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RESEARCH ARTICLE

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Reduced tillage intensity does not increase arbuscular mycorrhizal fungal diversity in European long-term experiments

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Abstract

Mechanical soil disturbance is one among the key factors influencing soil biodiversity in agriculture. Although many soil organisms are sensitive to soil disturbance, fungi could be highly impacted due to their sessile lifestyle, relatively slow growth and filamentous body structure. Arbuscular mycorrhizal (AM) fungi are of particular interest in arable lands, providing crop plants with numerous vital services such as nutrient acquisition and protection against abiotic and biotic stressors. Considering this, tillage practices that aim to reduce soil disturbance are often seen as a fungal-friendly alternative to conventional inversion tillage. Although local studies exist on the impacts of minimal tillage practices on AM fungi, the universality of this approach has been debated. Our objective was to assess the effects of reduced tillage intensity on AM fungi in comparison with conventional tillage. Using high-throughput sequencing techniques in long-term field experiments in five European countries, we show that the effects of reduced tillage intensity may not necessarily be positive on soil AM fungal diversity. Plots which were tilled using reduced tillage techniques had lower AM fungal richness in three countries, whereas in one of them, no significant differences were found. We also observed a shift in AM fungal communities where prevalence of taxa preferring root colonisation rather than soil exploration increased under reduced tillage regimes. Here, we argue that more detailed and long-term studies are needed to understand the factors that could make the reduction of soil disturbance more beneficial to AM fungi if agricultural sustainability goals are to be met.

KEYWORDS
AM fungi, conservation tillage, metabarcoding, soil biodiversity, tillage

1 | INTRODUCTION

Soil tillage has been a key element of agricultural land management since prehistoric times, enabling farmers to combat unwanted vegetation and to improve soil workability and structure. Therefore, research about how tillage practices affect soil properties also has a long history. Nonetheless, studies on the effects of tillage on soil microbiota are few, partly related to the relative novelty of high-throughput sequencing methods for studying microscopic soil organisms.

Soil fungal biodiversity has profound effects on the functions and services that soils provide (Delgado-Baquerizo et al., 2016). These may include regulating services, such as nutrient cycling, or provisioning services such as crop yield (Peay et al., 2016). At the same time, while intensive agricultural practices such as tillage enhance food production, they may decrease soil biodiversity (Adl et al., 2006; Tsiafouli et al., 2015), leading to a negative feedback loop requiring even more intensive management to maintain high yields. Because of these axioms, reducing soil disturbance has become a generalised recommendation for conservation agriculture and soil protection (FAO, 2023).

Arbuscular mycorrhizal fungi (AM fungi, subphylum Glomeromycotina; Spatafora et al., 2016) form a mutualistic symbiosis with over 80% of plant species worldwide, including most staple cereal crops (Sawers et al., 2018; Smith & Read, 2008). By extending their hyphae from plant roots into the soil matrix, AM fungi supply plants with phosphorus and nitrogen (Smith & Read, 2008) and also increase their resistance to biotic and abiotic stresses (Frew et al., 2022; Pozo et al., 2015). In return, plants provide their fungal partners with photosynthetic carbon (Keymer et al., 2017), allowing these obligate symbionts to complete their life cycle.

Owing to the role of AM fungi in plant nutrition and fitness, they have been under heightened interest regarding agricultural soil management. Tillage has been thought to be a major factor affecting fungal biota in arable soils, mainly because physical soil disruption severs extraradical hyphal networks (Jasper et al., 1991; Schnoor et al., 2011). Therefore, considering the potential benefits of AM fungi and the possible detrimental effects of conventional inversion tillage by ploughing, reduced tillage intensity practices have made their way into large-scale agriculture. Reduced tillage intensity practices aim to minimise soil disturbance by using less-invasive tillage methods or no tillage at all (Busari et al., 2015). Reduced tillage methods include using a chisel plough, where soil is tilled by chisels to a conventional ploughing depth of 30 cm, but without soil inversion. Minimum tillage is achieved using harrows that mix plant residues and soil only on the soil surface, usually within a depth of 10–15 cm. In direct seeding (or direct drilling), plant residues and soil are not purposefully mixed, and the only soil disturbance is caused by the direct seeding drill discs or tines making space for pushing the seeds into the soil (Busari et al., 2015).

Highlights

- Long-term experiments in five EU countries showed that reduced tillage intensity did not increase soil AM fungal diversity.
- In three countries, the AM fungal diversity was higher in conventionally ploughed plots.
- AM fungal communities became more rhizophilic under reduced tillage practices with possible functional implications to nutrient transfer.

Despite these trends, there is limited evidence of reduced tillage practices' effects on soil AM fungi. In field experiments, reduced tillage practices have often been shown to have a positive effect on AM fungal abundance (Castillo et al., 2006; Curaqueo et al., 2011; de la Cruz-Ortiz et al., 2020; Hydbom & Olsson, 2021; Moitinho et al., 2020; Rosner et al., 2020; Säle et al., 2015), while some mixed (Kabir, 2005) or negative (Gu et al., 2020) effects are also reported. Conversely, effects of reduced tillage on AM fungal diversity appear to be more variable: while Gu et al. (2020) and Säle et al. (2015) have shown positive effects of reduced tillage practices on AM fungal diversity, context-dependent (Kabir, 2005) or no positive effects (de la Cruz-Ortiz et al., 2020; Frøslev et al., 2022; Higo et al., 2020; Liu et al., 2022; Schalamuk et al., 2006; Wang et al., 2020) have also been reported. Therefore, while tillage practices may negatively impact AM fungal diversity during conversion from natural ecosystems to arable fields (Carneiro et al., 2019), the benefits of reduced tillage practices compared to conventional inversion tillage remain debated.

A particular knowledge gap relates to the effect of different tillage practices on the functional characteristics of AM fungi. One possible approach is to classify AM fungal families into guilds based on their general patterns of biomass allocation—'edaphophilic' (high allocation to extraradical hyphae, low allocation to intraradical hyphae), 'rhizophilic' (high allocation to intraradical

hyphae, with limited extraradical hyphae) and 'ancestral' (lower biomass and lack of allocation preference, hypothesised to be the ancestral condition for AM fungi by Powell et al., 2009) (Weber et al., 2019). Considering that the main effects of tillage would arise from AM fungal hyphal disruption, a shift towards rhizophilic or ancestral life history strategies could be expected, along with the corresponding functional implications.

Here, we used long-term field experiments from five European countries, focusing on the effects of reduced tillage intensity practices in comparison with conventional inversion tillage on AM fungal diversity. Specifically, our goals were to assess the effects of reduced tillage intensity on AM fungal species richness, diversity and community composition. Specifically, we hypothesised that (i) the effects of reduced tillage intensity would be positive on AM fungal diversity metrics, and (ii) the communities of AM fungi would be different under the tillage regimes, resulting in (iii) more edaphophilic AM fungi under reduced tillage intensities.

2 | MATERIALS AND METHODS

2.1 | Study areas

The study areas were in five European countries: France, Germany, Romania, Spain and Sweden. All study sites represent long-term experiments which had started approximately a decade before sampling, except for the field site located in Germany, set up in 1970. The main objective of these long-term experiments has been to test the effects of reduced soil disturbance on soil and crop properties. Recently, Engell et al. (2022) have shown the effects of tillage intensity on soil chemical properties and microbial biomass carbon from a subset of these sites.

All experimental sites were conventionally managed cereal rotations with occasional legume crops. In France, the experiment treatments were: (CT) conventional tillage with regular ploughing to a soil depth of 25 cm followed by seedbed preparation with a rotary harrow and shallow cultivation down to 6–8 cm depth with a rotary harrow, and (RT) reduced tillage with a rotary harrow down to 5–8 cm soil depth. The crops grown before sampling were winter wheat (*Triticum aestivum* L.) (2016), peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) mixture (2015), peas, oats and triticale (X *Triticosecale* Wittmack) followed by a cover crop (buckwheat, sunflower, phacelia and berseem clover) (2014), winter oilseed rape (*Brassica napus* L.) followed by a cover crop (blackseed) (2013) and triticale (2012).

In Germany, the experiment treatments were: (CT) conventional tillage with regular ploughing to a soil depth of 25 cm followed by a seedbed preparation with a rotary harrow and shallow cultivation down to 6–8 cm depth with a rotary harrow, and (RT) reduced tillage with rotary harrowing to 5–8 cm soil depth. Crop rotations were inconsistent and mainly based on cereals. Before sampling, winter wheat was grown in 2016 and a mixture of peas and oats was grown in 2015.

In Romania, the experiment treatments were: (CT) a conventional tillage treatment with ploughing to a soil depth of 25–30 cm with a seedbed preparation by a rotary harrow, and (RT) a reduced tillage treatment with chisel cultivation after maize and winter wheat and without tillage after soybean. The crop rotations of both tillage systems were soy (*Glycine max* L.), winter wheat and maize (*Zea mays* L.).

In Spain, the experiment treatments were: (CT) conventional tillage with a mouldboard plough to a soil depth of 25–30 cm and two cultivator passes of 15–20 cm depth and a disc harrowing to 15 cm depth, and (RT) reduced tillage treatment characterised by the absence of tillage operations (except for the crumbling of the sunflower stalks, the sowing was performed by direct drilling). The rotation consisted of cereals, sunflowers (*Helianthus annuus* L.) and legumes. The crops grown were winter durum wheat (*Triticum durum* L.) in 2017, broad bean (*Vicia faba* L.) in 2016 and winter durum wheat in 2015.

In Sweden, the experimental treatments were: (CT) conventional tillage with a mouldboard plough with a working depth of 23 cm, and (RT) reduced tillage with a cultivator with a working depth of 10–12 cm. The crop rotation consisted of winter wheat, oilseed rape (*Brassica napus* L.) and peas. Before sampling, winter wheat was grown in 2017 and 2016, and peas in 2015. Study area locations, agrometeorological, pedological and management descriptions are summarised in Table 1.

2.2 | Experimental design

The experimental design was similar throughout the five countries with some differences in design and plot areas (Figure 1). All experiments had the treatments of conventional tillage with soil inversion and a reduced tillage treatment as a replicate pair. The reduced tillage methods employed were chisel ploughing (Romania), harrowing (Sweden, Germany and France) and direct seeding or no tillage (Spain). In Sweden, Romania, Spain and France, both tillage treatments were replicated three times, whereas in Germany, it was four times. The agricultural management practices of the sites are summarised in Table 1 and are similar across countries. All long-term experiments in the five countries were under winter wheat prior to soil sampling.

2.3 | Soil sampling

Soil samples for AM fungal identification were collected from the experiments during spring 2017.

TABLE 1 Description of field sites.

	France	Germany	Romania	Spain	Sweden
Site	EFELE	Garte Süd	Turda	La Hampa	Säby
Coordinates	48°05′ N 1°48′ W	51°29′ N, 9°56′ E	46°35′ N 23°48′ E	37° 24′ N 5°35′ W	59°49′ N 17°42′ E
Soil type WRB	Luvisol-Redoxisol	Haplic Luvisol	Phaeozem	Calcic Fluvisol	Eutric Cambisol
Mean precipitation (mm)	700	621	550	580	547
CT[a] depth (cm)	25	25–30	25	25–30	23
RT[b] depth (cm)	6–8 (harrow)	8 (harrow)	25 (chisel)	0 (seeding cultivation only)	10–12 (harrow)
Yield[c] (t/ha)	8.3/8.6	6.6/6.1	5.6/5.2	5.3/5.4	8.9/8.9
No. of replicate plots per treatment	3	4	3	3	3
Fungicide 5 yr. avg[d]	0.6	1.6	1.4	0.2	0.4
Herbicide 5 yr. avg[d]	2.6	2.2	2	1	1.2
Insecticide 5 yr. avg[d]	0	1.4	1.2	0	0
pH (H_2O)[e]	5.4/5.4	7.2/7.2	6.8/7.1	8.3/8.2	5.7/5.5
P_{tot} (mg/kg soil)[e]	740/772	558/571	612/575	773/782	818/797
N_{tot} (g/kg soil)[e]	1.3/1.3	1.5/1.5	2.1/2.0	1.0/1.1	2.2/2.2
C_{org} (g/kg soil)[e]	12/12	15/16	22/20	8/9	26/26

[a]Conventional tillage.
[b]Reduced tillage.
[c]Yield of winter wheat in the year of sampling—values given for conventional tillage/reduced tillage, respectively.
[d]Number of applications as an average over preceding 5 years.
[e]Values given for conventional tillage/reduced tillage, respectively as a mean from the top 30 cm of soil.

In Germany, Romania, Spain and France, six sub-samples were uniformly collected from each plot in a 2 × 3 grid, considering the differing plot sizes between the countries and not sampling within a 1-m margin of the plots. In Sweden, nine sub-samples were collected in a 3 × 3 grid. From each sampling location, 10 g of soil were collected using sterile, single-use equipment, up to a depth of 10 cm and dried within 24 h using silica-gel at room temperature. Across countries, 105 samples were collected from each treatment, totalling 210 samples.

2.4 | Soil chemical analyses

One soil sample was collected in each of the plots per treatment with a 5 cm diameter split corer for soil chemical analyses. After collection, samples were sieved at 2 mm, dried at 105°C for 24 h and ball milled. Total carbon (C_{org}) and total nitrogen (N_{tot}) were measured by dry combustion (Elementar Vario El, Heraeus, Hanau, Germany). To the soil samples from Romania and Spain, HCl was added to remove inorganic C. No carbonates were detectable with the Scheibler method (Amelung et al., 2018) from other field sites, which means that the carbon content measured can be regarded as organic. The pH was measured in a soil and water mixture of 1:2.5. Total phosphorus (P_{tot}) was determined following the method of the Environmental Protection Agency (1997) by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Thermo Fischer Scientific GmbH, Dreieich, Germany).

2.5 | Molecular identification of AM fungi

DNA was extracted from 5 g of dried soil using a PowerMax® Soil DNA Isolation Kit (Qiagen) following modifications from Gazol et al. (2016). AM fungal sequences were amplified from soil DNA extracts using AM fungal-specific primers for the small subunit (SSU) ribosomal RNA gene: WANDA (Dumbrell et al., 2011) and AML2 (Lee et al., 2008) (see Data S1 for detailed description of molecular methods). Sequencing was done on the Illumina MiSeq platform,

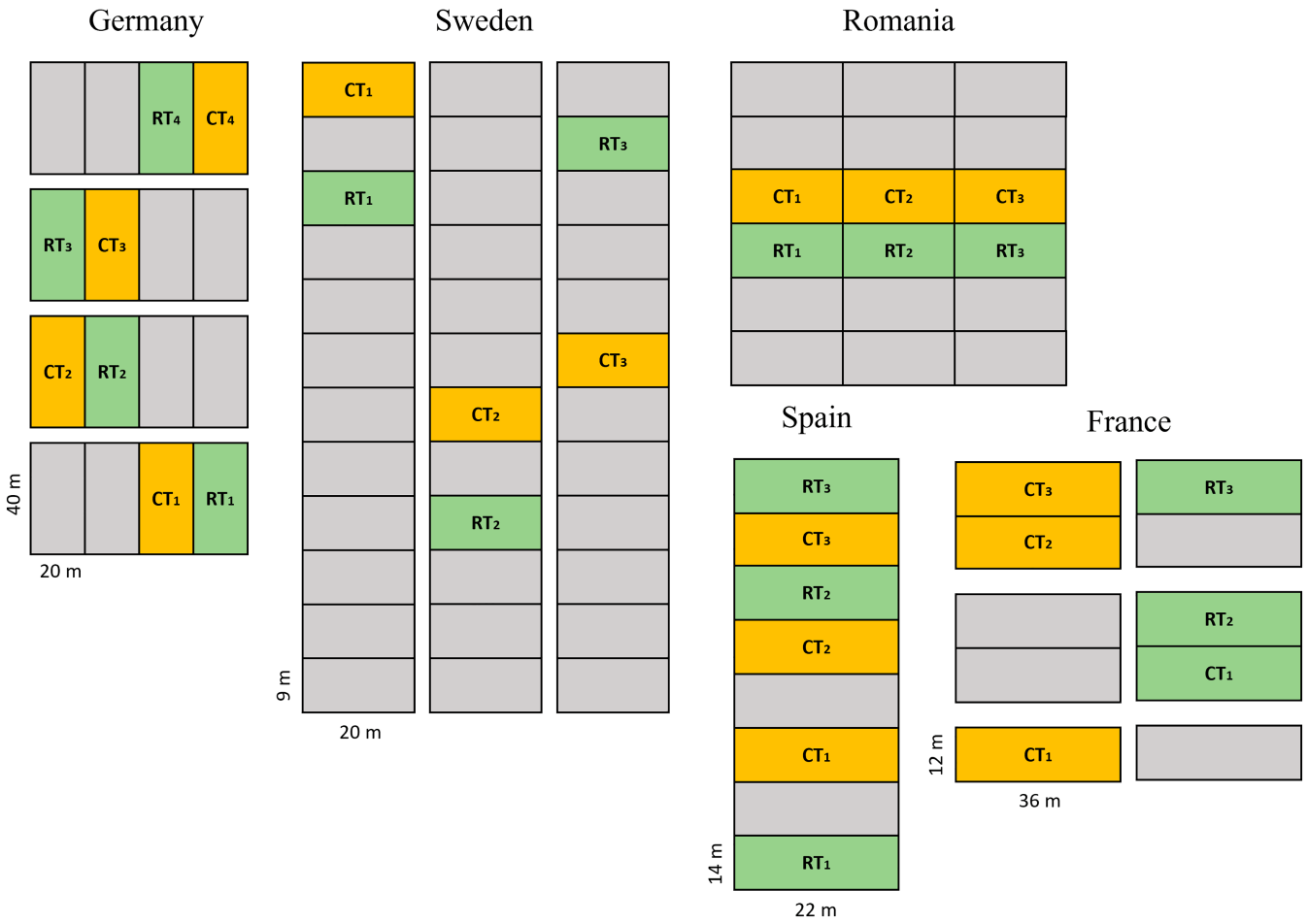

FIGURE 1 Designs of the long-term experiments in the studied countries. CT, conventional tillage; RT, reduced tillage. Grey plots indicate other experimental treatments not sampled in this study.

using a 2 × 300 bp pairedread sequencing approach, at Asper Biogene (Tartu, Estonia).

2.6 | Bioinformatics

Illumina MiSeq 2 × 300 bp paired-end raw reads (2 × 23,019,029 reads in total) were demultiplexed into samples and cleaned using a series of bioinformatic steps (Vasar et al., 2021) (see Data S1 for detailed bioinformatic procedures). Cleaned sequences were assigned to virtual taxa (VT) from the MaarjAM database (Öpik et al., 2010) using BLAST+ (v2.5.0, Camacho et al., 2009). VT are phylogenetically defined molecular taxa with the approximate taxonomic resolution of species or, in some clades, genera (Öpik et al., 2010). BLAST+ hits were filtered based on best hit using 97% identity and 95% alignment.

Raw reads from this Targeted Locus Study have been deposited in the National Center for Biotechnology Information Sequence Read Archive (BioProject PRJNA1007562).

2.7 | Statistical analyses

Because AM fungal sample taxon accumulation curves (calculated by random subsampling taxon occurrences from the sample sequence pool using the function *rarecurve (step = 20)* from R (R Core Team, 2019) package *vegan* (Oksanen et al., 2015; Figure S1)) showed no relationship between the number of sequences obtained from a sample and the taxon richness of the sample, we performed fungal richness analyses on unrarefied richness data. To visualise the sampling completeness for each country and tillage intensity combination, we also created taxon accumulation curves (i.e., cumulative VT richness based on each consecutive sample) using the same methodology as described for the sample accumulation curves, but using subsetting of samples instead of invidivual sequences. Diversity of AM fungi was calculated using the extrapolated asymptotic Shannon diversity, or Hill number order $q = 1$, using the R package *iNEXT* (Chao et al., 2014). The effect of tillage intensity both on mean sample VT richness and asymptotic Shannon diversity was tested using generalised linear

mixed-effects models (glmer and lmer from R package lme4; Bates et al., 2015) for the overall effect across countries and within each country separately. In models for all countries combined, tillage intensity nested in replicate ID nested in country was included as a random factor to account for the dependence between samples within a single tillage intensity replicate in a country (response ~ Tillage intensity + (1|Country/replicate ID/Tillage intensity)). In models for single countries, depending on the experimental setup, the replicate ID was included as a random factor for Germany, Romania and Sweden (response ~ Tillage intensity + (1|replicate ID)) because these countries followed a block design with treatment units within a single replicate ID being dependent among each other. For France and Spain, tillage intensity nested in replicate ID was included as a random factor (response ~ Tillage intensity + (1|replicate ID/Tillage intensity)) because the experimental setup was not a strict block design, and tillage intensity treatments were not clustered in replicate blocks. In models for all countries combined, replicate ID nested within country was included as a random factor to account for the experimental design. In models for single countries, the replicate ID was included as a random factor. Because VT richness, that is, species count, followed a Poisson rather than a normal distribution, we fit generalised linear mixed-effects models with an assumed Poisson distribution where the response variable was VT richness.

Although the effect of the sampling site with its particular soil conditions was accounted for in the models (replicate ID or tillage intensity nested in replicate ID as the random factor), in addition, we tested the additional overall effects of available soil parameters (pH, total N, total P, organic C) on the richness of AM fungal taxa by fitting generalised linear mixed-effects models (assuming a Poisson distribution of the response variable) with the soil parameter as the fixed-effects variable, and tillage intensity nested in replicate nested in country as a random variable (response ~ soil parameter + (1|Country/replicate ID/Tillage intensity)). We also tested the pairwise differences of country mean VT richness and Shannon diversity, fitting a (generalised in case of richness) linear mixed-effects model with tillage intensity and country as fixed effects and tillage intensity nested in replicate nested in country as a random variable (response ~ Tillage intensity + Country + (1|Country/replicate ID/Tillage intensity)). The pairwise differences were tested using the package emmeans (Lenth et al., 2018) in R.

To compare fungal communities between treatments, Bray–Curtis dissimilarity of Hellinger-transformed abundances was used as a measure of distance between communities. Non-metric multidimensional scaling (NMDS) using the *metaMDS* function, *vegan* R package version 2.4-1, was used to visualise the separation of communities. To test for differences in community composition, PERMANOVA with 999 permutations, was used (function *adonis2* from vegan R package), with Hellinger-transformed abundances of taxa in samples as the response matrix and tillage intensity and tillage intensity and country (including their interaction) as the explanatory variables. The permutations were constrained to within replicate ID-s in country adonis2 (response ~ Tillage intensity * Country, strata = Replicate ID in country). We also run PERMANOVA analyses on the AM fungal community differences between tillage intensities within each country separately (permutations constrained to within replicate ID-s). AM fungal functional guild assignments (ancestral, rhisophilic and edaphophilic) were retrieved from Weber et al. (2019). We used Random Forest analysis to assess the importance (functions randomForest() and importance() from R package randomForest; Liaw & Wiener, 2002) of the proportional abundance of fungal families in a sample in predicting the tillage intensity or country. The mean decrease in Gini index was used to evaluate the contribution of each fungal family to the predictive performance of the model. A higher mean decrease in Gini index means greater importance in distinguishing between different levels of tillage intensity or country.

3 | RESULTS

AM fungal VT richness in samples ranged from 3 to 31 with a mean of 16.5, Shannon diversity ranged from 1.5 to 12.2 with a mean of 6.7. Over all five countries sites, neither mean sample VT richness nor Shannon diversity significantly differed between tillage intensities (Figure 2). Countries differed significantly by their overall VT richness and Shannon diversity, but only VT richness was different between treatments within countries (Table S2).

The highest mean sample VT richness was found in Spain, under conventional tillage practices (21.1 ± 1.1 species), whereas the lowest VT richness was found in reduced tillage plots in France (6.33 ± 0.59 species) (Figure 2). Within countries, there were significant differences between conventional and reduced tillage intensities in France, Germany and Romania, where more taxa were found in plots that were conventionally tilled, and in Sweden, where higher VT richness was found in reduced tillage plots (Figure 2, Table S2). No differences in sample VT richness between soil tillage treatments was found in Spain. Shannon diversities were not significantly different within countries (Figure 2).

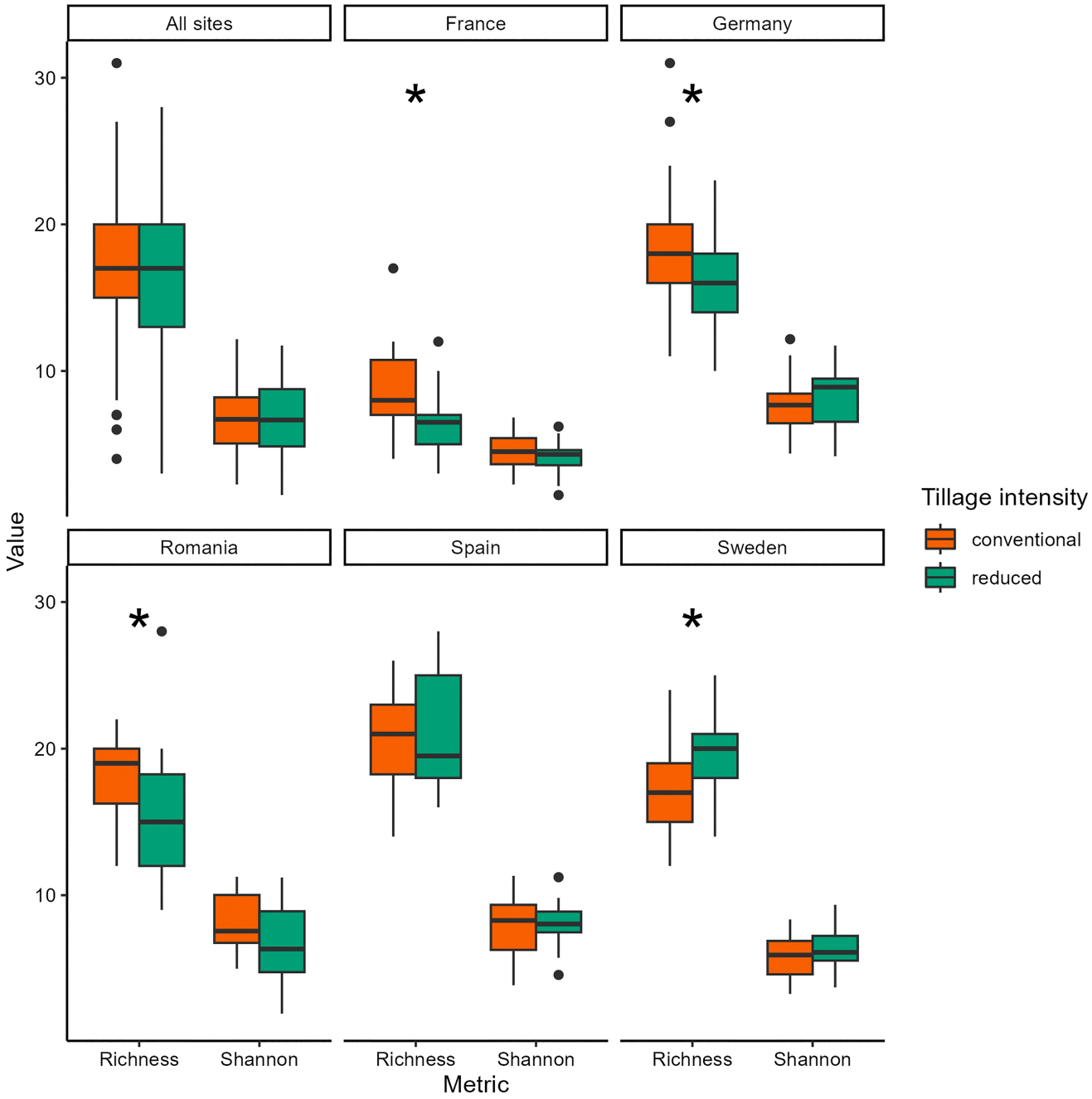

FIGURE 2 Mean sample virtual taxa richness and Shannon diversity in conventional and reduced tillage across and within all five countries' experimental sites. Significant pairwise differences (p <0.05) in arbuscular mycorrhizal fungal richness or diversity between tillage intensities are marked with an asterisk above the pair of treatment levels being compared. Central bars indicate the median; boxes indicate the interquartile range and whiskers indicate the minimum and maximum or 1.5 × interquartile range in case the minimum and maximum values lay outside this range.

Cumulative VT richness had a different trend than mean sample richness (Figure 3). The highest total number of unique VT's were found in Germany while conventionally tilled plots in France and Germany had more species in total than reduced tillage plots. In Sweden and Romania, reduced tillage intensity plots had more species in total than conventionally tilled plots.

3.1 | Soil AM fungal communities

Over all sites, the AM fungal communities in soil were clustered by the country of origin (PERMANOVA $R^2 = 0.62$, p <0.001) rather than the tillage practices, although a significant yet small (only 1.3% variance explained) difference was found between the tillage

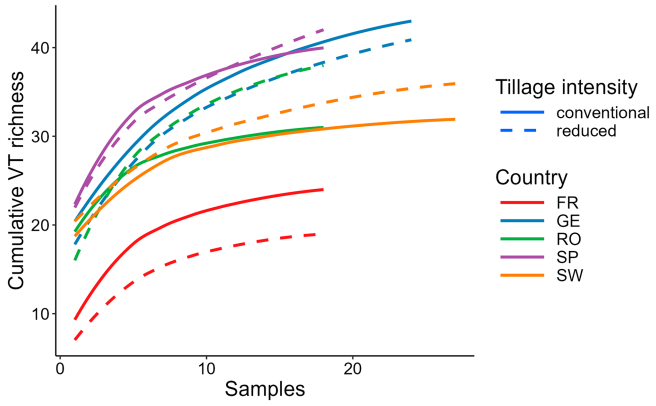

FIGURE 3 Cumulative virtual taxa (VT) richness in respect to sampling intensity. Solid lines indicate conventional tillage practices, dashed lines indicate reduced tillage practices. Colours represent different countries.

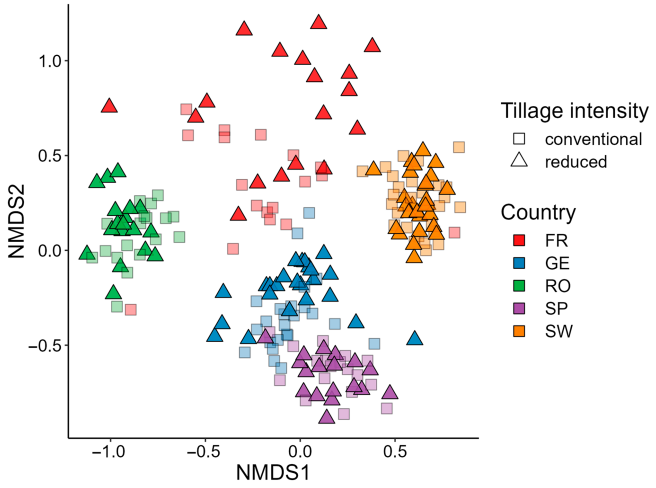

FIGURE 4 Arbuscular mycorrhizal fungal community composition in plots with different tillage intensities across five countries. Square symbols represent conventional triangles reduced tillage intensity treatments. Colours indicate countries.

treatments (PERMANOVA $R^2 = 0.013$, p <0.001) and the interaction between country and tillage treatment (PERMANOVA $R^2 = 0.032$, p <0.001; Figure 4). Because the effect of tillage intensity depended on the country, we also run permutational analyses within each country separately to separate the large influence of sampling location from the ecological effect of tillage intensity. Within countries, conventional and reduced tillage plots had significantly different AM fungal communities (Table S3).

The number of AM fungal sequences within a country and treatment varied substantially (Figure S2). Reduced tillage intensity plots in Sweden averaged about 6400 sequences per sample while conventionally tilled plots in France yielded only 218 AM fungal sequences, on average, per sample.

The most abundant AM fungal families varied between the countries and treatments but were generally either Paraglomeraceae or Glomeraceae (Figure 5a,b). Between conventional and reduced tillage treatments, the fungal families relative proportions that differed most were Diversisporaceae (−3%), Paraglomeraceae (+13%) and Glomeraceae (−10%). Between countries, Gigasporaceae and Claroideoglomeraceae were mostly contributing to the difference (Figure S3). Consistently, the proportional abundance of Paraglomeraceae was higher and that of Diversisporaceae and Glomeraceae families lower in reduced tillage compared with conventional tillage plots. In addition, by assigning AM fungal families into functional guilds according to their biomass allocation, we found that the rhizophilic guild was relatively more common in all the countries, and became more abundant in the reduced tillage plots, compared with edaphophilic and ancestral guilds, except in Spain (Figure 5c,d). The 20 most dominant VT in each country and tillage intensity are shown in Figure S4. With the exception of France, the dominant taxon in each country was dominant in both tillage regimes sampled.

No soil chemical parameter measured (pH, N_{tot}, P_{tot} and C_{org}) explained the variation, or lack thereof, of AM fungal VT richness in any country (Table S1).

4 | DISCUSSION

Our findings indicate that there were no universally positive effects of reduced tillage intensity on either AM fungal richness or Shannon diversity, thus disproving our first hypothesis. While these findings may be in contrast with the general theory of soil disturbance effects on soil biodiversity (Kladivko, 2001), they corroborate the results from previous single-site field experiments (de la Cruz-Ortiz et al., 2020; Higo et al., 2020; Liu et al., 2022; Schalamuk et al., 2006; Wang et al., 2020) and larger field surveys on production farmland (Frøslev et al., 2022; Vahter et al., 2022).

The effects of disturbance on biodiversity are generally characterised as being unimodal, with intermediate levels of disturbance having higher diversity than undisturbed and extremely disturbed habitats (Connell, 1978). Analogous findings have been reported for AM fungal diversity in soil, where anthropogenic disturbances increase diversity in species-poor communities and decrease diversity in naturally species-rich habitats (Garcia de Leon et al., 2018). While arable fields should be regarded as disturbed soil habitats, it is possible that the level of disturbance arising from conventional tillage, rather than reducing diversity,

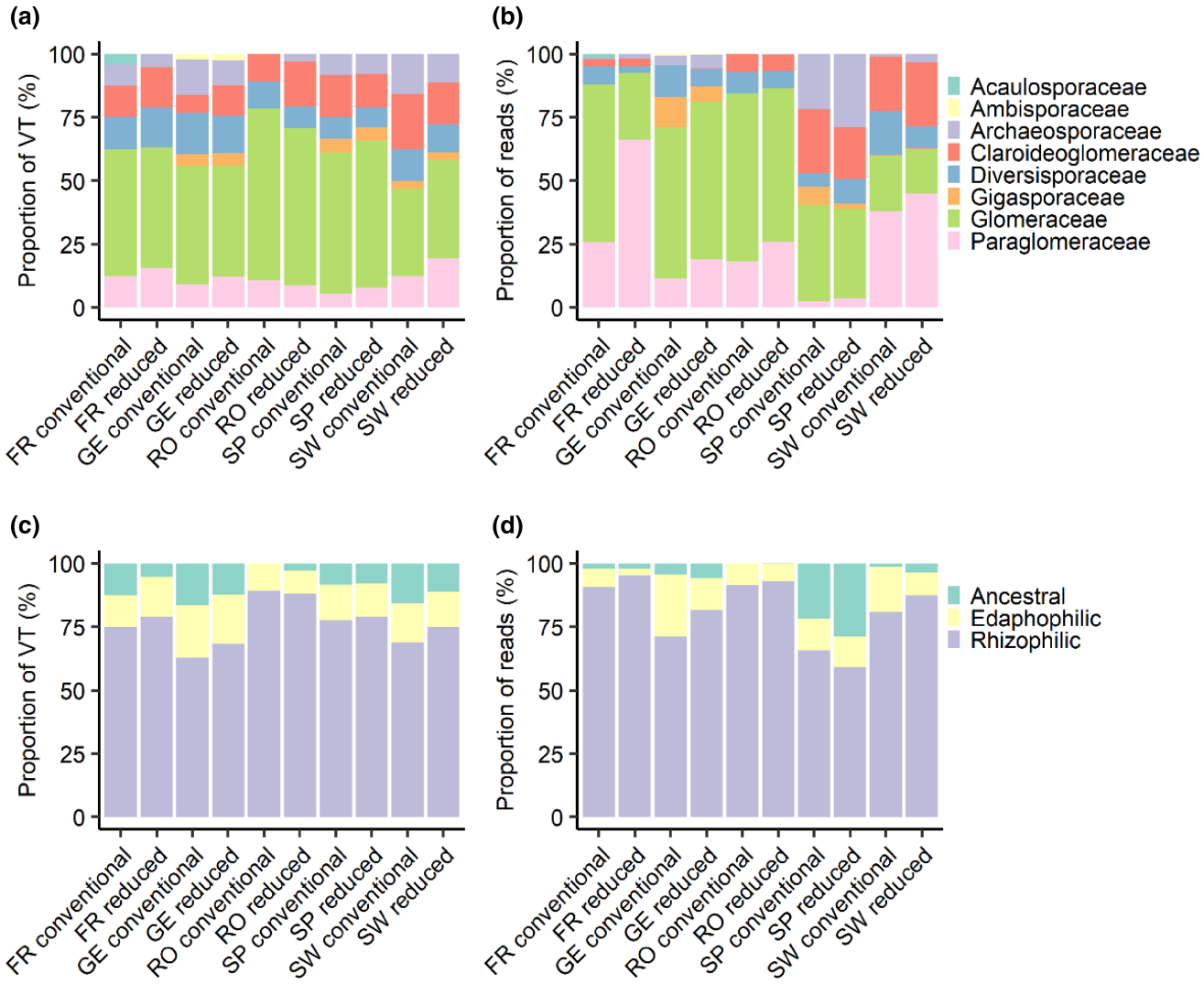

FIGURE 5 Proportion of arbuscular mycorrhizal (AM) fungal virtual taxas (VTs) (a) and reads (b) of AM fungal families in the experimental plots of each country and tillage intensity. Proportion of AMF VTs (c) and reads (d) belonging to different functional guilds in the experimental plots of each country and tillage intensity.

increases diversity or at least equalises the direct negative effects of physical hyphal disruption. While conventional inversion tillage is arguably an intensive form of soil disturbance, it is therefore possible that the soil AM fungal communities do not actually lose in species numbers.

Here, we observed a shift in soil AM fungal communities between conventional and reduced tillage practices, with some fungal families getting more abundant while others lose proportional presence, offering some evidence to support our second hypothesis. This can reflect the different life-history strategies of AM fungi under different disturbance regimes (Weber et al., 2019) as our results do indicate a taxonomic shift between the treatments. Still, over all experimental sites, geographic location was a more influential factor in describing the soil AM fungal community composition.

The shifts in AM fungal communities under reduced tillage regimes were represented by the proportional increases in Glomeraceae and Paraglomeraceae families' abundances, and a decrease of Diversisporaceae and Gigasporaceae representatives. This could reflect the tendency for rhizophilic species in Archaesporaceae, Glomeraceae and Paraglomeraceae, with higher investments into root intraradical hyphae, to become more abundant under reduced tillage regimes while edaphophilic Diversisporaceae and Gigasporaceae, with high amounts of soil hyphae production, become less abundant. This notion also disproves our third hypothesis as soil AM fungal communities became more rhizophilic, rather than edaphophilic under reduced tillage, contrary to what we expected initially.

It could be that the rhizophilic AM fungal species that are more prevalent in reduced tillage soil environments

and do not produce a lot of soil foraging hyphae are also less beneficial for the plants for nutrient acquisition. As soils with less physical mixing could retain more nutrients in the topmost layer of soil, it could enable crop plants to forage for nutrients effectively with their relatively small root systems in shallow soil depths (Sikes et al., 2010). This would make edaphophillic AM fungi with better nutrient foraging capacity less competitive, as the plant benefits from these fungi are reduced (Johnson, 1993). As reduced tillage practices are gaining popularity among farmers, this could direct selection pressure in agroecosystems to less-beneficial AM fungal taxa, with possible consequences to the functions and services these fungi provide.

Although total sequence numbers are not a direct proxy for biomass or abundance, we did observe a large variation in sequence numbers between the experimental sites. This may indicate varying agronomical and landscape contexts, with Central-, Western- and Southern-European sites generally yielding less AM fungal sequences per sample than the Swedish field site. It could be plausible that the landscapes around the experimental sites, to some extent, are sources for AM fungal propagules, especially of those species that could have affinity to a less disturbed soil environment. While the number of sites in this study does not allow for such an analysis, some support for the importance of surrounding landscapes species pools for general soil fungal diversity has been previously found (Vahter et al., 2022).

5 | CONCLUSIONS

In this study, we used long-term, tillage-oriented field experiments in France, Germany, Romania, Spain and Sweden, focusing on the effects of reduced soil disturbance in arable agroecosystems on AM fungal diversity and community composition. We found that, in comparison with conventional inversion tillage, reduced tillage intensity practices did not generally increase soil AM fungal richness or Shannon diversity, but rather resulted in the loss of some species and shifts in AM fungal community compositions towards rhizophilic AM fungi that are potentially less beneficial for nutrient acquisition. With the reduction of tillage intensity gaining traction for both economic and ecological reasons, the functional implications of subsequent changes in soil biotic communities need to be quantified.

AUTHOR CONTRIBUTIONS

Tanel Vahter: Conceptualization; data curation; formal analysis; methodology; project administration; visualisation; writing—original draft; writing. **Astrid R. Taylor, Blanca B. Landa, Guénola Pérès, Martin Potthoff, Mignon Sandor and Maarja Öpik:** Conceptualization; data curation; funding acquisition; methodology; project administration; supervision; writing. **Deborah Linsler and Kaisa A. Torppa:** Data curation; investigation; methodology; project administration; writing. **Engracia Maria Madejon Rodriguez, Francisco Giron Moreno and Luis F. Arias-Giraldo:** Data curation; investigation; writing. **Ilka Engell, Gema Guzmán and Vlad Stoian:** Data curation; investigation; methodology; writing. **Inga Hiiesalu and Siim-Kaarel Sepp:** Data curation; formal analysis; visualisation; writing. **Jan Bengtsson:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; supervision; writing. **Jane Oja and Martti Vasar:** Data curation; formal analysis; writing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest related to this article.

DATA AVAILABILITY STATEMENT

All data has been deposited to figshare DOI: 10.6084/m9.figshare.24182949. Raw sequencing reads from this Targeted Locus Study have been deposited in the NCBI SRA (BioProject PRJNA1007562).

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SUPPORTING INFORMATION

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