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Does apple replant disease affect the soil patch selection behaviour and population growth of Collembolans?

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Abstract

Apple replant disease (ARD) is common to all major apple-growing regions in the world. It occurs when new apple trees are replanted on sites where previously the same or closely related crop species were grown. Biotic (fungi, bacteria and nematodes) and abiotic soil factors (poor soil structure, nutrition) contribute to the development and severity of ARD. However, the aetiology of ARD and effects on higher trophic levels are still unknown. In that sense, Collembola might play an important role, since they are one of the dominant mesofauna groups in many soils. They act as decomposer, fungivores and predators, representing different trophic levels in soil food webs. Therefore, any effect of ARD on the occurrence of Collembola could have ecological impacts on the soil quality and health. Here, we examined the colonization behaviour of two Collembolan species, *Folsomia candida* and *Sinella curviseta*, in choice tests and population growth tests using Apple Replant Diseased soil (ARD) and non-ARD soil samples from different field sites and standardized laboratory bioassays. Additionally, Collembola behaviour was quantified by continuous video observations to investigate short-term behavioural changes. Results showed that both Collembolan species significantly preferred colonization of the non-ARD soils compared with ARD soils, independent of the origin of the soil samples or specific disinfection treatments. Moreover, the detailed video analysis of the foraging behaviour indicates rapid colonization of soil samples and low dispersal rates. Most likely, volatile compounds and to a lesser extent feeding stimulants play a vital role for the colonization process for both Collembolan species. Finally, results showed negative effects of ARD on population growth of both Collembolan species already after an 8-week period, implying strong nutritional deficiencies in ARD affected soils. The hypothesis that ARD causing microorganisms directly affected orientation, colonization and population development of Collembola is discussed.

KEYWORDS
choice test, *Folsomia candida*, foraging behaviour, microorganisms, population growth, *Sinella curviseta*, soil type

1 | INTRODUCTION

Apple replant disease had been a crucial problem to apple growers for more than 200 years and is currently found worldwide in apple-growing areas. ARD is also named in the literature as 'replant disorder', 'replant problem' or 'soil sickness' (Mai and Abawi, 1981; Utkhede, 2006). Recently Winkelmann et al. (2018) defined ARD as 'a phenomenon which accounted for the detrimental interruptions on the physiological and morphological reaction of apple plants in connection with soils where the changing of microbial communities occurs due to old apple cultures'. Primarily, replanting of new apple plants on a site, which is repeatedly used for cultivation, causes to ARD. Symptoms are long-lasting and can be consistently observed shortly, that is 1–3 months, after planting. Characteristic symptoms of ARD include severe stunting, shortened internodes, leaf rosetting, small root systems, discoloured roots, root necrosis, reduced root biomass, delayed and declined productivity and finally tree death (Leinfelder and Merwin, 2006; Mazzola and Manici, 2012).

So far known, ARD is accounted for by a disease complex. Both biotic and abiotic soil factors contribute to the severity of ARD. Biotic factors include fungi (i.e. *Cylindrocarpon* spp., Nectriaceae fungi) (Grunewaldt-Stöcker et al., 2019; Popp et al., 2020), oomycetes (i.e. *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Rhizoctonia* spp., *Trichoderma* spp.) (Mazzola, 1998), bacteria (Yim et al., 2013) and nematodes (i.e. *Pratylenchus* spp.) (Kanfra et al., 2018). Abiotic factors consist of poor soil structure (Willett et al., 1994), nutrition (Simon et al., 2020) and poor cultural practices related to irrigation and crop rotation (Mai and Abawi, 1981; Traquair, 1984; Willett et al., 1994). However, the exact aetiology of ARD is still undefined, and scientists believe that biotic components play a more important role in ARD than abiotic factors. This has been proved also through soil treatments such as fumigation (Mai and Abawi, 1981), pasteurization (Yim et al., 2013) and gamma radiation (Yim et al., 2015), where massive plant shoot and root growth occurred in disinfected ARD soil compared with diseased ARD soil. Moreover, the abundance and diversity of microorganisms (i.e. bacteria) differed largely between in disinfected ARD and ARD soils (Yim et al., 2015). Hence, soil disinfection treatments not only reduce the abundance of pathogenic microorganisms but also promote the recolonization with alternative microorganisms after the disinfection treatments.

However, there is no single strategy for controlling apple replant disease. Therefore, biological, chemical and physical properties of the soil should be considered through the combination with management practices to minimize ARD. Manipulation of microbial communities (De Corato, 2020) is also an upcoming alternative method to mitigate ARD with the intention to reduce pathogenic biotic components and promote beneficial organisms, such as soil mesofauna.

In the current study, the focus is on the arthropod group of Collembola, since they are one of the dominant mesofauna groups in the majority of soil ecosystem (Hopkin, 1997). They are tiny, only few millimetres long, wingless animals and act as decomposer, fungivores and predators, representing different trophic levels in soil food web (Hopkin, 1997). Their abundance, diversity, species composition and community structure are strongly affected by the status of the soil quality (i.e. biotic and abiotic), climate changes and the cropping system (Hopkin, 1997; Larink, 1997; Rusek, 1998).

Information available about impact of ARD on the soil mesofauna, such as Collembola, is scarce (Winkelmann et al., 2019). Since Collembola are known to show certain food preferences (Hopkin, 1997) and respond to a number of volatile organic substances in the soil (Werner et al., 2016; Salmon et al., 2019) direct as well as indirect impacts on ARD are likely. So far, own results already show a reduced biodiversity and abundance of Collembola species in ARD soil compared with non-ARD control sites (Michaelis, 2018). To our knowledge, nothing else is known in the literature about the specific relationship between ARD and Collembola, and therefore we investigated in the current study effects of ARD on the behavioural as well as on the population level. With dual choice experiments we first explored the attraction of Collembola to ARD and non-ARD soils. Based on the results, we investigate Collembola foraging in more detail with continuous video observations. Finally, population growth of Collembola was studied in microcosm experiments in ARD and non-ARD soil samples. As model organisms we select *Folsomia candida* and *Sinella curviseta* for several reasons: Both organisms are easy to rear under laboratory conditions, numerous studies are available in the literature for comparison, and both species were also present in soil samples from ARD field sites (Michaelis, 2018).

In general, we hypothesize that both Collembolan species prefer non-ARD soil patches over ARD soil patches for colonization, due to differences in food quality as a result of apple replant disease. Moreover, we also expect that population growth in ARD soil will be reduced compared with non-ARD soils.

2 | MATERIALS AND METHODS

2.1 | Rearing of Collembola

Folsomia candida Willem 1902 (Family Isotomidae) and *Sinella curviseta* Brook 1882 (Family Entomobryidae) species have been obtained from Göttingen University, Germany and reared at the entomology laboratory of the Section of Phytomedicine, Institute of Horticultural Production Systems, Leibniz Universität, Hannover. *Folsomia candida* and *S. curviseta* have been reared separately in plastic boxes (L = 26.5 cm, W = 16 cm, H = 9 cm) containing a 1.5 cm layer of plaster of Paris mixed with activated charcoal (20:1/w:w). Collembola were fed twice a week with 0.5–1.0 g dry bakers' yeast (*Saccharomyces cerevisiae*) and a few drops of distilled water. Rearing boxes were kept in climate cabinets at 23 ± 1°C in the dark. To obtain comparable results all specimens were reared to the same physiological age, that is young adults. Therefore, newly laid eggs were used to synchronize populations. Due to differences in development times *F. candida* needed 14-days and *S. curviseta* 18-days until reaching adulthood and maturity. All individuals were starved for 48 h before starting the experiments and were employed only once.

2.2 | Soil origin and preparation

Field sites available for this study are located in Ellerhoop (latitude 53.71435; longitude 9.770143 WGS 84, Schleswig-Holstein, Germany), Heidgraben (lat. 53.699199; long. 9.683171; WGS 84, Schleswig-Holstein, Germany) and Meckenheim (lat. 50.619028; long. 6.990389 WGS 84, North Rhine-Westphalia, Germany). On reference sites Ellerhoop and Heidgraben, ARD is repeatedly induced by new plantings of rootstock seedlings (cultivar 'Bittenfelder Sämling') every second year since 2009, while Meckenheim has been used for apple varieties grafted on the rootstock M9 since 2006 (Reim et al., 2020). Four randomly arranged ARD plots (10×10 m), as well as four grass-covered non-ARD control plots, are available in Ellerhoop and Heidgraben for sampling. Disease incidence and severity on field plots was confirmed by Mahnkopp et al. (2018). Results indicate that apple plant growth on the reference sites got halved over four replant generations. Additionally, standardized bio tests with apple seedlings in the greenhouse (see below) confirmed that ARD severity was highest at Heidgraben followed by Ellerhoop, underlined by a four-times (Heidgraben soil) and two times (Ellerhoop soil) increased plant growth in non-ARD (gamma treated) compared with ARD soil. Therefore, soil from different sites were used to demonstrate sensitivity of Collembola and repeatability of results.

From each location 10 L total volume of soil was collected at a depth of 0–25 cm in July and September 2017. Therefore, three subsamples were taken randomly from each plot. To receive a homogeneous soil substrate, subsamples from each plot were mixed carefully. Field-collected soil samples were directly used in dual choice tests, video analysis experiments and population growth experiments (see below).

The additional standardized soil samples originated from a greenhouse experiment which was designed as bio test to evaluate the expression of candidate genes in response to ARD in roots and leaves of apple plants and to quantify effects of ARD on plant growth (Reim et al., 2020). Briefly, soil samples from ARD field plots (Heidgraben and Meckenheim) were either left untreated, that is ARD, or were gamma treated with a minimal dose of 10 kGy, that is disinfected (Yim et al., 2015), to kill most microorganisms and animals (McNamara et al., 2003). Subsequently in vitro propagated apple seedlings were grown for a four-week period in the differently treated soils, that is non-ARD (gamma treated) vs ARD (untreated) soil. After 4 weeks, plant quality (root colour and habitus) and plant growth (shoot length, shoot and root fresh masses) were investigated and results showed expected negative effects of ARD on apple plant and root growth (Reim et al., 2020). For the current experiments with Collembola, the results of this biotest were a confirmation of the ARD and non-ARD soil status and compared with the field-collected soil samples provided a far more standardized substrate. Therefore, soil was collected at the end of this four-week experiment (September 2017) after removing apple plants and used for additional colonization experiments with Collembola, that is dual choice tests (see below).

FIGURE 1 Petri dish setup for dual choice experiment. In a layer of plaster of Paris two small Petri dishes are inserted which contain the soil samples. The red spot indicates the Collembola releasing point [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | Dual choice tests to investigate soil patch selection behaviour of Collembola

2.3.1 | Experimental design of dual choice tests

Dual choice tests were designed to investigate the effects of ARD on the colonization behaviour of two Collembolan species, that is *F. candida* and *S. curviseta*. Same amounts of non-ARD and ARD soil samples were offered in the dual choice tests for colonization by the Collembola.

The experimental arena was composed of a large (13.5 cm diameter, 1.9 cm height), and two smaller Petri dishes (3.5 cm diameter, 1 cm height) that were glued to the bottom of the large with 3.5 cm distance between the two smaller ones (Figure 1). The remaining area of the large dish was filled with a layer of plaster of Paris mixed with activated charcoal (20:1/ w:w). The large Petri dish was closed with a transparent lid while the small ones contained the soil samples and were left open for subsequent colonization.

Based on preliminary experiments moisture content was adjusted to 80% (w/w) in the plaster of Paris arena and 20% (w/w) in soil patches at the beginning of the experiment to optimize Collembola survival. To rule out any positional effects, that is systematic error in the later experiments, differences in distribution of both Collembola species in Petri dishes were also investigated in preliminary experiments. Results show that Collembola distributed evenly among two identical soil samples in the two small Petri dishes independent of the position (results not shown).

In the actual dual choice experiment, the two small Petri dishes were filled with 5 g of ARD or 5 g non-ARD soil samples (20% soil moisture), originating either from field samples or from the central greenhouse experiment (see above). A group of twenty synchronized *F. candida* or *S. curviseta* females were starved for 48 h and

then released near to the edge of the large arena for subsequent colonization of the soil patches (Figure 1). Four soil combinations were used: (1) field soil samples from Heidgraben non-ARD (grass control) vs ARD, (2) Ellerhoop non-ARD (grass control) vs ARD, (3) standardized soil samples from Meckenheim non-ARD (gamma treated) vs ARD and (4) Heidgraben non-ARD (gamma treated) vs ARD. Each combination was replicated at least 40 times for each species under controlled conditions in climate cabinets (23 ± 1°C, 80% RH, no light). Patch preference was estimated after 48 h by counting all individuals in the arena and in the soil, that is in each small Petri dish. Unfortunately, there were few dead Collembola in some arenas. Therefore, only alive individuals were considered and proportions of the population active in the arena (undecided individuals) or located on soil patches (decided individuals) were calculated for data analysis.

2.4 | Investigating Collembola behavioural decisions continuously via video analysis

2.4.1 | Experimental design

Since frequent migration processes between the two soil samples in the choice experiment could influence the single data points obtained after 48 h substantially, continuous observations of the foraging behaviour were realized by 48 h video recordings. Again, dual choice tests were carried out using 5 g of ARD or non-ARD (grass control) field soil samples from Ellerhoop (see above). Twenty synchronized *F. candida* or *S. curviseta* females were released into the arena (see above) and observed for 48 h by single video cameras. Therefore, cameras (Panasonic WV-BP322E) were focused on the experimental unit, which was covered with a transparent lid to control moisture and avoid disturbance, at a distance of 30–35 cm. The video recordings were done in a dark room at room temperature (23 ± 1°C) under infrared light illumination (4 LEDs; OS-5038F 940 nm, 30°) positioned above the arenas. Videos were stored on hard disk with a digital video recorder (ECOR-FHD-4F, EverFocus, Taiwan). Image processing and analysis were performed manually by using the build in EverFocus-EFP player. Each combination was replicated five times for each species. Room temperature and humidity was recorded using Tinytag data loggers (TGP-4017, Gemini Data Loggers, UK).

2.4.2 | Analysis of video recordings

Since tracking of individual Collembola was not possible inside the soil samples, number of Collembola moving in and out both soil patches during 48 h were counted manually while watching the recorded videos. For analysis, the mean number of Collembola located on each soil patch was counted at 0, 6, 12, 18, 24, 30, 36 and 48 h after introduction of Collembola into the experimental arena. Cumulative percentages of individuals in the soil patches were used for graphical and statistical analysis.

2.5 | Impact of ARD on population growth of Collembola

To investigate ARD effects on population growth of Collembola, plastic jars (h = 7 cm; d = 7.5 cm) covered with a fine-meshed lid were used as experimental units. Field soil samples from Ellerhoop and Heidgraben, that is non-ARD soil (grass control) and ARD soil, were used as substrate. Thereafter, 10 synchronized parthenogenetic *F. candida* or 10 synchronized males and 10 females *S. curviseta* were added to 50 g of soil (20% soil moisture) in each experimental unit. Each treatment was replicated twenty times for each species. Experimental units were kept in the climate cabinet (23 ± 01°C, RH ~80%, 24 h dark) for 8 weeks. Twice a week tap water (1–10 ml) was added to adjust the soil moisture content inside the experimental units based on the weight loss. At the end of the experiment, Collembola were extracted using a MacFadyen extractor within 9 days, in which soil samples were heated from above via hot air and cooled and slightly moistened by cool humidified air from below (see Michaelis, 2018, for details). Collembola moved towards the cool area and were sampled into the collecting vessels (70% alcohol) on the underside of the MacFadyen extractor. Total number of Collembola extracted were counted under a stereomicroscope.

2.6 | Data analysis

All analyses were performed using the statistical software SPSS (version 24). Significance level was always considered at $\alpha = 0.05$ (mean % ± SD). Proportions of *S. curviseta* and *F. candida* colonizing tested soil patches were compared using Wilcoxon signed rank test. Furthermore, the Binomial-logit model was employed to check the effects of different experimental sites (Heidgraben, Ellerhoop, Meckenheim) on colonization by *S. curviseta* and *F. candida*.

To analyse the migratory behaviour of Collembola between the two soil patches, percentages of individuals moving in and out patches were calculated per hour from manual counts on video recordings. All means were compared by Wilcoxon signed rank test.

The population growth rate (*pgr*) was estimated as the natural rate of increase, r, using following equation: $pgr = \log_e (N_t/N_0)/ t$, where N_0 is the initial number of Collembola at time zero, and N_t is the final number of Collembola (adults+juveniles) and t is the time (days) (Larsen et al., 2008). Declining populations are indicated by $r < 0$, while $r = 0$ indicates stable and $r > 0$ growing populations. The effects of site, soil type and their interaction on total population of *S. curviseta* and *F. candida* were investigated using Poisson-log models.

3 | RESULTS

3.1 | Dual choice tests with field soil samples and continuous video analysis

In general, both Collembola species responded in the dual choice experiments strongly to soils from Ellerhoop and Heidgraben. More than 80% of the surviving *S. curviseta* population colonized soil

patches (either non-ARD or ARD soil = decided Collembola) after 48 h (Figure 2). *Sinella curviseta* showed similar responsiveness for soils from both field sites ($W_T = 0.68$, df = 1, $p = 0.411$). In contrast, responsiveness of *F. candida* was lower and significantly differed between sites ($W_T = 75.57$, df = 1, $p < 0.01$). Although 75% of *F. candida* colonized soil patches from Ellerhoop, only 54% colonized soil patches from Heidgraben (Figure 2).

Comparison of colonization behaviour of Collembola towards field-collected soil samples in choice experiments showed that there is a strong preference of both Collembola species for non-ARD soil (grass control) (Figure 3). More than 75% of the population of *S. curviseta* as well as *F. candida* preferred colonization of non-ARD (grass control) compared with ARD soil patches. Moreover, both species colonized non-ARD (grass control) soil patches from both sites, that is Heidgraben and Ellerhoop, at similar rates (*S. curviseta*; $W_T = 0.061$, df = 1, $p = 0.63$ and *F. candida*; $W_T = 0.33$, df = 1, $p = 0.41$). Even though only 50% of the alive population of *F. candida* were found in the soil samples from Heidgraben after 48 h (see above), 75% of them significantly preferred non-ARD (grass control) soil from Heidgraben instead of ARD soil (Figure 3).

Investigating movements of Collembola between soil patches by continuous video recordings reveal that the overall colonization rate rapidly increased during the first day and remained on a plateau on the second day. Nevertheless, colonization rates differed slightly between the two Collembola species. The overall colonization rate of non-ARD soils by *S. curviseta* rapidly increased during the first 36 h (Figure 4) and remained constant, that is reaching a plateau, until the end of the observation period. In contrast, the colonization rate of the ARD soils increased only slightly with time and remained overall at a low level. Significantly more *S. curviseta* colonized the non-ARD soil (grass control) compared with ARD soil from 18 h (non-ARD 36.25% ± 12.50%, ARD 6.25% ± 7.50% mean number of *S. curviseta* / 18 h, $p = 0.042$) until the end of the experiment (non-ARD 57.50% ± 11.9%, ARD 15.00% ± 15.8% mean number of *S. curviseta* / 48 h, $p = 0.043$). In contrast, colonization rate of non-ARD (grass control) soils by *F. candida* rapidly increased during the first 24 h (non-ARD 67.00% ± 23.60%, ARD 9.00% ± 8.20%, mean number of *F. candida* / 24 h, $p = 0.043$) and remained at a high level until the end of the 48 h observational period (non-ARD 67.00% ± 21.40%, ARD 17.00% ± 14.40%, mean number of *F. candida* / 48 h, $p = 0.042$) (Figure 4). Moreover, pattern of colonization rate of the ARD soil by *F. candida* was similar to *S. curviseta*, increasing only slightly to a low level. Here again, result at 48 h was similar with the results from choice experiments (done in the climate cabinet) in which *F. candida* preferred significantly non-ARD soil (grass control) patches.

3.2 | Dual choice test with standardized soil samples from a greenhouse experiment

To validate the direct impact of ARD on Collembola behaviour and exclude effects from ground cover (grass control), dual choice experiments were repeated with soil samples from a highly standardized greenhouse experiment (see above). Compared with the previous choice test with field soil samples the overall responsiveness (based on decided Collembola) was similar but more consistent for samples from different locations for both Collembola species (Figure 5). On average more than 60% of the Collembola colonized the soil patches and both species showed a significant higher responsiveness to soils from Meckenheim (decided *S. curviseta* 82.24% ± 11.79, decided *F. candida* 74.55% ± 11.36) compared with Heidgraben. While *S. curviseta* responsiveness to soils from Meckenheim was higher than *F. candida*, both species responded similar to soils from Heidgraben (decided *S. curviseta* 67.45% ± 9.25, decided *F. candida* 65.76% ± 8.84). (Figure 5).

In the choice test both Collembola species clearly preferred non-ARD (gamma treated) soil over ARD soil. More than 70% of the

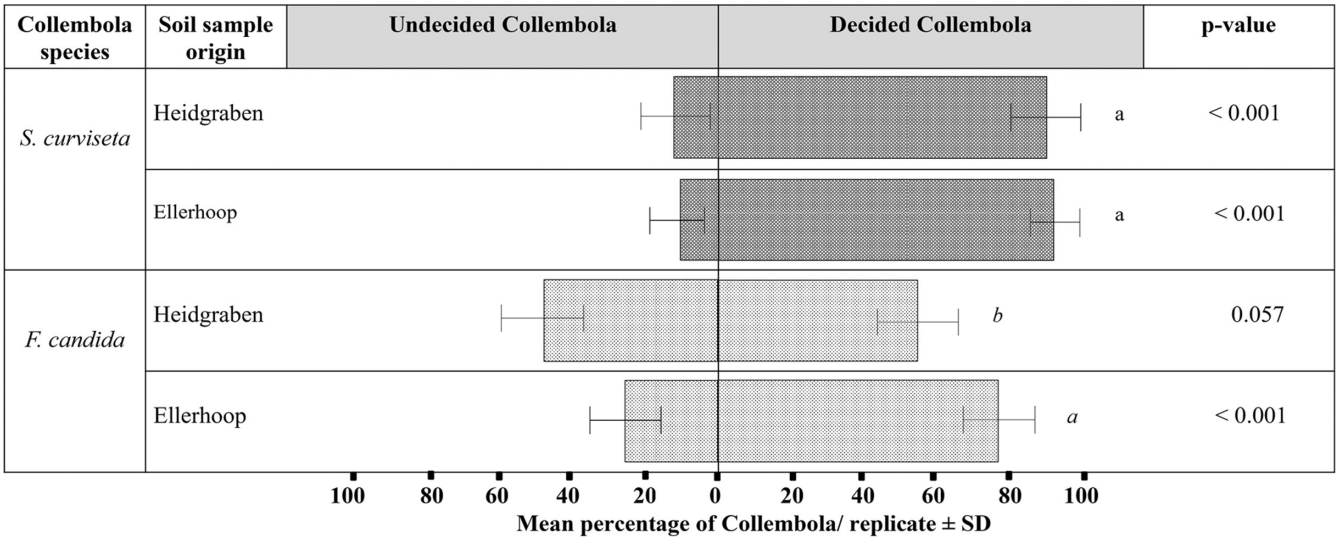

FIGURE 2 Average percentages (± SD) of decided (in the soil) and undecided (in the arena) adult Collembola in choice experiments with soil samples (5 g) from the field sites. P-values indicate significant differences between decided and undecided adult Collembola, while letters represent significant differences between decided Collembola across both species and sample origins (GLM-ANOVA)

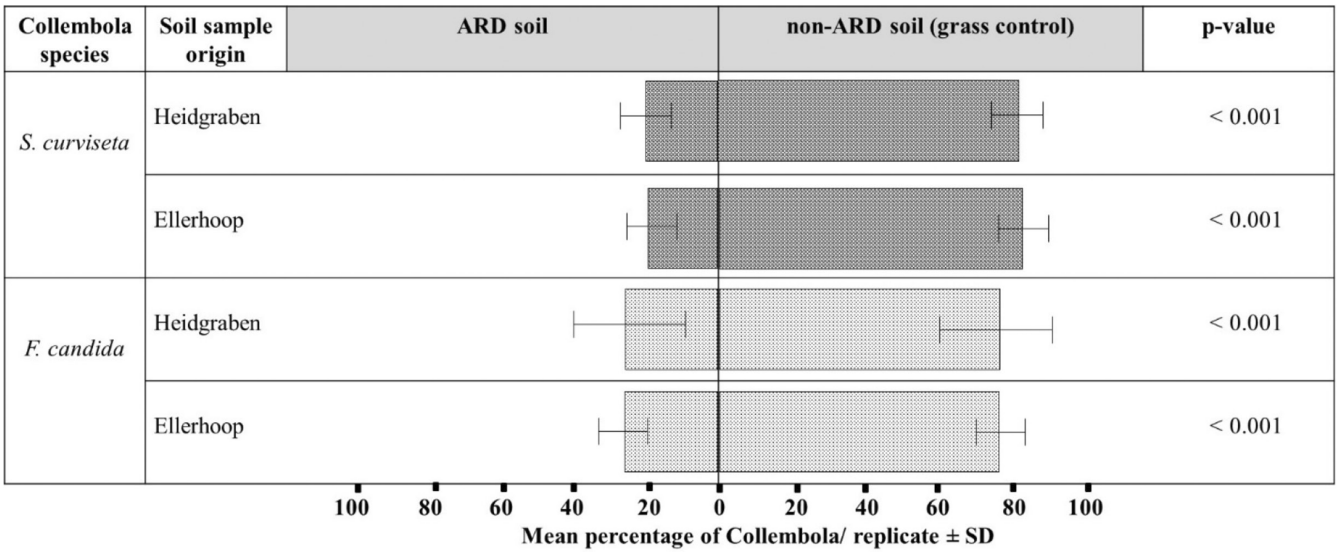

FIGURE 3 Average percentages (± SD) of adult Collembola colonizing apple replant disease soil (ARD) or non-ARD soil (grass control) in choice experiments with soil samples (5 g) from the field sites. *P*-values indicate significant differences in percentages of adult Collembola colonizing the two different soils (Wilcoxon signed ranks test)

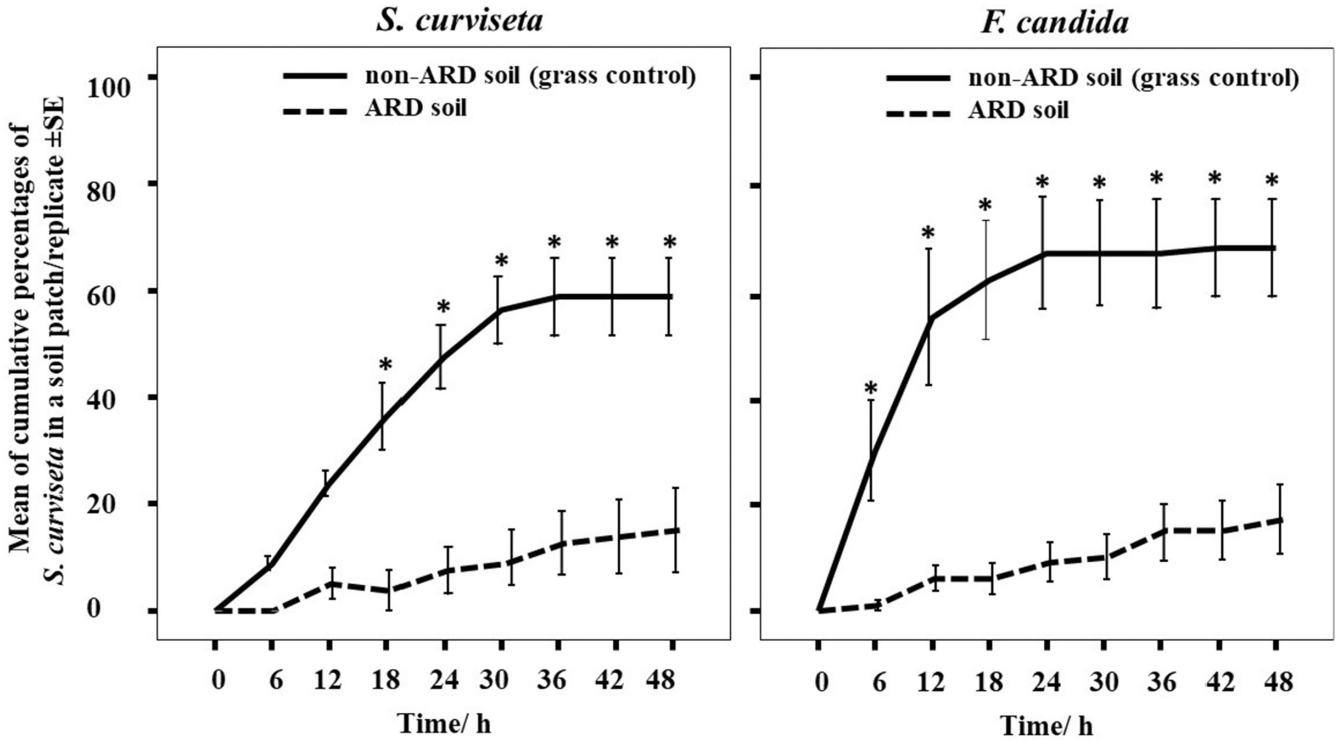

FIGURE 4 Average cumulative percentages of *Sinella curviseta* (left side) and *Folsomia candida* (right side) found in non-apple replant disease soil (non-ARD grass control) or ARD soil patches at 6 hour intervals ($n = 5$). Stars indicates statistically significant differences of values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

decided animals colonized non-ARD (gamma treated) soil irrespective of Collembola species or soil origin (Figure 6).

Moreover, a binomial-logit model confirmed that colonization of *S. curviseta* ($W_T = 2.184$, df = 1, $p = 0.139$) and *F. candida* ($W_T = 1.77$, df = 1, $p = 0.275$) of non-ARD (gamma treated) soil was not affected by the site location.

3.3 | Impact of ARD on population growth of Collembola

Population growth (*pgr*) of both species was more than two times higher in non-ARD (grass control) soil than ARD soil (*Folsomia candida* $W_T = 312.14$, df = 2, $p < 0.01$; *Sinella curviseta* $W_T = 255.677$, df = 2,

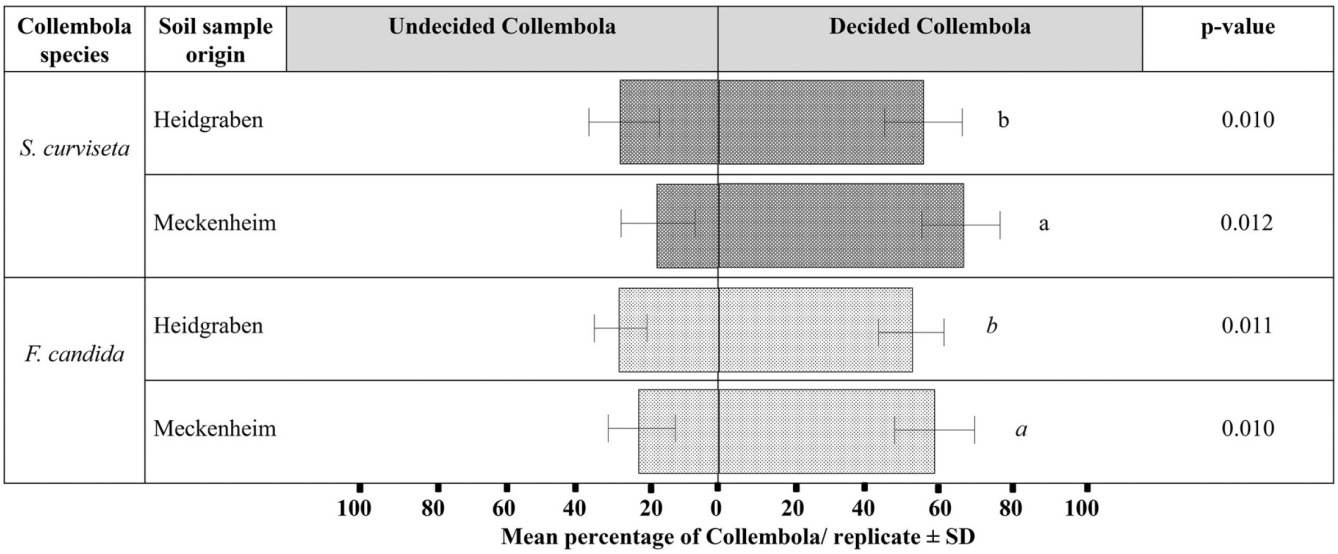

FIGURE 5 Average percentages (± SD) of decided (in the soil) and undecided (in the arena) adult Collembola in choice experiments with soil samples (5 g) from the central greenhouse experiment. *P*-values indicate significant differences between decided and undecided adult Collembola, while letters represent significant differences between decided Collembola across both species and sample origins (GLM-ANOVA)

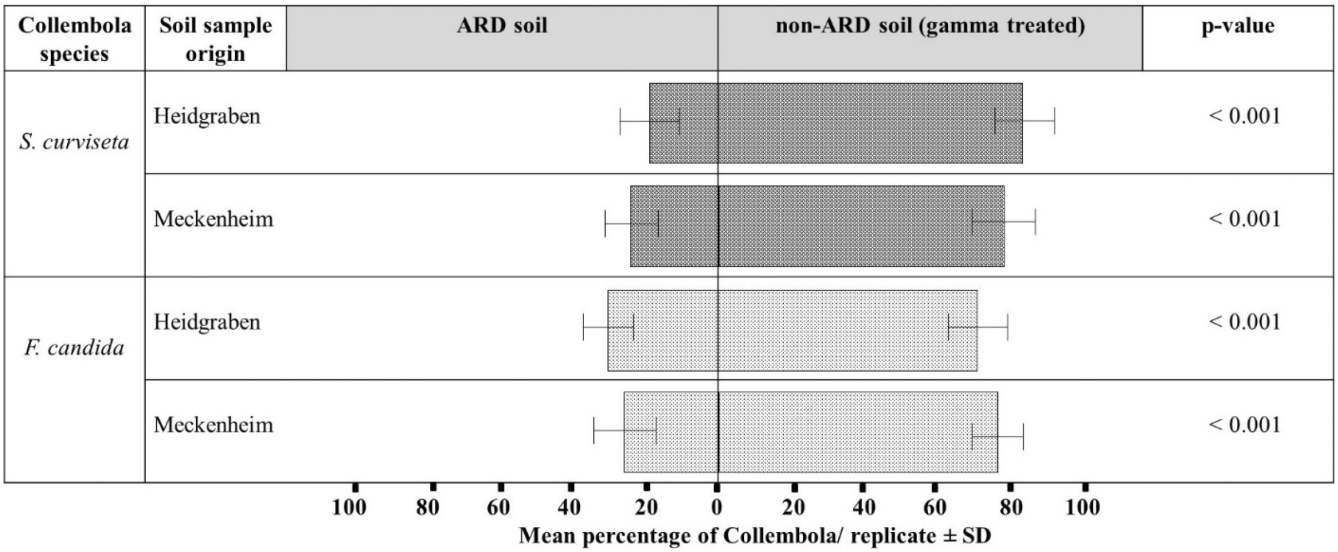

FIGURE 6 Average percentages (± SD) of adult Collembola colonizing apple replant disease (ARD) soil or non-ARD soil (gamma treated) in choice experiments with soil samples (5 g) from the central greenhouse experiment. P-values indicate significant differences in percentages of adult Collembola colonizing the two different soils (Wilcoxon signed ranks test)

$p < 0.01$). The highest population density of *S. curviseta* was recorded in non-ARD (grass control) soil from Ellerhoop (88.85 ± 14.52 individuals) followed by non-ARD (grass control) soil from Heidgraben (70.50 ± 15.01 individuals). Similarly, non-ARD (grass control) soil from Ellerhoop accounted for the highest population of *F. candida* (136.35 ± 22.42 individuals) followed by non-ARD (grass control) soil from Heidgraben (114.4 ± 19.55 individuals) (Figure 7).

Both species had positive *pgr* in both non-ARD (grass control) (*F. candida* Heidgraben: 2.561, Ellerhoop: 1.751 and *S. curviseta* Heidgraben: 1.335, Ellerhoop: 1.200) and ARD soil (*F. candida* Heidgraben: 1.540, Ellerhoop: 1.294 and *S. curviseta* Heidgraben: 1.156, Ellerhoop: 1.103). However, the *pgr* values were always higher in non-ARD soil than in ARD soil (*Folsomia candida* Ellerhoop, $p < 0.01$; *Folsomia candida* Heidgraben, $p < 0.01$; *Sinella curviseta* Ellerhoop, $p < 0.01$; *Sinella curviseta* Heidgraben, $p < 0.01$). Moreover, the differences in population growth of Collembola between non-ARD and ARD soil were slightly larger for Heidgraben than Ellerhoop soil (Figure 7).

4 | DISCUSSION

The main aim of the current research was to study the impact of ARD on the colonization behaviour and population growth of two Collembola species *S. curviseta* and *F. candida*. Overall, the strong adverse effect of ARD on the two Collembola species is similar in all experiments and are underlined by a strong negative impact of ARD on population growth of both Collembola species already after 8 weeks. Nevertheless, slight differences can be found for the number of undecided individuals after 48 h, which was higher for *F. candida* than for *S. curviseta* in experiments with field-collected soil samples. But in case of the highly standardized disinfected, that is gamma treated, soils from the greenhouse experiment with apple seedlings, the number of undecided individuals is similar for both species. Therefore, the experimental design of our choice test arena guarantees reliable results, since many undecided *F. candida* would indicate high disturbance in the small arena and therefore high reactivity to some unknown factors. On the contrary, it might also indicate low preference for any specific habitat in the experimental arena and therefore a low tendency of colonization. But especially the strong responsiveness with the standardized soil treatments of the second experiment indicate higher numbers of decided Collembola for both species.

In general, the composition of the bacterial community is different in the two field sites (i.e. Ellerhoop and Heidgraben), which is related to the site-dependent effect of microorganisms on Collembola behaviour documented in the current study. For example, bacterial genera *Streptomycetes*, *Bosea*, and *Methylophilaceae* were rich in ARD soils compared with non-ARD (grass control) soils from Ellerhoop (Suárez et al., 2018). Moreover, the fungal endophyte community in apple roots of ARD soil differed strongly from roots in non-ARD soil (grass control) with consequences along the root-soil interface (Popp et al., 2018). Among the discovered differences in endophytic organisms, especially members of the family *Nectriaceae* have the potential to act as causal agents of ARD due to blackening symptoms in the apple root system (Grunewaldt-Stöcker et al., 2019). Most likely differences in the bacterial and fungal community in the soil also affect the observed differences in Collembolan colonization behaviour. Several findings in the literature underline this hypothesis showing attraction to certain soils under experimental conditions. For example, Bengtsson et al. (1994) showed that the fungivorous Collembola species *Onychiurus armatus* had the highest dispersal in moor soils, that is F-layer from deciduous forests, enriched with the fungal species *Mortierella isabelline*.

In the current study, both Collembola species are found in higher numbers on the non-ARD soil compared with the ARD soil patches. Results are similar regardless of the origin of the soil samples (Heidgraben, Ellerhoop, Meckenheim) or the disinfection procedure by gamma radiation. Two non-exclusive mechanisms

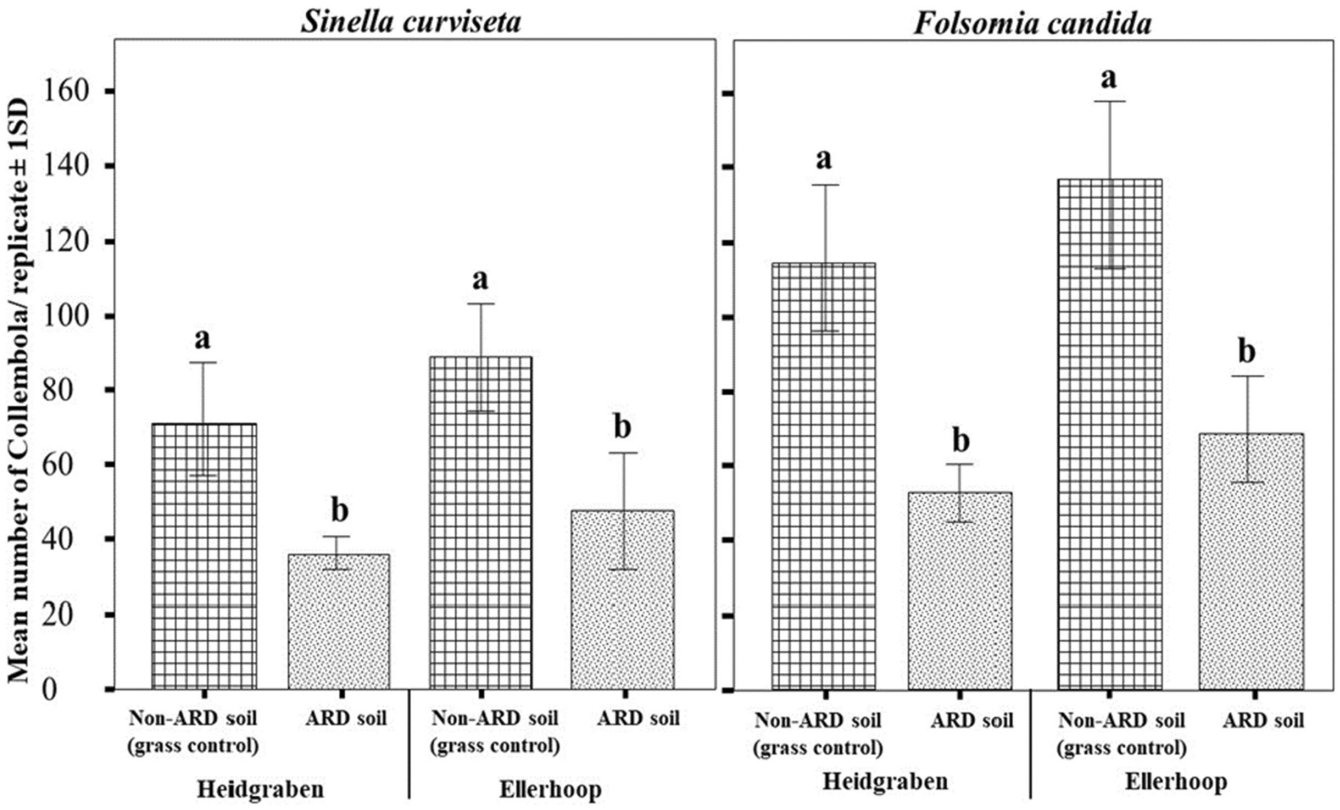

FIGURE 7 Mean numbers of Collembola after 8 weeks population growth in non-apple replant disease (non-ARD grass control soil) and ARD soil from different field sites. Mean numbers were separately compared using Poisson-log model (mean ± SD; $n = 20$; $p \leq 0.05$). Different letters are significant at $p < 0.05$

may account for the observed results: either attraction to the non-ARD soil or avoidance of the ARD soils. In principal, physical and chemical signals could play a role in Collembolan foraging behaviour. For Collembola most likely chemical signals are highly important and influence the foraging behaviour by volatile, contact or gustatory cues. It is known that volatile signals play a crucial role in food searching and trophic interactions among soil organisms (Pfeffer and Filser, 2010). Moreover, Collembola use info-chemicals in order to discriminate toxic fungal metabolites (Rohlfs et al., 2007) and to orientate their movement away from highly toxic fungi (Staaden et al., 2011). For example, on the basis of olfactory cues *F. candida* and two other Collembola species (*Heteromurus nitidus* and *Supraphorura furcifera*) were able to discriminate between toxic fungal strains (reactive metabolite: *sterigmatocystin*) and non-toxic *Aspergillus nidulans* (mutant for toxin production) and even discriminate ungrazed from previously grazed wild type-fungi (Rohlfs et al., 2007; Staaden et al., 2011). Moreover, Sabatini & Innocenti (2000) showed that propagules of the plant pathogenic fungi *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum* and *Rhizoctonia cerealis* were preferred by Collembola species *Mesaphorura krausbaueri* and that they avoided the hyphae of *Bipolaris sorokiniana*, which had a lethal effect. Therefore, it is very likely that olfactory cues are more important than gustatory stimuli for Collembolan (i.e. *Onychiurus armatus*) foraging behaviour, even at low volatile concentrations, that is 1 ng of fungi volatiles, such as 1-heptene and 1-octen-3-ol (Bengtsson et al., 1991). Additionally, Collembola are able to orientate towards the high microbial activity zones via sensing CO_2 sources (Hassall et al., 1986).

In the current study, field-collected soil samples and highly standardized soil samples obtained from a greenhouse experiment with apple seedlings (Weiß et al., 2017) lead to similar behavioural responses of all tested Collembola species. Although microflora/microorganisms or even plant root organic matter and soil type differed largely between all tested non-ARD soils, that is grass control or gamma treated soil, Collembola always preferred colonization of non-ARD soil instead of ARD soil patches. Therefore, it seems to be less likely that attractive cues in the various controls are responsible for the observed behavioural response of the Collembola. Instead, we hypothesize that repellent signals hinder Collembola from successful longer lasting colonization of ARD soils. Involved repellent signals are most likely volatile cues. Instead of equal colonization and distribution of both species on both soil patches, video analysis also indicates low colonization rate and therefore attractivity of ARD soils already early during the rather short observation period. Over time both species accumulate on the non-ARD soil patches in a similar way and without strong migration tendency between patches.

The impact of ARD on Collembola is also supported by the negative effect on population growth of both species independently of the soil origin. Although ARD disease severity is far more pronounced in Heidgraben (sandy soil) compared with Ellerhoop (loamy soil) (Mahnkoop et al. 2018; Winkelmann et al. 2019) only slight differences in population growth were detected in the current study, indicating adaptive behaviour for both species. Predominantly, Collembola have the capacity to shift their diets in response to availability or toxicity. For example, *F. candida* avoids heavy metal contaminated yeast even it had higher nutritional value and selects poor quality food (i.e. graphite) and *ivermectin*, that is an antiparasitic veterinary drug and has negative effects on population growth of *F. candida* only at higher concentrations (Noël et al., 2006). Moreover, if Collembola are exposed to toxic substances via their epidermis, ventral tube or by food ingestion, they can detoxify and excrete certain compounds through ecdysis (Fountain and Hopkin, 2005). They can also enrich their habitat with nutrients via decomposing dead organisms and depositing faecal pellets. Therefore, population growth even in ARD-contaminated soil is likely and was also detected over the 8-week period in the current study. Nevertheless, in the long run abundance and species diversity will be most likely affected and sensitive species might shift to more reliable habitats in the neighbourhood.

This hypothesis is supported also by the negative impact of ARD on mesofauna abundance and Collembola species biodiversity on selected field sites (Michaelis, 2018). Besides, Collembola have the capacity to alter microbial communities (Thimm et al., 1998) in natural habitats via grazing or enhancing microbial growth (i.e. spread of fungal spores). For example, *F. candida* enhanced ash (*Fraxinus pennsylvanica*) plant biomass via interaction with the arbuscular mycorrhizal fungus *Glomus intraradices* (Lussenhop and BassiriRad, 2005). Moreover, *F. candida* has the capacity to reduce nematode numbers in the laboratory or by feeding on a targeted slug- and insect-pathogenic nematodes species (*Phasmarhabditis hermaphrodita*, *Heterorhabditis megidis* and *Steinernema feltiae*) in the field (Read et al., 2006). Hence, advanced experiments will be conducted not only to investigate the interactions of Collembola with potential ARD causing agents, which have been intensively investigated by ORDIAmur project groups (www.ordiamur.de), but also to investigate the indirect effect of Collembola species on development of apple seedlings with the general aim to improve sustainable control strategies for ARD. In general, living conditions for Collembola have to be enhanced by adding organic matter and reducing application of harmful pesticides, while efficient Collembola species or species combinations can be inoculated to restore soil health. Especially the antagonistic role of several Collembola species, including *F. candida* and *S. curviseta*, against typical soil borne pathogens of crops (e.g. *Fusarium culmorum*, *Fusarium oxysporum*) was highlighted by several authors (Meyer Wolfarth et al. 2017, Sabatini & Innocenti 2000) and recently reviewed by Innocenti and Sabatini (2018).

5 | CONCLUSION

The results clearly showed that non-ARD soil was preferred over ARD soil by both species regardless of ARD severity, that is the soil origin. Moreover, population development of both species was negatively

affected by the presence of ARD. In combination with the detailed video observations our results also give rise to the assumption that repellent volatile signals emitted by ARD causing organism affect Collembola foraging behaviour. As a next step, volatile profiles from different soil samples from field sites will be sampled and analysed via GC–MS in order to identify relevant substances and responsible microorganisms.

AUTHOR CONTRIBUTIONS

Nilupuli Thushangi Wadu Thanthri and Rainer Meyhöfer were responsible for conceptualization, methodology and writing—review and editing. Nilupuli Thushangi Wadu Thanthri was responsible for validation, formal analysis, investigation, writing—original draft preparation and visualization. Rainer Meyhöfer provided resources, supervision, project administration and acquired funding. Both authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest and confirm that there are no disputes over the ownership of the data presented, and all contributions have been attributed appropriately.

DATA AVAILABILITY STATEMENT

The data that support the research findings of this study are currently available at LUH Data Repository (Wadu Thanthri & Meyhöfer, 2022): https://doi.org/10.25835/kg6tb8sd

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