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Title
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Permalink
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Journal

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Publication Date

DOI

Agricultural management and pesticide use reduce the functioning of beneficial plant symbionts

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Competing interest

The authors declare no competing interests.

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Abstract

Phosphorus (P) acquisition is key for plant growth. Arbuscular mycorrhizal fungi (AMF) help plants acquire P from soil. Understanding which factors drive AMF-supported nutrient uptake is essential to develop more sustainable agroecosystems. Here, we collected soils from 150 cereal fields and 60 non-cropped grassland sites across a 3,000 km trans-European gradient. In a greenhouse experiment, we tested the ability of AMF in these soils to forage for the radioisotope ^{33}P from a hyphal compartment. AMF communities in grassland soils were much more efficient in acquiring ^{33}P and transferred 64% more ^{33}P to plants compared to AMF in cropland soils. Fungicide application best explained hyphal ^{33}P transfer in cropland soils. The use of fungicides and subsequent decline in AMF richness in croplands reduced ^{33}P uptake by 43%. Our results suggest that land-use intensity and fungicide use are major deterrents to the functioning and natural nutrient uptake capacity of AMF in agroecosystems.

Introduction

With the global population growing, we need to find ways to promote crop production while minimizing environmental degradation [1,2]. Understanding and harnessing the natural functions provided by species above- and below-ground in the agricultural landscape is a promising approach to address both goals, thus paving the way to an ecological intensification of agroecosystems [3,4].

Arbuscular mycorrhizal fungi (AMF) inhabit the soils of virtually all terrestrial ecosystems and form symbiotic associations with most plants, including agricultural crops [5,6]. Plants deliver reduced carbon in the form of sugars and lipids to AMF in return for nutrients, especially phosphorus (P) and nitrogen, which AMF acquire through their extensive soil hyphal networks. AMF may supply up to 90% of the host plant's P requirements, especially in nutrient-poor and undisturbed vegetation [5,7]. Promoting the natural potential of AMF for crop P nutrition could therefore circumvent the adverse environmental effects of high P fertilization [8,9], reduce associated economic and environmental production costs, increase P availability in cropping systems with low fertilizer access, while contributing to other services provided by AMF such as soil aggregation [10]. The AMF-symbiosis is thus one key asset to improving P-use efficiency and to the design of sustainable agroecosystems [11–13]. However, whether current cropping systems support AMF functioning remains unclear, fueling the debate about the relevance of AMF for agricultural production [14–17].

The contribution of AMF to plant nutrition are context-dependent, given that AMF communities are shaped by local environmental conditions [18] and that the benefits of AMF to plant growth are cultivar dependent [19–21]. Some modern crop cultivars are less efficiently colonized by AMF and have been shown to only benefit from AMF under severe P limitation [22,23] likely since crop breeding and selection have not prioritized these symbiotic associations [24]. However, other studies indicate that AMF support crop yield and are a key factor to make agroecosystem more sustainable [3,10,13,17]. Apart from this, the abundance and diversity of AMF communities are negatively affected by intensive management practices [25–27], especially tillage, inorganic fertilization, and pesticide use [28]. Several studies indicate that AMF richness promotes plant productivity and plant P uptake [29,30]. Thus, a reduction in AMF richness due to intensive management may reduce the natural nutrient uptake capacity of agricultural soils. Whether management-induced changes in AMF communities subsequently impact their ability to acquire P for growing plants is still poorly understood. Until now, broad scale assessments of AMF functioning across different environmental conditions and land use types have not been performed, contributing to our lack of understanding of the drivers of hyphal P acquisition in intensively and extensively managed plant-soil systems.

To address these knowledge gaps, we collected 210 soils originating from cropland fields and neighboring non-cropped grassland sites across a 3,000 km European north-to-south gradient (ED Fig. 1). *Plantago lanceolata*, well known for its associations with a wide range of AMF and hence ideal for a broad screening of the potential AMF activity across various soil conditions [31], was then grown on these soils in a greenhouse experiment in which we measured the capacity of the associated AMF to mediate the uptake of the radioisotope ^{33}P from a labelled hyphal compartment (Fig. 1). This allowed us to 1) estimate the capacity of native AMF communities in different soils to provide plants with P, and 2) assess the drivers of hyphal P transfer by including the climatic background, biotic and abiotic soil properties, and legacy effects of land use and crop management practices in the analysis. We hypothesized that i) hyphal P transfer is strongly driven by the large gradient in climate and soil characteristics, ii) hyphal P transfer is lower in cropland compared to non-cropped grassland soils, and iii) intensive agricultural practices (pesticide use, tillage, and fertilization) and a reduction in AMF richness negatively impact hyphal P transfer. Ultimately, our research contributes to a better understanding of the main drivers of and constraints to AMF functioning in agroecosystems.

Results

Effect of land use on hyphal ^{33}P transfer and soil properties

The recovery of ^{33}P was 64% higher in pots filled with soils from grassland sites compared to the soils from croplands (p<0.001; Fig. 2). There was a large variation in hyphal ^{33}P transfer in both systems (1.07 – 17.6% in grassland soils; 0.003-14.2% in cropland soils), which was paralleled by significant biotic and abiotic differences between the soils originating from the grassland and cropland systems (Table 1). Especially the differences in microbial biomass C (-41% in cropland soils), AMF richness (-29% in cropland soils) and available soil P (Olsen P, +60% in cropland soils) were striking, and these factors correlated

significantly with hyphal ^{33}P transfer, when considering the whole dataset (Table 1, ED Fig. 2). Among the management factors considered in croplands, the number of fungicide application events showed the strongest correlation with hyphal ^{33}P transfer (Supplementary Table 1).

We assessed whether the observed difference in hyphal ^{33}P transfer in the two land-use systems had implications for plant nutrition. We found that plant growth and total P uptake did not differ between land use systems (Fig. 2 b and c), despite the greater P availability in cropland soils and a strong link between available soil P and total P uptake (R^2=0.404, p<0.001; Supplementary Fig. 1 b). Neither plant growth, total P uptake, nor the plant N:P ratio correlated significantly with the hyphal ^{33}P transfer when considering both cropland and grassland together (Supplementary Table 2). However, while hyphal ^{33}P transfer increased with increasing plant N:P ratios in grassland soils (ED Fig. 3 a), there was a weak negative correlation in cropland soils (ED Fig. 3 b).

Drivers of hyphal ^{33}P transfer in cropland vs. grassland soils

A multi-model inference approach used to assess the relative importance of climate, soil characteristics and management history on AMF functioning revealed that different predictors explained variation in ^{33}P recovery in cropland and non-cropped grassland soils (Fig. 3). In grassland soils, the most prominent and significant predictors were soil pH, followed by available P, aridity, and SOC (Fig. 3 a, Supplementary Table 3 a). These four factors explained most variation in the model selection approach (R^2adj=0.45; Supplementary Table 3 b). The strong correlation with soil pH and available P could also be observed using simple linear regression (Fig. 4).

Relative to grassland soils, less variation in hyphal ^{33}P recovery could be explained in the case of cropland soils (R^2adj.=0.16, Supplementary Table 4 b) despite the larger number of observations and the inclusion of various crop management predictors used in these soils (Table 1, Supplementary Table 1). The most important predictors for hyphal ^{33}P transfer in cropland soils were AMF richness and the number of fungicide application events during one year before sampling the soils from the fields (Figs. 3b and 5, Supplementary Table 4 a). Hyphal ^{33}P transfer in soils without fungicide application was, on average, 2.3 times higher compared to soils that received three fungicide applications. Multiple comparison analysis supported this observation, revealing that ^{33}P recovery rates significantly decreased with an increasing number of application events (Fig. 5 a), paralleling the results from simple linear and quantile regression analysis (Supplementary Fig. 2). Root AMF richness correlated positively with hyphal ^{33}P transfer in cropland soils (Fig. 5 b) showing that more ^{33}P was acquired in soils with high AMF richness. Interestingly, structural equation modelling revealed that also root AMF richness correlated negatively with fungicide application, leading to both direct and indirect effects of fungicide application on hyphal ^{33}P recovery (ED Fig. 4 c and d). On top of that, aridity influenced ^{33}P transfer indirectly though AMF richness, while the soil pH showed a positive direct as well as indirect relationship with ^{33}P transfer through AMF richness.

Discussion

Lower hyphal ³³P transfer in cropland vs. grassland soils

AMF in extensively managed or undisturbed soils are usually more abundant and diverse compared to intensively managed cropland soils that receive substantial amounts of fertilizers and pesticides [26,27,32–36]. However, the functional implications of such differences have not been investigated previously. This study demonstrated that AMF communities from non-cropped grassland sites are generally more active and transfer higher amounts of ³³P to host plants compared to AMF communities from cropland soils. Our observation, stemming from a vast diversity of different soil and climatic characteristics at a broad spatial scale, suggests that current cropping practices impair AMF functioning and that the capacity of AMF to support plant nutrition thus remains underexploited in European croplands.

Various mechanisms could explain the observed differences in AMF functioning between cropland and grassland soils. First, a range of studies showed that management practices associated with intensive land use, including soil tillage, pesticide and fertilizer use, reduce AMF abundance, spore abundance, AMF diversity and alter AMF community composition [37–39]. Our study confirmed that both microbial biomass as well as AMF richness were reduced in intensively managed croplands, and this was linked to a reduced hyphal ³³P transfer (Table 1). This suggests that hyphal P transfer is affected by intensive land-use to a similar extent as soil microbes, which have been shown to be sensitive indicators for land-use change [40–42]. AMF are estimated to contribute to 20-30% of the total soil microbial biomass [24] and it is likely that the positive link between ³³P recovery and microbial biomass is connected to AMF, especially because various studies showed that mycorrhizal hyphal density can correlate strongly with hyphal P transfer [43–45]. Interestingly, hyphal ³³P transfer was positively correlated with AMF richness in cropland soils, but not in grassland soils, perhaps indicating that functional diversity of AMF is more important under disturbed conditions like in cropland soils. Further studies are required to investigate this in more detail. It also should be noted that although earlier studies demonstrated that AMF are the main actors in the transfer of P from hyphal compartments to plants, the possibility that other microorganisms (*e.g.*, non-mycorrhizal hyphae) might have contributed to the transfer of P to the root zone cannot be excluded. Bacteria can also facilitate or suppress AMF activity in the soil [44]. Recent studies indicate that AMF fungal hyphae are colonized by specific bacterial communities [46], which in turn influence nutrient uptake, particularly from organic sources [47]. Finally, P transfer via hyphal networks depends on the distinct functional traits, activity, and foraging strategies of individual strains of AMF [48–51] and such aspects need more attention in future work. While we could not make these connections in this present study, further studies should investigate whether specific microbial groups that are affected by intensive management, also influence the ability of AMF to acquire P.

AMF compensate for lower P availability in grassland soils

In addition to direct effects, management can be indirectly linked with AMF-symbiosis through alterations of soil properties, such as available soil P [27]. High levels of available soil P, often accompanied by low soil N:P ratios, were shown to inhibit AMF root colonization and decrease the AMF's relative contribution to plant P nutrition [52–54]. In accordance

with these previous findings, we observed that AMF hyphal ^{33}P transfer was negatively associated with available soil P levels (Table 1, ED Fig. 2 c), which were on average 60% higher in the cropland soils. In turn, regarding implications for plant nutrition and growth, our findings indicate that AMF mediated P uptake compensated for the lower available P levels in the grassland soils, allowing for a similar average total P uptake and shoot biomass. This assumption is further supported by a negative correlation between the hyphal ^{33}P transfer and the soil available P in grassland soils, and a positive correlation with the plant N:P ratio, respectively (ED Fig. 3 a). This implies that when plant P demand in grassland is high and plant productivity is limited by P (*i.e.*, plants with N:P ratios above 16 [55]), AMF supply additional P to the plant. However, this trend could not be confirmed in the cropland soils where neither a link between available soil P, nor the plant N:P ratio, and hyphal P transfer was found, possibly indicating a dysfunctional symbiosis in croplands. Verbruggen *et al.* (2015) argued that the selective loss of AMF communities connected to soils with high N:P ratios might leave cropland fields with AMF of reduced symbiotic quality [34]. While we agree that this could be the case for a multitude of fields with high soil N:P ratios in this study, the relatively large range of soil N:P ratios in both land use systems suggests that this is not the only mechanisms at play. The observed inability of putatively P-limited plants to acquire P through AMF hyphal activity in cropland soils provides further evidence that AMF are heavily affected by crop management (e.g., fungicide application), inhibiting their potential contribution to plant P nutrition in current cropping systems.

Soil pH and P drive hyphal ^{33}P transfer in grassland soils

Using a multi-model inference approach, we were able to identify the main drivers of AMF hyphal ^{33}P transfer to growing plants. In grassland soils, we found that much of the observed variation in hyphal ^{33}P transfer could be explained by soil pH, available P, SOC content and climatic factors (*e.g.*, aridity). These results are in line with other studies demonstrating that these factors influence AMF abundance, colonization, and activity [44,52,56–58].

In addition to the above discussed influence of available soil P, our results suggest a suppression of hyphal ^{33}P transfer with decreasing pH, emphasizing the crucial role of soil pH for AMF activity, abundance, community structure as well as the occurrence of AMF host plants [32,44,59–62]. Van Aarle *et al.* (2002)[56] found reduced growth and activity of the AMF extraradical mycelium in low pH substrates, arguing that AMF are directly stressed by acidic environments. However, it is possible that indirect effects of pH on the activity of the AMF extraradical mycelium through interactions with other microbiota might be an even more important mechanism behind the suppressiveness of low pH soils[44].

Apart from the major importance of soil abiotic factors, our results indicate that increasing aridity decreased hyphal ^{33}P transfer in grassland systems. Although we did not manipulate water availability directly and observed only the legacy effects of aridity, our observation parallels various studies that determined direct negative effects of drought on AMF abundance and extraradical hyphae [57,58]. Additionally, plant communities, C inputs and SOC might be influenced by aridity, resulting in indirect effects on AMF, possibly due to reduced net photosynthesis and energy supply to AMF under water-limited conditions [63], corroborating the positive relationship between ^{33}P transfer and SOC observed in this

present study. Ultimately, these results suggest that the goals of promoting C sequestration and improving plant P nutrition through AMF go hand in hand, at least in non-cropped systems where SOC is generally higher (Table 1).

Fungicide application reduces the ability of AMF to acquire P

While a considerable amount of variation in hyphal ^{33}P transfer in grassland soils was explained by soil pH and P availability, we found that the number of fungicide application events was the most important predictor of hyphal ^{33}P transfer in cropland soil (Fig. 3). Interestingly, this reduced hyphal ^{33}P transfer in cropland soils was paralleled and partially mediated by a reduced AMF richness (ED Fig. 4 d) indicating that fungicides indirectly reduce hyphal mediated P uptake by reducing AMF richness. Prior work suggests that AMF richness can promote P uptake [29], providing further evidence that a reduction of AMF richness by fungicides may have implications for plant P nutrition. A recent study found that the abundance of AMF is negatively linked to the amount of pesticide residues in agricultural soils, and certain pesticide residues could be detected even decades after their last application [64]. This indicates that adverse effects of fungicides on AMF might be long-lasting, corresponding to observations by Pánková *et al.* (2018)[65], who showed that AMF infection rates of plants were reduced up to five years after the application of fungicides in a grassland site. These earlier studies focused on AMF abundance, whereas our results add a functional component to this debate and indicate that fungicide use in real agricultural contexts suppress both AMF diversity and functioning.

Given the great variety of amounts and compounds of the applied fungicide products (Supplementary Table 5), it is remarkable that the number of fungicide application events used as rough indicator in this study could capture the adverse effects posed by fungicide use on AMF. Although fungicides are applied to combat fungal diseases such as mildew and rusts, they often have non-target effects on other fungi, including beneficial AMF. Detrimental effects of various fungicides have repeatedly been reported for AMF biomass, spore density, root colonization, and alkaline phosphatase activity in internal and external hyphae [66–70]. The negative effects of some fungicides on AMF have also been previously used to assess the importance of AMF for plant community structure and diversity in grasslands (*e.g.*, [71,72]). However, the close links between fungicide application rate and AMF functioning in a wide range of cropland soils observed here have not been reported before. It further indicates that the common use of fungicides hampers the natural ability of soil organisms to provide crops with nutrients and supports the findings of Sallach *et al.* (2021) who showed that also fungicides applied unintentionally through wastewater or biosolids can decrease the ability of AMF to transfer P to plants [39]. Future studies under controlled conditions in the field now need to specifically test to which extent fungicides suppress the ability of AMF to support crop growth and test whether such effects are persistent. Our results also call for reconsidering the design of agricultural systems to be able to make full use of the potential of AMF-symbiosis for plant nutrition. For example, applying agroecological techniques, such as crop diversification, can be a promising way to reduce disease pressure [4] and hence the need to use pesticides, while at the same time promoting AMF richness [73] which could indirectly support plant P uptake (Fig 5 B) as well as to other benefits provided by AMF. In addition to the establishment of such

AMF-promoting practices, the breeding and use of AMF responsive crops, an aspect which hasn't been directly investigated in this study, is a way to promote AMF-supported crop production that requires further consideration in future research.

In conclusion, despite the wide range of different environmental conditions along the surveyed European gradient, the results show that the capacity of AMF to support plant P nutrition is impaired in croplands compared to non-cropped grasslands, particularly by the use of fungicides. Thus, we emphasize that there is a need to reconsider the design of agricultural systems to fully exploit the natural potential of AMF-symbiosis for a sustainable crop production.

Methods

Field sites

In spring 2017 we sampled soils from 150 croplands and 60 non-cropped grassland sites across a North-South gradient in Europe covering Sweden (n=34), Germany (n=48), Switzerland (n=57), France (n=39), and Spain (n=32) (ED Fig. 1). To minimize variation in AMF caused by different crop types, we selected cereal fields planted with wheat (*Triticum sp.*, n=119), or closely related cereals like barley (*Hordeum vulgare*, n=25) and oat (*Avena sativa*, n=6), when wheat fields were not available. Furthermore, we exclusively sampled plots where conventional tillage practices had been performed. Although 26 croplands (17%) in Switzerland and Southern Germany were organically managed, they did not statistically differ from their neighboring conventional fields in terms of hyphal ^{33}P transfer to plants (Supplementary Fig. 3), and therefore were kept in the dataset. The non-cropped grassland sites were located in the vicinity of the croplands to cover similar soil characteristics, and served as benchmark for AMF functioning, presumably providing less disturbed AMF communities, which were also supported by a greater plant diversity as compared to the croplands. These sites comprised extensively managed grasslands and marginal land (field strips) neighboring the croplands and were characterized by a permanent, predominantly herbaceous plant cover that was not part of a crop rotation. Most of these sites were unfertilized and occasionally mowed, although exact information on fertilization, grazing and mowing was not available for all of the sites. Following the classification by the Eurostat Land Use / Cover Area Frame Survey (LUCAS) [74], we refer to these plots as grassland sites.

Environmental data and location

To characterize the variation in climate along the sampled gradient, information on mean annual temperature (MAT) and mean annual precipitation (MAP) was extracted from the WorldClim Global Climate Data [75]. Aridity information was derived from the CGIAR-CSI database, where it is expressed as a function of MAP over the mean annual potential evapotranspiration [76]. In line with recent studies [77,78], we subtracted the aridity index from 1, to define aridity in our analyses such that higher aridity values indicate drier conditions. Additionally, the location of the fields along the gradients was described by the geographic distance from the S-W-most sampling site using the haversine formula [79].

Management practices

To determine the legacy effects of crop management on hyphal activity in the croplands, farmers and field managers provided information on management practices including fertilizer and pesticide use, tillage intensity (all during 2016, one year before sampling), as well as the crop rotational diversity and the duration of crop cover (during ten years before sampling) (Table 1). Fertilization intensity was assessed using the total amount of mineral N (ammonium and nitrate) applied through organic and/or mineral fertilization. Organic fertilization was additionally included as a binary variable in the analyses. The use of fungicides, herbicides and insecticides was summarized with the number of their respective application events. For fungicides, we further took into consideration the total amount and number of different active compounds added per hectare. However, these parameters were strongly linked to each other. Therefore, we focused our analysis on the number of application events, since they had the strongest correlation with our output variable of interest (Supplementary Table 5). Tillage intensity was estimated by averaging the number and maximum depth of tillage events (after normalization). For the crop rotations (see Supplementary Methods for more details), we calculated the proportion of time with plant cover and the crop diversity at each site according to a Shannon diversity index [80].

Soil sampling

To reduce variation between sites related to crop growth stage, all soils were collected during wheat flowering at each site (ranging from May in Spain to August in Sweden). At each site, eight soil samples were taken in a circular pattern within a 10 m radius using a five cm diameter auger and to a depth of 20 cm. Three of the soil cores were kept intact and stored at 4 °C before preparing them for the compartment experiment. The other soil cores were homogenized and sieved to 2-mm. A portion of this soil was air-dried for further processing of soil physical and chemical analyses. Soil texture, calcium carbonate ($CaCO_3$), pH, soil organic C (SOC), total nitrogen (N) and total phosphorus (P) were measured on the dried samples. Available phosphorus (Olsen P), and the microbial biomass C were measured on fresh soil samples within a few weeks after sampling. All above mentioned soil properties were measured following the Swiss standard protocols [81].

Soil microbial analyses

To assess the soil microbial diversity, DNA was extracted from 250 mg of each soil sample (stored at -19 °C) using the DNeasy PowerSoil-htp 96 well DNA isolation kit (Qiagen, France). Microbial diversity was further analyzed as described in Garland *et al.* (2021)[80] (also shown in the Supplementary Methods). Briefly, amplicons of bacterial and archaeal 16S rRNA genes were generated in two steps following Berry *et al.* (2011)[82] in two separate sequence data sets, one for each domain. The fungal ITS2 region was amplified using the PacBio SMRT Sequencing platform (Pacific Biosciences, CA) with the primers ITS1f (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) targeting the entire ITS region (~630 bp) [83,84]. Cercozoa diversity was estimated with a two-step PCR to amplify a fragment (c. 350 bp) of the V4 region of the 18S rRNA gene using the primers sets designed by Fiore-Donne *et al.* (2018)[85] for the specific amplification of cercozoa. Finally, the diversity of soil bacterial, fungal, archaeal, and cercozoan communities was

assessed using the Shannon-Weaver index of diversity, and richness was calculated as the number of observed operational taxonomic units (OTU).

AMF compartment system

To assess AMF hyphal ^{33}P transfer rates and hyphal activity in the collected field soils, we conducted an experiment starting in March 2018 using a compartment setup (Fig. 1) following, amongst others, Schweiger *et al.* (1999)[45] and Svenningsen *et al.* (2018)[44]. We used the radioisotope ^{33}P to trace P transport by fungal hyphae to *Plantago lanceolata* plants. To this end, the plant zone (compartment a; Fig. 1) of the compartment system was separated with a mesh (pore size 40 μm), hindering the plant roots to penetrate the hyphal zone (compartments b and c; Fig. 1). The buffer zone (compartment b; Fig. 1) had the purpose to minimize possible diffusion of ^{33}P injected into compartment c (Fig. 1) from entering the plant zone [45].

Plantago lanceolata was selected as the model plant in this experiment as it has been shown to be very AMF sensitive and unselective in terms of AMF species associations [31]. In this present study, a total of 252 different AMF taxa was found to associate with P. lanceolata roots, belonging to 11 known families (See 'Root AMF Microbiome' methods below, ED Fig. 5). Also, very different AMF communities were found to colonize Plantago roots in soils collected from different locations. This suggests that P. lanceolata could be colonized by a great diversity of AMF, both in grasslands and croplands (with observed AMF richness values up to 40 and 47, respectively). Forming a symbiotic relationship with a wide range of AMF was an important prerequisite to capture as many of the changes induced by management as possible and to estimate the potential AMF activity across this vast diversity of soils. However, it needs to be noted that AMF-mediated P transfer might depend strongly on the plant species considered and is likely to be lower in crop cultivars that depend less strongly on AMF for their nutrition [22,23].

The different field soils were filled into the compartments a and b to a total volume of 850 cm^3. To do so, three of the collected soil cores, which were kept intact and stored at 4 °C, were homogenized by gently removing stones and residues larger than 8 mm. A subsample was taken to assess the water content, available N and the exact weight of the soil at the start of the experiment. Furthermore, seven negative control pots were implemented using sterilized soils (which had been exposed to X-rays at a dose of 40.7 kGy for at least 17 hours at *Steris*, Däniken, Switzerland) originating from four croplands and three grassland sites representing a range of soil properties (pH 5.97 – 8.03, Olsen P 1.8 – 118 mg g^{-1}, Sand 18.34 – 33.53%), to verify that no or little ^{33}P would be found in plants when no AMF are present. Very small amounts of label (a median percentage of 0.24 as compared to the unsterilized pots) were detected in the plants grown in sterilized control pots (Fig. 2 a) demonstrating that fungal hyphae present in the field soil, rather than fine roots, mass flow, or microbial propagules in the greenhouse air, were responsible for the recorded ^{33}P uptake by plants. Additionally, assessments of AMF root colonization and AMF abundance in the hyphal compartment c (Fig. 1) in a subset of the soils (n=36; methods outlined in the Supplementary Methods section) showed that roots in pots filled with unsterile field soils were strongly colonized by AMF and that AMF accessed the hyphal compartment c in the

respective pots. In contrast, the sterilized control pots had little AMF root colonization and AMF abundance in the hyphal compartments was negligible (Supplementary Fig. 4 a and c).

The pots were watered to 60% of the soils' water-holding capacity and the soil volume was adjusted after two days if necessary. The hyphal compartment c (Fig. 1), into which the ^{33}P was injected later, was filled with a standardized, X-ray sterilized soil (58 g dry mass) from an extensively managed grassland soil at Agroscope Reckenholz, Zurich, Switzerland. Using a standardized soil in compartment c allowed for a similar sorption of ^{33}P at the injection spot, enabling us to focus our analyses on the hyphal activity solely, rather than the soils' P sorption capacity, which is highly variable across soils [86].

After three days of pre-incubation, five sterilized and pre-germinated seeds of *Plantago lanceolata* (cultivar *TONIC, 2012, Agricom NZ)* were planted in compartment a of each pot. To better distribute the random variation potentially caused by the genotypically non-identical seeds [21,87], five seeds were planted per pot, and seedlings were thinned to three seedlings of similar growth after one week. The pots were arranged randomly in the greenhouse. In the following 12 weeks of growth, the plants were watered three times a week to maintain a comparable water level ranging between 60 and 70% of the individual soil's water holding capacity. Every second week the pots were newly randomized. The temperature adjustments of the greenhouse were between 16 to 18 °C at night and between 19 and 21 °C during daytime, at a humidity around 70% water vapor saturation. Daytime was set at 16 hours with 30 klx lighting.

Plant growth, ^{33}P-injection, and analyses

After 10 weeks of plant growth, 600 µl of a carrier-free ^{33}PO$_4$$^{3-}$-solution (2333 kBq ml^{-1}), resulting in a total of 1399.8 kBq, were injected at three different locations within compartment c at a depth of 3-4 cm using a 200 µl pipette. The experiment was terminated with the harvest of Plantago shoots 12 days after ^{33}P injection, and 12 weeks after planting. The shoots were cut above the soil surface and placed in the oven at 60 °C for 48 hours. After assessment of the dry shoot biomass, the samples were milled and used for determination of P and N contained in shoots. Total plant N was measured with an elemental analyzer (Vario Pyro Cube, Elementar GmbH, Hanau, Germany). Incinerated samples were microwave-digested with nitric acid [88]. The P concentration in the extract was then determined colorimetrically using malachite green [89] and the activity of ^{33}P with liquid scintillation counting for 10 min per sample (TRI-CARB 2500 TR, liquid scintillation analyzer, Packard Instruments, Meriden, CT), using 5 ml scintillant (Ultima Gold™ AB, PerkinElmer, USA) for about 0.05 g of shoot sample. Due to the large dataset, samples were not replicated. The measured disintegrations per minute were corrected for the background activity, the counting efficiency to account for chemical and color quenching and were back-calculated to the time point of injection to correct for radioactive decay.

Finally, the relative amount of ^{33}P, which was recovered in the shoot material after transfer via AMF hyphae from the initially injected pool, *i.e.*, the recovery rate, was calculated using equation (1).

$$\text{Recovery rate}(\%) = \frac{r(Bq) * 100}{R(Bq)} \qquad (1)$$

Where r is the decay-corrected activity of ^{33}P in each sample (Bq g^{-1} shoots) multiplied by the total shoot weight per pot (g), and R refers to the total injected radioactivity (Bq). Following Frossard *et al.* (2011)[90], the recovery rate was then used as an estimate for the capacity of AMF to transfer P to growing plants, which we refer to as "hyphal ^{33}P transfer" in this study. However, it is important to note that the ^{33}P recovery rate is an indicator for but not an exact measurement of the relative contribution of AMF to plant P nutrition. This is because hyphae were able to take up non-radioactive P also in the compartments a and b, while ^{33}P could only be added to the hyphal compartment c.

Root AMF microbiome

After the harvesting of Plantago shoots, the roots were gently separated from the surrounding soil and thoroughly rinsed with deionized water to remove any remaining rhizosphere soil. Subsamples were taken from various parts of the root structure and stored at -20°C. Details on DNA extraction, sequencing, and characterization of the root AMF microbiome are explained in detail in the Supplementary Methods section. Briefly, DNA was extracted from the root samples, and an amplicon sequencing library was generated using the AMF-specific PCR primers AMV4.5NF and AMGDR [91]. The MiSeq library was prepared and sequenced in collaboration with the Genetic Diversity Center, ETH Zurich.

Sequences generated in this study will be deposited at the European Nucleotide Archive and be accessible at the time of publication. Paired-end reads were processed and denoised into amplicon sequence variants (ASVs) using the DADA2 plugin [92] implemented in QIIME2 [93]. The ASV reference sequences were clustered to the virtual taxa (VT) sequences of the AMF-specific taxonomy database MaarjAM [94](status June 2019) at 97% sequence similarity with the VSEARCH plug-in [95] in QIIME2. Sequences not clustering to a VT in the MaarjAM database were clustered de-novo into OTUs at 97% sequence similarity and taxonomically assigned with the SILVA database (v128)[96] to detect other AMF taxa not present in the MaarjAM database. The non-VT OTU taxonomy file and count table were then filtered to remove any OTU sequences not belonging to known AMF taxa. The count table and taxonomy assignments of the VT were combined with those of the AMF-OTUs identified by SILVA in order to produce the final count table and corresponding taxonomy assignments of the profiled AMF community.

For comparisons of AMF diversity within samples (alpha diversity), the OTU table was rarefied to 1000 sequences/sample, which was sufficient to capture the AMF richness of most of the samples (Supplementary Fig. 5). Eight samples were removed from subsequent analyses due to sequence counts less than 1000. The alpha diversity measures of observed richness and Shannon Diversity were calculated on the rarefied counts using the R package *phyloseq* [97].

Statistical analyses

Statistical analyses were performed using the software *R*, version 3.6.0 [98]. Individual missing values in the explanatory variables (1.04% of all investigated predictor values), except for AMF richness and diversity (as these were also used as dependent variables in some models), were replaced with the median value of the respective land use type and country. Consequently, when the AMF data was included in models, the number of observations changed from 150 to 146 in the cropland soils, and from 60 to 58 in the grassland soils. For all correlations (*i.e.*, between the different predictors and output variables) a Spearman rank correlation was applied. To assess the most important drivers of hyphal activity within the two land use systems, we performed a three-step model selection approach. First, we selected a wide range of available environmental and management data, under the condition that there is no strong co-correlation between the distinct variables. To do so, we scanned the correlations between all variables (Supplementary Tables 6 & 7) and reduced variables that were strongly correlated (*i.e.,* Spearman rank correlation coefficient $\rho > 0.8$). This approach resulted in a pool of 15 predictors used to explain variation in the grassland soils and 25 for the cropland soils. To better describe variation in the cropland soils, the latter also included nine predictors describing management practices (tillage, fertilization, pesticide use, crop diversity, crop cover). The choice of final variables is indicated in the Supplementary Tables 6 and 7, and all used predictors with corresponding units are shown in Table 1 and Supplementary Table 1. The second step of the model selection involved a multi-model inference approach using the R package *glmulti* (version 1.0.8)[99], resulting in a high number of possible linear models, ranked by the Akaine Information criterion (AICc). Using the best model, we explored whether model residuals were normally distributed and applied transformations to the data when needed. Logarithmic transformations were applied to the soil N:P ratio, Nmin:Pmin ratio, and plant N:P ratio, due to their strong non-normal distribution. Moreover, the hyphal ^{33}P transfer rates were log-transformed for analyses focusing on the grassland systems. In the last step, the best models that fell within an AIC range of 2 were averaged to obtain a conservative estimate and relative importance of the most important predictors following Cade (2015)[100] using the *MuMin* package (version 1.43.17)[101]. We used AIC values to compute the Akaike weight (*wi*) of each of the selected models, and *wi* was used to compute a weighted-average model[102]. The relative importance of each predictor was estimated taking all the models into consideration as the sum of the weights/probabilities for the models in which the variable appears.

Structural equation modeling (SEM) was performed using the R package *lavaan* [103] to test the validity of the multi-model inference approach and to investigate direct relationships between the identified predictors (Olsen-P, pH, Aridity, SOC; and in the cropland soils fungicide application) and hyphal ^{33}P transfer as well as indirect effects through changes in AMF richness. Initial SEMs comprised the complete set of possible correlation paths with hyphal ^{33}P transfer. A stepwise removal of non-significant correlation paths was performed until a maximum of model-fit parameters (Chi-squared) was reached.

The relationships between the most important predictors and the hyphal ^{33}P transfer were examined. Variables were transformed, if necessary, to reach a normal distribution of

model error and residuals. Furthermore, we compared linear, polynomial (2nd order) and logarithmic functions and, eventually, showed the best-fitting functions (highest R^2). For pair-wise comparisons between grassland and cropland soils, Wilcoxon rank test was used. For comparisons of more than two groups, a Kruskal-Wallis test followed by Dunn's test was applied to identify which groups are different.
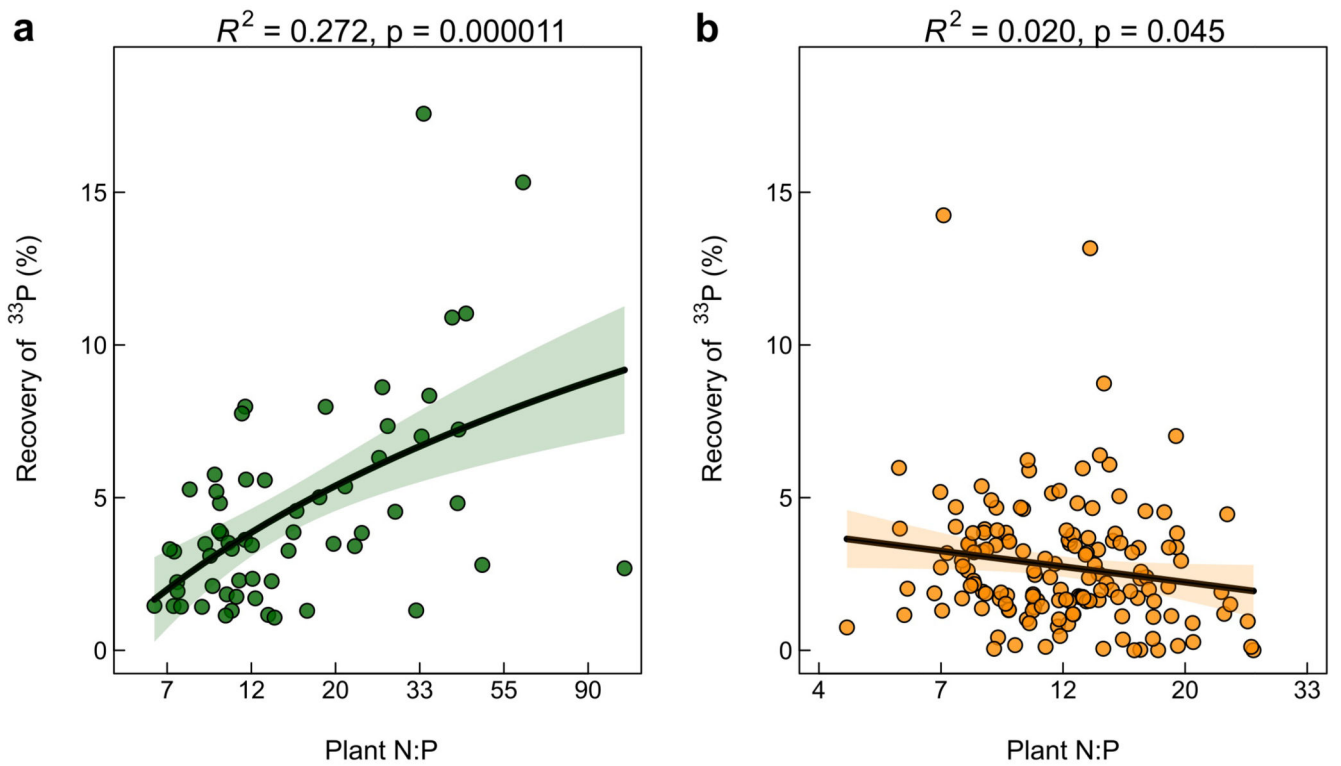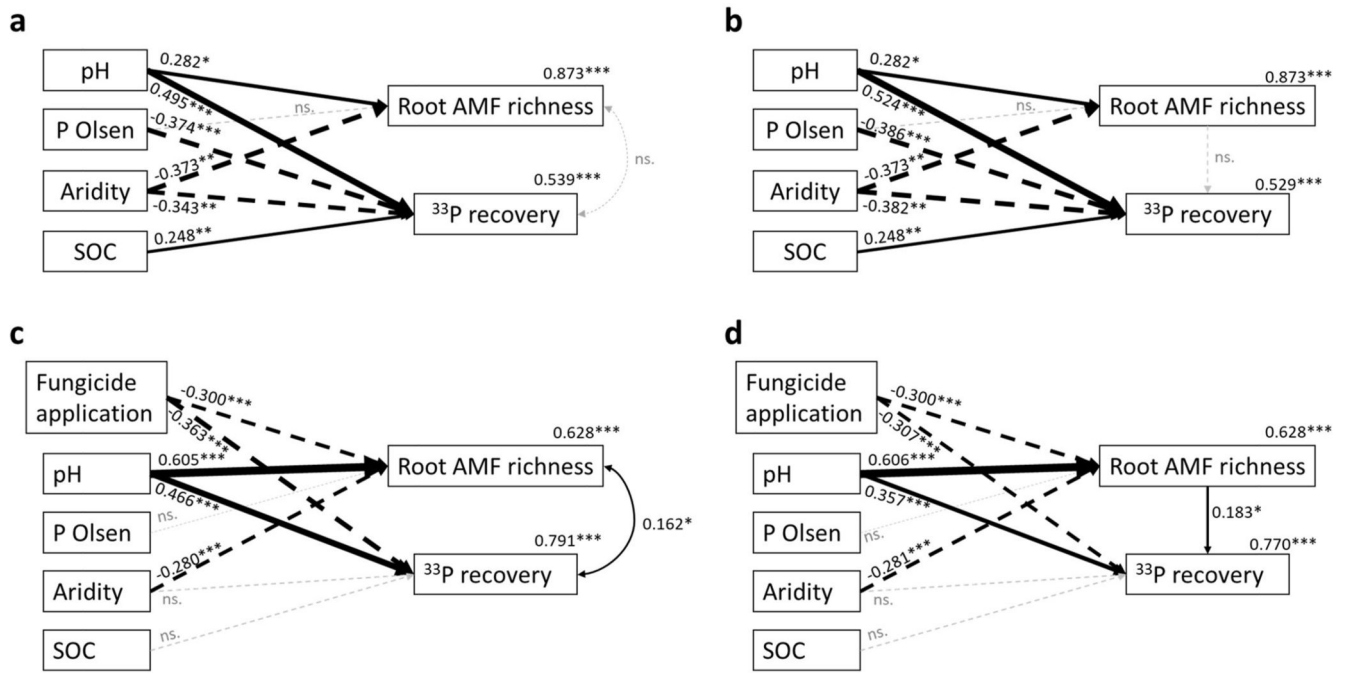
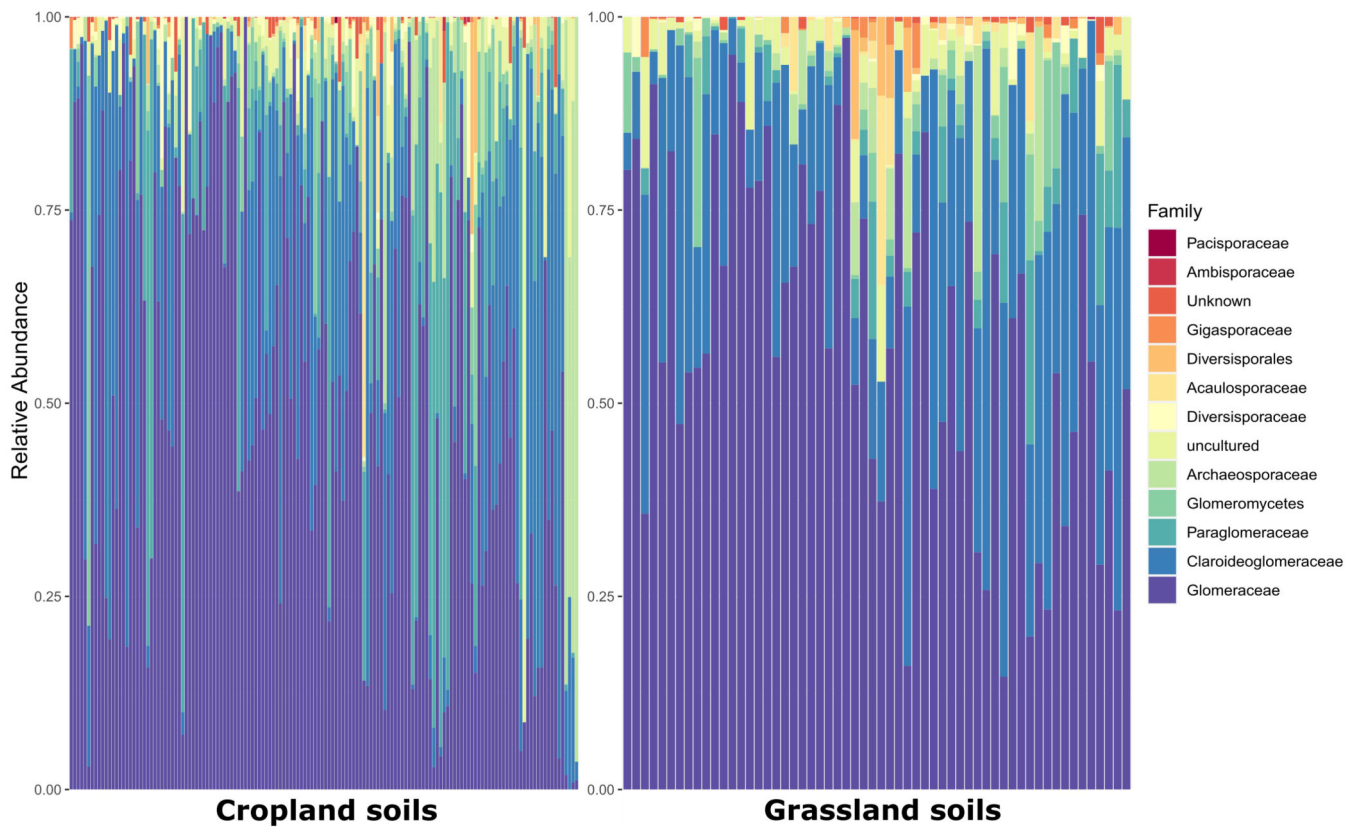## Extended Data
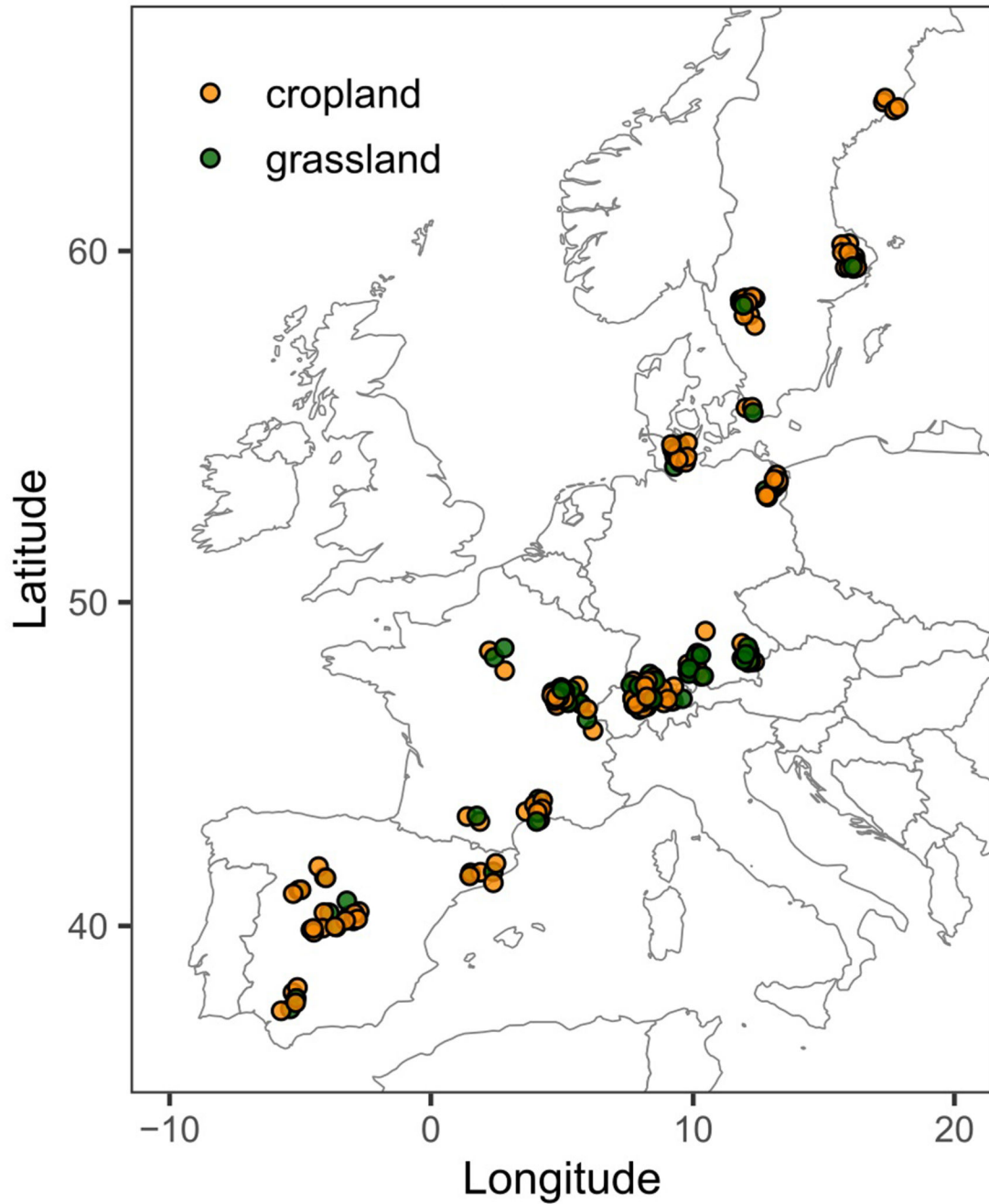
Extended Data Figure 1.
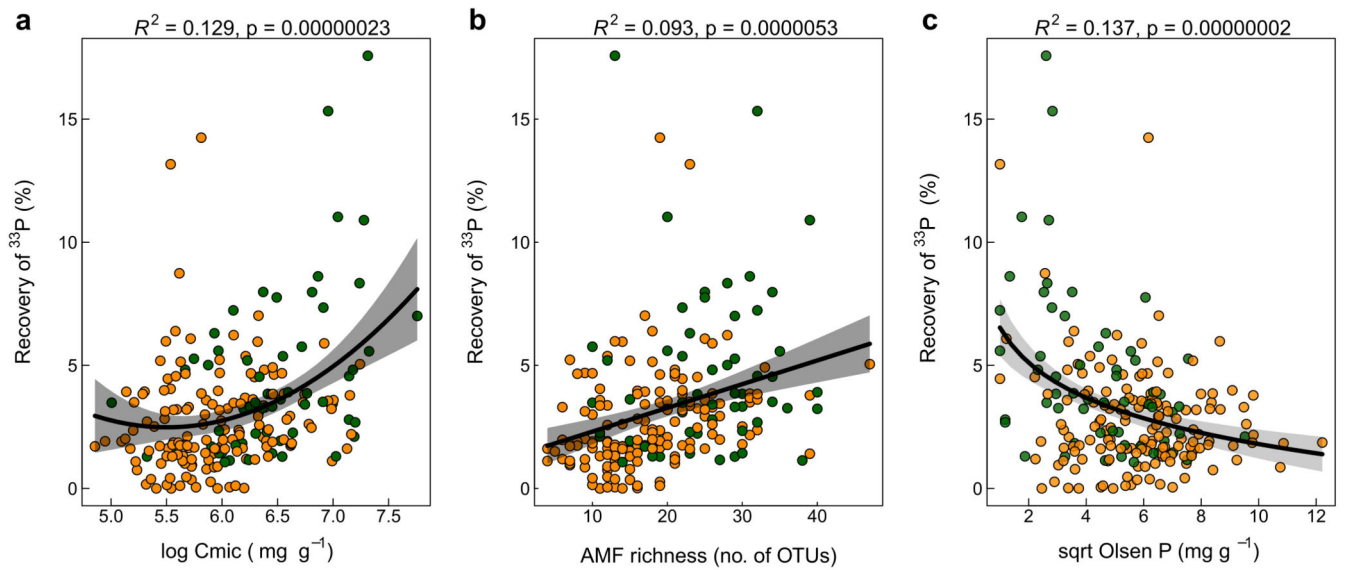
Extended Data Figure 2.

Extended Data Figure 3.

Extended Data Figure 4.

Extended Data Figure 5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Data availability

The data that support the findings of this study are available here: 10.6084/m9.figshare.15134328.

Code availability

The code used to analyze the data is available here: 10.6084/m9.figshare.15134670.

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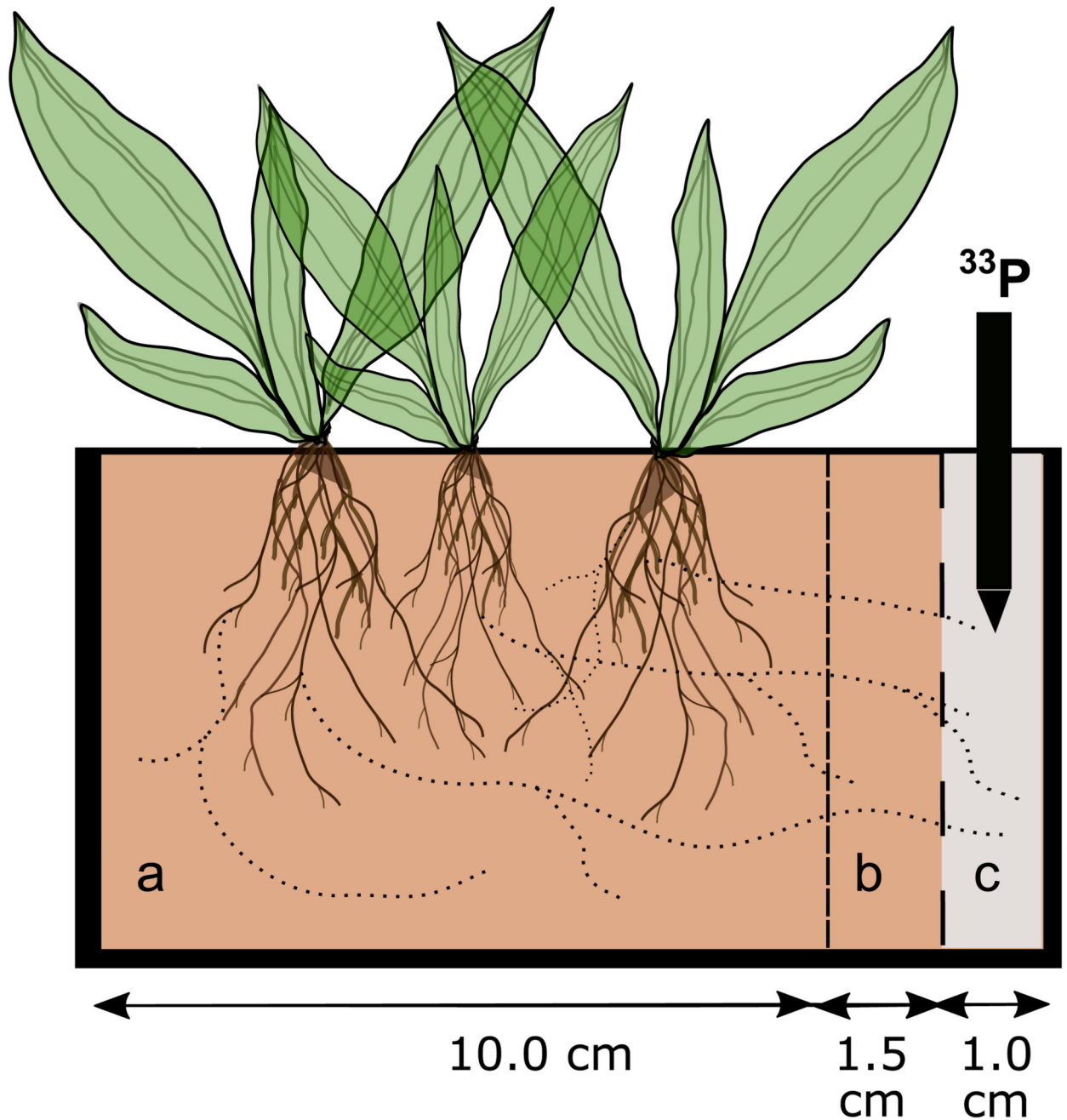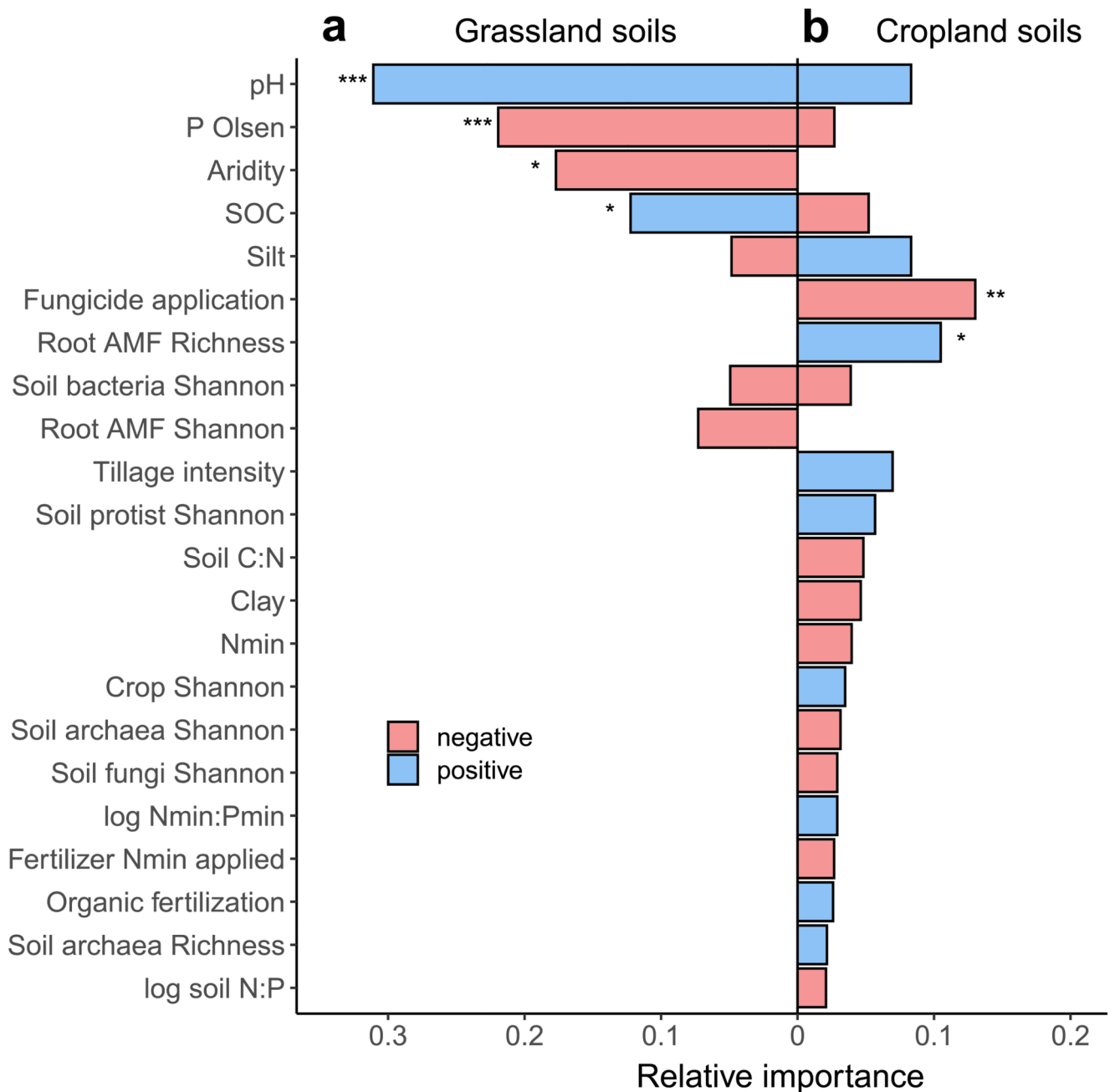
Figure 1.
Experimental set-up using *Plantago lanceolata* as model plant in pots (l = 12.5 cm, w = 8 cm, h = 8.5 cm) containing three compartments. The plant zone (compartment a) and the buffer zone (compartment b) were filled with the collected field soils. The compartment c contained a standardized, sterilized soil which was injected with the tracer ^{33}P. Compartment a was separated from b using a 40 μm mesh (narrow dashed line), restricting root penetration. The mash barrier between b and c compartments had a pore size of 500 μm (wide dashed line).
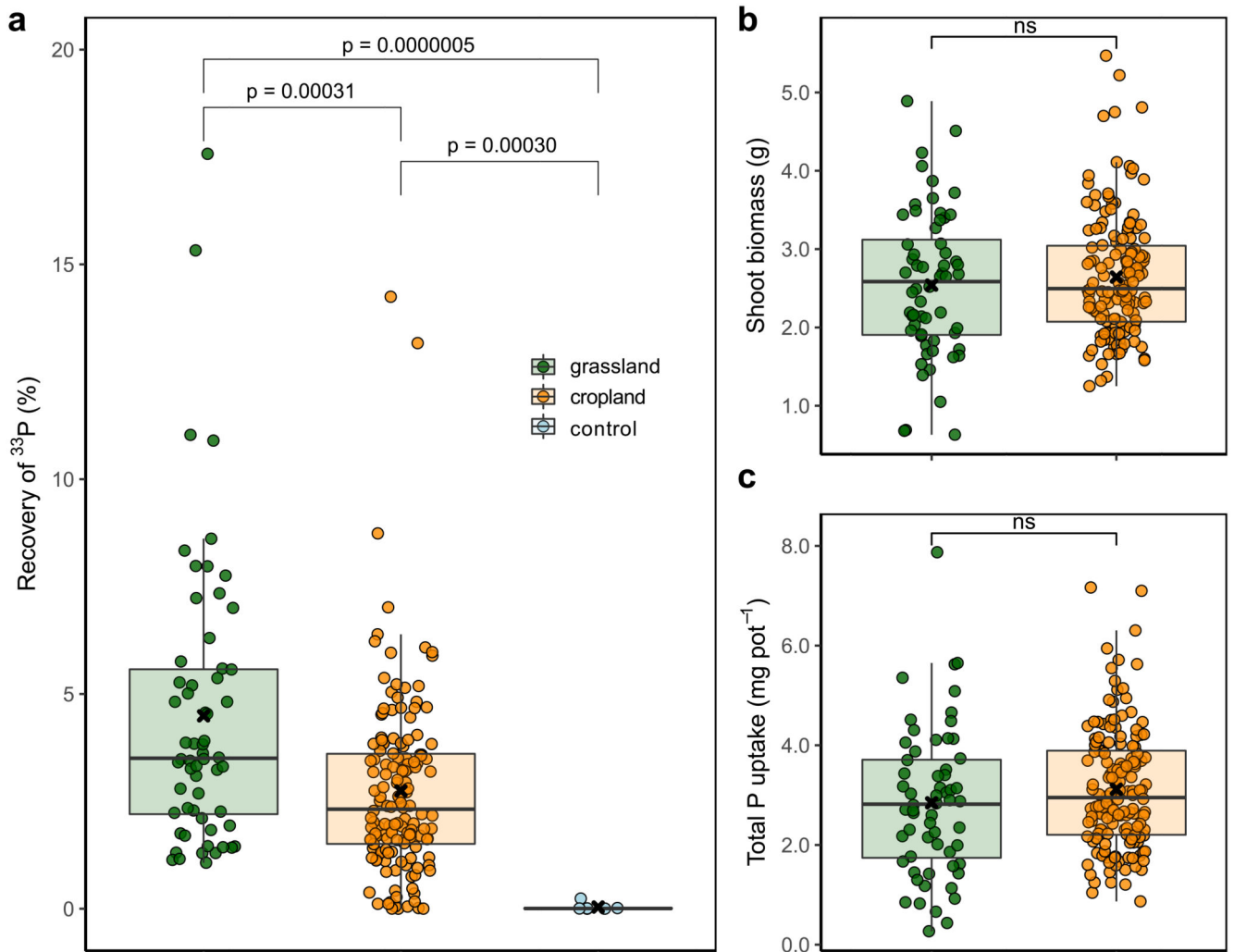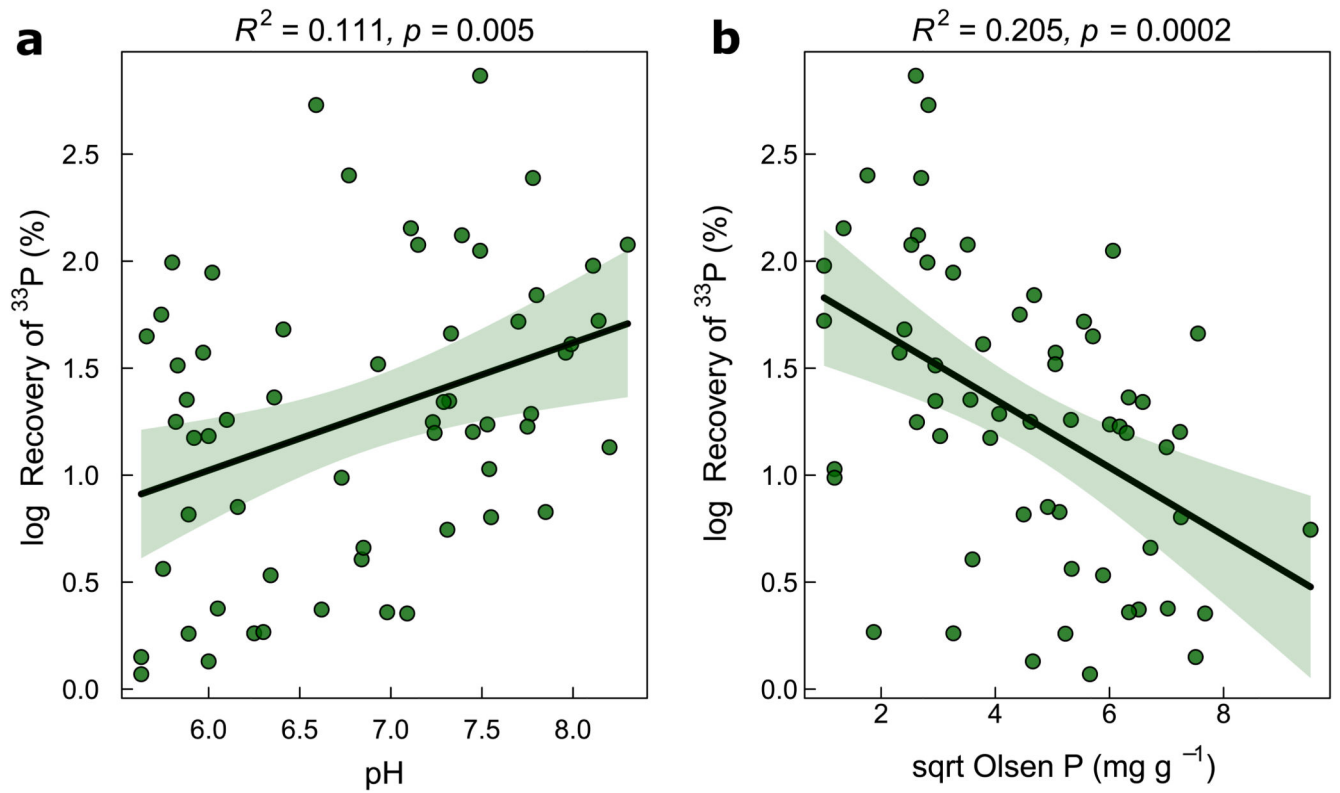
Figure 2.
Recovery of ^{33}P in the shoot material of Plantago lanceolata plants grown in grassland (green), cropland (orange) and sterilized control soils (blue) (**a**). The low ^{33}P recovery in sterilized control soils confirm that ^{33}P recovery corresponds to hyphal activity in the field soils. Shoot biomass (**b**) and total P uptake per pot (**c**) in the grassland vs. cropland soils. Boxes mark the interquartile range, vertical lines the whiskers, bold horizontal lines the median and "x" the mean values. Bonferroni corrected p-values <0.05 (based on two-sided Wilcoxon rank test) indicate significant differences between land use systems, "ns" means no significant difference.

Figure 3.

Relative importance of predictors for hyphal ^{33}P transfer, measured as ^{33}P recovery in plant shoots based on a multi-model inference analysis, in grassland (**a**, n=58) versus cropland soils (**b**, n=146). Note that the crop management predictors were used only in the cropland model. Predictors were ordered according to their total importance (*i.e.*, sum of both land use types). Negative and positive correlations are shown in red and blue, respectively. Asterisks indicate a significant correlation at p<0.001 (***), p<0.01 (**) and p<0.05 (*) based on the averaged model coefficients. Model coefficients, standard errors, z-values and exact p-values are reported in Supplementary Tables 6 a and 7 a.

Figure 4.
Correlation of ^{33}P recovery with soil pH (**a**), and available soil P (**b**) in the grassland sites (n=60). The green error bands mark the 95% confidence interval of the two-sided OLS regression models. $F_{1,58}$ = 8.375 (**a**) and $F_{1,58}$ = 16.21 (**b**). R^2 corresponds to the adjusted R^2 value.
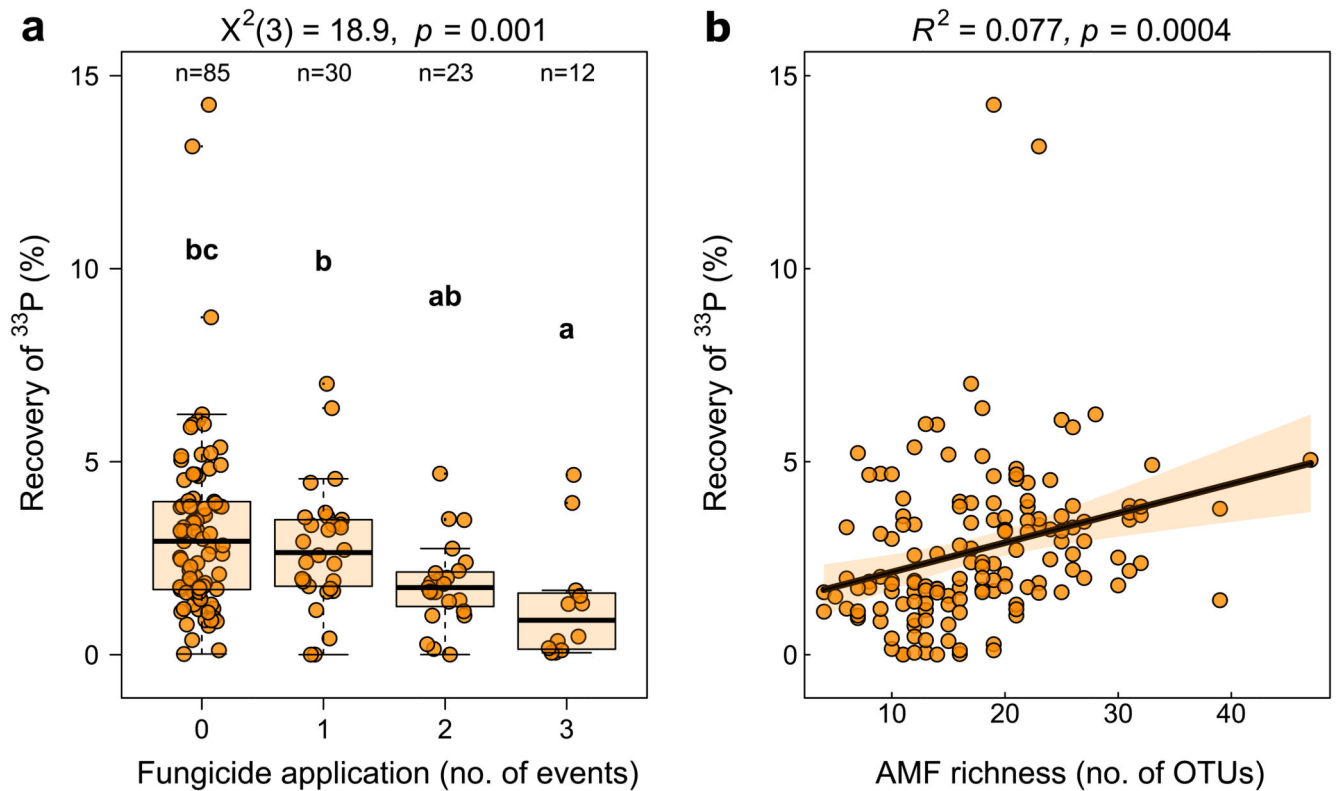
Figure 5.
Effects of an increasing number of fungicide application events in cropland soils on ^{33}P recovery (**a**). Significant differences between the number of applications are indicated with different letters examined using a Kruskal-Wallis rank test where X^2 represents the model fit with the degrees of freedom in brackets (n=150). Boxes mark the interquartile range, vertical lines the whiskers, bold horizontal linesthe median and "x" the mean values. The relationship of ^{33}P recovery and AMF richness in P. lanceolata roots (**b**) in the cropland soils (n=146), estimated using two-sided OLS regression ($F_{1,144} = 13.02$). The orange error band marks the 95% confidence interval. R^2 corresponds to the adjusted R^2 value.

Table 1

Mean values of climatic and edaphic factors (± standard error) in soils from croplands and grassland sites used in this experiment (n = 210). Differences between land use types were assessed using Wilcoxon's rank test. The last column shows the Spearman rank correlation coefficient of all variables with the ^{33}P recovery with p-values in brackets.

	Unit	Mean (± SEM)		p-value (Wilcoxon's rank test)	Correlation with ^{33}P recovery
		Grassland	Cropland		
Aridity	unitless	0.09 (±0.05)	0.11 (±0.03)	0.670	-0.14 (0.053)
Annual temperature	°C	9.74 (±0.36)	9.19 (±0.25)	0.180	0.03 (0.685)
Clay	%	27.3 (±1.48)	28.2 (±0.98)	0.740	0.12 (0.090)
Silt	%	33.6 (±1.67)	39.7 (±0.95)	0.003 [**]	0.00 (0.983)
pH	unitless	6.84 (±0.11)	7.11 (±0.06)	0.044 [*]	0.15 (0.027) [*]
Nmin	mg g^{-1}	14.6 (±1.12)	15.1 (±0.81)	0.810	-0.08 (0.267)
Olsen-P	mg g^{-1}	23.9 (±2.44)	38.2 (±2.07)	<0.001 [**]	-0.24 (<0.001) [**]
Soil C:N	g g^{-1}	8.61 (±0.33)	8.20 (±0.17)	0.230	-0.07 (0.348)
Soil N:P	g g^{-1}	0.62 (±0.59)	0.03 (±0.00)	<0.001 [**]	0.20 (0.005) [**]
Soil Nmin:Pmin	g g^{-1}	1.53 (±0.33)	0.72 (±0.10)	<0.001 [**]	0.18 (0.010) [*]
SOC	mg g^{-1}	20.2 (±1.56)	15.1 (±0.57)	0.003***	0.17 (0.013) [*]
Microbial biomass C	mg g^{-1}	0.75 (±0.05)	0.44 (±0.02)	<0.001 [**]	0.33 (<0.001) [**]
Root AMF Richness	No. OTUs	24.9 (±1.07)	17.7 (±0.64)	<0.001 [**]	0.41 (<0.001) [**]
Root AMF Shannon	unitless	2.27 (±0.05)	1.82 (±0.04)	<0.001 [**]	0.37 (<0.001) [**]
Soil bacteria	index	9.74 (±0.03)	9.88 (±0.02)	<0.001 [**]	-0.15 (0.029) [*]
Soil fungi Shannon	index	3.79 (±0.06)	3.44 (±0.04)	<0.001 [**]	0.19 (0.006) [**]
Soil fungi Richness	No. OTUs	198 (±5.06)	158 (±2.84)	<0.001 [**]	0.20 (0.004) [**]
Soil cercozoa Shannon	index	4.93 (±0.03)	4.96 (±0.02)	0.600	-0.07 (0.329)
Soil archaea Shannon	index	1.98 (±0.04)	2.06 (±0.03)	0.210	-0.02 (0.745)
Soil archaea Richness	No. OTUs	77.1 (±2.31)	77.9 (±1.68)	0.690	-0.07 (0.308)

[*] P<0.05;

[**] P<0.01