Research paper

Impact of plant diversity in potato-ley strip-cropping systems on soil microbial communities

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ABSTRACT

Crop diversification enhances agroecosystem productivity, yet underlying mechanisms, particularly those underground, remain unclear. Diversification practices, such as strip-cropping (alternating strips of different crops) and integrating legumes (plant-based fertilization), may improve nutrient uptake and reduce pathogen build-up via soil microbial community shifts. However, most evidence for plant diversity benefits arises from grasslands, and its impact in cropping systems remains underexplored. This study examined microbial communities in organically managed, long-term potato-ley strip-cropping systems, comparing mono-cropping and strip-cropping, and further examined the effect of replacing animal manure by integrating legumes and plant-based fertilization in strip-cropping systems. To assess changes in microbial community composition, bacterial, fungal and arbuscular mycorrhizal fungi (AMF) DNA was sequenced. In addition, AMF and potato fungal pathogens were quantified using quantitative PCR. Strip-cropping increased AMF and soil-borne potato pathogens at the strip edges, where ley and potato interacted, though edge effects were absent in plant-based strip-cropping systems. Despite higher pathogen abundance at the edges, these did not relate to more significant tuber disease symptoms or yield losses. Changes in soil abiotic properties, particularly lower pH, from plant-based management, influenced fungal community structure more than strip-cropping. Bacterial community structure, including N-fixing bacteria, remained unaffected by either practice. Our results indicate that legume legacy in plant-based systems more strongly affected fungal community than strip-cropping, while strip-cropping benefitted AMF abundances. Taken together, our findings suggest that for sustainable agricultural practices aiming to improve soil health and crop productivity, a comprehensive approach that considers crop diversity, soil management, and fertilization practices is necessary.

1. Introduction

Intensive agricultural practices, exemplified by large-scale monocultures, have significantly stressed soil ecosystem functions, like nutrient cycling and disease control, due to the use of high fertiliser and pesticide inputs and intensive tillage practices (de Graaff et al., 2019; Kopittke et al., 2019). Efforts to alleviate these issues have focused on decreasing mineral fertilizers and pesticide inputs, and reducing soil disturbance, but also on enhancing temporal crop diversification at the field level, with some success (Morugán-Coronado et al., 2022; Pearsons et al., 2023; Walder et al., 2023). Increasing crop diversity, another common characteristic of agroecosystems, may offer promise based on

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ecological theory, which links biodiversity and ecosystem functions (Ampt et al., 2019; Brooker et al., 2015; Gamfeldt and Roger, 2017; Weisser et al., 2017; Zavaleta et al., 2010). Strategies such as inter-cropping and including legumes in cover crops and mulches (i.e. plant-based systems) can support multiple ecosystem services, including improved nutrient cycles (e.g. nutrient availability), crop production, and pest and disease control (Beillouin et al., 2021; Brooker et al., 2015; Daryanto et al., 2018; Duchene et al., 2017; Tamburini et al., 2020; Wang et al., 2022). While such crop diversification practices have been shown to increase the productivity of agroecosystems, the underlying mechanisms remain to be elucidated (Stefan et al., 2021).

Investigating the composition and functions of soil microbial com-munities in the context of crop diversification is imperative for under-standing which agricultural practices can maintain soil health and productivity. While crop diversification generally enhances soil micro-bial biomass and diversity (Bargaz et al., 2017; Muhammad et al., 2021; Tang et al., 2014; Tian et al., 2019; Venter et al., 2016) and moderates microbial community composition (Santos and Olivares, 2021), it is less clear how it impacts microbes at the level of functional guilds (Venter et al., 2016). In grassland systems, the belowground benefits of plant diversity, such as improved nutrient bioavailability (Hacker et al., 2015; Honvault et al., 2021; Phoenix et al., 2020) and reduced prevalence of soil-borne pathogens (Ampt et al., 2022; Maciá-Vicente et al., 2023; Wang et al., 2023), have been linked to changes in soil microbial com-munities. The same links have also been suggested in agroecosystems (McDaniel et al., 2014; van der Putten et al., 2013). Crop diversification, in spatial and temporal patterns, can contribute to increased soil mi-crobial diversity and biomass, which are vital components in biogeo-chemical carbon and nutrient cycles (Crews and Peoples, 2005; Prommer et al., 2020). In addition, crop diversification has been sug-gested to counteract some of the downsides of continuous mono-cropping, such as the accumulation of soil pathogens and pests and reduced performance of beneficial soil microorganisms (Eisenhauer et al., 2012; Latz et al., 2012; Wang et al., 2021).

Crop diversity can also regulate specific community functions that, in turn, impact crop plant biomass and yield, such as decomposition, ni-trogen (N) fixation, phosphorous availability and occurrence of patho-gens. More specifically, intercropping, where two or more crops are grown together, can increase beneficial microbial communities such as N-fixing bacteria (rhizobia) and arbuscular mycorrhizal fungi (AMF), which are known to improve crop nutrient-use efficiency (Guzman et al., 2021; Lee et al., 2023; Zhang et al., 2020) and reduce diseases via pathogen dilution, interruption of pathogen life cycles, or spread (Ampt et al., 2022; Wang et al., 2021). However, as microbial communities are generally dispersal limited and crop diversity is limited to the edge of the strip, these positive effects of increased crop diversity on soil microbial communities might be localized to areas where crop species interact, spatially limiting crop diversity benefits on soil health and services. In strip-cropping systems with two or more crops grown in varying-width strips, a positive effect on soil communities might be localized to the edge of the strips where crop species interact. However, evidence for this is still missing. Understanding the small-scale spatial dynamics of soil microbial community structure and functions is crucial for elucidating the mechanisms underlying the benefits of crop diversity for soil health.

Another crop diversification practice that has received significant attention involves integrating legumes mixed cover crop mulches (crops grown overwinter and then tilled to fertilize the soil) with legumes due to their association with N-fixing Rhizobia and potential to reduce the incidence of certain soil pathogens (Daryanto et al., 2018; Duchene et al., 2017; Zhong et al., 2018). Plant-based cropping systems (htt ps://www.biocyclic-vegan.org/), which integrate legumes in cover crop mulches, enhance not only multiple ecosystem services, including N availability and subsequent crop yield, but also impact microbial communities (e.g. generally decreasing biomass of soil fungi and bene-fits saprotrophs (Bartelt-Ryser et al., 2005; Lange et al., 2014; Yu et al., 2022)) and interactions between microbes (e.g. promoting interactions

between Rhizobium and AMF, Hnini et al., 2024). However, the precise effects of combining multiple diversification practices on soil microbial communities and their functional groups remain underexplored. Research suggests that the presence of legumes, rather than the overall richness of plant diversity, plays a pivotal role in determining the structure of microbial communities in long-term grassland studies (Dassen et al., 2017; Schmid et al., 2021; Stephan et al., 2000). However, this point is still controversial (Cavalieri et al., 2020; Hortal et al., 2017). Therefore, evaluating the combined impacts of staking (i.e. combining) crop diversification practices on microbial communities is essential to fully understand the added benefits and potential trade-offs.

Here, we investigate how crop diversification, via strip-cropping and plant-based practices, affects soil bacterial and fungal community composition as well as beneficial and pathogenic fungal abundances. The system was an organically managed large-scale, long-term strip-cropping field system experiment, in which we first compared the cropping system's effect (mono- versus strip-cropping) and then the impact of replacing animal manure by plant-based practices in strip-cropping systems (Fig. 1). This was done using 3 m width potato-ley strip-cropping systems at the edge and the centre of the strip. We hy-pothesized, firstly, stronger impacts from crop diversification on mi-crobial community composition at the strip edge, where plant species spatially interact (*edge effects*), than at the strip centre and in the mono-cropping system. Secondly, we hypothesized that enhancing the diver-sification of strip-cropping systems by replacing animal manure with plant-based practices (i.e. legume cover crops mulches) would boost the abundance and diversity of beneficial microbes, such as AMF and N-fixing bacteria.

To test these hypotheses, we selected potato (*Solanum tuberosum*) as our targeted study crop, given its status as the world's third most important food crop (Gervais et al., 2021), which suffers substantially from pest and pathogen damage (Fiers et al., 2012). Apart from chemical and copper-based control measures, intercropping potatoes with leys have been found to reduce fungal pathogen spread without yield losses (Ditzler et al., 2021). Additionally, potato intercropping could offer a sustainable agro-ecological alternative to pesticide application.

2. Material and methods

2.1. Experimental site

Data were collected in the summer of 2021 and 2022 at the Droe-vendaal Experimental organic farm at Wageningen University & Research, the Netherlands (51°59′25.8″N 5°39′35.2″E) (https://www.wur.nl/en/project/strip-cropping.htm) (Appendix A). The soil across the experimental fields is sandy (2 % clay, 10 % silt, 84 % sand) with a pH of around 5.5 and an organic matter content of 2.5 %. The site has a mean annual rainfall of 70 ± 8 mm and a mean annual temperature of 11 ± 6 °C. The long-term strip-cropping experiment started in 2018 to assess the effects of strip-cropping and plant-based practices (htt ps://www.biocyclic-vegan.org/) for crops relevant to Netherlands farmers. The farming system experiment follows a 6-year rotation of cabbage (*Brassica oleracea*, var. Rivera), barley (*Hordeum vulgare*, var. RGT planet), potato (*Solanum tuberosum*, var. Agria), oat (*Avena sativa*, var. Symphony), pumpkin (*Cucurbita pepo*, var. Flexi kuri), and ley (grass (*Lolium multiflorum*, var. Turtertra) or grass-clover (*Trifolium resupina-tum*, var. Ciro). Potato-ley is one of the strip-cropping combinations; this combination was chosen to slow down the spread of *Phytophthora* in the potato strips (Ditzler et al., 2021) (Appendix A). Strip-cropping treat-ments (3 × 55 m strips) were either managed as the monocrop (9 × 9 m) control with manure fertilization (cow farmyard manure 35 tons/ha incorporated with cultivator, Table 1, Table A.1), or manure was replaced with plant-based practices, that include intercropping of clover with grass in the ley and mulching (mixed in the soil prior to crop planting) of overwintering grass-clover cover crop prior to crop planting (Fig. 1, Table 1). As the primary goal of our study was to assess the
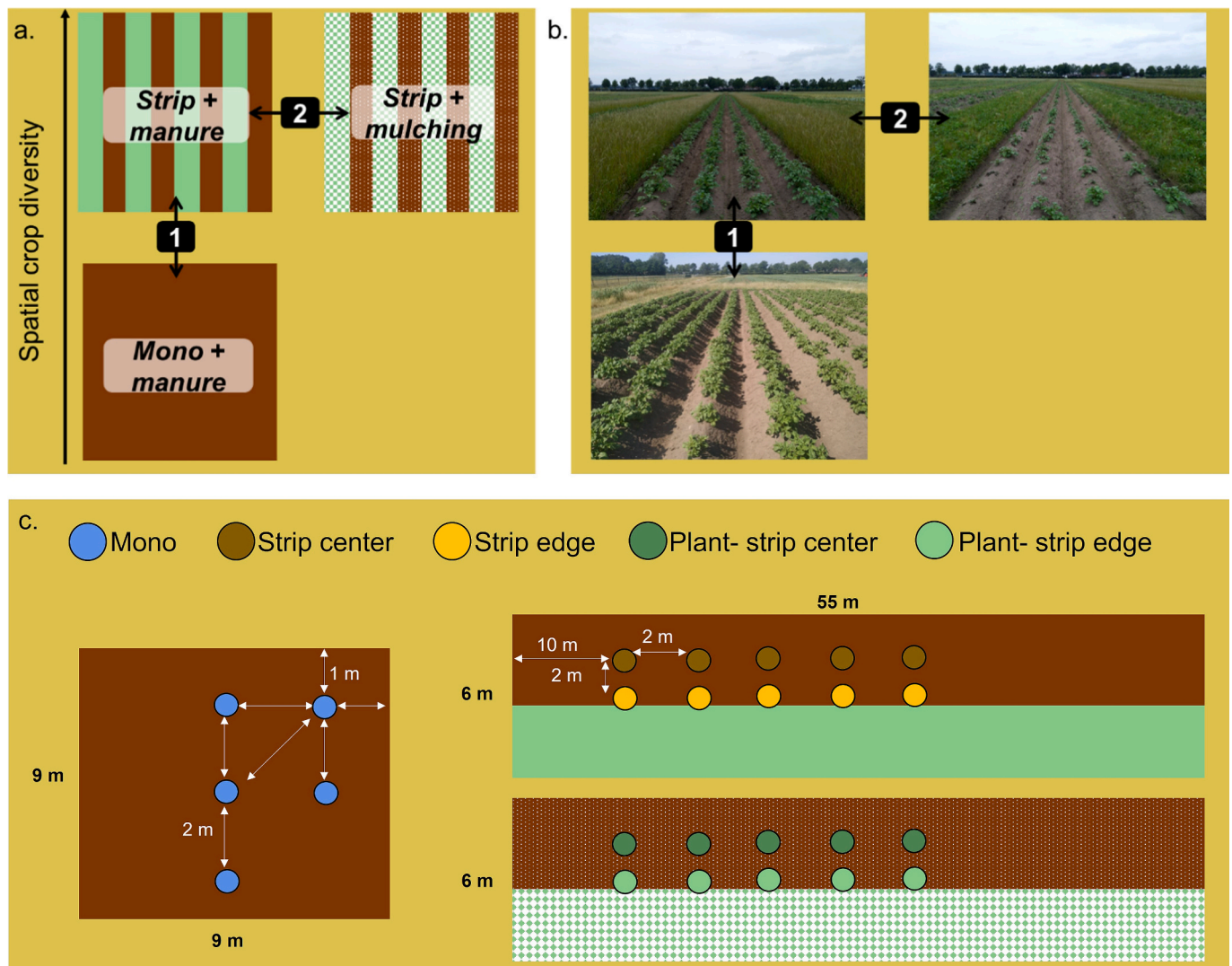
Fig. 1. Overview of the experimental design. **(a)** Schematic illustrating (1) the two distinct cropping systems (mono- and strip-cropping, fertilized with animal manure), and (2) the strip-cropping systems under animal-based (manure) or plant-based practices (legume integration in the ley and mulching in the potato strip). **(b)** Photographs of each system (11-12th of June 2020 and 2023, credits to Dirk van Apeldoorn and Anna de Rooij). **(c)** sampling design in on Block with each dot representing a sampling site in 2021.

Table 1
Agronomic information for each treatment. For more information regarding the management practices in each treatment, see Table A.1.

Treatment	Crop sequence (2018–21)	Green manures	Fertilization
Mono-cropping	grass, cabbage, barley, potato	rye (100 kg/ha)	Stable manure (35 tons/ha) in April
Strip-cropping	grass/potato, oat/cabbage, pumpkin/barley, potato/grass	rye (100 kg/ha)	Stable manure (35 tons/ha) in April
Plant-based Strip-cropping	grass-clover/potato, oat-faba bean/cabbage, pumpkin/barley-pea, potato/grass-clover	rye (100 kg/ha) and vetch (70 kg/ha)	Grass-clover (from the neighbouring strip, 18–20 tons/ha) in May

relative impact of strip-cropping versus monocropping on soil microbial communities. It is the relative differences between treatments that provide insights into the effects of crop diversity, which can be interpreted regardless of differences in plot sizes between treatments. The experiment is a randomized block design with three replicates of each

treatment (Fig. A.1). In both years, potatoes were planted at a within-row distance of 26 cm and a between-row distance of 75 cm. After planting, re-ridging and harrowing occurred to keep weeds under control (Table A.1). All treatments received the same planting density and management, except for the specific variables being tested (i.e., mono- versus strip-cropping and manure- versus plant-based fertilization), ensuring that any observed changes in microbial communities can be attributed to these treatments.

2.2. Soil chemical and plant analyses

Two soil cores (5×20 cm) were taken 5 m apart in each plot and combined ($n = 9$) respectively on the 15th and 20th June 2021 and 2022. Sampling timing coincided with potato tuber growth stage. The soil was homogenized and dried at 60 °C for three days. Then, it was divided into two subsamples of 20 g to assess pH and soil nutrients. pH was measured with a pH/mV meter in demineralized water, and soil-plant available Nitrogen (NO_3-N, $NH_4 +$ -N) and phosphate (PO_4-P) were quantified following the methods described Houba et al. (2000). Briefly, for soil nutrient quantifications, dry soil subsamples were extracted in 0.01 M $CaCl_2$ and measured spectrophotometrically with a

segmented-flow system (Skalar SAN++ system) (Table 2).

2.3. Soil sampling for microbial analyses

To investigate bacterial and fungal soil communities and fungal potato pathogens (*R. solani AG-3 and S. sclerotiorum*), five rhizosphere soil samples were taken in the centre of the mono-cropping, and in the centre and at the edge of the strip-cropping treatments in each block (n = 15 per block) on 15th June 2021 (potato tuber growth stage) (Fig. 1c). Samples within strip- and mono-cropping treatments were 2 m apart from each other (Fig. 1c). Additional sampling on the 20th of June 2022 was carried out in the same treatments as in 2021 (but in different fields) to quantify the same fungal pathogens along with AMF, using qPCR. In this second round of sampling, ten loose rhizosphere soil samples were taken in the centre of the mono-cropping, in the centre, and at the edge of the strip-cropping treatments in each block (*n* = 30 per block) on the 20th of June 2022. The soil was collected by uprooting potato plants and gently shaking off the soil adhering to the roots (Yanai et al., 2003). The soil was then dried at 40 °C for 72 h, and stored for 3 weeks with silica until extraction. In 2021 and 2022, different fields were sampled. However, fields are exact replicates of each other following the same management and 6-year crop rotation but starting with a different crop combination (Appendix A). Therefore, the potato fields sampled in 2021 and 2022 have the same crop and management history.

2.4. DNA extraction and PCR

Soil DNA was extracted from 200 mg of soil (dry weight) using the DNeasy Power Soil Kit (Qiagen). Genomic DNA was quantified using a Qubit 3.0 Fluorometer and a NanoDrop 2000 fluorospectrometer, and DNA quality was assessed using NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE). To identify bacteria and fungi, the V4 region of bacterial 16S (515F–806R (Caporaso et al., 2011)) and the ITS2 region of fungal ITS (gITS7ngs_201 and ITS4ng-sUni_201 (Nilsson et al., 2019)) were amplified. Detailed description of the PCR conditions and primers used is presented Appendix B and C.

2.5. Microbial sequencing and bioinformatics

The sequencing libraries were prepared and shipped for 250 PE sequencing on the Illumina NovaSeq 6000 Sequencing System at the Novogene Europe laboratory. LotuS2 (Özkurt et al., 2022) was used for processing metabarcoding data, including demultiplexing and denoising (using SDM), clustering (using Usearch), chimaera checking (using Vsearch, Rognes et al., 2016) ITS2 extraction (for Fungi) and taxonomic annotation (using Lambda, blastn and lowest common ancestor (LCA) algorithms against Unite (Kõljalg et al., 2013) and Silva (Quast et al., 2013) databases for fungi and bacteria, respectively) (Appendix B, C). For ITS2, 6,093,187 reads were processed, resulting in 1438 OTUs (1,277,036 reads). Respectively, for ITS and 16S, 8 and 10 out of 85 samples did not return sequences (< 50 reads) and were excluded from further analyses. For 16S, 3,811,893 reads were processed, resulting in 11,825 OTUs. OTUs assigned to Chloroplasts, eukaryotes and mito-chondria were omitted from the prokaryote dataset, and OTUs not assigned to fungi were omitted from the ITS dataset. This resulted in 948 and 10,170 fungal and bacterial OTUs, respectively. The fungal OTUs were assigned to guilds using the FungalTraits (Põlme et al., 2020), and the bacterial OTUs were assigned to functional groups based on meta-bolism using the FAPROTAX (Yang et al., 2022). Relative abundances of fungal OTUs and genera, bacterial OTUs and orders, and fungal (e.g. pathogen, AMF, saprotrophs) and bacteria (e.g. N-fixing) functional guilds were calculated for each treatment.

2.6. Fungal pathogens quantitative-PCR

Inhibition tests were performed on the DNA extracts by amplifying 1 × 104 copies of pGEM-16S plasmid (Promega, WI, USA) spiked into qPCR reactions with either DNA template or non-template controls and primers specific to amplify the plasmid (Appendix B, Table B.1). Real-time quantitative PCR (qPCR) was used to quantify the abundances of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* DNA (Fierer et al., 2005). PCR amplification of all samples (*n* = 75 in 2021 and *n* = 150 in 2022) was done in duplicates using a set of primers and standard DNA specific to each pathogen (Table B.1). The q-PCR was performed in a Master cycler ep realplex real-time PCR system (Eppendorf North America, Inc., Hauppauge, NY) (Appendix B). The number of copies was corrected per dilution, and the amount of soil used for DNA extraction.

2.7. AMF quantitative-PCR, sequencing and annotation

AMF were sequenced and quantified from DNA soil samples from 2022. Inhibition tests were performed as described for the pathogens qPCR as described. AMF DNA sequencing and qPCR were performed by amplifying the 18S rRNA region using the NS31 (Simon et al., 1992) and AML2 (Lee et al., 2008) primers (Appendix B, Table B.1). The AMF li-brary was sequenced using one Pacific Biosciences Sequel 2 cell. Se-quences were quality-filtered and clustered using the SCATA pipeline (https://scata.mykopat.slu.se/, accessed November 2023). Sequences shorter than 100 bases were removed, and the remaining sequences were screened for primers (requiring 90 % match) and sample tags (100 % match). Sequences with an average amplicon quality score of <20 or <5 at any position were omitted. Quantification values were corrected by subtracting the relative abundances of non-AMF taxa based on high-throughput DNA sequencing of the same region using the same primers (Appendix B).

2.8. Statistical analyses

The effect of strip-cropping and plant-based cropping systems on microbial communities was analysed using a (generalized) linear mixed model with the *glmmTMB* function. First, we compared manure fertilized strip- versus mono-cropping systems, and second, we compared animal-versus plant-based strip-cropping systems. Response variables included

Table 2
Soil characteristics (mean ± sd) per treatment in June 2021 and 2022. Different letters indicate significant differences ($p < 0.05$).

Treatment	pH	PO4 (mg/kg)	NH4 (mg/kg)	NO3 (mg/kg)
2021				
Mono-crop	6.03 ± 0.04 abc	2.09 ± 0.27 a	6.40 ± 0.52 a	1.54 ± 0.78 a
Strip-crop center	6.10 ± 0.06 c	1.83 ± 0.29 a	5.01 ± 0.24 ab	1.18 ± 0.38 a
Strip-crop edge	6.05 ± 0.07 bc	1.89 ± 0.06 a	4.40 ± 0.30 b	1.02 ± 0.35 a
Plant-based strip center	5.85 ± 0.02 ab	1.72 ± 0.13 a	4.82 ± 0.38 b	1.90 ± 0.98 a
Plant-based strip edge	5.84 ± 0.03 a	1.66 ± 0.13 a	4.40 ± 0.34 b	1.03 ± 0.32 a
2022				
Mono-crop	6.02 ± 0.22 a	2.91 ± 1.35 a	6.36 ± 0.34 a	5.39 ± 3.59 a
Strip-crop center	6.04 ± 0.26 a	2.53 ± 0.74 a	6.13 ± 0.72 ab	3.88 ± 0.65 a
Strip-crop edge	5.86 ± 0.20 a	2.75 ± 0.36 a	6.29 ± 0.86 ab	4.99 ± 2.87 a
Plant-based strip center	5.85 ± 0.20 a	1.73 ± 0.42 a	6.01 ± 0.23 ab	4.70 ± 0.89 a
Plant-based strip edge	5.86 ± 0.21 a	1.98 ± 0.22 a	5.51 ± 0.30 b	4.96 ± 0.78 a

fungal, AMF and bacterial OTU richness (chao index) and Shannon diversity, the relative abundance of N-fixing bacteria (out of total bacterial communities), the relative abundance of fungal plant pathogens and saprotrophs (out of total fungal communities), as well as abundances of specific potato fungal pathogens (*R. solani* and *S. sclerotiorum*) and AMF (log-transformed copy numbers per gram of soil). Shannon index and chao richness were estimated on rarefied bacterial and fungal communities using the *vegan* package using minimum sequencing depth per dataset, 3038 and 12,418 reads per sample, for fungi and bacteria, respectively (Oksanen et al., 2022). Chao richness response variables were analysed using a Conway-Maxwell-Poisson distribution as data was over-dispersed. Quantitative PCR and relative abundance response variables were analysed using a Gaussian distribution. Models included treatments as the fixed effect and block ($n = 3$) as a random effect. For *R. solani* and *S. sclerotiorum* models, the block and the year (i.e. 2021 and 2022) were included as random. Pathogen loads per treatment per year are presented in Fig. E.1. Model assumptions were assessed using the *DHARMa* package (Hartig and Lohse, 2020). In the case of significant ANOVA results, posthoc tests were performed using *means* (Lenth et al., 2023).

Principal component analysis (PCA) was used to visualize the variation in microbial community composition data between treatments with Euclidian distances. The effect of strip-cropping and plant-based systems on microbial communities was analysed separately. Relative abundances of fungal OTUs, genera and functional guilds (e.g. AMF, pathogens, saprotrophs), AMF OTUs and genera, and bacterial OTUs, order and functional guilds (e.g. N-fixing) were centred and scaled (divided by standard deviation) prior to analyses. A Hellinger transformation was applied to analyse non-aggregated OTU community data accounting for zero inflation. To evaluate differences between the cropping systems (mono versus strip-cropping) and between animal- and plant-based strip-cropping systems, two PERMANOVAs tests were conducted using the '*betadisper*' and '*adonis*' functions with 999 permutations from the *vegan* package (Oksanen et al., 2022), followed by the '*pairwise.adonis2*' function to investigate post-hoc comparison including block as strata. When significant treatment effects were found, indicator species analyses using the *interspecies* package were used to associate fungal/bacterial groups to treatments. Redundancy analysis (RDA) was performed through the vegan package to visualize correlations between bacterial order or fungal genera and soil (pH and nutrients) characteristics. Soil parameters were averaged per treatment within block. All analyses were performed in R (version 4.3.2).

3. Results

3.1. Fungal community structure

No differences in bacterial richness, Shannon index, community composition and relative abundance of N-fixing bacteria were found (Fig. D.1, D.2); therefore, results and discussion focus on fungal and AMF community composition. Fungal richness and Shannon diversity index were not affected by cropping systems but only by fertilization treatments, with lower OTUs richness and Shannon diversity in plant-based strip-cropping systems fertilized with legume green mulch compared to animal-based strip-cropping systems fertilized with manure (Fig. 2, Fig. D.3, Table D.1). Ordination analyses showed that communities both at OTU level (Fig. D.3) and genus level differed between animal- and plant-based treatments ($R = 0.32$ $p = 0.001$, Fig. 3a, Table D.2) but not between cropping systems ($R = 0.03$, $p = 0.119$, Fig. D.3) nor between strip edge and center. RDA analyses showed that soil abiotic factors explained 19.5 % (R^2 adjusted) of the variation in fungal genus-level composition between these treatments, with lower pH ($p = 0.001$) and higher nitrate ($p = 0.011$) associated with plant-based systems (Fig. 3c). The composition of functional fungal guilds showed significant differences between animal- and plant-based systems (Fig. 3, $R = 0.24$, $p = 0.001$). Relative abundances of pathogens were marginally lower and richness higher in plant- compared to animal-based strip-cropping systems; these differences were significant at the edges (Fig. 4c,e, Table D.1.). By contrast, fungal saprotrophs had higher relative abundances in plant-compared to animal-based strip-cropping systems; these differences were more significant at the centre of the strips (Fig. 4d,f, Table D.1). Indeed, indicator species analyses revealed strong associations between fertilization treatments and certain fungal genera (Table D.3), with noticeable associations of the potential plant pathogenic genera *Alternaria* associated with plant-based treatments and *Fusarium* with animal-based fertilization (Fig. 4a, Table D.3). The composition of fungal genus-level and bacterial order-level communities is shown in Fig. D.4.

3.2. AMF community composition

AMF OTU richness was affected by plant-based practices but not by strip-cropping treatments, with, on average, one more AMF taxon in the centre of plant-based strip-cropping systems compared to the edge (Fig. 5b). AMF OTU and genus community composition were affected by both cropping system ($R^2 = 0.07$ and 0.10 respectively for OTUs and
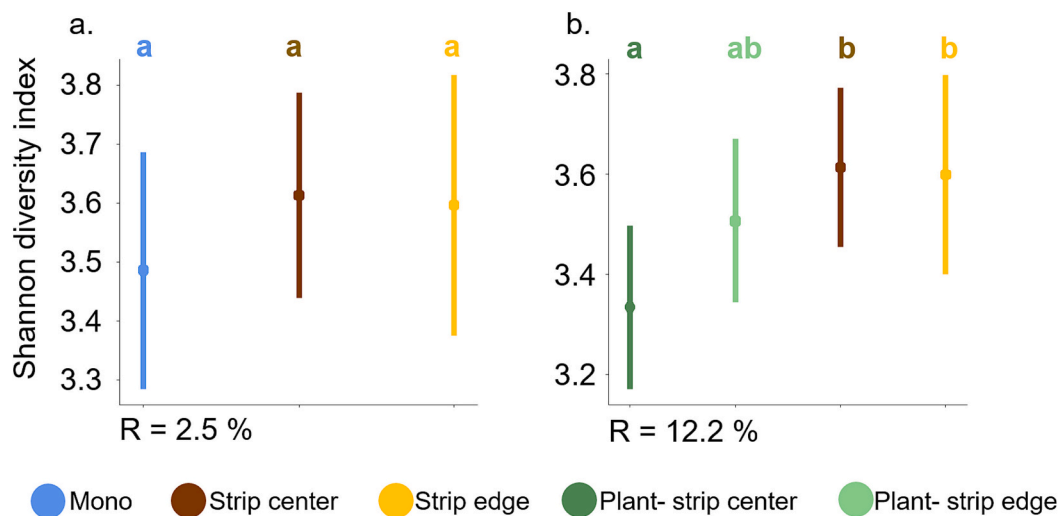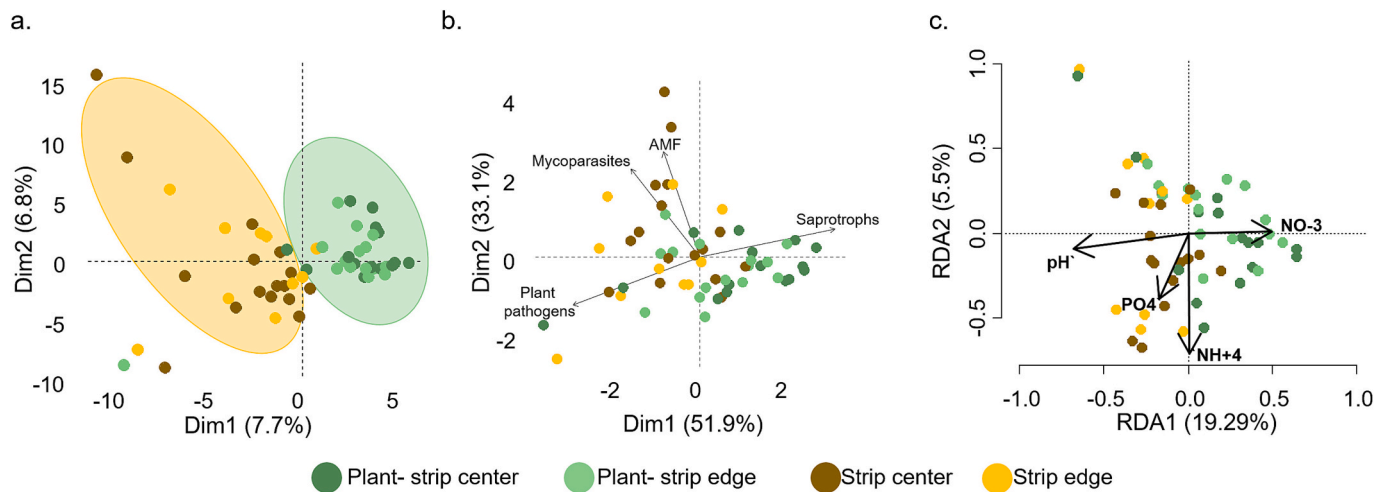

Fig. 2. Impact of strip-cropping on fungal diversity. Model estimates showing the effect of **(a)** cropping system and **(b)** plant-based systems on fungal diversity, calculated based on Shannon diversity of fungal OTUs generated derived from amplicon sequencing analysis. R is the marginal R^2 of the model.

Fig. 3. Impact of plant- and animal-based strip-cropping on the composition of fungal communities and functional guilds. PCA ordination sample plots representing **(a)** fungal genus-level communities (ellipse show 95CI for animal- (yellow) and plant-based (green) systems), and **(b)** fungal functional guild-level communities (with arrows showing significantly associated fungal functional guilds) characterized based on amplicon sequencing. **(c)** RDA analysis of fungal genus-level communities and soil properties indicate that plant-based systems were associated with lower pH and higher nitrate (NO₃).

genera PCAs, strip (edge and centre) versus monoculture, Fig. 5c) and plant-based systems (R^2 = 0.09 and 0.15 respectively for OTUs and genera PCA, plant- versus animal-based, Fig. 5d) treatments (Fig. D.4). Strip-cropping edge communities were more similar, as shown by a narrower ellipse (0.95 CI), and appear nested within the other treatments (Fig. 5c,d). While, treatments did not significantly associate with specific AMF OTUs nor genera, LMMs indicate that there is relatively more *Claroideoglomus* in the plant- compared to animal-based strip-cropping systems t (est = 0.72, se = 0.21, *p* = 0.005, Fig. D.4).

3.3. Abundance of pathogens and beneficial microbes

Edge effects increased the abundance of fungal potato pathogens, with relatively more *R. solani* and *S. sclerotiorum* strip-cropping edges compared to the centre, especially in animal-based systems (Fig. 6a,b, Table D.1). Edge effects were also detected for AMF with greater abundances in strip-crop edge compared to strip-cropping centre and mono-cropping systems (Fig. 6c, Table D.1) but only in manure fertilized treatments. Marginal edge effects (0.05 < *p*-value <0.08) were found with slightly greater relative abundances of fungal potato pathogens at the edge compared to the centre of the strip-cropping systems (est = −0.09, se = 0.03, *p* = 0.063, Fig. 4c).

4. Discussion

This study demonstrated that strip-cropping increases the abundance of beneficial fungi at the strip edge, where crop plants interacted, as evidenced by higher AMF abundance at the edge compared to the centre and mono-cropping systems. Similarly, soil-borne fungal potato pathogens loads were higher at the strip's edge, indicating a general increase in fungal pathogen abundances. However, compared to mono-cropping, crop diversity did not increase microbial richness or impact microbial communities in a potato-ley organic system. Fungal communities were mainly influenced by plant-based fertilization, with legume integration and mulching reducing overall fungal richness and shifting the relative abundance of fungal groups. Our data indicate that the fungal community structure in the long-term strip-cropping system was more affected by soil abiotic changes from plant-based practices than by increased spatial crop diversity. These results highlight the complexity of soil microbial dynamics, where multiple factors, including crop diversity, fertilization, and environmental conditions, collectively shape the microbial community structure.

4.1. Both beneficial and harmful fungi were more abundant in strip-cropping edge

We found that strip-cropping positively associated with AMF and pathogenic fungi abundances at the edge of the potato strip. In contrast, no effects of crop diversity nor legume integration in plant-based systems were found on N-fixing bacteria. Positive edge effects on AMF abundances and richness were expected due to greater plant richness at the edge of the strip, providing a more diverse set of plant hosts for AMF. Polycultures of pumpkin and eggplant have been found to enhance AMF richness and change AMF communities (Guzman et al., 2021). We found no differences in AMF OTU richness per se between cropping systems despite differences in community composition, likely driven by lower variation in AMF communities composition in strip- compared to mono-cropping systems. The lack of AMF richness effects in our study compared to Guzman et al. (2021) might be due to (1) a lower number of different crop mixtures investigated in our study and (2) sandy soil conditions decreasing AMF diversity in our system (Verbruggen and Toby Kiers, 2010).

We found positive edge effects on AMF abundances in manure but not in plant-based treatments. The presence of leys sown in autumn likely benefited AMF abundance in the neighbouring strip. Legumes can increase AMF diversity and spore numbers (Oehl et al., 2004). In line with this, we observed positive effects of legumes on AMF richness in the strip center. However, this did not translate to an increase in AMF abundance in the legume treatments. This might be due to changes in soil abiotic properties (i.e. reduced pH) in plant-based treatments or mulching of the green manure disrupting AMF (Table A.1). *Claroideoglomus* AMF species were relatively more abundant in plan- than in animal-based strip-cropping systems. *Claroideoglomus* have been classified as rhizophilic (i.e. high allocation to intraradical hyphae, with limited extraradical hyphae), and suggested to play a role in reducing root pathogen infection (Weber et al., 2019). Differences in community composition between cropping systems and fertilization treatments coupled with greater AMF abundances at the edge of the strip, where potato and ley interacted, could indicate differences in AMF functionality with implications for potato yield.

We found positive edge effects on the abundance of soil fungal potato pathogens. *R. solani* was more abundant at the edge on the strip in both animal- and plant-based treatments, whereas for *S. sclerotiorum*, positive edge effects were found only in the manure treatments. Positive edge effects on soil pathogen loads in strip-cropping systems can be explained
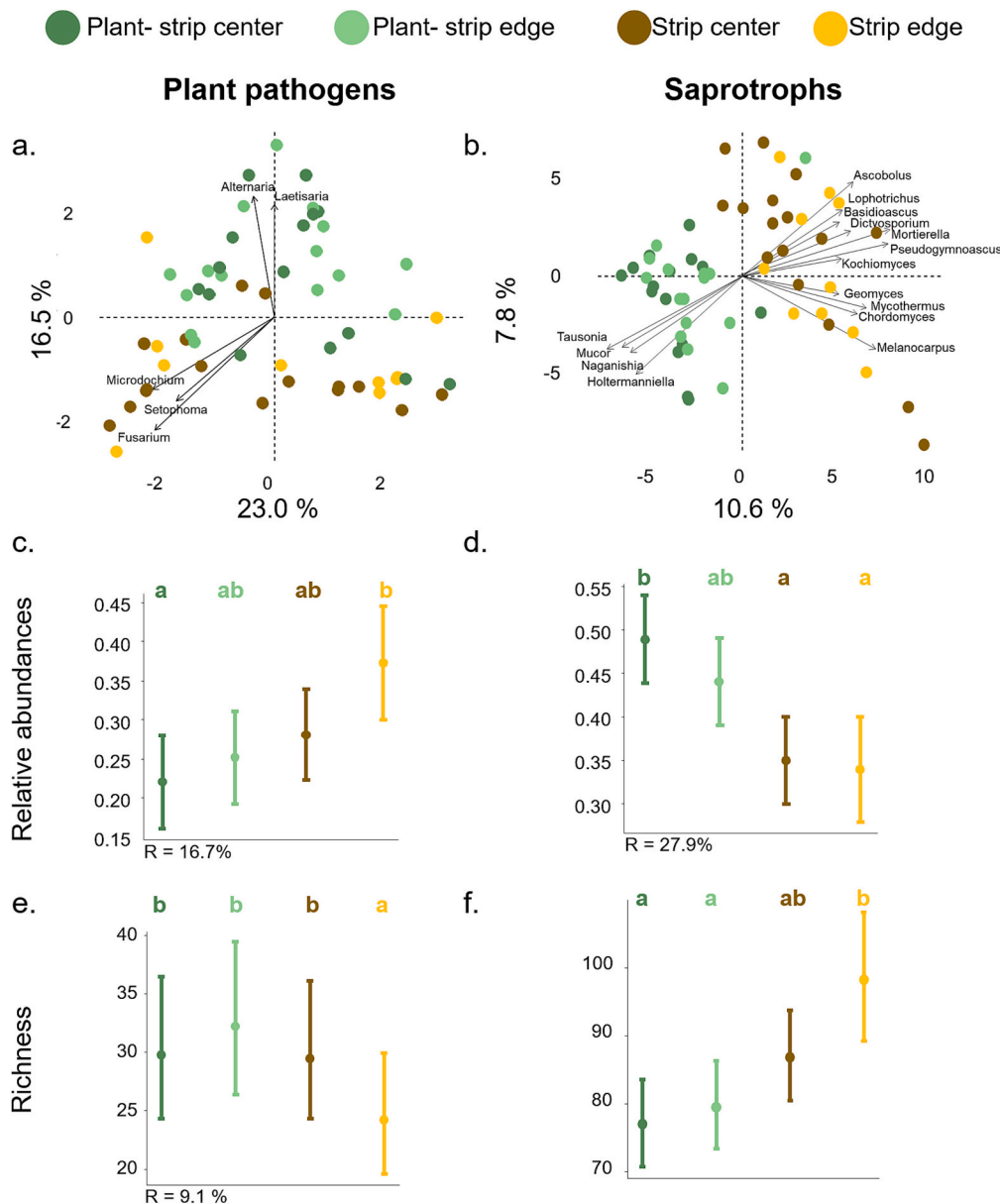
Fig. 4. **Impact of plant- and animal-based strip-cropping on the relative abundance and richness of fungal pathogens and saprotrophs**. PCA ordination plots of the potential **(a)** plant pathogenic and **(b)** saprotrophic fungal genera (with arrows showing significantly associated genera) derived from amplicon sequencing analysis. **(c)** relative abundance of fungal plant pathogenic OTUs. **(d)** Relative abundance of fungal saprotrophic OTUs. **(e)** Richness of fungal plant pathogenic OTUs. **(f)** Richness of fungal saprotrophic OTUs. R is the marginal R^2 of the model not presented for Conway-Maxwell-Poisson distributions (f).

by shorter return times in strip-compared to mono-cropping systems. In a mono-cropping system, the return time of each crop is a full rotation cycle (6-year in this experiment), whereas in a strip-cropping system, the same crop will return to the field, in the neighbouring strip, after a shorter cycle (4-year in this experiment). Potatoes had been cultivated in the neighbouring strip in 2018. This finding suggests that the dormancy of soil pathogens could increase abundance in strip-cropping systems, where the return time for the same crop is shorter compared to mono-cropping systems, potentially enhancing the edge effects on soil pathogen loads for various crop combinations. The absence of edge effects for *S. sclerotiorum* in plant-based systems could be due to suppression by biocontrol agents in the rhizosphere of leguminous plants (Giorgio et al., 2015; Han et al., 2023). While *R. solani AG-3* is specific to potatoes, *S. sclerotiorum* is a ubiquitous pathogen with a broad host range, which could benefit from diverse hosts in the rotation (Vleugels et al., 2013). Interestingly, positive edge effects on pathogen loads did not translate to greater potato tuber disease occurrence or yield losses (Fig. E.2, E.3).

This is in line with findings that increased cabbage root fly oviposition in cabbage-oat strip-cropping systems, did not cascade to greater pest damage, likely due to higher pest mortality (Karssemeijer et al., 2023). While the focus of our study was not to asses yield, harvest data from the Experimental farm indicates that strip-cropping did not associate to greater incidence of tuber fungal damage or yield loss at harvest (Fig. E.2, E.3). Nevertheless, the increase in pathogen load at the edge of a strip is worrisome for strip-cropping implementation, and impacts of strip-cropping on plant tolerance and resistance to disease and yield quality should be further evaluated (Pagán and García-Arenal, 2018).

The co-increased abundance of AMF and pathogens at the edge of the strip crop could represent a strategic cropping systems adaptation to enhance crop plant resilience. Increased plant diversity, through mechanisms such as promoting beneficial soil microbes, like AMF, that compete with and have a potential biocontrol effect on pathogens (Jung et al., 2012; Liu et al., 2024), could contribute to increased plant resistance and tolerance against pathogens in cropping systems (Harrier and
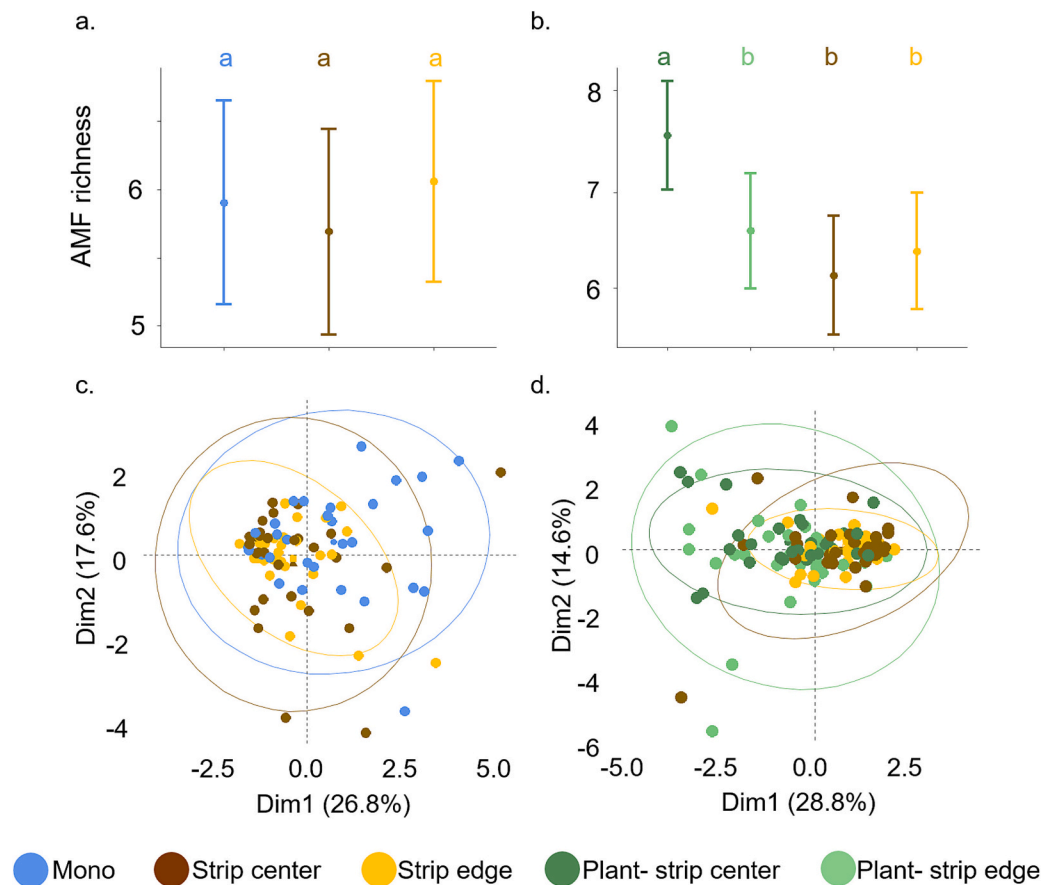
Fig. 5. Impact of cropping system and plant-based system on AMF richness and community composition. Model estimates showing the effect of **(a)** cropping systems and **(b)** plant-based treatment on AMF OTUs richness, characterized based on amplicon sequencing analysis. PCA ordination plots for AMF genera between **(c)** cropping systems ($R^2 = 10.7$ %, p-value = 0.001) and **(d)** plant-based systems ($R^2 = 15.9$ %, p-value = 0.001). Ellipses represent 95% CI.

Watson, 2004). This is in line with a lack of noticeable edge effects on fungal disease symptom occurrence on tubers despite greater fungal pathogen loads (Fig. E.3). While higher levels of AMF at the edge of the strip may mitigate tuber disease occurrence, additional factors such as favourable microclimatic conditions and soil nutrient characteristics are likely influencing the incidence of fungal diseases. To improve the mechanistic understanding linking crop diversity to disease reduction, future studies should investigate the link between plant diversity, AMF root colonization, soil pathogen loads and plant pathogenic rate. In addition, future research should take a system approach, by assessing microbial communities not only in the focal crop but also in the neighbouring stripped crop to better understand edge effect.

*4.2. Diversification practices effects on soil microbial diversity and composition*

Previous studies showed that increased plant richness is an important driver of microbial richness and community composition in grassland (Bartelt-Ryser et al., 2005; Lange et al., 2015; Stephan et al., 2000) and in mixed-cropping systems (Horner et al., 2019; Xiao et al., 2023). However, we found no differences in fungal or bacterial richness and community composition between mono- and strip-cropping systems. We propose that the lack of differences between strip- and mono-cropping on microbial communities in our systems could be due to three non-exclusive factors: (1) insufficient increase in plant richness, (2) stronger legume effects in plant-based systems, and (3) bulk versus root microbial community sampling, which we further develop below.

Increasing plant richness from one to two species, typically in strip-cropping systems, shows mixed and weak impacts on fungal and especially bacterial communities compared to larger plant species richness gradients (Lange et al., 2015; Loranger-Merciris et al., 2006). In strip-cropping systems, the effects of crop species richness on microbial communities result from temporal (rotation) and spatial (strip-cropping) plant diversity effects. Over four years since the start of the experiment, the strip-crop fields had been planted with green manures and a full rotation of six crop species across four functional groups (i.e. grasses (barley, oats and grass), cabbage, pumpkin and potato), and mono-crop fields with four species and three functional groups (i.e. grasses (barley and grass), cabbage and potato). Therefore, across years, functional plant diversity differed only slightly between mono- and trip-cropping systems, and changes in plant richness might have been too subtle to influence the richness and composition of soil microbial communities. On the other hand, changes in soil abiotic characteristics due to presence of legumes in the plant-based systems, appear to have been a stronger determinant of soil microbial richness and community composition than crop diversity in our systems.

Long-term legume addition in the rotation by integrating mixed cover crops and grass-clover mulches was associated with lower soil pH and ammonium content than manure-fertilized treatments. Changes in soil nitrogen could directly and indirectly affect soil microbial communities by changing soil carbon availability, C/N ratio, and soil pH (Wang et al., 2018). Studies indicate that soil abiotic variables and plant functional group identity, particularly legumes, have a stronger impact on bacterial and fungal community composition than plant richness (Dassen et al., 2017; Schmid et al., 2021; Stephan et al., 2000). This aligns with our results, whereby integration of legumes in the strip-cropping systems via legume cultivation and mulching, rather than increased cash crop richness, affected soil fungal richness and
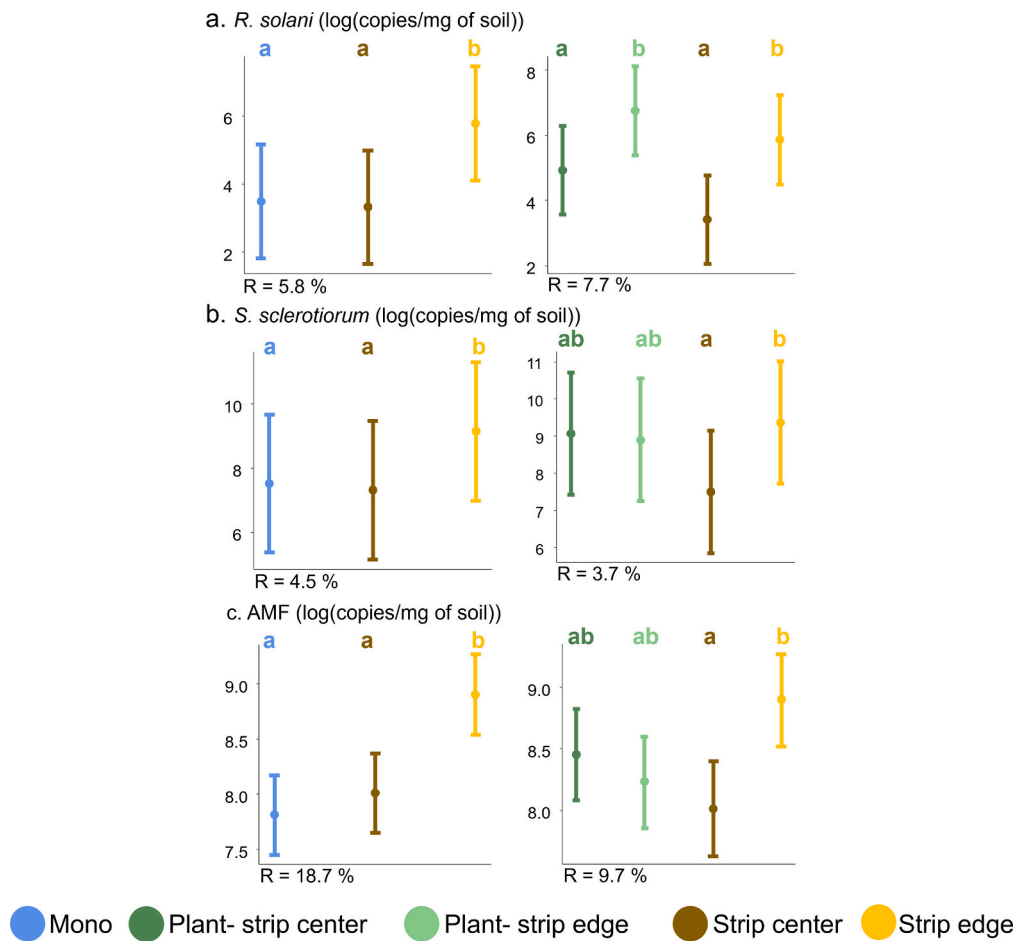
Fig. 6. Edge effect promotes the absolute abundance of AMF and pathogens in the soils. Model outputs for the qPCR analyses for the abundance (number of copies per gram of soil) of **(a)** *Rhizoctonia solani* and **(b)** *Sclerotinia sclerotiorum* (n = 225 per pathogen, n = 85 in 2021, with 5 replicates per treatment, and n = 150 in 2022 with 10 replicates per treatment) and for **(c)** AMF (n = 150 in 2022, 10 replicates per treatment). R indicates the marginal R^2 of the model.

communities. Lower soil fungal diversity in plant-based systems might be partially due to greater relative abundances of fewer fungal saprotroph species in plant- compared to animal-based treatments. In particular, saprotrophs from the *Mucor* genus, known to utilize simple carbohydrate molecules rapidly, were associated with plant-based treatments. Interestingly, despite the potential of legumes to promote microbial symbionts (rhizobia and AMF) (Scheublin and van der Heijden, 2006), plant-based systems were not associated with N-fixing bacteria or AMF. Some effects of legumes might have been diluted over time, as legume cover crops and mulching of grass-clover leys were applied over a month before sampling (Fox et al., 2020; Hannula et al., 2021).

Bulk soil microbial communities have been suggested to be less strongly influenced by plant species composition than rhizosphere microbial communities, with rhizosphere soils changing faster but with less long-lasting legacy and changes in bulk soil being slower but becoming apparent as longer-lasting soil legacy effects (Kowalchuk et al., 2002; Schmid et al., 2021; Soonvald et al., 2020). We sampled loose rhizosphere soil around the roots and thus might have missed faster changes in soil microbial composition. The lack of plant diversity affects soil microbial richness and community composition in certain studies has also been attributed to short experiment duration (Chen et al., 2019). We exclude this as the driving factor in this study as the experiment was part of a four-year organic strip-cropping farming system experiment, which falls within the time frame of other grassland diversity experiments. Ultimately, different farming practices, such as organic management and reduced soil tillage, might benefit fungal communities and

richness more than diversification practices (i.e. crop diversity and legume integration) (Karlsson et al., 2017; Morugán-Coronado et al., 2022; Verbruggen and Toby Kiers, 2010). A key challenge to disentangle the benefit of plant richness and legumes from other farming practices in agricultural systems will be to move from artificial grassland plant communities towards more farming system factorial experiments integrating farming practices and soil disturbances characteristic of arable systems. Furthermore, further work should assess the impact of cropping systems on microbial communities and functions across years, to better understand emerging trends.

4.3. Conclusions

Using a long-term strip-cropping experiment, we found that the legacy effects of integrating legume cover crops and mulching — rather than increasing cash crop richness via strip-cropping — significantly impact the overall structure of fungal communities in organic potato-ley systems but not bacterial communities. Our findings, however, suggest that strip-cropping could enhance beneficial fungi like AMF, thereby supporting crop yield in organic systems, albeit mainly in the strip edge. Future studies assessing root colonization by AMF and crop growth rates are needed to evaluate how such an increase in AMF abundance is translated in crop functions. Finally, despite positive edge effects on potato fungal pathogens in strip-cropping systems, these did not relate to more significant tuber disease symptoms or yield losses. This finding might indicate greater soil suppressive effects against soil-borne pathogens at the edge of the strip, where crop plants interact, and warrant

further investigation. Taken together, our findings suggest that for sustainable agricultural practices aiming to improve soil health and crop productivity, a comprehensive approach that considers crop diversity, soil management practices, and environmental conditions is necessary.

Statement on inclusion

Our study brings together authors from a number of different countries, and research disciplines. The authors were engaged with the research to ensure that the diverse sets of perspectives they represent were considered.

CRediT authorship contribution statement

L.G.A. Riggi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **T. Ranheim Sveen:** Formal analysis, Writing – review & editing. **C. Castaño:** Data curation, Formal analysis, Writing – review & editing. **P. Onorati:** Data curation, Writing – review & editing. **D.F. van Apeldoorn:** Conceptualization, Methodology, Writing – review & editing. **M. Berri:** Writing – review & editing. **L. Mommer:** Conceptualization, Writing – review & editing. **K.E. Clemmensen:** Data curation, Methodology, Writing – review & editing. **M. Bahram:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105777.

Data availability

Data to support the findings of this study is available via Zenodo (https://doi.org/10.5281/zenodo.14211468).

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