al., 1987)	mg kg^{-1} soil	Trier University (DE)
Microbial biomass nitrogen (MBN)	Fumigation extraction method (Vance et al., 1987)	mg kg^{-1} soil	Trier University (DE)
Soil respiration	Incubation of soil at 25 °C for 72 h in thermostat bath	µg h^{-1} g^{-1} soil	University Miguel Hernandez (ES)
Earthworm abundance and biomass	Hand sorting from 30*30*30 cm^3 monolith	Number and fresh weight (g m^{-2})	Field assessment by LTE owners
Tea bag decomposition	Tea bag incubation (tea bag index) (Keuskamp et al., 2013)	g mass loss	Field assessment by LTE owners
Labile carbon fractions			
Dissolved organic carbon (DOC)	Extraction with ultrapure water and filtration at 0.45 µm filters.	mg kg^{-1} soil	Wageningen University (NL)
Hydrophilic dissolved organic carbon (Hy-DOC)	Fractionation of DOC with DAX-8 resin (Van Zomeren and Comans, 2007).	mg kg^{-1} soil	Wageningen University (NL)
Dissolved organic carbon and hydrophilic dissolved organic carbon specific ultraviolet absorbance (DOC SUVA and Hy SUVA)	Analysis of DOC and Hy solution with spectrophotometer at 254 nm (Weishaar et al., 2003; Amery et al., 2008).	L g C^{-1} cm^{-1}	Wageningen University (NL)
Permanganate oxidizable carbon (POXC)	Oxidation with K_2MnO_4 (Weil et al., 2003).	mg kg^{-1} soil	Wageningen University (NL)
Hot water extractable carbon (HWEC)	Extraction with hot water (80 °C) for 16 h and filtration at 0.45 µm filters (Ghani et al., 2003).	mg kg^{-1} soil	Wageningen University (NL)
Particulate organic matter carbon (POMC)	Suspension in NaCl for 15 h, wet-sieving through a 53 µm sieve and calculation of POM by loss on ignition (Salas et al., 2003).	mg kg^{-1} soil	FiBL (CH)

follows. Ten days before sowing the cress, inoculum of *P. ultimum* (culture code: Py1, 2005) originally isolated from tomato (provided and stored by Biointeraction and Plant Health, Wageningen Plant Research, The Netherlands) was produced on millet (24 g of sterile millet used as a substrate plus 20 ml of demineralized water) and incubated in the dark at 20 °C. Nine days before sowing the cress, autoclaved and non-autoclaved soil (see 2.4.1 and 2.4.2) was taken out of the cold room and incubated at 20 °C for one week to acclimatize and permit the re-activation of microorganisms. After eight days of mycelium growth, and two days before sowing the cress, the mycelium/millet culture was chopped and homogenized with a sterilized metal spatula. The homogenized *P. ultimum*/millet culture was then mixed with sand (1:80 (w/w)) to allow for a homogeneous distribution of *P. ultimum* in the soil. Subsequently, 10 g of the *P. ultimum*/millet/sand mixture was mixed per litre of soil to obtain a final concentration of 0.125 g of *P. ultimum*/millet culture per litre of soil. The test soils did not receive any fertilization.

The soil suppressiveness bioassays were run with two types of soil samples: (a) pooled LTE samples (section 2.4.1) and (b) management treatment samples (section 2.4.2.). All the bioassays were run in the laboratory facilities of Unifarm, Wageningen University and Research and executed by the first author.

2.4.1. Soil suppressiveness bioassay with pooled LTE samples

To assess the soil status before pathogen inoculation and the soil suppressiveness in the different LTEs, equal parts of soil (approximately 100 ml) were collected from each treatment replicate in a given LTE. These samples from different treatments were pooled and mixed to

obtain 1 L of soil for each LTE (further called 'pooled LTE samples'). This resulted in 10 pooled LTE samples, one for each of the 10 LTEs. To confirm the biological nature of soil suppressiveness, half of each pooled LTE sample (0.5 L) was autoclaved at 121 °C for 20 min to exclude the majority of the soil microorganisms, including soil pathogens (Trevors, 1996). The other half was not manipulated and both 0.5 L samples were stored for up to 2 day at 3 °C before conducting biosassays. One week before the inoculation, autoclaved and not autoclaved soils were placed in a climate chamber at 20 °C to permit stabilization of the microbial communities (soil equilibration).

The experimental setup included 10 autoclaved and 10 non-autoclaved pooled LTE samples, two dosages of *P. ultimum* (0, i.e. no *P. ultimum* added, and 0.125 g L^{-1} of *P. ultimum*/millet/sand mixture added), and 4 replicates per *P. ultimum* inoculum concentration (a total of 160 pots). The inoculated and non-inoculated soils were placed in plastic polypropylene containers (Ø 133 cm, 0.5 L) perforated at the top and pre-incubated in the dark at 20 °C for two days. After this pre-incubation, each soil was used to fill 4 replicate pots (Ø 6 cm, 95 ml). Each pot was sown with 0.5 g untreated biological seeds of *L. sativum* (De Bolster, Epe, The Netherlands). The pots were placed on individual plant saucers to avoid cross-contamination between different soils and treatments. Pots were completely randomized and incubated in a growth chamber at 23 °C (day) and 18 °C (night) with a day-length of 16 h and 80% relative humidity (Unifarm, Wageningen University, The Netherlands). For the first two days after sowing, a plastic sheet covered the pots to prevent evaporation and ensure 100% relative humidity for germination. After two days, the plastic sheet was removed and the pots were irrigated from below when needed. Seven days after sowing, shoot fresh weight in each pot was assessed by cutting the shoots with scissors directly above the ground.

2.4.2. Soil suppressiveness bioassay with management treatment samples to compare management treatments within individual LTEs

To assess the effect of management treatments on soil suppressiveness, bioassays were run in 10 separate batches, one for each LTE. The procedure was identical to that for the pooled samples, with the exception that no autoclaved soils were included. For each LTE, all soil samples collected in the field (i.e. the number of management treatments X number of treatment replicates, resulting in a total number of 101 samples for all the LTEs) were tested with two dosages of *P. ultimum* (0 and 0.125 g L^{-1}) with four replicate pots per *P. ultimum* inoculum concentration (this resulted in a total of 808 pots across all the bioassays performed with the management treatment samples). Trial CH3 was repeated in order to check the reproducibility of the bioassay (Fig. S2, Table S6). In the statistical analyses, the mean of the four replicate pots per *P. ultimum* inoculum concentration was used.

2.4.3. Calculation of soil health and soil suppressiveness indices

To characterise the soil status before inoculation, a soil health index was calculated for pooled LTE samples as follows:

$$SHI\ (\%) = 100 * (Wn * Wa^{-1}) \quad (1)$$

where *Wn* = shoot weight of cress in pots with natural soil not inoculated with *P. ultimum*, and *Wa* = mean cress weight in autoclaved soil not inoculated with *P. ultimum*.

In our study we use the term soil health not as a synonym for soil quality, but we use it taking into account its association with soil biota (Bünemann et al., 2018). We consider a soil as healthy in which disease outbreaks are limited (similarly to Janvier et al. (2007)). In our case the autoclaved soils showed the possible growth in the absence of pathogens.

As a measure for robustness of soils towards inoculation with *P. ultimum*, soil suppressiveness indices were calculated as follows:

(a) For the non-autoclaved pooled LTE samples and the non-autoclaved management treatment samples,

$$SSni\ (\%) = 100 * (Wni * Wn^{-1}) \quad (2)$$

where *Wni* = shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and *Wn* = mean shoot weight in natural soil not inoculated with *P. ultimum*.

(b) For autoclaved pooled LTE samples,

$$SSai\ (\%) = 100 * (Wai * Wa^{-1}) \quad (3)$$

where *Wai* = shoot weight of cress in pots with autoclaved soil inoculated with *P. ultimum*, and *Wa* = mean cress weight in autoclaved soil not inoculated with *P. ultimum*.

2.5. Statistical analysis

All statistical calculations were performed using R version 3.3.2 (R Development Core Team, 2013). For the linear mixed effects model and the generalized least square model, the packages *nlme* (Pinheiro et al., 2018), and *emmeans* (Lenth et al., 2018) were used, for the multiple linear regression and the correlation analysis the packages *car* and *stats* were used. For the structural equation model the *lavaan* and *piecewiseSEM* package was used (Rosseel, 2012; Lefcheck, 2018).

For each pooled LTE sample, the effect of the four different soil treatments (natural soil, natural soil with *Pythium,* autoclaved soil, and autoclaved soil with *Pythium*) on the fresh weight of cress was analysed with one-way ANOVA followed by a Tukey's HSD post-hoc test to assess significant differences between treatments.

The effects of the agricultural treatments on the soil suppressiveness (SSni) were assessed by linear mixed effect models (LMEs). The LMEs were run independently for each LTE. Tillage and/or organic matter addition were included as fixed factors while, depending on the trial, block, main plot and subplot were introduced as random factors to take the nested design of the experiments into account. In addition, a model merging all the LTEs, and one merging only the trials were tillage was part of the management factor (CH1,CH2,NL1,NL2,SL1, ES4 and HU4) was run to test the effect of tillage and organic matter addition on soil suppressiveness. In this case tillage and organic matter addition were included as fixed factors while, LTE, main plot and subplot were introduced as random factors. The results were considered statistically significant at $p \leq 0.05$. The effects of tillage and organic matter addition and their interaction on soil suppressiveness (SSni) were assessed by analysis of variance (function *anova*) on the linear mixed effect model. For all the models, normality and homogeneity of variances of the residuals were checked both visually (plotting sample quantiles *versus* theoretical quantiles and residuals *versus* fitted values) and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). For these tests, results were considered statistically significant at $p \leq 0.05$. When the ANOVA indicated a statistically significant effect at $p \leq 0.05$, Tukey's HSD post-hoc test was used to assess significant differences between treatments.

Spearman's rank order correlation was used to examine relationships between soil suppressiveness (SSni) and biological, physical, and chemical soil quality parameters, including labile carbon fractions (bivariate correlations). For the correlation analyses, data from the management treatments samples were used (n = 101). The relationship between soil suppressiveness and other soil parameters was validated using partial correlations, correcting for variation caused by the intrinsic differences of the LTEs (pedoclimatic zones). To rank the relative importance of the variables in predicting soil suppressiveness (SSni), we standardized all the variables by subtracting the mean and dividing the result by the standard deviation. Thereafter we performed linear mixed model regression with SSni as the dependent variable and the chemical, physical and biological parameters as explanatory variables, checking one after the other. To take the nested structure of the experimental design of the LTEs (Table S1) into account, we allowed the slope and the intercept to vary depending on the LTE (random slope and intercept model) (Zuur, 2009). The variables that resulted to be significant

($p \leq 0.05$) in explaining variation in SSni were selected and used in multiple mixed model regression, but only after discarding variables which were highly correlated ($\rho > 0.80$). T-values are reported to quantify the contribution of each predictor to the model (Field et al., 2012). We applied manual stepwise regression, and we selected the final model with the *anova* function and the Akaike Information Criterion (AIC) (Field et al., 2012). We used a multiple regression model with only the LTEs as random intercept, because it appeared that this model did not differ significantly from a model with random slope and intercept. All the models were checked for normality and homogeneity of the residuals.

Piecewise structural equation modelling (SEM) was used to evaluate the direct and the indirect effects of the labile carbon fractions on SSni, taking into account the dependent structure of the data coming from the same LTE (Lefcheck, 2016). For this reason, the LTE was used as random factor in the analysis. We established an *a priori* model including the main physical, chemical and biological variables and labile carbon fractions that appeared to be of importance for SSni according to the results obtained in the correlation and the multiple regression model analyses and according to ecological mechanisms (Fig. S1). The hypothesised relationships acted as a framework for the optimization of the piecewise SEM. The data matrix was fitted using the log-transformed variables, and SSni was logit transformed. The evaluation of the AIC was used to estimate the robustness of the models and to select the appropriate final model (Shipley, 2013). The Fisher Chi-square test (χ^2; the model has a good fit when $0 \leq \chi^2/\text{d.f.} \leq 2$ and $p \geq 0.05$) was used to test the overall goodness of fit of the model (Lefcheck, 2016). We calculated and reported the total standardized effects of the predictors on soil suppressiveness (SSni).

3. Results

3.1. Characterisation of sites (pooled LTE samples)

3.1.1. Soil health status

The growth of cress in native and autoclaved pooled LTE samples (without inoculation) was compared (Fig. 2) to characterise the 'health status' of soils, and a soil health index (SHI) was calculated (relative growth of cress in natural soils compared to the growth of cress in soils after removal of the majority of microorganisms by autoclaving).

Growth of cress on natural pooled LTE samples showed high variability between LTEs. After autoclaving, growth of cress was similarly high in all pooled LTE samples (fresh weight about 3 g, Fig. 2), with the exception of CH1, where autoclaving decreased the cress weight compared to the natural soil (−79%, $p < 0.05$) (Fig. 2), mirrored in a soil health index above 100% (Fig. 3A). Cress grew very poorly on natural soils from PT1, ES4 and NL2 (fresh weight below 2 g) (Fig. 2), and the related soil health indices were all below 50% (Fig. 3A, Table S2). On natural soil from SL1, cress showed intermediate growth (average fresh weight 2.2 g) (Fig. 2) and the related soil health index was 79% (Fig. 3). In natural soils from CH1, CH2, NL1, HU1 and HU4, cress showed good and similar (Fig. 2, n compared to a) growth (fresh weight > 2.5 g), and soil health indices were between 87% and 107%, with the exception of CH1 (SHI of 180%, see above).

3.1.2. Soil suppressiveness to Pythium ultimum

In natural pooled LTE samples inoculated with *P. ultimum*, cress reached on average 68% of the biomass of non-inoculated natural soils (mean soil suppressiveness index of natural soils, SSni) (Fig. 2, n compared to ni, Fig. 3B). In autoclaved pooled LTE samples inoculated with *P. ultimum*, cress reached between 0 and 20% of the biomass compared to non-inoculated soils (soil suppressiveness index of autoclaved soils, SSai) (Fig. 2, a compared to ai, Fig. 3C).

In natural soils, ES4 and HU4 showed the highest soil suppressivenes indices SSni (90% and 78%, Fig. 3B). However, the fresh weight of cress showed different situations: in ES4 we observed low fresh weight in non-inoculated soil and comparable low fresh weight in inoculated soils, while in HU4 the cress fresh weight was high in non-inoculated soil and comparably high in inoculated soils (Fig. 2, n compared to ni). In all other LTEs, cress weight was significantly reduced in natural inoculated compared to non-inoculated soils ($p < 0.05$) (Fig. 2). Soil suppressiveness indices SSni were lowest in soils from SL1, NL2 and CH3 (Fig. 3B) (average SSni of 46%, 57%, and 60%, respectively, see also Table S2).

3.2. Influence of management treatments on soil suppressiveness

We tested the effect of tillage and organic matter-based additions on soil suppressiveness in each LTE separately. Tillage did neither affect cress fresh weight in non-inoculated soils nor the soil suppressiveness index (SSni) in any of the six LTEs including tillage as a management factor (CH1, CH2, NL1, NL2, SL1 and HU4) separately (Table 2). However, reduced tillage resulted in higher yield in natural soils and higher SSni than conventional tillage, when testing the effect of tillage in an overall model with all the LTEs included (Table 3, $p = 0.05$) and a model with only the LTEs including tillage in the management factor (Table S3, $p = 0.01$).

In two (CH3, ES4) out of three system comparison trials (ES4, CH3 and NL2), significant effects of management were observed. In ES4, soil suppressiveness to *P. ultimum* (SSni) as well as the fresh weight of cress in natural, non-inoculated soils was higher in plots that were managed organically compared to plots that were managed conventionally ($p = 0.04$ and $p = 0.008$, respectively). Similar results were found in CH3, with significantly higher weights of cress in soil from the biodynamic than from the conventional treatment (Table 2). At the same time, however, soil suppressiveness in CH3 was not affected by soil management (Table 2). In one (HU1) out of four organic matter addition trials (PT1, HU1, NL1, and SL1), significant management effects on performance of cress were found. In HU1, SSni was significantly higher ($p = 0.005$) in plots that had received mineral N fertilization either alone or in combination with organic fertilizers (farmyard manure or straw plus green manure). In NL1 and SL1, the cut and carry fertilizer and the bio-waste application, respectively, did neither affect SSni nor growth of cress on native non-inoculated soils (Table 2). In PT1, we found a tendency ($p = 0.06$) towards lower SSni when biochar (either alone or in combination with compost) was added to the soil as compared to the non-amended control soil (Table 2). In the overall model taking into account all the LTEs we did not observe an effect of organic matter additions on the fresh weight of plants in natural soil nor on the soil suppressiveness (SSni) (Table 3).

3.3. Correlations of soil suppressiveness with soil parameters

Bivariate correlation analysis showed that soil suppressiveness (SSni) (calculated from the management treatment samples) was positively associated with higher values of various chemical (pH, total N, cation exchange capacity (CEC), Ca and K), physical (water holding capacity (WHC), silt, clay, penetration resistance), microbial parameters (microbial biomass C and N (MBC and MBN), soil respiration (SR), microbial quotient (qMic), tea bag decomposition, earthworm number and biomass, and labile carbon fractions (hydrophilic dissolved organic carbon (Hy-DOC), permanganate oxidizable carbon (POXC) and hot water extractable carbon (HWEC)) (Table S4). In contrast, we found negative correlations with C to N ratio (C/N), bulk density (BD), sand, dissolved organic carbon and hydrophilic organic carbon specific ultraviolet absorbance (DOC SUVA and Hy SUVA). The partial correlation showed that after normalization for structural differences between the LTEs (i.e. for the pedoclimatic characteristics) higher values of total N, MBC, soil respiration, qMic, earthworm number, Hy SUVA, POXC, HWEC and carbon in the particulate organic matter (POMC) were associated with higher values of SSni, while higher values of C to N ratio, tea bag decomposition and DOC SUVA were associated with lower

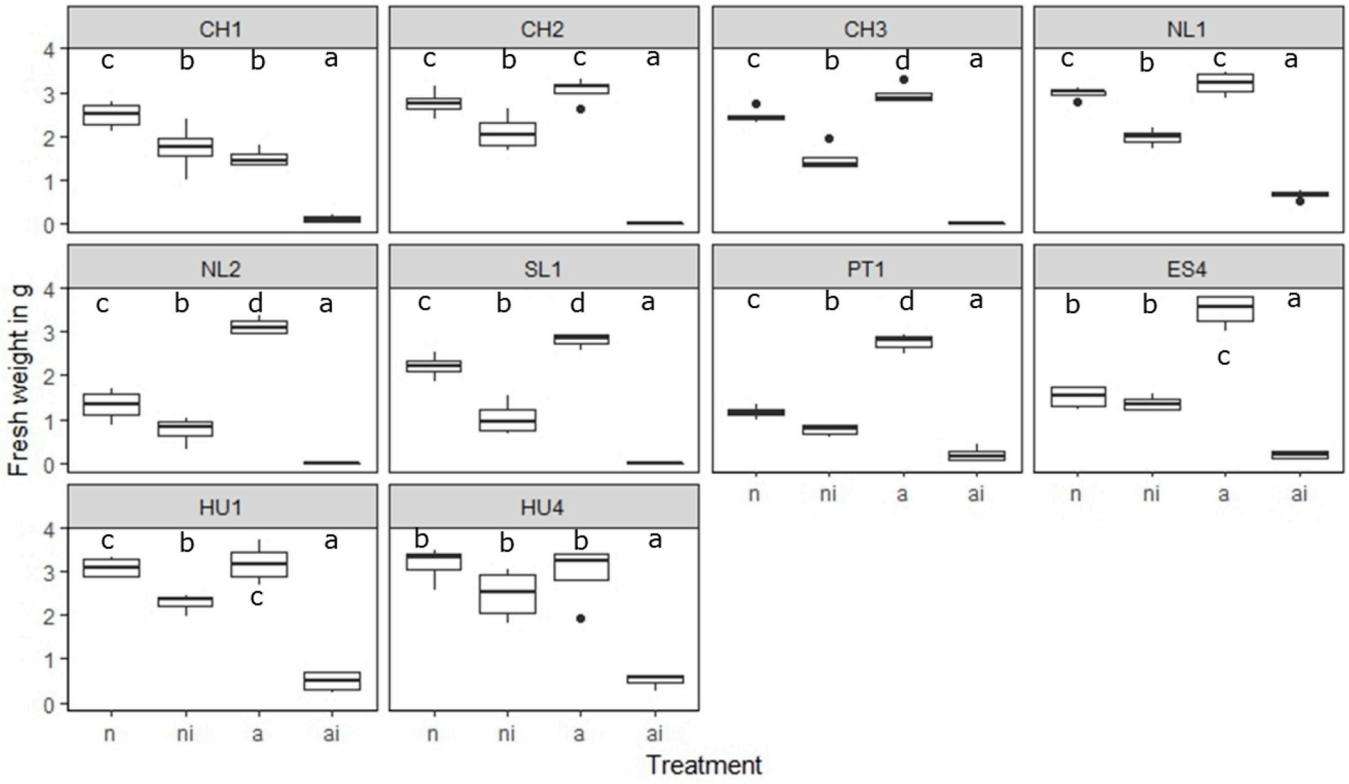

Fig. 2. Shoot fresh weight of *L. sativum* grown in natural or autoclaved LTE pooled soil samples not inoculated or inoculated with *P. ultimum*. *n* = natural soil, *ni* = natural soil inoculated with *P. ultimum*, *a* = autoclaved soil, *ai* = autoclaved soil inoculated with *P. ultimum*. *CH1* Frick trial, *CH2* Aesch trial, *CH3* DOK trial, *NL1* BASIS trial, *NL2* De Peel trial, SL1 Tillorg trial, *PT1* Vitichar trial, *ES4* Pago trial. *HU1* Keszthely trial, *HU4* Keszthely trial. For a detailed description of the trials we refer to Table S1. The boxes in the graph summarize the results of 4 individual pot replicates and represent the values between the 25th and the 75th percentiles, the horizontal line within a box is the median, and the extending lines represent the minimum and the maximum values. The black dots close to the boxes are observations which are considered outliers. Letters indicate significant differences between treatments in each long-term field experiment at $p \leq 0.05$ tested with ANOVA followed by a Tukey HSD post-hoc test.

values of SSni (Table 4).

3.4. Multiple regression and structural equation model (SEM) with soil parameters and soil suppressiveness

The mixed linear regression models carried out for each soil parameter revealed that the variables C to N ratio, sand and silt, WHC, MBC and MBN, and HWEC (Table 5) significantly explained the variation in SSni in the LTEs.

Since sand was highly correlated with silt and WHC and MBC was highly correlated with MBN ($\rho > 0.80$), only WHC, MBC, the C to N ratio and HWEC were retained for the multiple mixed linear model. The most important variable for explaining SSni resulted to be MBC (Table 6).

The structural equation model (SEM) fitted to investigate the direct and indirect effects of the labile carbon fractions on the SSni indicated that the HWEC, POXC and water holding capacity (WHC) had an indirect positive effect on SSni through their positive effects on microbial biomass carbon (MBC) (Fig. 4). In particular, within the labile carbon fractions only the POXC revealed a highly significant ($p = 0.0007$) positive effect on the microbial biomass carbon. The piecewise SEM explained 25% of variation in the SSni.

4. Discussion

4.1. Quality of the bioassay

The quality of the bioassay was considered good as we obtained relatively low variability between replicate plots and highly reproducible results (Fig. S2 and Table S6). This is in line with results from Thuerig et al. (2009). Shoot fresh weight is a good measure for the combined effect of *P.ultimum* on germination and growth of cress. In the short time of the bioassay (7 d) we expect that differences in the level of nutrients have been negligible and did not affect the results of the bioassay.

4.2. Soil health and suppressiveness indeces in the pooled LTE samples

Cress fresh weight in native non-inoculated soils from pooled LTE samples differed significantly between the LTEs, with low yields in NL2, PT1 and ES4, high yields for CH1, CH2, CH3, NL1, HU1, HU4 and an intermediate yield in SL1. After autoclaving of soils, the fresh weight was high and similar in all - except one (CH1) - LTEs. It is well known that autoclaving (as any other type of sterilization) can make nutrients available by killing organisms (Trevors, 1996). Nevertheless, autoclaving of soils has been used extensively before to assess the effect of living microorganisms and/or pathogens on growth/suppressiveness (van Os and van Ginkel, 2001; Medvecky et al., 2007; Mitsuboshi et al., 2018). Yet, the facts that (i) none of the soils was nutrient-deficient before autoclaving (Table S5) (ii) the cress bioassay is very short (6 days in total) and consequently does not require a lot of external nutrients (cress can even been grown on simple filter paper, as done in many germination experiments (Buss and Masek, 2014; Luo et al., 2018)), and (iii) all soils reached similar levels of biomass after autoclaving of soils (Fig. 2, a) indicate that the main growth-limiting factor for cress in native soils is of biological nature.Thus, we hypothesize that the observed yields in natural soils reflect mainly the outcome of the competition between putatively present soil-borne pathogens and

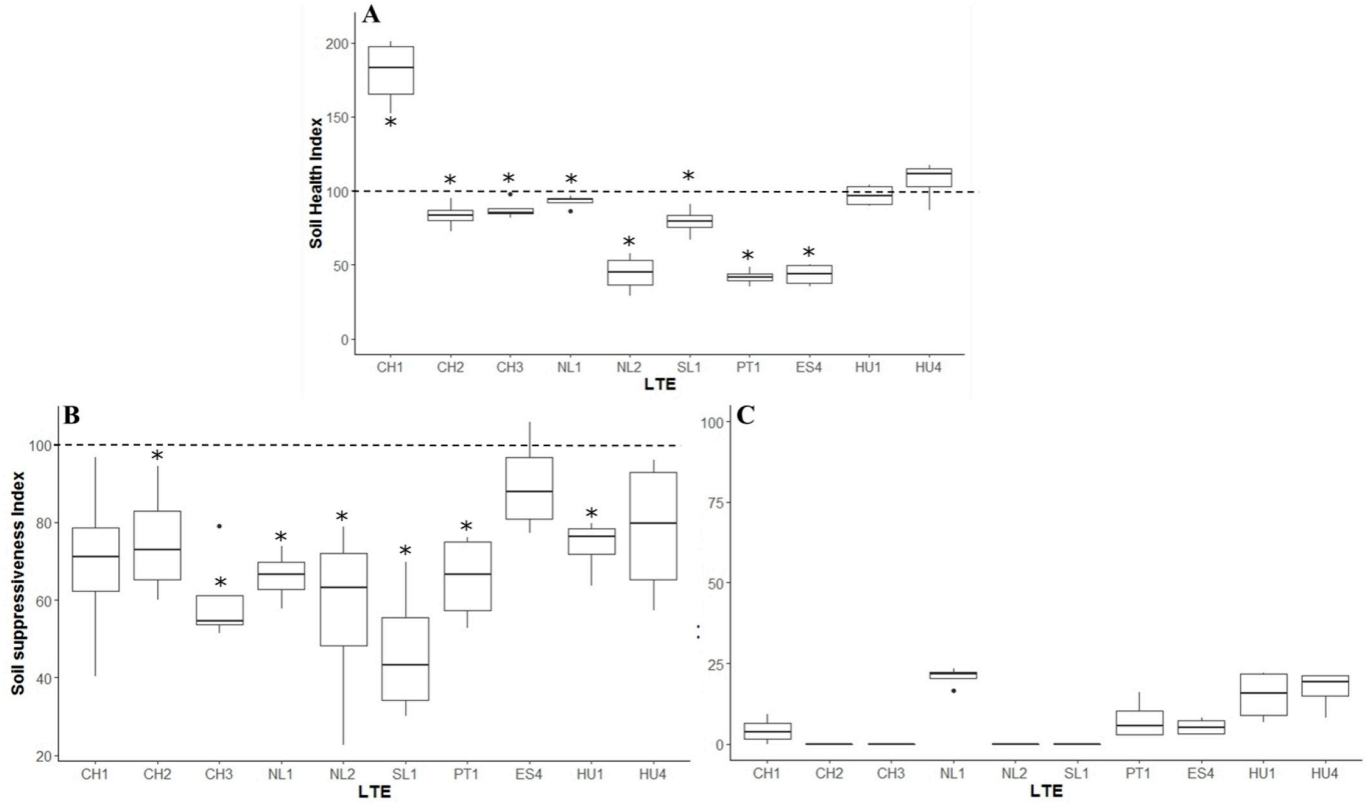

Fig. 3. Soil Health Index in the pooled LTE samples calculated for A) natural non-inoculated soils relative to autoclaved non-inoculated soils (Soil Health Index, a measure of the natural disease pressure in the soil). Soil suppressiveness index in the pooled LTE samples was calculated for B) natural soils inoculated with *P. ultimum* relative to natural non-inoculated soil (Soil Suppressiveness Index in natural inoculated soil (in the text SSni), a measure of soil suppressiveness upon pathogen addition), and C) inoculated autoclaved soils relative to non-inoculated autoclaved soils (Soil Suppressiveness Index in autoclaved inoculated soil (in the text SSai)). The boxes in the graph depict the results of four individual pots and represent the values between the 25th and the 75th percentiles, the horizontal line within a box is the median, and the extending lines represent the minimum and the maximum values. The black dots close to the boxes are observations that are considered outliers. The asterisks in panel A and C indicate statistical significant difference ($p \leq 0.05$) from the line which is set to 100. *CH1* Frick trial, *CH2* Aesch trial, *CH3* DOK trial, *NL2* De Peel trial, *NL1* BASIS trial, *SL1* Tillorg trial, *PT1* Vitichar trial, *ES4* Pago trial, *HU1* Keszthely trial, *HU4* Keszthely trial.

beneficial soil microbiota.

CH1 was the only site where the cress yield in autoclaved soils was lower than in natural non-inoculated soils. We speculate that the autoclaving process either released some toxic elements (i.e. manganese, aluminium), ammonium (NH_4−N), nitrite or organic compounds. Autoclaving soils is known to reduce soil organisms, but also nutrients and salts are released, and the soil structure is disrupted (Razavi Darbar and Lakzian, 2007). The high values of organic matter, total nitrogen and labile organic carbon present in CH1 (Table S5) could have facilitated the release of toxic elements or substances during autoclaving (Jager et al., 1968; Sonneveld and Mulder, 1979).

Native soils from pooled LTE samples differed in their capacity to mitigate the impact of inoculation with *P. ultimum*. The suppressiveness index (SSni) ranged between 46% and 90% on natural soils, and these values are in the same range as those found by Thuerig et al. (2009) and Tamm et al. (2010) for the same (CH3) or other natural soils. In the autoclaved soils, soil suppressiveness was dramatically reduced after inoculating the soil with *P. ultimum* as reported before in other studies (van Os and van Ginkel, 2001; Knudsen et al., 2002; Thuerig et al., 2009; Gravel et al., 2014; Löbmann et al., 2016), confirming the biological nature of soil suppressiveness against *P. ultimum*. Soil suppressiveness to *Pythium* spp. has been often reported and ascribed to mechanisms of both general and specific soil suppressiveness (Postma et al., 2005; Adiobo et al., 2007; Alfano et al., 2011; Oberhaensli et al., 2017), and also to abiotic mechanisms such as nutrient availability and physical properties (Adiobo et al., 2007; Löbmann et al., 2016).

Taken together, our results underline that soils from different fields have specific characteristics (chemical, physical but in particular biological) which have a diverse potential to interact with pathogens.

In this study we found that high soil suppressiveness can coincide with high yield (HU1 and HU4), but also with low yield in non-inoculated natural soils (ES4), resulting in large differences in yields in natural inoculated soils. These results emphasize the importance of taking into account both parameters (yield in natural non-inoculated soil together with measures of soil suppressiveness) when assessing suppressiveness of soils. For agriculture, the ideal soil is a soil combining high initial yield and high suppressiveness, as observed for HU1 and HU4.

The calculation and evaluation of soil health and suppressiveness indices from the pooled LTE samples, permitted a rapid and general characterisation of differences in soil health between sites, and for the assessment of the biological nature of the phenomenon of soil suppressiveness.

4.3. Effect of soil management practices on soil suppressiveness

We found several significant long-term management effects on yield (fresh weight in non-inoculated soils) and SSni within sites (Table 2, Table 3). However, these effects were smaller than the differences between the sites. This result is in accordance with previous studies (Knudsen et al., 2002; Tamm et al., 2010; Löbmann et al., 2016).

We found higher values of cress shoot fresh weight and soil suppressiveness in reduced tillage compared to conventional tillage when combining the trials together in overall models, which is in accordance

Table 2
Effect of different tillage (T) and organic matter additions (OM) on cress shoot yield (g) in natural non-inoculated soil and soil suppressiveness index (SSni). Least square means, standard errors (in parentheses) and *F* and *p* values for mixed linear effect models are reported for the different type of tillage and fertilization. Mean and standard errors were calculated from the biological (spatial) replicates in each long-term field experiment (LTE). Differences are considered significant at $p \leq 0.05$ (values ≤ 0.05 are given in bold).

Long term field experiment (LTE)	Management		Fresh weight in non-inoculated soil (g)	Soil suppressiveness index (SSni)* (%)
CH1	CT		2.72 (0.1)	81 (5.5)
	RT		2.67 (0.1)	78 (5.5)
	Tillage (T)	F	0.13	0.14
		p	0.73	0.72
CH2	CT		3.02 (0.2)	90 (3.3)
	RT		2.98 (0.2)	87 (3.3)
	Tillage (T)	F	0.04	0.54
		p	0.84	0.49
NL1	CT		3.98 (0.1)	51 (5.5)
	CT-Cut and carry fertilizer		4.18 (0.1)	59 (5.5)
	RT		4.25 (0.1)	60 (5.5)
	RT-Cut and carry fertilizer		4.35 (0.1)	68 (5.5)
	Tillage (T)	F	5.74	1.94
	Organic matter (OM)	*p*	0.14	0.30
		F	3.22	1.82
		p	0.13	0.23
NL2	CT-Conventional		1.80 (0.3)	19 (3.5)
	CT-Integrated		1.76 (0.3)	20 (3.5)
	RT-Conventional		2.43 (0.3)	21 (3.5)
	RT-Integrated		2.07 (0.3)	22 (3.5)
	Tillage (T)	F	3.06	0.15
	Organic matter (OM)	*p*	0.14	0.71
		F	0.26	0.02
		p	0.63	0.88
SL1	CT-Mineral		2.20 (0.2)	51 (3.5)
	CT-Biowaste		2.26 (0.2)	46 (3.5)
	RT-Mineral		2.67 (0.2)	55 (3.5)
	RT-Biowaste		2.37 (0.2)	50 (3.5)
	Tillage (T)	F	1.79	0.82
	Organic matter (OM)	*p*	0.31	0.46
		F	0.34	4.28
		p	0.59	0.09
CH3	Conventional		1.96 (0.2)	64 (7.1)
	Biodynamic		3.16 (0.2)	60 (7.1)
	Farming system	F	20.08	0.27
		p	**0.02**	0.63
ES4	Conventional system		1.21 (0.16)	63 (6.2)
	Organic system		2.63 (0.16)	88 (6.2)
	Farming system	F	114.13	22.80
		p	**0.008**	**0.04**
PT1	Control		2.52 (0.15)	60 (14)
	Biochar		2.49 (0.15)	20 (14)
	Biochar + compost		2.07 (0.15)	29 (14)
	Organic fertilization	F	2.66	6.03
		p	0.18	0.06
HU1	Control		2.98 (0.2)	43 (5.2)
	Control + Nitrogen		3.18 (0.2)	61 (5.2)
	Farmyard manure		2.70 (0.2)	43 (5.2)
	Farmyard manure + Nitrogen		3.60 (0.2)	61 (5.2)
	Straw		2.04 (0.2)	50 (5.2)
	Straw + Nitrogen		3.10 (0.2)	68 (5.2)
	Mineral fertilization	F	10.67	11.90
	Organic fertilization	*p*	**0.006**	**0.005**
		F	3.09	0.93
		p	0.08	0.42
HU4	CT		3.25 (0.19)	62 (6.4)
	RT		3.46 (0.19)	81 (6.4)
	Tillage (T)	F	1.21	6.80
		p	0.35	0.08

CT conventional tillage, *RT* reduced tillage.
*Calculated as. $SSni\ (\%) = 100 * (Wni * Wn^{-1})$
where *Wni* = shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and *Wn* = mean shoot weight in natural soil not inoculated with *P. ultimum*.

Table 3
Effect of different tillage (T) and organic matter additions (OM) on cress shoot yield (g) in natural non-inoculated soil and soil suppressiveness index (SSni) for all the trials as analysed with mixed linear effect models (number of observations = 101). Least square means, standard errors (in parentheses) and *F* and *p* values for mixed linear effect models are reported for the different types of tillage and organic matter additions. Differences are considered significant at $p \leq 0.05$ (values ≤ 0.05 are given in bold).

		Fresh weight in non-inoculated soil (g)	Soil Suppressiveness index (SSni)* (%)
CT- LOW		2.57 (0.22)	57.90 (6.69)
RT- LOW		2.97 (0.24)	65.08 (7.26)
CT- HIGH		2.69 (0.23)	56.83 (6.78)
RT- HIGH		2.97 (0.24)	63.60 (7.26)
Tillage(T)	F	8.05	3.59
	p	**0.008**	**0.05**
OM	F	1.10	0.05
	p	0.30	0.81
T X OM	F	0.41	0.004
	p	0.53	0.94

LOW low organic matter input, *HIGH* high organic matter input, *CT* conventional tillage, *RT* reduced tillage, *OM* organic matter addition, *T* tillage.
*Calculated as. $SSni\ (\%) = 100 * (Wni * Wn^{-1})$
where *Wni* = shoot weight of cress in pots with natural soil inoculated with *P. ultimum*, and *Wn* = mean shoot weight in natural soil not inoculated with *P. ultimum*.

with our expectations. Reduced tillage is known to have a positive effect on soil properties (e.g. water stable aggregates, total organic carbon, bulk density) which can create a favourable environment for microorganisms (D'Hose et al., 2018), antagonists of pathogens (Peters et al., 2003) and plant growth. It is well known that soil microbial biomass and total soil organic carbon are enriched in the uppermost soil layer due to vertical stratification effects after reduced tillage, which was demonstrated also for the Frick trial (CH1) (Gadermaier et al., 2012; Krauss et al., 2017). As shown in previous studies and in this study, these factors, and in particular the microbiological properties, can favour soil suppressiveness (Thuerig et al., 2009).

Farming systems (organic versus conventional agriculture) showed a significant impact on soil suppressiveness in one out of three long-term trials (ES4), with a higher SSni in organic than in the conventional system. This agrees with other studies that found higher soil suppressiveness in organic compared to conventional farming systems (Manici et al., 2003; He et al., 2010; Tamm et al., 2010; Bonanomi et al., 2018a). This could be due to the positive effect of organic management on various soil chemical, physical and biological parameters, such as nutrients, organic carbon, water-stable aggregates, microbial biomass and activity (Biswas et al., 2014; Lori et al., 2017) and to the retention

Table 4
Partial correlation coefficients (ρ) between the soil suppressiveness index (SSni) and chemical, physical and biological parameters used as dependent variables, corrected for the long-term field experiments (LTEs). The number of samples used in the analyses was 101.

Chemical parameters							
TOC	pH	TN	C/N	CEC	Ca	Mg	K
0.06	−0.10	**0.21***	**−0.32***	0.01	−0.08	−0.03	0.02
Physical parameters							
WSA	WHC	Bulk density	Silt	Clay	Sand	Penetration resistance	
0.10	−0.15	0.005	0.06	−0.07	0.14	–	
Biological parameters							
MBC	MBN	Soil respiration	qCO_2	qMic	Earthworm number	Earthworm biomass	Tea bag decomposition
0.26*	0.18	**0.25***	−0.04	**0.27***	**0.35****	**0.16**	**−0.21***
Labile carbon fractions							
Hy	Hy SUVA	DOC	DOC SUVA	POXC	HWEC	POMC	
0.09	**0.23***	0.04	**−0.32***	**0.27***	**0.26***	**0.21***	

TOC total organic carbon, *TN* total nitrogen, *C/N* carbon to nitrogen ratio, *CEC* cation exchange capacity, *WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, qCO_2 metabolic quotient (soil respiration/MBC), *qMic* microbial quotient (microbial biomass carbon/TOC), *Hy* hydrophilic carbon, *Hy SUVA* specific ultraviolet absorbance of hydrophilic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.
$*p \leq 0.05$, $**p \leq 0.001$, $***p \leq 0.0001$.

Table 5
Simple mixed linear model with random slope and intercept for each LTE determined from soil parameters measured in the 101 soil samples. The dependent variable was the soil suppressiveness index (SSni). The explanatory variables were chemical, physical and biological indicators. In the table estimates, standard error, t-value, *p*-value and marginal and conditional R^2 (R^2_m and R^2_c respectively) are reported. Differences are considered significant at $p \leq 0.05$ (significant parameters are given in bold).

	Estimate	Std. error	t value	*p*-value	R^2_m	R^2_c
Chemical parameters						
TOC	0.03	0.19	0.2	0.87	0.001	0.75
TN	0.38	0.22	1.7	0.14	0.12	0.75
pH	0.007	0.16	0.04	0.96	< 0.0001	< 0.0001
CEC	0.13	0.19	0.7	0.50	0.02	0.68
C/N	**−1.58**	**0.52**	**−3.0**	**0.03**	**0.52**	**0.93**
Ca	0.22	0.14	1.6	0.16	0.05	0.59
Mg	0.04	0.26	0.2	0.88	0.001	0.69
K	0.10	0.12	0.8	0.60	0.01	0.68
Physical parameters						
WSA	0.22	0.19	1.11	0.37	0.04	0.71
WHC	**0.72**	**0.11**	**6.3**	**0.002**	**0.49**	**0.64**
BD	−0.07	0.18	−0.39	0.69	0.004	0.68
Clay	0.10	0.27	0.4	0.72	0.01	0.67
Sand	**−0.78**	**0.11**	**−7.2**	**0.003**	**0.52**	**0.68**
Silt	**0.70**	**0.23**	**4.4**	**0.03**	**0.37**	**0.73**
Biological parameters						
MBC	**0.52**	**0.13**	**3.9**	**0.005**	**0.25**	**0.71**
MBN	**0.37**	**0.11**	**2.1**	**0.04**	**0.14**	**0.66**
SR	0.30	0.30	1.0	0.44	0.07	0.75
qCO_2	−0.22	0.18	−1.2	0.50	0.04	0.69
qMic	0.46	0.22	2.0	0.12	0.19	0.73
Earthworm number	0.88	0.56	1.58	0.22	0.20	0.92
Earthworm biomass	0.21	0.13	1.63	0.21	0.05	0.65
Tea bag decomposition	−0.11	0.31	−1.2	0.22	0.01	0.74
Labile carbon fractions						
Hy	0.06	0.11	0.5	0.60	0.004	0.69
Hy SUVA	0.16	0.09	1.7	0.09	0.02	0.78
DOC	−0.05	0.18	−0.3	0.77	0.002	0.81
DOC SUVA	−0.30	0.11	−2.6	0.12	0.08	0.71
POXC	0.24	0.13	1.8	0.09	0.05	0.71
HWEC	**0.34**	**0.13**	**2.6**	**0.05**	**0.11**	**0.68**
POMC	0.41	0.31	1.3	0.23	0.08	0.86

Table 6
Multiple mixed linear model determined from soil parameters measured in the 101 soil samples. The dependent variable was the soil suppressiveness index (SSni). Differences are considered significant at $p \leq 0.05$. The Akaike information criterion (AIC) is an estimator of the quality of the statistical model, the R_m^2 (marginal coefficient of determination) indicates the proportion of the variation explained by the predictor variables and the R_c^2 (conditional coefficient of determination) indicates the variation explained by both the fixed and the random factors.

Dependent variable	Starting model	Final model	Model type	Significant parameters	AIC	R_m^2	R_c^2
Soil suppressiveness (%, SSni)	~WHC_scaled + MBC_scaled + HWEC_scaled + C.N_scaled + (1|LTE)	~MBC_scaled + (1|LTE)	Multiple mixed linear model	MBC_sclaled (0.0001; 4)	208	0.25	0.70

WHC water holding capacity, *MBC* microbial biomass carbon, *HWEC* hot water extractable carbon, *C/N* carbon to nitrogen ratio.

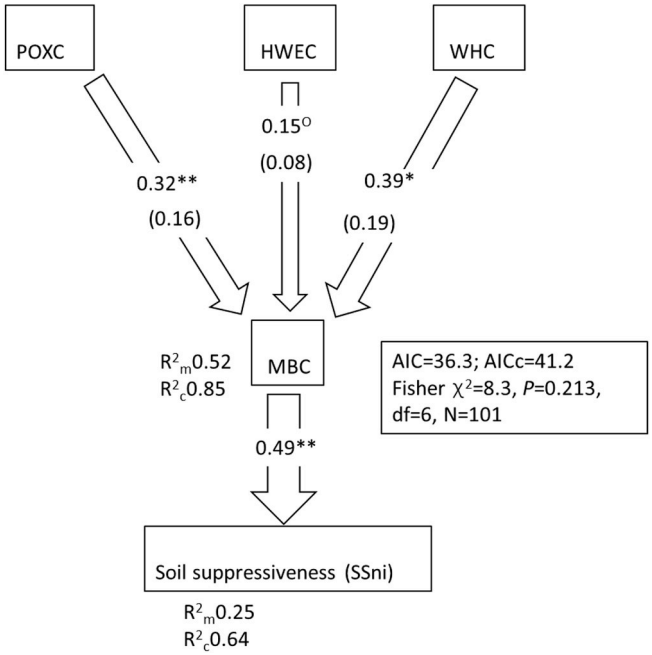

Fig. 4. Piecewise structural equation model (SEM) of soil quality parameters as predictor of soil suppressiveness (SSni). Boxes represent measured variables and arrows represent the unidirectional relationship between the parameters. Numbers on the side of the arrows indicate standardized effect size (reported as path coefficients) and the width of the arrow is proportional to the strength of the path coefficient. The numbers close to the boxes of the response variables are R_m^2 (marginal coefficient of determination) and R_c^2 (conditional coefficient of determination) indicating the proportion of the variation explained by the fixed predictor variables and the proportion of the variation explained by the fixed and random predictor variables. Variables lacking the R_m^2 and the R_c^2 acted only as predictor. Values in parentheses are the indirect effects strength on SSni. In the box adjacent to the figure the Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AIC_C), Fisher chi-square (Fisher χ^2), *p* value (P) of the test, degrees of freedom (df), and the number of observation used for the analysis (N) are indicated. SEM models with a χ^2 with a $p \geq 0.05$ are considered to be statistically significant. *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *WHC* water holding capacity, *MBC* microbial biomass carbon. ^{O} $p \leq 0.1$, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

of both readily available and complex organic substrates in the organic system. Some complex substrates, for example lignocellulosic substrates, can increase the presence of natural antagonists like other *Pythium* spp. and *Trichoderma* spp., and more readily available substrates can increase general microbial activity (Medvecky et al., 2007). In the present study, in the organic management treatment in ES4, we found a higher concentration of labile carbon, which is positively related to microbial biomass and activity (Bongiorno et al., 2019), cation exchange capacity ($p = 0.01$), water-stable aggregates ($p = 0.02$), microbial biomass carbon ($p = 0.003$) and soil respiration ($p = 0.004$). The concentration of lignocellulosic substrates was not measured.

We did not find an effect of organic matter additions on the SSni neither in the individual nor in the overall models. Organic matter additions have been reported to have, in the short-term, positive, negative or neutral effects on soil suppressiveness (Bonanomi et al., 2007a), but studies reporting positive effects predominate (Bailey and Lazarovits, 2003). Variable results could be partly explained by the fact that the chemical composition of the organic matter added to the soil is crucial for soil suppressiveness (Bonanomi et al., 2018b). Organic matter should preferably be decomposed, but not excessively, in order to support soil suppressiveness (Litterick et al., 2004). These observations suggests that changes in the nature of the organic matter (i.e. chemistry, quality and stage of decomposition, time of application, temporal effects) and in the soil environment are central for soil suppressiveness.

Furthermore, the suppressive capacity of organic material added to the soil can disappear some months after its application and it can differ between different batches of the same material and depending on the frequency of application (Litterick et al., 2004; Bonanomi et al., 2018b). For example, Darby et al. (2006) found that the disease severity of root rot of sweet corn increased with time (after 6 months - 1 year) in soil which received organic amendment and slightly decomposed free particulate organic matter (free-POM). Therefore, it is possible that in the present study, organic matter additions had a short-term effect that was lost some months after their application (we sampled in spring, before any agricultural management was applied), or that they lacked readily available substrates which are favourable for antagonistic and competitive microbial activity.

The positive effect of the mineral fertilization found in HU1 can possibly be ascribed to its enhancing effect on plant biomass (Table 2), which increases also root biomass and in turn can have a stimulatory effect on microbial activity.

All the soil management measures investigated in the current study have been applied at the end of summer or in the autumn. In order to focus on long-term effects of soil management rather than on short-term effects, soil sampling was done in spring. This time lapse might have played a role in the non-significant effect of soil management on soil suppressiveness found in various LTEs. To compare short-term to long-term effects and to study the development of the studied parameters throughout the year, soils should be sampled at several times.

4.4. Relationship between soil suppressiveness and soil parameters

Suppression of *Pythium* spp. has often been associated with the biomass and activity of the entire microbial community (van Os and van Ginkel, 2001; Scheuerell et al., 2005; Gravel et al., 2014). In this study, we assessed the relationship between soil suppressiveness and relevant soil biological parameters (microbial biomass, soil respiration, qMic), soil parameters routinely used in soil quality assessment (e.g. TOC, pH, TN, WSA) and in addition labile organic carbon fractions (HyDOC, DOC, POXC, HWEC and POMC). Only occasionally, soil suppressiveness has been related before with labile organic carbon fractions (Pane et al., 2011; Cao et al., 2016; De Corato et al., 2018).

Using a multiple regression model we found that microbial biomass C was the most important parameter for explaining soil suppressiveness. The importance of biotic factors in this study is also reflected in the positive correlations (bivariate and partial) between soil suppressiveness and the biological parameters measured in the study, i.e. microbial biomass C and N, soil respiration and qMic. These observations suggest that increased microbial populations and activity are associated with a decreased disease severity, and support the hypothesis that soil biota, and in particular microbial communities, are involved in soil suppressiveness against *P. ultimum*.

In our study, we found correlations of soil suppressiveness with various labile carbon fractions (positive correlations for POMC, HWEC and POXC), but not with TOC. Both organic matter and labile carbon fractions were found to be positively correlated to soil suppressiveness, an effect that is ascribed to their positive impact on the competitive potential of soil microbial communities against pathogens (Mazzola, 2004; Schlatter et al., 2017). Labile carbon is considered the primary energy source for microorganisms, and probably contains part of the microbial biomass and microbial by-products. Therefore, labile organic carbon can favour soil suppressiveness supporting an active soil microbial community, which will compete for nutrients and space and can thrive on nutrients released by the plant during attack by the pathogen (Pascual et al., 2002; De Corato et al., 2018). This hypothesis is supported by our structural equation model (SEM), where POXC and water holding capacity (WHC) had a significant indirect positive effect on soil suppressiveness through a direct positive effect on microbial biomass. Our results support the hypothesis that the quality of the organic matter (in our case labile carbon fractions) and its effect on soil microorganisms are more important in explaining soil suppressiveness, than just soil organic matter quantity (Hoitink and Boehm, 1999).

We could explain only part of variability in soil suppressiveness with several measured soil parameters. Additional measures of microbial activity, for example fluorescein diacetate hydrolysis (De Corato et al., 2018) or other enzymatic activities (Pane et al., 2011), might add to the model and help in predicting soil suppressiveness. In addition, soil microorganisms are known to contribute to soil suppressiveness with various mechanisms, such as competition for nutrients and space, parasitism, predation, production of specific compounds (e.g. fungistats, siderophores, enzymes), and host mediated resistance (Mazzola, 2002; Charest et al., 2005; Pane et al., 2011; Van Agtmaal et al., 2017). Therefore, the composition of the microbial community and the presence and activity of specific microbial groups or taxa will affect soil suppressiveness (Mazzola, 2002; Trivedi et al., 2017). For example, the presence of *Bacillus* (Erhart et al., 1999; De Corato et al., 2018) and Acidobacteria and Cystobasidiomycetes has been found to be positively associated with *Pythium* suppressiveness (Yu et al., 2015). Therefore, elucidating the composition of soil microbial communities during soil suppressiveness assessment using molecular methods such as next generation sequencing and DNA microarrays, and coupling this with the detection of metabolites or genes that contribute to suppressiveness, and with functional bioassays, might further contribute to the understanding of the role of microorganisms in soil suppressiveness.

5. Conclusions

We found clear differences in soil suppressiveness between sites, whereas the effects of long-term agricultural practices on soil suppressiveness were less pronounced. Tillage had a positive effect on suppressiveness of the soil taking into account all the trials together. Organic farming and mineral fertilization increased soil suppressiveness in some LTEs, however the effect of organic matter addition across all the LTEs was not significant.

Soil suppressiveness across LTEs was linked mainly to microbial biomass and soil organic carbon quality (labile carbon, and in particular HWEC and POXC), but not to total soil organic matter content. We conclude that labile carbon is important for the maintenance of an abundant and active soil microbial community, which is essential for the expression of soil suppressiveness.

Soil suppressiveness could only partly (25%) be explained by the soil parameters measured and used in the SEM, suggesting that other mechanisms contribute to soil suppressiveness, such as the presence and activity of specific bacterial and fungal taxa, the activity of specific enzymes, or the presence of specific compounds with a detrimental effect on the pathogen.

Competing interests

The authors declare that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.03.012.

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