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Disentangling the abiotic and biotic components of AMF suppressive soils

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are important in plant nutrient uptake, but their function is prone to environmental constraints including soil factors that may suppress AMF transfer of phosphorus (P) from the soil to the plant. The objective of this study was to disentangle the biotic and abiotic components of AMF-suppressive soils. Suppression was measured in terms of AMF-mediated plant uptake of ^{33}P mixed into a patch of soil and treatments included soil sterilization, soil mixing, pH manipulation and inoculation with isolated soil fungi. The degree of suppression was compared to volatile organic compound (VOC) production by isolated fungi and to multi-element analysis of soils. For a selected suppressive soil, sterilization and soil mixing experiments confirmed a biotic component of suppression. A *Fusarium* isolate from that soil suppressed the AMF activity and produced greater amounts than other fungal isolates of the antimicrobial VOC trichodiene (a trichothecene toxin precursor), beta-chamigrene, alpha-cuprenene and p-xylene. These metabolites deserve further attention when unravelling the chemical background behind the suppression of AMF activity by soil microorganisms. For the abiotic component of suppression, soil liming and acidification experiments confirmed that suppression was strongest at low pH. The pH effect might be associated with changed availability of specific suppressive elements. Indeed ^{33}P uptake from the soil patches correlated negatively to Al levels and Al toxicity seems to play a major role in the AMF suppressiveness at pH below 5.0–5.2. However, the documentation of a biotic component of suppression for both low and high pH soils leads to the conclusion that biotic and abiotic components of suppression may act in parallel in some soils. The current insight into the components of soil suppressiveness of the AMF activity aids to develop management practices that allow for optimization of AMF functionality.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the majority of known plants (Smith and Read, 2008). They are important to ecosystem functions through effects on carbon and mineral nutrient cycles, plant stress tolerance, plant diversity and soil aggregation (van der Heijden et al., 2015). The extra-radical mycelium (ERM) is a key structural component of the AMF symbiosis as it links colonized roots with the soil matrix where nutrients such as phosphorus (P) are taken up and transferred to the plant (Leake et al., 2004).

Most experiments addressing the function of the ERM in nutrient uptake and transfer have used (semi)-sterile soils to ensure that observed effects on plant nutrition could be attributed to the introduced AMF, and not to other members of the soil biota. However, in natural (non-sterile) soil a multitude of organisms interacts with the AMF and modulates their function. For example, the growth of ERM was suppressed in response to the addition of a bacterial soil filtrate (Leigh et al., 2011) and P uptake by AMF-colonized roots was much smaller from natural (non-sterile) soil than from semi-sterile soil (Hetrick et al., 1988).

Such suppression of AMF activity in non-sterile soils was investigated in more detail in two recent papers using one subset of soils from 16 Scandinavian cultivated field sites (Svenningsen et al., 2018) and another subset from 19 Danish non-cultivated sites (Cruz-Paredes et al., 2019). Both subsets covered a range of soil characteristics for texture, pH and nutrient availability. For the experiments, a mesh-enclosed patch of each soil was labelled with ^{33}P and embedded in semi-sterile

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soil with plants colonized by a model AMF. The AMF activity was measured as the uptake and transfer of ^{33}P from each patch and into the plants. Suppression of the AMF activity varied greatly for both soil subsets and both biotic and abiotic soil factors appeared to be involved (Cruz-Paredes et al., 2019; Svenningsen et al., 2018).

Suppression of the AMF activity is mitigated by soil pasteurization and has a dominating biotic component (Cruz-Paredes et al., 2019; Svenningsen et al., 2018). Indeed, those authors found that bacterial and fungal communities differ between suppressive and non-suppressive soils, but also observed so-called general suppression, which is often expressed as a continuum of suppression levels and involves the common action of several taxa. The bacterial taxa that contributed most to the variation between suppressive and non-suppressive soils were *Acidobacteria* subgroup 1 and Xanthomonadacea, and both groups have been associated with soils suppressive towards fungal pathogens as *Rhizoctonia*, *Fusarium* and *Gaeumannomyces* (Campos et al., 2016; Mendes et al., 2011; Sanguin et al., 2009; Shen et al., 2015). Moreover, Cruz-Paredes et al. (2019) found that fungi such as *Mycena*, *Mortierella* and Archaeorhizomycetes species were more abundant in AMF-suppressive soils. *Mycena* spp. produces organic acids (Rosling et al., 2004), which might contribute to AMF-suppression, while Archaeorhizomycetes co-occurs with AMF (Choma et al., 2016), but there is limited knowledge about their lifestyle and ecological role (Rosling et al., 2011).

Examples of antagonism by microorganisms towards AMF include fungal parasitism of AMF spores and hyphae (Paulitz and Menge, 1986; Rousseau et al., 1996) as well as disruption of the ERM in the presence of *Trichoderma harzianum* (de Jaeger et al., 2011). The latter study even reported that the AMF activity was reduced (general metabolic activity as well as specific ^{33}P translocation within the AMF mycelium and consequently the transfer of ^{33}P to the host plant). Soil microorganisms may adversely affect AMF activities in soil e.g., by competing for resources, or by producing unfavorable metabolites, but the mechanism behind the above antagonism was not identified. For other soils that are suppressive towards plant pathogenic fungi (Hol et al., 2015; Raaijmakers and Mazzola, 2016) suppression is sometimes caused by secondary metabolites produced by soil microorganisms including volatile organic compounds (VOC's; Cha et al., 2016; Cordovez et al., 2015). These metabolites are involved in signaling and antagonism (Kanchiswamy, 2015) and will help the producer organism to survive abiotic and biotic stress (Khan et al., 2018). While some VOCs produced by microorganisms can indeed suppress growth of fungi (De Boer et al., 2019; Effmert et al., 2012; Garbeva et al., 2011; Garbeva and Weisskopf, 2020), their effect on growth and activity of AMF remains unknown.

The suppression of the AMF activity is generally higher in low pH soils (Svenningsen et al., 2018; Cruz-Paredes et al., 2019) and can be mitigated by liming (Svenningsen et al., 2018). Soil pH plays a major role in shaping microbial communities (Lauber et al., 2009; Rousk et al, 2009, 2010). Consequently, soil pH may affect soil suppressiveness towards AMF through indirect effects of the microbial community composition. Soil pH also directly affects the root colonization intensity and ERM production by AMF (van Aarle et al., 2002; Wang et al., 1993). In this context, Cruz-Paredes et al. (2020) found that AMF isolates differed in their susceptibility to suppression, but for most isolates, some soils could be identified as suppressive and others as non-suppressive.

Moreover, soil pH directly affects the amount of nutrients and chemicals in the soil solution and hence their biological availability to the soil microbiota. For example, the availability of B, Cu, Fe, Mn, and Zn usually increases, and Mo availability decreases as soil pH decreases (Fageria and Nascente, 2014). Some of these elements are potentially toxic to AMF. Hence, growth of AMF is severely inhibited by bioavailable Cu at concentrations that do not affect growth of other main microbial groups in soil (Hagerberg et al., 2011). Even Al toxicity is usually restricted to acidic conditions (Illmer and Buttinger, 2006) and Göransson et al. (2008) found that high levels of easily reacting Al showed a strong negative correlation with root colonization by AMF.

The present work aims to disentangle the abiotic and biotic components of AMF-suppressive soils and to identify some underlying mechanisms; the following hypotheses were tested: 1) biotic suppression may be alleviated by soil sterilization, shift of soil pH or mixing with non-suppressive soil, 2) suppressive soil fungi may be identified after inoculation of sterile soil with fungi isolated from a suppressive soil, 3) suppression may be related to microbial production of AMF-toxic VOCs and 4) abiotic suppression is caused by specific toxic elements in addition to direct pH effects. Patches of soils to be tested were labelled with ^{33}P, enclosed by meshes and placed in irradiated soil with AMF-colonized plants. This model system served to assess the degree of suppression of AMF by quantifying the hyphal transfer of ^{33}P from soil patches to the plant.

2. Materials and methods

2.1. Model system, soils, plants and AMF

The model system was modified from Smith et al. (2003) (Supplementary, methods and Fig. S1). In brief, *Medicago truncatula* colonized by *Rhizophagus irregularis* and growing in pots with semi-sterile soil served as donor association for the production of ERM, with the aim to study the ERM uptake up of ^{33}P from an unsterile mesh-enclosed soil patch. Six experiments were carried out using soils selected from a subset of Scandinavian cultivated soils and a subset of non-cultivated soils from the Danish BIOWIDE project (http://www.biowide.dk/) that were already tested for suppressiveness against AMF in previous experiments (Svenningsen et al., 2018; Cruz-Parades et al., 2019). See Table S1 for geographical and physiochemical details for soils used in this study. Each experiment had three replicate pots per soil or soil treatment. The plants were harvested after 35 days and the shoot ^{33}P content was quantified and used as a proxy for the P uptake activity of the ERM (Supplementary, methods).

2.2. Modifications of soils in mesh-enclosed soil patches

2.2.1. Experiment 1: pasteurization and irradiation

The aim of this experiment was to use pasteurization (water bath at 80 °C for 1h) and/or irradiation (2 × 18 kGy electron beam) to remove the biotic components from the soils and determine whether biotic soil factors were responsible for suppression of the AMF activity. Four suppressive soils were selected and were exposed to both sterilization treatments (Moystad E2 and Rodekro, cultivated) or to irradiation alone (Toftlund, cultivated and SV93, heathland). Two non-suppressive soils served as controls and were also irradiated (VO40, grassland and VO41, dune; Table S1 provides soil characteristics).

2.2.2. Experiment 2: mixing of soils

Different soil mixing treatments were used to investigate transferability of suppression and hence further address whether AMF suppression has a biological basis. Pasteurized E2 soil was mixed with the suppressive E2 or with the non-suppressive Trelleborg soil (Table S1) in the following ratios: 0:1, 1:9, 1:1, 9:1, and 1:0. These ratios were also used for mixing the suppressive E2 soil and the non-suppressive Trelleborg soil.

2.2.3. Experiment 3: soil liming or acidification

To determine whether a decrease in soil pH induces soil suppressive activity, the non-suppressive soils, Risø stored (Table S1) and Trelleborg, which had baseline pH of 7.3 and 5.9 respectively, were supplemented with HCl to target new soil pH levels of 4.8 and 4.4 respectively. Furthermore, it was determined if the AMF-suppressive effect of the E2 soil could be mitigated by increasing soil pH from 4.4 to 7.1 by adding 4.0 g of $CaCO_3$ per kg soil as in Svenningsen et al. (2018).

2.2.4. Experiment 4: inoculation with fungal isolates from E2 soil

Fungi were isolated from the suppressive E2 soil with the aim to screen for the ability of specific isolates to suppress AMF activity. Pure cultures of fungi growing on potato dextrose agar (PDA) were obtained (Supplementary, methods) and their preliminary identity was determined by morphology. This identification was further detailed by sequencing of the ITS region of rDNA using the primers ITS1-F and ITS4 (Gardes and Bruns, 1993; White et al., 1990) (Supplementary, methods). Fungal isolates of *Archaeorhizomycetes* and *Mycena* were also obtained from private culture collections as these groups were apparently enriched in suppressive non-cultivated soils (Cruz-Paredes et al., 2019). *Mycena galopus* and *M. epipterygia* were provided by Ella Thoen, University of Oslo and *Archaeorhizomyces finlayi* and *A. borealis* by Anna Rosling, Uppsala University. The *Mycena* isolates were cultured on PDA while *Archaeorhizomyces* isolates were cultured in liquid modified Melin Norkrans media (Supplementary, methods).

Inoculum of the E2 and the *Mycena* isolates was prepared as follows. Mycelium from a two week old culture on PDA was scraped from the surface of the agar, combined with 5 ml sterile distilled water and fragmented using a ball bearing homogenizer. Sterile distilled water was then added to the mycelium slurry to give a final volume of 40 ml. Serial dilutions of the inocula were plated on PDA and colony forming units were counted after 5–10 days growth. For the *Archaeorhizomyces* species, inoculum was prepared by filtering mycelium from liquid cultures on sterile filter paper and homogenizing as described above.

The inoculum of each fungus (Table 1) was introduced into electron beam-irradiated (2 × 18 kGy) E2 soil after the soil had been labelled with ^{33}P and filled into the plastic cylinders. Each soil patch was inoculated with 5 ml of the 40 ml suspension of fungal fragments or spores in sterile distilled water and the influence of the inocula on AMF activity was determined. A suppressive *Fusarium* isolate was identified and used in a second experiment including two- and three-fold dilutions. That experiment also included a mixture of the *Fusarium* isolate and a tentatively suppressive *Penicillium* isolate to test for synergetic effects.

2.3. Experiment 5: VOCs produced by fungal isolates from E2 soil

The fungi isolated from the suppressive E2 soil (Experiment 4) were analyzed for production of volatile organic compounds (VOCs) in order to screen for potentially AMF-toxic VOCs. Particular attention was paid to VOCs produced by fungi found to be AMF suppressive. Fungi were grown from 2 mm (diameter) agar plugs placed on PDA in pre-autoclaved 20 ml headspace vials. The vials were closed with 18 mm, magnetic HDSP caps (PTFE/Sil, Supelco, Bellefonte, PA, United States). The fungal cultures were grown at room temperature for six weeks and the headspace vials were then transferred to the GC-MS autosampler for analysis of VOCs, Sampling was performed by solid-phase microextraction (SPME) and VOCs were analyzed by GC-MS using a 7890A GC interfaced to a 5973N MS (Agilent technologies, CA, USA) (Supplementary, methods). For three out of the 23 fungal cultures little or no growth was observed in the four replicates, which was later confirmed by the VOC analysis. Results for these cultures were therefore left out of the statistical analysis.

Table 1
Fungal isolates from the E2 soil and existing fungal isolates assessed for their AMF-suppressiveness. Isolates were submitted in GenBank with submission ID: 2445755.

Fungal isolate used	Source	Genbank Accession No.
Archaeorhizomyces borealis	Culture collection	
Archaeorhizomyces finlayi	Culture collection	
Fusarium cf. *solani*	Isolated from E2 soil	MW837838
Fusarium sp. 1	Isolated from E2 soil	MW837831
Fusarium sp. 2	Isolated from E2 soil	No Sequence
Fusarium sp. 3	Isolated from E2 soil	MW837836
Mortierella sp. 1	Isolated from E2 soil	MW837833
Mortierella sp. 2	Isolated from E2 soil	MW837834
Mortierella sp. 3	Isolated from E2 soil	MW837840
Mycena epipterygia	Culture collection	
Mycena galopus	Culture collection	
Penicillium sp. 1	Isolated from E2 soil	MW837835
Penicillium sp. 2	Isolated from E2 soil	MW837837
Penicillium sp. 3	Isolated from E2 soil	MW837839
Sterile culture 1	Isolated from E2 soil	No Sequence
Sterile culture 2	Isolated from E2 soil	No Sequence
Sterile culture 3	Isolated from E2 soil	No Sequence
Sterile culture 4	Isolated from E2 soil	No Sequence
Sterile culture 5	Isolated from E2 soil	No Sequence
Sterile culture 6	Isolated from E2 soil	No Sequence
Talaromyces sp. 1	Isolated from E2 soil	MW837832
Trichocladium sp. 1	Isolated from E2 soil	MW837830
Trichoderma sp. 1	Isolated from E2 soil	No Sequence
Trichoderma sp. 2	Isolated from E2 soil	No Sequence

2.4. Experiment 6: multi-elemental analysis of cultivated and non-cultivated soils

The possible role of specific elements as abiotic components behind AMF suppression was analyzed by correlating the concentration of elements extracted from cultivated and non-cultivated soils (Table S3) against the shoot ^{33}P content as previously determined in Svenningsen et al. (2018) and Cruz-Paredes et al. (2019). Four suppressive and six non-suppressive, cultivated soils (Tables S1 and S3) were extracted by shaking 8 g of soil in 16 ml of 0.01 M $CaCl_2$ for 16 h. Samples were then centrifuged for 15 min at 1800 g. After centrifugation, the supernatant was passed through 0.45 μm Q-Max syringe filters (Frisenette, Knebel, Denmark) and extracts were kept frozen until analysis. The elemental composition of all extracts was measured by ICP-OES (Optima 5300DV, PerkinElmer, MA, USA).

Six suppressive and 12 non-suppressive, non-cultivated soils (Tables S1 and S3) were extracted by shaking 100 mg of soil in 0.5 ml 0.5 M NH_4Ac. Samples were centrifuged for 15–20 min at 10,000 g and the supernatant collected. The residual soil pellet was re-extracted and the two supernatants were pooled, filtered through 0.2 μm nylon spin filters (Spin-X, Costar, Merck, Darmstadt, Germany), centrifuged for 8 min as above and acidified with HNO_3 to an acid percentage of 3.5% followed by ICP-MS analysis (Agilent 7900, CA, USA).

2.5. Data analysis

Student's t-tests were used to compare shoot ^{33}P content in plants grown in soils with the original and the modified pH and to compare between soils inoculated with candidate fungi and uninoculated soils (control). One-way analysis of variance (ANOVA) was conducted to compare the shoot ^{33}P content in plants grown in the soil pasteurization, irradiation, and transfer experiments, with Tukey's post hoc tests implemented when appropriate. Non-normally distributed data (as per Shapiro–Wilk test), were log-transformed prior to statistical analyses. Data was also checked for homogeneity of variances (as per Bartlett's test). Pearson correlation was used to find relationships between ^{33}P and the different element concentration from the elemental analysis. Statistical analyses were performed using R (R Core Team, 2020).

For VOC analyses, peak detection and deconvolution in the GC-MS SCAN data was done in AMDIS 2.73 (Mallard, 2014) providing a peak list of retention times, quantifier, qualifier ions etc. VOCs were identified by comparison of the deconvoluted mass spectra with a reference spectral library (NIST14) and their linear retention index. Compounds were considered tentatively identified if they had a NIST mass spectral match factor >80% and a deviation in the retention index between 1 and 2% from reported values in literature (e.g., NIST14). The AMDIS peak list and raw GC-MS data were then imported into MATLAB 9.3.0 (R2017b, MathWork Inc., USA) using the graphical user interface Gavin3 0.96 (Behrends et al., 2011) for visual confirmation and adjustment of the retention time, and peak areas for the quantified ion.

VOCs detected in the fungal samples were compared to VOCs in the

growth media ('blank sample'). Among VOCs showing significantly ($p < 0.05$, *t*-test) larger peak area of the compounds in the fungal samples compared to the blank sample, a total of 330 compounds with at least 10 times higher peak areas were included in the intensity peak table. The intensity peak table was cube root transformed, and Pareto scaled (van den Berg et al., 2006). The data distribution was assessed according to its skewness, kurtosis, and visual inspection of histograms (Vinaixa et al., 2012) for each compound. Box-whisker plots (Filipiak et al., 2012) were used to identify compounds with significantly larger intensities in *Fusarium* cf. *solani* cultures than in cultures of the other fungi. Mann-Whitney *U* test calculated in R, with the function "Wilcox.test" was used to determine the significance of individual compounds. Principal component analysis (PCA) was performed on the cube root transformed, and Pareto scaled intensity peak table (Bro and Smilde, 2014). The data filtration was set to exclude compounds with more than 70% missing data, resulting in 73 compounds in the final peak table. The reliability of the contribution from each of the compounds was calculated by jackknife confidence intervals, based on a 7-fold full cross-validation. Partial least squares regression (PLS) was used to investigate the relationship between VOC profiles and suppression using the filtered peak intensity table as X and range scaled values for the suppression; i.e. differences from the control shoot ^{33}P content.

3. Results

3.1. Biotic component of suppression of AMF activity in the E2 soil

3.1.1. Pasteurization, irradiation and mixing of soils

Initially, the highly suppressive E2 soil was exposed to pasteurization as well as irradiation. The ^{33}P shoot content was significantly ($p < 0.001$) higher for irradiated and pasteurized soil than for not treated soil (Fig. 1) supporting that a biotic component suppressed AMF activity in this soil. However, pH of the E2 soil increased by up to 0.4 pH units due to pasteurization or irradiation (Fig. 1). In four of the soils, the ^{33}P shoot content was not enhanced by pasteurization or irradiation; this will be considered in section 3.2.

Subsequently, transfer of suppressiveness was studied by mixing E2 soil with pasteurized E2 soil or non-suppressive Trelleborg soil. In both cases, the AMF activity, i.e. shoot ^{33}P content, was gradually suppressed with increasing proportions of E2 soil. This suppression had reached 80–90% ($p < 0.001$) when unsterile E2 soil constituted 50% of the mixture with pasteurized E2 soil (Fig. 2A) or non-suppressive Trelleborg soil (Fig. 2B), respectively. The mixing of pasteurized E2 soil into the non-suppressive Trelleborg soil also led to decreases in shoot ^{33}P content; however, these decreases followed a more linear pattern and were significant only when pasteurized E2 soil constituted as much as 90% of the mixture (Fig. 2C). We noted that although soil pH was consistently lowered by increasing the proportion of the E2 soil (Fig. 2) the AMF activity was significantly more reduced ($p = 0.01$) by the E2 soil than by the pasteurized E2 soil at similar pH (compare 1:1 mixing ratios in Fig. 2B and C).

3.1.2. Suppression of AMF activity by fungi isolated from E2 soil

Both the soil pasteurization and the mixing experiments indicated that a biotic component of the E2 soil was involved in the suppression of AMF activity and a collection of soil fungi was therefore established from this soil (Table 1). Briefly, we obtained several isolates from *Trichocladium*, *Talaromyces*, *Fusarium*, *Penicillium*, *Trichoderma*, *Mortierella* and several sterile cultures. The obtained sequences were submitted to GenBank with the submission ID: 2445755.

Inoculation of the fungal isolates into the soil patches of the plant model system had in most cases no significant effect on the shoot ^{33}P content when compared to uptake from uninoculated irradiated soil (Control) (Fig. 3A). However, the shoot ^{33}P content was consistently reduced by the isolate *Fusarium* cf. *solani* ($p = 0.008$) when tested at variable inoculum strength ($p < 0.001$; $p = 0.01$), and in mixture with *Penicillium* sp.1 ($p < 0.001$), as compared to uninoculated irradiated soil (Fig. 3B).

3.1.3. VOC production by fungal isolates

The total number of detected fungal VOCs was 330, of which 189 compounds were tentatively identified (see Table S2). The number of detected VOCs for the 20 individual fungal strains is shown in Fig. S2. Many of the 141 unidentified compounds exhibited mass spectral

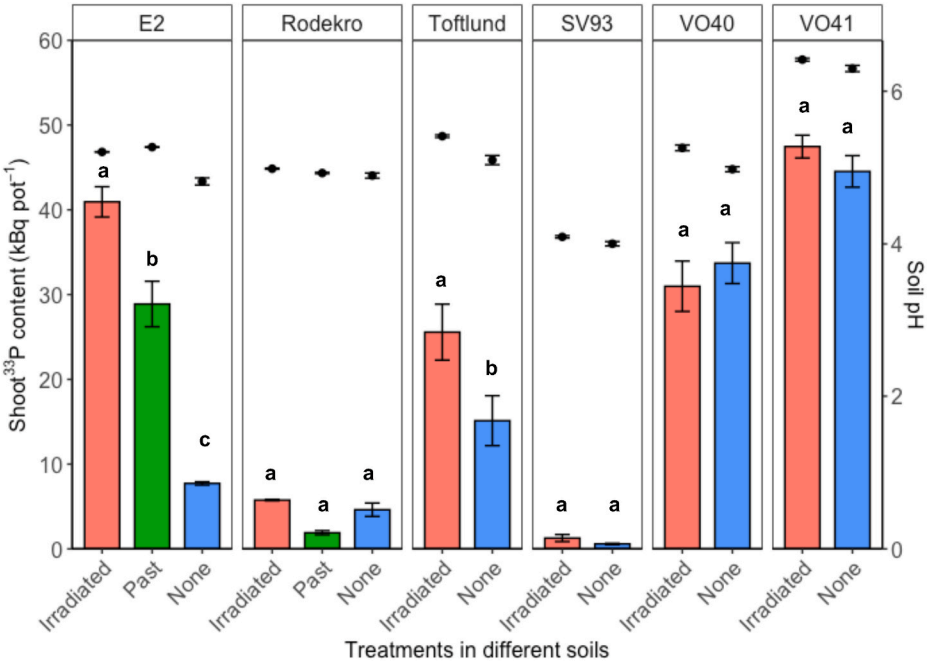

Fig. 1. Shoot uptake of ^{33}P by arbuscular mycorrhizal fungi from soil patches exposed to irradiation (irradiated) and/or pasteurization (past) compared to shoot ^{33}P uptake from untreated soils (none) are shown as bars. Soil pH values from soil patches at the end of the experiment are shown as (●). Data are presented as mean values ± SEM (n = 3). Different letters indicate significant differences in shoot uptake of ^{33}P ($p < 0.05$).

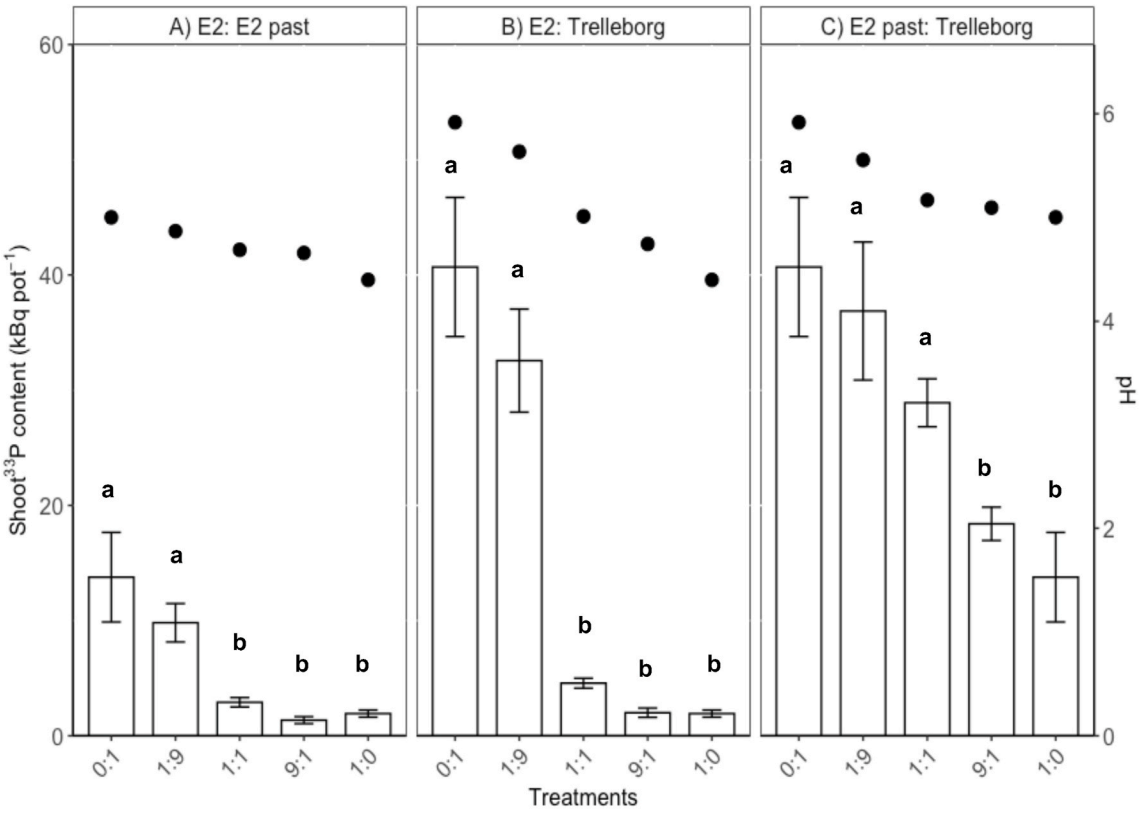

Fig. 2. Shoot uptake of ^{33}P by arbuscular mycorrhizal fungi (bars) from soil patches containing **A)** suppressive E2 and pasteurized suppressive E2 soils mixed at different ratios (1:0, 9:1, 1:1, 1:9, 0:1); **B)** suppressive E2 soil and non-suppressive Trelleborg soil mixed at different ratios; and **C)** pasteurized suppressive E2 soil and non-suppressive Trelleborg soil mixed at different ratios. For all panels, soil pH values from soil patches at the end of the experiment are shown as (●). Data are presented as means ± SEM (n = 3). Different letters indicate significant differences in shoot uptake of ^{33}P ($p < 0.05$).*.

similarity (>80%) to mono- and sesquiterpenes, but with a deviation in the retention index >1–2%; which indicate other isomers than the ones in the NIST14 library.

PCA was used to investigate differences and similarities in the VOC profiles of the 20 fungi (Fig. 4). PC1 and PC3 seemed to describe relevant chemical information on the VOCs while for PC2 the confidence intervals indicated that this component is unreliable. PC1 explained 31.6% of the variation; the separation can be explained by negative loading coefficients for acetic acid, while positive contribution was found for several alcohols and ketones such as 1-pentanol, 2-nonanone, phenyl ethyl alcohol, ethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, and their esters. PC1 is related to fungal VOC production, so that fungi with high scores generally have high intensities (high production) and produce a variety of VOCs. PC3 explained 10.3% of the variation, and negative loadings were dominated by 1-octen-3-ol, 2,3 butanol, acetoin, octane, while the positive loadings were dominated by 2-heptanol, ethyl acetate, butanoic acid ethyl ester, 2-nonanol, and beta-phellandrene. The PC1 and PC3 loading plots are shown in Fig. S3. However, the PCA did not enable identification of specific compounds related to the observed suppression.

To identify specific compounds related to the suppression mediated by *Fusarium* cf. *solani*, we compared the peak area of individual compounds produced by *Fusarium* cf. *solani* with median peak area measured for the rest of the isolates (Fig. 5). We found significantly higher intensity of p-xylene and several terpenes including the sesquiterpene trichodiene and the compounds beta-chamigrene and alpha-cuprenene. Furthermore, an unidentified sesquiterpene ($C_{17}H_{28}O_2$), with retention index = 1721 and high spectral similarity to cedryl acetate, was only found for *Fusarium* cf. *solani*.

3.2. Abiotic components of suppression of AMF activity in soil

3.2.1. Irradiation did not alleviate suppression in all soils

Among the soils subjected to pasteurization and/or irradiation, four soils showed no significant increase in ^{33}P shoot content, which was in contrast to the increase seen for the suppressive E2 soil (Fig. 1). Two of these, VO40 and VO41, could be assigned as non-suppressive as ^{33}P uptake was high also from unsterile soil patches. Besides, the strongly suppressive SV93 had a very low pH. The general observation that pH tended to be higher in pasteurized or irradiated soils than in untreated soils (Fig. 1), prompted us to investigate the effect of soil pH manipulation on AMF activity (Fig. S4). Liming of the E2 suppressive soil from pH 4.4 to 7.0 increased the shoot ^{33}P content significantly ($p < 0.001$). In contrast, HCl-mediated acidification of two non-suppressive soils, selected for their high pH, resulted in shoot ^{33}P contents being significantly decreased for the Trelleborg soil ($p = 0.01$), but not for the Risø stored soil with higher pH values. Similar decreases in shoot ^{33}P contents were obtained when the two soils had been pasteurized before acidification (data not shown). In this experiment, the acidification-generated pH values (4.8 for Risø and 4.4 for Trelleborg) had increased to 5.9 and 5.0 at the end of the experiment. The observed abiotic effects of pH on AMF suppression in some soils led us to investigate whether the pH effect was associated with changed availability of specific suppressive elements.

3.2.2. Multi-elemental analysis of soils

The correlation analyses of the concentration of elements extracted from cultivated and non-cultivated soils (Table S3) and the shoot ^{33}P uptake showed for the cultivated soils that correlations were significant and negative between ^{33}P shoot content and Al ($r = -0.50$, $p = 0.009$), K ($r = -0.47$, $p = 0.01$), Mn ($r = -0.53$, $p = 0.005$), S ($r = -0.41$, $p =$

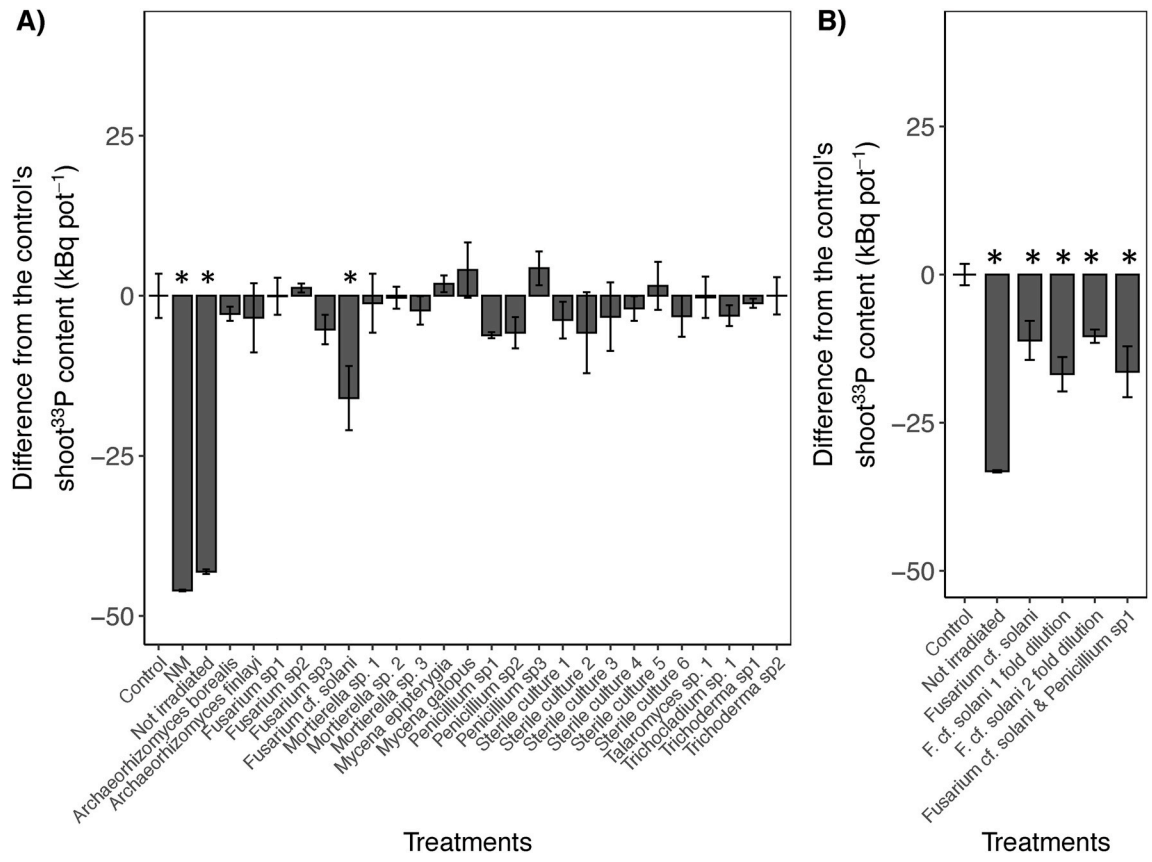

Fig. 3. Differences in shoot ^{33}P uptake by arbuscular mycorrhizal fungi from soil pathces containing control soil (irradiated E2 soil) and soil patches inoculated with fungal isolates obtained from E2 soil. **A)** All fungal isolates, non-mycorrhizal (NM) and E2 soil with no irradiation treatment, **B)** Dilutions and mixtures of selected fungal isolates. Data are presented as means ± SEM (n = 3). Significant differences are indicated by * ($p < 0.05$).

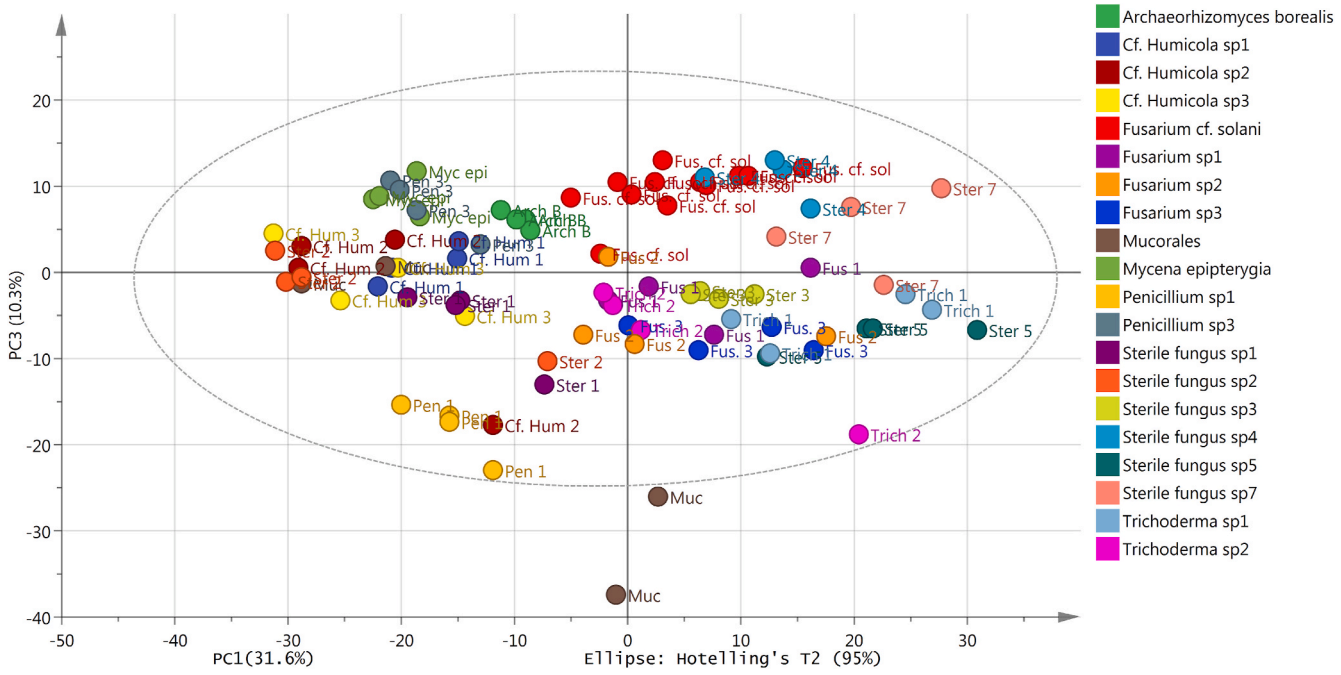

Fig. 4. VOCs produced by 20 fungal strains isolated from suppressive E2 soil. The PCA score plot shows PC1 and PC3 for the dataset consisting of VOCs detected in cultures of 20 fungi strains with four biological replicates and of *Fusarium* cf. *solani* with12 biological replicates. The data were cube root transformed, and Pareto scaled (van den Berg et al., 2006). The dataset was filtered to exclude compounds with more than 70% missing values. PC1 explaning 31.6% and PC3 10.3% of variation.

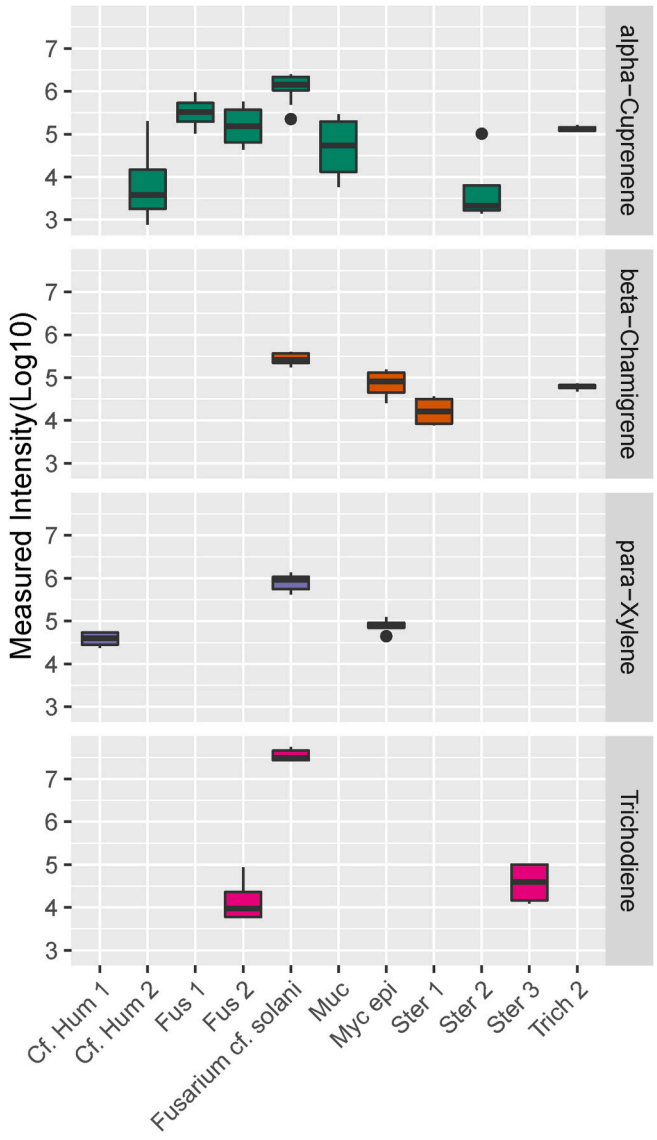

Fig. 5. Box-Whisker plot showing Log 10 transformed measured intensities for alpha-Cuprenene, beta-Chamigrene, para-Xylene and Trichodiene which had significantly higher ($p < 0.05$) intensity in Fusarium cf. solani than in any other fungal culture. The box plots show the median, the two hinges that correspond to the 25th and the 75th percentiles, and the upper and lower whiskers, which extend from the higher and lower hinges to the largest and smallest values no further than 1.5 times the inter-quartile range. The black points represent outliers.

0.03) and Zn ($r = -0.53$, $p = 0.005$) (Fig. 6A). For the non-cultivated soils, significant negative correlations were observed for Al ($r = -0.70$, $p < 0.001$) and Pb ($r = -0.51$, $p = 0.03$) (Fig. 6B). Hence, high soil Al levels clearly correlated with suppression of AMF activity across all cultivated and non-cultivated soils. Since Al toxicity prevails in low pH soils, the correlations between ^{33}P uptake, pH and Al were further explored (Fig. 7). In general, ^{33}P uptake was low at pH < 5.0–5.2 (Fig. 7A, D) and this was associated with markedly increased Al levels (Fig. 7B, E). In consequence, ^{33}P uptake from the soil patches correlated negatively to Al levels (Fig. 7C, F). It appears that Al becomes toxic to the activity of *R. irregularis* at 1–2 μg Al g^{-1} soil or higher, depending on the extraction agent (Fig. 7).

4. Discussion

Soil-associated suppression of AMF activity was demonstrated in this study by means of a plant-AMF model system with a mesh-enclosed patch of ^{33}P-labelled unsterile field soils. As the soil patches could be accessed by AMF hyphae only and not by roots, suppression could be investigated without confounding influence from the rhizosphere (e.g. pH changes and root exudation) and without the impact of the AMF suppressive soil on root colonization, which in all cases was higher than 90%, by the model AMF *R. irregularis*. The model system proved suitable to show that the degree of suppression varies between soils and that the suppression of the AMF activity involves both abiotic and biotic components (Svenningsen et al., 2018; Cruz-Paredes et al., 2019). Disentangling the abiotic and biotic components of AMF-suppressive soils and understanding some underlying mechanisms were the focal points of this study.

4.1. Biotic factors behind suppression

The experiments aiming at identifying a biotic component of suppression involved the E2 soil previously shown to be suppressive (Svenningsen et al., 2018). In disease-suppressive soils, suppression is eliminated by pasteurization (Garbeva et al., 2011). Likewise, for the E2 soil, we here show that pasteurization as well as irradiation mitigates suppression, hence confirming our first hypothesis and expanding previous observations made for this soil (Svenningsen et al., 2018). Transfer of the suppressive effect of E2 soil to pasteurized E2 soil and to the conducive Trelleborg soil confirmed a biotic component, but transfer of suppression occurred only at a 1:1 ratio. For disease-suppressive soils, a distinction is made between specific and general suppression. Specific suppression is caused by a single microorganism and is transferable by adding small amounts (1–10%) of suppressive soil to a non-suppressive soil (Henry, 1931; Schlatter et al., 2017). Hence, the current results indicate that the suppression of the AMF activity is a general suppression. General suppression does not depend on the presence of the target organism, here AMF (Schlatter et al., 2017), and involves the collective competitive and antagonistic activity of the soil microbiota (Weller et al., 2002). Accordingly, Svenningsen et al. (2018) and Cruz-Paredes et al. (2019) compared the composition of bacterial and fungal communities for selected AMF-suppressive versus AMF-conducive soils and found several taxa that had higher relative abundance in suppressive soils and therefore might contribute to suppression.

The second hypothesis was confirmed by our identification of *Fusarium* cf. *solani* as an isolate that significantly suppressed the AMF activity when it was introduced into soil patches. This result differs from most previous reports on negative interactions between saprotrophic fungi and AMF, which mostly reported effects of soil fungi on AMF spore germination and growth (Paulitz and Menge, 1984, 1986), abundance and viability of AMF hyphae (De Jaeger et al., 2011; Rousseau et al., 1996) and root colonization (McAllister et al., 1994; Ravnskov et al., 2006). Taxa belonging to the genus *Fusarium* are widespread in soil but their occurrence and their ability to cause plant disease vary between soils (Orr and Nelson, 2018; Venkatesh and Keller, 2019). This variability is often caused by biotic (antagonistic) interactions, but these biotic interactions even depend on the abiotic characteristics of the soil such as the pH, the availability of elements, and the soil texture (Orr and Nelson, 2018; Siegel-Hertz et al., 2018). Moreover, the impact of *Fusarium* on the AMF-to-plant transfer of ^{33}P seems inconsistent. One report points to suppression of growth and P uptake in AMF colonized chickpea by *F. oxysporum* (Shukla et al., 2015), while another found no suppression of AMF growth or P transport by *F. culmorum* in a model system resembling the one used in the current study (Larsen et al., 1998). Hence, more studies are needed to confirm a general role of *Fusarium* as part of AMF-suppressive consortia in other soils that the E2 soil currently investigated. The variable results for antagonism of *Fusarium* against the AMF activity might even depend on the metabolites produced by the specific strains under study.

In the current study, we identified 189 fungal VOCs produced by 20 different fungal cultures. The VOCs produced by these fungi include the

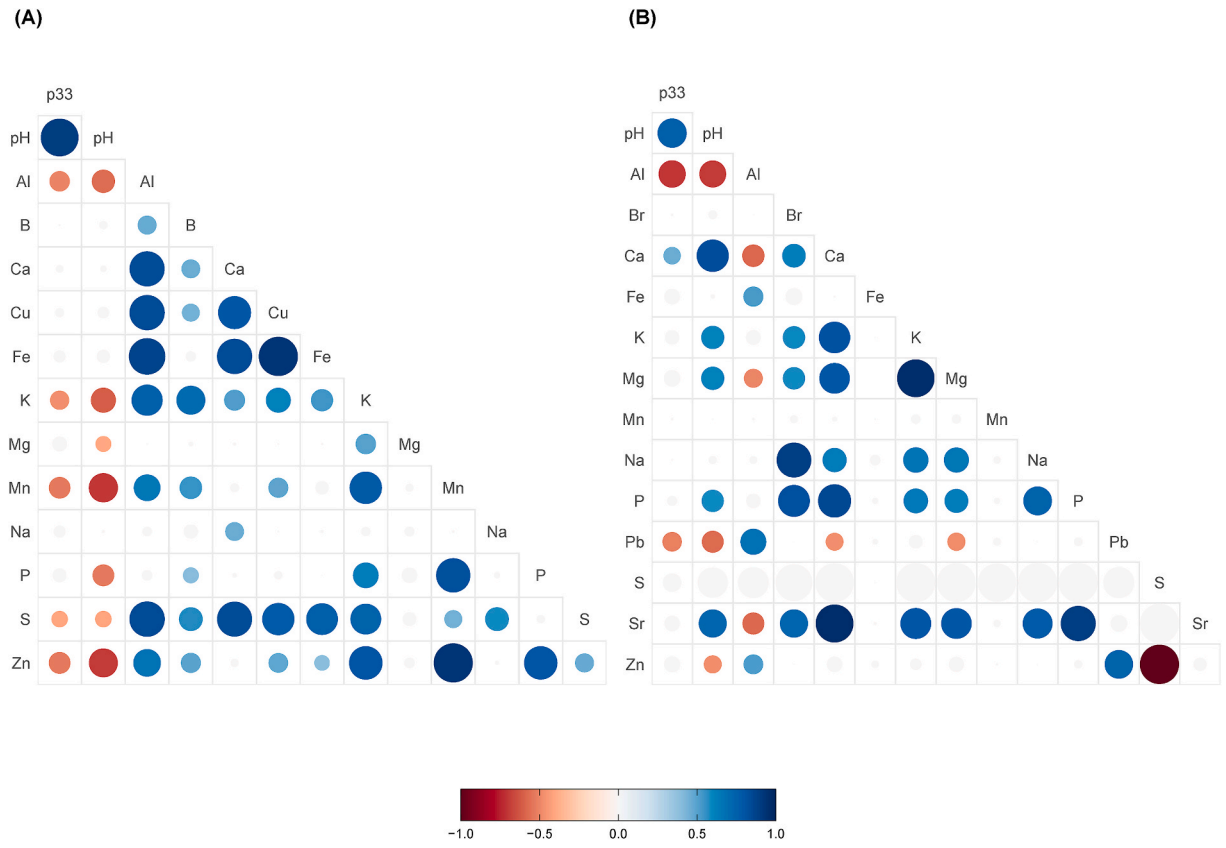

Fig. 6. Pearson correlations between ^{33}P shoot content and soil elements measured by ICP-MS for experiments in **A)** 0.01M $CaCl_2$ extracts of cultivated soils and **B)** 0.5M NH_4Ac extracts of non-cultivated soils. Circles show significant correlations, red circles represent negative correlations and blue circles represent positive correlations, the size of the circles reflects r-values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dominating compounds 1-pentanol, 2-nonanone, phenyl ethyl alcohol, ethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, and their esters which are commonly produced by soil fungi and detected in fungal VOC experiments (Dickschat, 2014; Kanchiswamy, 2015).

Fusarium cf. *solani* produced significantly higher amounts of the volatile sesquiterpenes trichodiene, beta-chamigrene, and alpha-cuprenene, which are related to trichodiene synthesis (Vedula et al., 2008). Trichodiene has previously been isolated from a number of *Fusarium* species, and is one of the first intermediates in the synthesis of trichothecene toxins (Perkowski et al., 2008), and trichodiene production has been correlated to the production of these toxins (Kramer and Abraham, 2012). Beta-chamigrene production has also been documented for *Fusarium* and some fungi produce mixtures of VOCs including beta-chamigrene for defense against enemies (Kramer and Abraham, 2012). Hence, these results point to a potential role of the above volatile sesquiterpenes as well as of trichothecene toxins in the antagonism of *Fusarium* cf. *solani* towards AMF. This potential mechanism of antagonism is in line with the third hypothesis and points to the need for future effect studies, which should even address broader ecological interactions since other volatile sesquiterpenes from *Fusarium* play important roles in e.g. antagonism against nematodes and promotion of growth and disease resistance in plants (Li et al., 2016; Werner et al., 2016).

The trichothecenes is a group of potent mycotoxins with prominent antibiotic properties (Peres de Carvalho et al., 2016). In accordance, antifungal activity of trichothecenes from *Fusarium* towards other fungi has previously been documented (Campos et al., 2011), and it has been proposed that these metabolites play a role in securing the environmental niche of the producer (Venkatesh and Keller, 2019). In support of this notion, production of deoxynivalenol by *F. culmorum* was upregulated during co-culture with the fungus *Alternaria tennuissima* (Venkatesh and Keller, 2019). The interactions between *Fusarium* and AMF may be more complex as the AMF *Glomus (Rhizophagus) irregulare* can down-regulate production of the trichothecene toxin 4,15 diacetoxyscirpenol by a plant pathogenic *F. sambucinum* (Ismail et al., 2013). Future studies should consequently address how the chemical dialogue between AMF and *Fusarium* affect the fitness of the AMF, and even have attention on the plant symbiont as trichothecenes may be highly phytotoxic (Venkatesh and Keller, 2019).

While the above experiments clearly documented a biotic component of suppression, at least for the E2 soil, several of our observations point to an additional role for pH as previously observed by (Svenningsen et al., 2018). In brief, both the current transfer experiments with pasteurized versus field E2 soil, the pH adjustment experiments for suppressive and non-suppressive soils, and the lack of an effect of soil irradiation/pasteurization for several soils support a role for pH or pH-dependent factors in AMF activity suppression or reduced P availability across a wider panel of soils.

4.2. Abiotic factors behind suppression

Low pH might be suppressive due to the direct toxicity of H^+, due to pH-related toxicity factors such as Al^{3+} or deficiency of nutrients such as P. Plants growth is often inhibited by H^+ toxicity in organic, acid soils, low in Al and with pH below ~4; in contrast, Al^{3+} toxicity and/or P deficiency are the limiting factors in mineral soils at pH 4–5 (Kidd and Proctor, 2001; Rahman et al., 2018). This is the pH range where cation exchange buffering shifts to Al buffering. In the present study, AMF suppression was severe in soils with pH ~5 or less and the highly significant negative correlations between extractable Al and pH in

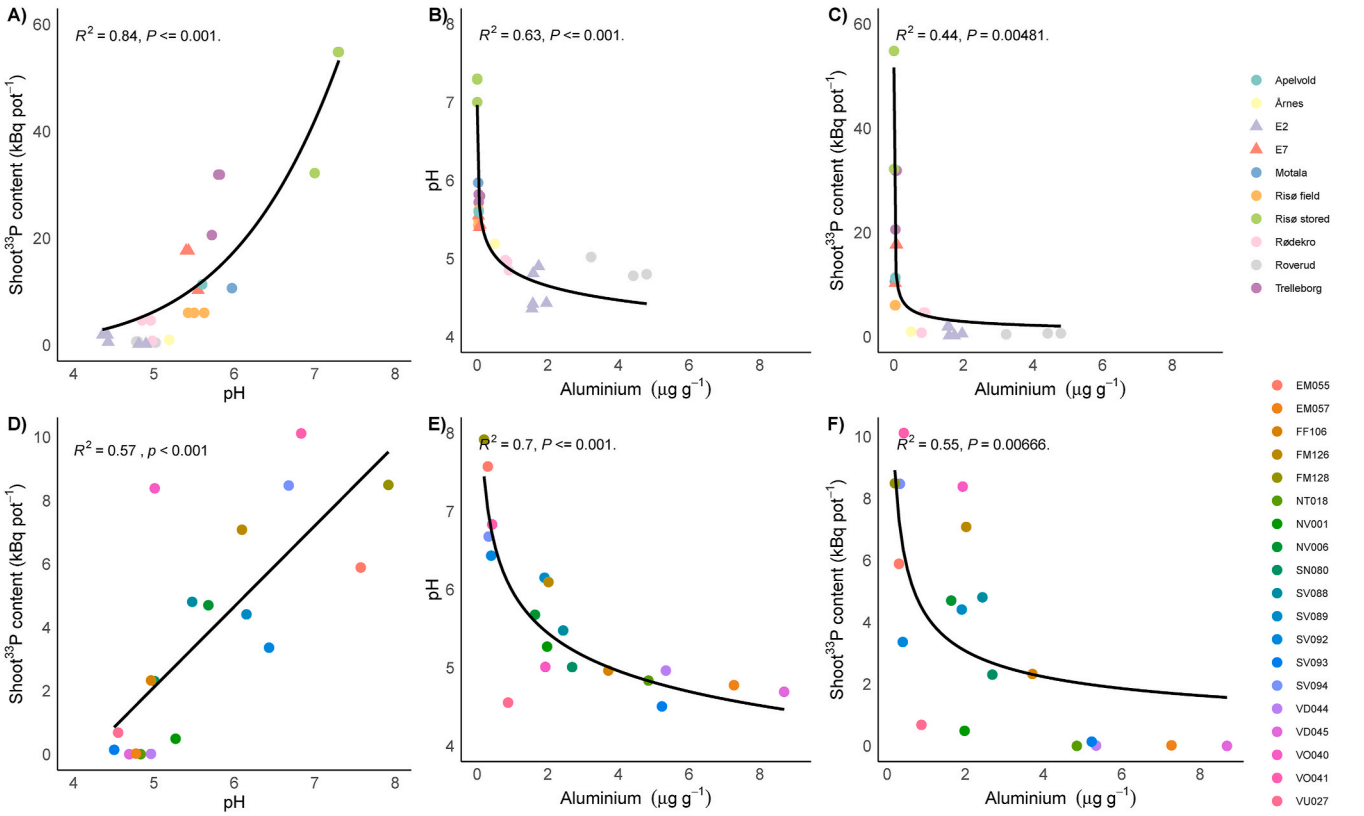

Fig. 7. Pearson correlations between ^{33}P shoot content and soil pH (**A, D**), soil pH and soil aluminium concentration (**B,E**) and ^{33}P shoot content and soil aluminium concentration (**C,F**) in cultivated (**A-C**) and non-cultivated soils (**D-F**). Different colors indicate different soils. Cultivated soils E2 (▲) and E7 (▲) were sampled from the same long-term field experiment, but received NPK fertilizer and manure, respectively. Al was extracted with 0.01 M $CaCl_2$ in cultivated soils and with 0.5 M NH_4Ac in non-cultivated soils. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cultivated as well as non-cultivated soils had a 'break-point' around pH 5.0–5.2. This suggests a role for Al in the suppression of AMF activity (^{33}P uptake) which was indeed negatively correlated to extractable Al. The correlative evidence for Al toxicity-related suppression of AMF function in P uptake in low pH soils supports our fourth hypothesis and extends previous reports on low pH effects on AMF colonization and abundance (Aliasgharzad et al., 2010; Göransson et al., 2008; Zhang et al., 2015).

Sorption of P and ^{33}P to Al and Fe oxides/hydroxides represents an alternative potential explanation for the suppressed ^{33}P uptake in low pH soils. However, ^{33}P uptake did not correlate with P availability (Svenningsen et al., 2018) as confirmed by data from the suppressive E2 (pH 4.4) and the non-suppressive E7 soils (pH 5.6) of the Moystad long-term field experiment. Aqueous extracts of these soils contained twice as much P for E2 than for E7 (12.6 vs 6.4 µg P g^{-1} soil) and also the most ^{33}P in extracts from E2 (533 vs 427 Bq g^{-1} soil). Since the other soils represented in Fig. 7 were also poorly weathered it can be assumed that the low uptake of ^{33}P from low pH and high Al soils was probably not caused by fixation of the added ^{33}P, and hence by a lower availability of ^{33}P for AMF uptake and transport.

Rhizophagus irregularis BEG87 was the AMF model strain throughout this study and an involvement of Al in suppression of P uptake by this strain corroborates previous observations where Al^{3+} was highly toxic towards colonization of soybean roots by another isolate of the same AMF species (Zhang et al., 2015). Indeed, a previous study reported a limited variation in soil suppression of AMF activity among five *R. irregularis* isolates, while three other *Rhizophagus* species showed different patterns (Cruz-Paredes et al., 2020). Such variation among AMF in their tolerance to low soil pH has been demonstrated for spore germination, hyphal growth and root colonization (Bartolome-Esteban and Schenck, 1994; Kelly et al., 2005; Rohyadi et al., 2004; Zhang et al., 2015). Nevertheless, for *R. irregularis* we can generally distinguish between suppressive and non-suppressive soils and propose that the current results are relevant for a wide range of AMF inoculants.

5. Conclusions and outlook

This study extends our previous reports on soil-borne suppression of AMF P transfer from soil to plant (Svenningsen et al., 2018; Cruz-Paredes, 2019) by unequivocally showing the involvement of both biotic and abiotic factors. The demonstrated suppression of AMF activity by *Fusarium* cf. *solani* isolated from an AMF suppressive soil is novel, as is our identification of *Fusarium*-derived trichodiene, an intermediate for potentially AMF-toxic trichothecenes. Future work in this area will hopefully improve our understanding of the chemical dialogue underpinning the interactions between AMF and other soil fungi. However, our confirmation of a general suppression even shows that the interplay between several microorganisms in the soil microbial web needs to be resolved in more detail. This study focused on suppression of AMF in terms of P transfer only and it will be relevant to investigate the suppression of other AMF characteristics such as growth, sporulation and transport of other nutrients by the ERM.

Our finding that suppression of AMF function is most severe in low pH soils, and related to Al toxicity, has relevance for the management of AMF towards optimal function under field conditions. In case the low pH dependent suppression occurs in the field, the soil pH should be kept higher than 5.0–5.2 by liming and fertilizing. These standard management practices might have a hitherto overlooked potential for enhancing the function of native and introduced AMF in the field.

Declaration of competing interest

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

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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.2021.108305.

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