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Inconsistent effects of agricultural practices on soil fungal communities across 12 European long-term experiments

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Abstract

Cropping practices have a great potential to improve soil quality through changes in soil biota. Yet the effects of these soil-improving cropping systems on soil fungal communities are not well known. Here, we analysed soil fungal communities using standardized measurements in 12 long-term experiments and 20 agricultural treatments across Europe. We were interested in whether the same practices (i.e., tillage, fertilization, organic amendments and cover crops) applied across different sites have predictable and repeatable effects on soil fungal communities and guilds. The fungal communities were very variable across sites located in different soil types and climatic regions. The arbuscular mycorrhizal fungi (AMF) were the fungal guild with most unique species in individual sites, whereas plant pathogenic fungi were most shared between the sites. The fungal communities responded to the cropping practices differently in different sites and only fertilization showed a consistent effect on AMF and plant pathogenic fungi, whereas the responses to tillage, cover crops and organic amendments were site, soil and crop-species specific. We further show that the crop yield is negatively affected by cropping practices aimed at improving soil health. Yet, we show that these practices have the potential to change the fungal communities and that change in plant pathogenic fungi and

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in AMF is linked to the yield. We further link the soil fungal community and guilds to soil abiotic characteristics and reveal that especially Mn, K, Mg and pH affect the composition of fungi across sites. In summary, we show that fungal communities vary considerably between sites and that there are no clear directional responses in fungi or fungal guilds across sites to soil-improving cropping systems, but that the responses vary based on soil abiotic conditions, crop type and climatic conditions.

Highlights:

- Soil fungi were analysed using standardized measurements in 12 long-term experiments and 20 agricultural treatments
- Fungal communities responded to the cropping practices differently at different sites
- Only reduced fertilization showed a consistent effect on AMF and plant pathogenic fungi, whereas the responses to tillage, cover crops and organic amendments were site specific.
- Fungal community structure varied significantly between sites, crops and climate conditions; therefore, more cross-site studies are needed in order to manage beneficial soil fungi in agricultural systems.

KEYWORDS

long-term experiments, organic amendments, soil fungi, soil-improving cropping systems, tillage

1 | INTRODUCTION

Soil fungi are responsible for many ecosystem functions, such as nutrient and carbon cycling, biological control and soil aggregate stability (Frąc et al., Frąc, Hannula, Belka, & Jędryczka, 2018). They are often found to be less abundant in agricultural systems compared to natural systems (Hannula et al., 2017; Hannula, De Boer, & Van Veen, 2012); however, in agricultural soils they also play important roles as mutualists (Lekberg & Koide, 2005; Verbruggen, van der Heijden, Rillig, & Kiers, 2013), decomposers (Clocchiatti, Hannula, van den Berg, Korthals, & de Boer, 2020) and biocontrol agents (Vinale et al., 2008). Furthermore, soil-borne plant pathogenic fungi can have devastating effects on performance of crop plants and hence have a severe effect on agroecosystem functioning (Corredor-Moreno & Saunders, 2020). Hence, the composition of the fungal community and balance between different functional groups of fungi (Hannula & Träger, 2020) are important in arable soils.

Many factors are known to affect soil fungal community structure and function. It has been suggested that the soil fungal community composition at both global and regional scales is mainly driven by abiotic factors such as soil C:N ratio (Lauber, Strickland, Bradford, & Fierer, 2008; Thomson et al., 2015), pH (Dumbrell, Nelson, Helgason, Dytham, & Fitter, 2010; Sun et al., 2016; Tedersoo et al., 2015), soil organic carbon content, soil texture, overall climatic conditions, and land use (Creamer et al., 2016; Frac, Jerzy, Bogusław, Karolina, & Małgorzata, 2020; Sun et al., 2016; Thomson et al., 2015). Additionally, at more local scales, plant species identity (Hannula, Ma, Pérez-Jaramillo, Pineda, & Bezemer, 2020) and agricultural practices (Bender, Wagg, & van der Heijden, 2016; Frąc et al., 2018; Oehl, Laczko, Oberholzer, Jansa, & Egli, 2017; Rillig et al., 2016) have been identified as important factors affecting fungi. More specifically, studies have shown that soil tillage (Schmidt, Mitchell, & Scow, 2019; Sharma-Poudyal, Schlatter, Yin, Hulbert, & Paulitz, 2017; Sommermann et al., 2018), fertilizer regime (Qin et al., 2015; Wang, Rhodes, Huang, & Shen, 2018; Zhu et al., 2016), cover crops (Benitez, Taheri, & Lehman, 2016; Detheridge et al., 2016), selection of crop species and rotation (Sommermann et al., 2018) and organic amendments (Lourenço, Suleiman, Pijl, Cantarella, & Kuramae, 2020; Qin et al., 2015) change the composition of soil fungal communities. However, it is likely that these effects are at least partially dependent on local conditions, including soil type, climatic conditions and soil abiotic factors

(Tedersoo et al., 2014). A recent meta-analysis showed that tillage affected significantly and consistently the soil bacterial community, whereas there were no consistent effects on fungi (Li et al., 2020). Yet, different methodologies (i.e., primers and DNA extraction kits) used in different studies hamper the inference of conclusions across studies (Ramirez et al., 2018). Therefore, it is still unclear how large the effects of the above-mentioned agricultural practices are compared to each other and how applicable the concepts and conclusions of the studies are across soil types.

The mechanisms of how agricultural practices affect the soil fungal communities differ between practices and between the fungal groups (and functional guilds) examined. For example, the negative effects of tillage on soil (saprotrophic) fungal communities are often attributed to physically damaging the hyphae (Kabir, 2005; Kihara et al., 2012), and can be thought of also as an ultimate disturbance that resets the community succession and favours fast-growing fungal species (Sharma-Poudyal et al., 2017) while at the same time negatively affecting its stability (Wagg, Dudenhöffer, Widmer, & van der Heijden, 2018). Fertilizer type (e.g., organic vs. inorganic fertilizers and their ratios) is shown to affect mainly the arbuscular mycorrhizal communities (Verbruggen et al., 2013). High N fertilizer additions can lead to a decrease in soil fungal biomass (de Vries, Hoffland, van Eekeren, Brussaard, & Bloem, 2006). On the other hand, following a reduction or stop in mineral fertilization, an increase in the abundance of fungi is often observed (Morrien et al. Morriën et al., 2017; de Vries et al., 2006 & de Vries, van Groenigen, Hoffland, & Bloem, 2011; Gordon, Haygarth, & Bardgett, 2008) and these conditions favour the growth and efficiency of fungi (Di Lonardo, van der Wal, Harkes, & de Boer, 2020). Organic amendments and green manure have been shown to influence the saprotrophic fungal community (Clocchiatti et al., 2020), due to the capacity of fungi to utilize a wide fraction of the added organic materials (Heijboer et al., 2016). Furthermore, competition, antagonism and hyperparasitism play important roles in affecting the fungal population density, dynamics and metabolic activities (Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moënne-Loccoz, 2009). A change in one functional guild of fungi can affect other functional guilds via interaction effects between fungal species (Kepler, Maul, & Rehner, 2017; Lendzemo, Kuyper, Kropff, & van Ast, 2005; Xiong et al., 2017), through effects via other microbes (de Boer et al., 2015; Van Beneden et al., 2010) or through soil food–web interactions (Moore, 1994).

To study the variation in soil fungi caused by the agricultural practices across soils, we performed standardized measurements on soil chemistry and soil fungal communities across four countries, eight locations and 12 experiments, testing a total of 20 agricultural practices that were grouped in four main categories: (a) tillage, (b) fertilization, (c) organic amendments and (d) cover crops. Our hypotheses were that (a) different agricultural management practices change the soil fungal communities in a predictable and consistent manner across the sites and (b) arbuscular mycorrhizal fungi (AMF) are most affected by soil nutrient levels, type and plant species identity, whereas tillage and organic amendments (such as straw) affect the saprotrophic community most. We further investigated whether the same fungi are shared and affected similarly by the agricultural sites and if we could find operational taxonomic units (OTUs) responding consistently to the agricultural practice; hence, they could be considered potentially as biological indicator OTUs (of specific agricultural management practices).

2 | MATERIAL AND METHODS

2.1 | Sampling sites

We sampled 12 long-term experiments evaluating different soil improving treatments (Table 1). For each experimental treatment there were replicated reference (control) plots at the same location at each sampling, so that the effect of treatment could be evaluated locally, as well as across countries. The longest running experiment was started in 1894 (DEN2) and the newest in 2015 (UK2; Table 1). The field experiments were located in four different European countries (Belgium, Denmark, Hungary and the UK) and covered a range of climates and soil types. Treatments investigated in these studies varied but could be generally divided into four main categories: (a) tillage, (b) fertilization, (c) organic amendments and (d) cover crops and avoidance of bare fallow. Here we evaluated the change in fungal community, crop yield and soil chemical parameters in response to the treatments. We compared the changes in such a way that "conventional" treatment is the control and the treatment(s) are the activities intended to enhance the soil life. This meant that for tillage, conventional tillage is the control and reduced and no-tillage are the treatments. For fertilization, normal fertilization levels (control) were compared to adding no or reduced fertilizers (treatments). Similarly, no organic amendments (control) was compared with organic amendments such as straw, compost or manure (treatments); mineral fertilizer (control) was compared to manure and compost amendment (treatments); and fallow (control) was compared with using cover crops or wider rotation (treatments). In experiments in which several treatments were performed in combination, the comparisons were always made with the control

TABLE 1 Description of the sites included in the study. For further details, see Supporting Information

Experiment	Name	Location	Set-up year of the experiment (sampled in 2016)	Treatment	Type of experiment	Levels of treatment	Number of replicates	Number of samples	Crop 2016	Soil type (FAO classification)	Further information
Belgium 1	BE1	Huldenberg, Lubbeek	2001 (Huldenberg) and 2004 (Lubbeek)	Tillage	Replicated over fields	Fields with conventional tillage and fields with reduced (inversion) tillage	2	4	Maize, potato	Luvisols	
Belgium 2	BE2	Boutersem	1997 (compost addition started 2013)	Compost/ inorganic fertilizer/ fallow	Replicated in a field	T1 = control, T2 = mineral according to advice, T5 = triannual compost amendment (45 t), T9 = annual compost (15 t), T11 = annual compost (45 t), T12 = left fallow	4	20	Winter wheat	Luvisols	
Denmark 1	DEN1	Jyndevad	1942	Liming/P addition	Replicated in a field	Lime (0, 4, 8 and 12 t) and P (0 or 15 kg P/ha) and barley or permanent fallow	3	48	Spring barley/ fallow	Orthic Haplohumod	Azeez et al., 2020.
Denmark 2	DEN2	Askov	1980	Straw incorporation/ catch crop	Replicated in a field	Straw (0, 12 t) and ryegrass/ryegrass + clover	3	12	Spring barley	Aric Haplic Luvisol	Hemkemeyer et al., 2019; Christensen et al., 2019
Denmark 3	DEN3	Askov	1894	Fertilization	Replicated in a field	Control, 1 NPK, 1 AM (animal manure)	3	9	Spring barley	Aric Haplic Luvisol	Hemkemeyer et al., 2019
Denmark 4	DEN4	Foulum	2002/cover crops 2008	Tillage/cover crop	Replicated in a field	Three levels of tillage (conventional/harrow/ direct drill) and cover crop (fodder radish)	4	48	Oat	Mollic Luvisol	Hansen, Munkholm, Olesen, & Melander, 2015; Abdollahi et al., 2013
Hungary 1	HUN1	Keszthely	1983	Inorganic fertilization, organic amendment	Replicated in a field	Two crops (maize-wheat), 3 levels of inorganic N (N0, N2, N4), 3 levels of organic N (0, organic manure, straw/stalk incorporation)	3	54	Maize and winter wheat	Eutric Cambisol	
Hungary 2	HUN2	Keszthely	1972	Tillage/ fertilization	Replicated in a field	Soil tillage system (conventional, shallow, minimum) and N-fertilization rates (N0, N2, N4)	4	32	Maize	Eutric Cambisol	

TABLE 1 (Continued)

Experiment	Name	Location	Set-up year of the experiment (sampled in 2016)	Treatment	Type of experiment	Levels of treatment	Number of replicates	Number of samples	Crop 2016	Soil type (FAO classification)	Further information
Hungary 3	HUN3	Keszthely	1963	Fertilization/ crop rotation	Replicated in a field	Two different crops at two different rotations*, 3 levels of fertilizers (0, 2080 NPK5 $year^{-1}$, 2080 NPK5 $year^{-1}$ + manure)	4	48	Maize and winter wheat	Eutric Cambisol	
Hungary 4	HUN4	Keszthely	1969	Fertilization/ timing of fertilization	Replicated in a field	Fertilizer rate (0 or 900 NPK) and timing of fertilization (spring/ autumn, spring*2)	4	32	Maize	Eutric Cambisol	
United Kingdom 1	UK1	Loddington, Normanton, Stonton, Goadby	Field trials by farmers	Tillage	Replicated over fields	Fields with conventional tillage and fields with no-tillage	8	16	Varies	Luvisol/ Cambisol	
United Kingdom 2	UK2	Loddington	2015	Cover crops	Replicated over fields	Three different cover crop mixtures, control	3	12	Spring oats	Luvisol/ Cambisol	Crotty & Stoate, 2019

for the desired treatment with the same level in the other parameter (e.g., in experiment HUN2 the minimum tillage is compared to conventional tillage in non-fertilized plots and in fertilized plots separately).

2.2 | Soil sampling

Soils were sampled using the same standardized method in all locations. Between April and October 2016, from each field, 1 kg of soil was collected at 0–20-cm depth. Ten subsamples per plot were collected randomly and pooled to form one sample per plot. All samples were kept cool during transportation. For the chemical analyses, soils were dried at 50°C and sieved (2 mm) to remove roots and rocks. The soil was crushed manually. Tubes of 50 mL were filled with roughly 30 g of soil and stored. For the molecular analyses, subsets of fresh soil samples were taken after homogenization by hand and stored at −20°C until further processing (DNA extractions).

2.3 | Soil chemical analysis

Chemical soil properties were determined by AgroCares BV (Wageningen, the Netherlands). Soils for chemical analysis were dried at 50°C using fruit dryers, crushed and sieved (2 mm sieve). One part of the soil sample was homogenized and pulverized (<0.2 mm) using a planetary micro mill with 10 clean metal balls for 3 min with speed 500 rpm. This sample was used to measure the total C and N by heating it to 900°C in the presence of O_2, forming CO_2 and N_2, which were quantitatively measured with a thermal conductivity detector. Peak areas are correlated with validated calibration curves, to obtain element weight for C and N, which is recalculated to percentage by considering the sample mass. Total organic carbon (TOC) was measured using the Elementar Rapid CS cube (Elementar Analysensysteme, Germany) after removal and quantification of the total inorganic carbon (TIC) fraction as carbonates through acid (1 M HCl) treatment. Samples for soil texture were weighed and treated with 30% H_2O_2 for the removal of organic material, treated with dithionite solution (40 g/L $Na_2S_2O_4$ in 0.3 M NaOAc, pH 3.8) for the removal of iron oxide, and treated with 1 M HCl for the removal of carbonates. After this sample treatment, the samples were measured with the Mastersizer 3,000 (Malvern Panalytical B.V., Almelo, the Netherlands) to determine the particle size distribution using laser diffraction. Soil pH (KCl) was determined using a pH electrode.

The procedure for the extraction of soils using Mehlich-3 solution as extractant was validated and executed according to Wolf and Beegle (2011), with one exception, the shaking time was increased from 5 to 10 min. The measurement of samples for the determination of bulk multi-element concentrations in dry soil samples (RT: Real Totals) was carried out using the PANalytical Epsilon 3 energy dispersive x-ray fluorescence (ED-XRF) (Malvern Panalytical B.V., Almelo, the Netherlands). The procedure is in accordance with ISO18227:2014 and validated. The samples were prepared as pellets with a soil to wax ratio of 9:1. Lastly, cation exchange capacity (CEC) and the content of exchangeable cations (Al^{3+}, Ca^{2+}, Fe^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, B^+, Cu^{2+}, Mo^{2+}, Ni^{2+} and Zn^{2++}) and anions (S^{2-}, P^{3-}) in soils were determined after extraction with hexamminecobalt trichloride solution. The procedure was validated and is in accordance with ISO 23470:2007.

2.4 | Yield

We obtained yield information from most of the plots with crop plants in them for the year of sampling and 2 years prior. As there were different crops in the field and measurements are not fully comparable, we calculated relative change in yield due to agricultural practice (M2) compared to control (M1) using Cohen's d (M2-M1/SD pooled). If information from multiple years (and hence multiple crops) was available, we averaged out the effects between years to obtain one index for each treatment.

2.5 | Molecular analysis

DNA was extracted using the modified Power Soil protocol (Harkes et al., 2019), with 0.25 g soil per sample and Lysing matrix E beads tubes (MP Biomedicals, Irvine, CA, USA). Fungal DNA was amplified using primers ITS4ngs and ITS3mix1-5 (Tedersoo et al., 2014, 2015) and purified using AMPure magnetic beads (Beckman Coulter, Brea, CA, USA). Polymerase chain reactions (PCRs) were performed with 12.5-μL Hotstart ready mix (Fisher scientific, Waltham, MA, USA) and approximately 50 ng of DNA per reaction. Dual tags were added to samples (Illumina Nextera XT dual indexing kits v1-3, San Diego, CA, USA) using seven cycles of PCR. PCR products were further purified using magnetic beads. The DNA was quantified using a Qubit fluorometer and equimolar pooled into libraries of 285 and 250 samples each. Mock community samples with eight fungal strains were sequenced along with the experimental samples. Sequencing was performed using Illumina MiSeq pair-end 2x300bp.

The reads were assigned to samples based on tags at both ends. No mismatches were allowed. The reads were processed using PIPITs (Gweon et al., 2015). In short, first

paired end reads were joined and low quality and non-paired reads were filtered out using PEAR and FASTX-TOOLKIT (Zhang, Kobert, Flouri, & Stamatakis, 2014). Then the ITS region was extracted using ITSx (Bengtsson-Palme et al., 2013), which uses HMMER3 (Mistry, Finn, Eddy, Bateman, & Punta, 2013), and sequences were reoriented and reinflated to reflect their original abundances. The resulting sequences were clustered using PARSE (Edgar, 2013), taxonomy was assigned using the UNITE database (Nilsson et al., 2019) with a 97% similarity threshold and chimeric sequences were removed. Sequences from non-fungal origin were also removed from the dataset. The ecology of each OTU was assigned according to FunGuild (Nguyen et al., 2016) and an in-house database of plant pathogens when possible (i.e., taxonomy assignment could be carried out to higher than genus level). Samples with less than 2,000 or more than 60,000 OTUs were removed from the dataset in order to standardize the data. Furthermore, OTUs found in less than three samples with relative abundance of <0.001% were removed.

2.6 | Statistical analysis

Statistical analysis was performed in R using packages "phyloseq" and "vegan" (McMurdie & Holmes, 2013; Oksanen et al., 2013). Proportion of a read from total reads was used to correct for differences in number of reads between samples. Non-metric multidimensional scaling (NMDS) with Bray-Curtis transformation was used to explore the clustering of the samples. Permutational analysis of variance (PERMANOVA), command ("adonis") with Bray-Curtis transformation was used to compare the community composition between treatments and "simper" to detect which OTUs contribute most in explaining differences between treatments. Analysis of variance (ANOVA) (with Tukey post-hoc) was used to compare differences in number of sequences and/or OTUs between treatments. ENVFIT analysis and canonical correlation analysis (CCA) in vegan were used to investigate the effects of soil chemistry on community structure of fungi.

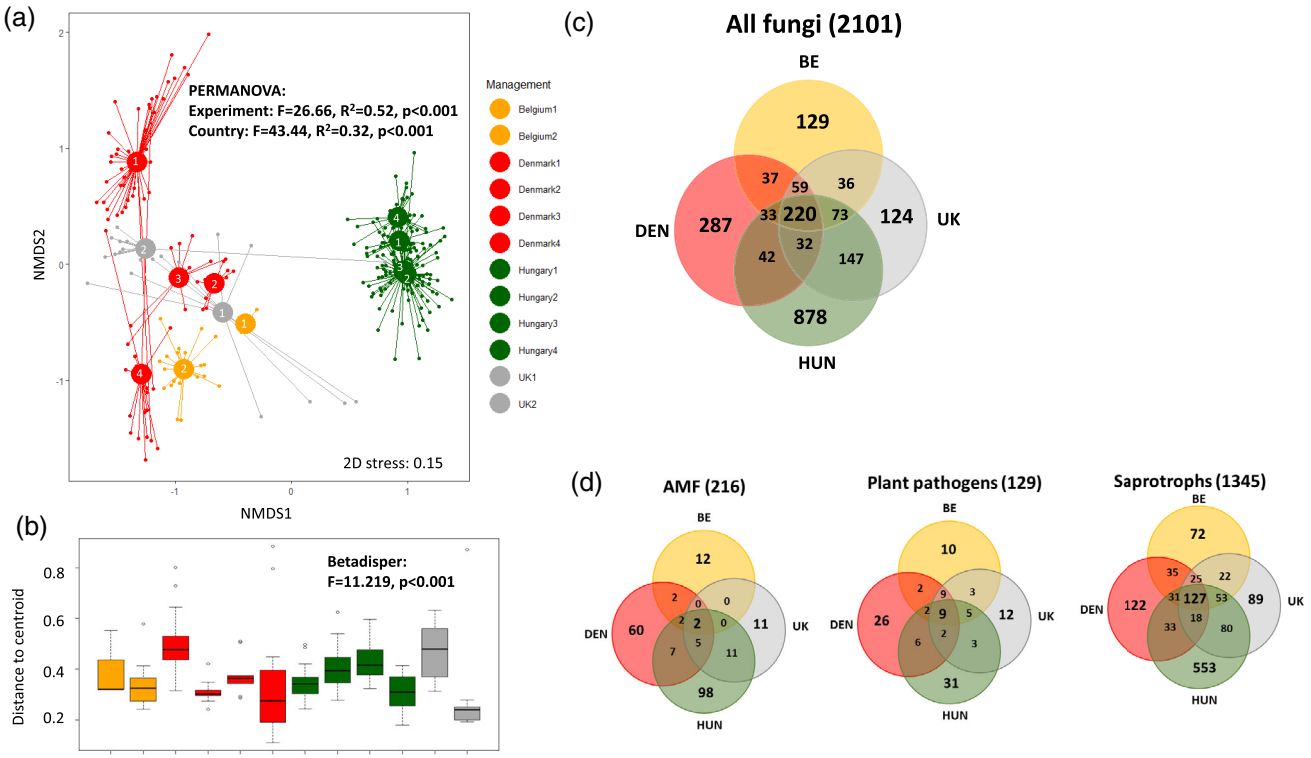


FIGURE 1 The community structure and beta dispersion of soil fungi across countries and experiments and shared fungal operational taxonomic units (OTUs) between countries. (a) The Non-metric multidimensional scaling (NMDS) ordination was calculated using Bray-Curtis distance and shows the centroids and variation of each experiment in all countries. For further information on the experiments see Table 1. (b) Beta dispersion of the same experiments depicts how much the treatments within an experiment vary from each other. (c & d) Partial Venn-diagram of OTUs that are unique and shared between experiments for all fungi (c) and for the main fungal guilds (d). For c & d, only OTUs that were present in at least 5% of the samples from that country were included. In all figures, colours depict country of the experimental plot (yellow = Belgium, red = Denmark, green = Hungary and grey = the UK). The comparisons between Denmark (DEN) and the UK and Hungary (HUN) and Belgium (BEL) are not shown for simplicity but were in the same magnitude as the other two-way comparisons. AMF, arbuscular mycorrhizal fungi [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Identity of the arbuscular mycorrhizal fungi (AMF), plant pathogenic and saprotrophic fungal operational taxonomic units (OTUs) shared across all field locations depicted in Figure 1D. All fungal OTUs are identified to the highest accuracy possible ("unknown" indicates species that were not able to be identified). For saprotrophs the sum of multiple OTUs with the same classification are marked in the column "#OTUs"

Guild	# OTUs	Phylum	Class	Order	Genera/species
AMF	1	Mucoromycota	Glomeromycetes	Paraglomerales	*Paraglomus*
AMF	1	Mucoromycota	Glomeromycetes	Diversisporales	Gigasporaceae
Potential plant pathogen	2	Ascomycota	Dothideomycetes	Capnodiales	*Devriesia* sp.
Potential plant pathogen/endophyte	1	Ascomycota	Dothideomycetes	Pleosporales	*Dendryphion nanum*
Potential plant pathogen	1	Ascomycota	Dothideomycetes	Pleosporales	*Drechslera* sp.
Potential plant pathogen	1	Ascomycota	Sordariomycetes	Hypocreales	*Fusarium poae*
Potential plant pathogen	1	Ascomycota	Sordariomycetes	Hypocreales	*Ilyonectria robusta*
Potential plant pathogen	1	Ascomycota	Sordariomycetes	Hypocreales	*Fusarium* sp.
Potential plant pathogen	1	Ascomycota	Taphrinomycetes	Taphrinales	*Protomyces inouyei*
Potential plant pathogen	1	Chytridiomycota	Chytridiomycetes	Rhizophydiales	*Rhizophydium* sp
Saprotroph	3	Ascomycota	Dothideomycetes	Pleosporales	*Pyrenochaetopsis* sp.
Saprotroph	1	Ascomycota	Dothideomycetes	Pleosporales	*Trematosphaeria hydrela*
Saprotroph	2	Ascomycota	Dothideomycetes	Pleosporales	*Pyrenochaeta* sp
Saprotroph	3	Ascomycota	Eurotiomycetes	Chaetothyriales	*Exophiala*
Saprotroph	11	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae (including *Aspergillus* & *Penicillium*)
Saprotroph	1	Ascomycota	Eurotiomycetes	Onygenales	*Auxarthron umbrinum*
Saprotroph	1	Ascomycota	Leotiomycetes	Helotiales	*Neobulgaria* sp.
Saprotroph	1	Ascomycota	Leotiomycetes	Helotiales	*Scytalidium lignicola*
Saprotroph	2	Ascomycota	Orbiliomycetes	Orbiliales	*Arthrobotrys* sp.
Saprotroph	1	Ascomycota	Pezizomycetes	Pezizales	*Ascobolus* sp.
Saprotroph	1	Ascomycota	Pezizomycetes	Pezizales	*Byssonectria fusispora*
Saprotroph	2	Ascomycota	Pezizomycetes	Pezizales	*Pseudaleuria* sp.
Saprotroph	2	Ascomycota	Pezizomycetes	Pezizales	*Scutellinia scutellata*
Saprotroph	1	Ascomycota	Saccharomycetes	Saccharomycetales	Unknown
Saprotroph	1	Ascomycota	Sordariomycetes	Chaetosphaeriales	*Chaetosphaeria*
Saprotroph	19	Ascomycota	Sordariomycetes	Hypocreales	Unknown
Saprotroph	5	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae
Saprotroph	11	Ascomycota	Sordariomycetes	Hypocreales	*Trichoderma*
Saprotroph	2	Ascomycota	Sordariomycetes	Hypocreales	Unknown
Saprotroph	1	Ascomycota	Srdariomycetes	Hypocreales	*Acremonium persicinum*
Saprotroph	1	Ascomycota	Sordariomycetes	Hypocreales	*Acremonium rutilum*
Saprotroph	1	Ascomycota	Sordariomycetes	Hypocreales	*Stachybotrys eucylindrospora*
Saprotroph	13	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae

TABLE 2 (Continued)

Guild	# OTUs	Phylum	Class	Order	Genera/species
Saprotroph	1	Ascomycota	Sordariomycetes	Sordariales	*Chaetomium funicola*
Saprotroph	3	Ascomycota	Sordariomycetes	Sordariales	*Cercophora* sp.
Saprotroph	2	Ascomycota	Sordariomycetes	Sordariales	*Podospora* sp.
Saprotroph	1	Ascomycota	Sordariomycetes	Sordariales	*Sordaria*
Saprotroph	1	Basidiomycota	Agaricomycetes	Agaricales	*Lycoperdon*
Saprotroph	1	Basidiomycota	Agaricomycetes	Agaricales	*Coprinellus micaceus*
Saprotroph	1	Basidiomycota	Agaricomycetes	Agaricales	*Coprinopsis narcotica*
Saprotroph	1	Basidiomycota	Agaricomycetes	Agaricales	*Hypholoma fasciculare*
Saprotroph	1	Chytridiomycota	Chytridiomycetes	Rhizophydiales	*Rhizophlyctis rosea*
Saprotroph	23	Mucoromycota	Mortierellomycotina	Mortierellales	*Mortierella*
Saprotroph	3	Mucoromycota	Mortierellomycotina	Mortierellales	*Mortierella exigua*
Saprotroph	1	Mucoromycota	Mortierellomycotina	Mortierellales	*Mortierella alpina*
Saprotroph	1	Mucoromycota	Mortierellomycotina	Mortierellales	*Mortierella amoeboidea*
Saprotroph	1	Mucoromycota	Mortierellomycotina	Mortierellales	*Mortierella clonocystis*

Effect sizes compared to control were calculated using Cohen's d (M2-M1/SD pooled) to standardize between differences in relative abundances of microbial groups.

To explore fungal OTUs that were significantly affected by treatments, the package DEseq2 was used (Love, Huber, & Anders, 2014). Only OTUs with over log2-fold change and with p values <0.05 after Benjamini-Hochberg multiple-inference correction are reported.

3 | RESULTS

3.1 | Change in fungal communities due to agricultural practices

We first quantified the variance in soil fungal communities explained by country and experiment. We found that the fungal communities in soils of the experiments in Hungary (Eutric cambisol) were the most dissimilar, whereas other locations clustered more together (Figure 1a). Both experiment (pseudo-F = 26.66, $R^2 = 0.52$, $p < 0.001$) and country (pseudo-F = 43.33, $R^2 = 0.32$, $p < 0.001$) significantly affected soil fungal community composition. Also, beta dispersion was significantly affected by both experiment and country ($F = 11.22$, $p < 0.001$; Figure 1b), making direct comparisons between experiments difficult. We further investigated if the same fungi were present in all sites and if they could potentially be used as an indicator organism of disturbances across sites. Over two-thirds (68% of 2,101 OTUs detected) of the fungal species were present only in one country, whereas around 20% were shared by three or more countries (Figure 1c). Hungary had the highest number of unique OTUs, as 67% (878 of 1,304 OTUs in total) of OTUs detected in Hungary were unique to Hungary. When looking at specific fungal guilds, we saw that out of 216 detected AMF OTUs, 84% were present only in the soil of one country, whereas just over 3% were shared by three or more countries. Of potentially plant pathogenic fungi, around 21% were found in at least three countries, whereas 61% were specific to a country. Similarly, for saprotrophic fungi, around 19% were found in soils of at least three countries and 62% were unique to one of the countries (Figure 1d). Looking at the identity of the OTUs shared between all sites (Table 2), we noted that the shared potentially plant pathogenic OTUs were mainly from ascomycete orders Pleosporales and Hypocreales and the saprotrophic OTUs shared between all sites were mainly identified as Hypocreales (53 OTUs) and Mortierellales (Mucoromycota; 29 OTUs). Yet, only a small proportion of OTUs were shared between sites and hence, instead of comparing soils on the OTU level or community structures to each other, we decided to use change in fungal communities due to agricultural practice compared to a local control as a measure.

When we investigated the effects of treatments on fungal communities using Bray–Curtis dissimilarity of communities as a distance of the treatment to the control as a proxy for change, we detected that the change in fungal community dissimilarity was largest in DEN1, and especially in the lime addition treatment

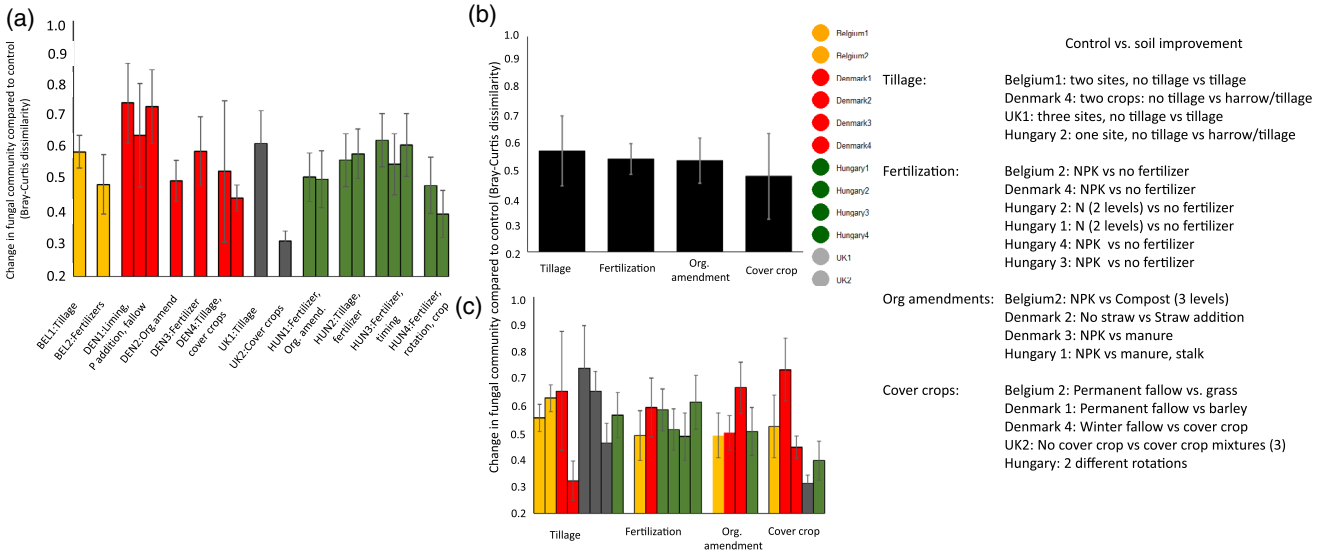


FIGURE 2 Degree of change in fungal communities compared to the control plots measured with Bray–Curtis dissimilarity across experiments (a & c) and averaged by treatment category (b). The bars represent average distance between each control and treatment plot in a respective experiment and error bars depict standard error for calculations across the plots. Colours depict country of the experimental plot (yellow = Belgium, red = Denmark, green = Hungary, grey = the UK). (d) The treatments used and comparisons made in this figure and throughout the article [Color figure can be viewed at wileyonlinelibrary.com]

(average dissimilarity between controls and limed plots: for 4 t lime/ha 0.62, 8 t/ha 0.71 and 12 t/ha 0.75, respectively). Adding lime was also the treatment that was most significantly affecting the community structure measured with PERMANOVA (Figure 2, pseudo-$F = 3.637$, $R^2 = 0.23$, $p < 0.001$; Figure 2a). The treatment affecting soil communities the least was identity of cover crop mixtures in experiment UK2 (Figure 2, pseudo-$F = 0.93$, $R^2 = 0.01$, $p = 0.56$; Figure 2a).

We did not detect a significant difference in magnitude of change in soil fungal communities between larger categories of the replicated agricultural treatments (i.e., tillage, fertilization, organic amendments and cover crops) and the detected differences were context dependent. In general, tillage had the largest effect on fungal community composition (Figure 2), yet the magnitude of change varied largely across sites and crops. Especially striking was the detected interaction effect between cover cropping and tillage within one soil type in the experiment Denmark 4 (DEN4): tillage in soils with cover crops had a strong effect on soil communities (average Bray–Curtis dissimilarity between tillage and no-tillage 0.65), whereas in soils kept bare in winters since 2008 the effect of tillage was much smaller (average Bray Curtis dissimilarity 0.32).

For tillage and fertilization experiments we further investigated if the effect depends on the intensity of tillage or the amount of fertilizer added (Figure S1). In the three experiments in which shallow disk/harrow was used, their effect on fungal community structure was slightly but not statistically significantly smaller than for conventional tillage. Fertilization level did not make a difference to the fungal community structure when it was compared to the control.

3.2 | Change in fungal guilds due to agricultural practices

Non-fertilized plots had consistently more AMF than their fertilized counterparts across all experiments investigating fertilization (Figure 3a). Furthermore, most plots with organic amendments had more AMF than the plots fertilized with inorganic fertilizers only. This was most clear for plots treated with manure or compost. Also, cover crops had an effect on the relative abundance of AMF in two of the three experiments. Tillage did not cause significant, consistent, shifts in the relative abundance of AMF. The largest decrease in relative numbers of AMF due to mineral fertilization was noted in the Danish experiment (DEN3) and Belgian treatments with added mineral nutrients or compost (BEL2). In both studies, in the control plots without fertilization the relative abundance of AMF was double the abundance compared to the plots with added manure, compost or NPK. Fertilizers also changed the community structure of AMF the most when measured with R^2 values compared to control based on Bray-Curtis distance (Figure 3d). Fertilizer type had also a significant direct effect on the AMF community

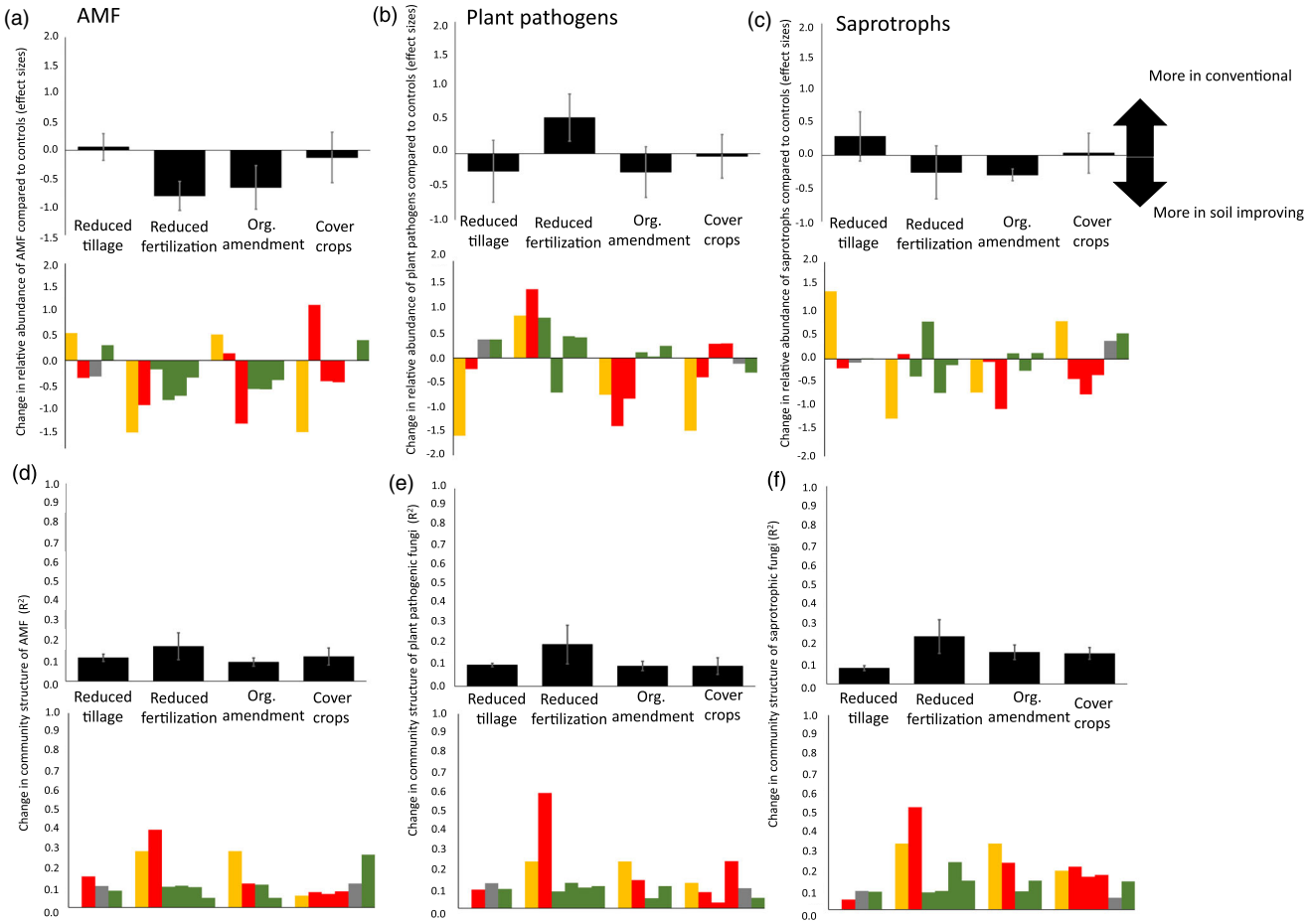


FIGURE 3 Degree of change in relative abundance of fungal guilds (a–c) and in their community structure (d–f) compared to the control plots sorted by treatment category (see Figure 1d; tillage, fertilization, organic amendment and cover crops). Positive values indicate in a–c that a group is more abundant in conventional treatment plots than in treatment plots, whereas negative values mean that it is increased in the soil improvement plots compared to conventional plots. Conventional treatments are: tillage (soil-improving treatment no-tillage), inorganic fertilization (contrary to no fertilization), inorganic fertilization or nothing (compared to organic amendments) and cover crops/cover (compared to fallow). Colours depict country of the experimental plot (yellow = Belgium, red = Denmark, green = Hungary, grey = the UK). For the organic amendments also adding nutrients (manure, compost), the change in communities is calculated compared to the mineral fertilized plots. The coloured bars represent average change in relative abundance (measured as %; a–c) and average change in community structure (measured as R^2 values; d–f) of fungal functional guilds between control and treatment plots. Black bars represent averages of aforementioned values across experiments and error bars show calculated standard error. AMF, arbuscular mycorrhizal fungi [Color figure can be viewed at wileyonlinelibrary.com]

(PERMANOVA across experiments pseudo-F = 2.22, $R^2 = 0.05$, $p < 0.001$). As for the relative abundance of AMF, the largest shifts in the AMF community were observed in experiments DEN3 and BEL2.

The relative abundance of plant pathogenic fungi was higher in plots with mineral fertilization and was only reduced consistently in the no-fertilizer treatments (Figure 3b). We observed increases in plant pathogens due to fertilization in all the sites except for one site in Hungary (HUN1), where there was relatively less plant pathogenic fungi in fertilized control treatments than in non-fertilized plots. Yet, in that experiment also stalk and manure were added in combination with fertilizer. There were no specific pathogens that increased across sites (Figure 1d), but the effect was rather attributed to total increase in relative numbers of potentially plant pathogenic fungi in soils. Tillage, organic amendments and cover crops had inconsistent effects across sites in most cases, relative abundance of plant pathogenic fungi was decreased as a result of adding organic substances and reducing tillage and due to cover crops in around half of the sites, whereas the abundance of plant pathogenic fungi increased in some sites as a result of soil-improving practices (Figure 3b). The strongest increase in plant pathogenic fungi was observed due to no-tillage in BEL1, which was mainly a result of increases in *Ilyonectria*

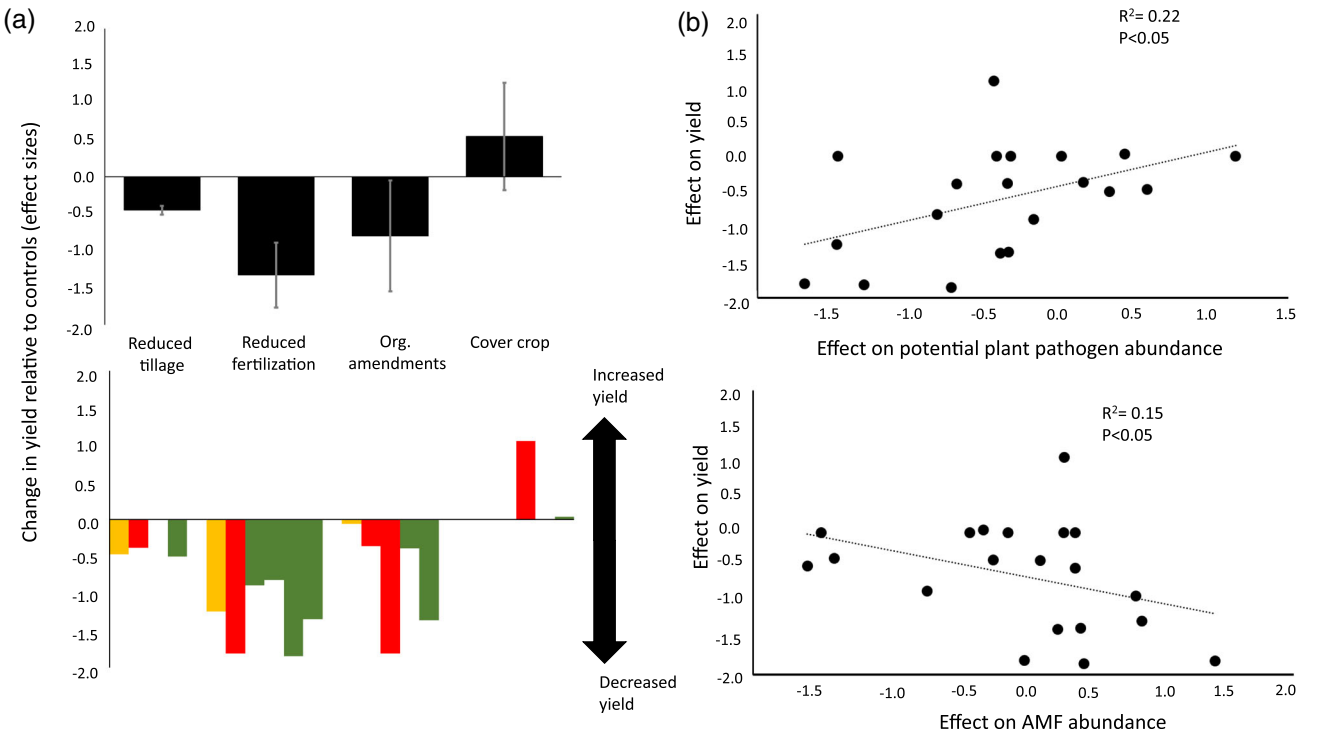


FIGURE 4 Relative change in yield across experiments by soil-improving cropping system (a). Colours depict country of the experimental plot (yellow = Belgium, red = Denmark, green = Hungary, grey = the UK). (b) The change in yield correlated with the change in relative abundance of arbuscular mycorrhizal fungi (AMF) and plant pathogenic fungi in the same experiments. For the organic amendments also adding nutrients (manure, compost), the change in yield is calculated compared to the mineral fertilized plots. In (a) the coloured bars represent average change in yield between control and treatment plots, black bars represent averages in yield across experiments and error bars show calculated standard error. AMF, arbuscular mycorrhizal fungi [Color figure can be viewed at wileyonlinelibrary.com]

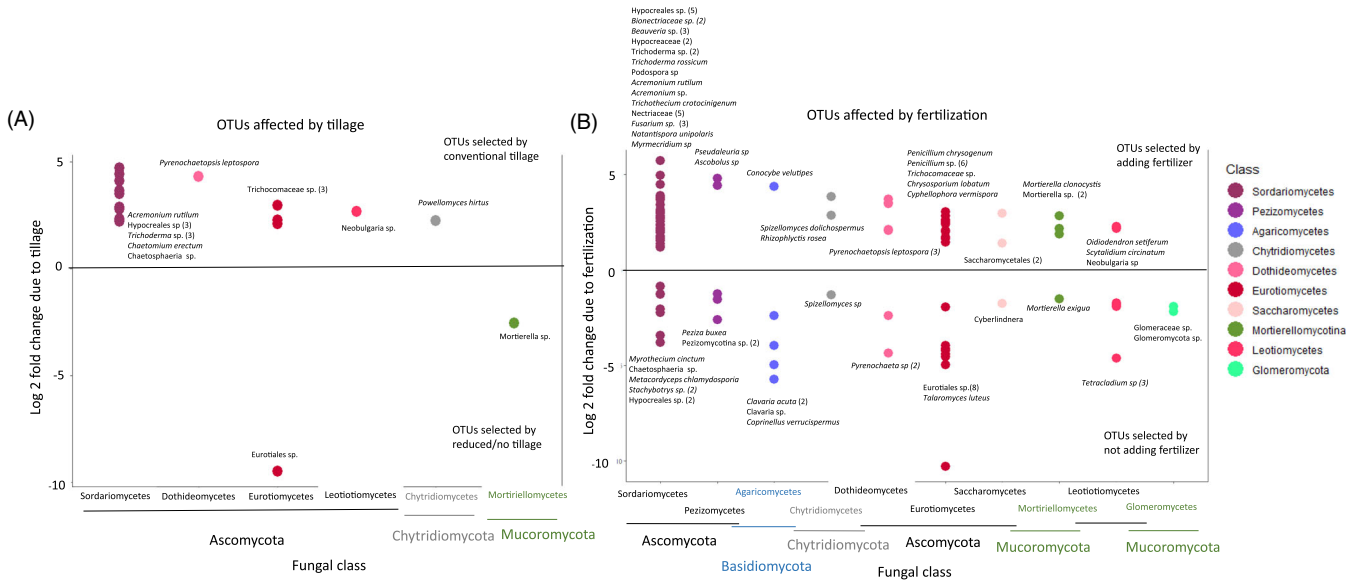


FIGURE 5 The fungal taxa most responsive to tillage (a) and fertilizer (b) treatment. Only operational taxonomic units (OTUs) significantly affected by treatment (compared to control) after false discovery rate (FDR) correction are included. OTUs are divided and coloured by fungal class [Color figure can be viewed at wileyonlinelibrary.com]

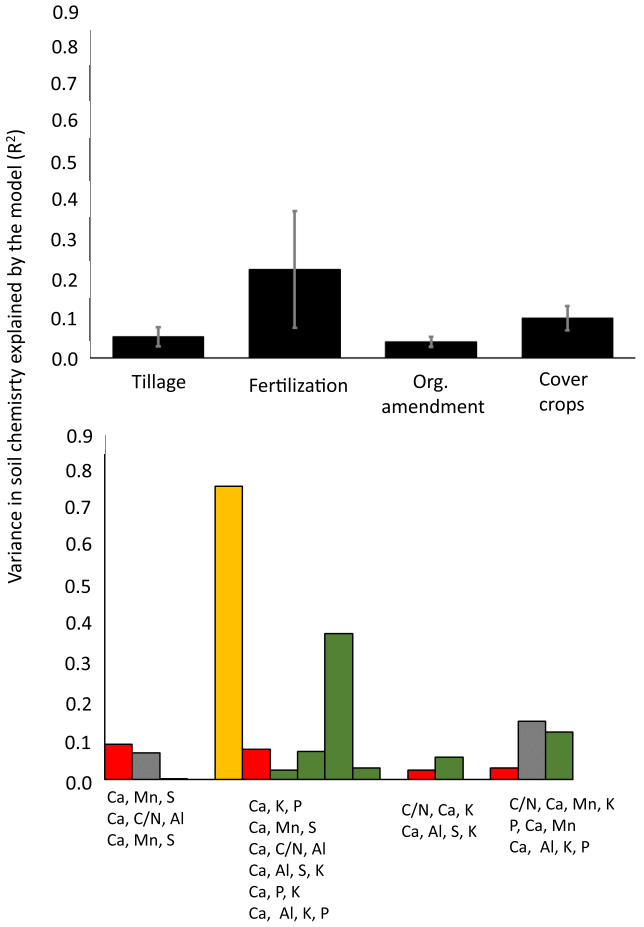


FIGURE 6 Variation in soil chemistry in treatments compared to control, organized per bigger treatment category (a) and divided per experiment (b). In (b) colours depict country of the experimental plot (yellow = Belgium, red = Denmark, green = Hungary, grey = the UK). The absolute values for selected macronutrients per crop (fertilizers) and per country (tillage) are shown in Figure S2. The coloured bars represent variation explained by soil chemistry in the model, black bars represent averages of these values across experiments and error bars show calculated standard error [Color figure can be viewed at wileyonlinelibrary.com]

robusta, a pathogen causing root rot. Community structure of plant pathogenic fungi was affected mostly by mineral fertilization, and again, the experiment DEN3 caused the strongest effect on the community structure (Figure 3e). Adding organic substances in the form of manure or compost compared to adding the nutrients in inorganic form reduced the number of potential plant pathogens in Belgium and Denmark but resulted in a slight increase in the sites in Hungary.

The relative abundances of soil saprotrophic fungi were most variable and no trends in large categories of agricultural practices were detected (Figure 3c). For example, organic amendments tended to have a positive effect on relative abundance of saprotrophic fungi but this was not consistent. However, when the effects of stalk and straw were separated from the effects of manure, there was a consistent increase in saprotrophs (effect sizes of −0.244 for HUN1 and −0.054 for DEN2) in straw treatments. Manure addition seemed to have negative effects on the relative abundance of saprotrophs compared to inorganically fertilized plots in some of the sites (effect sizes of 0.120 for HUN1 and 0.218 for HUN3) and positive effects in others (effect size of −1.028 for DEN3). These tendencies can be explained by better availability of fresh straw (as a labile form of organic carbon) for decomposition processes, than a more stable form of organic substances after a fermentation process during maturation of farmyard manure (FYM). The saprotrophic fungal community structure was most affected by inorganic/mineral fertilizer but also by organic amendments and cover crops, especially at the Danish sites (Figure 3f).

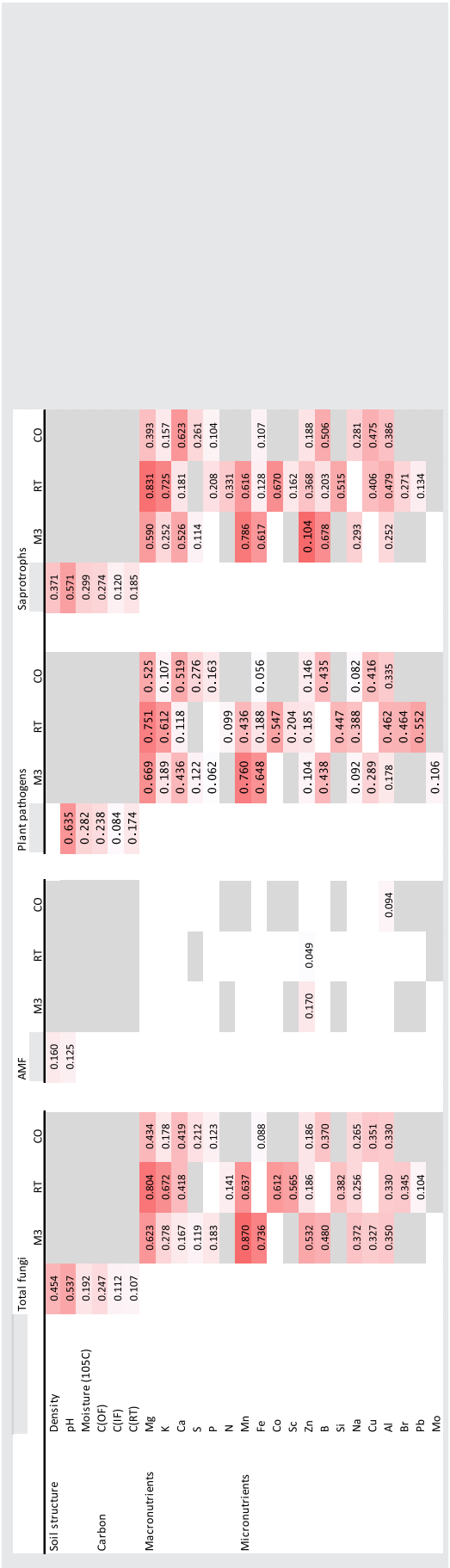
3.3 | The effect of agricultural practice on yield

We further investigated yield data collected at the harvests, to assess what kind of consequences the agricultural practices have on crop yield. Generally, all intensive/conventional agricultural practices lead to higher crop yield, whereas the agricultural practices selected for soil health decreased the yield. Cover crop addition and hence avoidance of fallow was the only practice that had a slight positive effect on the yield of the main crop (Figure 4a). This was mainly due to an increase in spring barley grain production following grass-clover cover cropping (compared to fallow) in experiment DEN2. The largest negative effect on the yield was detected for the no-fertilizer treatments, and reduced tillage and amendment of soils with organic materials also reduced the yield compared to the control. We further found that change in relative abundance of AMF and plant pathogenic fungi due to the treatments was correlated with relative change in the yield (Figure 4b). When an agricultural treatment had a specifically negative effect on the relative abundance of plant pathogenic fungi, the effect on yield was less pronounced, whereas a positive effect on AMF abundance alleviated the generally negative effects and the relative effect on AMF was positively correlated with change in yield.

3.4 | Fungal taxa as indicators of agricultural practices

One of our aims was to see if the abundance of certain taxa of fungi would consistently increase or decrease due

TABLE 3 Soil parameters significantly related to soil fungal community structure and structure of each fungal guild. Only significant R^2 values derived from ENVFIT analyses after false discovery rate (FDR) correction are shown, and the darker red colour means the stronger the relationship. Not measured parameters are marked with grey, whereas non-significant values are marked with white. The nutrients are ordered based on the strength of interaction for the total fungal community. M3 marks Mehlich-3 extraction, RT marks real totals measured with x-ray fluorescence (XRF), and CO extraction with hexamminecobalt trichloride solution represents exchangeable cations and anions



to agricultural practices across sites. For this purpose, we focused only on the main categories of disturbances as presented earlier. After correction for false discoveries, 16 fungal OTUs showed a consistent response to tillage across sites (Figure 5a). Most (14 OTUs) were significantly enriched in sites with conventional tillage, whereas two were enriched in sites with reduced or no-tillage. Most of the "tillage-tolerant" OTUs belonged to classes of Ascomycota, such as Sordariomycetes (9 OTUs), Eurotiomycetes (3 OTUs), Dothideomycetes (1 OTU) and Leotiomycetes (1 OTU). There was furthermore one chytridiomycete OTU (*Powellomyces hirtus*) that was increased with conventional tillage. The OTUs sensitive to tillage were 1 OTU of Eurotiomycetes and 1 of *Mortierella* sp.

Compared to the tillage treatments, many more OTUs were consistently responding to the soil fertilization. Thirty-five OTUs were sensitive to fertilization (i.e., found more in non-fertilized plots than in plots with fertilizers; Figure 5b). These OTUs included 2 OTUs of Glomeromycotina, 4 OTUs of Basidiomycota (Agaricomycetes), 27 OTUs of Ascomycota (Sordariomycetes, Pezizomycetes, Dothideomycetes, Eurotiomycetes, Saccharomycetes and Leotiomycetes), 1 OTU assigned as Chytridiomycota and 1 as Mortierellomycotina. We detected a further 59 OTUs that were preferring conditions of added nutrients over control plots. These OTUs belonged to the same classes as the fertilizer-sensitive OTUs, except that no Glomeromycotina were detected. The most notable class that was enriched in fertilized plots was the Sordariomycetes (31 OTUs).

3.5 | Change in soil chemistry due to agricultural practices

As we treated the fungal community as a whole, we adopted a similar approach to soil chemistry. Fertilization affected the soil chemistry the most (Figure 6) and the effect was particularly strong in Belgium and Hungary. Across sites, tillage, cover crops and organic amendments did not have significant predictable effects on soil chemical structure. Of all the treatments (Figure S2), liming and individual fertilization experiments had the largest effects. The elements most affected by the treatments varied slightly between experiments and countries but calcium (Ca) content was significantly changed in all sites. Tillage further affected mainly manganese (Mn) and sulphur (S) content, and fertilization affected phosphorus (P) and potassium (K) content (Figure 6). Organic amendments and cover crops changed the C/N ratio and K content (Figure 6). We further evaluated the effects of fertilization and tillage on soil chemical elements. Tillage did not significantly affect any of the elements measured across the sites (with country as a random factor),

TABLE 4 Soil parameters significantly related to soil fungal community structure in each of the countries sampled. Only significant R^2 values after false discovery rate (FDR) correction are shown, and the darker red colour means the stronger the relationship. Not measured parameters are marked with grey, whereas non-significant values are marked with white. The nutrients are ordered based on the strength of interaction for the total fungal community across sites. M3 marks Mehlich-3 extraction, RT marks real totals measured with XRF, and CO extraction with hexamminecobalt trichloride solution represents exchangeable cations and anions

		Hungary				Belgium				Denmark				UK			
			M3	RT	CO		M3	RT	CO		M3	RT	CO		M3	RT	CO
Soil structure	Density	0.211								0.653							
	pH	0.333				0.387				0.285				0.436			
	Moisture (105C)									0.601							
Carbon	C(OF)	0.472				0.349				0.245							
	C(IF)	0.214								0.151							
	C(RT)	0.497								0.342							
Macronutrients	Mg			0.363				0.610			0.421	0.790	0.482				0.606
	K			0.183			0.346		0.486		0.689	0.801	0.649				
	Ca		0.275	0.219	0.536		0.592	0.476	0.596		0.616	0.792	0.687		0.511		
	S								0.459		0.381						
	P		0.133								0.589	0.722					
	N			0.476								0.675					
Micronutrients	Mn						0.660	0.566			0.511	0.735			0.606		
	Fe		0.383	0.258	0.140				0.468		0.482	0.745			0.526		
	Co						0.479				0.309	0.767					
	Sc			0.308								0.745					
	Zn			0.142	0.141						0.729	0.714					
	B		0.507		0.141				0.523		0.703						
	Si			0.430				0.597				0.781					
	Na		0.110	0.101			0.758		0.714		0.649	0.690	0.596				
	Cu				0.137				0.478		0.508	0.464					
	Al		0.191								0.466	0.776	0.362			0.414	
	Br			0.665				0.765								0.765	
	Pb																
	Mo		0.1732								0.2316						

TABLE 5 Correlations between relative abundance of the major fungal guilds and soil structure and nutrients. Only significant Pearson correlation values after false discovery rate (FDR) correction are shown, and the darker red colour means the stronger positive relationships, whereas blue colours represent strong negative correlations. Not measured parameters are marked with grey, whereas non-significant values are marked with white. M3 marks Mehlich-3 extraction, RT marks real totals measured with XRF, and CO extraction with hexamminecobalt trichloride solution represents exchangeable cations and anions

		AMF			Plant pathogens			Saprotrophs		
		M3	RT	CO	M3	RT	CO	M3	RT	CO
Soil structure	Density									
	pH				-0.39			0.52		
	Moisture (105C)							0.34		
Carbon	C(OF)									
	C(IF)				-0.13					
	C(RT)									
					-0.33					
Macronutrients	Mg					-0.33	-0.41	-0.26	0.63	0.65	0.57
	K	-0.20		-0.20			-0.39	-0.19	0.49	0.73	0.40
	Ca					-0.35	-0.17	-0.36			0.43
	S										0.34
	P		-0.22	-0.17			-0.17				0.30
	N										
Micronutrients	Mn					-0.38	-0.34	0.19	0.77	0.66	
	Fe						-0.18		-0.57		
	Co						-0.38			0.63	
	Sc						-0.20				
	Zn	-0.14						0.15	0.28	0.39	
	B					-0.39		-0.33	0.47		0.44
	Si						0.35			-0.43	
	Na									0.57	
	Cu			-0.15			-0.38	-0.34		0.40	
	Al					0.21	-0.33		-0.41	0.46	-0.36
	Br						0.17			-0.75	
	Pb										
	Mo										

whereas fertilization increased the concentrations of N, P and K and also soil carbon content and this was detected across countries and crops.

3.6 | Interplay between soil chemistry and soil fungal communities

We further investigated which components of soil chemistry were most likely to be affecting soil fungal community structure. ENVFIT revealed that soil chemistry was affecting the soil fungal community structure significantly across the sites ($F = 1.945$, $p < 0.001$). We further looked at specific chemical components using CCA and many of the measured factors were significantly explaining fungal community across sites after correction of the false discovery rate (FDR) (Table 3). Most soil abiotic factors were linked to soil fungal community structure; the strongest effects were detected between Mn, Mg, Fe, K and pH, and soil fungal community (Figure S3). Within each country different chemical elements significantly affected the soil fungal community composition. The strongest link was detected between soil chemistry and the soil fungal community in DEN sites, whereas weakest connections (measured with fewest elements connected to soil fungi) were detected in the UK. Only pH affected fungal community across sites, whereas Ca-Mehlich was the only macronutrient shown to be related to soil fungal community structure across the sites (Table 4).

We further investigated if one of the fungal guilds would be more sensitive to soil chemistry by repeating the ENVFIT analysis for the major fungal guilds. Community structure of AMF across sites and treatments was significantly affected by soil chemistry ($F = 1.745$, $p < 0.001$) and most affected by soil density ($R^2 = 0.16$, $p = 0.001$), soil pH ($R^2 = 0.13$, $p = 0.001$) and Zn content ($R^2 = 0.17$, $p = 0.001$; Table 3). Relative abundance of AMF from total fungi was affected by soil P, K, Cu and Zn (Table 5). Although the AMF community's response to soil cues was relatively small, the effect of soil chemistry was large on the soil plant pathogen community structure ($F = 2.4036$, $p < 0.001$), and elements most related to plant pathogenic fungi were Ni, Mn, Mg, Fe and K, but also soil pH was linked to community structure of plant pathogens (Table 3). The relative abundance of plant pathogenic fungi was also affected by many soil parameters including pH and CEC. The saprotrophic fungal community was also strongly linked to soil chemistry ($F = 1.785$, $p < 0.001$) and most chemical elements studied were linked to the community structure. The strongest links were detected between the community

structure of soil saprotrophs and concentrations of Mg, Mn and K (Table 3). The relative abundance of saprotrophs showed an opposing pattern to plant pathogens that were mainly negatively correlated with nutrients and most elements measured were positively correlated with the relative abundance of soil saprotrophs (Table 5). Also, pH and soil moisture affected the relative abundance of saprotrophs, whereas soil carbon content or density did not.

4 | DISCUSSION

This study shows that comparisons of soil fungal communities between sites, even when the same technology is used and samples are processed together (Ramirez et al., 2018), is hampered by the small amount of shared OTUs between the experiments, which also prevents us, to a large extent, from detecting possible indicator species (Schloter, Nannipieri, Sørensen, & van Elsas, 2018). Only less than a quarter of the OTUs detected in this study were present in three or more countries and less than 10% in all sites. Surprisingly, the potentially plant pathogenic fungi were the group with least specialization to certain soils and countries, whereas AMF communities and OTUs varied the most between the sites. The relatively small degree of specialization of plant pathogens could be due to their strong host-relatedness and the fact that the range of agronomical crops grown is quite limited. This could also be related to the wide distribution patterns of soil-borne plant pathogenic fungi (Corredor-Moreno & Saunders, 2020). On the other hand, AMF often have wide host ranges, and the fact that they vary between sites indicates potentially their dependence on soil parameters such as available P. Recently, it was shown that ectomycorrhizal fungi have narrower climatic tolerance than plant pathogenic fungi (Větrovský et al., 2019). Our study was conducted in similar (Atlantic) climatic conditions, with the exception of the sites in Hungary, and we show an opposing pattern, that AMF was more variable between sites, whereas plant pathogens were more commonly shared. We expected the saprotrophic fungi to be more widely distributed due to their assumed generalist role in agricultural soils (Kohn, 2005), but this was not the case and most of the saprotrophs were specific to certain soils or even to certain treatments. From mushroom-forming saprotrophic fungi, it is known that they vary both in composition and phenology across Europe due to differences in climate and edaphic factors (Andrew et al., 2017; Krah et al., 2019) and we confer here that also the saprotrophic fungi in agricultural soils differ between soils and climatic zones.

Despite the low numbers of shared OTUs between experiments, we could still find a small number of OTUs that responded in the same way to the tillage and fertilizer treatments across countries (Figure 5). Due to the small overlap in OTUs, we focused more on indicator functions and used a broader level of identification in the analysis here. We urge the scientific community to consider the guild, ecology and traits of the organisms rather than their identity (Daws et al., 2020; Hannula & Träger, 2020), especially when focusing on large-scale patterns.

Many studies performed on one soil have found tillage to affect fungal community structures (Hartmann, Frey, Mayer, Mäder, & Widmer, 2015; Legrand et al., 2018; Sharma-Poudyal et al., 2017; Sommermann et al., 2018; Wang, Chen, Liu, Wen, & Liao, 2016), whereas a global meta-analysis showed that fungal diversity was not consistently affected by tillage (Li et al., 2020). The effects of tillage have been linked to disturbances and changes in ecosystem stability (Wagg et al., 2018). For example, a recent study has found that long-term tillage shifts the ratio between saprotrophs and symbiotrophs but did not affect pathotrophs (Schmidt et al., 2019). Here we found that conventional tillage when compared to reduced tillage or no-tillage does change the community structure of fungi but that the magnitude of change depends on the soil type and, in the case of Denmark, the cover crop usage. Furthermore, in our study long-term tillage in some of the soils increased the relative abundance of AMF, but at the same time it also increased the abundance of saprotrophs in the same soils, while having variable effects on plant pathogenic fungi. We did not detect consistent effects of tillage on the relative abundance of any of the fungal guilds studied, saprotrophs being the group most affected. We acknowledge, however, that saprotrophic fungi are a large group of fungi with varying physiology (e.g., decomposers of cellulose and diverse types of lignin) (Daws et al., 2020). Also, we can speculate that, for example, mycelia-forming saprotrophs would be more affected by tillage than unicellular fungi. We could detect only two fungi that were sensitive to tillage across the soils across countries, whereas abundance of many more fungi was consistently increased by tillage. The OTUs sensitive to tillage were Eurotiomycetes spp. and *Mortierella* sp., both generally characterized as fast-growing hyphal fungi. We detected that also fertilization and the type of fertilization changed the soil fungal community structure and that the magnitude of change is dependent on the experiment and soil. Compared to tillage, fertilization had more constant change in soil fungal communities and caused an increase in relative abundance of plant pathogens and a decrease in relative abundance of AMF.

The type of fertilizer and amount of fertilizer added are known to affect the fungal community structure and especially AMF are known to be sensitive to the added nutrients (Hartmann et al., 2015; Oehl et al., 2004; Verbruggen, Kiers, Bakelaar, Röling, & van der Heijden, 2012). It is speculated that adding organic fertilizers or leaving a soil unfertilized can boost its suppressiveness against fungal plant pathogens (Chen et al., 2020), yet if crop plants are weakened due to lack of suitable nutrients, they may also be more susceptible to pathogens. Here we did not test the suppressiveness of soils but show that the relative proportion of plant pathogenic fungi is diminished in soils with no added (inorganic or organic) fertilizer as compared to the control. This can potentially also be related to the increase of relative abundance of AMF and as both of these taxa have relative abundances of less than 5 %, we believe this is a genuine soil interaction effect. Recently, it has been shown that fertilization and soil fertility can cause changes in the interactions between different fungal guilds and especially change their ratios (Chen et al., 2020; Hannula & Träger, 2020). Here we can only speculate on the reason for detecting less potential plant pathogenic fungi in no-fertilizer treatments across experiments but see this as a potential avenue for more natural agroecosystems that support soil resilience and are naturally buffering against diseases. As we used here methods that detect only the identity of the species and not their activity or function (Hannula, Morriën, van der Putten, & de Boer, 2020), we cannot say if the community functioning has changed in response to agricultural practices such as tillage. Further studies should also look into the functioning of the soil microbial communities across soils.

In general, we detected that any potentially soil-improving agricultural practice reduced the yield of the main crop, with the exception of cover cropping. It has been shown earlier that soil-improving cropping practices can lead to a reduction in current yields (Seufert, Ramankutty, & Foley, 2012) but might lead to savings in energy and inputs (Smith, Williams, & Pearce, 2015) and increases in future long-term yields (Schrama, de Haan, Kroonen, Verstegen, & van der Putten, 2018). Here we were specifically interested in links between soil fungi and the yield and detected that in soils where agricultural practice managed to promote the AMF and suppress plant pathogenic fungi, the yield reduction was less than in practices without large changes in the abundance of these fungal groups. Even if soil-improving agricultural practices have negative effects on yield, these effects can be alleviated by the positive effects on soil fungal communities. This presents an interesting new avenue to investigate further and try to find a balance in soil-improving cropping practices that produce the best possible fungal community, especially the notion that decreasing fertilization rates increase the relative abundance of beneficial fungi such as AMF while decreasing the pathogen load. Further investigations of fertilization levels that could sustain profitable yield but also promote soil fungal community functions are needed.

We concur with earlier findings that soil chemistry greatly affects fungal communities (Tedersoo et al., 2014). This is seen within experiments due to, for example, fertilization treatments, but also between experiments, although in this case also climatic factors play an important role. Not all fungal functional guilds were equally affected by soil chemistry and surprisingly the AMF community structure was least affected by the chemistry outside the obvious effects due to fertilization with P. Previously it has been indicated that Ca (Tedersoo et al., 2014) is the element most affecting soil fungi at a global scale, which is in line with our study. In addition, also other micro- and macronutrients such as Mn, Mg, Fe and K have significant effects on fungal community structure and different fungal guilds respond to different soil nutrients. Most of the nutrient contents are interlinked with other soil parameters and especially with pH, which also was affecting soil fungi, so we cannot say if these are direct interactions between fungi and the nutrients or indirect interactions between, for example, soil types. The role of fungi in potassium (K) cycling in the soils is well documented and fungi are known to increase the plant-available K in agricultural soils (Meena, Maurya, & Verma, 2014). Furthermore, fungi play a role in the cycling and transformation of Mn in the soils (Thompson, Huber, Guest, & Schulze, 2005). However, as most studies have focused on the macronutrients in the soils, little is known on the effect of micronutrients on soil fungal communities. Notably, increases in soil Mg, Mn and K lead to decreases in the relative abundance of plant pathogenic fungi and increase the relative abundance of saprotrophic fungi. For AMF abundance, only (negative) effects of K were detected and this is probably linked with NPK fertilization. Managing soil (micro) nutrient levels as a way to promote saprotrophic fungi and decrease plant pathogenic fungi is an intriguing idea, yet more research and manipulative experiments on the topic are needed to explore if these interactions are indirect or direct and how they play out at the field scale.

5 | CONCLUSIONS

In conclusion, we show that the magnitude of responses of fungal communities and fungal functional guilds to agricultural practices differs between long-term

experiments even if the same agricultural practices are compared using standardized methodology. We further show that in long-term experiments fungal communities are strongly affected by soil-improving agricultural practices, yet the effects on specific guilds such as AMF and plant pathogens vary between the sites. Yield was in most cases negatively associated with practices aiming to improve the yield and the change in plant pathogenic fungi and in AMF is linked to the yield. We further link the soil fungal community and guilds to soil abiotic characteristics and reveal that especially Mn, K, Mg and pH affect the composition of fungi across sites.

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AUTHOR CONTRIBUTIONS

Silja Emilia Hannula: Conceptualization; formal analysis; methodology; visualization; writing-original draft; writing-review and editing. **Dominico Paolo Di Lonardo:** Writing-original draft; writing-review and editing. **Bent T. Christensen:** Resources; writing-review and editing. **Felicity V. Crotty:** Resources; writing-review and editing. **Annemie Elsen:** Resources; writing-review and editing. **Peter J. van Erp:** Funding acquisition; project administration; writing-review and editing. **Elly M. Hansen:** Resources; writing-review and editing. **Gitte H. Rubaek:** Resources; writing-review and editing. **Mia Tits:** Resources; writing-review and editing. **Zoltan Toth:** Resources; writing-review and editing. **Aad J. Termoshuizen:** Conceptualization; funding acquisition; project administration; writing-original draft; writing-review and editing. Emilia Silja Hannula, Peter J. van Erp and Aad J. Termorshuizen designed the sampling strategy together with all site managers (Bent T. Christensen, Felicity V. Crotty, Annemie Elsen, Elly M. Hansen, Gitte H. Rubæk, Mia Tits and Zoltan Toth). Site managers (Bent T. Christensen, Felicity V. Crotty, Annemie Elsen, Elly M. Hansen, Gitte H. Rubæk, Mia Tits and Zoltan Toth) allowed access to the long-term sites and provided data on the site and the yield. Emilia Silja Hannula analysed the data and wrote the first draft of the manuscript with Dominico Paolo Di Lonardo and Aad J. Termorshuizen. All authors commented on and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data is archived in Dryad in accession https://doi.org/10.5061/dryad.kh189324t

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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