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RESEARCH ARTICLE

Strip-cropping legacy enhances potato plant defence responses to aphids via soil-mediated mechanisms

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Abstract

1. Intensive agriculture often comes at the expense of soil health. A shift towards practices that foster soil health will support yield and defences against pests and diseases. Growing crops in monoculture is the standard in modern agriculture, though strip-cropping, in which different crops are planted in strips, is a promising strategy in the transition towards sustainable agriculture. Increasing crop diversity is hypothesized to positively influence arbuscular mycorrhizal fungi (AMF), thereby enhancing soil health, but the mechanisms by which AMF-plant symbioses contribute to higher yields and reduced pest populations in strip-cropping systems remain unclear.
2. We used a green-house experiment with potato plants to explore the soil legacy effects of mono-cropping versus strip-cropping systems, AMF inoculation and aphid infestation on AMF root colonization, the induction of plant defences (untargeted LCMS-based metabolomics), aphid population size and potato yield.
3. We found that potato plants grown in strip-cropping soil had higher AMF colonization than plants grown in mono-cropping soil. Potato plants grown in strip-cropping soil also had higher shoot nitrogen content, increased solamargine levels, and reduced aphid populations. AMF root colonization was only enhanced by the addition of commercial AMF in mono-cropping soils. Potato plant metabolites were affected by strip-cropping soil, including jasmonic acid (JA) derivatives. Structural equation models revealed that strip-cropping soil directly reduced aphid populations and also had a negative direct effect on the JA precursor OPC-8, and hydroxyJA-glucosides, indicating complex effects of strip-cropping soils on JA-inducible plant defences. Indirect benefits of strip-cropping soil and AMF

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inoculation on tuber yield were mediated by their direct positive effects on plant nitrogen content.

4. Our results emphasize the potential of strip-cropping to enhance AMF root colonization in the field. We show that soil legacy effects of strip-cropping alter the plant metabolome in ways that suppress of aphid populations. Strip-cropping legacy effects are the result of crop diversity, crop neighbour and edge effects resulting from crop management practices. While the mechanisms by which soil from strip-cropping supresses pest populations still need to be identified, our study underscores the potential for strip-cropping to enhance pest control and yield via soil mediated processes.

KEYWORDS
arbuscular mycorrhizal fungi, biological inoculation, crop diversification, jasmonic acid, metabolomics, pest population growth, soil health, *Solanum tuberosum*

1 | INTRODUCTION

Intensive agricultural production often comes at high environmental costs to soil health, resulting in decreased soil biodiversity and organic matter, with consequent increased soil erosion, and nutrient leaching (Yang et al., 2020). These environmental costs decrease beneficial below-ground interactions that foster plant growth and resilience against stress and increase the reliance of crop plants on external inputs for fertilization and pest management (Morris et al., 2007). To support the duelling priorities of farmers who are interested in growing crops using less environmentally harmful methods without yield costs, sustainable management alternatives are needed. Before the green revolution, diverse crop rotations and intercropping systems were common practices used to enhance soil health, increase plant resilience, and maintain crop productivity. The combined stressors of agricultural intensification and global climate change, resulting in more frequent pest outbreaks are causing extensive crop failures (Deutsch et al., 2018). Agricultural practices that benefit soil–plant interactions, thereby increasing plant resilience and crop yield, need to be restored (Rillig et al., 2019) and the mechanisms by which these practices confer benefit need clarification.

The importance of below-ground interactions between plants and soil biota is recognized in both natural and managed ecosystems (Bender et al., 2016). Soil microorganisms that associate with plant roots play a critical role in nutrient acquisition as well as increasing resistance to biotic stress, such as herbivorous pest insects (Friman et al., 2021; Larimer et al., 2010; Morris et al., 2007; Porter et al., 2020; Rho et al., 2018). Arbuscular mycorrhizal fungi (AMF), which engage in symbiotic interactions with more than 60% of terrestrial vascular plant species, can be used to improve the sustainable management of cropping systems (Kiers & van der Heijden, 2006; Rillig et al., 2019). AMF provide plants with nutrients, and in return, receive carbohydrates and fatty acids from their host (Lanfranco et al., 2018). In the context of crop production, AMF are typically valued for their potential to enhance plant growth, nutrient uptake, and yield (Garcia de Leon et al., 2020). Yet, they induce a range of changes in their host plant growth and metabolism which can alter plant tolerance to stress (Balog et al., 2017; Borowicz, 2013; Hartley & Gange, 2009; Song et al., 2015; Vannette & Rasmann, 2012), or plant defences against herbivorous insects (Jung et al., 2012; Koricheva et al., 2009; Pozo & Azcón-Aguilar, 2007). Under more stressful biotic and abiotic conditions, such as drought or herbivory, the benefits AMF confers on their plant hosts are expected to become more critical, reflecting a positive synergy (Porter et al., 2020).

Many widely grown crops form AMF symbioses (Hijri, 2016; Tran et al., 2019), but the potential of optimizing these beneficial interactions to reduce pest damage in agricultural systems remains largely unexploited. Indeed, soil inoculation using commercial AMF strains have often failed, as they do not capture the natural diversity in AMF communities (Hart et al., 2018; Jansa et al., 2006). Given that AMF responses are the product of complex interactions between plants, AMF species, the environment and farm management practices, this is not surprising (Ryan & Graham, 2018). While studies focusing on cosmopolitan AMF taxa, such as *Rizophagus irregularis* (Malik, 2018), are essential in advancing our fundamental knowledge of AMF-plant interactions, they provide only a partial understanding of crop interactions with AMF in the field. To realize the potential of AMF to enhance sustainable agriculture practices, how natural assemblages of AMF differentially shape crop plant-herbivore interactions needs to be examined (Rillig et al., 2019; Thirkell et al., 2017).

Diverse plant communities have been shown to enhance AMF communities in natural (Hiiesalu et al., 2014) and agricultural systems (Guzman et al., 2021; Montesinos-Navarro et al., 2012). In addition, crop diversity has been suggested to alleviate stresses associated with 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). Benefits of crop diversity on soil communities are expected to accumulate over time and profit the next generation of crop plants (i.e. legacy effects; Eisenhauer et al., 2012; Wang et al., 2021). Spatial crop diversification practices, such as strip-cropping, in which different crops are planted in alternating strips, can enhance ecosystem services, including pest and disease control, biodiversity and yield (Beillouin et al., 2021). Though crop diversification enhances soil health and beneficial plant–soil interactions, it remains unclear which mechanisms drive the positive effects on pest control in diversified cropping systems, and how increased AMF diversity and abundance contribute to bottom-up pest control.

Potato (*Solanum tuberosum*), the third most important food crop in the world (Gervais et al., 2021), suffers from aphid-transmitted viruses (Dedryver et al., 2010). Intercropping potato with cereals or leys reduces aphid populations and fungal pathogens (Bouws & Finckh, 2008; Nakahira et al., 2012), and could increase AMF diversity and abundance (Guzman et al., 2021; Montesinos-Navarro et al., 2012). Potato readily associates with several AMF strains. AMF inoculation increases potato yield (Douds et al., 2007; Hijri, 2016) and improves resistance to pathogens (Alaux et al., 2018, 2020; Bharadwaj et al., 2008; Gallou et al., 2011; Ismail et al., 2012; Yao et al., 2002). The AMF-mediated plant resistance to pests and pathogens is proposed to be supported by the metabolization (i.e. induced production) of phytohormones, including salicylic acid, jasmonic acid (JA) and their derivates (Miozzi et al., 2019). In another Solanaceae crop, AMF inoculation reduced the number of aphids by increasing plant-induced anti-nutritive defences (Balog et al., 2017).

Despite its potential, strip-cropping legacy effects on AMF abundance and its downstream effects on herbivorous insects have seldom been quantified (but see: Zhang et al., 2024). Strip-cropping legacy effects are the result of complex interactions between crop diversity, crop-combination identity and crop management on soil. This study explored the effects of soil collected from long-term organic mono- and strip-cropping farming systems and AMF inoculation on potato plant growth and the induction of defences against the major vector of potato virus Y, the green peach aphid, *Myzus persicae*. Specifically, these experiments aimed to test the hypotheses: (1) that strip-cropping results in higher AMF root colonization of potato than commercial AMF inoculation, (2) that greater AMF root colonization in the strip-cropping soils increases tuber yield, particularly under herbivory stress (*positive synergy*) and (3) that greater AMF root colonization in the strip-cropping soils affects above-ground potato metabolites, and induces plant defences that decrease pest population size. These hypotheses were assessed using structural equation models to disentangle the direct and indirect pathways between AMF root colonization, pest abundance and yield. In addition, interactions between AMF inoculation, herbivory and strip-cropping soil legacy were further validated using generalized mixed models.

2 | MATERIALS AND METHODS

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Individuals (plants)	Individuals	80 individuals/10 replicates per treatment combination

2.1 | Greenhouse experiment on aphid populations and plant performance

We conducted a complete randomized block design greenhouse factorial pot experiment with eight treatments and 10 replicates per treatment ($n=80$) with one potato plant (*S. tuberosum*, 'Fontane') per 5L pot, at Wageningen University, Netherlands, between February and April 2022. The experiment consisted of three factors, 'Strip-cropping legacy' (with two levels), 'AMF inoculation' (with two levels) and 'Herbivore' (with two levels). The greenhouse was organized in 10 spatial blocks and each of the eight treatments was present in each block.

For the strip-cropping legacy treatments, plants were grown either in soil from the middle of 100 m^2 mono-cropped plots (*mono*) or from the edge of strip-cropped plots (*strip*), collected from the long-term organic strip-cropping experiment at Wageningen University (51°59′33.06″ N, 5°39′43.56″ E; Supplementary Material, Figure S1). The pre-crops were the same in each soil treatment (i.e. barley), while pumpkin was grown in the neighbouring strip in the strip-cropping fields. Soil from each treatment was homogenized, air-dried and sieved prior to potting. To explore AMF communities in each homogenized soil inoculum, fungal communities were assessed via sequencing following the protocol of Liu et al. (2023, Supplementary Material S2). Soils were classified as sandy and did not differ in nutrients and organic matter content (Table S1).

The AMF inoculation treatment consisted of plants with (AMF+) and without (AMF0) commercial AMF (*Rhizophagus irregularis* spores in liquid solution inoculated at planting, Koppert Biological Systems, Supplementary Material S3). AMF colonization can be very rapid, especially under green-house pot conditions. To avoid missing differences in colonization between treatments and to assess effectiveness of AMF inoculation in mono- and strip-cropping soils prior to harvest, a full-factorial experiment was set-up with eight potato plants randomly assigned to a soil legacy treatment and AMF inoculation treatment ($n=32$ pots). Four plants per treatment combination were destructively sampled 40 and 60 days after planting to assess AMF colonization prior to harvest (method described below).

The herbivore treatment consisted of plants with and without aphid infestation (*Myzus persicae*). Plants were infested with aphids 6 weeks after planting of the seed potato. Herbivore infestation was done on two scales: on the leaf, to assess treatments effects on the plant metabolome, and on the whole plant, to assess treatments effect on yield and aphid population size (Supplementary Material S4). At the leaf scale, four late instar nymphs were placed on a fully expanded leaf just below the growth apex of the plant and covered with a leaf bag, the leaf bag, including the leaf, were collected after 2 weeks for metabolomics analyses. At the plant scale, three late-instar and two early instar nymphs were placed randomly on the other parts of the plants and the whole plant was covered with a mesh bag. The aphid infestation at the whole-plant scale lasted 4 weeks. All plants, including control plants, without herbivores, were covered with both the leaf and plant bag to keep them under the same light conditions (Supplementary Material S4).

Four weeks after aphid infestation (90 days after planting), whole potato plants were harvested and number of aphids counted at the whole-plant scale. Potato shoots and roots were separated at harvest. An equal weight subsample of roots per plant was stored in 70% alcohol for AMF root colonization analyses (Supplementary Material S5). Shoot and root dry weight were determined after 96 h of drying at 65°C. To quantify AMF root colonization, potato root subsamples before harvest (40 and 60 days after planting, $n=32$) and at harvest (90 days after planting, $n=80$) were stained using ink and vinegar and the proportion of AMF root colonization (including all AMF structures: hyphae, arbuscules, and vesicles) was determined using 100 gridline intersect method (Giovannetti & Mosse, 1980; Supplementary Material S5). After measuring dry weight, leaf nitrogen (N) and phosphorus (P) content were spectrophotometrically determined in grounded leaves following H2SO4-Se digestion, according to the protocol of Houba et al. (2000). Tuber number and fresh weight per plant were quantified at harvest, and used to calculate total tuber weight per plant (g) and mean individual tuber weight per plant (g).

2.2 | Liquid chromatography mass spectrometry

To assess treatment effects on the plant metabolome, bagged leaves were collected from the control and herbivore treatments and flash-frozen in liquid nitrogen 2 weeks after aphid infestation and stored at −80°C. Frozen leaf material was ground into a fine powder under liquid nitrogen using a ball shaker device (74 of the 80 leaves were recovered for further analyses, as 6 leaves were thawed and therefore discarded). A quality control sample was prepared by pooling the powders from a random mixture of leaf samples from each treatment. The samples were extracted and submitted for untargeted liquid chromatography-mass spectrometry (LC–MS) profiling as described in Garrido et al. (2021) (for protocol see Supplementary Material S6).

LC–MS results were processed and aligned using Metalign software (Lommen, 2009) following the procedure described by Garrido et al. (2021). After filtering, mass peaks were clustered using MSClust software (Tikunov et al., 2012). This clustering resulted in relative intensity data for 1169 putative compounds across samples. The compound intensity was calculated from the sum of intensities of all mass peaks in its cluster, that is total ion count. The base peak mass was automatically searched in the KNApSAcK metabolite database (http://kanaya.naist.jp/knapsack) with '*Solanaceae*' as a filter, for possible elemental formula and annotation using a maximum mass deviation of 5 ppm. Detailed information regarding LC–MS data processing and metabolite annotations can be found in Supplementary Material S7. In addition, linear mixed effect models (LMMs), and a random forest approach were used to identify compounds that strongly associated to treatments (Supplementary Material S8). These methods confirmed 85 compounds of interest, which were subsequently manually checked and annotated based on in-house databases and indicative in-source fragments, if present. Using this combination of methods, 184 compounds were putatively annotated, with 37 compounds annotated manually (Supplementary Material S8).

2.3 | Statistical analyses

To investigate the hypothesis that soil with a strip-cropping legacy will benefit AMF root colonization more than commercial AMF inoculation, we analysed the effect of AMF inoculation and strip-cropping legacy treatment on AMF root colonization before and at harvest, using two models. Before harvest, a model with AMF inoculation (AMF+/AMF0), soil legacy (mono/strip), and days after planting (i.e. 40 [$n=16$], 60 [$n=16$] days after planting) and their three-way interaction was analysed using a linear model. At harvest, fixed factors included soil legacy, AMF inoculation and herbivore treatments and their three-way interactions, with block as a random effect in *glmmTMB* (Magnusson et al., 2021). AMF root colonization proportion models were fitted with a binomial distribution. Model assumptions were checked using *DhArma* (Hartig & Lohse, 2020). At harvest, an individual level random effect was included to account for overdispersion in the AMF colonization data. Standard model reduction with AICc and step-wise removal of non-significant variables was applied. When significant effects were found, post-hoc tests using *emmeans* (Lenth et al., 2023) were carried out.

To investigate the treatment effects on potato leaf metabolome (i.e. metabolite composition), metabolomic data were log-transformed (0's were replaced by half of the minimal observed value for each compound, Wei et al., 2018) and scaled and analysed using NMDS (Garrido et al., 2021). PERMANOVA was used to determine the statistical significance of strip-cropping legacy, AMF inoculation and herbivore treatments alone and in combination using *adonis* function from the *vegan* package (Oksanen et al., 2022). To assess treatment effect on plant metabolites, the 184 putatively annotated metabolites were analysed for treatments effects using general linear mixed effect models (*glmmTMB*), with individual metabolite intensity as response variable and interactions between treatments as explanatory variables and block as random effect. To account for multiple comparisons, a false discovery rate correction was applied (Benjamini & Hochberg, 1995), and for significant models, post-hoc tests were run. Out of the 184 initially annotated putative compounds, 29 compounds were significantly affected by treatments (Supplementary Material S8). These compounds were grouped into six biochemical classes (i.e. primary metabolites and respiratory products, indolic compounds, terpenoids, phenylpropanoids, steroids and octadecanoid compounds, associated with jasmonic acids, Figure S8) and were assessed for inclusion in the piecewise structural equation model (pSEM) analyses.

Direct and indirect effects of strip-cropping legacy and AMF inoculation at harvest on potato tuber yield and aphid population size were investigated using pSEMs (*piecewiseSEM*, Lefcheck, 2016). Two pSEMs models were built to capture the hypothesized hierarchical relationships of treatments on tuber yield ($n=80$) and on aphid population size (proxy for resistance to pest infestations, $n=40$; Figure 1). Detailed information regarding data processing and analyse for the pSEMs can be found in Supplementary Material S9. To determine standardized pSEM path coefficients, raw coefficients were scaled. Fischer's C statistic, based on p-values calculated from a test of directed separation, was used to evaluate full model fit and missing paths, that is paths not defined in the component models, were included when these improved model fit. A chi-squared test of Fischer's C with $p>0.05$ indicates that the full model represents the data well and that there are no missing paths. If significant missing paths were identified during analysis, or when bidirectional effects were expected, the alternate model was compared using AICc.

Finally, to further investigate all potential two- and three-way interactions between strip-cropping legacy, herbivory and AMF inoculation treatment on plant and aphid population size, we used generalized linear mixed effect models (GLMMs, *glmmTMB*). These analyses complement the pSEMs models that are not as robust as GLMMs to assess interactions between several factors. To test the hypothesis that soil with a strip-cropping legacy will lead to higher plant growth, particularly under herbivory stress, we assessed the interactive effects of strip-cropping legacy, AMF inoculation and herbivory at harvest (90 days after planting) and their two- and three-way interactions on plant response variables—including shoot and root dry weight, tuber count per plant, total and mean individual tuber weight per plant, and shoot N and P content. To account for differences in sizes of the planted potato tuber on biomass and yield measurements, weight of the original potato tuber was included as a co-variate in these models. All plant response variables were fitted with a Gaussian distribution except for tuber count per plant fitted with a Conway-Maxwell Poisson distribution to correct for overdispersion. To assess the hypothesis that greater AMF root colonization in the soils with a strip-cropping legacy will decrease pest population size, aphid count data were analysed on the subset of potato plants infested with aphids ($n=40$), using a Poisson distribution after checking model fit and overdispersion. Fixed factors included soil legacy (mono/strip), AMF inoculation (AMF0/AMF+) and their two-way interaction. Block was included in all models as a random effect. R^2 was calculated using *r.squaredGLMM* (MuMIN, Barton, 2018). All analyses were done in R (version 4.2.1).

3 | RESULTS

3.1 | AMF colonization

At 40 and 60 days after planting, potatoes grown in soil with the strip-cropping legacy showed more natural AMF root colonization in treatments without additional inoculation (control AMF0) compared to potatoes grown in soil with the mono-cropping legacy (root colonization of 33.0 and 32.2% in strip-cropping soil vs. 14.8 and 13.9% in mono-cropping soil at 40 and 60 days after planting, respectively; Figure 2, Table S11). The additional AMF inoculation treatment (AMF+) resulted in significant increase in the proportion of AMF root colonization compared to the control for potatoes grown in soils with a mono-cropping legacy, but not a strip-cropping legacy (Figure 2, Table S11). At harvest, 90 days after

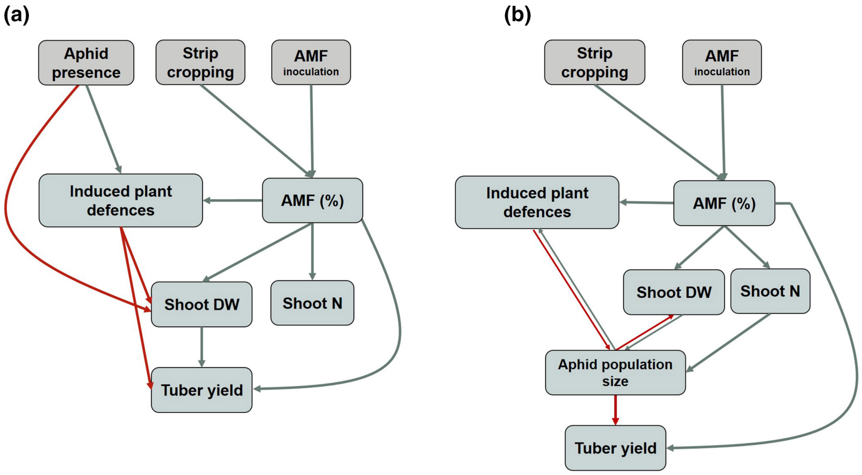

FIGURE 1 The diagram shows the hypothesized effects that were tested in the preliminary component mixed models for (a) tuber yield ($n=80$) and (b) aphid population size ($n=40$) pSEMs. (a) We hypothesized that aphid presence would induce plant defences, which are costly to produce and thus negatively affect shoot biomass (dry weight, DW) and yield. However, we expected strip-cropping soil and AMF inoculation to positively affect yield and biomass via enhanced AMF root colonization. (b) When aphids were present, we expected strip-cropping soil and AMF inoculation to up-regulate plant defences and positively affect biomass and yield via AMF root colonization, leading to reduced aphid population size and increased yield. Grey and red lines represent hypothesized positive and negative effects respectively, double arrows show relations that are bidirectional. Shoot nitrogen (N, mmol/kg) and phosphorous (P) were positively correlated (rho = 0.6, $p<0.001$) for both pSEMs models and therefore only shoot N was included in the models. Only plant compounds that were selected by the *dredge* method were included as “induced plant defence” (Supplementary Material S9). These pSEMs were built to specifically test for hypothesized effects of treatments on yield and aphid population size rather than to test all possible effects of metabolites and AMF root colonization on potato yield and aphids.

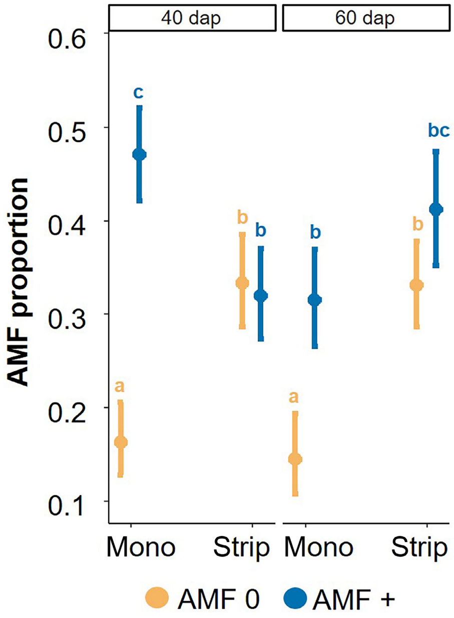

FIGURE 2 Model estimates of the effects of soil (strip- (strip) vs. mono-cropping (mono) soils) and AMF inoculation (control conditions [AMF 0] vs. AMF inoculated [AMF +]), on the proportion of AMF root colonization of potato plants before harvest (40 and 60 days after planting [dap]). Letters indicate significant post-hoc test differences ($p < 0.05$), dots represent estimated mean values and bars show 95% confidence intervals (CI).

planting, AMF root colonization was not significantly affected by strip-cropping legacy or AMF inoculation (Figure S2). Fungal community sequencing indicated greater AMF relative abundance in the homogenized strip-cropping compared to mono-cropping soil inoculum (Figure S2).

3.2 | Metabolome differences between treatments

Leaf metabolites significantly differed between soils with a mono- and strip-cropping legacy (sum of squares: 0.003, F: 2.60, R^2: 0.03; p: 0.044) and aphid infested and uninfested plants (sum of squares: 0.008, F: 7.14, R^2: 0.09; p: 0.001), independent of AMF inoculation treatment (Figure 3A,B). Although the first axis of the NMDS did not relate to any higher order variables (i.e. shoot dry weight, aphid control, shoot N and tuber weight) in either pSEM model, 29 out of 184 annotated metabolites were affected by treatments based on GLMMs (Figure S8). Strip-cropping affected six compounds in total, including a reduction in a compound putatively identified as 3-Oxo-2-(2-entenyl) cyclopentaneoctanoic acid (OPC-8), a precursor of the plant defence hormone jasmonic acid (JA) (Züst & Agrawal, 2016). AMF inoculation resulted in a reduction in the inactive JA-forms hydroxyl-JA and methylhydroxy-JA glucosides in strip-cropping soils, possibly an indication of weaker downregulation of downstream JA responses compared to mono-cropping soil (Miersch et al., 2008). AMF inoculation also resulted in a reduction in the terpenoid-glycoside linalool-3-rutinoside independent of soil type. An increase in solamargine, a steroidal glycoalkaloid known for its cytotoxic effects leading to reduced aphid reproduction (Güntner et al., 2000), was observed in strip-cropping, but not in mono-cropping soils. Independent of soil type or AMF inoculation, aphid presence down-regulated the terpenoid-glycoside linalool-3-rutinoside and up-regulated compounds in the phenylpropanoid pathway, known to be involved in antimicrobial activity and defence signalling (Bauters et al., 2021), via an increase in several phenylpropenoic acid conjugates (caffeoyl dopamine, coumaroyl alcohol glucoside, feruloylquinic acid). In locally infested leaves, aphids also up-regulated torvonin A and Indole-3-acetic acid (IAA) alanine, the latter indicative of plant growth modulation via the auxin phytohormonal pathway (Chou & Huang, 2005). Tuberonic acid glucoside, involved in tuber formation, also significantly decreased during aphid infestation, indicating that below-ground growth was halted by herbivory. For more insights in treatment effects on metabolites see Supplementary Material S6.

3.3 | Direct and indirect effects of treatments on tuber yield and aphid population

Two piecewise SEMs were used to explore the network of correlations between AMF inoculation, aphid infestation and soil treatment on tuber yield and aphid population size separately. Seven putatively annotated plant metabolites were identified as the most relevant non-correlated metabolites as explained in the methods (Supplementary Material S9). These included: methylhydroxy-JA glucoside, α-ketoglutarate (AKG), N-(phenylmethyl) adenosine, L-tyrosine, coumaryl alcohol glucoside, the steroidal glycoalkaloid torvosid H and linalool-3-rutinoside, a terpenoid-glycoside.

In the full pSEM model, all treatments indirectly affected yield by influencing plant nitrogen which positively related to tuber yield. There was a negative relationship between aphid presence and plant nitrogen, while strip-cropping soil and AMF inoculation were positively associated to plant nitrogen (Figure 4a). There was a negative effect of aphid presence on tuber yield via the downregulation of α-ketoglutaric acid, which plays a key role in energy metabolism in the tricarboxylic acid cycle (Wu et al., 2016). AMF inoculation had an overall positive effect on shoot biomass (sum of direct and indirect effects = 0.13) and aphid presence and strip-cropping soil had an overall negative impact on shoot biomass (−0.37 and −0.11, respectively; Figure S10).

With regard to the aphid population model, strip-cropping soil had contrasting direct and indirect effects on aphid population size (Figure 4b). Positive indirect effects of strip-cropping on aphid population size were associated with a reduction in methylhydroxy-JA glucoside, but the direct negative effect of strip-cropping legacy on aphid population size was greater than the indirect positive effect (−0.43 and 0.22 respectively), resulting in an overall negative effect (−0.21). We also found indirect negative effects of AMF root colonization on aphid population size through increased methylhydroxy-JA glucoside accumulation.

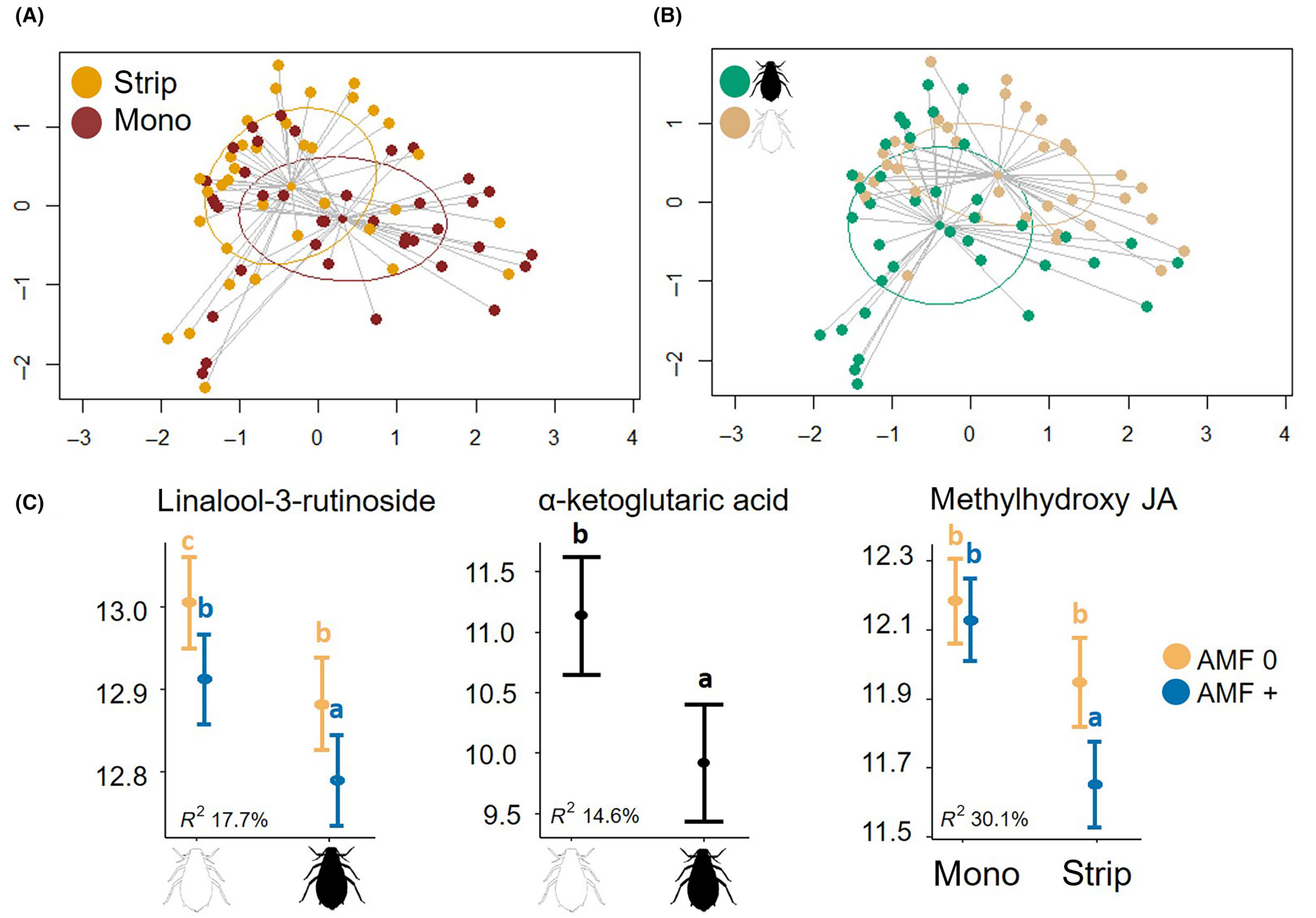

FIGURE 3 Non-metric multidimensional scaling ordination plots showing differences in potato leaf metabolome assemblages between (A) strip- and mono-cropping soils and (B) herbivore and no-herbivore treatments (NMDS stress: 0.148, dots corresponds to leaf metabolome assemblage per plant). (C) LMMs model outputs showing the effect of herbivore presence, soil legacy and AMF inoculation treatments on the intensity (i.e. total ion counts per scan at chromatographic peak height) of the three plant induced compounds (log-transformed) involved in the pSEMs models (Figure 4). White aphids represent no aphid infestation, and black aphids represent aphid infestation treatments.

3.4 | Effects of strip-cropping soil and AMF on plant and aphid performance

GLMMs analyses confirmed the pSEMs result that, potato shoots grown in soil with a strip-cropping legacy had higher nitrogen content than potato shoots grown in soil with mono-cropping legacy (mmol/kg, est. ± SE = 148 ± 57.5; $p = 0.012$, Figure S11, Table S11). In addition, in line with the pSEMs, the addition of AMF inoculate to mono- and strip-cropping legacy soils increased mean tuber weight compared to the control (AMF0) (grams (g), est. ± SE = 6.59 ± 3.09; $p = 0.036$, Figure 5A, Table S11). When plants were exposed to herbivory, AMF inoculation buffered the negative effects of aphid presence on shoot biomass (Figure S11) and shoot nitrogen content (Figure 5B, Table S11). Potato root biomass (i.e. excluding tubers) was marginally increased by AMF inoculation (g, est. ± SE = 0.13 ± 0.06; $p = 0.062$), and 20% lower in plants infested with herbivores (g, est. ± SE = −0.21 ± 0.07; $p = 0.004$; Figure S11). In line with the pSEM results, aphid population size was higher on potato plants grown in soils with a mono-cropping legacy than strip-cropping legacy (est ± SE = 0.39 ± 0.14; $p = 0.045$), but inoculation with AMF mitigated this effect with comparable aphid population sizes between soils with a mono- and strip-cropping legacy (Figure 5C). These results indicate positive cascading effects of strip-cropping legacy soil on the capacity for potato plants to supress aphids, and positive effects of AMF inoculation on tuber yield and shoot biomass, particularly under aphid herbivory.

4 | DISCUSSION

The legacy effects of increasing crop diversity through strip-cropping have been shown to support AMF communities, plant health, and pest control, but the complex relationship between these is poorly understood in agricultural soils. Here, we begin to elucidate the mechanisms by which strip-cropping and the presence of AMF confer benefits to crop plants. Our data showed that potato plants grown in soil with a strip-cropping legacy engaged

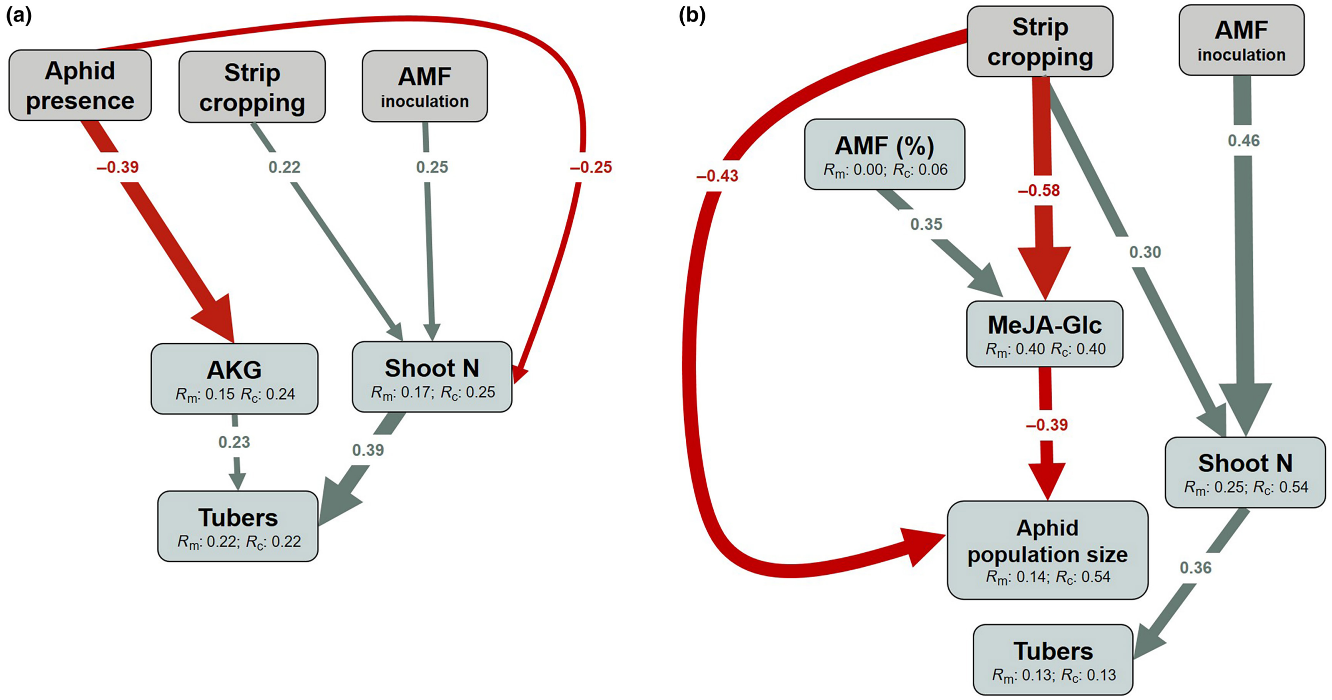

FIGURE 4 Piecewise structural equation models showing the direct and indirect effects of treatments on (a) total tuber weight per plant (Chisq = 12.93, $p = 0.93$, $n = 22$; Fisher's $C = 41.67$, $p = 0.572$ and $n = 44$) and (b) aphid population size (Chisq = 8.04, $p = 0.999$, $n = 25$; Fisher's $C = 59.30$, $p = 0.173$ and $n = 50$). Standardized coefficients are provided for each relationship and marginal (R_m) and conditional (R_c) R^2 are provided for each response variable. Grey and red arrows indicate significant ($p \leq 0.05$) positive and negative relationships respectively. The width of each arrow is proportional to the standardized path coefficients. Only compounds that related to higher order variables were included to avoid overfitting the models. MeJA-Glc, methylhydroxy jasmonic glucoside; AKG, α-ketoglutaric acid. All variables and hierarchical mixed models used in the analyses are summarized in Supplementary Material S8 and full pSEM including biomass data are presented in Figure S11.

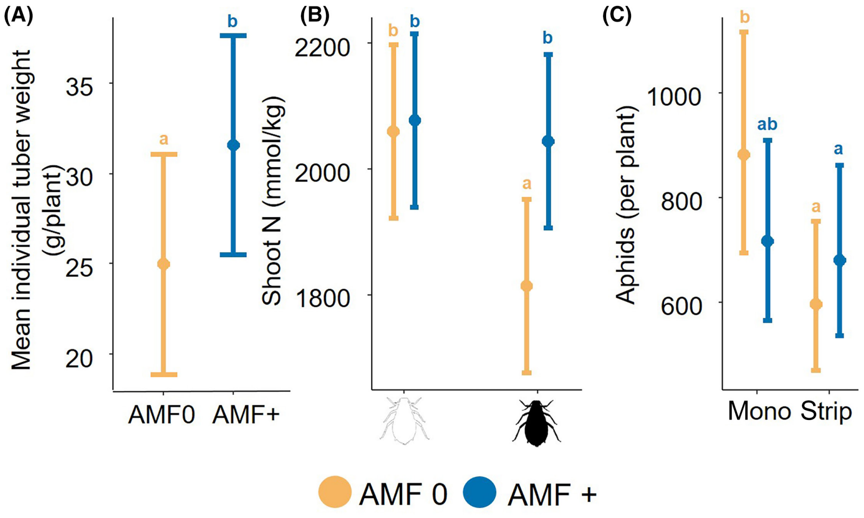

FIGURE 5 Models estimates of the main and interactive effects of AMF inoculation on plant and aphid response variables on (A) mean individual tuber weight (g); (B) shoot N content (mmol/kg) and (C) aphid population size at harvest. Letters indicate significant post-hoc test differences ($p < 0.05$) and bars show 95% CI. White aphids represent no aphid infestation, and black aphids represent aphid infestation treatments.

more in AMF symbiosis and had greater AMF root colonization than plants grown in mono-cropping soil during the early establishment phase. Inoculating AMF benefited AMF root colonization more in mono- than in strip-cropping soil up to 60 days after planting. Both direct and indirect positive effects of strip-cropping soil on potato yield and aphid pest management were found. We highlight indirect benefits of strip-cropping and AMF inoculation on tuber weight via positive effects on shoot nitrogen, as well as complex interactions between strip-cropping soil and plant metabolites involved in plant defence pathways. Finally, we show, that strip-cropping legacy alters the potato metabolome in ways that are ecologically significant for aphid population growth and tuber yield, with overall positive effects of strip-cropping soil legacy on tuber yield and aphid population control.

4.1 | Strip-cropping legacy increases natural AMF colonization

In recent years, there has been debate about the effectiveness of commercial AMF products to colonize crops and promote crop health in the field due to environmental factors influencing the performance of commercial inoculants (Verbruggen et al., 2013). Our results indicate that commercial AMF inoculation is most useful when there is not yet AMF-friendly management (e.g. crop diversity). Indeed, we find that commercial AMF inoculation was associated to greater AMF root colonization in mono- but not in strip-cropping soils in the early stages of plant growth. Two critical environmental factors determining the success of commercial AMF inoculants are the availability of plant-accessible phosphorus and the presence of indigenous mycorrhizae communities (Jiang et al., 2021; Verbruggen et al., 2013), however, there were no differences in plant-available soil phosphorus between strip- and mono-cropping soils (Supplementary Material S1). We hypothesize that the success of commercial AMF inoculation in colonizing the roots of potato plants in mono-cropping soils is linked to the lack of native AMF communities in soil with a monocrop legacy. Higher plant richness is often associated with more abundant and diverse AMF communities (Guzman et al., 2021; König et al., 2010). In line with this, the relative abundance of AMF taxa sequence reads was higher in the strip- compared to the mono-cropping soil inoculum (Supplementary Material S2). Therefore, AMF colonization was faster in plants grown in strip- compared to mono-cropping soils, giving plants grown in strip-cropping soils a head start early in their growth. Commercial AMF inoculation did benefit mean individual tuber weight, potentially indicating a change in growth regulators in AMF inoculated treatments (Dutt et al., 2017). This might be due to the specific AMF strain inoculated, *R. irregularis*, which has been shown to benefit potato size, possibly via changes in tuber growth regulators (Hijri, 2016). Augmenting field AMF communities and crop root colonization could be achieved by increasing crop species richness as well as other AMF-friendly management practices such as reduced tillage and fungicide applications. While further studies are needed to clarify mechanisms, and define the impact of strip-cropping on AMF communities, our results showed that AMF inoculation might not be as effective in farming systems already adopting AMF-friendly management practices like strip-cropping.

4.2 | AMF inoculation benefits are more pronounced under herbivory

Under stressful pest infestation conditions our results indicate greater plant nutrient resource acquisition in plants inoculated with AMF. AMF inoculation was positively associated with plant nitrogen and biomass in plants infested with aphids. This supports the hypothesis that the benefits of AMF (e.g. nutrient uptake and plant growth) are more pronounced under stressful conditions, such as herbivory (*positive synergy*; Porter et al., 2020). As expected, we found that positive effects of AMF inoculation on tuber yield were mediated by higher shoot N. The same pathway, and of similar strength, was found for the positive effect of strip-cropping soil on tuber yield. Positive associations of strip-cropping on plant nutrients might also be linked to earlier AMF root colonization in strip- compared to mono-cropping soils. Complex interactive effects between aphids and AMF inoculation on total tuber yield might have been missed as tuber growth was constrained by pot size and early harvest. AMF symbiosis and nutrient allocation in relation to induced stressed conditions is a complex and dynamic process and future studies should assess plant nutrient allocation in parallel to AMF root colonization over time.

4.3 | Strip-cropping legacy and herbivory affects plant metabolome

The impacts of strip-cropping legacy on the potato leaf metabolome were likely due to changes in micro-biota and soil resources. This is in line with studies in other mixed-cropping systems, where changes in secondary metabolite leaf composition in intercropped treatments enhanced plant defences against herbivory and crop quality (Duan et al., 2021; Wu et al., 2021; Zhang et al., 2024). Our untargeted metabolome study revealed that strip-cropping soil legacy modulates both compounds required for de novo biosynthesis of JA as well as downstream JA conjugates and JA-induced secondary metabolites. Specifically, strip-cropping soil legacy reduced the putatively annotated compound OPC-8, a precursor of jasmonic acid (Züst & Agrawal, 2016) and structural equation models showed that strip-cropping soil legacy down-regulated JAglucosides and some terpenoids. Although it is disputable how to interpret these findings without insights in other modulations along the JA pathway, they seem to indicate that at the sampling time, which was 2 weeks after aphid infestation, plants in strip-cropping soil attenuated de novo biosynthesis of JA, but retained existing JA activities by suppressing JA hydroxylation and glucosylation (Caarls et al., 2017; Miersch et al., 2008). Exact implications need further characterization of JA molecules on different time points. Production of secondary metabolites is regarded as costly and can inhibit plant growth (Erb & Kliebenstein, 2020). Up-regulation of JA-glucosides in leaves in potato plants grown in mono-cropping soils and in plants that did not receive AMF inoculation might indicate a stronger switch off of costly defences compared to plants grown in strip-cropping soil and AMF inoculated plants. Apart from soil-mediated effects, aphid presence significantly altered the leaf metabolome. Hydroxy-JA glucoside and several phenylpropanoids were up-regulated when aphids were present, indicating on one hand down-regulation of previous JA accumulation via hydroxylation and glucosylation (Caarls et al., 2017; Miersch et al., 2008), but also an accumulation of other plant defence compounds. Aphid infestation had a detrimental effect on potato yield and biomass, which could be linked to removal of photosynthates via feeding, induction of costly defence responses, and/or aphid-induced changes in source-sink relationships (Watts et al., 2023). We found indirect negative effects of herbivory on tuber yield via decreased shoot nitrogen content and via down-regulation of α-ketoglutaric acid, an important metabolite implicated in the energy metabolism (Cheng et al., 2022). Herbivore damage often decreases the concentrations of nitrogen in shoots (Karban & Myers, 1989; Newingham et al., 2007). Reduced shoot nitrogen in plants infested with aphids could be due to plant reallocation of nitrogen away from the sites of damage (*plant defence mechanisms*; Newingham et al., 2007). A negative effect of aphids on plant nitrogen appears to be compensated for by AMF inoculation and strip-cropping practices that enhance AMF populations which directly facilitate increased plant uptake of nitrogen. To assess how herbivory and AMF interactively affect nitrogen allocation more detailed components of aphid performance (e.g. individual fecundity) and plant nitrogen budget (e.g. nitrogen in the roots and tubers) should be assessed (Newingham et al., 2007).

4.4 | Strip-cropping legacy affects pest population size

Soils with a strip-cropping legacy suppressed aphid population size. The mechanisms driving this direct negative effect could not be associated with effects on plant nutrients or specific plant metabolites The direct negative effect of strip-cropping legacy on aphid population size is likely due to soil-mediated changes in induced plant defences or plant tolerance that were not captured in the analyses performed here. When potato plants were exposed to aphid herbivory, we observed a higher abundance of solamargine in plants grown in soil with a strip-cropping legacy compared to mono-cropping soil. This glycoalkaloid is known to have negative effects on the reproduction of the potato aphid *Macrosiphum euphorbiae* (Güntner et al., 2000) which may explain the observed reduction in *M. persicae* aphid population size in strip-cropping soils. In contrast to the strip-cropping, we did find indications that AMF could affect aphid performance via the JA pathway. Greater AMF root colonization during the earlier stages of plant growth in strip-cropping soil might have negatively affected aphid reproduction at plant harvest. This is in line with the finding that, greater AMF root colonization at harvest positively affected methylhydroxy-JA glucoside accumulation (Figure 4), which is indicative for the downregulation of a stronger JA induction during the previous infestation weeks, which may have supressed aphid population growth (Aslam et al., 2022; Cao et al., 2014). The indirect negative effect of AMF on aphid population in our experiment supports results found in other studies of negative effects of AMF on aphid population size which is associated to changes in plant induced defences (Bezemer & van Dam, 2005; Gehring & Whitham, 2003; Guerrieri et al., 2004; Hempel et al., 2009), though there are inconsistencies in the literature (Babikova et al., 2014; Hartley & Gange, 2009; Koricheva et al., 2009). With this, our study indicates that a strip-cropping legacy affects the leaf metabolome and alters plant defence responses (i.e. upregulation of solamargine and downregulation of OPC-8) via soil-mediated mechanisms. These results are consistent with other studies in other mixed-cropping systems (Zhang et al., 2024). Strip-cropping legacy effects could result from crop diversity, the identity of the neighbouring crop or/and crop management practices at the edge of the strip. In our study, pumpkin was grown adjacent to the barley strip, and *Cucurbitaceae*-specific secondary metabolites, such as cucurbitacin, may also have exerted an effect on the recruitment of soil microbes (Kusstatscher et al., 2021). In future studies, targeted quantification of JA, salicylic acid and their conjugates as well as other defence metabolites and expression of defence-related genes in diverse cropping systems will help us to understand the underlying mechanisms of soil-mediated suppression of aphid pests in these systems.

5 | CONCLUSIONS

Alternative cropping strategies that increase plant resilience to stressors are needed to transition towards sustainable agriculture. We found that potatoes grown in strip-cropping soil exhibited increased natural AMF symbiosis compared to those in mono-cropping soil, possibly explaining the higher shoot nitrogen content and reduced aphid population size in strip-cropping soil. The direct positive effects of strip-cropping legacy on pest management could also be attributed to alterations in other soil microbiota and resources that impact plant defences. As AMF symbioses are dynamic, future studies need to assess these relations over time to unravel the complex relationships between AMF, plant nutrient status and yield. Strip-cropping legacy effects could result from crop diversity, the identity of the neighbouring crop or/and crop management practices at the edge of the strip. While this study does not allow to disentangle the relative contribution of these different effects, our results emphasize the potential of strip-cropping to enhance AMF root colonization in the field. Additionally, commercial AMF inoculation countered the lack of AMF root colonization in mono-cropping soils in early stages of plant growth, and benefited plant nutrient acquisition and biomass, particularly under aphid infestation, supporting the idea that AMF benefits are more pronounced in stressful conditions. This research also identifies changes in plant metabolome and defence responses, influenced by strip-cropping legacy effects and AMF inoculation, which lead to reduced aphid populations. Specifically, we highlight complex relationships between strip-cropping and the JA pathway. While mechanisms supporting the direct benefits of strip-cropping soil on pest control still need to be uncovered and tested across soil types and crop combinations, our study underscores the potential for integrated approaches like strip-cropping, to enhance pest control and overall plant health via soil mediated mechanisms.

AUTHOR CONTRIBUTIONS

Laura G. A. Riggi, Karen J. Kloth, Roland Mumm, Thijs P. M. Fijen, Dirk F. van Apeldoorn and Liesje Mommer conceived the idea. Laura

G. A. Riggi, Aandi N. Dirham, Onikepe R. Akangbe; Ric C. H. de Vos, Jeroen van Arkel, Roland Mumm; Karen J. Kloth planned and executed the experiment. Laura G. A. Riggi, Aandi N. Dirham, Onikepe R. Akangbe, Ric C. H. de Vos; Jeroen van Arkel, Roland Mumm processed the data. Laura G. A. Riggi; Aandi N. Dirham; Onikepe R. Akangbe, Sara E. Emery analysed the data. Laura G. A. Riggi and Karen J. Kloth wrote the manuscript. All co-authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Data to support the findings for this study and the metabolomics data produced are available from the Swedish National Data repository https://doi.org/10.5878/kr3s-6v85 (Riggi et al., 2024).

STATEMENT ON INCLUSION

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

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SUPPORTING INFORMATION

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

Supplementary Material S1. Experimental site and soil characteristics.

Supplementary Material S2. AMF communities and AMF structures.

Supplementary Material S3. AMF treatments.

Supplementary Material S4. Aphid infestation and assessment.

Supplementary Material S5. Step-by-Step root staining protocol.

Supplementary Material S6. Liquid Chromatography Mass Spectrometry protocol.

Supplementary Material S7. LC-MS data processing and metabolite annotations.

Supplementary Material S8. Compounds annotations and metabolic pathways.

Supplementary Material S9. Piecewise SEMs analyses.

Supplementary Material S10. pSEMs results.

Supplementary Material S11. GLMMs results.

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