

# Distance to semi-natural habitats matters for arbuscular mycorrhizal fungi in wheat roots and wheat performance in a temperate agricultural landscape

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## Abstract

**Introduction:** The proximity of semi-natural habitats and agricultural fields in an agricultural landscape leads to unavoidable biological, chemical, and physical interactions. Fungi can negatively influence, but also support crop growth in agricultural fields. Therefore, in this field study we investigated the colonisation of arbuscular mycorrhizal (AM) fungi and non-AM fungi in winter-wheat roots as well as winter-wheat performance in distance to semi-natural habitats.

**Materials and Methods:** We sampled in an intensively managed agricultural landscape in North-east Germany along agricultural transition zones, that is, along 50 m-transects from semi-natural habitats like hedgerows and glacially created in-field ponds—so-called kettle holes—into agricultural fields.

**Results:** To our knowledge, we show for the first time that AM fungal colonisation in winter-wheat roots decreased linearly with increasing distance to semi-natural habitats while non-AM fungal root colonisation did not change. Winter-wheat grain yield and biomass slightly increased with increasing distance to hedgerows but not to kettle holes. This clearly shows that there is a difference between different crop performance parameters. Random forest machine learning algorithms confirmed the particular importance of distance to semi-natural habitats for AM fungal root colonisation and for winter-wheat grain yield. Less intensive agricultural management close to semi-natural habitats, for example, no herbicide and pesticide applications as a result of nature protection regulations, may partly explain this pattern. However, spatial response patterns of AM but not of non-AM fungi in wheat roots also point to changed ecological interactions close to semi-natural habitats.

**Conclusion:** Semi-natural and natural habitats in agricultural landscapes are slowly recognised not only to be important for biodiversity conservation, but also for sustainable crop production. Additionally, they may also be a tool for farmers and policy makers to improve sustainable landscape management. And agricultural transition zones are spatially and temporally complex dynamic ecosystems that should be the focus of further investigations.

Jasmin Joshi and Matthias C. Rillig contributed equally to this study.

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## KEYWORDS

fungal root endophytes, hedgerows, kettle holes, random forest analysis, transition zone

## 1 | INTRODUCTION

Agricultural landscapes in Europe present a mosaic of agricultural fields, natural and semi-natural habitats. Due to the proximity of semi-natural habitats and agricultural fields these transition zones are not only of concern for policy due to potential land-use conflicts and regulations for land-use practices but also hotspots for biological, chemical and physical interactions. We define the area of an agricultural field that is influenced by neighbouring landscape patches, for example, natural, or semi-natural habitats, neighbouring agricultural fields, as an agricultural transition zone (Kerneckner et al., 2022). The ecological uniqueness of transition zones (ecotones) has long been recognised as they capture an environmental gradient representing the mixture of the two distinct habitats (Clements, 1905; Livingstone, 1903; Schmidt et al., 2017). Meta-ecosystem theory highlights that different ecosystems in proximity are connected via spatial flows of energy, materials and organisms (Gounand et al., 2018) and this exchange would be particularly intense in these transition zones. Nonetheless, transition zones in agricultural landscapes also play an important role in policy issues because for example, in the European Union semi-natural habitats can fall under the so-called ecological focus areas (EFA) of the European Common Agricultural Policy (CAP). This means that farmers can apply for compensation subsidies when using practices in these areas that are beneficial for the climate and the environment. Because EFAs are often important habitats for nature conservation, but also occupy a part of farmland (e.g., for kettle holes: (Kalettka & Rudat, 2006), these areas can be conflict zones of diverging interests of farming and nature conservation. A profound ecological understanding of the conflict zone is needed to ameliorate the partly ineffective CAP, where for example, EFAs lack management criteria (Pe'er et al., 2017).

At the field scale, agricultural management selects for certain soil microorganisms and functions, hence shifting organism diversity and abundance (Bowles et al., 2017; Hartmann et al., 2015; Sommermann et al., 2018). Therefore, agricultural fields support a certain diversity and abundance of microorganisms, which may be fundamentally different from those in natural or semi-natural areas. At the landscape scale, homogeneous areas with only few natural or semi-natural habitats enlarge the area for agricultural management and hence food production and are easier to manage for the farmer compared with more complex areas with a higher amount and diversity of interspersed natural or semi-natural habitats. However, the change from complex to simple agricultural landscapes is not only a major driver of biodiversity decline (Billeter et al., 2008; Newbold et al., 2015), but may also result in the loss of important regulating and supporting ecosystem functions and services (e.g., nutrient cycling or water retention) derived from semi-natural habitats (Garibaldi et al., 2011; Holden et al., 2019). Holden et al. (2019), for example, showed that soils under hedgerows can support important ecosystem functions, like carbon and runoff storage.

Arbuscular mycorrhizal (AM) fungi are among the most important fungi in terrestrial ecosystems as they form arbuscular mycorrhizal associations with the majority of all vascular plants (Brundrett & Tedersoo, 2018). AM fungi can affect plant growth directly via improving plant nutrition (Smith & Read, 2008), pathogen protection (Veresoglou & Rillig, 2012), and stress tolerance (Kakouridis et al. nd; Smith & Read, 2008) and indirectly via changing soil structure (Lehmann et al., 2017; Leifheit et al., 2014). These direct and indirect positive impacts of AM fungi on plant performance make them suitable tools for sustainable agriculture (Rillig et al., 2016, 2019). While greenhouse studies on the impact of AM fungi on plant performance are numerous, there are not many field studies on AM fungi and plant performance at larger spatial scales, especially the landscape scale (Lekberg and Helgason, 2018). In addition to AM fungi, plant roots can harbour other endophytic fungi with positive, negative, neutral and unknown effects on the functioning of plants and plant communities (Aguilar-Trigueros et al., 2014; Geisen et al., 2017). Endophytic fungi in plant roots can interact with AM fungi and can have a positive (Andrade-Linares and Franken, 2013; Jia et al., 2016) or variable (Gan et al., 2017) impact on plants and plant communities.

Some studies showed that agricultural land in proximity to certain semi-natural habitats can have reduced crop yield (Raatz et al., 2019; Van Vooren et al., 2018) and may contain a different soil microbial diversity (Dacunto et al., 2014; Holden et al., 2019). Furthermore, Holden et al. (2019) found that AM fungal richness was lower in agricultural soil compared to hedgerow, field margin and pasture soil. Battie-Laclau et al. (2020) investigated an agroforestry system in France where the perennial components, trees, and herbaceous vegetation below the trees, had a positive effect on AM fungi abundance in neighbouring wheat roots. Nevertheless, the spatial patterns of AM and non-AM fungal abundance in crop roots across agricultural transition zones and how they are associated with different crop performance parameters are poorly described. Holland et al. (2017) point out in their review that crop yield is rarely measured in studies of semi-natural habitats. However, for the evaluation of semi-natural habitats for sustainable agricultural landscape management it is important to also take agronomic parameters into account. Raatz et al. (2019) showed that yield losses are relevant mainly close to woody landscape patches. In that study they focused on two different agricultural areas in Germany over several years, however, using only grain yield as crop performance parameter. Therefore, in the present field study, our objective was to quantify AM and non-AM fungal abundance in crop roots as well as a number of crop performance parameters along 50-m transects from semi-natural habitats into agricultural fields in an agricultural landscape in North-eastern Germany. We addressed the following questions and hypotheses:

- (1) Does distance to semi-natural habitats matter for AM and non-AM fungal root colonisation in winter-wheat agricultural transition zones? We hypothesise that AM and non-AM fungal root colonisation decreases with increasing distance from semi-natural habitat because semi-natural habitats may be reservoirs

of belowground AM and non-AM fungi and agricultural management, for example, tillage, was shown to decrease AM fungal abundance (Bowles et al., 2017).

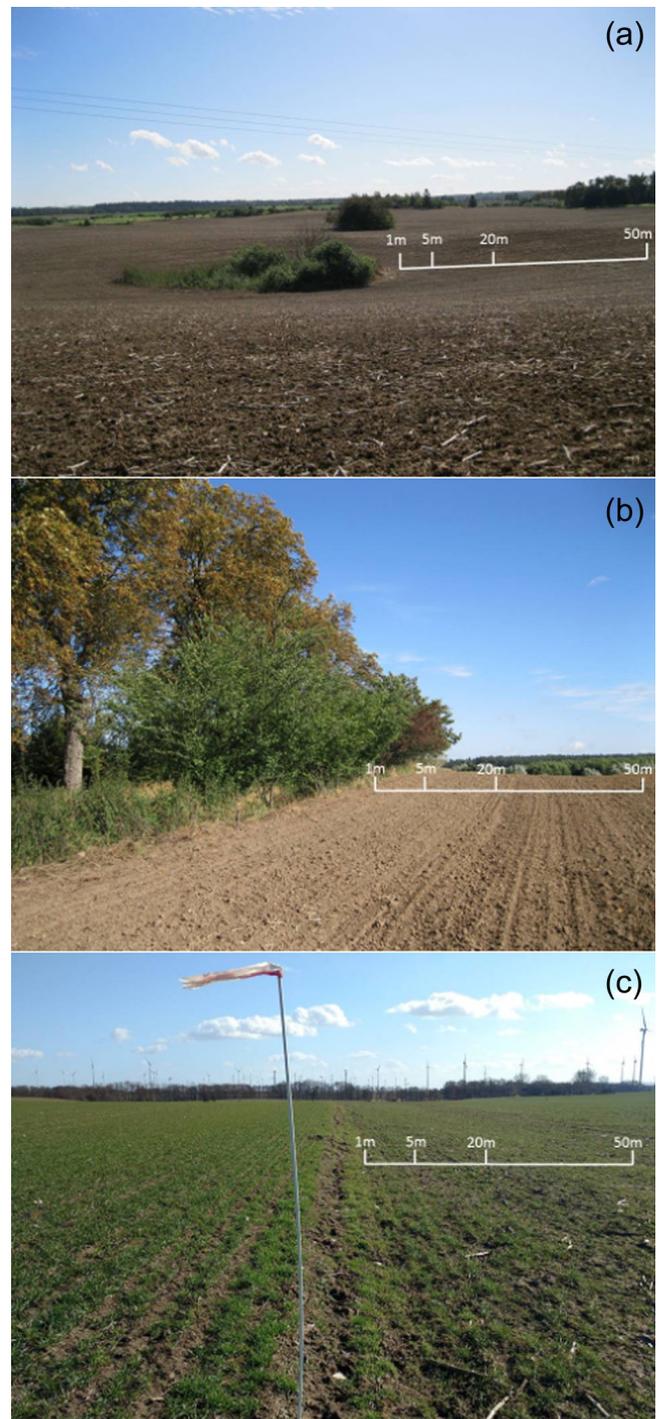
- (2) Does distance to semi-natural habitats matter for different crop performance parameters, that is, biomass at wheat flowering and at final harvest, grain yield and thousand kernel weight? Knowing from Raatz et al. (2019), that with increasing distance from woody landscape patches, grain yield increased, we hypothesise that different crop performance parameters are influenced differently by the distance to semi-natural habitats due to changing interactions between agricultural field and semi-natural habitats during the growing season.
- (3) Which abiotic and biotic variables have an important influence on AM and non-AM fungal root colonisation and crop performance parameters? We hypothesise that several abiotic and biotic variables affect AM and non-AM fungal root colonisation and crop performance parameters due to the very dynamic nature of agricultural transition zones.

## 2 | MATERIALS AND METHODS

### 2.1 | Site and study design

We carried out the study in the agricultural landscape of the “Quillow” river catchment in North-east Germany, in the State of Brandenburg (53° 35' N; 13° 68' E) in 2016. The study region is characterised by a continental climate with a 30-year precipitation average of 493 mm per year and a 30-year temperature average of 8.6°C. The study region covers approximately 290 km<sup>2</sup> and is used as the long-term research platform called “AgroScapeLab Quillow” (AGRIcultural landSCAPE LABo-ratory) of the Leibniz Center for Agricultural Landscape Research (ZALF) and the Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB). The main crops of the 62% arable land are conventionally grown maize, oilseed rape and cereals for food and energy production. The rest of the land use comprises small forest patches, grassland and some small villages and scattered single houses. The “AgroScapeLab Quillow” agricultural landscape contains two main semi-natural habitat types: hedgerows and in-field ponds, so-called kettle holes. Kettle holes are small glacially created wetlands embedded in the moraine landscapes of for example, north-eastern Germany and North America (Euliss & Mushet, 1996; Kalettka & Rudat, 2006).

We established transects from hedgerows ( $n = 7$ ) and kettle holes ( $n = 6$ ) into agricultural fields to be able to test the effect of distance of semi-natural habitats on different variables measured in the agricultural field. To be able to distinguish between the effect of edges and the effect of semi-natural landscape patches on our response variables we also established “control” transects ( $n = 4$ ) from the boundary between cereal fields and the studied agricultural fields. The neighbouring agricultural fields were typically managed by different farmers and had, for example, a different crop rotation. We selected four sampling points along the transects: 1, 5, 20, and 50 m from the edge of one of the two semi-natural habitats (Figure 1).



**FIGURE 1** Examples of the transects used in this field study. Along each transect, four sampling points were established at 1, 5, 20 and 50-m distance from the edge of a semi-natural habitat (a: kettle hole and b: hedgerow) and from the edge of an adjacent agricultural field (c: control). Distance units are not exact values due to different scales.

We focused our study on agricultural fields that were cultivated with oilseed rape in the previous year and winter wheat during the study year because it is the most common crop rotation of the study region. To select winter-wheat fields of 2016 with oilseed rape as the main crop in the previous cropping season, we used land-use maps from ZALF with spatial and temporal information of crop types in the

“AgroScapeLab Quillow” region. In ArcMap 10.4.1 (ESRI, 2013), we combined the land-use map and the natural-habitat map of Brandenburg (Ministerium für ländliche Entwicklung, 2014) and selected winter-wheat fields that bordered hedgerows or had embedded kettle holes.

We selected kettle holes in winter-wheat fields using the following criteria: size (up to 1 ha), hydrology (permanently filled with water), plant-community type around the water body (no trees, but shrubs and grassland), slope of transect (lowest change of slope possible) and direction of soil management, e.g., ploughing (perpendicular to transect) to avoid the mixing of soil from two data points of the transect. We selected hedgerows that bordered winter-wheat fields, with the following characteristics of the hedgerows: plant community (trees of maximum 14 m height, shrubs and grassland with similar vegetation for the different hedgerows), width (~8 m) and direction of soil management, for example, ploughing (perpendicular to transect).

## 2.2 | Soil, plant and fungi sampling

### 2.2.1 | Soil parameters

At each sampling point we took five soil samples (~15 cm depth) in a star pattern approximately 20 cm apart from each other, mixed the samples and divided it into different aliquots. Fresh soil was cooled and delivered to different laboratories for the determination of physical, chemical and isotopic soil and plant characteristics (Supporting Information: Table S1):

Soil aggregation (>250 µm macroaggregates) was determined by wet sieving (Cambardella and Elliott, 1993) and corrected for sand content (Elliott et al., 1991). Available water holding capacity (AWHC) was estimated using ceramic pressure plates (Richards, 1965) as the difference between the gravimetric water content at field capacity (~33 kPa) and wilting point (~1500 kPa). Soil moisture was measured gravimetrically following DIN ISO 11465. Electrical conductivity was measured via conductometry using a TitraMaster85 (HFA D77.1.4.1, -2, DIN EN 27888, DIN ISO 11265). Soil nitrogen content was measured by elemental analysis using a TruSpec CNS (Leco Instruments GmbH; DIN ISO 13878; HFA D58.1.3.1). Soil phosphorous and potassium content was measured by aqua regia extraction, using an ICP-OES by ThermoFisher SCIENTIFIC GmbH (VDLUF A MB Volume 1 paragraph 2.4.3.1; HFA A3.3.3, HFA Part D, following DIN ISO 11466 and DIN EN 16274). Plant available soil phosphorous was measured after a double lactat extraction, photometrically, on a GalleryTM Plus from Microgenics GmbH (VDLUF A MB Volume 1 paragraph 6.2.1.2). Fractionated simultaneous determination of total (TC) organic (TOC) and inorganic (TIC) carbon was done on a RC 612 from Leco Instruments GmbH (DIN ISO 10694). Soil pH was measured potentiometrically with a TitraMaster85 (HFA D76.1.4.1, -2, -3, DIN 38404-5, DIN ISO 10390). <sup>15</sup>N/<sup>14</sup>N ratio was measured to determine the isotopic soil N signature using an Elemental Analyzer (EA) Flash 2000 HT (Thermo Fisher Scientific), coupled with a Delta V

isotope ratio mass spectrometer (IRMS) via a ConFlo IV interface (Thermo Fisher Scientific) and equipped with a Costech Zero-Blank 50-position autosampler (Costech International). Particle size distribution was assessed by Bouyoucos hydrometer (Day, 1965) and broad texture classes were established according to Hollis et al. (2006). Soil ammonium and nitrate content were measured spectrophotometrically after extraction with CaCl<sub>2</sub>-solution (VDLUF A MB Bd. 1 Volume 6.1.4.1 DIN ISO 14256) with a CFA-SAN from Skalaranalytic GmbH. Soil microbial biomass carbon was measured by using the chloroform fumigation–extraction method modified by Gregorich et al. (1990). Carbonate content in the soil was measured by gas volumetric determination after Scheibler (VDLUF A MB Bd. 1 Kap. 5.3.1; DIN ISO 10693; HFA D31.3.1.1) with a Carmhomat 12D from Wösthoff Messtechnik GmbH. Soil bulk density was determined using soil cores (5–15 cm soil depth) with a 100 ml volume metal corer. After drying the soil to constant weight, we weighed the sample and calculated the density in g cm<sup>-3</sup>.

### 2.2.2 | AM fungi and non-AM fungal root colonisation

Two winter-wheat plants were harvested along the transects at 1, 5, 20 and 50 m away from the hedgerow/kettle hole/agricultural land edge (Figure 1) to determine AM fungi and non-AM fungal colonisation in the roots. After removal from the field, we washed the roots and kept them cool until further processing. In the laboratory, we placed roots in test tubes and covered them with 10% potassium hydroxide (KOH) solution. Then they were heated in a heating block for 35 min at 90°C. Thereafter, we removed the KOH solution, washed the roots thoroughly with 3.7% hydrochloric acid (HCl) solution for 10 min. After that, the HCl solution was removed and lactophenol blue solution was added until the roots were fully covered. After incubation at room temperature for 90 min, the lactophenol blue solution was removed and we added 50% lactoglycerol to destain the roots and prepare them for storage. Two hundred root segments per sample were investigated at 200x magnification (McGonigle et al., 1990). Stained segments with intraradical AM fungal structures were counted, like arbuscules, intercellular hyphae and vesicles and presented as the percentage of AM fungal root colonisation (Vierheilig et al., 2005). All other fungal structures were counted as non-AM fungi. Finally, the ratio between the number of total segments and segments colonised with AM fungal and non-AM fungal structures resulted in the percentage of root colonised by AM fungi and non-AM fungi, respectively. We calculated the average percentage root colonisation of the two winter-wheat plants per sampling point for further statistical analyses.

### 2.2.3 | Plant parameters

To determine crop performance, we collected winter-wheat plant biomass twice: at the flowering time (May 23rd to May 27th, 2016) and at the stage when winter-wheat grains were almost mature (July

11th to July 20th 2016). At the second harvest, we also determined grain yield and thousand kernel weight. Thousand kernel weight is a wheat trait, which is used in the agricultural sector, for example, for breeding programs, to describe the kernel size of a cereal (Baril, 1992). We determined thousand kernel weight by randomly picking 100 kernels from the one square metre grain yield harvest, weighing them and multiplying them by 10. At both harvest times, we determined the phenological development stage of five winter-wheat plants on the harvested square metre plot, referring to the BBCH-scale that starts from 00 (dry seed) up to 99 (harvested product; Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; (Meier, 2018). For winter-wheat biomass at flowering, we had a BBCH-scale range between 45 and 55 and at final harvest a BBCH-scale between 77 and 92. We cut all winter-wheat plants within one square metre with a sickle and dried them until constant dry weight. Some grains were still slightly green at the second harvest because we had to make sure that we cut the plants before the farmer harvested the whole field. Therefore, after harvesting we let them mature for some days before threshing. Grain yield and thousand kernel weight were determined from mature grains (July harvest) after threshing with a threshing machine.

The following procedures are used to analyse additional plant parameters for the random forest analyses: Plant nitrogen content was measured by elemental analysis using a TruSpec CNS (Leco Instruments GmbH; DIN ISO 13878; HFA D58.1.3.1).  $^{15}\text{N}/^{14}\text{N}$  ratio and  $^{13}\text{C}/^{12}\text{C}$  ratio was measured to determine the isotopic soil N signature using an Elemental Analyzer (EA) Flash 2000 HT (Thermo Fisher Scientific), coupled with a Delta V IRMS via a ConFlo IV interface (Thermo Fisher Scientific), and equipped with a Costech Zero-Blank 50-position autosampler (Costech International). Fractionated simultaneous determination of total carbon (TC) was done on a RC 612 from Leco Instruments GmbH (DIN ISO 10694). Weed cover was measured using a (Braun-Blanquet, 1951) scale in six 1 m<sup>2</sup> vegetation quadrats close to each experimental plot at a wheat growth stage of 37–45 with three quadrats to the left and right of the plot, parallel to the field border. Single scores were converted to percentage values and averaged per experimental plot.

## 2.3 | Data analyses

In field studies, we cannot control important parameters that may affect response variables. For that reason, we performed, on the one hand, a linear mixed-effects model analysis that specifically tested for questions 1 and 2. On the other hand, we used a machine learning approach to investigate the relative importance of a large set of measured explanatory variables (i.e., question 3).

### 2.3.1 | Mixed-effects models

We applied a linear mixed-effects model, using the “lme” function from the “nlme” package (Lindstrom & Bates, 1990) in R, for each of

the following response variables: AM fungal root colonisation, non-AM fungal root colonisation, biomass at wheat flowering, biomass at final wheat harvest, wheat grain yield and thousand kernel weight. We checked the residual plots from all the sampling points of the respective response variable for normal distribution before modelling, and AM and non-AM fungal root colonisation rates were square root transformed to meet the assumptions of linear mixed models. To test if there is a difference in the effect on the response variables between different types of neighbouring landscape patches, we used the factor “neighbouring landscape patch” that comprised three levels (i.e., kettle hole, hedgerow, and agricultural field), distance (i.e., 1, 5, 20, 50-m distance from the semi-natural/agricultural edge), and its interaction term as explanatory variables. To account for the heterogeneity across agricultural fields (e.g., long-term crop rotation history), we used “field” as the random factor. To minimise the potential bias from spatial autocorrelation among sampling points and transects, we applied the distance-based Moran’s Eigenvector Map (MEM) approach using the geographic coordinates of the sampling points (Blanchet et al., 2008; Borcard & Legendre, 2002; Dray et al., 2006; Legendre & Gauthier, 2014). Six orthogonal eigenvectors with a positive eigenvalue were obtained from the distance matrix of the sampling sites representing possible spatial structuring across different spatial scales (MEM1, MEM2, ..., MEM6). We used the “dbmem” function from the “adespatial” package (Dray et al., 2017) in the R software package for statistical computing (R Core Team, 2020).

### 2.3.2 | Random forest analyses

Each response variable (AM and non-AM fungal root colonisation, biomass at wheat flowering and final harvest, grain yield, thousand kernel weight) was modelled with all predictors (Supporting Information: Table S2), using the Random Forest machine learning algorithm (Breiman, 2001) of Hapfelmeier and Ulm (Hapfelmeier & Ulm, 2013). The algorithm estimates the relative importance of each predictor (i.e., how much each predictor contributes to model accuracy) as well as statistical significance (i.e., *p*-value) for each predictor based on a permutation approach ( $\alpha = 0.05$ ). In this test, a *p*-value represents a probability that the predictor contributes to a model accuracy by chance (Ryo & Rillig, 2017).

The algorithm is a non-parametric modelling approach that alleviates the multicollinearity issue for quantifying variable importance (Nicodemus et al., 2010). In addition, it can build a model even if the number of predictors is larger than the number of samples (Breiman, 2001). The hyper-parameters, that is, the numbers of trees and permutations that affect algorithm performance, were set to 1000 and 4000, respectively, after confirming that these numbers are large enough to stabilise the results.

We used 50 explanatory variables for AM and non-AM fungal root colonisation, and 55 explanatory variables for biomass at flowering, biomass at final harvest, grain yield and thousand kernel weight (Supporting Information: Table S1), to identify key factors explaining their variability and to evaluate if the main variables of

interest are included (i.e., the type of neighbouring landscape patch and distance from the edge). The explanatory variables contained (besides distance from the edge and type of neighbouring landscape patch) plant and soil chemical, physical and biological characteristics, the six orthogonal eigenvectors taking the spatial distribution of the sampling points into account, landscape characteristics, which were determined using Fragstats 4.2 (McGarigal & Marks, 1995), for example, Shannon diversity Index, percentage of arable land in a 250 m radius landscape around the sampling points, the owners of the agricultural fields, wheat variety and weed abundance (Supporting Information: Table S1).

Only variables with an  $R^2 > 0.05$  are reported. We do not report the variables explaining random or spatial variation, for example, "field," "MEM 1–6" and those which have many missing values, for example, "wheat variety," because explaining their importance would be beyond the scope of our study. We used the partial dependence plot approach (Trevor et al., 2009), which visualises how each predictor is associated with the respective response variable while accounting for the effects of the other predictors. We used the packages "party" (Strobl et al., 2009) and "mlr" (Bischl et al., 2016) in R.

### 3 | RESULTS

#### 3.1 | AM and non-AM fungal wheat root colonisation with increasing distance to semi-natural habitats

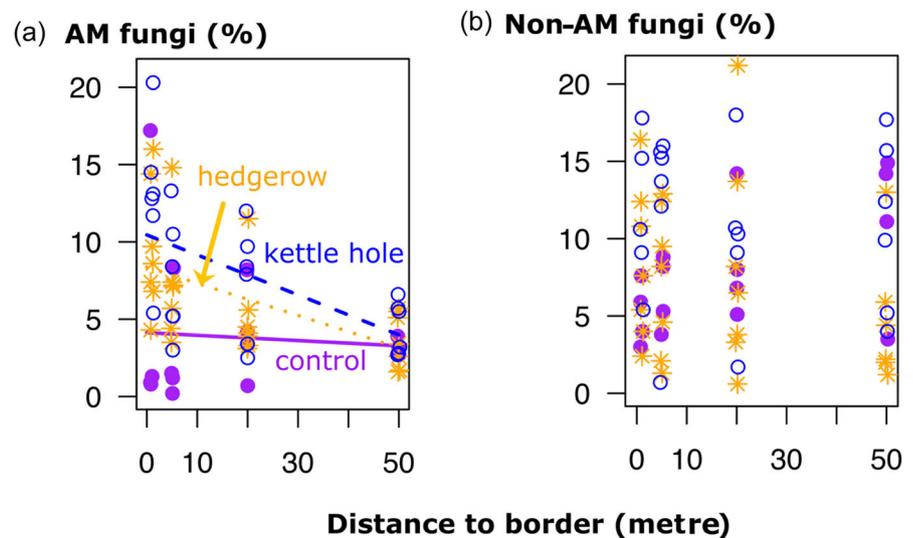
Arbuscular mycorrhizal root colonisation in winter-wheat roots over all sampling points ( $n = 68$ ) showed maximum values of 20.3%, minimum values of 0.2%, and a mean and standard error of  $6.4\% \pm 0.5\%$ . Non-AM fungal root colonisation in winter-wheat roots over all sampling points ( $n = 68$ ) showed maximum values of 31.2%, minimum values of 0.6% and a mean and standard error of  $9.5 \pm 0.8\%$ .

AM fungal root colonisation of winter-wheat plants decreased linearly with the distance from kettle holes and hedgerows while there was no such change in transects bordering another agricultural field (i.e., controls; Figure 2a). For AM fungal root colonisation of winter-wheat plants, we found an interaction effect of "landscape patch  $\times$  distance" ( $p = 0.01$ ) and their independent effects (distance:  $p < 0.001$  and landscape patch: ( $p < 0.001$ ; Table 1). By contrast, we did not find such associations for non-AM fungal root colonisation (all  $p > 0.05$ , Table 1; Figure 2b).

Random forest exploratory analyses indicated that only a few factors explain the variation in the response variables. The variation in AM fungal root colonisation was mostly explained by the type of neighbouring landscape patch ( $R^2 = 0.16$ ), followed by the distance to the neighbouring landscape patch ( $R^2 = 0.10$ ; Supporting Information: Table S2), and the Shannon-diversity of landscape patches within a 250-m radius landscape area around the sampling points ( $R^2 = 0.05$ ; Table 2 and Supporting Information: Table S2). We could explain very little of the variation in non-AM fungal root colonisation. Most of the variation was correlated with the type of neighbouring landscape patch ( $R^2 = 0.06$ ) as well as by the percentage of arable land in a landscape area of 250-m radius around the sampling points ( $R^2 = 0.05$ ; Table 2 and Supporting Information: Table S2).

#### 3.2 | Crop performance with increasing distance to semi-natural habitats

For all sampling points ( $n = 68$ ), winter-wheat biomass at flowering time showed maximum values of  $5.295 \text{ t ha}^{-1}$ , minimum values of  $0.490 \text{ t ha}^{-1}$  and an average and standard error of  $3.204 \pm 0.120 \text{ t ha}^{-1}$ ; winter-wheat biomass at final harvest showed maximum values of  $22.486 \text{ t ha}^{-1}$ , minimum values of  $6.743 \text{ t ha}^{-1}$  and an average and standard error of  $17.032 \pm 0.379 \text{ t ha}^{-1}$ ; winter-wheat grain yield showed maximum values of  $9.970 \text{ t ha}^{-1}$ , minimum values of  $1.615 \text{ t ha}^{-1}$  and an average and standard error of  $7.036 \pm 0.194 \text{ t ha}^{-1}$ ; and,



**FIGURE 2** Percentage of arbuscular mycorrhizal (AM) fungal root colonisation (a) and non-AM fungal root colonisation (b) as a function of distance from the agricultural field border for transects starting at kettle holes (circles with a dashed line;  $n = 6$ ), hedgerows (asterisks with a dotted line;  $n = 7$ ) and at neighbouring agricultural fields (filled circles with a continuous line;  $n = 4$ ). The regression line is not shown for panel b because the mixed model analysis for non-AM fungal root colonisation was not significant (see Table 1).

**TABLE 1** Linear mixed-effects model statistics results (degrees of freedom = DF; *F*-values; *p*-values) for the response variables percentage of AM fungal (AMF) and non-AM fungal (non-AMF) winter-wheat root colonisation, winter-wheat biomass at flowering time (biomass at flowering) and at final harvest (biomass at final harvest), winter-wheat grain yield (grain yield), thousand kernel weight and the test parameters type of landscape patch (i.e., kettle hole, hedgerow, agricultural field; "Landscape patch type") and distance to neighbouring landscape patch (i.e., 1, 5, 20, 50-m distance from neighbouring landscape patch type; "distance"), their two-way interactions and Moran's eigenvector maps (MEM1-6) (*p*-values lower than 0.05 are in bold and in grey)

Factor	DF	AMF		Non-AMF		Biomass at flowering		Biomass at final harvest		Grain yield		Thousand kernel weight	
		<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Landscape patch type	2	12.39	<b>&lt;0.001</b>	0.86	0.431	0.44	0.647	1.12	0.335	3.70	<b>0.033</b>	5.18	<b>0.010</b>
Distance	1	23.28	<b>&lt;0.001</b>	0.002	0.965	5.74	<b>0.021</b>	12.01	<b>0.001</b>	15.09	<b>&lt;0.001</b>	1.20	0.280
MEM1	1	1.07	0.307	0.80	0.377	22.61	<b>&lt;0.001</b>	11.10	<b>0.002</b>	4.27	<b>0.045</b>	3.72	0.060
MEM2	1	0.48	0.492	0.65	0.423	6.76	<b>0.013</b>	2.53	0.119	10.92	<b>0.002</b>	5.93	<b>0.019</b>
MEM3	1	0.07	0.793	6.44	<b>0.015</b>	5.31	<b>0.027</b>	0.18	0.676	1.84	0.182	0.00	0.978
MEM4	1	0.001	0.971	3.58	0.065	6.90	<b>0.012</b>	0.42	0.520	2.96	0.093	1.55	0.220
MEM5	1	0.19	0.670	0.20	0.661	14.79	<b>&lt;0.001</b>	8.34	<b>0.006</b>	4.03	0.051	7.00	<b>0.011</b>
MEM6	1	1.40	0.243	13.60	<b>&lt;0.001</b>	0.98	0.329	0.95	0.336	0.33	0.567	0.28	0.600
Landscape patch type x Distance	2	5.11	<b>0.010</b>	2.54	0.090	1.34	0.273	1.81	0.176	3.94	<b>0.027</b>	1.57	0.220
Residuals	1	195.65	<b>&lt;0.001</b>	120.47	<b>&lt;0.001</b>	1132.60	<b>&lt;0.001</b>	2893.60	<b>&lt;0.001</b>	1778.68	<b>&lt;0.001</b>	1985.66	<b>&lt;0.001</b>

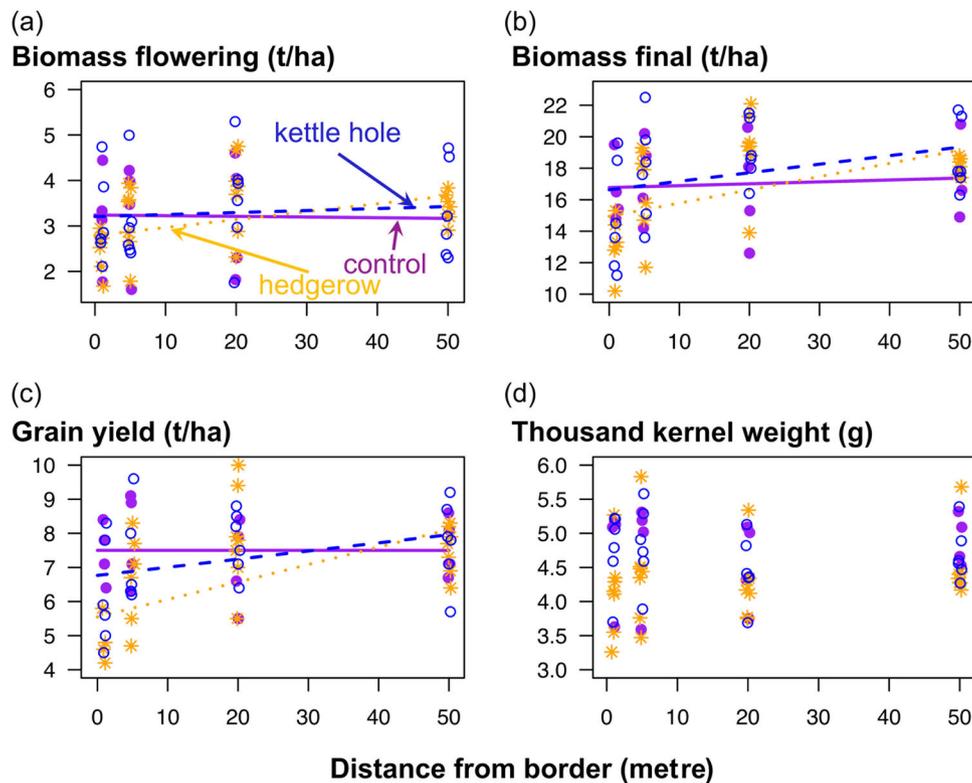
**TABLE 2** Variable importance of the main explanatory variables from the random forest analyses for biomass at flowering and at final harvest, grain yield, thousand kernel weight, arbuscular mycorrhizal (AM), and non-AM fungal root colonisation (see detailed description for all explanatory variables in Table S1. "Not incl." describes variables that were not included for this specific analysis)

	Variable importance (contribution to R <sup>2</sup> -fitting)					
	Biomass flowering	Biomass final	Grain yield	1000 kernel weight	AM fungi	non-AM fungi
R <sup>2</sup> -fitting	0.797	0.812	0.852	0.846	0.803	0.749
R <sup>2</sup> -validation	0.494	0.521	0.597	0.619	0.431	0.448
Weed cover	0.077	0.275	0.337	0.000	0.013	0.000
Distance	0.000	0.152	0.135	0.000	0.101	0.000
Neighboring patch	0.000	0.000	0.013	0.014	0.162	0.060
Soil aggregation	0.035	0.101	0.066	0.000	0.000	0.000
AM fungi	0.000	0.067	0.064	0.000	not included	not included
Shannon Diversity Index	0.005	0.000	0.000	0.031	0.053	0.043
Percentage arable land	0.005	0.000	0.000	0.024	0.025	0.054
Edge density	0.018	0.000	0.000	0.064	0.000	0.014
Patch richness	0.014	0.000	0.000	0.052	0.000	0.000
Soil C/N ratio	0.071	0.000	0.005	0.000	0.000	0.000
AWHC	0.056	0.000	0.000	0.000	0.008	0.000

thousand kernel weight showed maximum values of 58.3 g, minimum values of 32.6 g and an average and standard error of 45.5 ± 0.07 g.

The mixed-effects model analyses (Table 1) showed a significant effect of distance on all crop performance parameters besides

thousand kernel weight (biomass at flowering: *p* = 0.021; biomass at final harvest: *p* = 0.001; grain yield: *p* < 0.001; Figure 3a–d). The type of neighbouring landscape patch influenced both grain yield (*p* = 0.033) and thousand kernel weight (*p* = 0.01). Transects starting



**FIGURE 3** Winter-wheat biomass at wheat flowering time (a), winter-wheat biomass at final harvest (b), winter-wheat grain yield (c) and thousand kernel weight (d) depending on distance from the agricultural field border for transects starting at kettle holes (open circles with a dashed line;  $n = 6$ ), hedgerows (asterisks with a dotted line;  $n = 7$ ) and at neighbouring agricultural fields (filled circles with a continuous line;  $n = 4$ ).

at hedgerows showed lower grain yield and thousand kernel weight compared to those starting at kettle holes (Table 1; Figure 3c,d). The interaction of “landscape patch  $\times$  distance” was only significant for grain yield at final harvest ( $p = 0.027$ ). This means that the change of grain yield close to semi-natural landscape patches and especially hedgerows, was steeper than along transects neighbouring other cereal fields (Figure 3c). However, grain yield at a 20-m or further distance from the border between the agricultural field and the semi-natural landscape patches showed a marginal difference from that of controls (Figure 3c).

In the random forest analyses the variability in biomass at wheat flowering was explained by weed abundance ( $R^2 = 0.08$ ), C:N ratio measured in June ( $R^2 = 0.07$ ), and the available water holding capacity ( $R^2 = 0.06$ ; Table 2 and Supporting Information: Table S2). The main explanatory variables for wheat biomass and grain yield at final harvest were the same: weed abundance ( $R^2 = 0.28$  and  $0.34$ , respectively; Table 2 and Supporting Information: Table S2), the distance to the landscape patch border ( $R^2 = 0.15$  and  $0.14$ ), macroaggregates ( $R^2 = 0.10$  and  $0.07$ ) and AM fungal root colonisation ( $R^2 = 0.07$  and  $0.06$ ; Table 2 and Supporting Information: Table S2). Unlike the other response variables, the variability in thousand kernel weight was uniquely explained by edge density ( $R^2 = 0.06$ ) and landscape patch-richness in a circle of 250-m radius ( $R^2 = 0.05$ ; Table 2 and Supporting Information: Table S2).

## 4 | DISCUSSION

To our knowledge, we show for the first time that AM fungal root colonisation of wheat decreases with increasing distance from semi-natural habitats. This relationship remained strong even when many covariables were added in a random forest analysis. We did not observe such a pattern for non-AM fungal root colonisation. In addition, several crop performance parameters presented different patterns with distance from semi-natural habitats. Grain yield was affected the most, mainly increasing with distance from hedgerows.

### 4.1 | AM and non-AM fungal wheat root colonisation with increasing distance to semi-natural habitats

AM fungal root colonisation in winter-wheat decreased with distance to semi-natural habitats. This is an opportunity for management, as AM fungi are not only known to facilitate plants in accessing soil nutrients for plant-derived carbon, but also to ameliorate their tolerance against abiotic and biotic stress and improving soil quality, for example, soil aggregation, water retention and soil carbon and nutrient cycling (Bardgett & van der Putten, 2014). Several factors may be causing the decrease in AM fungal root colonisation with

increasing distance from semi-natural habitats: Agricultural management may be the strongest factor. The farmers in our area manage their winter-wheat fields conventionally with plant protection applications and soil management that can cause a reduction in AM fungi diversity and abundance (Bowles et al., 2017; Helgason et al., 1998; Manoharan et al., 2017; Pellegrino et al., 2020; Verbruggen et al., 2010). Plant protection measures for winter wheat entail among others, fungicides against *Fusarium* and *Alternaria* fungi and glyphosate application, which may have a negative effect on the AM fungal community (Hage-Ahmed et al., 2019; Rivera-Becerril et al., 2017). In addition, in our study area, nature protection regulations request land managers to keep between 5-to-25-m distance from semi-natural habitats like hedgerows and kettle holes depending on the crop protection substances (Baer et al., 2018). Therefore, we suggest that the AM fungal root colonisation gradient could mainly be a result of an agricultural management gradient along the transects. Raatz et al. (2021) showed greater weed abundances close to kettle holes and hedgerows at the same transects and during the same year which they suggest being the result of reduced herbicide application near semi-natural habitats. Many weed species are hosts of AM fungi (Vatovec et al., 2005). Hence, the pattern of decreasing AM fungal root colonisation with increasing distance from semi-natural habitats may partly also be the result of decreasing weed abundance with distance to the semi-natural habitats. Weed abundance was, however, not a strong predictor for AM fungal root colonisation in the random forest analyses (Table 2 and Supporting Information: Table S2).

We chose the transects in the field to be perpendicular to the working direction of the land managers (e.g., ploughing direction) to avoid the mixing of soil between sampling points at varying distances. Ecological interactions between semi-natural habitats and agricultural fields, such as light competition (Schmidt et al., 2017) and spatial flows of energy, material and organisms (Gounand et al., 2018; Loreau et al., 2003), may also have played important roles in our study. Meta-ecosystem theory describes how different ecosystems are connected by spatial flows of energy, materials and organisms across ecosystem boundaries (Loreau et al., 2003). This framework may help to understand ecosystem dynamics (Gounand et al., 2018) as well as biodiversity-ecosystem functioning across different ecosystems (Scherer-Lorenzen et al., 2022). We did not specifically study spatial flows along the transects, however, greater soil carbon and nitrogen as well as soil moisture close to semi-natural habitats compared with in-field datapoints may be the result of such spatial flows (Supporting Information: Table S3, Figure S1). For example, leaves from kettle hole and hedgerow vegetation can fall on agricultural field edges, kettle hole water can wet agricultural soil around the pond and tall kettle hole and hedgerow vegetation can shade the agricultural transition zone, leading to less evaporation and hence greater soil humidity at field borders (Schmidt et al., 2017). Such abiotic and biotic flows across landscape patch borders do not happen at field-to-field borders. And these flows can influence soil biotic characteristics, and thus, also belowground AM fungi (Bainard et al., 2014). Landscape patch type (i.e., kettle hole, hedgerow, agricultural field) was a strong predictor for AM fungal root colonisation in the mixed model and random forest

analyses (Tables 1 and 2). Consequently, we suggest that agricultural management as well as spatial flows of energy, material and organisms to contribute to the AM fungi pattern.

AM fungi can disperse actively (i.e., extraradical hyphae) and passively, that is, by abiotic (e.g., wind, water) and biotic (e.g., small mammals, earthworms) agents (Paz et al., 2021 and references therein). However, further research is needed to determine the contribution of such processes to the observed AM fungal root colonisation pattern in agricultural transition zones.

Non-AM fungal root colonisation did not show any dependence on the distance to semi-natural habitats. However, our results indicate that transects starting at kettle holes tended to have greater non-AM fungal root colonisation than the other transects. Kettle holes of this study were permanently filled with water and agricultural soil close to the kettle holes showed higher soil-water content (Supporting Information: Table S3, Figure S1 and Pirhofer Walzl, 2022) compared with further away from the kettle holes, which may have positively affected the presence of fungi (Müller et al., 2016).

Different studies showed greater AM fungal diversity and abundance under hedgerows (Holden et al., 2019) or perennial agroforestry vegetation (Battie-Laclau et al., 2020) in comparison to agricultural fields. Therefore, semi-natural habitats may be a valuable reservoir of AM fungi. When these AM fungi spill over in the agricultural field, they can be potentially beneficial for agricultural production, especially when crops are exposed to severe disturbances. AM fungi can increase resilience of plants against resource scarcity for example, during droughts (Chareesri et al., 2020; Kakouridis et al.; Li et al., 2019), which will become more frequent in the future (Masson-Delmotte et al., 2021). Moreover, AM fungi root colonisation and thus, soil and plant quality, may also be improved within the agricultural field by specific management practices favouring AM fungi, like no-till and legume cover-cropping (Bowles et al., 2017).

## 4.2 | Crop performance with increasing distance to semi-natural habitats

In line with our results, several studies have shown an increase of yield with increasing distance to semi-natural landscape patches (e.g., Laura et al., 2017; Raatz et al., 2019). Raatz et al. (2019) showed for two different agricultural areas in Germany and during several years, including the grain yield of our transects during the same year, that grain yield increased with distance to semi-natural habitats, but mainly at wood landscape patches. While most studies have not measured crop yield when evaluating semi-natural habitats in agricultural landscapes (Holland et al., 2017), some focused only on one crop performance parameter, for example, Raatz et al. (2019). We present different temporal and descriptive crop performance parameters (i.e., wheat plant biomass at flowering and at final harvest, grain yield and thousand kernel weight) that show varying results. The effect of distance to the neighbouring landscape patches on crop performance increased from winter-wheat biomass at flowering to

final harvest to grain yield while thousand kernel weight was not affected from the distance. However, only grain yield varied with distance depending on the landscape patch, namely increased with distance, especially at hedgerow transects (Table 1). As for AM and non-AM fungal wheat root colonisation patterns along agricultural transition zones, crop performance patterns can also be explained by a combination of agricultural management, ecological interactions and nature protection regulations. And these factors are often naturally interlinked with each other.

Weed abundance turned out to be a key explanatory variable for wheat biomass at flowering and at final harvest and grain yield in the random forest analyses. Our results for grain yield confirm Raatz et al. (2021), who show negative effects of weed abundance for grain yields from experimental plots along the same transects as used for the present study at 1 and 5 m distances from semi-natural habitats. However, Raatz et al. (2021) studied wheat plants that they sowed into the farmers' fields while we harvested wheat plants sown by the farmers. Incidental plants, like weeds, compete with crops for space, nutrients, water and light (Zimdahl, 2004) and hence can reduce crop biomass and grain yield effectively, especially close to semi-natural habitats where herbicides could not be sprayed due to nature protection regulations (Baer et al., 2018). In addition, seed dispersal from hedgerow and kettle hole vegetation into the agricultural field may also be a reason for greater abundance of incidental plants in the proximity to semi-natural habitats (Lozada-Gobilard et al., 2019). Competition between crop plants and semi-natural habitat vegetation may be another explanation for reduced biomass and yield at the edges. As nutrients, for example, nitrogen and phosphorus, and soil water were available in greater quantities close to semi-natural habitats (Supporting Information: Table S3, Figure S1 and Pirhofer Walzl, 2022), nutrient and water competition cannot be the cause of yield and crop biomass reduction close to the edge in our study. Light competition was shown earlier to be the cause of low crop yield close to semi-natural habitats, for example, in agroforestry systems (Dufour et al., 2013) and near hedgerows (Laura et al., 2017; Raatz et al., 2019) and thus, can also be one explanation in our study. Nonetheless, semi-natural habitats, especially those with taller vegetation such as hedgerows and forest fragments, can also positively influence crops in several ways: for example, through soil conservation, increase of soil moisture, and protection from physical damage (Kort, 1988; Nuberg, 1998). In our transects, increased soil water and nutrients close to hedgerows and kettle holes suggest a soil protection effect (Supporting Information: Table S3, Figure S1 and Pirhofer Walzl, 2022). We did not evaluate the direction of our transects and the wind to determine the evapotranspiration effect of semi-natural habitats on our crop plants. Yet, in our study region, the shelter effect of semi-natural habitats on yield was more pronounced during the dry 2016 year in comparison with 2017 (Raatz et al., 2019). This sheds light on the possible importance of semi-natural habitats as climate and weather buffer since climate scenarios predict more dry years and more intense weather and climate extremes (Masson-Delmotte et al., 2021). Crop management and nature protection regulations (EU and German regulations, No. 1107/2009, Bundestag, 2012) may have strongly impacted the crop performance pattern we see along agricultural transition zones. Those regulations differ depending on

the active substance, the type of neighbouring landscape patch and the amount of natural or semi-natural landscape patches in the surrounding landscape (Baer et al., 2018). Low or no application of herbicides might have led to increased growth of incidental plants close to semi-natural habitats (see above). Low or no application of plant protection measures against pests, like fungi and herbivores, close to semi-natural patches may have caused increased pest infestations in vicinity to hedgerows and kettle holes, as well as pests from neighbouring semi-natural habitats can spill over into agricultural fields. Nonetheless, Raatz et al. (2021) showed no effect of fungal seed, fungal leaf pathogens and herbivory of cereal leaf beetles on grain yield in the same winter-wheat transects as in this study. However, there are other wheat pests that could have been the culprit for reduced yield close to semi-natural habitats, for example, aphids.

In addition, low or no application of fertilisers close to water bodies (Baer et al., 2018), may have lowered the yield close to kettle holes. However, the soil nutrient measurements in our study that were taken twice during the growing season, showed high values close to semi-natural habitats (Supporting Information: Table S3, Figure S1 and Pirhofer Walzl, 2022).

The random forest analyses showed that, in addition to weed abundance and distance to a neighbouring landscape patch, the percentage of macroaggregates in the soil positively affected biomass at final harvest and grain yield and C/N ratio positively affected biomass at wheat flowering. Aggregation of soil particles is an important soil quality parameter relevant for soil water dynamics and nutrient cycling, hence for crop yield. And soil biota, especially bacteria and fungi, play an important role in increasing the abundance of micro- and macroaggregates in the soil (Lehmann et al., 2017) as well as in soil C/N ratio dynamics.

## 5 | CONCLUSION

Our study sheds light on patterns of crop performance and fungal root colonisation in wheat plants in distance to semi-natural habitats. We show for the first time that AM fungal root colonisation decreased with distance to semi-natural habitats and crop performance, that is, grain yield, increased. Our study also shows that there is a difference between different crop performance parameters. Therefore, semi-natural habitats may be a valuable reservoir of AM fungi. Agricultural management (especially weed control measures), which is strongly influenced by nature protection regulations, may be a strong driver for our results. Agricultural transition zones are spatially and temporally complex dynamic ecosystems that need more empirical investigations in the field. Field studies should look at the effect of ecological interactions (e.g., competition, nutrient flows etc.) and agricultural management on above- and below-ground ecosystem functions and agronomic parameters along agricultural transition zones. This knowledge may help improve CAP regulations as well as local conflicts between nature protection agencies and farmers (Kerneck et al., 2022). Small semi-natural habitats in agricultural landscapes are slowly recognised not only to be important for biodiversity conservation (Herzon et al., 2022; Tschardt et al., 2021; Wintle et al.,

2019), but also for sustainable crop production (Pywell et al., 2015). Farmers are under pressure to produce high yields as well as contribute to sustainable landscape management. The maintenance and implementation of natural or semi-natural landscape patches may be a tool for farmers and policy to improve the contribution of the farmers to sustainable soil, crop, and landscape management.

## AUTHOR CONTRIBUTIONS

Karin Pirhofer Walzl: Conceptualisation; data collection; methodology; formal analysis; investigation; data curation; writing—original draught preparation; visualisation. Masahiro Ryo: Methodology; formal analysis; investigation; data curation; visualisation; writing. Larissa Raatz: Conceptualisation; data collection; methodology; writing – review and editing. Jana S. Petermann: Methodology; formal analysis; investigation; data curation; visualisation; writing—review and editing. Arthur Gessler: Conceptualisation; writing—review and editing; supervision; project administration. Jasmin Joshi: Conceptualisation; writing—review and editing; supervision; project administration. Matthias C. Rillig: Conceptualisation; writing—review and editing; supervision; project administration. All authors read and approved the final manuscript.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the BONARES repository at <https://doi.org/10.4228/d7y2-tw27>.

## ETHICS STATEMENT

The authors declare that they followed the ethics policies of the journal.

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## REFERENCES

Aguilar-Trigueros CA, Powell JR, Anderson IC, Antonovics J, Rillig MC. Ecological understanding of root-infecting fungi using trait-based approaches. *Trends Plant Sci.* 2014;19(7):432–8.

- Andrade-Linares D, Franken P. Fungal endophytes in plant roots: taxonomy, colonization patterns, and functions. In: Aroca R, editor. *Symbiotic Endophytes Soil Biology*. Berlin Heidelberg: Springer-Verlag; 2013. p. 311–34.
- Baer H, Bergmann E, Dittrich R, Ewert K, Goessner K, Goetz R, et al. *Pflanzenschutz im Ackerbau und Grünland*. Brandenburg, Sachsen, Sachsen-Anhalt und Thüringen: Eine Information der Pflanzenschutzdienste der Länder Berlin; 2018.
- Bainard LD, Bainard JD, Hamel C, Gan Y. Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. *FEMS Microbiol Ecol.* 2014;88(2):333–44.
- Bardgett RD, van der Putten WH. Belowground biodiversity and ecosystem functioning. *Nature.* 2014;515(7528):505–11.
- Baril CP. Factor regression for interpreting genotype-environment interaction in bread-wheat trials. *Theor Appl Genet.* 1992;83(8):1022–6.
- Battie-Laclau P, Taschen E, Plassard C, Dezette D, Abadie J, Arnal D, et al. Role of trees and herbaceous vegetation beneath trees in maintaining arbuscular mycorrhizal communities in temperate alley cropping systems. *Plant Soil.* 2020;453:1–19.
- Billeter R, Liira J, Bailey D, Bugter R, Arens P, Augenstein I, et al. Indicators for biodiversity in agricultural landscapes: a pan-European study. *J Appl Ecol.* 2008;45(1):141–50.
- Bischi B, Lang M, Kotthoff L, Schiffner J, Richter J, Studerus E, et al. Mlr: machine learning in R. *J Mach Learn Res.* 2016;17(1):5938–42.
- Blanchet FG, Legendre P, Borcard D. Modelling directional spatial processes in ecological data. *Ecol Model.* 2008;215(4):325–36.
- Borcard D, Legendre P. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol Model.* 2002;153(1):51–68.
- Bowles TM, Jackson LE, Loeher M, Cavigliaro TR. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *J Appl Ecol.* 2017;54(6):1785–93.
- Braun-Blanquet J. *The plant communities of Mediterranean France*. Paris: C.N.R.S.; 1951.
- Breiman L. Random forests. *Mach Learn.* 2001;45(1):5–32.
- Brundrett MC, Tedersoo L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* 2018;220(4):1108–15.
- Bundestag D. Gesetz zum Schutz der Kulturpflanzen (Pflanzenschutzgesetz). [Internet]. 2012. Available from [www.gesetze-im-internet.de/pflschg\\_2012](http://www.gesetze-im-internet.de/pflschg_2012)
- Cambardella CA, Elliott ET. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. *Soil Sci Am J.* 1993;57(4):1071–6.
- Chareesri A, De Deyn GB, Sergeeva L, Polthanee A, Kuyper TW. Increased arbuscular mycorrhizal fungal colonization reduces yield loss of rice (*Oryza sativa* L.) under drought. *Mycorrhiza.* 2020; 30(2):315–28.
- Clements FE. *Research methods in ecology*. Cornell Univ Lib. 1905:364.
- Dacunto L, Semmartin M, Ghera C. Uncropped field margins to mitigate soil carbon losses in agricultural landscapes. *Agric Ecosyst Environ.* 2014;183:60–8.
- Day, PR. Particle Fractionation and Particle Size Analysis. *Methods of Soil Analysis*. Part 1. In: *Methods of soil analysis* [Internet]. 1st ed. Wisconsin, USA: American Society of Agronomy, Inc; 1965. p. 545–67. Available from <https://dl.sciencesocieties.org/publications/books/pdfs/agronymonogra/methodsofsoilnb/frontmatter>
- Dray S, Legendre P, Peres-Neto PR. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol Model.* 2006;196(3):483–93.
- Dray S, Blanchet FG, Borcard D, Guenard G, Jombart T, Larocque G. *adespatial: Multivariate multiscale spatial analysis*. R package version 0.0-8. 2017.
- Dufour L, Metay A, Talbot G, Dupraz C. Assessing light competition for cereal production in temperate agroforestry systems using experimentation and crop modelling. *J Agron Crop Sci.* 2013;199(3):217–27.

- Elliott ET, Palm CA, Reuss DE, Monz CA. Organic matter contained in soil aggregates from a tropical chronosequence: correction for sand and light fraction. *Agric Ecosyst Environ.* 1991;34(1):443–51.
- Euliss NH, Mushet DM. Water-level fluctuation in wetlands as a function of landscape condition in the prairie pothole region. *Wetlands.* 1996;16(4):587–93.
- Gan H, Churchill ACL, Wickings K. Invisible but consequential: root endophytic fungi have variable effects on belowground plant–insect interactions. *Ecosphere.* 2017;8(3):e01710.
- Garibaldi LA, Steffan-Dewenter I, Kremen C, Morales JM, Bommarco R, Cunningham SA, et al. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecol Lett.* 2011;14(10):1062–72.
- Geisen S, Kostenko O, Cossen MC, Ten Hooven FC, Vreš B, van der Putten WH. Seed and root endophytic fungi in a range expanding and a related plant species. *Front Microbiol.* 2017;8:1645.
- Gounand I, Harvey E, Little CJ, Altermatt F. Meta-Ecosystems 2.0: rooting the theory into the field. *Trends Ecol Evol.* 2018;33(1):36–46.
- ESRI. ARCMAP. ArcGIS. 10.2. Redland, California: Environmental Systems Research Institute; 2013.
- Gregorich EG, Wen G, Voroney RP, Kachanoski RG. Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass C. *Soil Biol Biochem.* 1990;22(7):1009–11.
- Hage-Ahmed K, Rosner K, Steinkellner S. Arbuscular mycorrhizal fungi and their response to pesticides. *Pest Manag Sci.* 2019;75(3):583–90.
- Häpfelmeier A, Ulm K. A new variable selection approach using random forests. *Comput Stat Data Anal.* 2013;60(C):50–69.
- Hartmann M, Frey B, Mayer J, Mäder P, Widmer F. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* 2015;9(5):1177–94.
- Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. Ploughing up the wood-wide web? *Nature.* 1998;394(6692):431.
- Herzon I, Raatikainen KJ, Helm A, Rūsiņa S, Wehn S, Eriksson O. Semi-natural habitats in the European boreal region: Caught in the socio-ecological extinction vortex? *Ambio* [Internet]. 2022. Available from: <https://doi.org/10.1007/s13280-022-01705-3>
- Holden J, Grayson RP, Berdeni D, Bird S, Chapman PJ, Edmondson JL, et al. The role of hedgerows in soil functioning within agricultural landscapes. *Agric Ecosyst Environ.* 2019;273:1–12.
- Holland JM, Douma JC, Crowley L, James L, Kor L, Stevenson DRW, et al. Semi-natural habitats support biological control, pollination and soil conservation in Europe: a review. *Agron Sustain Dev.* 2017;37(4):31.
- Hollis JM, Jones RJA, Marshall CJ, Holden A, Van de Veen JR, Montanarella L. The soil profile analytical database for Europe. version 1.0. European Soil Bureau Research Report No.19, EUR 22127 EN [Internet]. Luxembourg: Office for Official Publications of European Communities; 2006. Available from [https://esdac.jrc.ec.europa.eu/Esdb\\_Archive/eusoils\\_docs/esb\\_rr/n19\\_EUR22127.pdf](https://esdac.jrc.ec.europa.eu/Esdb_Archive/eusoils_docs/esb_rr/n19_EUR22127.pdf)
- Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, et al. A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Front Microbiol.* 2016;7:906.
- Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldman LJ, Herman DJ, et al. Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *New Phytologist* [Internet]. Available from: <https://nph.onlinelibrary.wiley.com/doi/abs/10.1111/nph.18281>
- Kaletka T, Rudat C. Hydrogeomorphic types of glacially created kettle holes in North-East Germany. *Limnol Ecol Manag Inland Waters.* 2006;36:54–64.
- Kerneck M, Fienitz M, Nendel C, Pätzig M, Pirhofer Walzl K, Raatz L, et al. Transition zones across agricultural field boundaries for integrated landscape research and management of biodiversity and yields. *Ecol Sol Evid.* 2022;3(1):e12122.
- Kort J. 9. benefits of windbreaks to field and forage crops. *Agric Ecosyst Environ.* 1988;22–23:165–90.
- Laura VV, Bert R, Steven B, Pieter DF, Victoria N, Paul P, et al. Ecosystem service delivery of agri-environment measures: a synthesis for hedgerows and grass strips on arable land. *Agric Ecosyst Environ.* 2017;244:32–51.
- Legendre P, Gauthier O. Statistical methods for temporal and space-time analysis of community composition data†. *Proc R Soc B.* 2014;281(1778):20132728.
- Lehmann A, Zheng W, Rillig MC. Soil biota contributions to soil aggregation. *Nat Ecol Evol.* 2017;1(12):1828–35.
- Leifheit EF, Veresoglou SD, Lehmann A, Morris EK, Rillig MC. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation—a meta-analysis. *Plant Soil.* 2014;374(1):523–37.
- Lekberg Y, Helgason T. In situ mycorrhizal function: knowledge gaps and future directions. *New Phytol.* 2018;220(4):957–62.
- Li J, Meng B, Chai H, Yang X, Song W, Li S, et al. Arbuscular mycorrhizal fungi alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses via altering antioxidant enzyme activities and photosynthesis. *Front Plant Sci.* 2019;10:499.
- Lindstrom MJ, Bates DM. Nonlinear mixed effects models for repeated measures data. *Biometrics.* 1990;46(3):673–87.
- Livingstone B. The distribution of the upland societies of Kent country. *Bot Gaz.* 1903;35(1):36–55.
- Loreau M, Mouquet N, Holt RD. Meta-ecosystems: a theoretical framework for a spatial ecosystem ecology. *Ecol Lett.* 2003;6(8):673–9.
- Lozada-Gobilard S, Stang S, Pirhofer-Walzl K, Kalettka T, Heinken T, Schröder B, et al. Environmental filtering predicts plant–community trait distribution and diversity: kettle holes as models of meta-community systems. *Ecol Evol.* 2019;9(4):1898–910.
- Manoharan L, Rosenstock NP, Williams A, Hedlund K. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. *Appl Soil Ecol.* 2017;115:53–9.
- Masson-Delmotte V, Zhai P, Pirani SLC, Péan C, Berger S, Caud N, et al. IPCC - Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press; 2021.
- McGarigal K, Marks BJ. FRAGSTATS: Spatial pattern analysis program for quantifying landscape structure. USDA Forest Service General Technical Report PNW-351, Corvallis.; 1995.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytol.* 1990;115(3):495–501.
- Meier U. Growth stages of mono- and dicotyle- donous plants BBCH Monograph [Internet]. 1st ed. Quedlingburg, Germany: Julius Kühn-Institut (JKI) Quedlinburg, Germany; 2018. Available from <https://www.julius-kuehn.de/media/Veroeffentlichungen/bbch%20epaper%20en/page.pdf>
- Ministerium für ländliche Entwicklung U und L des LB (MLUL). Flächendeckende Biotop- und Landnutzungskartierung (BTLN) im Land Brandenburg - CIR - Biotoptypen 2009 [Internet]. 2014. Available from: <http://www.mlul.brandenburg.de/cms/detail.php/bb1.c.310474.de>
- Müller MEH, Koszinski S, Bangs DE, Wehrhan M, Ulrich A, Verch G, et al. Crop biomass and humidity related factors reflect the spatial distribution of phytopathogenic *Fusarium fungi* and their mycotoxins in heterogeneous fields and landscapes. *Precision Agriculture.* 2016;17(6):698–720.
- Newbold T, Hudson LN, Hill SL, Contu S, Lysenko I, Senior RA, et al. Global effects of land use on local terrestrial biodiversity. *Nature.* 2015;520(7545):45–50.

- Nicodemus KK, Malley JD, Strobl C, Ziegler A. The behaviour of random forest permutation-based variable importance measures under predictor correlation. *BMC Bioinformatics*. 2010;11(1):110.
- Nuberg IK. Effect of shelter on temperate crops: a review to define research for Australian conditions. *Agroforestry Systems*. 1998;41(1):3–34.
- Paz C, Öpik M, Bulascoschi L, Bueno CG, Galetti M. Dispersal of arbuscular mycorrhizal fungi: evidence and insights for ecological studies. *Microb Ecol*. 2021;81(2):283–92.
- Pe'er G, Lakner S, Müller R, Passoni G, Bontzorlos V, Clough D, et al. Is the CAP fit for purpose? An evidence-based fitness check assessment. Leipzig, German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig; 2017.
- Pellegrino E, Gamper HA, Ciccolini V, Ercoli L. Forage rotations conserve diversity of arbuscular mycorrhizal fungi and soil fertility. *Front Microbiol*. 2020;10:2969.
- Pirhofer Walzl K. Chemical, physical and biological soil and plant/crop parameters in distance to agricultural field edges [Internet]. 2022. Available from: <https://doi.org/10.4228/d7y2-tw27>
- Pywell RF, Heard MS, Woodcock BA, Hinsley S, Ridding L, Nowakowski M, et al. Wildlife-friendly farming increases crop yield: evidence for ecological intensification. *Proc R Soc B*. 2015;282(1816):20151740.
- Raatz L, Pirhofer Walzl K, Müller MEH, Scherber C, Joshi J. Who is the culprit: is pest infestation responsible for crop yield losses close to semi-natural habitats? *Ecol Evol*. 2021;11(19):13232–46.
- Raatz L, Bacchi N, Pirhofer Walzl K, Glemnitz M, Müller MEH, Joshi J, et al. How much do we really lose?—yield losses in the proximity of natural landscape elements in agricultural landscapes. *Ecol Evol*. 2019;9(13):7838–48.
- R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; [Internet] 2020. Available from <https://www.R-project.org/>
- Richards LA. Physical Condition of Water in Soil. In: *Methods of Soil Analysis* [Internet]. John Wiley & Sons, Ltd; 1965. p. 128–52. Available from <https://access.onlinelibrary.wiley.com/doi/abs/10.2134/agronmonogr9.1.c8>
- Rillig MC, Sosa-Hernández MA, Roy J, Aguilar-Trigueros CA, Vályi K, Lehmann A. Towards an integrated mycorrhizal technology: harnessing mycorrhiza for sustainable intensification in agriculture. *Front Plant Sci*. 2016;7:1625.
- Rillig MC, Aguilar-Trigueros CA, Camenzind T, Cavigliaro TR, Degrune F, Hohmann P, et al. Why farmers should manage the arbuscular mycorrhizal symbiosis. *New Phytol*. 2019;222(3):1171–5.
- Rivera-Becerril F, van Tuinen D, Chatagnier O, Rouard N, Béguet J, Kuszala C, et al. Impact of a pesticide cocktail (fenhexamid, folpel, deltamethrin) on the abundance of Glomeromycota in two agricultural soils. *Sci Total Environ*. 2017;577:84–93.
- Ryo M, Rillig MC. Statistically reinforced machine learning for nonlinear patterns and variable interactions. *Ecosphere*. 2017;8(11):e01976.
- Scherer-Lorenzen M, Gessner MO, Beisner BE, Messier C, Paquette A, Petermann JS, et al. Pathways for cross-boundary effects of biodiversity on ecosystem functioning. *Trends Ecol Evol*. 2022;37:454–67.
- Schmidt M, Jochheim H, Kersebaum KC, Lischeid G, Nendel C. Gradients of microclimate, carbon and nitrogen in transition zones of fragmented landscapes: a review. *Agricult Forest Meteorol*. 2017;232:659–71.
- Smith SE, Read D. 2-Colonization of roots and anatomy of arbuscular mycorrhizas. In: Smith SE, Read D editors. *Mycorrhizal Symbiosis (Third Edition)* [Internet]. Third Edition. London: Academic Press; 2008. p. 42–90. Available from: <https://www.sciencedirect.com/science/article/pii/B9780123705266500040>
- Sommermann L, Geistlinger J, Wibberg D, Deubel A, Zwanzig J, Babin D, et al. Fungal community profiles in agricultural soils of a long-term field trial under different tillage, fertilization and crop rotation conditions analyzed by high-throughput ITS-amplicon sequencing. *PLoS One*. 2018;13(4):1–32.
- Strobl C, Hothorn T, Zeileis A. Party on! A new, conditional variable importance measure for random forests available in the party package [Internet]. 2009. Available from: <https://epub.ub.uni-muenchen.de/9387/1/techreport.pdf>
- Trevor Hastie, Tibshirani R, Friedman J. The elements of statistical learning: Data mining, inference, and prediction. [Internet]. 2nd ed. Springer; 2009. p. 745. Available from <https://www.springer.com/gp/book/9780387848570>
- Tscharntke T, Grass I, Wanger TC, Westphal C, Batáry P. Beyond organic farming – harnessing biodiversity-friendly landscapes. *Trends in Ecology & Evolution* [Internet]. Available from: <https://doi.org/10.1016/j.tree.2021.06.010>
- Van Vooren L, Reubens B, Ampoorter E, Broekx S, Pardon P, Van Waes C, et al. Monitoring the impact of hedgerows and grass strips on the performance of multiple ecosystem service indicators. *Environ Manage*. 2018; 62(2):241–59.
- Vatovec C, Jordan N, Huerd S. Responsiveness of certain agronomic weed species to arbuscular mycorrhizal fungi. *Renew Agricult Food Syst*. 2005;20(3):181–9.
- Verbruggen E, Rölting WFM, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MGA. Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytol*. 2010;186(4):968–79.
- Veresoglou SD, Rillig MC. Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal fungi. *Biol Lett*. 2012;8(2):214–7.
- Vierheilig H, Schweiger P, Brundrett M. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots†. *Physiol Plant*. 2005;125(4):393–404.
- Wintle BA, Kujala H, Whitehead A, Cameron A, Veloz S, Kukkala A, et al. Global synthesis of conservation studies reveals the importance of small habitat patches for biodiversity. *Proc Natl Acad Sci USA*. 2019;Jan 15 116(3):909–14.
- Zimdahl RL. Influence of Competition on the Plant. In: *Weed-Crop Competition* [Internet]. John Wiley & Sons, Ltd; 2004. p. 19–26. Available from <https://onlinelibrary.wiley.com/doi/abs/10.1002/9780470290224.ch4>

## SUPPORTING INFORMATION

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

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