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EDITED BY
Kevin King,
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Agharkar Research Institute, India
Pavel Matusinsky,
Palacký University, Olomouc, Czechia

*CORRESPONDENCE
Jürgen Köhl
jurgen.kohl@wur.nl

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Population dynamics of *Alternaria solani*, *Cercospora beticola*, *Ramularia beticola*, and *Stemphylium beticola* in residues of host crops, non-host crops, and weeds in Dutch rotation systems

Jürgen Köhl[1]*, Georgina Elena[1], Bram Hanse[2], Ilse Houwers[1], Lia Groenenboom-de Haas[1], Ezra de Lange[1], Harry Verstegen[1] and Albartus Evenhuis[1]

[1]Wageningen Plant Research, Wageningen University & Research, Wageningen, Netherlands, [2]Institute for Sugar Beet Research (IRS), Dinteloord, Netherlands

Crop residues colonized saprophytically by necrotrophic plant pathogens are an important primary inoculum source for epidemics of foliar diseases. Residues of crops, weeds, and litter were systematically sampled in a complex crop rotation experiment. Concentrations of DNA of major pathogens of the grown crops, *Alternaria solani* in potato and *Cercospora beticola, Ramularia beticola*, and *Stemphylium beticola* in sugar beet, were quantified in the residues using newly developed qPCR assays. Repeated field trials gave additional insights into the dynamics of *A. solani* in potato foliage residues for 2 years. The overall results demonstrate that the *A. solani* and *C. beticola* colonized crop residues of their host crops initially after harvest at high densities whereas *R. beticola* and *S. beticola* were almost absent in the field. Within several months, amounts of available host residues decreased substantially and concentrations of pathogens in the remaining host residues decreased steeply. Alternative substrates, residues of non-host crops including cover crops and weeds, were colonized saprophytically by the necrotrophic pathogens *A. solani* and *C. beticola.* It can be concluded that residues of non-hosts can potentially serve as an important bridge for pathogen populations during host-free cropping seasons in crop rotation systems. These findings contribute to the development of rational crop residue management strategies aiming at disease prevention by lowering the inoculum potential in crop rotation systems.

KEYWORDS

foliar pathogens, *Alternaria solani*, *Cercospora beticola*, crop residues, crop rotation, inoculum sources, potato, sugar beet

1 Introduction

Foliar diseases of potato caused by *Alternaria solani* Sorauer and of sugar beet caused by *Cercospora beticola* Sacc., *Ramularia beticola* Fautr. & Lamb, or *Stemphylium beticola* Woudenb. & Hanse regularly lead to significant yield losses (Abuley et al., 2018; Shane and Teng, 1992; Hanse et al., 2015). Multiple fungicide applications per season are common to control the diseases (Jindo et al., 2021; Khan and Smith, 2005; Vereijssen et al., 2007). *Alternaria solani* infecting *Solanaceae* including common weeds such as *Solanum nigrum* but also non-solanaceous host species of *Asteraceae* and *Fabaceae* (Woudenberg et al., 2014), as well as wild cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*), and zinnia (*Zinnia elegans*) (reviewed by van der Waals et al., 2001), has been described. Host ranges of the pathogens *C. beticola*, *R. beticola*, and *S. beticola* are also narrow with sugar beet and other *Chenopodiaceae* including the common weed *Chenopodium album*, but for *C. beticola* and *S. beticola*, several other plant genera have also been described as host (Hanse et al., 2015; Knight et al., 2019). Common to all four pathogens is their necrotrophic lifestyle and life cycles that include survival on crop residues in and on soil during host-free periods.

The role of residues of host crops for survival of pathogen populations and as potential inoculum sources in subsequent host crops has been studied for many pathogen–host combinations. Examples are *Venturia inaequalis* in apple (MacHardy et al., 2001), toxigenic *Fusarium* spp. in maize and wheat (Cotton and Munkvold, 1998; Köhl et al., 2007; Mourelos et al., 2024), and *A. brassicicola*, *A. brassicae*, *Mycosphaerella brassicicola*, and *Xanthomonas campestris* pv. *campestris* in cabbage (Humpherson-Jones, 1989; Köhl et al., 2011). This knowledge can be used to develop crop residue management strategies to reduce inoculum survival and thus to reduce the amounts of primary pathogen inoculum in subsequent cropping seasons. For example, physical, chemical, or biological treatments of overwintering apple leaves have been investigated in apple orchards to reduce the survival of *V. inaequalis* in the leaf residues during winter and to delay the onset of apple scab epidemics in spring (Gomez et al., 2007).

Crop residues of infected crops can be considered as the main substrate for pathogen populations, allowing survival during host-free periods. However, other saprophytic microorganisms will compete with the pathogens for nutrients and space in the residues, and residues will be degraded during time. Saprophytic colonization of residues of non-host crops or weeds present in the field may allow an additional route for pathogen survival. An example for such a survival strategy is the colonization of residues of non-host weeds and grasses by pear-pathogenic *S. vesicarium*, resulting in significant contributions of such alternative inoculum sources in brown spot epidemics in pear orchards (Rossi et al., 2008; Köhl et al., 2013). Mourelos et al. (2024) also observed that soybean residues were an inoculum reservoir for *Fusarium graminearum* causing Fusarium head blight in wheat. They found correlations between the pathogen DNA quantified in soybean residues compared to the amount of inoculum found in the previous cereal crop, suggesting that the inoculum is perpetuating in the fields and increases the chances of infection development.

The objective of our study was to contribute to the development of rational crop residue management strategies aiming at disease prevention in crop rotation systems. The colonization of residues of host crops by *A. solani*, *C. beticola*, *R. beticola*, and *S. beticola* was quantified using species-specific qPCR assays. Residues of non-host crops and cover crops grown in rotation as well as weeds and organic litter on the soil surface were also assessed to elucidate their role in pathogen population dynamics during host-free periods in arable systems with crop rotation. Pathogen populations were measured in field plots with various crop sequences during three winter seasons without growing a main crop (except winter barley) with sampling breaks during the two corresponding growing seasons. This was executed in replicated field plots of a crop rotation field trial with potato (1), winter or spring barley, sugar beet (late harvest), carrots, potato (2), maize, sugar beet (early harvest), and spring sown onion grown in rotation in two different crop management systems, comparing common practice in the Southern parts of the Netherlands with an integrated crop management (ICM) strategy based on minimizing chemical crop control (Riemens et al., 2022).

2 Materials and methods

2.1 Fungal isolates

Fungal isolates used in the study are listed in Table 1. Isolates are from the collection of Wageningen University & Research unless indicated otherwise.

2.2 Crop rotation experiment

2.2.1 Experimental design

The field experiment started in 2020 at Wageningen University & Research, Vredepeel (51.54 N; 5.85 E) on sandy soil with an organic matter content, varying from 4.4% to 4.8%. Crops were grown according to two plant protection strategies. The reference strategy (REF) controls weeds, pests, and diseases according to common practice in the southeast region of the Netherlands. The ICM strategy was based on minimizing chemical crop protection and refraining from pesticides, which are listed by the European Union as candidates for substitution (CfS) (European Commission, 2015). For example, potato varieties with better resistance against late blight (*Phytophthora infestans*) were grown in ICM to minimize pesticide input and to avoid fluopicolide (CfS). Since difenoconazole as a CfS was not available for early blight control, options were limited in the first years. To control Cercospora leaf spot, no synthetic fungicides were available, since these all contained an active ingredient listed as CfS. Details of the fungicide applications in potato aiming at early blight control and in sugar beet aiming at the control of foliar diseases are given in Table 2.

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TABLE 1 Fungal isolates used in the study and their origin.

Fungal species	Isolate number	Source	Country	Year
Alternaria allii	CBS 114.38 [a]	*Allium cepa*, seed	Denmark	1937
Alternaria alternata	CBS 154.31 [a]	*Staphylea trifolia*	USA	1931
Alternaria bataticola	CBS 531.63 [a]	Unknown	Unknown	1960
Alternaria brassicae	PRI424	*Brassica oleracea*, seed	Netherlands	Unknown
Alternaria brassicicola	PRI177	*B. oleracea*, seed	Netherlands	Unknown
Alternaria dauci	CBS 106.48 [a]	*Daucus carota*, seed	Unknown	1948
A. dauci	PRI 757	*D. carota*, seed	Unknown	1999
Alternaria mali	T3e	*Lamium purpureum*	Netherlands	2014
A. mali	T6b	Unknown grass	Netherlands	2014
Alternaria porri	PRI 1058	*Allium porrum*	Netherlands	Unknown
Alternaria radicina	PRI 756	*D. carota*, seed	Unknown	1999
A. radicina	PRI 846	*D. carota*, seed	Unknown	Unknown
A. radicina	CBS 245.67 [a]	*D. carota*	USA	Unknown
Alternaria solani	2020VR001	*Solanum tuberosum*	Netherlands	2020
A. solani	NL15002	*S. tuberosum*	Netherlands	2015
A. solani	NL17003	*S. tuberosum*	Netherlands	2017
A. solani	AltNL05047	*S. tuberosum*	Netherlands	2005
A. solani	AltNL04008d	*S. tuberosum*	Netherlands	2004
A. solani	AltNl0629	*S. tuberosum*	Netherlands	2006
A. solani	AltNL09011	*S. tuberosum*	Netherlands	2009
A. solani	AltNL11002	*S. tuberosum*	Netherlands	2011
Aphanomyces cochlioides	K20Aph1 [b]	*Beta vulgaris*	Netherlands	2020
Aureobasidium pullulans	H2	*Malus domestica*	Netherlands	2006
Botrytis aclada/allii	PRI 1078	*Allium ascalonicum*	Netherlands	Unknown
B. aclada/allii	PRI 007	*Allium cepa*	Netherlands	Unknown
Botrytis cinerea	PRI 700	*Gerbera* sp.	Netherlands	1986
Cercospora beticola	09-40	*B. vulgaris*	USA	2009
C. beticola	18-533-3 [b]	*B. vulgaris*	Netherlands	2018
C. beticola	18-516-3 [b]	*B. vulgaris*	Netherlands	2018
C. beticola	18-539-2 [b]	*B. vulgaris*	Netherlands	2018
C. beticola	18-518-3 [b]	*B. vulgaris*	Netherlands	2018
C. beticola	18-389-3c2 [b]	*B. vulgaris*	Netherlands	2018
Cladosporium allcinum	BN111	*M. domestica*	Netherlands	2019
Cladosporium cladosporoides	BN124	*M. domestica*	Germany	2019
Cladosporium delicatum	BN109	*M. domestica*	Netherlands	2019
Cladosporium europaeum	BN105	*M. domestica*	Netherlands	2019
Cladosporium pseudocladosporoides	BN119	*M. domestica*	Germany	2019
Cladosporium weserdijkiae	BN126	*M. domestica*	Germany	2019

(Continued)

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TABLE 1 Continued

Fungal species	Isolate number	Source	Country	Year
Coniothyrium cereale	H33	*M. domestica*	Germany	2006
Cystobasidium laryngis	54-1PDAA	*M. domestica*	Norway	2019
Epicoccum nigrum	L45x	*Galium aparine*	Netherlands	2014
E. nigrum	T5d	Unknown grass	Netherlands	2014
Fusarium culmorum	CBS 173.31 [a]	*Avena sativa*	Canada	1927
Fusarium graminearum	PD 88/790	*Dianthus* sp.	Netherlands	1988
Fusarium oxysporum f.sp *betae*	20-073a [b]	*B. vulgaris*	Netherlands	2020
Fusarium proliferatum	Item 2287	*Zea mays*	USA	Unknown
Fusarium verticillioides	CBS 576.78 [a]	*Mycofilic*	USSR	Unknown
Helminthosporium carbonum	CBS 209.79 [a]	*Z. mays*	Romania	1976
Helminthosporium turcicum	CBS 690.71 [a]	*Z. mays*	Germany	Unknown
Mycosphaerella brassicicola	CBS 174.88 [a]	*B. oleracea*	Germany	1987
Penicillium expansum	P8b	*Triticum aestivum*	Netherlands	2014
Phoma betae	Ph4 [b]	*B. vulgaris*	Unknown	Unknown
P. betae	20-006a [b]	*B. vulgaris*	Netherlands	2020
Phoma exiqua	T1a	*Taraxacon* sp.	Netherlands	2014
Phoma herbarum	J33a	Unknown grass	Germany	2014
Phoma macrostoma	L44d	*Papaver* sp.	Netherlands	2014
Phoma pinodella	H3	*M. domestica*	Germany	2006
Ramularia beticola	11rr04	*B. vulgaris*	Denmark	2011
R. beticola	11rr06	*B. vulgaris*	Denmark	2011
R. beticola	11rr09	*B. vulgaris*	Denmark	2011
R. beticola	12rr04	*B. vulgaris*	Denmark	2012
R. beticola	12rr05	*B. vulgaris*	Denmark	2012
Rhexocercosporidium carotae	PRI 783	*D. carota*	Netherlands	2001
Rhizoctonia solani	14-188a [b]	*B. vulgaris*	Netherlands	2014
R. solani	19-287a [b]	*B. vulgaris*	Netherlands	2019
Rhodotorula glutinis	BN093	*M. domestica*	Netherlands	2019
Sporidiobolus pararoseus	53PDAC	*M. domestica*	Germany	2019
Stemphylium amaranthi	NL17041	*Amaranthus retroflexus*	China	2008
Stemphylium beticola	GV14-250-1[b]	*B. vulgaris*	Netherlands	2014
S. beticola	GV13-425a1[b]	*B. vulgaris*	Netherlands	2013
S. beticola	17-285c1[b]	*B. vulgaris*	Netherlands	2017
S. beticola	NL17120	*B. vulgaris*	Netherlands	2017
S. beticola	NL17059	*Chenopodium album*	Netherlands	2017
Stemphylium eturmiunum	NL17028	*Capsicum annuum*	China	Unknown
Stemphylium vesicarium	NL 17054	*S. tuberosum*	Netherlands	2017
S. vesicarium	NL 17180	*B. vulgaris*	Netherlands	2017
S. vesicarium	GV15-362b1[b]	*Cichorium intybus*	Netherlands	2015

(Continued)

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TABLE 1 Continued

Fungal species	Isolate number	Source	Country	Year
Verticillium dahliae	GN12-470a1[b]	*B. vulgaris*	Netherlands	2012
Vishniacozyma tephrensis	BN129	*M. domestica*	Germany	2019
Vishniacozyma victoriae	BN128	*M. domestica*	Germany	2019

[a]Obtained from the Westerdijk Fungal Biodiversity Institute (CBS-KNAW), Utrecht, Netherlands.
[b]Provided by Institute for Sugar Beet Research, IRS, Dinteloord, Netherlands.

The crops grown were potato, winter or spring barley, sugar beet, carrots, maize, and spring sown onion. The crop rotation was one in eight except for potato and sugar beet which was one in four. The rotation order of the crops in both strategies was potato (1), winter or spring barley, sugar beet (late harvest), carrots, potato (2), maize, sugar beet (early harvest), and spring sown onion (Figure 1; Supplementary Table 1). Cover crops depended on main crops and the crop protection strategy (Supplementary Table 1). In total, the experiment consisted of four main blocks (replicates), each split into two plots with the different cropping systems, each plot with eight sub-plots for randomly distributed crops (REF1 to REF8; ICM 1 to ICM 8). Sub-plot size was 24 m × 24 m. Disease severity of early blight in potato and Cercospora leaf spot in sugar beet was weekly estimated visually on a scale of 0%–100% of leaf area affected. The area under the disease progress curve (AUDPC) was calculated according to Madden et al. (2007) and standardized by dividing by the duration of the epidemic (days). The resulting StAUDPC values for potato and sugar beet crops grown in the different cropping systems are shown in Table 3. Symptoms of infections by *R. beticola* were not observed in sugar beet plots and only few symptoms typical for *S. beticola* infections were found.

Weeds were controlled in the various crops by a combination of mechanical control and use of herbicides, depending on crop, crop stage, and control strategy (REF or ICM). Nevertheless, weed development was never completely controlled such that it always remained possible to take senescent weed samples.

2.2.2 Sampling of crop residues

Residues were sampled three or four times in a season depending on the grown crop. Samples were taken (1) directly after harvest; (2) approximately 3 weeks after harvest but before tillage or seeding of the subsequent crop; (3) at the end of the year just before the onset of winter (if possible); and (4) in spring before tillage, planting, or sowing of the subsequent crop. In some cases, 3 weeks after crop harvest coincided with the end of the year. In that case, only one sample was taken per plot at that time. Figure 1 illustrates crop rotation and sampling periods in the eight different crop rotation plots and Supplementary Table 1 gives an overview of sowing dates, harvesting dates, and sampling dates in the various crops.

Three different types of crop residues were sampled from the soil surface at each sampling time: (1) clearly recognizable debris of the grown main crop or the cover crop, (2) weed debris, and (3) litter scraped using a small blade together with some top soil from

TABLE 2 Application dates of fungicides and dose rates in (A) potato and (B) sugar beet crops in the reference cropping system (REF) and the integrated crop management (ICM) system.

(A) Early blight control strategies in potato.		
Date	Reference cropping system	Integrated crop management system
Cropping season 2020		
29-06-2020	Narita (difenoconazole 250 g/L) 0.5 L/ha	–
17-07-2020	Carial star (difenoconazole 250 g/L + mandipropamid 250 g/L) 0.6 L/ha	–
31-07-2020	Narita (difenoconazole 250 g/L) 0.5 L/ha	Amistar (azoxystrobin 250 g/L) 0.25 L/ha
Cropping season 2021		
09-07-2021	Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 0.4 L/ha	–
23-07-2021	Narita (difenoconazole 250 g/L) 0.5 L/ha	–
21-07-2021	–	Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 0.45 L/ha
04-08-2021	Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 0.4 L/ha	–
Cropping season 2022		
05-07-2022	Carial star (difenoconazole 250 g/L + mandipropamid 250 g/L) 0.6 L/ha	–
22-07-2022		Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 0.5 L/ha
25-07-2022	Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 0.4 L/ha	–
04-08-2022	–	Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 0.5 L/ha
09-08-2022	Narita (difenoconazole 250 g/L) 0.5 L/ha	–

(Continued)

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TABLE 2 Continued

(B) Control strategies for leaf diseases in sugar beet.		
Date	Reference cropping system	Integrated crop management system
Cropping season 2020		
03-07-2020	Spyrale (difenoconazole 100 g/L + fenpropidin 375 g/L) 1.0 L/ha	–
27-07-2020	–	Serenade (*Bacillus amyloliquefaciens*) 4.0 L/ha + Hi-Wett [a] 0.1 L/ha
28-07-2020	Retengo Plust (epoxiconazole 50 g/L + pyraclostrobin 133 g/L) 1.0 L/ha	–
13-08-2020	–	Serenade (*Bacillus amyloliquefaciens)* 4.0 L/ha + Hi-Wett 0.1 L/ha
24-08-2020	–	Serenade (*Bacillus amyloliquefaciens)* 4.0 L/ha + Hi-Wett 0.1 L/ha
27-08-2020	Sphere (trifloxstrobin 375 g/L + cyproconazole 160 g/L) 0.3 L/ha	–
04-09-2020	–	Serenade (*Bacillus amyloliquefaciens*) 4.0 L/ha + Hi-Wett 0.1 L/ha
26-09-2020	Sphere (trifloxstrobin 375 g/L + cyproconazole 160 g/L) 0.3 L/ha	–
25-09-2020	–	Serenade (*Bacillus amyloliquefaciens)* 4.0 L/ha + Hi-Wett 0.1 L/ha
Cropping season 2021		
25-06-2021	Spyrale (difenoconazole 100 g/L + fenpropidin 375 g/L) 1.0 L/ha	Charge (chitosan hydrochloride) 3.0 kg/ha
21-07-2021	–	Charge (chitosan hydrochloride) 3.0 kg/ha (early harvest) Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 1.2 L/ha (late harvest)
27-07-2021	Bicanta (azoxystrobin 125 g/L + difenoconazole 125 g/L) 1.0 L/ha	
12-08-2021	–	Charge (chitosan hydrochloride) 3.0 kg/ha (early harvest)
20-08-2021	Spyrale (difenoconazole 100 g/L + fenpropidin 375 g/L) 1.0 L/ha	Propulse (prothioconazole 125 g/L + fluopyram 125 g/L) 1.2 L/ha (late harvest)
03-09-2021	–	
08-09-2021	–	Charge (chitosan hydrochloride) 3.0 kg/ha (early harvest)

(Continued)

TABLE 2 Continued

(B) Control strategies for leaf diseases in sugar beet.		
Date	Reference cropping system	Integrated crop management system
Cropping season 2021		
21-09-2021	–	Charge (chitosan hydrochloride) 3.0 kg/ha (late harvest)
Cropping season 2022		
23-06-2022	–	Charge (chitosan hydrochloride) 2.0 kg/ha + Promotor [b] 0.4 L/ha
28-06-2022	Spyrale (difenoconazole 100 g/L + fenpropidin 375 g/L) 1.0 L/ha	–
08-07-2022	–	Microthiol (sulfur 825 g/L) 5.0 L/ha
20-07-2022	–	Microthiol (sulfur 825 g/L) 5.0 L/ha
27-07-2022	Bicanta (azoxystrobin 125 g/L + difenoconazole 125 g/L) 1.0 L/ha	–
09-08-2022	–	Charge (chitosan hydrochloride) 2.0 kg/ha + Promotor 0.4 L/ha
18-08-2022	Sphere (trifloxstrobin 375 g/L + cyproconazole 160 g/L) 0.3 L/ha	–
22-08-2022	–	Charge (chitosan hydrochloride) 2.0 kg/ha + Promotor 0.4 L/ha
23-09-2022	Spyrale (difenoconazole 100 g/L + fenpropidin 375 g/L) 1.0 L/ha (late harvest)	–

[a]Added as surfactant based on trisiloxane.
[b]Added as surfactant based on sugar derivative.

the ground surface. Only plant material that was already senescent was taken. Plant material, which was still alive, was not sampled. Samples were taken from each plot at approximately 20 different sites randomly distributed over the plot. Between sampling of the plots, the scissors, knives, and small spades used were cleaned and disinfected with 70% alcohol to prevent contamination of the samples between plots. Sample size per plot varied but was generally at least 10 g fresh weight. The samples were stored at −18°C in plastic bags.

2.2.3 Crop residue management experiment

A potato crop cv. Fontane showing early blight symptoms at the end of the season was selected as the source of potato canopy residues for the experiment. The percentage leaf surface affected by early blight (disease severity) was 7.5%. Potato canopy residues were collected at growth stage BBCH 95-97 on 19 August 2020. Residues were treated as follows: no treatment, cut in 2-cm pieces with a pruning shears, and cut in 10-cm pieces. For a fourth treatment, canopy residues were

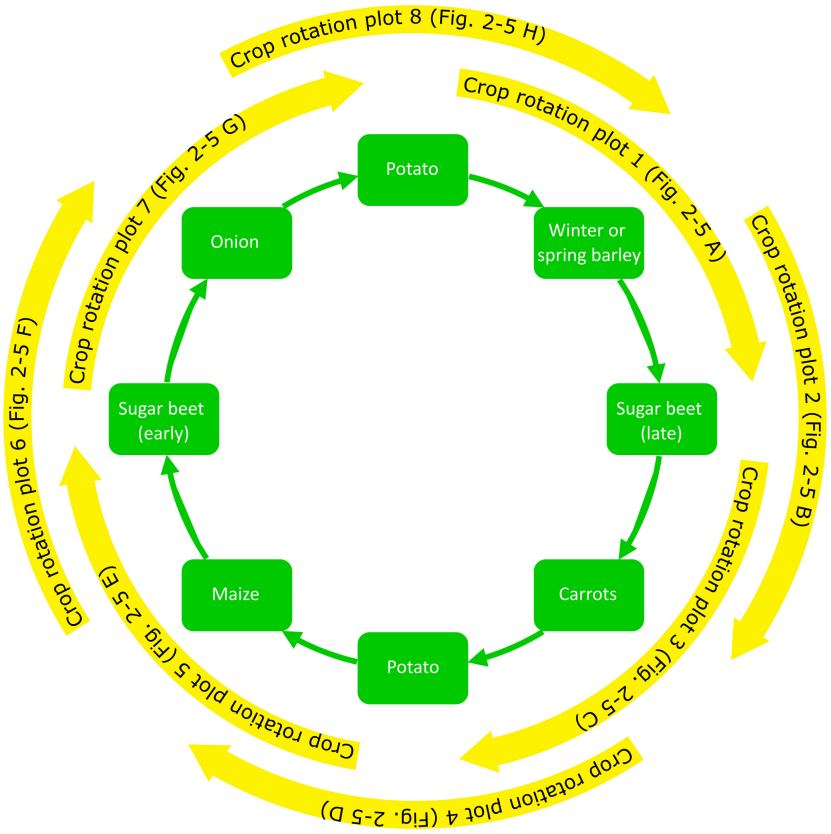

FIGURE 1
Diagram showing crop rotation and sampling periods in the eight crop rotation plots. Corresponding results are shown in Figures 2–5.

collected on a stroke of the same field where the canopy had been treated by a flailing machine, Grimme KS 3000 (Damme, Germany), 30 min before collecting the samples. Net bags (size 25 × 30 cm) were filled with subsamples of residues of each of the four treatments with approximately 250 g fresh weight per bag. In total, 40 bags per treatment were prepared and placed in a field not grown with a crop at Wageningen University & Research, Vredepeel.

There were two main plots sized 3.1 × 4.8 m, one with net bags buried horizontally in the soil at 10 cm depth and one with net bags placed on top of the soil. Within each main plot, there were five blocks (replicates), each consisting of four plots with net bags belonging to the four different treatments, arranged fully randomized within the block. In each of the plots, four bags with the same treatment were placed with 0.1-m distance between bags. One of the bags was selected randomly per sampling time, approximately every 6 months on 15 April 2021, 23 September 2021, 29 April 2022, and 23 September 2022. Ten additional samples, each with approximately 250 g fresh weight, were taken at the beginning of the experiments on 19 August 2020 from the canopy residues used for treatments 1–3 and 10 samples from the flailing-treated field stroke. All samples were stored at −20°C before processing.

The experiment was repeated in the same field. Canopy residue samples were collected on 23 September 2021 from a potato crop cv. Cammeo at growth stage BBCH 99. Early blight disease symptoms were found on 5% of the canopy surface on 15 September 2021. Sampling dates of bags containing canopy residues were 23 April 2022, 29 September 2022, 1 May 2023, and 20 September 2023.

2.3 Sample processing

Frozen field samples of plant residues of main crops, cover crops, and weed debris collected in the crop rotation experiment were shredded and subsamples of approximately 150 mL were freeze-dried using the Alpha 1-4 LSC basic (Martin Christ, Osterode am Harz, Germany). Thereafter, the dried material was pulverized using a CT 293 Cyclotec laboratory mill with a 1-mm mesh sieve (Foss, Hillerød, Denmark) and stored at −20°C.

The frozen litter samples were transferred to 1-L plastic beakers and 600 mL of tap water was added. After complete thawing, the water with the sample was vigorously stirred with a disinfected metal spoon to create an aqueous mixture and left for 1 h to completely saturate and allow sand to settle at the bottom. The supernatant, including most of the litter, was carefully poured through a funnel containing a monodur filter (200 µm). Pouring of the supernatant was stopped before sand particles could flow out. To increase the yield of litter, this process was repeated once, this time with a settling time of 10 s. The litter residues in the monodur

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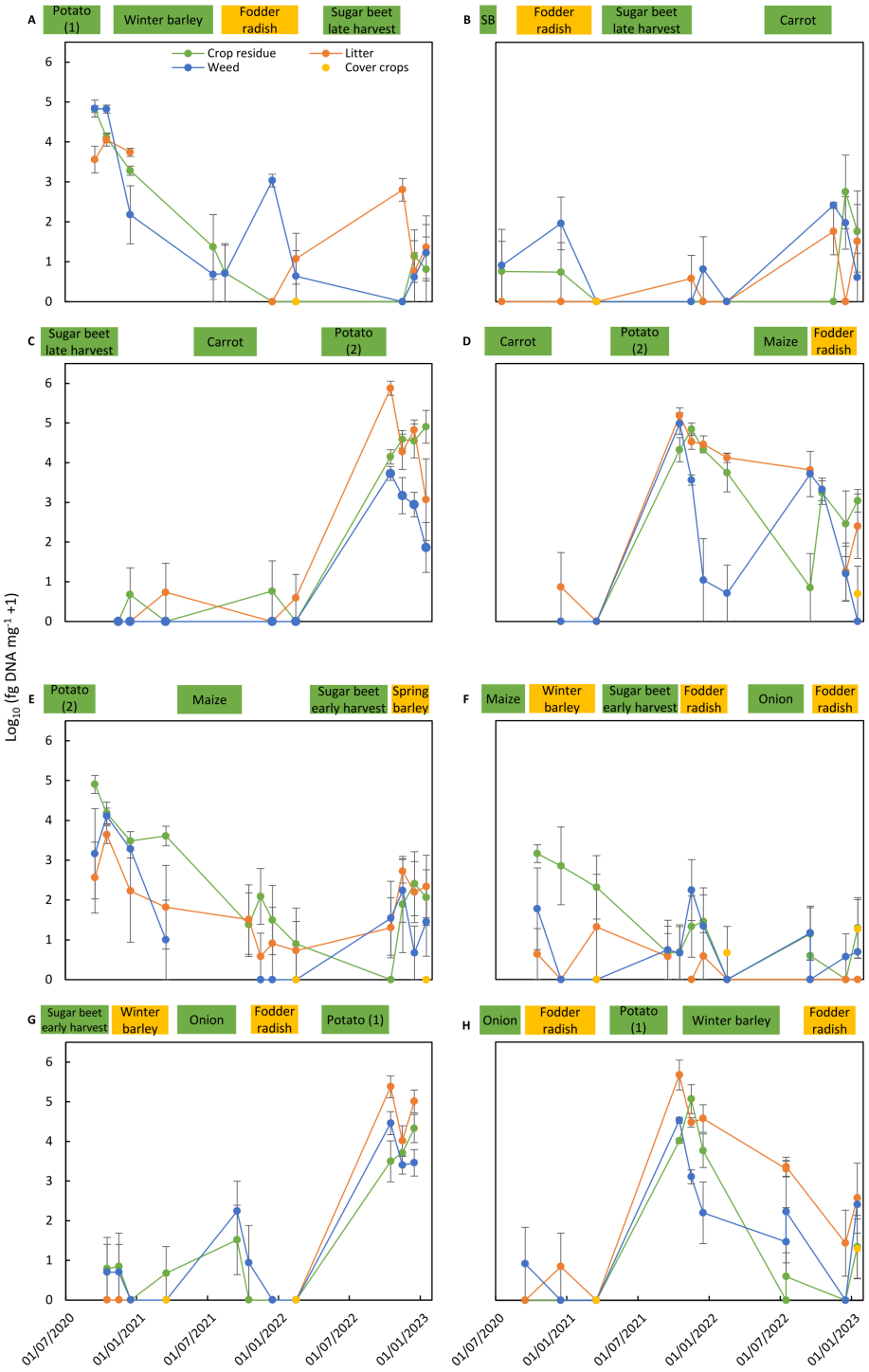

FIGURE 2
Colonization of residues of main crops, cover crops, and weeds and of litter by *Alternaria solani* in the eight crop rotation plots **(A–H)** of the reference cropping system (REF). *Alternaria solani* colonization is expressed as the mean of log_{10}-transformed data from four replicate sub-plots (fg of DNA of *A. solani* mg^{-1} dry material – 1). Bars correspond to the standard error of the means. Cropping periods of main crops (in green) and cover crops (in yellow) in the crop rotation plot are shown on top of each graph. SB in graph B means spring barley.

filter were carefully rinsed with tap water to ensure all small particles were removed. The remaining litter was collected in a 15-mL Precellys tube (Bertin Technologies, Montigny-le-Bretonneux, France) and two 6.35-mm RVS beads (Vanem, Hoek van Holland, the Netherlands) were added. The tubes including the litter samples were freeze-dried using the Epsilon 1-4 LSC plus (Martin Christ, Osterorde am Harz, Germany). The freeze-dried samples were pulverized using the Precellys Evolution

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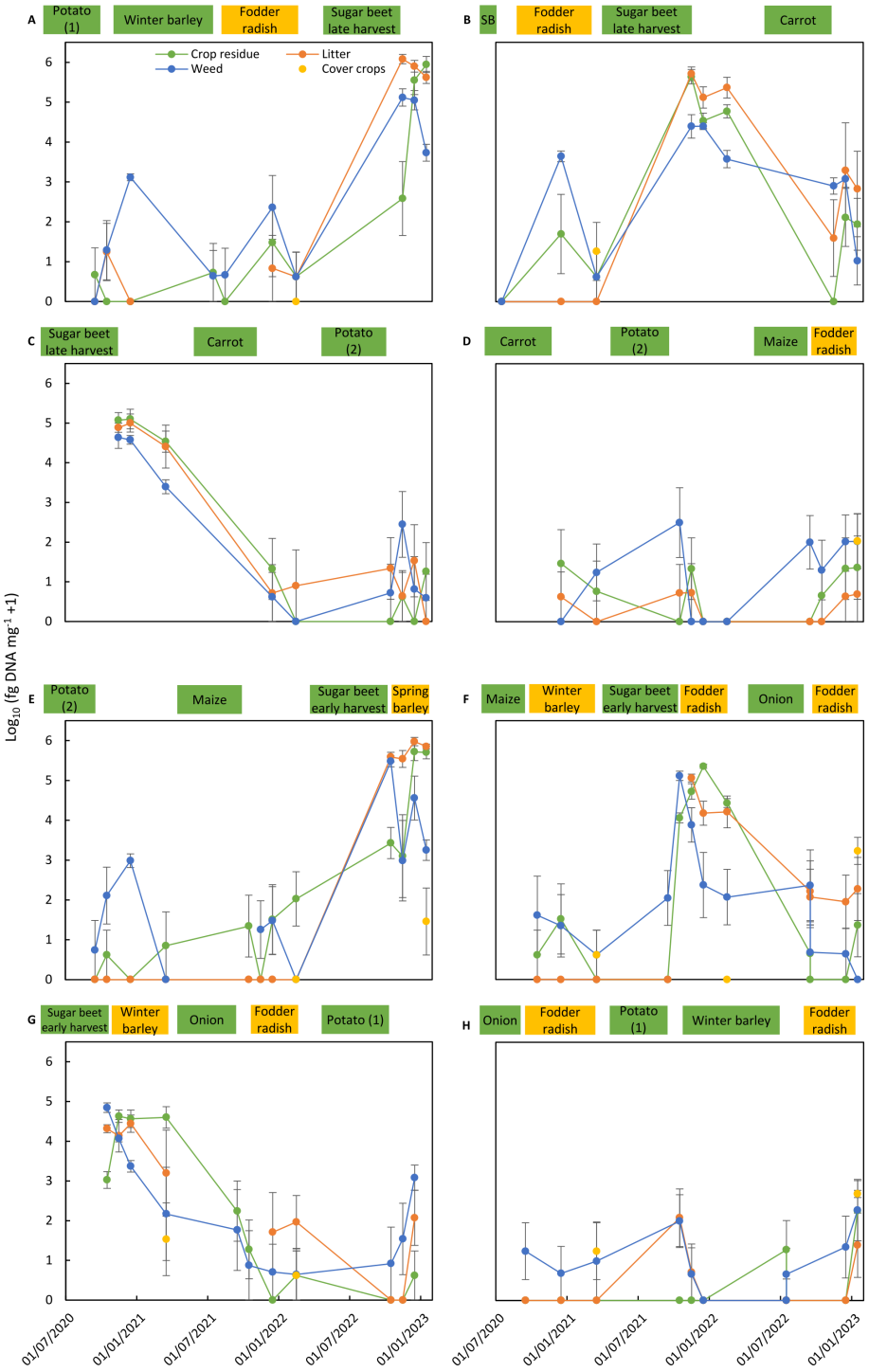

FIGURE 3
Colonization of residues of main crops, cover crops, and weeds and of litter by *Cercospora beticola* in the eight crop rotation plots **(A–H)** of the reference cropping system (REF). *Cercospora beticola* colonization is expressed as the mean of $\log_{10}$-transformed data from four replicate sub-plots ($\log_{10}$ fg of DNA of *C. beticola* mg^{-1} dry material + 1). Bars correspond to the standard error of the means. Cropping periods of main crops (in green) and cover crops (in yellow) in the crop rotation plot are shown on top of each graph. SB in graph B means spring barley.

Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6,000 rpm for 2 × 15 s with a 10-s pause. Milled samples were stored at −20°C.

Frozen samples of the potato canopy residues collected from the crop residue management experiments were transferred to 1-L plastic beakers and 600 mL of tap water was added. After complete thawing, the water with the sample was vigorously stirred with a disinfected metal spoon to create an aqueous mixture. The complete mixture was poured on a metal sieve (mesh size, 2.8 mm) and carefully rinsed with tap water to ensure all small particles were removed. The residue was

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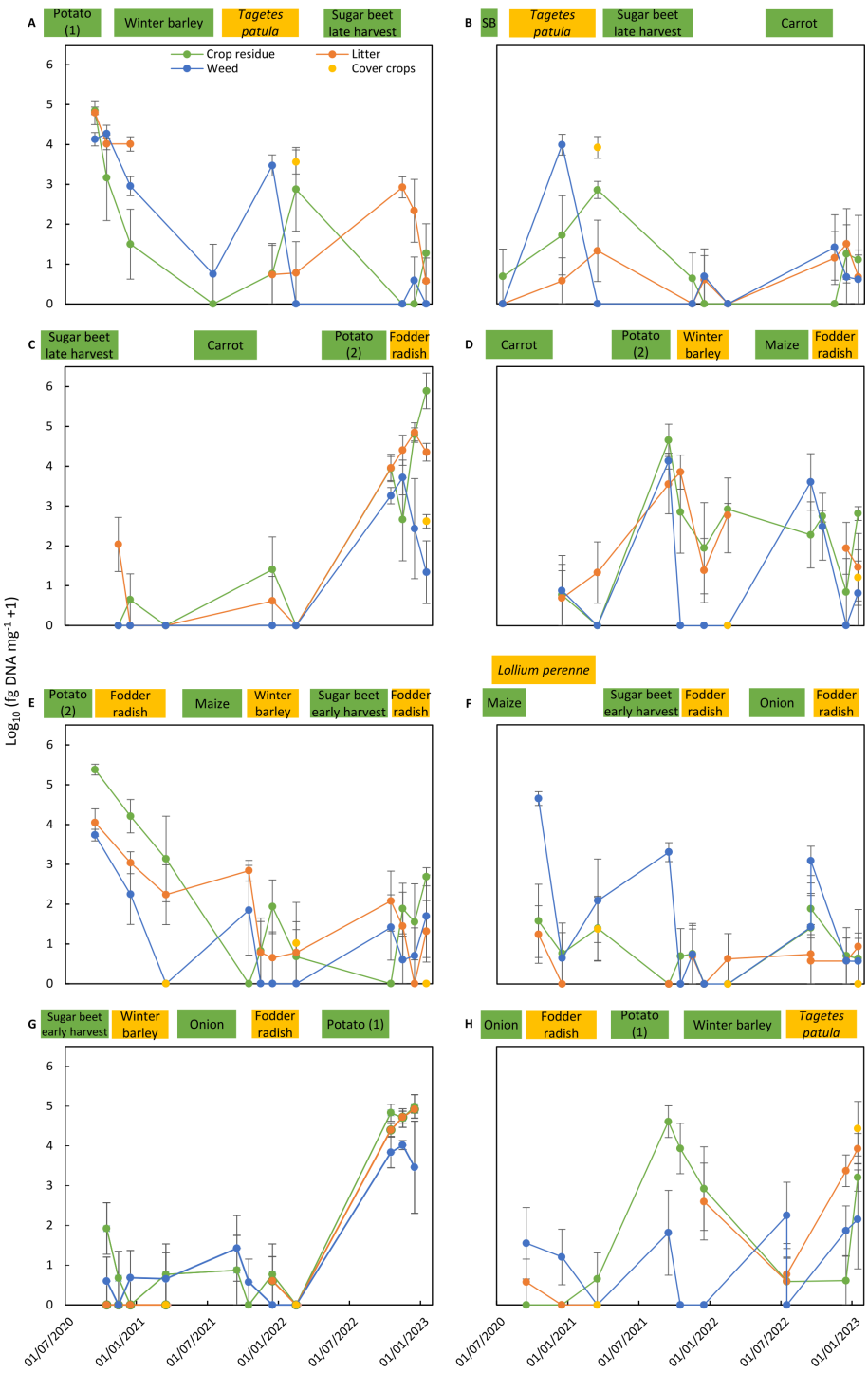

FIGURE 4
Colonization of residues of main crops, cover crops, and weeds and of litter by *Alternaria solani* in the eight crop rotation plots **(A–H)** of the integrated crop management system (ICM). *Alternaria solani* colonization is expressed as the mean of $\log_{10}$-transformed data from four replicate sub-plots ($\log_{10}$ fg of DNA of *A. solani* mg^{-1} dry material + 1). Bars correspond to the standard error of the means. Cropping period of main crops (in green) and cover crops (in yellow) in the crop rotation plot are shown on top of each graph. SB in graph B means spring barley.

sorted out and contaminants such as weeds, insects, or material other than potato residue were removed. The remaining potato residue was collected, freeze-dried using the Alpha 1-4 LSC basic and the total dry weight was noted. Finally, the samples were pulverized in the CT 293 Cycloteca laboratory mill with a 1-mm mesh sieve and stored at −20°C.

2.4 DNA extraction

2.4.1 DNA extraction from fungal isolates

Fungal isolates were grown on potato dextrose agar (PDA) for 2 weeks at 20°C in the dark except for *R. beticola* isolates, which were

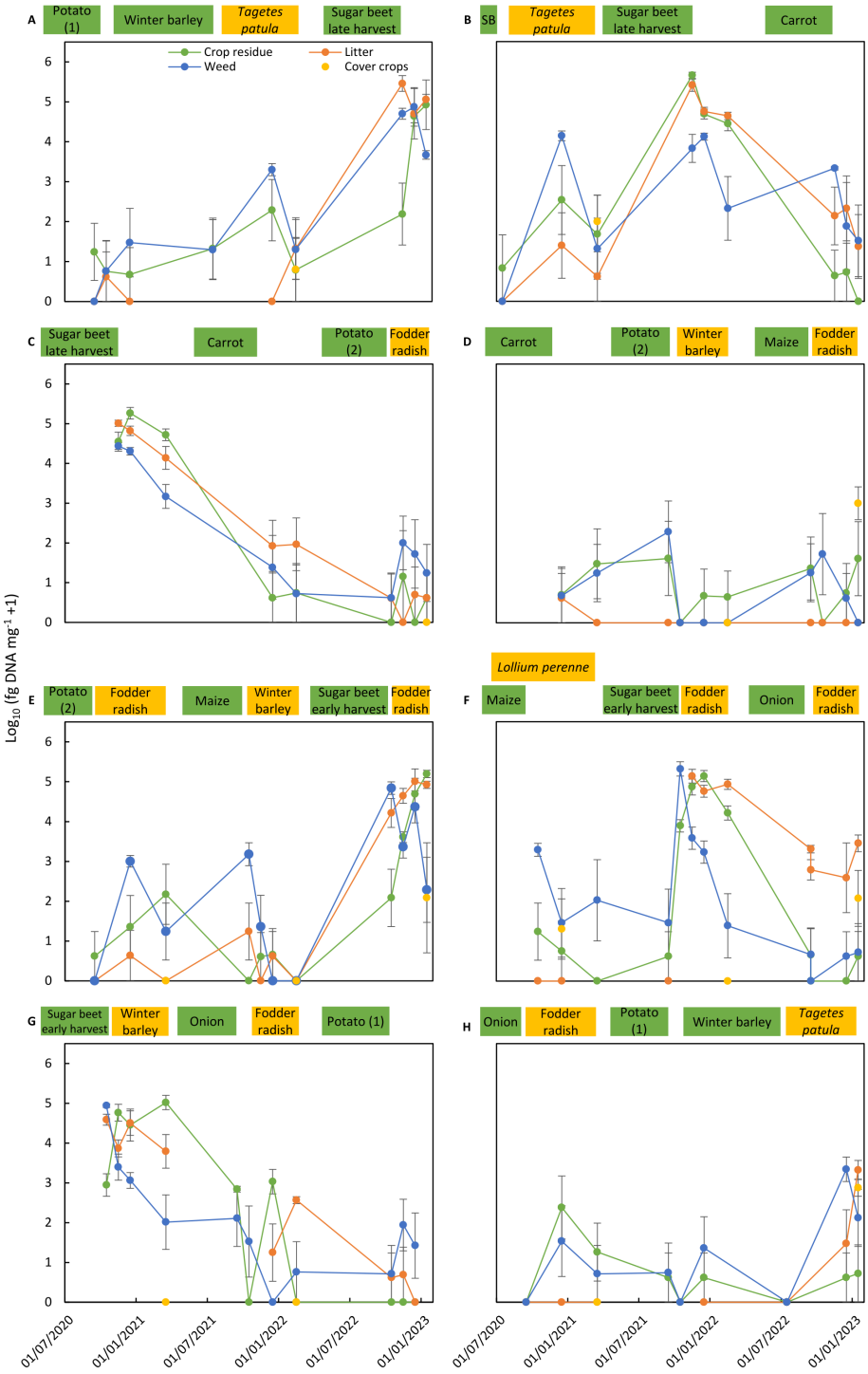

FIGURE 5
Colonization of residues of main crops, cover crops, and weeds and of litter by *Cercospora beticola* in the eight crop rotation plots **(A–H)** of the integrated crop management system (ICM). *Cercospora beticola* colonization is expressed as the mean of log_{10}-transformed data from four replicate sub-plots (log_{10} fg of DNA of *C. beticola* mg^{-1} dry material + 1). Bars correspond to the standard error of the means. Cropping periods of main crops (in green) and cover crops (in yellow) in the crop rotation plot are shown on top of each graph. SB in graph B means spring barley.

grown for 4 weeks (Table 1). Mycelium and spores of cultured isolates were scraped from the agar surface, freeze-dried, and disrupted using the TissueLyser II (Qiagen) and one stainless bead (3.2 mm diameter) for 30 s at 30 Hz. After disruption, 200 µL of lysis buffer from the Sbeadex maxi plant kit (LGC, 175 Teddington, UK) was added to each sample and further DNA extraction was done automatically according to the protocol supplied by the manufacturer with the KingFisher™ Flex

TABLE 3 Disease rating (StAUDPC) for early blight caused by *Alternaria solani* in potato crops and Cercospora leaf spot caused by *Cercospora beticola* in sugar beet crops grown in rotation plots in 2020–2023.

Year	Cropping system	StAUDPC Alternaria *		StAUDPC Cercospora *	
		Potato (1)	Potato (2)	Early harvested sugar beet	Late harvested sugar beet
2020	REF	0.13 ± 0.07	0.12 ± 0.04	5.20 ± 0.51	9.35 ± 1.16
	ICM	2.23 ± 2.90	11.50 ± 7.74	12.46 ± 1.63	14.88 ± 2.16
2021	REF	1.69 ± 1.15	0.53 ± 0.30	3.83 ± 2.58	7.50 ± 2.52
	ICM	0.85 ± 0.46	1.61 ± 0.96	9.75 ± 2.57	5.17 ± 4.09
2022	REF	0.09 ± 0.06	0.27 ± 0.21	4.57 ± 0.48	17.30 ± 1.15
	ICM	0.46 ± 0.14	0.73 ± 0.19	1.21 ± 0.66	7.91 ± 1.67

* Mean and standard error of the mean of four replicate sub-plots.

Purification System pipetting robot (Thermo Fisher Scientific, Waltham, MA, USA). DNA concentration was measured using PicoGreen in a fluorescence plate reader (TECAN).

2.4.2 DNA extraction from crop residue samples

DNA was extracted from weighted freeze-dried and pulverized crop residue samples (approximately 50 mg) using the Sbeadex maxi plant kit according to the manufacturer's protocol with the following modifications: lysis was done at 65°C for 1 h with 700 µL of lysis buffer, and after centrifugation, 200 µL of the supernatant was used in the subsequent protocol. The KingFisher™ Flex Purification System pipetting robot was used to automate the extraction. DNA extracts were stored at −20°C.

2.5 Development of species-specific Taqman qPCR assays

Four different Taqman qPCR assays were developed for the detection and quantification of *A. solani*, *C. beticola*, *R. beticola*, and *S. beticola*. For primer and probe design, alignments were made with CLC genomics workbench 12.0 (CLC bio, Denmark) based on target and non-target *Alt a 1* sequences from Woudenberg et al. (2014) for *A. solani*, *gapdh* sequences from Bakhshi et al. (2018) for *C. beticola*, ITS sequences from Videira et al. (2016) for *R. beticola*, and *cmdA* sequences from Woudenberg et al. (2017) for *S. beticola*. The species-specific primers and probe were designed with CLC genomic workbench and primer express v.3.0 (Thermo Fisher Scientific, USA).

The specificity of developed Taqman qPCR assays was first tested *in silico* using blastn search in NCBI and, if specific, the designed primers and probe were assessed with synthetic DNA and with 1 ng of DNA of all target and non-target fungi listed in Table 1. The test was considered as specific if, for the non-target fungi, no reactions or C_t values were measured that were higher than the C_t value for the target fungus at the lowest concentration of the dynamic range of the TaqMan PCR. The sensitivity was determined by a 10-fold dilution series from 1 ng to 10 fg for five replicates of *A. solani* isolate 2020Vr001, *C. beticola* isolate 18-539-2, *R. beticola* isolate 11rr04, and *S. beticola* isolate 14-250-1.

2.6 Taqman qPCR assays

Separate qPCR assays were performed in a 384-well format in CFX 384 (Bio-Rad) Real-Time PCR Detection System to quantify *A. solani, C. beticola, R. beticola, S. beticola*, and green fluorescent protein (GFP) serving as an amplification control (AC) (Klerks et al., 2004). For each TaqMan PCR, 1 µL of sample was mixed with 9 µL of reaction mix containing 5 µL of 2× PerfeCTa Toughmix no ROX (Quantabio), 100 nM probe, and 300 nM of each forward and reverse primer (Table 4). For *C. beticola*, 150 nM probe was used. The reaction conditions were as follows: 95°C for 2 min, 40 cycles of 95°C for 15 s, followed by 60°C for 60 s.

TABLE 4 Taqman qPCR primers and probes used for species-specific quantification of *Alternaria solani, Cercospora beticola, Ramularia beticola*, and *Stemphylium beticola* in environmental samples.

Fungal species	Forward primer[a] (5′-3′)	Reverse primer[a] (5′-3′)	Taqman probe[b] (5′-3′)
Alternaria solani	cggcctgctcctgaa	taggtgatgctggaaaagc	tgccctCACgctt
Cercospora beticola	atgtacgcctggaggacat	acactacgctgtaagttgtcttcga	ctcatAGCCgagcagc
Ramularia beticola	tctccggctgtctgattca	tcaccggtccaaactcctctac	cCTAgcgttgTAAcaaa
Stemphylium beticola	gcgatggttagtagcctctttt	ggagctggcttcgggtagc	CaaTtcaCATccgcgc

[a]Primers from Integrated DNA Technologies (IDT).
[b]Labeled at 5′ end with fluorescein label, 6-FAM and at the 3′ end with BHQ1 (IDT); LNA nucleotides in uppercase letters.

A 10-fold serial dilution range of genomic DNA was included in each 384-well plate for reference. The concentrations of extracted pathogen DNA in the samples were calculated from the derivative cycle threshold values (C_t values) of TaqMan PCRs for the DNA dilution series and for DNA extracts of plant samples, and expressed as fg of DNA of pathogen DNA per mg of plant residue (dry weight).

2.7 Statistical analysis

For the crop rotation experiment, means and standard errors of the means of the four replicates were calculated for $\log_{10}$-transformed DNA concentrations [fg of DNA of pathogen DNA per mg of plant residue (dry weight)] of *A. solani*, *C. beticola*, *R. beticola*, and *S. beticola* per sampling date for the different types of crop residues obtained in each crop–cropping system combination of the crop rotation experiment.

For the crop residue management experiment, means and standard errors of the means of the replicates (10 replicates at the beginning of the experiments and 5 replicates for each sampling date) were calculated for $\log_{10}$-transformed DNA concentrations of *A. solani* (expressed as fg of DNA of *A. solani* mg^{-1} dry material +1) on each experiment per sampling date, treatment, and soil surface/buried placement. DNA of *A. solani* was also calculated as the $\log_{10}$-transformed DNA concentrations remaining in the original samples placed in the bag (expressed as pg of DNA of *A. solani* in the remains of 1 g of dry material at the beginning of the experiment + 1). Decomposition was expressed as the means and standard errors of the means of the percent dry weight decomposed at each sampling date compared to the beginning of the experiments.

Statistical analysis was performed for the crop residue management experiment using the R software version 4.0.3 (R Core Team, 2020). For the analysis, linear mixed models as implemented in the R package version 3.1-3 *lmerTest* (Kuznetsova et al., 2017) were used. The responses in the analyses were respectively percent dry weight decomposed, the $\log_{10}$ transformation of *A. solani* DNA (fg of DNA of *A. solani* mg^{-1} dry material +1), and the $\log_{10}$ transformation of *A. solani* DNA present in the remains of the original samples placed in the bags [expressed as pg of A.s. DNA initial g^{-1} canopy residue (dw)]. The assumptions of the underlying model were checked with diagnostic plots. In order to include the initial amount of both DNA responses at the beginning of the experiments, the responses were transformed into the difference between the mean amount at the beginning and the actual measured amount at the four sampling dates. Percent dry weight decomposed was analysed as is. The model included the treatment as well as the placement as nominal variables in the fixed part of the model. Additionally, the four sampling times were included as a factor in the model. Initially, the model(s) also comprised the interactions between these 3 factors. However, they were reduced to a more parsimonious model when (some or all of) the interactions were not significantly contributing to the model. The random part of the model(s) accommodates corrections for the experimental design and controls for the variation associated with the experiments and the blocks nested within the experiments.

3 Results

3.1 Specificity and sensitivity of Taqman qPCR assays

Primers and probes developed for the quantification of *A. solani*, *C. beticola*, *R. beticola*, or *S. beticola* are given in Table 4. The specificities of the four qPCR assays were evaluated *in silico* by blastn research (https://www.ncbi.nlm.nih.gov/BLAST). The DNA of the host plants *Solanum tuberosum* (biosample SAMN02981305) and *Beta vulgaris* subsp. *vulgaris* (biosample SAMN23167344) could not be detected by the different primers and probes. Blast search of *R. beticola* and *S. beticola*-specific primers and probes gave a 100% match with the respective target and no match with the closest related species. In contrast, primers and probe for the detection of the *A. solani* were not specific to the targeted species but also detected *A. alternariacida* sp. nov, *A. catananches* sp. nov., *A. cichorii*, *A. conidiophora*, *A. dichondrae*, *A. grandis*, *A. linicola*, and *A. protenta*. For *C. beticola*, there was also a 100% match with *C. plantaginis*.

The specificities of the four developed Taqman qPCR assays were confirmed with synthetic DNA of the targets and the phylogenetically closest non-targets and genomic DNA (1 ng) of isolates listed in Table 1. Positive Taqman qPCR results were found for the respective assay with all targeted eight *A. solani*, six *C. beticola*, five *R. beticola*, or five *S. beticola* isolates. For non-targeted hyphal fungi and yeasts listed in Table 1, C_t values were higher than 40 or higher than C_t values of the lowest target concentration (see below). Calibration curves each consisting of five replicate dilution series of DNA of its target were used to determine the sensitivity. Linearity, efficiency, and limit of detection were estimated from the standard curves of *A. solani* 2020VR001, *C. beticola* 18-539-2, and *S. beticola* GV14-250-1 ranging from 1,000 to 0.1 pg of genomic DNA per reaction and from 1,000 to 0.01 pg for *R. beticola* 11rr04 (Supplementary Figure 1).

3.2 Population dynamics of *Alternaria solani*, *Cercospora beticola*, *Ramularia beticola*, and *Stemphylium beticola* in crop residues in crop rotation systems

Alternaria solani was consistently detected in 80% to 100% of the assessed samples of residues of the two potato crops at high concentrations above 10,000 fg of A.s. DNA mg^{-1} (Tables 5, 6). The pathogen was also consistently found in the litter on the soil surface after potato crops. Residue samples of weeds collected after potato crops contained for 50%–80% *A. solani*, but colonization was less compared to pathogen concentrations present in potato residues. The incidence of *A. solani*, defined as percentage of samples with positive detection of the targeted pathogen, in crop residue samples

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TABLE 5 Colonization of residues of main crops, cover crops, and weeds and of litter by *Alternaria solani* and *Cercospora beticola* in crops grown with the reference cropping system strategy (REF).

Crop	Type of residue	*Alternaria solani*				*Cercospora beticola*			
		Concentration[1]				Concentration[1]			
		Number of samples	Mean[2]	Maximum	Incidence (0–100)	Number of samples	Mean[2]	Maximum	Incidence (0–100)
Potato 1	Crop residues	36	12,634	438,211	100	36	0	494	6
	Litter	36	50,184	4,294,366	100	36	4	2,359	25
	Weeds	36	5,554	266,404	94	36	35	9,354	47
Winter/ Spring barley	Crop residues	44	2	1,507	18	44	5	6,481	27
	Litter	35	142	9,589	35	36	2	2,107	13
	Weeds	43	19	33,539	43	43	19	9,420	40
Sugar beet late harvest	Crop residues	36	1	1,750	11	36	74,861	2,057,300	97
	Litter	35	15	4,072	25	35	348,097	2,333,333	100
	Weeds	36	1	1,783	11	36	24,023	523,762	100
Carrot	Crop residues	28	11	8,659	18	28	12	2,172	38
	Litter	28	5	5,539	22	28	125	413,446	35
	Weeds	28	15	563	22	28	72	2,995	40
Potato 2	Crop residues	48	22,217	582,105	100	48	1	2,523	15
	Litter	48	23,563	1,667,726	88	48	5	3,741	15
	Weeds	47	750	368,731	79	47	17	11,738	37
Maize	Crop residues	48	152	26,211	65	48	8	1,365	31
	Litter	41	41	192,500	39	48	1	592	4
	Weeds	44	39	163,122	29	44	33	9,627	56
Sugar beet early harvest	Crop residues	48	16	111,694	33	48	30,139	1,499,416	98
	Litter	44	46	3,589	28	44	187,701	1,766,342	98
	Weeds	47	14	5,739	34	47	6,358	628,225	92
Onion	Crop residues	43	2	1,090	15	43	4	2,129	19
	Litter	36	0	2,460	3	36	31	6,644	46
	Weeds	43	3	5,768	20	43	8	20,116	33
Fodder radish	Cover crop	28	4	605	14	28	154	12,292	52
Winter barley	Cover crop	20	0	466	4	20	13	4,415	29

Mean values and maximum values for pathogen DNA concentration and incidence of *A. solani* and *C. beticola* in individual samples.
[1]fg of DNA of *A. solani* or *C. beticola* mg^{-1} dry material − 1.
[2]Backtransformed from mean of log_{10}-transformed data from all samples.

of barley, sugar beet, carrot, maize, or onion plots ranged between 10% and 60%, and mean concentrations of the pathogen were three to four orders of magnitudes lower compared to concentrations in potato residues collected during the first months after potato harvest. A similar pattern was observed in residues of weeds and in litter samples obtained from the same plots. Maximum concentrations indicate that in exceptional cases, crop residues, weed residues, or litter from plots not grown with potato but other

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TABLE 6 Colonization of residues of main crops, cover crops, and weeds and of litter by *Alternaria solani* and *Cercospora beticola* in crops grown with the integrated crop management (ICM) strategy.

		Alternaria solani				*Cercospora beticola*			
				Concentr-ation[1]		Concentration[1]			
Crop	Type of residue	Number of samples	Mean[2]	Maximum	Incidence (0–100)	Number of samples	Mean[2]	Maximum	Incidence (0–100)
Potato 1	Crop residues	36	25,902	402,990	89	36	3	1,062	8
	Litter	36	22,423	305,455	92	28	1	580	17
	Weeds	36	4,017	76,510	69	36	10	1,478	33
Winter/ Spring barley	Crop residues	40	29	109,214	45	40	26	5,772	26
	Litter	36	51	34,544	38	36	3	6,677	38
	Weeds	40	27	28,686	42	40	60	23,047	53
Sugar beet late harvest	Crop residues	36	1	468	11	36	54,513	729,878	100
	Litter	36	31	2,537	33	36	83,353	980,287	97
	Weeds	36	0	564	6	36	12,663	548,380	97
Carrot	Crop residues	28	4	27,224	21	28	6	2,785	51
	Litter	28	7	2,814	31	28	58	3,810	26
	Weeds	28	3	3,223	15	28	64	10,129	47
Potato 2	Crop residues	44	13,250	12,706,061	89	44	10	41,049	9
	Litter	36	8,848	329,595	91	44	1	614	29
	Weeds	43	204	41,772	54	43	17	2,560	41
Maize	Crop residues	48	53	7,866	46	48	4	1,861	6
	Litter	36	23	2,500	41	44	1	300	23
	Weeds	47	188	414,212	45	47	45	9,637	45
Sugar beet early harvest	Crop residues	43	13	8,190	33	48	20,437	307,376	100
	Litter	48	6	3,995	20	43	46,710	424,383	98
	Weeds	48	5	16,926	21	48	3,286	405,736	92
Onion	Crop residues	43	5	1,200	22	42	11	3,458	56
	Litter	35	2	5,462	16	35	72	6,805	32
	Weeds	44	11	7,776	34	44	6	2,556	27
Fodder radish	Cover crop	32	3	1,193	13	32	68	679,144	35
Tagetes patula	Cover crop	12	13,026	263,439	100	12	286	3,232	67
Winter barley	Cover crop	12	1	12,374	6	12	2	300	6
Lolium perenne	Cover crop	4	23	884	25	4	19	550	25

Mean values and maximum values for pathogen DNA concentration and incidence of *A. solani* and *C. beticola* in individual samples.
[1]fg of DNA of *A. solani* or *C. beticola* mg^{-1} dry material – 1.
[2]Backtransformed from mean of log_{10}-transformed data from all samples.

crops contained *A. solani* at high concentrations at 1,000 to 100,000 fg of A.s. DNA mg^{-1}. In residues of fodder radish, winter barley, and *Lolium perenne* grown as cover crops, the incidence of *A. solani* in samples was 25% or lower with only few cases of concentrations above 100 fg of A.s. DNA mg^{-1}. A distinct exception was found for *Tagetes patula*, grown as a cover crop after winter or spring barley in the ICM cropping system. *A. solani* was detected in all samples of *T. patula* residues at a high mean concentration of 13,000 fg of A.s. DNA mg^{-1}, similar to concentrations measured in residues of potato.

A similar pattern as found for *A. solani* in potato was found for *C. beticola* in early or late harvested sugar beet crops (Tables 5, 6). The incidence of *C. beticola* was >90% in sugar beet residues, weed residues, and litter collected in plots after harvest of sugar beet. Pathogen DNA concentrations were generally two times higher in plots where sugar beets had been harvested late compared to early harvested sugar beet. The concentrations in litter reached levels similar to or above the levels in sugar beet residues. In samples obtained from plots grown with barley, carrot, potato, maize, or onion, the incidence of *C. beticola* ranged between 4% and 56% and concentrations were three to four orders of magnitudes lower compared to the concentrations measured in residues from sugar beet plots. Exceptional high concentrations of *C. beticola* DNA were found in individual samples of weeds collected in crops grown in rotation with sugar beets. Concentrations above 10,000 fg of DNA of *C. beticola* mg^{-1} dry material were also found in individual crop residue samples collected in plots grown with fodder radish as cover crop.

Ramularia beticola was detected in 64 of 168 samples of sugar beet residues at average concentrations of 94 fg of R.b. DNA mg^{-1} (backtransformed values) and in 60 of 577 samples of other crop residues at on average 1 fg of R.b. DNA mg^{-1}. *Stemphylium beticola* was detected in 39 of 168 samples of sugar beet residues at an average concentration of 14 fg of S.b. DNA mg^{-1} and in 25 of 612 samples of other crop residues at an average concentration of 3 fg of S.b. DNA mg^{-1}. In litter, *R. beticola* was detected in 65 of 659 samples (average concentration of 28 fg of R.b. DNA mg^{-1}) and *S. beticola* was detected in 64 of 659 samples (average concentration of 35 fg of S.b. DNA mg^{-1}). In weeds, *R. beticola* was detected in 66 of 701 samples (average concentration of 13 fg of R.b. DNA mg^{-1}) and *S. beticola* was detected in 39 of 701 samples (average concentration of 2 fg of S.b. DNA mg^{-1}).

The dynamics of pathogen colonization during 2.5 years in residues of main crops, cover crops, and weeds and in litter present in the four replicated sub-plots are shown for all rotation plots of both crop management systems for *A. solani* (Figures 2, 4) and *C. beticola* (Figures 3, 5). Figures also include information on cropping periods of main crops and cover crops before and during the sampling period of 2.5 years and time points of residue sampling (for details, see Supplementary Table 1).

Main trends as summarized in Tables 5, 6 are shown, such as high concentrations of the pathogens in residues of their host crops and of weeds and in litter collected in plots during the months after harvest of the host crop. A decrease of *A. solani* concentration of up to two orders of magnitude for a few months after potato harvest was found in most cases, whereas no decrease or even an increase in colonization by one to two orders of magnitude during the months after harvest was observed in two cases (Figures 2C, G). These patterns in plots after potato cropping were similar for potato residues as well as for weed residues and litter. A similar rapid decrease was also found for *C. beticola* in residues of sugar beet and weeds and litter collected in plots during the months after harvest of the host crop.

During sampling periods following non-host crops, concentrations of *A. solani* were magnitudes lower than found in samples collected after potato growing, and the observed pattern was inconsistent. Values fluctuated between sampling dates, between replicate plots (resulting in larger standard error of the means), and between substrate types. Despite the inconsistent results for the various individual assessments, a common pattern occurred for all sampling periods following cropping periods of non-host crops: *A. solani* was present in all plots (at low levels) during periods without host crops. The highest variation including occasionally higher values of *A. solani* concentrations was found for residues of weeds, indicating that some weeds present in non-host crops may function as a host for *A. solani*. A similar pattern was found for *C. beticola* concentrations in non-host plots with fluctuating values between sampling dates, replicate plots, and substrate types, with the highest variation and highest values in weed samples.

Pathogens showed the same behavior in both crop management systems (Figures 2–5). Similar levels of colonization and similar patterns in population dynamics were observed except for the periods with *T. patula* growth in the ICM system. *A. solani* reached high concentrations not only in residues of this cover crop, but also in residues of the preceding main crop and weeds and in litter present in plots with *T. patula* cover crops.

3.3 Effect of crop residue treatment on decomposition and dynamics of *Alternaria solani* in potato crop residues

Flailed potato canopy residues buried in soil decomposed for 95% for 6 months in the first experiment started after potato harvest in 2020 (Figure 6A). Canopy residues left untreated or cut into 10-cm or 2-cm pieces decomposed for 92% to 93%. After the rapid decomposition during the initial 6 months of the experiment, further decomposition was slower. After 24 months, 1%–2% of the originally canopy residues were recovered. Residues were found in 75% of the buried bags (representing all four treatments), and in the remaining 25% of the bags, no residues could be recovered. Canopy residues that had been exposed in the field on the soil surface decomposed slower (Figure 6C). During 6 months, 93% of flailed residues were decomposed, whereas untreated residues or residues cut into 10-cm or 2-cm pieces decomposed less by 84%–87%. After 24 months, 3% of the flailed residues and 7%–9% of the residues cut into 10-cm or 2-cm pieces were still present. In the second experiment, started after harvest in 2021, decomposition of potato canopy residues in the soil was initially slower (Figure 6B) than in the first experiment. After 6 months, 80% of the untreated residues were decomposed. Higher decomposition was found for the other three treatments with 85%–88% decomposition. Further

decomposition was slower with no significant differences between treatments, and after 24 months, only 1%–4% of the residues were still present in the buried bags. In addition, residues placed on the soil surface decomposed slower compared to the first experiment (Figure 6D). After 6 months, 80% of the residues were decomposed without differences between treatments. Further decomposition was slow, but flailed residues tended to decompose faster than untreated or cut residues. After 24 months, 4% of the flailed residues were present, whereas a higher proportion of 10% of the residues cut into 2-cm pieces was still found. Statistically significant treatment effects were found only in a few cases for the two experiments. Flailed residues decomposed significantly faster compared to the untreated residues (p = 0.0045) and the residues cut in 2-cm pieces (p = 0.0090). However, there was a significant difference between bags buried in soil versus bags placed on the soil surface (p < 2.2e-16). Decomposition was significantly higher during the first two sampling dates (p < 0.0001), compared to the decomposition rates after the first year. In summary, decomposition of potato canopy residues showed similar pattern in both experiments for all "cutting" treatments.

The concentration of *A. solani* DNA steeply decreased in canopy residues buried in soil during the initial 6 months by three to four orders of magnitude with no differences between the treatments in the first experiment (Figure 7A). *A. solani* DNA concentration further decreased during the following months and no *A. solani* could be detected on the remaining residues after 24 months. Similar patterns were found in the second experiment (Figure 7B). However, higher concentrations of *A. solani* DNA were found in residues cut into 2-cm pieces after 12-month field exposition with 3,568 fg of A.s. DNA mg^{-1} (dw) (backtransformed values) compared to <50 fg of A.s. DNA mg^{-1} (dw) in the other treatments. At the end of the experiment after 24 months, traces of *A. solani* DNA were found in few residue samples. For residues on the field surface, different patterns compared to residues buried in soil and between the two experiments were observed (Figures 7C, D). In the first experiment, *A. solani* DNA concentration decreased by two orders of magnitude in flailed residues and residues cut into 2-cm pieces, whereas for the other treatments, such a decrease in concentration was not found during the initial 6 months. DNA concentration decreased in flailed residues during the following months, and after 24 months, *A. solani* could not be detected in such residues. In untreated residues and residues cut into 10-cm pieces, higher concentrations were found at the subsequent sampling dates, and finally after 24 months, low concentrations of 55–225 fg of A.s. DNA mg^{-1} (dw) were detected. In the second experiment, no treatment effect was observed during the first year (Figure 7D). Flailed residues still contained 3,568 fg of A.s. DNA mg^{-1} (dw) after 18 months of field exposition on the soil surface and 87 fg of A.s. DNA mg^{-1} (dw) after 24 months. At the end of the experiment, traces of *A. solani* were also still found in residues of the other treatments. Statistical analysis of both experiments showed no significant differences in any of the treatments (p = 0.2338). However, the decomposition of the canopy samples left on the soil surface was significantly slower

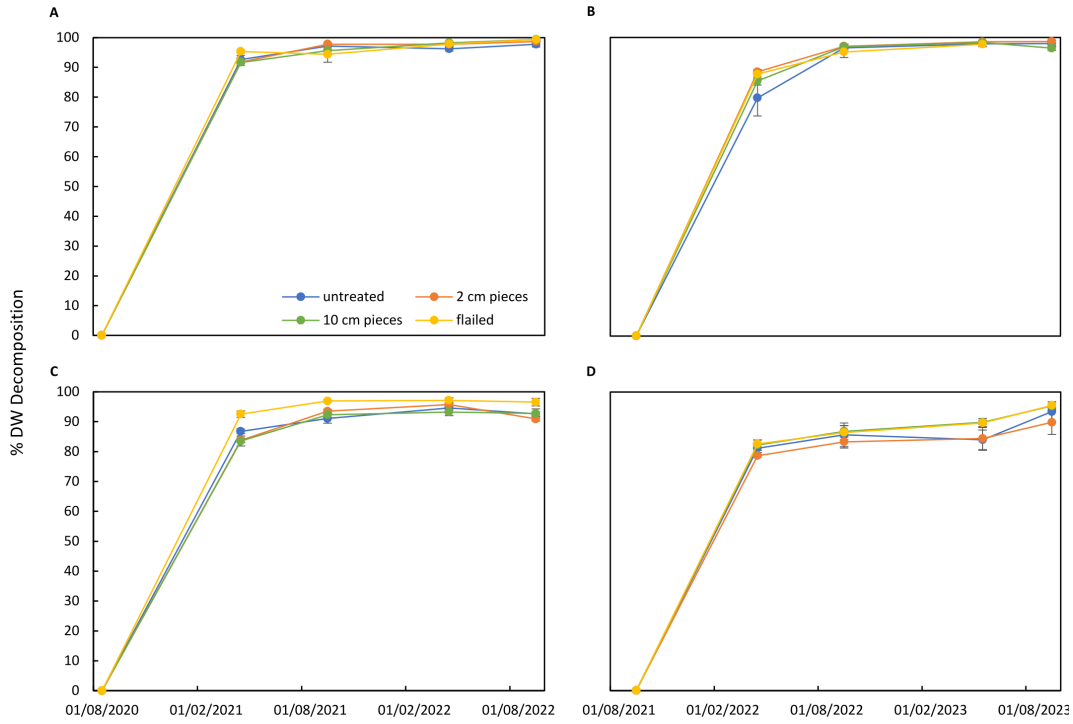

FIGURE 6
Decomposition (%) of potato canopy residues treated by flailing or cutting tissues in 2-cm or 10-cm pieces in comparison to untreated residues. Residues were buried in soil **(A, B)** or exposed to soil surface **(C, D)**. Two experiments started after potato harvest in 2020 **(A, C)** and in 2021 **(B, D)**. Decomposition was expressed as the percent dry weight decomposed at each sampling date compared to the beginning of the experiments.

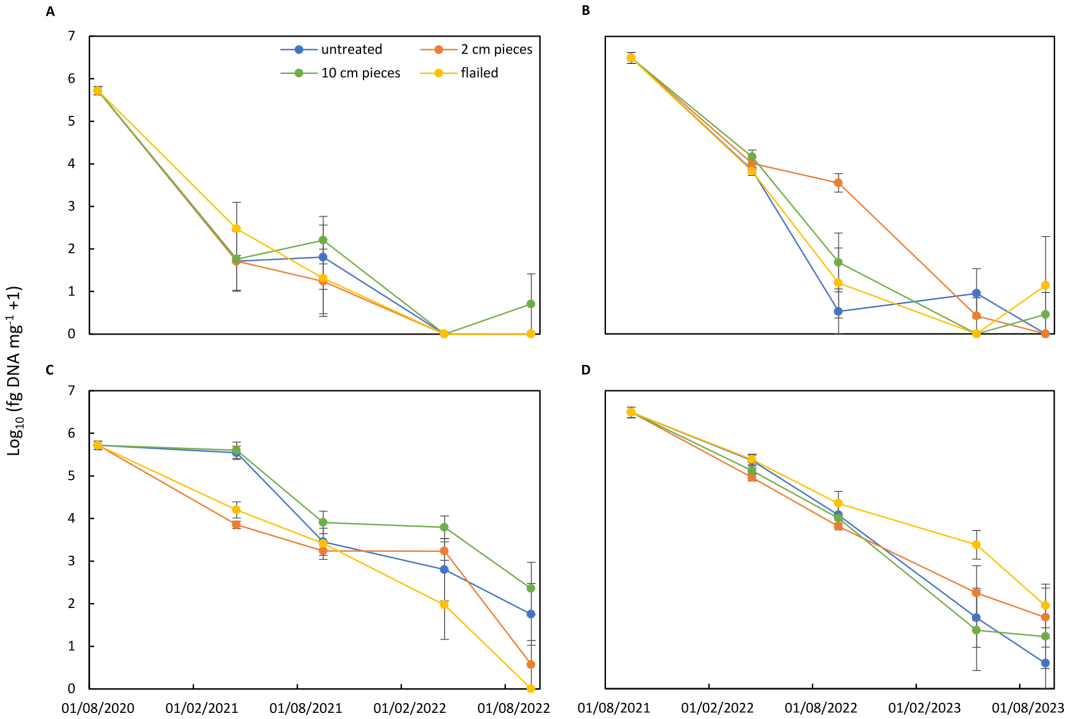

FIGURE 7
Concentration of *Alternaria solani* (log_{10} fg of DNA of *A. solani* mg^{-1} dry material + 1) in potato canopy residues treated by flailing or cutting tissues in 2-cm or 10-cm pieces in comparison to untreated residues. Residues were buried in soil **(A, B)** or exposed to soil surface **(C, D)**. Two experiments started after potato harvest in 2020 **(A, C)** and in 2021 **(B, D)**. *Alternaria solani* DNA concentration is expressed as the mean of log_{10}-transformed data from 10 replicates at the beginning of the experiments and five replicates for each sampling date. Bars correspond to the standard error of the means.

in all sampling periods, compared to the samples buried in the soil ($p = 2.369e^{-08}$). In summary, the *A. solani* DNA concentrations decreased in all treatments by two to four orders of magnitude during the initial 6 months and down to traces or undetectable concentrations after 24 months in the remaining residues. Decrease in concentrations in residues on soil surfaces was generally slower but reached similar low levels after 24 months as found in buried residues.

Combining the data on residue decomposition and the DNA concentration of *A. solani* in the remaining residues resulted in the amounts of *A. solani* DNA being present in the remains of the original samples placed in the bags (expressed as pg of DNA of *A. solani* in the remains of 1 g of dry material at the beginning of the experiment − 1) (Figure 8). Amounts of *A. solani* DNA present in the remains of canopy residues decreased during the initial 6 months by five orders of magnitude in residues buried in soil in the first experiment and two orders of magnitude in the second experiment (Figures 8A, B) without consistent treatment effects. In residues exposed to field surface, treatment effect was found in the first experiment, which was mainly caused by the treatment effects on DNA concentration of *A. solani* rather than treatment effects on decomposition (Figure 8C). In the second experiment, the decrease of *A. solani* DNA amounts followed the same pattern (Figure 8D). Statistical analysis showed no significant differences between any of the treatments ($p = 0.5428$). Significant differences were found between soil surface/buried placement ($p < 2e^{-16}$) and between sampling periods ($p < 2e^{-16}$).

4 Discussion

Residues of crops, weeds, and litter were systematically sampled in a complex crop rotation experiment. Concentrations of DNA of major pathogens of the grown crops, *A. solani*, *C. beticola*, *R. beticola*, and *S. beticola*, were quantified in the residues using newly developed qPCR assays. *Alternaria solani* and *C. beticola* were consistently detected, whereas *R. beticola* and *S. beticola* were found only a few samples at low concentrations. Repeated field trials gave additional insights into the dynamics of *A. solani* in potato foliage residues during 2 years. The overall results demonstrated that *A. solani* and *C. beticola* colonized crop residues of their host crops initially after harvest at high densities. Within several months, amounts of available host residues decreased substantially (as found for potato canopy residues) and concentrations of pathogens in the remaining host residues decreased steeply. Alternative substrates, residues of non-host crops including cover crops and weeds, were colonized saprophytically by necrotrophic pathogens. It can be concluded that such residues can potentially serve as a bridge for pathogen populations during host-free cropping seasons in crop rotation systems.

Quantitative Taqman PCR assays are powerful tools for such assessments because they can potentially quantify the concentration of the targeted DNA in environmental samples reliably within a large dynamic range. Original samples and extracted DNA samples can be stored, allowing the organization of workflows with high

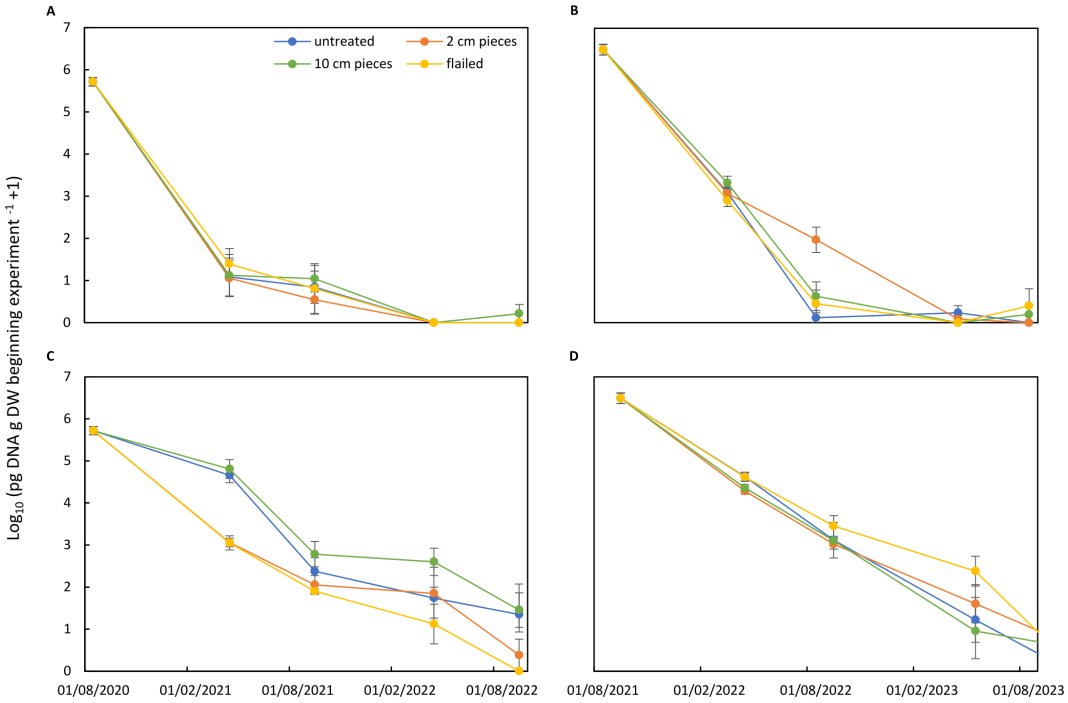

FIGURE 8
Amount of *Alternaria solani* $\log_{10}$ pg of DNA of *A. solani* in remains of 1 g of dry material at the beginning of the experiment +1 in potato canopy residues treated by flailing or cutting tissues in 2-cm or 10-cm pieces in comparison to untreated residues. Residues were buried in soil **(A, B)** or exposed to soil surface **(C, D)**. Two experiments started after potato harvest in 2020 **(A, C)** and in 2021 **(B, D)**. Values were calculated by considering residue decomposition (Figure 6) and *A. solani* concentration (Figure 7).

numbers of assessments in cost-effective formats, e.g., automated DNA extraction in combination with a 384-well format for real-time PCR measurements. Furthermore, storage of DNA samples allows future measurement of additional important pathogens, e.g., of toxigenic *Fusarium* spp. as pathogens of maize and barley, *A. dauci* and *A. radicina* as pathogens of carrots, *Botrytis squamosa* and *B. aclada/allii* as pathogens of onions, and *C. apii* as a pathogen of sugar beet, to enhance the view on the role of crop residues in pathogen population dynamics.

Several PCR assays for the quantification of *A. solani* have been described. The previously reported qPCR assay by Leiminger et al. (2015) showed a weak cross-reactivity with *P. infestans* and several other potato pathogens as well as with fungi not pathogenic in potato. The assay reported by Lees et al. (2019) shows cross-reaction with *A. dauci*, which is a pathogen in carrots present in our rotation experiment. The Syber green-based assay developed by Khan et al. (2018) for the detection of *A. solani* needs extra run and analyses time for melting curve analysis. Our new qPCR assay includes a Taqman probe, eliminating the use of a melting curve analyses. It also has a higher sensitivity with 100 fg per reaction compared to Khan et al. (2018), which has a sensitivity of 1,000 fg per reaction. However, our Taqman-PCR assay developed for quantification of *A. solani* also detects the closely related *A. alternariacida* sp. nov, *A. catananches* sp. nov., *A. cichorii*, *A. conidiophora*, *A. dichondrae*, *A. grandis*, *A. linicola*, and *A. protenta*. *A. grandis* and *A. protenta* are causing early blight in potato and are members of the European Alternaria population on potato (Landschoot et al., 2017). In addition, *A. alternariacida* sp. nov, newly described by Woudenberg et al. (2014) for an isolate from a fruit of *Solanum lycopersicum* in UK, has recently been reported as a causal agent of potato leaf blight in Russia (Kokaeva and Elansky, 2023). The other *Alternaria* species have distinct host ranges and have not been associated with potato or other crops grown in the rotation experiment. The recently newly described species *A. catananches* sp. nov. has been found on *Catananche caerulea* (Cupid's dart, *Asteraceae*) in the Netherlands, and *A. conidiophora* has been found on an unspecified host (Woudenberg et al., 2014). *A. cichorii* is causing leaf spot in endive and other Asteraceae (Barreto et al., 2008). *A. dichondrae* is a leaf spot pathogen of *Dichondra repens* (*Convolvulaceae*) (Cardin et al., 2005). *A. linicola* is causing seedling blight in flax (Corlett and Corlett, 1999). It is thus not likely that DNA of *A. catananches*, *A. conidiophore*, *A. cichorii*, *A. dichondrae*, or *A. linicola* was present in the investigated residue samples in amounts interfering with results obtained for *A. solani*. For members of the *Alternaria* complex on potato causing early blight, *A. grandis* and *A. protenta*, and possibly *A. alternariacida*, it is expected that they are present in the Netherlands, but this has not been confirmed yet.

Unexpected high concentrations of DNA of *A. solani* were detected in residues of *Tagetes patula* cover crops. Tagetes is not a known host of *A. solani*. However, *A. tagetica* has been described as a pathogen of *T. patula* causing foliar necrotic lesions (Tomioka et al., 2000). Sequence information on *A. tagetica* revealed that this pathogen is not detected by the applied Taqman-PCR assay. Another pathogen of *T. patula*, *A. patula* has been described as a new species in 2005 (Wu and Wu, 2005, 2019). Published

information on this species closely related to *A. tagetica* is limited and sequence information is not available. *Alternaria* spp. were frequently isolated from residues of *T. patula* in three samples collected in February 2023 from the rotation experiment (data not presented). The majority of the isolates as identified by *alta 1* and *gpd1* DNA sequence information belonged to *A. alternata*, a species that is not detected by the applied Taqman-PCR assay. Isolates of *A. solani* were also isolated from two of the three samples. Only one of the obtained various *Alternaria* spp. isolates belonged to another species, *A. cinerariae*.

The Taqman-PCR developed for the quantification of *C. beticola* described by Knight and Pethybridge (2020) also detects *C.* cf. *flagellaris* albeit with low sensitivity. The presence of *C.* cf. *flagellaris* on *B. vulgaris* has been reported by Groenewald et al. (2006) and Vaghefi et al. (2018), respectively. Our newly developed Taqman-PCR is not detecting *C.* cf. *flagellaris* and achieves a 10-fold increased sensitivity compared to the qPCR described by Knight and Pethybridge (2020). However, our Taqman-PCR assays developed for quantification of *C. beticola* also detect *C. plantaginis*. *C. plantaginis* has been associated with *Plantago* (Groenewald et al., 2006) and reported on *Plantago lanceolata* (Bakhshi et al., 2018). *P. lanceolata* (buckhorn plantain) is a ubiquitous species in the Netherlands that is commonly not growing in arable fields. There are no reports of *C. plantaginis* on sugar beet or other crops grown in the rotation experiment or other plant genera. It is thus not very likely that DNA of *C. plantaginis* was present in the investigated residue samples in amounts interfering with obtained for *C. beticola*. Besides *C. beticola*, *C. apii* is causing Cercospora leaf spot in sugar beet (Groenewald et al., 2006). This species is not detected by the used Taqman PCR assay. *C. apii* has recently been isolated from leaf spots on sugar beet leaves in Dutch fields including the experimental field in Vredepeel. In 2021, *C. beticola* was mainly isolated from leaf spots and only few isolates of *C. apii* were obtained from cv. Reforma KWS. However, in 2022, approximately 50% of isolates obtained from cv. Reforma KWS grown at the experimental field or from various cultivars grown at different locations in the Netherlands belonged to *C. apii* (Hanse, 2023). A new detection assay for quantification of *C. apii* is thus needed to complete the study on the role of crop residues on Cercospora leaf spot.

Quantifying the DNA concentration of a targeted fungal pathogen in environmental samples for a certain duration gives insights into its population dynamics. However, a careful interpretation should consider that such measurements do not distinguish between DNA derived from living or dead cells. Especially in situations when populations are decreasing, the decrease may thus be underestimated since the DNA of dead cells may be detectable for certain time spans. Furthermore, fungi can produce different cell types with different importance in population survival, e.g., mycelial cells, spores, resting spore such chlamydospores, sclerotia, and fruiting bodies. Knowledge on the biology of the pathogen is thus essential to allow adequate conclusions. *Alternaria solani* generally survives as mycelium and conidia, protected by dark pigmentation from environmental stress factors. Chlamydospores have been reported, but found infrequently (Kemmit, 2002). For *C. beticola*, it is reported that the pathogen survives in infested crop debris through formation of pseudostromata and that the pathogen can also survive on dead plant tissue as a necrotroph regardless of host status (Sharma et al., 2022).

The assessments of pathogen DNA in plant residues including litter present in the upper soil layer in the rotation experiment revealed a common pattern for both pathogens *A. solani* and *C. beticola*. Pathogen DNA concentrations were high in crop residues, weed residues, and residues in litter after harvest of the host crop. Concentrations decreased substantially during the following months. Pathogen DNA was found at low concentration in plant residues from plots grown with non-host crops. However, such results varied in time and between replicate plots. Specific variation and occasionally high concentrations were measured in residues of weeds, most likely because particular weeds occurring in the rotation plots acted as hosts, e.g., *C. album* for *C. beticola* or *S. nigrum* for *A. solani*. The risk that residues of weeds acting as hosts of the studied pathogens contribute to pathogen survival in a rotation system may increase in future cropping systems with reduced input of herbicides. Although not yet observed in the comparison between the reference cropping system and the ICM cropping system during the first years of the experiment, it is expected that occurrence of certain weeds will increase after several years of implementation of ICM systems with reduced herbicide use.

Our results suggest that residues of non-host crops may play an important role, allowing pathogens to bridge periods in the field without host crops and rapidly decaying residues of host crops. The amount of particular types of residues was not quantified when residues in the crop rotation experiment were sampled in our study. The relative importance of residues for colonization by pathogens depends on the amount and concentration of pathogens in the particular substrate. Residues will be present at harvest of a crop at high amounts and decrease in subsequent months as demonstrated for potato canopy residues in the additional experiments on residue treatments. This fluctuation of substrate availability will result in considerable fluctuations of pathogen populations. Cover crops included in crop rotation schemes may play a particular role since they produce biomass that entirely remains in the field and converts into plant residues. Differences in pathogen colonization between cover crops were observed in our study with unexpected high concentrations of *A. solani* in tagetes residues. Tagetes residues may be a particular suitable substrate for *A. solani*. Possibly, the senescence of the Tagetes crop coincidenced in certain periods with high *A. solani* densities on other residues leads to a rapid and intense colonization of Tagetes residues. Since cover crops produce huge amounts of residues, their particular role in the dynamics of pathogen populations needs attention in further studies.

The hypothesis for our field trial with treatments of potato canopy residues was that *A. solani* concentrations decrease faster in smaller canopy pieces since smaller pieces may be faster colonized by saprophytes outcompeting the pathogen. Effects of canopy residue treatments on *A. solani* during time could not be found. Further experiments are needed to better understand the colonization of residue pieces by saprophytes and how to support such microbiome functions. A clear main effect was demonstrated in the experiments. Canopy residues buried in soil decayed significantly faster and the concentration of *A. solani* also decreased faster compared to residues on the soil surface. This

may be due to different environmental conditions with higher and more constant water availability in buried substrates in combination with different composition of the invading microbiome developing in the residues. Burying crop residues, e.g., by ploughing, may not only enhance decomposition and reduce pathogen colonization but will also block transfer of pathogen from host residues to residues present after harvest of the subsequent non-host crop. This possible advantage of tillage in pathogen management needs further attention in future studies on tillage systems (Kerdraon et al., 2019; Wang et al., 2020).

Different inoculum sources can potentially initiate new epidemics once a susceptible host crop is established in the field. Pathogen populations may be present, colonizing necrotic plant tissues saprophytically as assessed in our study. Another source within a field can be volunteer plants of the host and host weeds that survived in subsequent crops and harbor the pathogen actively infecting the volunteer plants and weeds as pathogen (Thompson et al., 2015). Sources from outside the field can also play a role, e.g., pathogen spores present on seeds or airborne spores originating from fields where epidemics are already ongoing, or from saprophytically colonized plants and infected volunteer plants in the neighborhood of the field. The relative importance of the different potential sources depends on the biology of pathogen as well as on regional cropping systems and crop management. Experimental data on the source of spores initiating epidemics are limited and hard to obtain in field studies (Suffert and Sache, 2011). Experimental manipulations, e.g., by removal of known inoculum sources, that result in delayed epidemics give insight into the roles of certain sources (Köhl et al., 1995).

Future reduced tillage and non-tillage systems aiming at reduced CO_2 emissions, increase of organic matter in soil, and improved soil structure will result in increasing amounts of crop residues. However, there is evidence, including the results of this study, that crop residues within the field are a major source of pathogen inoculum, e.g., in cereal production (Kerdraon et al., 2019). Crop residue management—not interfering with the general goals of increasing organic matter in arable systems—is thus an option to lower the inoculum potential and subsequently delay or even prevent disease outbreaks. Crop residue treatments like shredding, application of urea, or physical removal have been reported for fruit production (Gomez et al., 2007). For prevention of early blight caused by *A. solani*, the removal of potentially infected materials such as wines and fruits (in case of tomato) is considered (Kemmit, 2002). Scientific reports on the effects of such sanitation measures for arable crops are limited, and reports considering the dynamics of pathogens in residues of subsequently grown crops including non-host crops in crop rotation systems are missing. Understanding the temporal dynamics of substrate quantities and quality will open opportunities for disease prevention through crop residue management in arable rotation systems. Understanding the biotic mechanisms will be important. Plant debris is colonized by different types of organisms in succession, depending on nutrient availability in degrading tissues, the nutritional needs of the potential colonizers, and their ability to utilize the remaining more complex organic compounds (Hudson, 1971; Hudson and Webster, 1958; Köhl and Fokkema, 1998; Kerdraon et al., 2019). Pathogens, being present already at senescence of the tissue, have an advantage in the early stages of decomposition during competitive substrate colonization. Substrate composition changes caused by nutrient consumption by initial colonizers result in more complex organic substrates, favoring specialists with appropriate physiological properties and leading to typical microbial successions in substrate colonization. Pathogens in this situation may be outcompeted and need to survive through the formation of resting structures or the colonization of alterative plant debris.

Crop residue management aiming at disease prevention thus needs to focus on the early stage of substrate colonization. Collection and physical removal of crop residues from the field is a realistic option if crop residues such as straw can be used as side products with an economic value. Field management will always focus on pathogens outcompeting during the early stages of decomposition, enhancement of early decomposition, or burying residues into the soil to block the transfer to alternative substrates and additionally to create a microbial buffer preventing substrate colonization by pathogens at later stages. Decomposition processes are the result of microbial activity, determined by the microbiome present at the beginning of decomposition processes and microbiome dynamics during decomposition processes (Kerdraon et al., 2019). Knowledge on microbiome functions and options for shaping microbiomes in the "residue sphere", defined as the microhabitat consisting of all crop residues on and in soil (Kerdraon et al., 2019), is essential to develop rational strategies for crop residue management including microbiome-based biocontrol solutions. Additionally, the role of the mesofauna in early decomposition of necrotic plant tissues, often neglected by plant pathologists, needs particular attention.

Crop residues of non-host crops grown in rotation as alternative routes for pathogens make pathogen life cycles more complex and considerations of sanitation more difficult. On the other hand, sanitation measures targeting crop debris shortly after harvest will not only block the development of populations of pathogens of this particular crop but also affect pathogens of other crops grown in the rotation. This increases the value of targeted crop residue management and may allow an integral sanitation of the crop rotation system rather than sanitation directed at a single pathogen.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JK: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. GE: Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. BH: Conceptualization, Funding acquisition, Investigation, Writing – original draft, Writing – review

& editing. IH: Data curation, Investigation, Methodology, Writing – original draft. LG: Data curation, Investigation, Methodology, Writing – original draft. EL: Data curation, Investigation, Methodology, Writing – original draft. HV: Data curation, Investigation, Methodology, Writing – original draft. AE: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2024.1470598/full#supplementary-material

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