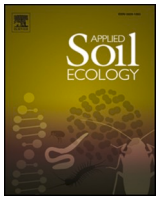
Applied Soil Ecology 181 (2023) 104646

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Reduced tillage increases soil biological properties but not suppressiveness against *Rhizoctonia solani* and *Streptomyces scabies*

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ARTICLE INFO

Keywords:
Tillage
Disease suppression
Rhizoctonia solani AG2-2IIIB
Streptomyces scabies
Microbial biomass
Lysobacter spp.
Arbuscular mycorrhiza

ABSTRACT

Intensive agricultural crop production can lead to a decline in biological soil characteristics and functions, such as soil microbial biomass and activity, carbon and nutrient cycling and soil suppressiveness, important for the sustainable production of food and feed. There is a need to understand how those soil functions can be improved by agricultural practices. In a long-term field study, we assessed whether reduced tillage could enhance soil biological parameters and soil suppressiveness against the plant-pathogenic fungus *Rhizoctonia solani* AG2-2IIIB and bacterium *Streptomyces scabies*. Soil suppressiveness was assessed in bioassays with a susceptible crop while adding pathogen inoculum. Undisturbed soil cores (0–12 cm) were used in these bioassays to include the soil structural aspects, which were likely affected by the tillage treatments. Reduced i.e., non-inversion, tillage was compared with conventional ploughing treatment over 6 years. We found that reduced tillage led to an increase in bacterial and fungal biomass, labile carbon and nitrogen and an increase in the abundance of potential bacterial antagonists, compared to conventional tillage in the upper 12 cm. However, the increase of these microbial parameters did not lead to consistent changes in soil suppressiveness against both *R. solani* and *S. scabies* in response to the tillage treatment. Rather, disease suppressiveness varied significantly between field and year of sampling but was not correlated to any of the assessed soil parameters. Thus while reduced tillage can be beneficial for soil biology, other measures will have to be investigated for inducing *R. solani* and *S. scabies* disease suppressiveness.

1. Introduction

The organisms inhabiting soil, collectively referred to as the soil biota, are the drivers of many functions that are essential for food production and other ecosystem services (Bender et al., 2016; Brussaard, 2012), such as the provision of clean water, nutrient cycling and carbon transfer through decomposition and mineralization (Bardgett and Chan, 1999), carbon sequestration (Six et al., 2006), maintaining soil structure and preventing erosion (Rillig and Mummey, 2006). Soil biodiversity as a whole, as well as specific species, have also been implied to convey resistance against one or several diseases, generating what is called soil suppressiveness (Altieri, 1999). However, commonly applied agricultural practices can have a negative impact on soil biota and their functions (Köhl et al., 2014). In this study, we investigate how two different tillage intensities affect soil biological properties, and especially soil suppressiveness after several years of the treatment regimes.

Suppressive soils are soils in which pathogens cause no or little disease or in which disease incidence declines over time (Weller et al., 2002). Suppressiveness of soils is thought to be a combination of two different mechanisms, with specific suppressiveness superimposed on a background of general suppressiveness (Weller et al., 2002). General suppressiveness is conveyed by the general activity of soil microorganisms that compete with pathogens for space and nutrients. Pathogens known to be susceptible to general suppressiveness include for example *Pythium ultimum* (Chen et al., 1988). Specific suppressiveness, on the other hand, involves antagonistic microbial species, which convey

Abbreviations: RT, Reduced tillage; CT, Conventional tillage.

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https://doi.org/10.1016/j.apsoil.2022.104646
Received 13 April 2022; Received in revised form 15 July 2022; Accepted 21 August 2022
Available online 27 August 2022

biocontrol for example by competition, parasitism or antibiosis. Specific suppressiveness of soils has been reported for a range of pathogens and is associated with several microbial taxa (Gómez Expósito et al., 2017; Weller et al., 2002). *Rhizoctonia solani* and *Streptomyces scabies* are examples of two relevant pathogens in Dutch arable rotations for which soil suppressiveness has been described. Specific suppression against *R. solani* AG2-2IIIB, a pathogen on important crops such as sugar beet, carrot and maize, has been attributed to the presence of species of the genus *Lysobacter* (Postma et al., 2008) or other antagonistic microbes (Andreo-Jimenez et al., 2021; De Corato et al., 2019; Gómez Expósito et al., 2017). Similarly, suppressiveness against *S. scabies*, causing common scab on potatoes, has been associated with a high abundance of antagonistic *Streptomyces* species (Postma et al., 2008; Weller et al., 2002) as well as few other taxa (Gómez Expósito et al., 2017). Treatments, such as the addition of green manures and compost have been found to increase suppressiveness against these pathogens by enhancing populations of such antagonists (Kinkel et al., 2012; Pérez-Piqueres et al., 2006). Many studies have been performed on the effect of organic matter management (Bonanomi et al., 2010) or organic farming systems (van Bruggen and Finckh, 2016) on soil suppressiveness against different soil-borne pathogens. However, the effect of tillage on soil suppressiveness, already demonstrated in 1985 in no-tilled wheat fields with *R. solani* AG8 (Roget, 1995), has been studied less intentive.

Farmers and other stakeholders currently aim for a change in practices that include less soil disturbance, since deep tillage, such as moldboard ploughing, is known to lead to soil degradation and soil organic carbon loss by aggregate disruption (Garcia-Franco et al., 2015). Reduced tillage (RT) was shown to increase the number of macroaggregates and soil organic matter content (Crittenden et al., 2015). In addition, reduced tillage was reported to increase microbial biomass and activity, alter microbial community composition and the prevalence of arbuscular mycorrhizal fungi (Hungria et al., 2009; Köhl et al., 2014; Tyler, 2019; van Groenigen et al., 2010). It can be expected that an increase in soil microbiological activity contributes to the suppression of soil-borne diseases. Accordingly, reduced tillage has been found to increase soil suppressiveness against *P. ultimum* (Bongiorno et al., 2019b), a pathogen which is susceptible to general suppressiveness. Also for *Fusarium graminearum* (Campos et al., 2016), *Phytophthora erithroseptica* (Peters et al., 2003), *Gaeumannomyces graminis* var. *tritici* (Pankhurst et al., 2002), and *R. solani* AG8 (Roget, 1995; Pankhurst et al., 2002) suppressiveness were found to be increased with reduced tillage. Since mechanisms of disease suppression differ per pathogen, the effect of tillage on suppressiveness is also likely to be pathogen specific. Moreover, there are indications that several years of reduced tillage intensity are needed for an increase in suppressiveness (Roget, 1995). In the current study we are especially interested in the effect of tillage on soil suppressiveness against *R. solani* AG2-2IIIB and *S. scabies* which are expected to be influenced, at least partially, by specific suppressiveness.

In contrast to suppressiveness, more often the effect of tillage on pathogen levels in the field and disease incidence has been determined. For example, a pathogen build up in the upper layers was demonstrated for *Fusarium* spp. (Hofgaard et al., 2016; Kadziene et al., 2020) and *R. solani* AG8 (Paulitz, 2006; Rovira, 1986) with reduced tillage in continuously grown cereals, while systems with a crop rotation were not leading to more disease (Peters et al., 2003). However, to asses disease suppressiveness of soils, not the pathogen level in the field itself, but the effect of a certain pathogen dosage on disease development is measured. This is generally assessed in pot experiments under controlled environmental conditions, by adding pathogen inoculum to the soil and using a susceptible (model) crop. Usually, mixed soil samples are taken from the field (Postma et al., 2008), which can be sieved to removed stones and other bigger particles (Roget, 1995; Schroeder and Paulitz, 2008; Wen et al., 2017). However, this disturbance of soil is known to disrupt soil structure (horizontal layers as well as aggregates) and consequently alter chemical and physical properties (Powers and Skidmore, 1984). In a study, which intends to look at the effect of tillage, a practice that strongly influences soil structure, the use of undisturbed soil cores is desirable. Few studies so far have used undisturbed soil cores for determining suppressiveness (Lucas et al., 1993).

In this study we assessed the effect of two different tillage intensities on soil biological parameters and soil suppressiveness against *R. solani* AG2-2IIIB and *S. scabies*, two relevant pathogens in Dutch arable rotations of which it is known that soils can achieve suppressiveness. To this end we compared soil samples from an experimental site with an arable rotation where five fields were treated with conventional tillage (CT) and reduced tillage (RT) in adjacent plots in four replicates. Several parameters were measured that are assumed to influence soil suppressiveness: fungal and bacterial biomass, which have been demonstrated to be increased with general suppressiveness; HWC and PMN, which are labile fractions of soil C and N respectively and can be used as rapid indicators of changes in these measures, in addition they are increasingly used as proximate measures for microbial biomass; *Streptomyces* and *Lysobacter*, which have been shown to be potentially antagonistic taxa against the tested pathogens and might convey specific suppressiveness; AMF, which can also have disease suppressive effects. Undisturbed soil cores were used in the bioassays (pot experiments with inoculated pathogens) to assess disease suppressiveness of the different field soils. Ten years after the start of the tillage treatments some parameters in the respective fields were measured again to assess long-term effects. We hypothesized that reduced tillage would increase microbial biomass and activity and/or the presence of specific antagonists, and that this might subsequently affect suppressiveness against the pathogens *R. solani* AG2-2IIIB and *S. scabies*.

2. Material and methods

2.1. Long term field experiment

The experiment was carried out with samples from the long-term field experiment BASIS (Broekemahoeve Applied Soil Innovation Systems) at WUR Field Crops in Lelystad, the Netherlands (52° 32′N, 5° 34′E), where the different soil tillage treatments started in 2009. This field experiment is situated in a polder reclaimed in 1957, having a characteristic homogeneous soil composed of 61 % sand, 22 % silt and 17 % clay which corresponds to a sandy loam soil. Soil pH-KCl ranges between 7.2 and 7.4 and soil organic matter (SOM) ranges from 3.4 to 3.8 % (organic) and 3.2 to 3.5 % (conventional) in the 0–25 cm soil layer.

Five fields (size: 280 × 90 m) within the experimental site were treated with conventional tillage (CT) and reduced tillage (RT) in adjacent plots in a randomized block design with four blocks each. The subplots with the different tillage treatments were 85 m long and 12.6 m wide. The detailed set-up and organization of this field experiment has been described by Crittenden et al. (2015). Three of these fields were maintained under organic farming (certified since 2004) and two fields were maintained under conventional farming (Table 1). The organic fields had a six-year crop rotation of potato, grass clover, cabbage, spring wheat, carrots and faba beans/spring wheat and were sampled in 2010, 2013 and 2015 during the carrot crop. The conventional fields had a four-year crop rotation of wheat/barley, onion, potato and sugar beet and were sampled in 2010, 2014 and 2015 during the onion crop. All fields received both types of tillage systems, conventional tillage (CT) which was moldboard ploughing up to 23–25 cm depth, and reduced i.e., non-inversion, tillage (RT) using a Kongskilde Paragrubber up to a depth of 18–23 cm. In organic as well as conventional farming crop residues were left in the field. Fertilization in the organic fields was performed with a combination of animal manure and other fertilizer types, based on standard organic practices as prescribed in EU organic regulations (EG Nr.834.2007, IFOAM-eu.org, 2018). In the conventional fields, fertilization was performed with mineral fertilizers, based on actual fertilization advises for arable cropping (Commissie Bemesting Akkerbouw en Vollegrondsgroenteteelt (CBAV)). Fertilization was based

Table 1
Crop and organic addition of the sampled fields per year, each field having four subplots with conventional tillage (CT) and reduced tillage (RT). Organic amendments previous to the main crop were: NA, no addition; CC, Cover crop; Co, Compost; CCF, Cut & carry fertilizer; GC, Grass-clover. Fields were always sampled in September when the crop was still present.

Field	Farming system	Crop and organic addition of sampled fields per year *)				
		2010	2013	2014	2015	2019
J9-4	Conventional	Onion CC	-	Onion CC	-	Potato CC
J9-6	Conventional	–	–	–	Onion NA	Pea CC
		–	–	–	Onion Co	Pea CC + Co
J10-3	Organic	–	Carrot NA	–	–	Carrot CC
		–	–	–	–	Carrot CC+CCF
J10-4	Organic	–	–	–	Carrot CC	Pumpkin GC
J10-6	Organic	Carrot CC	–	–	–	Grass-clover GC

* In 2010–2015 all analyses were performed; in 2019 only *R. solani* soil suppressiveness and HWC and PMN were analyzed.

on crop demand, soil properties and legislation. This resulted in a fertilization (sum of all crops in rotation) of 668 (N), 343 (P_2O_5) and 864 (K_2O) kg ha^{-1} in the organic system and 431, 243 and 555 kg ha^{-1} for the conventional system for N, P_2O_5 and K_2O respectively. Fertilization schemes between cropping systems differed because of different crop rotations resulting in varying crop demands. Fertilization schemes in CT and RT were the same in terms of type, rate and timing. Cover crops were sown in organic and conventional fields when time and weather conditions allowed. In CT treatments, the cover crops were killed by ploughing (November), whereas RT treatments had cover crops until the following sowing or planting (March–April). Additional subplots were used to evaluate the effect of compost (green waste compost; 20 ton ha^{-1}) on a conventional field (J9-6) in 2015 in combination with the tillage treatments.

In 2019, sampling was repeated for all five fields to assess the long-term effect of tillage on *R. solani* AG2-2IIIB suppressiveness, since suppressiveness results in 2015 tended to show differences between the tillage treatments. Also, a selection of soil biological indicators was measured, i.e. HWC and PMN. The fields with their crops are mentioned in Table 1. Two fields had additional subplots with organic amendments: compost in field J9-6 (fruit and vegetable waste compost; 20 ton ha^{-1}) and cut and carry fertilizer in field J10-3 (1.6 ton dry matter ha^{-1}) (Table 1). Field J9-4 had to be omitted from the suppressiveness analysis due to growth abnormalities of the sugar beet seedlings in the bioassay, which might have been caused by the application of chemicals for the potato haulm killing.

2.2. Soil sampling

Soil of the selected fields (Table 1) was always sampled in September when the crop was still present. A schematic overview of the sampling and subsequent analyses is given in Fig. 1. Soil samples (approximately 1 kg) were taken up to 12 cm depth at several locations between the plants and mixed in the bag before subsampling for biological and chemical analysis. Soil was stored at 4 °C until use. Physicochemical soil parameters were analyzed at CBLB (Wageningen University and Research, the Netherlands). Biotic characteristics of the soil samples were analyzed as described below. Part of the soil was stored at −20 °C for molecular analysis.

For all bioassays, undisturbed soil cores of 12 cm depth were taken from the same location with a template of the same size as the pots (10 cm diameter) or rectangular containers (4 × 25 cm) used for the bioassays. Pots and containers were carefully filled in the field with the undisturbed soil samples and transported to the growth chamber and kept at room temperature (approx. 20 °C) in the dark. Bioassays were performed as described below, as soon as possible but always within 1 month after sampling.

2.3. Microbial biomass - bacterial and fungal biomass

Bacteria were measured by confocal laser scanning microscopy and automatic image analysis, after staining of soil smears with DTAF, a fluorescent dye which binds to proteins (Bloem and Vos, 2004). From the number and cell volumes, bacterial biomass was calculated and expressed as μg C g^{-1} soil. Fungi in soil smears were stained with a

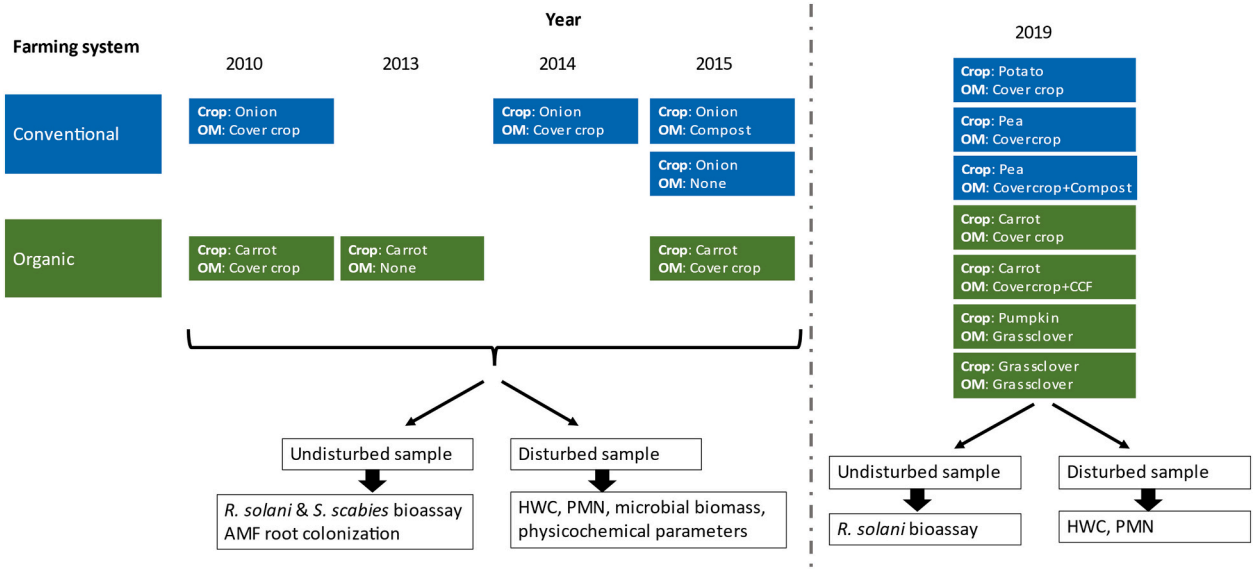

Fig. 1. Schematic overview of soil samples per year and per crop in the conventional or organic farming system and the analyses performed on these samples. Each field included conventional (CT) and reduced tillage (RT) in adjacent plots in a randomized block design with four blocks each. OM is the organic matter amended previous to the main crop: none, cover crop, compost, cut & carry fertilizer (CCF), grass-clover or a combination. Fields were always sampled in September when the main crop was still present.

mixture of two stains: fluorescent brightener (blue), which binds to cell walls (polysaccharides), and europium chelate (red), which binds to nucleic acids (DNA and RNA) (Bloem and Vos, 2004). Thus, active and inactive hyphae were distinguished. In addition, unstained hyphae were counted by switching to transmitted light. The total hyphal length measured under the microscope was used to calculate fungal biomass in terms of μg C g^{-1} soil.

2.4. Labile N and C-PMN and HWC

Potentially mineralizable N (PMN) was determined by anaerobic incubation of a soil sample under water for 1 week at 40 °C (Canali and Benedetti, 2006; Keeney and Nelson, 1983). These warm and anoxic conditions are optimal for a quick mineralization of organic matter by anaerobic bacteria. The lack of oxygen prevents conversion of released NH_4^+ to NO_3^- (nitrification) and uncontrolled N losses by denitrification cannot occur. The amount of mineral nitrogen (NH_4-N) released is a measure of labile organic nitrogen.

Hot Water extractable Carbon (HWC) was determined as the amount of dissolved organic carbon that is released during incubation of a soil sample in hot water during 16 h at 80 °C (Ghani et al., 2003). This is a measure of easily decomposable (labile) organic carbon. The HWC fraction of organic matter is rich in amorphous polysaccharides (mucigel) which originate mainly from microbial exudates and to a lesser extent from plant exudates. This fraction is highly available to microorganisms and is also regarded as one of the key labile components of organic matter responsible for soil micro-aggregation which is an important soil physical parameter to consider in terms of soil quality (Ghani et al., 2003; Haynes, 2005).

2.5. Beneficial microorganisms: Streptomyces, Lysobacter and Mycorrhiza

Streptomycetes were enumerated on chitin oatmeal agar (COA; 2 g colloid chitin [Sigma] purified in 37 % HCl, 0.7 g K_2HPO_4, 0.3 g KH_2PO_4, 18 g oatmeal agar [Difco Laboratories, Detroit], 12 g agar and 100 mg Delvocid per liter deionized water) similar as described in Postma et al. (2008). Appropriate dilutions were spread in duplicate on a sterilized nitro-cellulose filter with 0.2 μm pores (Ø 82 mm, OPTITRAN BA-S 83; Schleicher & Schuell) which was placed on the COA. The LOG mode of the spiral plater (WASP, Don Whitley Scientific Ltd., Shipley, UK) was used. Plates were incubated for 5 days at 20 °C in darkness. Then the filters were removed from the COA plates, and the plates were further incubated for another 5 days before counting.

Lysobacter was quantified with TaqMan, a specific and quantitative DNA detection method. DNA was isolated using PowerSoil® DNA Isolation Kit (MoBio Laboratories). TaqMan real-time quantitative PCR was performed with a primer-probe combination which was designed for specific quantification of three closely related *Lysobacter* species, *L. antibioticus*, *L. capsici* and *L. gummosus* (Postma et al., 2011). The primers were Fw_Lyso_guanxl (5′-CAACGCGAAGAACCTTACC-3′) and Rv_Lyso_guanxl (5′-TGCAGCACCTGTCTCAC-3′). The probe was P_Lyso_guanxl (5′-TGACATCCACGGAACTTTC-3′, underlined letter is an LNA base). The probe was 5′ labelled with FAM as reporter dye and 3′ labelled with BHQ1 as quencher. Primers and probes were manufactured by Biolegio BV (Nijmegen, the Netherlands). For quantification, a dilution series of DNA template from freshly cultured cells of *L. gummosus* 3.2.11 with known density was added to every TaqMan run (Postma and Schilder, 2015).

Presence of arbuscular mycorrhizal fungi (AMF) in soil was evaluated by determining the amount of mycorrhiza that colonized young onion plants during 6 weeks in a pot experiment. Seeds of onion cultivars were sown in steamed peat soil and sand (2:1 v/v ratio). Undisturbed soil samples (12 cm depth) of approximately 1 kg were taken from each of the four replicate plots per treatment and placed in pots of 10 cm diameter and 20 cm height with 5 cm florist's foam blocks at the bottom in order to keep a constant moisture content in the soil. In each pot, three onion seedlings were carefully planted avoiding soil disturbance. The assay was performed in a growth chamber at 23/18 °C (day/night), 60 % humidity and a day/night regime of 8 h dark and 16 h light (230 μMol m^{-2} s^{-2} photo-active light; TL280HF). The four replicates were placed according a randomized block design. After 6 weeks of growth, roots were washed, cut in 2-cm pieces and processed as described by McGonigle et al. (1990) with slight modifications. Root colonization was estimated for each individual plant independently, applying the magnified intersection method, after staining with trypan blue. For each sample, at least 100 observation points were evaluated.

2.6. Soil suppressiveness against Rhizoctonia solani and Streptomyces scabies

Disease suppression against *R. solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) was analyzed by measuring the disease spread of *R. solani* AG2-2IIIB isolate 02–337 from sugar beet (IRS, Bergen op Zoom, the Netherlands) causing damping-off and black root rot in young sugar beet plants (Bakker et al., 2005) as well as carrot seedlings (Postma et al., 2014). Tanks with an internal size of 4 × 25 × 30 cm were used with florist's foam blocks (Van Dillewijn Verpakkingen BV, Aalsmeer, the Netherlands; Water holding capacity ≈ 55 %) of 4 × 25 × 17 cm at the bottom. In each tank, 12 cm soil (undisturbed sample) was packed on top of the rinsed and water saturated foam blocks. The soil water matric potential was automatically regulated at −50 mbar (pF 1.7) (Oyarzun et al., 1994). The assay was performed in a growth chamber at 23/18 °C (day/night), 60 % humidity and a day/night regime of 8 h dark and 16 h light (230 μMol m^{-2} s^{-2} photo-active light; TL280HF). The four replicates were placed according a randomized block design.

Sugar beet seeds susceptible to *R. solani* (cultivar Coyote, SESVanderHave N.V./S.A.; treated with hymexazol and the insecticide Gaucho®) were seeded in two rows 2 cm deep and with 2 cm distance, 22 seeds per tank. The tanks were watered and covered with plastic foil for 1 week. Then the soil in each tank was inoculated with five oat kernels colonized with *R. solani*, 20 mm in front of the seedling rows, just under the soil. The inoculum was prepared with organically grown oat kernels as described previously (Postma et al., 2008). Disease spread was determined 7, 14, 21 and 25 days after inoculation by scoring the number of seedlings displaying damping off or brown-grey lesions on the stem at soil level, as well as their distance to the inoculation point. A control with sterilized soil was included to check pathogenicity of the inoculum. Disease spread 21 days after inoculation was used for comparison of data, since maximum disease spread was reached in some of the tanks in this period.

Streptomyces scabies (Thaxter) Lambert and Loria, isolate Scab1 from potato, is a bacterial pathogen causing scab on radish, potato and few other crops. A bioassay with radish was adapted after Wanner (2004) (Postma et al., 2008). The pathogen was grown on PDA with 100 mg l^{-1} Delvocid to avoid fungal contamination during 3 weeks at 25 °C. A spore suspension was prepared in sterile demineralized water with 0.2 % Silwet and added on top of the soil at a concentration of approximately 5×10^8 CFU per pot, which was checked by plate counting. Undisturbed soil samples of approximately 1 kg were taken per replicate per treatment and placed in pots of 10 cm diameter and 20 cm height with 5 cm florist's foam blocks at the bottom. Per pot, five seeds of radish cultivar Master Red were sown. The test was performed in a growth chamber with the conditions as described above. Pots were watered twice a week from the bottom, but kept relatively dry the last weeks before harvest to stimulate symptom expression. The assay was carried out in a randomized block design. After 6 weeks, plants were harvested, weighed and scab symptoms on the radishes were scored with the following index: 0 = no scab, 1 = 1 lesion, 2 ≤ 10 % of the surface with scab, 3 = 10–25 % of the surface with scab, 4 = 25–50 % of the surface with scab, 5 ≥ 50 % of the surface with scab. The mean value of the indices was calculated

per pot. In addition, the fresh weight of the radish plants was determined.

2.7. Statistical analysis

All statistical analyses were carried out in RStudio vs. 1.4 (RStudio, 2016). First it was tested which of the factors "Tillage", "Crop (Farming system)", "Organic amendment" (compost and cover crop) and "Year"

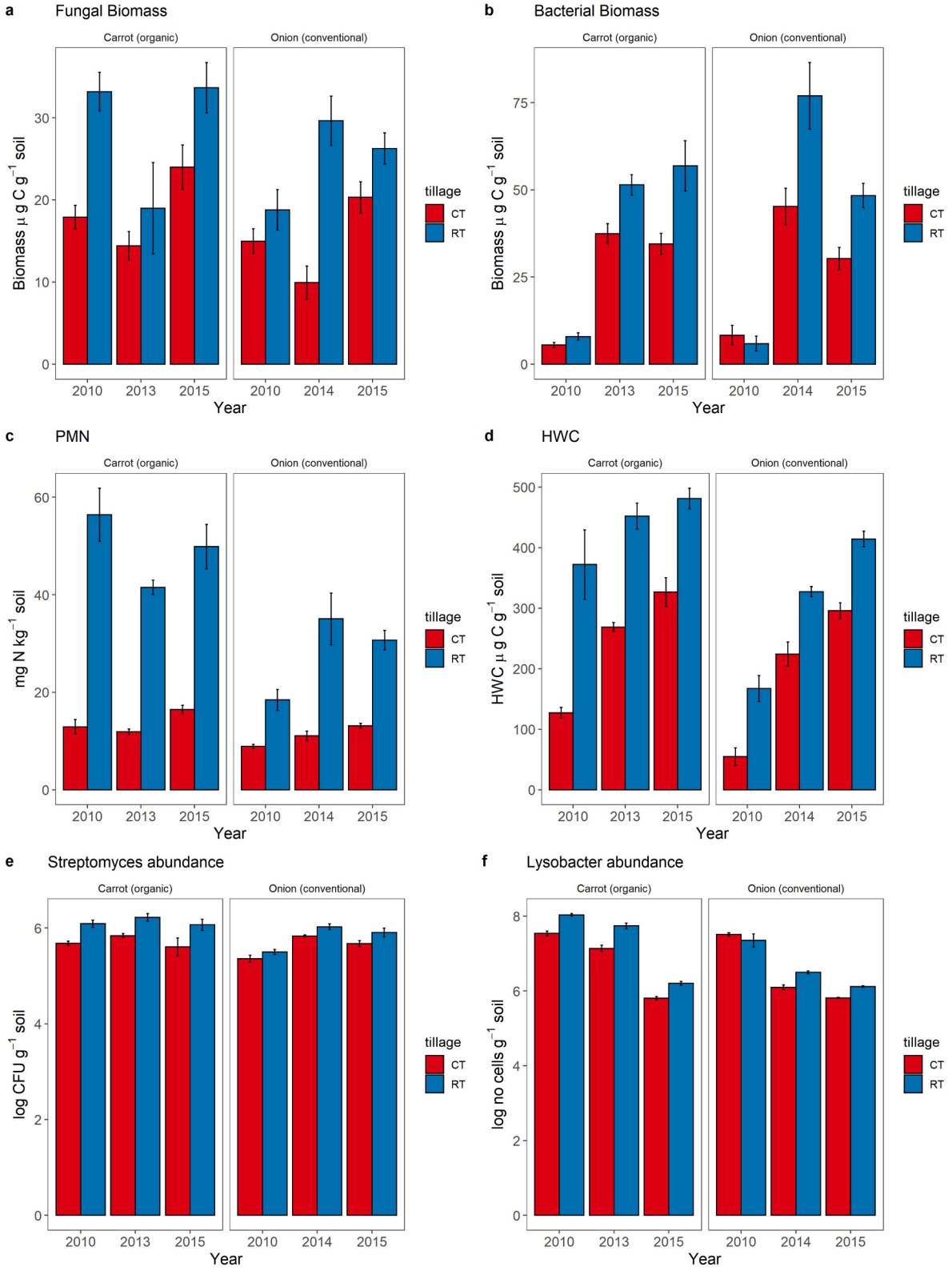

Fig. 2. Soil biological parameters per year, crop and tillage treatment (conventional (CT) and reduced (RT) tillage), averaged over replicate in the years 2010–2015 (n = 4); a) fungal biomass, b) bacterial biomass, c) PMN, d) HWC, e) Streptomyces abundance, f) Lysobacter abundance; error bars represent the standard error.

significantly affected the measured response variables. This was done using linear regression and both forward and backward selection with the stepAIC() function from the package MASS (Venables and Ripley, 2002). The stepAIC() function allows to determine which variables are necessary for the best performing model. The combination of forward and backward selection starts with no variables and stepwise adds variables that contribute most to prediction. Subsequently, after the addition of the most contributing variables, any variables that no longer improve the model are removed again. This analysis showed that the variable "Organic matter" did not contribute significantly to the prediction of any of the response variables. Therefore, this factor was dropped from the analysis. Subsequently, for each response variable the best minimal model was chosen. Thus, for models in which year was a significant predictor, year was included in the final model and the same was done for each explanatory variable. For all used models see Table S1. The emmeans() function from the package emmeans was used for pairwise comparisons between the explanatory variables (Lenth et al., 2018). In case of three-way interactions, the interaction between "Year" and "Tillage" was analyzed separately for the two crops as otherwise pairwise interactions could not be assessed. Correlation analysis between the measured parameters over all years, crops and tillage treatments was done with the cor.test() function.

R. solani disease suppressiveness in 2019 was analyzed separately, as unlike the previous years, it was measured on all fields with several different crops. Also, for this parameter, a stepwise selection was performed as described above. However, no variables showed a significant effect.

3. Results

3.1. Soil biological parameters

Both fungal and bacterial biomass were increased by 35 % in the RT treatment compared to the CT treatment averaged over all years and crop (farming system) (Fig. 2a, b) with tillage as a significant factor (for statistical results see Table S2). Bacterial biomass also differed between years (Table S3). In both the CT and RT treatment, bacterial biomass was significantly lower in 2010 compared to the later years and the difference between RT and CT was not significant in 2010. In addition, in the RT treatment bacterial biomass in the carrot plots was higher in 2015 compared to 2013, but lower in the onion plots in 2015 compared to 2014. For fungal biomass there was an interaction between tillage and year. Fungal biomass in the CT treatment was higher in 2015 compared to 2013 and 2014. In the RT treatment, fungal biomass was lowest in 2013 (Table S3). The difference between RT and CT was not significant in 2013 for fungal biomass. Differences between years can be the result of different weather conditions, the sampled fields (consequence of crop rotation), and the development of the soil characteristics since 2009 when the tillage experiment started.

In addition, RT had a positive effect on the amount of PMN and HWC in soil (Fig. 2c, d) (Table S2), with an average increase of 150 % in PMN and an increase of 39 % in HWC compared to the CT treatment averaged over all years and crop (farming system). HWC was lower in 2010 compared to all other years and highest in 2015. The latter effect was significant only in the onion crop (Table S4). For PMN there was an interaction between tillage, year and crop (Table S5). In 2019, PMN was increased on average 131 % in the RT treatment compared to the CT treatment and HWC was increased 61 % (Fig. S1b, c).

The abundances of both potentially antagonistic *Streptomyces* spp. and *Lysobacter* spp. were significantly increased in the RT treatment compared to the CT treatment averaged over all years and crop (farming system) (Fig. 2e, f) (Table S2). In addition, *Streptomyces* abundances were higher in 2014 and 2015 than in 2010 in the onion crop (Table S5) and *Lysobacter* abundances decreased from 2010 to 2015 (Table S6). Only in 2010 in the onion crop, the difference in *Lysobacter* abundances between CT and RT was not significant.

There was no overall effect of tillage on potential AMF root colonization measured in a bioassay with young onion plants (Fig. 3) (Table S2). However, there was a significant interaction of tillage with crop (farming system). In the onion crop (conventional farming) RT was associated with higher AMF colonization, whereas there were no significant differences between RT and CT in the carrot crop (organic farming) (Table S7) leading to no significant differences on average. In the carrot crop, AMF colonization increased over the years, whereas in the onion crop, colonization was lower in 2015 compared to 2014.

Several soil biological parameters were positively correlated with PMN and HWC, namely bacterial biomass ($R = 0.41$, $p < 0.01$ and $R = 0.62$, $p < 0.01$), fungal biomass ($R = 0.66$, $p < 0.01$ and $R = 0.57$, $p < 0.01$) and *Streptomyces* abundance ($R = 0.71$, $p < 0.01$ and $R = 0.69$, $p < 0.01$) (Fig. 4).

3.2. Suppressiveness against Rhizoctonia solani and Streptomyces scabies

There was no overall effect of tillage on disease spread of *R. solani* AG2-2IIIB in the bioassay as the effects of tillage differed between years and crops (Fig. 5a) (Table S2). On average, the soil from the onion crop showed an increased disease spread in the RT treatment, while there was no difference in the soil of the carrot crop (Table S8). In 2010 *R. solani* disease spread was reduced in the RT treatment, while in 2013 and 2015, disease spread was significantly reduced in the CT treatment compared to RT (Table S8). To assess if this trend would continue, it was decided to take additional measurements in 2019. The repetition of the experiment in 2019 with soil from the same fields but with other crops due to the rotation did not show any effect of tillage on disease spread ($\chi^2 = 0.1$, $p = 0.75$) (Fig. S1a).

Scab symptoms in radish were not affected by tillage, but the disease incidence was significantly higher in 2015 than in 2010 and 2014 in the soil of the carrot crop and higher in 2014 and 2015 than in 2010 in the soil of the onion crop (Fig. 5b) (Table S8).

3.3. Physicochemical soil parameters

Tillage had a significant effect on soil pH, NH_4, NO_3, total N, PO_4 and SOM in the sampled layer of 0–12 cm in 2014 and 2015 ($\chi^2 = 93.49$, $p < 0.01$; $\chi^2 = 8.90$, $p < 0.01$; $\chi^2 = 9.31$, $p < 0.01$; $\chi^2 = 12.14$, $p < 0.01$; $\chi^2 = 64.02$, $p < 0.01$; and $\chi^2 = 98.57$, $p < 0.01$, respectively). While NH_4, NO_3, total N, PO_4 and SOM were significantly increased in RT treatments (15 % for SOM from 3.17 % in CT to 3.73 % in RT), pH was slightly reduced from 7.55 to 7.46 (Fig. S2).

4. Discussion

4.1. Effect of tillage on soil biological properties

The aim of this study was to determine the effects of reduced tillage on soil biological parameters and disease suppressiveness in order to assess how soil functions can be improved by changing management practices. In support of our hypothesis, we found that reduced tillage had a positive effect on several soil biological parameters in the upper 12-cm soil layer. HWC, PMN and both bacterial and fungal biomass were increased significantly under reduced tillage compared to conventional tillage. A positive correlation between microbial biomass and HWC and PMN was found, confirming that these measurements can be used as indicators for microbial soil life (Ghani et al., 2003). Soil HWC and PMN are commonly used as rapid indicators of changes in soil C and N pools. Increases in these measures have been found to be related to an increase in soil quality (Bongiorno et al., 2019a; Chen et al., 2009). Several studies found an increase in both HWC and PMN in response to reduced tillage compared to conventional tillage (Bongiorno et al., 2019a; Mahal et al., 2018). This is supposed to be due to disruption of soil aggregates by tillage and the following increased decomposition of previously protected soil organic matter. Reduced tillage on the other hand leads to

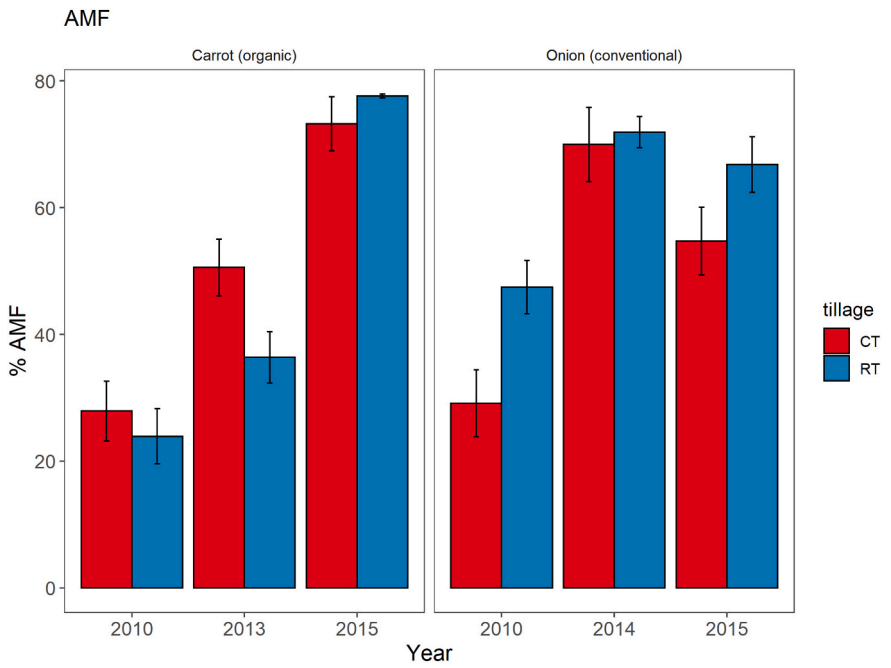

Fig. 3. AMF root colonization (%) per year, crop and tillage treatment (conventional (CT) and reduced (RT) tillage), averaged over replicate in the years 2010–2015 (n = 4); error bars represent the standard error.

an accumulation of labile carbon and nitrogen in the upper soil layer and therefore increase in microbial activity. Accordingly, we found a positive correlation between microbial biomass and HWC (Hazarika et al., 2009).

The increase of microbial biomass with reduced tillage as observed in the present study is in agreement with previous studies (Balesdent et al., 2000; Doran, 1987; Man et al., 2021) and is likely stimulated by increased retention of organic residues, such as residues from the previous crops including cover crops, in the upper soil layer (Höflich et al., 1999). In addition, previous studies have observed that in the long term, reduced tillage and the increased microbial sequestration of nutrients and C are linked to an increase in SOM (Cooper et al., 2016; Crittenden et al., 2015). Accordingly, we found an increase in SOM in the upper 12-cm soil layer in the RT treatment compared to CT. Increased SOM is linked to increased soil health and productivity (Chatskikh et al., 2008; Lehmann and Kleber, 2015), emphasizing that reduction of tillage can play an important role in maintaining important soil functions and mitigation of carbon loss.

Changes in quality and quantity of SOM as well as microbial abundance, as have been found to occur under reduced tillage, are invariably linked to microbial community composition (Bu et al., 2020). Accordingly, a number of studies found reduced tillage practices to be linked to differences in microbial community composition (Sengupta and Dick, 2015; Tyler, 2019). In the present study microbial community composition was not assessed. However, the abundances of *Streptomyces* spp. and *Lysobacter* spp. were measured as these are known as antagonists against the two pathogens used in this study. Both taxa were increased under reduced tillage, which might simply be a consequence of overall increased bacterial biomass. On the other hand, there are indications that an increase of these genera is more directly linked to tillage. Actinobacteria and specifically members of the genus *Streptomyces* were found to be increased in abundance under reduced tillage regimes, which was likely due to increased residues of organic material on the soil surface (Shanmugam et al., 2021; Zaitlin et al., 2004). Also, *Lysobacter* has been found to be increased in abundance under non-inversion tillage and was positively correlated to the C/N ratio (Sun et al., 2020). Still, it is difficult to compare these studies to the present results since most are done by microbiome sequencing, which is less accurate with regard to taxon abundances when compared to qPCR.

In contrast, the colonization potential of AMF overall was not increased in response to reduced tillage, while previous studies frequently reported increased amounts of AMF colonization of cash crop roots in reduced tillage systems, with no-till having the strongest influence (Bowles et al., 2017). This is assumed to be due to a lack of disruption of hyphal networks as a result of reduced soil disturbance (Säle et al., 2015). As AMF colonization in our bioassay was slightly increased in the soil of the onion crop under reduced tillage, but not in the soil of the carrot crop, it is possible that ridge formation for carrot growth still disrupted hyphal networks considerably. The rotation in the present study also contained crops such as potato, sugar beet and carrots where harvesting tubers or roots cause substantial soil disturbance. Accordingly, in the study of Säle et al. (2015) where higher numbers of AMF spores were found in reduced tillage systems compared to conventional mouldboard ploughing, only crops for which the soil is not disturbed at harvest were included, i.e. cereals, maize, sunflower and grass-clover. Another factor that could have reduced the effect of tillage on AMF was the general use of cover crops in the rotation. Presence of cover crops was found to be at least as important for AMF colonization as decreased disturbance of the soil according to a meta-analysis including 54 fields; i.e. cover cropping appeared to counteract some of the negative impacts of soil disturbance on AMF formation (Bowles et al., 2017). In our study cover crops are included in both CT and RT tillage regimes.

4.2. *Effect of tillage on disease suppression*

Increased microbial biomass is often an indicator of general suppressiveness because of a presumed increase in competition for nutrients leading to decreased pathogen growth (Schlatter et al., 2017). Recent studies found positive effects of reduced tillage on disease suppressiveness. Bongiorno et al. (2019b) reported increase in *P. ultimum* disease suppressiveness lined to increased soil microbial biomass. Also, few other studies demonstrated elevated soil suppressiveness levels with reduced tillage against *Fusarium graminearum* (Campos et al., 2016), *Phytophthora erithroseptica* (Peters et al., 2003), *Gaeumannomyces graminis* var. *tritici* (Pankhurst et al., 2002) and *R. solani* AG8 (Pankhurst et al., 2002; Roget, 1995). In our study, reduced tillage did not increase

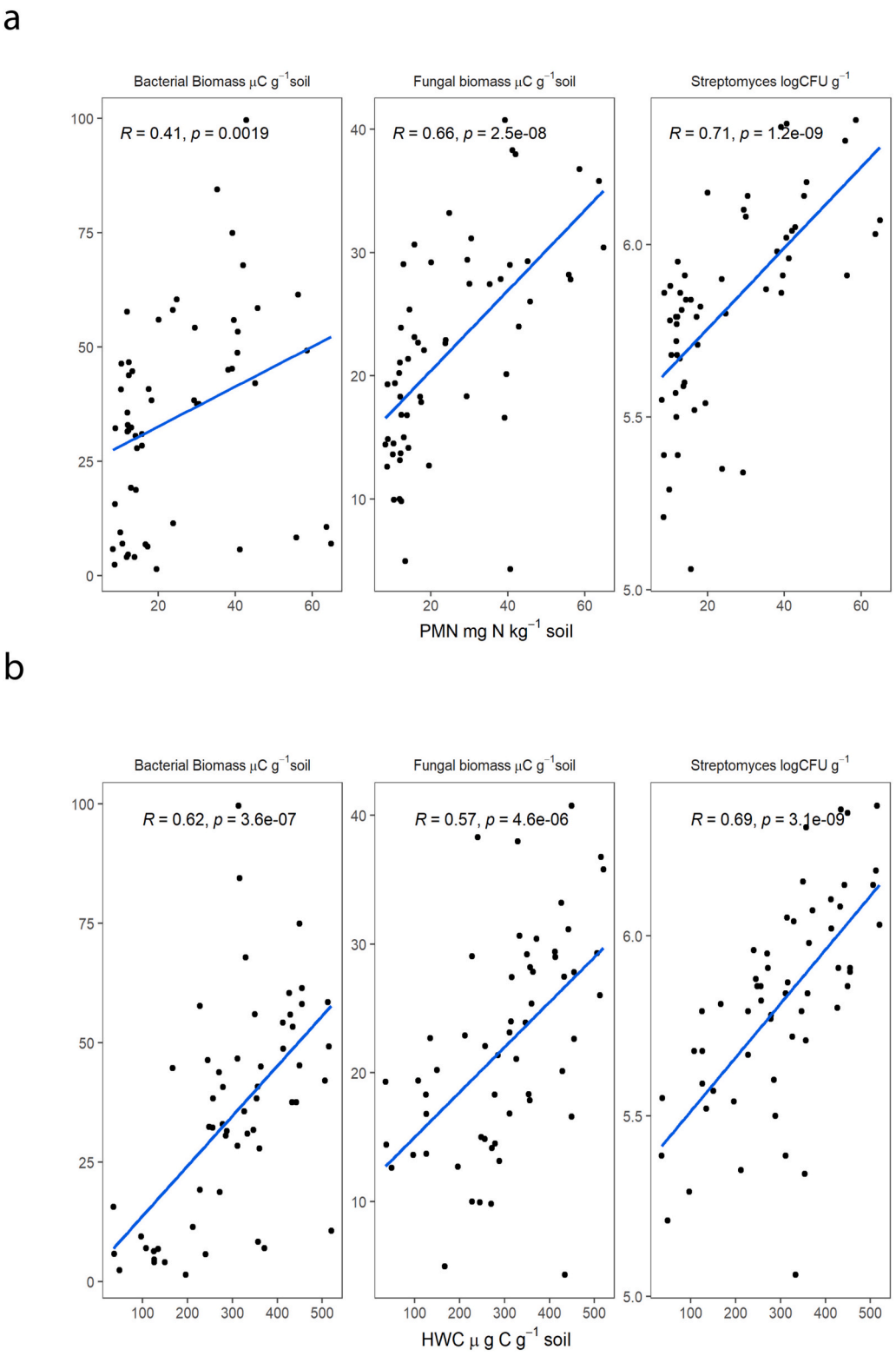

Fig. 4. Spearman correlations of bacterial biomass, fungal biomass, and Streptomyces abundance with a) PMN and b) HWC; results of carrot and onion crop in the years 2010–2015.

R. solani AG2-2IIIB suppressiveness, indicating that this *R. solani* pathogen is not or not sufficiently affected by general suppressiveness, which is in accordance with earlier results showing that general suppressiveness alone was not effective against either *R. solani* or *S. scabies* (Mazzola, 2002). In contrast, for both pathogens, the effectiveness of specific suppressiveness has been described. *Streptomyces* suppressiveness increased with the presence of antagonistic *Streptomyces* species and the application of specific *Streptomyces* strains decreased scab symptoms (Hiltunen et al., 2017; Postma et al., 2008). Suppressiveness against *R. solani* AG2-2IIIB was correlated with higher numbers of *Lysobacter* sp. (Gómez Expósito et al., 2015; Postma et al., 2010). But while the abundance of *Streptomyces* and *Lysobacter* were increased under reduced

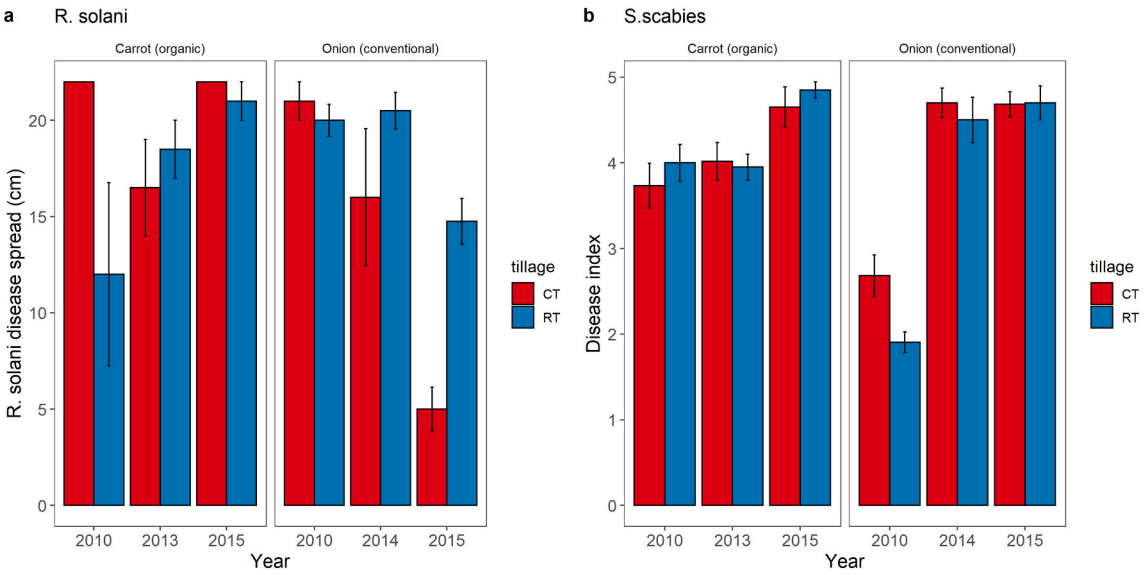

Fig. 5. Disease suppressiveness in bioassays per year, crop and tillage treatment (conventional (CT) and reduced (RT) tillage), averaged over replicate in the years 2010–2015 (n = 4); a) spread of *R. solani* AG2-2IIIB disease, and b) *Streptomyces* scab disease index; error bars represent the standard error.

tillage, no increase in suppressiveness was found. Possibly, the presence of *Lysobacter* and *Streptomyces* alone was not sufficient for suppressiveness. A large number of other taxa has been suggested to be involved in disease suppressiveness, such as *Trichoderma* spp. (Henis et al., 1979), several fungi (Penton et al., 2014), *Pseudomonas* spp. and *Bacillus* spp. (Sturz et al., 2004). However, these taxa have not been measured in the current study.

In addition to soil suppressiveness, research has been done on the effect of tillage on the occurrence of the pathogens in the field. Tillage does not only affect soil biology, but it also alters the distribution of crop residues in and on the soil, which can have a direct impact on the growth of (saprotrophic) fungal pathogens. For *R. solani* AG8 in cereals this is extensively described: several studies demonstrated increased *R. solani* AG8 infections in no-till systems, especially in continuous cropping of cereals (Roget, 1995; Paulitz, 2006; Rovira, 1986). A decline of the disease occurred after several years of wheat growth, and the soil became suppressive particularly in the no-till continuous wheat plots (Roget, 1995). *R. solani* suppressiveness has been demonstrated to develop in reaction of the presence of the pathogen itself for several crop – AG combinations, known as disease decline (Postma et al., 2010). However, in the current experiment no significant damage was observed in the field for both pathogens in susceptible crops in the rotation. Moreover, different crops were rotated and reduced tillage in this crop rotation with potato and/or carrot included extensive soil disturbance to prepare ridges or to harvest the crop and is entirely different from the no-till cereal systems. Accordingly, delayed tillage in a field trial with a potato rotation did not affect black scurf (*R. solani* AG3) and common scab (*S. scabies*) (Griffin et al., 2009).

4.3. Additional organic amendments

Some fields received additional organic matter in the form of compost or cover crops. Several studies indicate that organic matter amendment in combination with reduced tillage can lead to even stronger increase in microbial biomass and SOM (Garcia-Franco et al., 2015) and organic matter amendment has been demonstrated to be able to increase soil suppressiveness (Bonanomi et al., 2010; Lehmann and Kleber, 2015). Nevertheless, these effects could not be supported in the present study, since the few fields with additional organic matter amendments did not result in a change in disease suppression for the tested pathogens. The lack of positive effects of organic amendments on soil suppressiveness can have different reasons: the presence of cover crops differed per year and compost was added incidental, while repeated treatments might be needed to obtain effects under field conditions. Also, the type of organic matter is relevant, with chitin- and keratin-based products being most promising to enhance disease suppression against *R. solani* AG2-2IIIB (Andreo-Jimenez et al., 2021; Postma and Schilder, 2015). Most consistent results with such organic matter products are obtained in pot experiments under controlled conditions, while there is still room to improve the efficacy under field conditions.

5. Conclusions

We conclude that reduced tillage can lead to a rapid improvement of soil biology in the upper soil layer as measured by microbial biomass, HWC and PMN, and to increases in the abundance of bacterial groups with potential antagonistic activity. However, suppressiveness against *R. solani* AG2-2IIIB or *S. scabies* assessed in bioassays with artificial pathogen inoculation was not consistently affected by tillage in the short or long term, even though undisturbed soil cores were used. This demonstrates that stimulating soil biology is not necessarily improving soil suppressiveness of the two soil-borne pathogens assessed in this study. Enhancement of the soil suppression against *R. solani* AG2-2IIIB or *S. scabies* requires other treatments effecting the specific community responsible for the disease suppression.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgements

We thank Maarten Holdinga and Jolanda Tom for their support with the soil sampling and performing bioassays, Wim Dimmers for the microbiological analyses and Marieke Förch and Betty Henken for mycorrhiza counts. This research was supported by the Dutch Ministry of Agriculture, Nature and Food Quality in subsequent research projects

Functional Biodiversity (BO-12.03-004-012), PPS Improved Soil Management (BO-56-001-005), and the Knowledge Base project on soil biology (KB-34-008-001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104646.

References

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. In: Paoletti, M. G. (Ed.), Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes. Elsevier, pp. 19–31.

Andreo-Jimenez, B., Schilder, M.T., Nijhuis, E.H., te Beest, D.E., Bloem, J., Visser, J.H.M., van Os, G., Brolsma, K., de Boer, W., Postma, J., 2021. Chitin- and keratin-rich soil amendments suppress *Rhizoctonia solani* disease via changes to the soil microbial community. Appl. Environ. Microbiol. 87, e00318-00321.

Bakker, Y., Van Loon, F., Schneider, J., 2005. Soil suppressiveness to *Rhizoctonia solani* and microbial diversity. Commun. Agric. Appl. Biol. Sci. 70, 29–33.

Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil Tillage Res. 53, 215–230.

Bardgett, R.D., Chan, K.F., 1999. Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. Soil Biol. Biochem. 31, 1007–1014.

Bender, S.F., Wagg, C., van der Heijden, M.G.A., 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol. Evol. 31, 440–452.

Bloem, J., Vos, A., 2004. Fluorescent staining of microbes for total direct counts. In: Kowalchuk, G.A., De Bruijn, F.J., Headm, I.M., Akkermans, A.D.L., van Elsas, J.D. (Eds.), Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 861–873. Volumes 1 and 2.

Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F., 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biol. Biochem. 42, 136–144.

Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 2019a. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecol. Indic. 99, 38–50.

Bongiorno, G., Postma, J., Bünemann, E.K., Brussaard, L., de Goede, R.G., Mäder, P., Tamm, L., Thuerig, B., 2019b. Soil suppressiveness to *Pythium ultimum* in ten european long-term field experiments and its relation with soil parameters. Soil Biol. Biochem. 133, 174–187.

Bowles, T.M., Jackson, L.E., Loeher, M., Cavagnaro, T.R., 2017. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. J. Appl. Ecol. 54, 1785–1793.

van Bruggen, A.H.C., Finckh, M.R., 2016. Plant diseases and management approaches in organic farming systems. Annu. Rev. Phytopathol. 54, 25–54.

Brussaard, L., 2012. Ecosystem services provided by the soil biota. In: Wall, D.H., Bardgett, R.D., Behan-Pelletier, V., Herrick, J.E., Jones, T.H., Ritz, K., Six, J., Strong, D.R., van Der Putten, W.H. (Eds.), Soil Ecology and Ecosystem Services. Oxford University Press, USA, pp. 45–58.

Bu, R., Ren, T., Lei, M., Liu, B., Li, X., Cong, R., Zhang, Y., Lu, J., 2020. Tillage and straw-returning practices effect on soil dissolved organic matter, aggregate fraction and bacteria community under rice-rice-rapeseed rotation system. Agric. Ecosyst. Environ. 287, 106681.

Campos, S.B., Lisboa, B.B., Camargo, F.A.O., Bayer, C., Sczyrba, A., Dirksen, P., Albersmeier, A., Kalinowski, J., Beneduzi, A., Costa, P.B., Passaglia, L.M.P., Vargas, L.K., Wendisch, V.F., 2016. Soil suppressiveness and its relations with the microbial community in a brazilian subtropical agroecosystem under different management systems. Soil Biol. Biochem. 96, 191–197.

Canali, S., Benedetti, A., 2006. Soil nitrogen mineralization. In: Microbiological Methods for Assessing Soil Quality, pp. 23–49.

Chatskikh, D., Olesen, J.E., Hansen, E.M., Elsgaard, L., Petersen, B.M., 2008. Effects of reduced tillage on net greenhouse gas fluxes from loamy sand soil under winter crops in Denmark. Agric. Ecosyst. Environ. 128, 117–126.

Chen, H., Hou, R., Gong, Y., Li, H., Fan, M., Kuzyakov, Y., 2009. Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in loess plateau of China. Soil Tillage Res. 106, 85–94.

Chen, W., Hoitink, H., Madden, L., 1988. Microbial activity and biomass in container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Phytopathology 78, 1447–1450.

Cooper, J., Baranski, M., Stewart, G., Nobel-de Lange, M., Bàrberi, P., Fließbach, A., Peigné, J., Berner, A., Brock, C., Casagrande, M., 2016. Shallow non-inversion tillage in organic farming maintains crop yields and increases soil C stocks: a meta-analysis. Agron. Sustain. Dev. 36, 22.

Crittenden, S., Poot, N., Heinen, M., Van Balen, D., Pulleman, M., 2015. Soil physical quality in contrasting tillage systems in organic and conventional farming. Soil Tillage Res. 154, 136–144.

De Corato, U., Patruno, L., Avella, N., Lacolla, G., Cucci, G., 2019. Composts from green sources show an increased suppressiveness to soilborne plant pathogenic fungi: relationships between physicochemical properties, disease suppression, and the microbiome. Crop Prot. 124, 104870.

Doran, J.W., 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biol. Fertil. Soils 5, 68–75.

Garcia-Franco, N., Albaladejo, J., Almagro, M., Martínez-Mena, M., 2015. Beneficial effects of reduced tillage and green manure on soil aggregation and stabilization of organic carbon in a Mediterranean agroecosystem. Soil Tillage Res. 153, 66–75.

Ghani, A., Dexter, M., Perrott, K., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243.

Gómez Expósito, R., Postma, J., Raaijmakers, J.M., De Bruijn, I., 2015. Diversity and activity of *Lysobacter* species from disease suppressive soils. Front. Microbiol. 6, 1243.

Gómez Expósito, R., De Bruijn, I., Postma, J., Raaijmakers, J.M., 2017. Current insights into the role of rhizosphere bacteria in disease suppressive soils. Front. Microbiol. 8, 2529.

Griffin, T.S., Larkin, R.P., Honeycutt, C.W., 2009. Delayed tillage and cover crop effects in potato systems. Am. J. Potato Res. 86, 79–87.

van Groenigen, K.-J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., Jones, M.B., 2010. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biol. Biochem. 42, 48–55.

Haynes, R., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: anoverview. Adv. Agron. 5, 221–268.

Hazarika, S., Parkinson, R., Bol, R., Dixon, L., Russell, P., Donovan, S., Allen, D., 2009. Effect of tillage system and straw management on organic matter dynamics. Agron. Sustain. Dev. 29, 525–533.

Henis, Y., Ghaffar, A., Baker, R., 1979. Factors affecting suppressiveness to rhizoctonia solani in soil. Phytopathology 69, 1164–1169.

Hiltunen, L.H., Kelloniemi, J., Valkonen, J.P., 2017. Repeated applications of a nonpathogenic *Streptomyces* strain enhance development of suppressiveness to potato common scab. Plant Dis. 101, 224–232.

Hofgaard, I.S., Seehusen, T., Aamot, H.U., Riley, H., Razzaghian, J., Le, V.H., Hjelkrem, A.-G.R., Dill-Macky, R., Brodal, G., 2016. Inoculum potential of *Fusarium* spp. relates to tillage and straw management in norwegian fields of spring oats. Front. Microbiol. 7, 556.

Höflich, G., Tauschke, M., Kühn, G., Werner, K., Frielinghaus, M., Höhn, W., 1999. Influence of long-term conservation tillage on soil and rhizosphere microorganisms. Biol. Fertil. Soils 29, 81–86.

Hungria, M., Franchini, J.C., Brandão-Junior, O., Kaschuk, G., Souza, R.A., 2009. Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. Appl. Soil Ecol. 42, 288–296.

Kadziene, G., Suproniene, S., Auskalniene, O., Pranaitiene, S., Svegzda, P., Versuliene, A., Ceseviciene, J., Janusauskaite, D., Feiza, V., 2020. Tillage and cover crop influence on weed pressure and *Fusarium* infection in spring cereals. Crop Prot. 127, 104966.

Keeney, D.R., Nelson, D.W., 1983. Nitrogen—inorganic forms. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 2 ed. American Society of Agronomy, Inc., pp. 643–698

Kinkel, L.L., Schlatter, D.C., Bakker, M.G., Arenz, B.E., 2012. *Streptomyces* competition and co-evolution in relation to plant disease suppression. Res. Microbiol. 163, 490–499.

Köhl, L., Oehl, F., van der Heijden, M.G.A., 2014. Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecol. Appl. 24, 1842–1853.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Emmeans: Estimated Marginal Means, AKA Least-square Means, 1.2.3 ed.

Lucas, P., Smiley, R., Collins, H., 1993. Decline of rhizoctonia root rot on wheat in soils infested with *Rhizoctonia solani* AG-8. Phytopathology 83, 260–265.

Mahal, N.K., Castellano, M.J., Miguez, F.E., 2018. Conservation agriculture practices increase potentially mineralizable nitrogen: a meta-analysis. Soil Sci. Soc. Am. J. 82, 1270–1278.

Man, M., Wagner-Riddle, C., Dunfield, K.E., Deen, B., Simpson, M.J., 2021. Long-term crop rotation and different tillage practices alter soil organic matter composition and degradation. Soil Tillage Res. 209, 104960.

Mazzola, M., 2002. Mechanisms of natural soil suppressiveness to soilborne diseases. Antonie Van Leeuwenhoek 81, 557–564.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.

Oyarzun, P., Van Der Zalm, T., Roelofsen, H., Dijst, G., 1994. Computerized equipment to control soil water matric potential and soil temperature in inoculum potential assays of soil-borne pathogens. Plant Soil 166, 125–135.

Pankhurst, C., McDonald, H., Hawke, B., Kirkby, C., 2002. Effect of tillage and stubble management on chemical and microbiological properties and the development of suppression towards cereal root disease in soils from two sites in NSWAustralia. Soil Biol. Biochem. 34, 833–840.

Paulitz, T.C., 2006. Low input no-till cereal production in the Pacific northwest of the US: the challenges of root diseases. Eur. J. Plant Pathol. 115, 271–281.

Penton, C.R., Gupta, V.V.S.R., Tiedje, J.M., Neate, S.M., Ophel-Keller, K., Gillings, M., Harvey, P., Pham, A., Roget, D.K., 2014. Fungal community structure in disease suppressive soils assessed by 28S LSU gene sequencing. PLOS ONE 9, e93893.

Pérez-Piqueres, A., Edel-Hermann, V., Alabouvette, C., Steinberg, C., 2006. Response of soil microbial communities to compost amendments. Soil Biol. Biochem. 38, 460–470.

Peters, R.D., Sturz, A.V., Carter, M.R., Sanderson, J.B., 2003. Developing disease-suppressive soils through crop rotation and tillage management practices. Soil Tillage Res. 72, 181–192.

Postma, J., Schilder, M.T., 2015. Enhancement of soil suppressiveness against *Rhizoctonia solani* in sugar beet by organic amendments. Appl. Soil Ecol. 94, 72–79.
Postma, J., Schilder, M.T., Bloem, J., van Leeuwen-Haagsma, W.K., 2008. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. Soil Biol. Biochem. 40, 2394–2406.
Postma, J., Scheper, R., Schilder, M., 2010. Effect of successive cauliflower plantings and *Rhizoctonia solani* AG 2–1 inoculations on disease suppressiveness of a suppressive and a conducive soil. Soil Biol. Biochem. 42, 804–812.
Postma, J., Schilder, M.T., van Hoof, R.A., 2011. Indigenous populations of three closely related *Lysobacter* spp. in agricultural soils using real-time PCR. Microb. Ecol. 62, 948–958.
Postma, J., Schilder, M.T., Stevens, L.H., 2014. The potential of organic amendments to enhance soil suppressiveness against *Rhizoctonia solani* disease in different soils and crops. ISHS Acta Hortic. 1044, 127–132.
Powers, D.H., Skidmore, E.L., 1984. Soil structure as influenced by simulated tillage. Soil Sci. Soc. Am. J. 48, 879–884.
Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytol. 171, 41–53.
Roget, D., 1995. Decline in root rot (*Rhizoctonia solani* AG-8) in wheat in a tillage and rotation experiment at Avon, South Australia. Aust. J. Exp. Agric. 35, 1009–1013.
Rovira, A.D., 1986. Influence of crop rotation and tillage on rhizoctonia bare patch of wheat. Phytopathology 76, 669–673.
RStudio, 2016. RStudio: Integrated Development Environment for R. RStudio Inc., Boston, MA. http://www.rstudio.com/.
Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M. G., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 84, 38–52.
Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., Paulitz, T., 2017. Disease suppressive soils: new insights from the soil microbiome. Phytopathology 107, 1284–1297.
Schroeder, K.L., Paulitz, T.C., 2008. Effect of inoculum density and soil tillage on the development and severity of rhizoctonia root rot. Phytopathology 98, 304–314.
Sengupta, A., Dick, W.A., 2015. Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. Microb. Ecol. 70, 853–859.
Shanmugam, S.G., Buehring, N.W., Prevost, J.D., Kingery, W.L., 2021. Soil bacterial community diversity and composition as affected by tillage intensity treatments in corn-soybean production systems. Microbiol. Res. 12, 157–172.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.
Sturz, A.V., Ryan, D.A.J., Coffin, A.D., Matheson, B.G., Arsenault, W.J., Kimpinski, J., Christie, B.R., 2004. Stimulating disease suppression in soils: sulphate fertilizers can increase biodiversity and antibiosis ability of root zone bacteria against streptomyces scabies. Soil Biol. Biochem. 36, 343–352.
Sun, L., Li, J., Wang, Q., Zhang, Y., Xu, Z., Wang, R., Wang, X., Jia, G., Zhang, X., 2020. The effects of eight years of conservation tillage on the soil physicochemical properties and bacterial communities in a rain-fed agroecosystem of the loess plateau, China. Land Degrad. Dev. 31, 2475–2489.
Tyler, H.L., 2019. Bacterial community composition under long-term reduced tillage and no till management. Adv. Appl. Microbiol. 126, 1797–1807.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics With S-PLUS, Fourth ed. Springer, New York.
Wanner, L.A., 2004. Field isolates of *Streptomyces* differ in pathogenicity and virulence on radish. Plant Dis. 88, 785–796.
Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40, 309–348.
Wen, L., Lee-Marzano, S., Ortiz-Ribbing, L.M., Gruver, J., Hartman, G.L., Eastburn, D., 2017. Suppression of soilborne diseases of soybean with cover crops. Plant Dis. 101, 1918–1928.
Zaitlin, B., Turkington, K., Parkinson, D., Clayton, G., 2004. Effects of tillage and inorganic fertilizers on culturable soil actinomycete communities and inhibion of fungi by specific actinomycetes. Appl. Soil Ecol. 26, 53–62.