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Conservation agriculture in organic farming: Impacts on weeds and plant-parasitic nematodes during the transition

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CONSERVATION AGRICULTURE IN ORGANIC FARMING:

IMPACTS ON WEEDS AND PLANT-PARASITIC NEMATODES DURING THE TRANSITION

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List of Abbreviations

ANOVA	Analysis of variance
C	Control; weedy fallow
CA	Conservation agriculture
CC	Cover crops
CCA	Constrained correspondence analysis
CT	Conventional tillage; plough tillage
DM	Dry matter
LSD	Least significant difference
NT	No tillage
LM	Living Mulches
OR; OR/ BO	Oilseed radish/ black oat mixture (*Raphanus sativus/ Avena strigosa*)
P	Probability (statistics)
Pf	Final population
Pi	Initial population
RAI	Relative abundance index
RDA	Redundancy analysis
RT	Reduced tillage; non-inversion tillage;
V	Vetch (Vicia sativa)
WF	Weedy Fallow
YWC	Yard waste compost

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1 General Introduction

Agriculture of the 21st century is confronted with a globally increasing human population and climate change. Thus, there is a need to increase agricultural productivity either for food safety or for alternative energy production such as biofuels. However, agricultural intensification based on intensive soil tillage, mineral fertilizer, and pesticides have deeply affected soils and environments in the past decades. For example, humus contents in agricultural soils generally declined and more soil erosion events occurred due to low soil organic matter, soil kept bare, and increasing extreme weather events (Lal, 2015). Furthermore, there is a broad uncertainty about longevity of phosphorus rock deposits ranging from 45 to 10,000 years (Ulrich & Frossard, 2014). Soil degradation will inevitably affect agricultural productivity in the future and, therefore, agricultural intensification will have to be sustainable based on conservation agriculture (CA) systems (Lal, 2015). The latter is defined by a minimum of soil disturbance and a permanent soil cover by living (intercrops, cover crops) and dead mulches (residues on soil surface, transferred mulch). Both measures need to be integrated in site specific crop rotations (Hobbs et al., 2008; FAO, 2015).

Switching from conventional to conservation agriculture can be harsh for farmers. The transistion process is well described by four phases (Figure 1.1). The 4th phase of transition is reached after 6-7 years of consequent performance of CA resulting in an agricultural equilibrium with higher profits and outputs than before. Why then, is CA so unpopular in Europe?

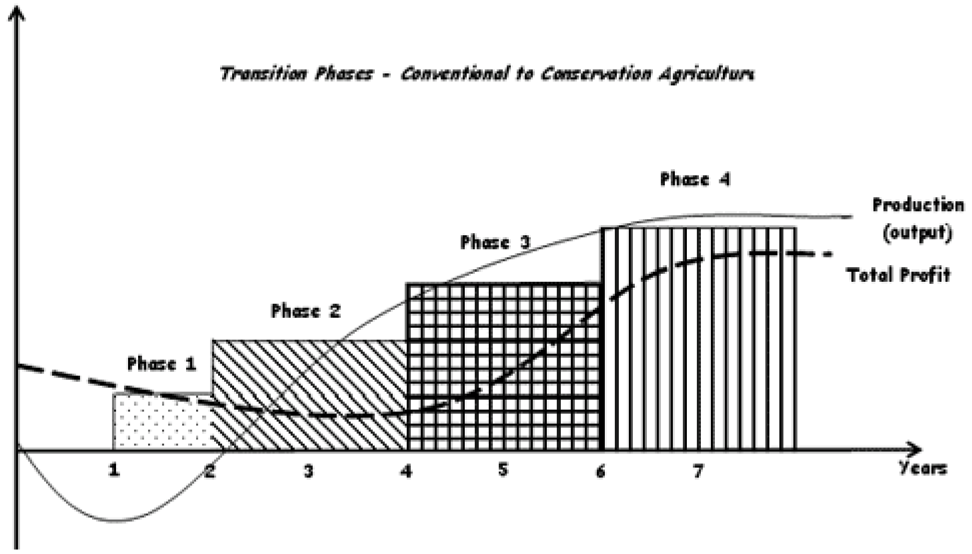

Figure 1.1 The transition phases of conservation agriculture adoption (FAO, 2015).

“First Phase - Improvement of tillage techniques: During this first phase, no increase in farm output is foreseen. But decreases in: labour; time; draught animal or motorised power (reduction of production costs) would occur. An increase in agro-chemical use, especially to control weeds may be required. Furthermore, there may be an increase in family expenses to compensate a probable (but not certain!) reduction of production in comparison with the conventional agriculture;

Second Phase - Improvement of soil conditions and fertility. Decreases in labour, time and draught animal and motorised power (reduction of production costs). Increases in yields and consequently increase in net farm income;

Third Phase - Diversification of cropping pattern. Increased and more stable yields. Increased net farm income and soil fertility.

Fourth Phase - The integrated farming system is functioning smoothly. Stability in production and productivity. The full technical and economic advantages of conservation agriculture can be appreciated by the farmer.”

1.1 Conservation Agriculture in the World and Europe

Conservation agriculture has been widely adopted in North and South America, especially since the Dust Bowl era in the “Dirty Thirties” (Figure 1.2, Figure 1.3). During that time, wind erosion caused up to 75% losses of the fertile top soil in the Great Plains of the USA, particularly if soils had been kept free from vegetation previously for food production (McLeman et al., 2014; Hornbeck, 2012).

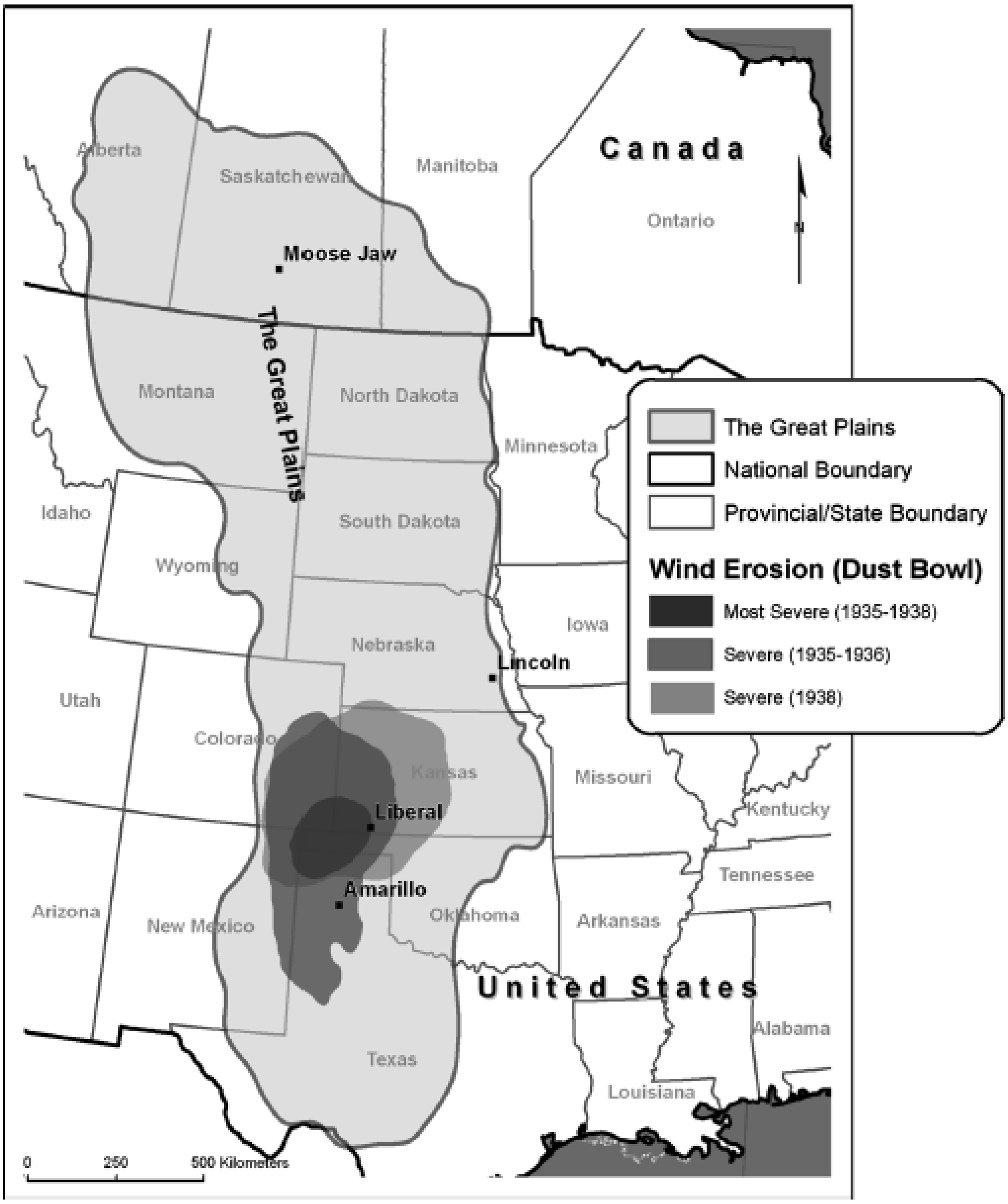

Figure 1.2 The Great Plains and the Dust Bowl areas . Great Plains boundaries based on McLeman et al. (2014).

Figure 1.3 Soil drifting over hog house. South Dakota (Rosebud Photo, 1935). Farm Security Administration/Office of War Information, Black-and-White Negatives, catalog no. LC-USF344-001610-ZB (b&w film nitrate neg.). http://www.loc.gov/pictures/item/fsa1998018168/PP/

Besides wind erosion, soil erosion by water run-off can be a similar threat to agriculture where strong rainfalls occur. For example, in Brazil in the state of Paraná, average annual rainfall ranges between 1250 and 1900 mm with a maximum of 60 mm per hour (Derpsch et al., 1986). As a consequence, annual soil losses (Figure 1.4) average between 10 and 40 t ha^{-1} but can reach up to 700 t ha^{-1}. Depending on the soil type, 2.5 to 15 t (average 10 t) soil losses (ha year)$^{-1}$ are tolerable which is equivalent to 0.2 to 1.2 mm (average 0.8 mm) year^{-1} of the soil layer (Bertol & Almeida, 2000).

Figure 1.4 Soil erosion through water run-off in Brazil. Source: A. Calegari (2012): Presentation held at the OSCAR Kick-off meeting in Witzenhausen: Foto by Assmann, IAPAR, Est. Experim. Pato Branco-PR 2010

In North and South America, the damage caused by these extreme climatic conditions has led to a general revision of common farming practices followed by governmental interventions. In the US, conservation tillage, no-tillage and crop rotations including cover crops were promoted. Furthermore, farmers got direct payments for taking (unproductive) land out of cultivation. In addition, a federally guaranteed crop insurance was introduced (McLeman et al., 2014).

According to the FAO (2016) and the World Bank (2016), in the early 21st century 23.5 and 41.8% of the arable land in the US and Brazil, respectively, was under CA (data from 2009-2013). In contrast, CA is only practiced on 3% of the arable land in Europe (2012) indicating that European farmers are not yet convinced from the manifold benefits of CA (Friedrich et al., 2014). What are the reasons for it?

Maybe the transition phase is not as simple in Europe as pictured in Figure 1.1. In the rainfed and cool region of north-west Europe, CA may not result in higher total profits as low soil movement and plant residues on the soil surface delays soil warming in spring. As a result, nitrogen mineralization is reduced and seedling growth delayed. Thus, the European situation might not be comparable with the situation in North and South America with their oftentimes higher temperatures which generally favor CA.

The European countries do not support CA very well financially and some times schedules of subsidy requests for CA purposes do not match the schedule required for proper agronomic practices. For example, delayed information of certain CA practices to be done for financial support resulted in no application of CA methods in 2008 (Friedrich et al., 2014). Also, the financially very powerful “plough lobby” is hardly interested in promoting CA. Thus, potentially interested farmers may receive a positive or negative feedback depending if the information source is a CA pioneer or a plough manufacturer, respectively (Friedrich et al., 2014). The need for new machinery, the lack of know-how, and concerns about probable yield losses during the transition phase due to CA may also reduce popularity of CA in the absence of incentives.

Another reason for the low adoption of CA in Europe may be the mild climate that until now has not affected financial incomes of farmers as strongly as in North and South America. There, crop yields were largly affected by more frequent extreme weather events that often also resulted in soil losses by wind and water erosion. Thus, rethinking their own agricultural practice was more crucial for farmers in the Americas than for farmers in Europe. In addition, relatively cold winter and spring temperatures in Europe compared to North and South America reduce nitrogen mineralization and therefore yields, particularly if soils are covered with residues (Mäder & Berner, 2012).

Nonetheless, studies throughout Europe have shown numerous benefits gained from CA such as lower environmental harm due to nitrate leaching, less soil erosion and water pollution, lower greenhouse gas production due to less traffic and higher carbon sequestration in soil, and higher nutrient efficiency allowing lower fertilizer requirements (Friedrich et al., 2014).

1.2 Conservation Agriculture and Pests and Diseases

Weeds and certain pests and diseases may change in importance in CA and management approaches need to be adapted. The impact of reduced tillage and crop rotations on soil and residue borne diseases have been reviewed by several authors (Sumner et al., 1981; Sturz et al., 1997; Bockus & Shroyer, 1998) and general trends were rarely found, not even in long-term experiments. Sumner et al. (1981) concluded that regional effects of tillage and crop rotation on plant diseases cannot be foreseen without experimental data. This might be different for weeds. CA does not incorporate weed seeds into deeper soil layers and weed seeds accumulate near the soil surface. Light-dependent species, such as many grasses, will benefit from this situation and dominate the weed spectrum (Tørresen et al., 2003; Moonen & Bàrberi, 2004). Furthermore, low disturbance of perennial weeds can increase their competition with crops for light and nutrients (Mäder & Berner, 2012). Herbicides are widely used to control such weeds in conventional agriculture. However, herbicide restrictions in organic agriculture can lead to increased weed infestations which may further increase the yield gap between conservation tillage and plough based systems (Mäder & Berner, 2012).

Organic agricultural systems are generally less affected by host specific soil borne pests and diseases than conventional agriculture due to longer and more diverse rotations including fodder crops, and organic fertilizer amendments (van Bruggen & Semenov, 2015). However, some pests and diseases can either persist long periods in soil or have a broad host spectrum reducing the effectiveness of rotations in their control and threatening both organically and conventionally managed systems (van Bruggen & Semenov, 2015; Hallmann & Kiewnick, 2015). Out of these, plant-parasitic nematodes are cosmopolitan occurring obligate plant feeders with high damage potential (Hallmann & Kiewnick, 2015). In particular, endoparasitic species such as root-knot (*Meloidogyne* spp.) and root-lesion (*Pratylenchus* spp.) nematodes can be highly damaging and have broad host ranges (Agrios, 2005). In addition, also ectoparasitic species such as *Helicotylenchus*, *Paratylenchus*, and *Tylenchorhynchus* commonly occur in farmed land but have a lower damage potential (Hallmann et al., 2007; Hallmann & Kiewnick, 2015). All those genera parasitize a large number of plant species, including weeds, while they are considered generalists in comparison to specialists that have a narrow host range (Sharma, 1971; Wouts & Yeates, 1994; Knight et al., 1997). Those generalists may be especially promoted if CA is introduced into organic agriculture due to the broad rotations, frequent cropping of good nematode hosts such as legumes, and often times high weed pressure providing green bridges. Over time, these nematodes may build up to high densities causing yield reduction in susceptible crops.

1.3 Specific Aims of this Study

This study focuses on the effects of CA in the first 2 to 4 years after transitioning. The study includes up to four agri-environments throughout Europe in organically and conventionally farmed systems. While economic impacts of CA have been sufficiently researched (Figure 1.1), knowledge is lacking regarding phytomedical challenges, particular in organic farming systems.

Here, the effects of weeds and plant-parasitic nematodes were studied as these were expected:

1) to respond rapidly to changes in farm management;
2) to be of increasing importance especially in organic CA probably limiting production; and

3) to interact with each other, as weeds can serve as additional hosts for plant-parasitic nematodes.

To investigate the impact of CA during the the first two years after transitions on weeds, combined data of the viable soil seed bank and field vegetation (cover, biomass) were used. Although CA generally affects both parameters, annual conditions such as weather, tillage timing and intensity can lead to opposed effects of both investigation methods (Albrecht, 2005). Thus, the weed seed bank analysis can be used for long-term investigations, while field vegetation assessments are useful for short-term evaluations. The latter is important when annual impacts on yields or other parameters, such as plant-parasitic nematodes are investigated.

Nematode evaluation was done for the soil following standard practice. Plant roots were not evaluated for nematodes as being difficult to standardize on a hectar basis. However, the fact that endoparasitic species occur inside roots and there numbers might be underestimated in this study, is considered in the discussion.

Weed and plant-parasitic nematode communities are good indicators for the degree of farming system sustainability. Although long-term investigations are indispensable, impacts on the dynamics of these organisms in the initial phase of CA can be used to assess the risk of future infestations and to adapt a system towards more sustainability. For these reasons, the overall aims of the study were to:

1) Identify phytomedical benefits arising with CA practices used in this study;
2) Predict future threats for agricultural sustainability;
3) Deduct approaches to increase sustainability of CA concerning weed and plant-parasitic nematode management;
4) Provide the data background for long-term investigations of weeds and plant-parasitic nematodes.

1.4 Structure

In Chapter 2, the status quo analysis of the weed seed bank is compared with the field vegetation in the first experimental year under wheat in two long-term organic fields. Effect of tillage and compost with high carbon:nitrogen ratio is analysed.

This is continued in Chapter 3, in which the results of the initial weed seed bank community, described in Chapter 2, are compared with the community after the wheat-potato cropping sequence. Furthermore, the effects of conservation tillage as well as the effects of subsidiary crops and compost on the weed seed bank are evaluated.

Chapter 4 presents the early effects of non-inversion tillage and a grass-clover-wheat-potato rotation including subsidiary crops after wheat, either applied as cover crops or living mulches, on plant parasitic nematode dynamics. Nematodes were evaluated after clover-grass, wheat, subsidiary crops, and potato to follow their dynamic over time in dependence of crops and the tillage system. The effects of the weeds documented in Chapter 2 is also partially discussed here.

In Chapter 5, the early effects of tillage, crop rotations including subsidiary crops, and fertilizer on plant-parasitic nematodes are compared among four distinct European agro-environmental zones. These range from Sweden (Nemoral) to Italy (Mediterranean North) and differ further in their management (organic, conventional), pedo-climatic conditions, soil structure and organic matter contents.

Each of these chapters is introduced and discussed separately as they cover distinct agricultural problems. In Chapter 6, the observations will be discussed in general and a future outlook is provided.

2 Early Effects of Reduced Tillage and Compost on Weeds in Organic Winter Wheat

2.1 Abstract

One of the major obstacles to conservation tillage, particularly in organic farming is a large increase in weed populations and weed seed banks following transition to reduced tillage. We investigated the initial effects of tillage reduction and compost application in organically-managed winter wheat on weed populations and seedling emergence from the seed bank over two successive years in two independent field experiments. Conventional moldboard (30 cm) or chisel ploughing (reduced tillage, 5-15 cm) was applied after two years of grass-clover before winter wheat. Half of the plots of both tillage regimes received 5 t ha^{-1} of yard waste compost as an organic amendment. Prior to sowing the wheat, the soil was sampled from 0-15 and from 15-30 cm to determine the weed seed bank. Weed assessments were conducted directly in the field and in case of the soil samples in an unheated glasshouse throughout the winter wheat growing season. In the field, species richness was 27% higher under reduced tillage than under conventional tillage. Weed biomass was between 1.7 and 6.4-fold higher under reduced tillage. Although applied in limited amounts, surface incorporated compost consistently reduced weed cover and weed biomass under field conditions in the two experiments by 12 and 24%, respectively. In the unheated glasshouse, the number of emerged seedlings of *Aphanes arvensis*, *Matricaria* spp., and *Myosotis arvensis* was 35-55% lower in soil samples from reduced tillage compared to conventional tillage plots suggesting effects of tillage on germination behaviour of these species. Our results are promising in that the build-up of weeds during transition to reduced tillage in organic farming can be minimized by management practices.

Nomenclature: parsley-piert, *Aphanes arvensis* L.; shepherds purse, *Capsella bursa-pastoris* (L.) Medicus; common lambsquarters, *Chenopodium album* L.; couch grass, *Elymus repens* (L.) Gould; common fumitory, *Fumaria officinalis* L.; catchweed bedstraw, *Galium aparine* L.; dead-nettles, *Lamium* spp. L.; perennial rye-grass, *Lolium perenne* L.; mayweeds, *Matricaria* spp. L.; field forget-me-not, *Myosotis arvensis* (L.) Hill; annual bluegrass, *Poa annua* L.; wild buckwheat, *Fallopia convolvulus* (L.) Á. Löve; common chickweed, *Stellaria media* (L.) Vill.; annual sowthistle, *Sonchus oleraceus* L.; field pennycress, *Thlaspi arvense* L.; wheat, *Triticum aestivum* L. ‘Achat’; ivy-leaved speedwell, *Veronica hederifolia* L.; speedwells, *Veronica* spp. L.; field pansy, *Viola arvensis* Murray.

Keywords: non-inversion tillage, organic farming, seed bank, weed infestation, yard waste compost.

2.2 Introduction

During the first few years of transition to reduced tillage, agricultural fields suffer from increased infestation by annual and perennial weeds, probably due to increased weed emergence from seed banks (Hegarty, 1978; Milberg, 1997; Thompson et al., 1977). Patterns of weed abundance observed in response to reduced tillage in conventional farming have been reviewed by Moyer *et al.* (1994) and Nichols *et al.* (2015). These include increased volunteer and grass weeds (e.g. *Avena fatua*, *Bromus* spp., *Poa annua*, *Lolium rigidum*) and decreased annual broad leafed weeds adapted to intensive tillage (e.g. *Fallopia convolvulus*, *Fumaria officinalis*, *Thlaspi arven*se). Under long term reduced tillage, weed seed abundance declines exponentially with soil depth, whereas ploughing results in a more uniform distribution of weed seeds throughout the tilled horizon (Cardina et al., 2002). Over the longer term, weed emergence from the seed bank in no-tillage systems is low due to constraints on weed emergence, and high levels of seed predation and disease in intact soil (Ehlers & Claupein, 1994; Pekrun & Claupein, 2006). Together with herbicide application, these processes lead to reduced weed emergence over time in conventional systems.

In organic farming, where crop rotations are different and no herbicides are used, the weed seed bank is often larger and has a different species composition than conventionally-managed fields. The differences depend on field history and pedo-climatic conditions. In one study in Germany, the weed seed bank was twice as large with higher diversity six years after transition to organic farming (Albrecht, 2005), while in a study in Michigan, U.S.A., total weed seeds and species number in the seed bank were similar under long-term organic and conventional management (Davis et al., 2005). In both cases, however, the weed biomass and its corresponding species density and diversity were considerably higher under organic management. Little is known about the dynamics of the weed seed bank when converting to reduced tillage under organic management.

In herbicide-free systems, cover crops either in between main crops or as living mulches in combination with main crops are crucial for adequate weed control (Hartwig & Ammon, 2002). In addition, the application of high quality composts, i.e. composts that had undergone certain heating phases during composting ensuring sanitation from weeds and pathogens, has been proposed as a weed management tool. For example, increasing the microbial activity of soils by adding compost can enhance weed seed decay (Bàrberi, 2002; Kremer & Li, 2003). In one study, however, the weeds benefitted more than wheat from composts with low C:N ratios, which during microbial degradation released nitrogen to the soil (Blackshaw et al., 2005). This suggests that surface-incorporated composts with high C:N ratios may have the opposite effect. Thus, weed seed dormancy may be enhanced due to reduced nitrogen availability in the top soil layer due to increased microbial fixation (Flavel & Murphy, 2006; Milberg, 1997).

In 2010 and 2011, we established two long-term field trials to investigate the transition to reduced tillage in organic farming. Organic fields that had been managed organically since 1989 were used to compare a typical plough-based tillage system with reduced or non-inversion tillage. Additional experimental factors were repeated surface-incorporation of relatively small amounts (on average 5 t DM per ha and year) of high quality compost as a long-term soil improvement strategy, and a variety of cover crops and/or living mulches.

The experiments started with two years of grass-clover. After the first application of differential tillage in 2012 and 2013, we assessed the viable weed seed bank prior to sowing of winter wheat at two depths covering the minimally tilled horizon (0-15 cm) and the horizon only reached by ploughing (15-30 cm), as well as weed soil cover and biomass during the wheat growing period in the field. The specific aims of this study were to determine the species in the seed bank and their vertical distribution and to follow the weed development in the fields as affected by field history, tillage and compost application. We hypothesized (i) there will be no differences in total seed numbers, only changes in the vertical seed distribution due to differential tillage; (ii) that reduced tillage will change the dominance of weeds in the field and overall increase weed cover; and (iii) that the application of surface-incorporated compost with high C:N ratios will reduce weed pressure.

2.3 Materials and Methods

The field experiments were set up in 2010 (Expt 1) and in 2011 (Expt 2) in adjacent fields located on the organic experimental farm of the University of Kassel in Neu-Eichenberg (51°22'51''N, 9°54'44''E, 231 m ASL with an eastern incline of 3 %). The soil type is a Haplic Luvisol with 3.3 % sand, 83.4 % silt and 13.3 % clay (USDA classification Zc). Both experiments started with two years of grass-clover, which was mulched repeatedly. In the years preceding the grass-clover, the soil had been ploughed 20-25 cm deep regularly. The experiments consisted of a split-split-plot design with four replicates. The main factor (12 x 90 m) was reduced tillage by chisel ploughing (RT) versus conventional moldboard ploughing (CT) to terminate the grass-clover. Tillage treatments were split into eight 6 x 15 m² plots per

Table 2.1 Cropping history (2008) and field operations during wheat production of both experimental fields (Expt 1: 2012-2013, Expt 2: 2013-2014) for main factors before wheat harvest at the experimental farm in Neu-Eichenberg. RT: reduced tillage; CT: conventional tillage

Operations	Expt 1	Expt 2
Cropping history since 2008	Grass-clover – sunflower or maize - winter wheat/mustard cover crop – grass-clover (2 years)	Grass-clover (2 years) - rape seed or turnip rape - spring wheat – grass-clover (2 years)
Grass-clover termination RT	3 x chisel plough 10-15 cm, 09/14/2012 and 2 x 09/17/2012	Undercutting 3-5 cm, 09/13/2013
Grass-clover termination CT	Chisel plough, 09/14/2012; ploughing 20-25 cm, 10/02/2012	Chisel plough, 09/13/2013; ploughing 20-25 cm 09/24/2013
Appl. of yard waste compost	10/04/2012	09/25/2013
Soil seed bank sampling	10/04/2012	09/26/2013
Seed bed preparation (5 cm)	10/12/2012	09/25/2013
Sowing of winter wheat	10/12/2012, 350 seeds m²	09/27/2013, 350 seeds m²
Sowing of clover living mulches	10/19/2012, 7 kg ha (*T. repens*), 19 kg ha (*T. subterraneum)*	09/27/2013, 7 kg ha (*T. repens*), 19 kg ha (*T. subterraneum*)
Field soil cover assessments	11/22/2012; 04/19/2013; 05/24/2013	11/18/2013; 02/28/2014; 04/01/2014; 05/08/2014
Hoeing, harrowing clover resowing	04/25/2013; 05/03/2013; 05/06/2013	-
Weed and wheat DM assessments	06/03/2013 - 06/06/2013	06/02/2014 - 06/03/2014
Wheat harvest	08/15/2013	08/09/2014

replicate. As second factor, two clover species were undersown in the winter wheat as "living mulch" (*Trifolium repens* L. and *T. subterraneum* L.) in half of the plots. The third factor was 5 t dry matter (DM) ha^{-1} yard waste compost, applied manually after soil tillage before sowing wheat in 2012 and 2013 versus no compost. In Expt 1, the clover was sown one week after the wheat; in Expt 2, wheat and clover were sown together (Table 2.1).

Due to unsatisfactory winter survival of clover, they were resown in spring in Expt 1 after mechanical weeding. However, both living mulch species failed in both experiments and did not play a role in the further analysis.

Approximately 3-month-old yard waste compost (referred to hereafter as compost) was obtained from a municipal composting plant in both years and added to the soil. Dry matter and carbon content of the compost was high and bulk densities low (Table 2.2) compared with municipal household compost or sludge (Debosz et al., 2002; Forster et al., 1993).

Table 2.2 Chemical characteristics, including dry matter (DM), bulk density, pH, electrical conductivity (EC), potassium (K), phosphorous (P), total nitrogen (N), carbon (C) and the C/N ratio, of three-month-old yard waste composts (≤ 20 mm sieved) from municipal trees and shrubbery used in 2012 and 2013

Year	DM (%)	Bulk density (g L^{-1})	pH	EC (µS cm^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Total N (%)	Total C (%)	C/N ratio
2012	85	389	7.5	498	3104	541	1.81	29.01	16.04
2013	81	282	6.4	778	NA	807	1.47	37.44	25.5

2.3.1 Weed Seed Bank Analysis and Field Weed Assessments

Twenty evenly-distributed soil cores were taken from the central 10 m x 3 m section of each plot after seedbed preparation and compost application, but before sowing of winter wheat (Table 2.1). Soil corers with a diameter of 2.4 cm were used and each core was divided into 0-15 cm and 15-30 cm layers. Plastic trays (200 cm^2) were filled with 600 ml soil and placed in an unheated greenhouse with screenwalls and a plastic roof (coldhouse). There were 128 trays representing the trial plus 4 pure compost controls. The soil in the trays was kept moist, but watering was suspended when frost occurred. From October until June emerged seedlings were identified, counted, and removed whenever the majority of seedlings reached the two-leaf stage. In May, when the emergence rate was decreasing, the soil in the plastic trays was mixed to simulate field disturbance and to break compacted soil clods. Vegetative reproductive parts of perennial weeds were not assessed as they were very rare after two years of grass-clover ley.

Weed populations in the field were assessed several times throughout the winter wheat growing period until the end of stem elongation (Table 2.1). In each plot, two to three 0.1 m^2 quadrats were assessed visually, and the total ground cover by crops, living mulches, and weeds were estimated. In addition, the four most common weed species were identified and ranked by soil cover. Other species were rare and lumped as “others”. At wheat flowering, aboveground biomass of wheat, living mulches, and weeds in two 0.5 m² quadrats per plot were harvested, separated, and samples were oven dried at 105°C, and weighed to obtain dry mass (DM).

2.3.2 Data Processing and Statistical Analysis.

Statistical analyses were performed with R version 3.2.2 (R Core Team, 2013) following instructions of Dormann and Kühn (2009) and Hedderich and Sachs (2012), using the packages *Vegan* (Oksanen et al., 2015) for multivariate analysis of data sets and *Agricolae* (Mendiburu, 2016) for post-hoc tests. Normal distribution of residuals and variance homoscedasticity were visually assessed using QQ-plots and comparing residuals versus fitted values, respectively. For experimental field data, square-root (x+1)- transformations were performed if data did not meet the requirements of analysis of variance. A protected Fisher-LSD test was performed for multiple comparisons of treatments.

For the comparison of RT and CT treatments in the weed seed bank, emerged seedling densities from plastic trays (200 cm²) were extrapolated to 1 m²:

$$Seeds\ m^{-2} = seeds\ (tray) \times \frac{10000\ cm^2 \times volume\ (core)}{volume\ (tray) \times area(core)}$$

Due to non-homogeneity of variance in the RT and CT weed seed banks, statistical analyses were performed on untransformed data for each soil depth separately with the Wilcoxon rank sum test with continuity correction.

A dominance index (DI) was calculated from the ordinal field assessment data and the metric data from the coldhouse trays. Weed data from the cold house were ranked based on weed abundance per plot. For example, if there were seven species observed, the most frequent one obtained the rank number 1 and the least frequent one rank number 7. The highest rank number in this case was then set to 8 for all weeds observed in this study but not found in the sample. The DI for species i in plot j was calculated for both field and coldhouse data sets as follows:

$$DI_{ij} = 1 - \frac{rank\ number_i}{highest\ rank\ number_j}$$

In the example given, the weed with rank number 7 has a DI of 0.125, while most frequent weed has a DI of 0.875. Weeds not occurring in a treatment have a DI of 0, while DI = 1.0 in cases in which only a single weed species occurred. Thus, data values ranged between 0 and 1. Based on results of the detrended correspondence analysis with the R function "decorana" (Dormann & Kühn, 2009), we performed a partial constrained correspondence analysis (CCA) with the DI’s from coldhouse (0-15 cm depth) and field of both experimental years. Data were not transformed as they were already scaled between 0 and 1. Nominally scaled factors were experiment (Expt 1, Expt 2), site (field, coldhouse) and tillage (RT, CT). Compost application (not significant according to permutation test) and the four replicates were inserted as co-variables. Significances of the CCA model and factors were calculated using a permutation test with 999 permutations.

The weed species richness was calculated from field and coldhouse data (both depths) based on dominance indices. Other indices (e.g. Shannon-index) were not shown due to high correlations with the species richness.

2.4 Results and Discussion

2.4.1 Weed Seed Bank Analysis

The total number of seedlings m^{-2} was 9100 and 6600 in Expt 1 and Expt 2, respectively (Figure 2.1). Although higher numbers of seedlings were counted in Expt 1 these consisted only of 21 species in contrast to 37 species found in Expt 2. The composts applied to both field experiments were free of weed seedlings (data not shown). In general, the size of the total seed bank observed in our study was similar to other experiments conducted under conventional or organic management (Blackshaw et al., 2005; Dessaint et al., 1997; Menalled et al., 2001; Moonen & Bàrberi, 2004; Ruisi et al., 2015). The seed bank size after two years grass-clover under organic management in a Norwegian study was slightly higher (~12000 seeds m^{-2}) than here (Sjursen, 2001).

Expt	Expt 1		Expt 2	
tillage treatment	CT		CT	
soil horizon	0-15	15-30	0-15	15-30
Total (CT vs. RT)	5138	4621	3745	3435
Thlaspi arvense	834	741	117	111
Aphanes arvensis	872	703	360	247
Stellaria media	769	591	162	124
Fallopia convolvulus	441	469	242	202
Veronica spp.	497	319	272	260
Veronica hederaefolia	309	272	162	124
Sonchus oleraceus	150	319	100	84
Lamium spp.	188	178	268	182
Viola arvensis	94	169	13	20
Matricaria spp.	103	150	673	775
Chenopodium album	84	141	371	318
Fumaria officinalis	75	94	27	65
Myosotis arvensis	94	47	253	176
Poa annua	19	38	48	33
Galium aparine	28	19	121	85
Capsella bursa-pastoris	0	0	433	404
Lolium perenne	0	0	0	0
other	581	497	124	228

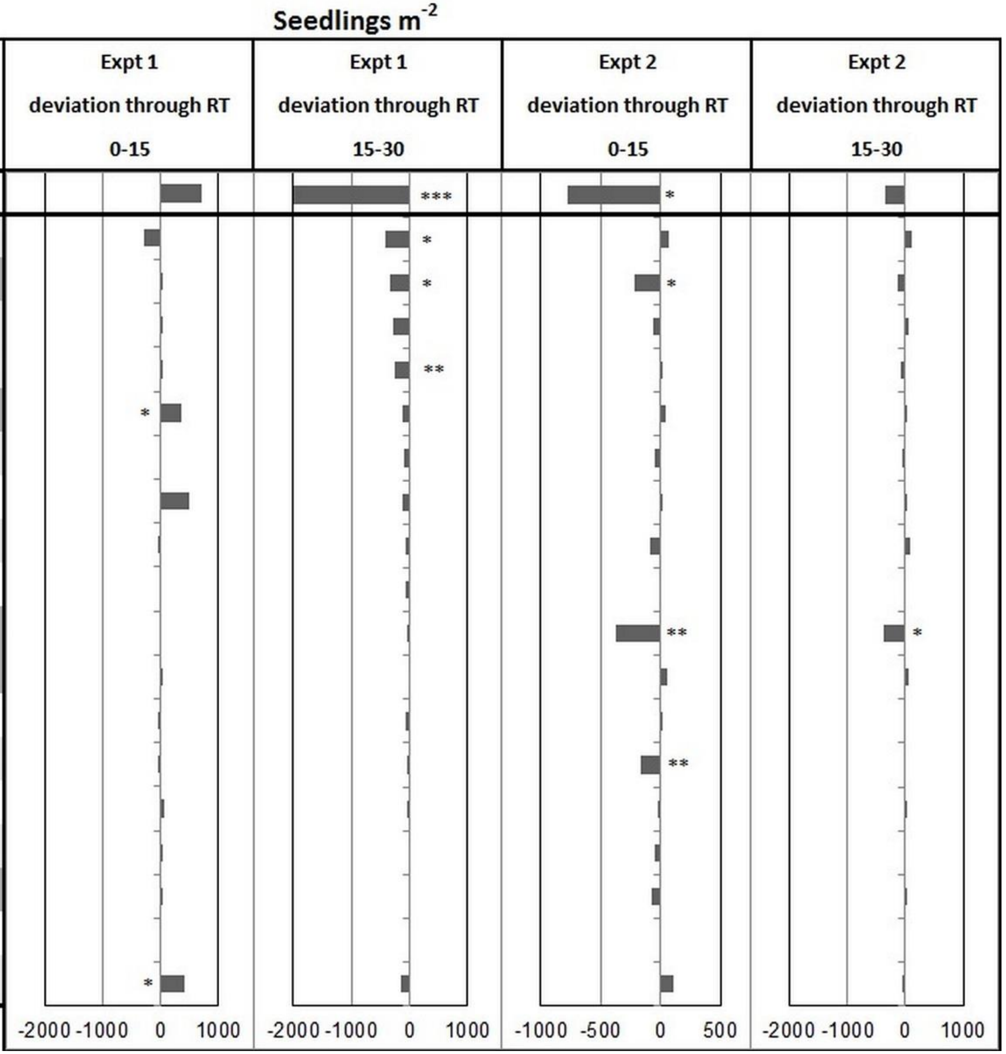

Figure 2.1 Total weed seed bank densities (number of seedlings m^{-2}) and densities of the 17 most frequently identified weed species in the field and seed bank study. Totals are shown for conventional tillage (CT) in 0-15 and 15-30 cm depth (left table) and absolute deviations under reduced tillage (RT) from CT in 0-15 cm and 15-30 cm depth in Expt 1 and Expt 2 (bar graphs). White, light grey, and dark grey backgrounds of seedling numbers under CT indicate <200, 200-500, and >500 seedlings per 0-30 cm depth. Exact Wilcoxon signed rank test indicates whether deviation through RT is not equal to 0 with *P*<0.05 (*), *P*<0.01 (**), and *P*<0.001 (***).

Seventeen species occurred commonly in both years and locations (field, seed bank), representing 90% of the identified weed flora in the field and the seed bank. Weed species that occurred sporadically or could not be identified are not considered in detail and are grouped as “other” (Figure 2.1). *Lolium perenne* occurred only in the field and not in the seed bank.

Dominating species in Expt 1 were A. arvensis, T. arvense F. convolvulus, S. media, V. persica, and S. oleraceus while Matricaria spp., M. arvensis, C. album, and C. bursa-pastoris were the most frequent in Expt 2 (Figure 2.1). The differences in species in the two fields is probably due to the different crop rotation history of the two fields. Aphanes arvensis, S. media and Veronica spp. are usually associated with winter cereals or sunflower (Albrecht, 2005) that were cropped three and four years prior to Expt 1. Thus, large initial soil seed banks of these species could be expected in Expt 1. In contrast, pre-crops such as turnip, rape, or spring wheat increase appearance of Matricaria spp. and summer annual weeds, particularly C. album (Eyre et al., 2011) as observed in Expt 2. All the above-mentioned weed species are known to persist in soil at least 2-5 years (Klaassen & Freitag, 2004; Thompson et al., 1993) and were not eradicated by the 2 years of grass-clover-cropping preceding the experiments. Furthermore, soil movement and seed exposure to light probably affected seed dormancy throughout the tillage and sampling processes. Small-seeded weeds that require light induction to germinate, such as S. media, Matricaria spp., Poa spp., V. persica, were therefore abundant in the seed bank samples (Froud-Williams et al., 1984; Wesson & Wareing, 1969). Hence, without information of field history tillage effects on weeds can hardly be considered (Carr et al., 2013; Pekrun et al., 2003). For example, grass weeds with low seed persistence in soil that are expected to be more prominent in RT than CT may be efficiently controled by perennial grass-clover leys.

Species richness ranged between 5.4 and 8.5 depending on soil depth and year (Table 2.3). This are comparatively high values as in other seed bank studies values between 1.8 and 6 have been reported (Davis et al., 2005; Menalled et al., 2001; Thorne et al., 2007). It is likely that summer and winter crops in the rotation history and reliance on physical weed control contributed to the high weed diversity (Albrecht, 2005). In contrast, Ruisi et al. (2015) observed between 9.0 and 18 weed species under conventional management in semi-arid Sicily, Italy, when sampling larger surface areas depending on tillage intensity, crop rotation, and soil depth. Clearly, the surface area sampled for seed bank analysis as well as site specific climatic and soil conditions affect the outcome.

Table 2.3. Species richness under field (0-15 cm) and seed bank (0-15 cm, 15-30 cm) conditions (Location) in both experimental years (Expt 1, Expt 2) for conventional (CT) and reduced (RT) tillage. N.s., *, **, * indicate not significant, *P*<0.05, *P*<0.01, *P*<0.001 differences, respectively, between conventional and reduced tillage according to analysis of variance. Exact *P*-values were shown for single effects and interactions of experimental years (Year) and the location.**

		Year/Expt	Expt 1		Expt 2		P (>F)		
	Depth (cm)	Tillage/ Location	Field	Seed bank	Field	Seed bank	Year	Location	Year* Location
Species richness (S')	0-15	CT	9.09	7.56	11.63	8.19	0.093	<0.001	0.013
		RT	13.03	8.50	12.94	7.41			
		sign.	***	n.s.	***	n.s.			
	15-30	CT	-	7.50	-	7.78	0.109	-	-
		RT	-	5.44	-	6.66			
		sign.		***		n.s.			

[1] Df for each CT-RT comparison is 59.

2.4.1.1 *Weed Seed Bank Affected by Tillage*

As expected, the composition of the weed seed bank differed depending on tillage intensity and soil layer. CT homogenized the total number of seeds in the two soil layers in both experiments which is also reflected in the similar species richness between CT and the seed bank (Figure 2.1, Table 2.3). RT in Expt 1 resulted in 14% higher weed seed density in the upper soil layer compared to CT while in the lower layer it was 43% lower with lower species richness (Figure 2.1, Table 2.3). This suggests that there were considerably more seeds present in the upper layer before ploughing the field. In Expt 2, however, no significant differences in weed density occurred between the two soil layers under RT, suggesting relatively uniform seed densities in the total sampled soil horizon before ploughing. In contrast to Expt 1, seedling numbers were 21% lower in the upper layer after RT compared to CT in Expt 2 (*P*< 0.05; Figure 2.1).

Contrary to our expectations, the total number of seedlings was 13 and 16% lower under RT compared to CT in Expt 1 and Expt 2, respectively, suggesting that either seed viability or dormancy was affected by the differential tillage within the two to three weeks that the field had been tilled before sampling (see Table 2.1 for dates). Light induction and nitrogen mineralization due to intensive soil movement under CT may have disrupted seed dormancy more than under RT (Pekrun et al., 2003). It is therefore possible that the different tillage equipment used for RT had contrasting effects and this could explain the differences in seedling emergence in the upper layer in Expt 2. In Expt 1, RT was achieved by chisel ploughing the top 12 cm while in Expt 2, a specialized undercutting equipment was used at 4 cm depth (Table 2.1). Two years soil cover by grass-clover followed by minimal disturbance in Expt 2 may have suppressed weed germination by shading and kept buried seeds in dormancy (Albrecht, 2005; Egley & Duke, 1985).

RT did not affect the species richness in the top 15 cm soil. This is in line with studies from Canada and Italy, where effects of tillage on the seed bank diversity were low (Légère et al., 2005; Ruisi et al., 2015). The authors hypothesized that tillage affects seed bank communities more than the overall species diversity. In contrast, in the 15-30 cm soil layer, RT reduced species richness by 27% and 15% in Expt 1 and Expt 2, respectively (Table 2.3). CT homogenized the seed bank to a depth of 30 cm and transferred non-dormant seeds to the deeper soil layers, thus increasing the viable seed bank in the 15-30 cm layer compared to RT where non-dormant seeds were kept in the upper layer.

RT reduced or increased some of the dominant species depending on year and depth. In Expt 1 at 0-15 cm RT reduced *T. arvense* but increased *Veronica* spp. and *S. oleraceus* (Figure 1). At 15-30 cm, RT reduced *A. arvensis, T. arvense,* and *F. convolvulus*. In Expt 2, RT reduced the dominant small seeded winter annuals *A. arvensis, Matricaria* spp., and *M. arvensis* significantly at 0-15 cm. Reductions at 15-30 cm were only significant for *Matricaria* spp. (Figure 2.1). In contrast, species with a large seed size, such as *G. aparine, F. convolvulus,* or *V. hederifolia,* were not much affected by tillage as expected, presumably because they are capable of withstanding unfavorable germination conditions (Pekrun et al., 2003).

2.4.1.2 No Compost Effects on the Seed Bank

The total weed seed bank in 0-30 cm was on average across both tillage treatments about 5% lower in compost amended compared to unfertilized treatments (9028 and 6345 seedlings m^{-2} in Expt 1 and 2, respectively). Some differences in weed germination did occur between the two tillage treatments in the top layers, however. Thus, in compost treated top layers from the CT plots emergence of weeds was about 12% lower (4800 and 3517 seedlings m^{-2} in Expt 1 and Expt 2, respectively) than in unfertilized soils. In contrast, in compost amended soils from the RT plots, weed germination was about 15% higher (6150 and 3220 seedlings m^{-2} in Expt 1 and Expt 2, respectively) compared with unamended soils (data not shown). In the 15-30 cm layers, effects were similar for the two tillage treatments within year but differed between years (reduction of 4 and 2% in Expt 1 and 17 and 15% in Expt 2 under CT and RT, respectively). Sampling was done after seed bed preparation immediately after compost application in Expt 1 and one day after in Expt 2. Thus, compost particles were sampled randomly with the 0-15 cm soil layer and could not have interacted with the seeds in the field. The amount of compost applied was 500 g m^{-2} dry matter and thus, only small amounts of compost would have been present in the samples. It is therefore considered unlikely that the observed effects in the coldhouse were related to compost application.

2.4.2 Field Performance of Crops and Weeds

2.4.2.1 Large Year Effects

In Expt 1, a dry autumn was followed by mean temperatures below zero with almost constant snow cover from January until early April (Figure 2.2A). For this reason, weed growth in the fall was low while early spring weed assessments could not be performed as weeds were hidden by snow layers of 5-10 cm (Figure 2.2B). High temperatures from April on led to strong wheat growth, and the last non-destructive soil cover assessments at wheat BBCH 41 was done on May 24 2013. In contrast, precipitation in autumn in Expt 2 was adequate, leading to higher weed growth before winter than in Expt 1. This was followed by a very mild winter with 5°C higher temperatures and no snow. The growing period started already at the end of February and weeds were therefore assessed earlier than in Expt 1 and BBCH 41 of the wheat was reached by May 8 2014. The differences in climatic conditions and sowing dates between both experiments led to large differences in weed and wheat biomass (Figure 2.3). In Expt 2, weed biomass and its variance was much higher at wheat flowering than in Expt 1 (Figure 2.3A). Similarly, wheat DM yield at flowering in Expt 2 was more than double than in Expt 1 (Figure 2.3B). Weed species with high soil cover were *G. aparine, Lamium* spp., *V. hederifolia, Veronica* spp., and perennial grasses (*Dactylis glomerata*, *Festuca pratensis,* and *L. perenne).* Highly variable total weed biomass in wheat was also observed in Michigan, U.S.A. (Davis et al., 2005; Menalled et al., 2001). However, the weed biomass differed only by 0.1 and 0.6 t ha^{-1} across 12 experimental years while the weed biomass differed by 0.1 and 1.3 t ha^{-1} between both experimental years in our study. Therefore, separation of both experiments was essential for the detailed analysis of treatment effects.

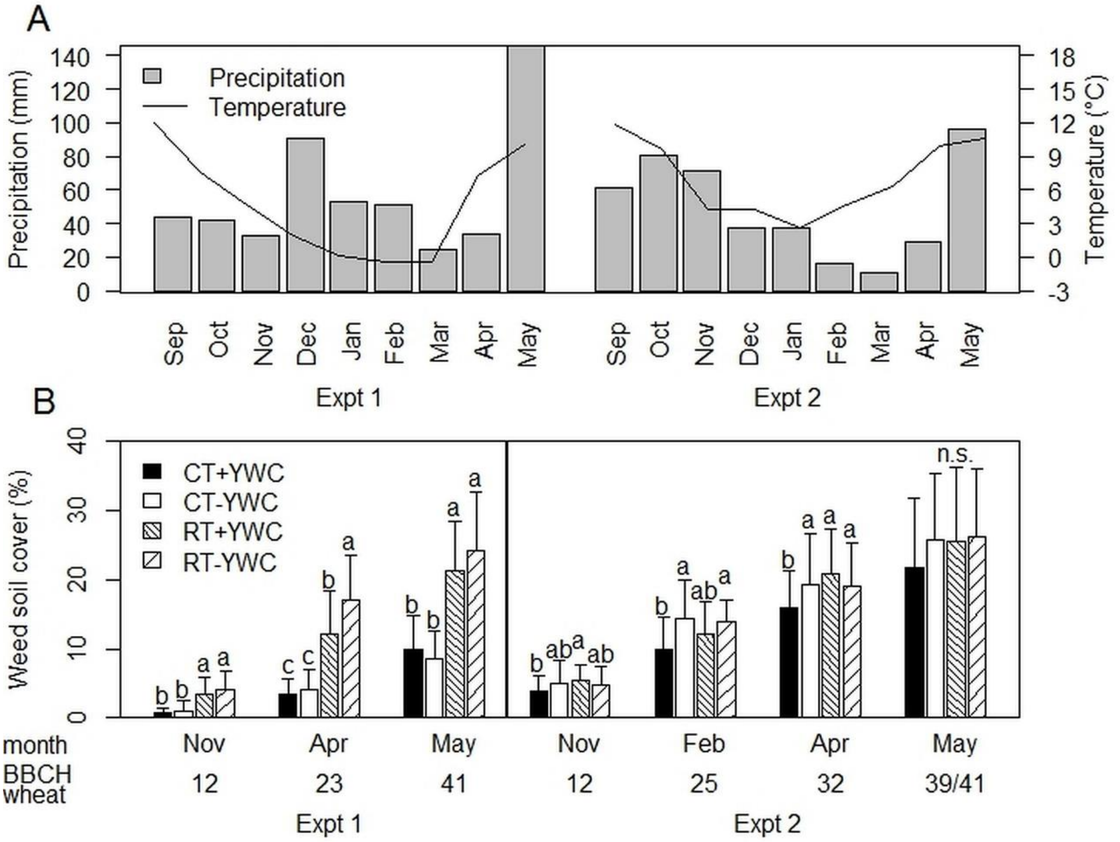

Figure 2.2 (A) Monthly precipitation and mean temperature measured at the experimental station during the assessment time of both experiments. (B) Soil cover by weeds (%) from November until May with means and standard deviation for the treatments conventional (CT) and reduced (RT) tillage with (+YWC) and without (-YWC) yard waste compost for Expt 1 (2012/13) and Expt 2 (2013/14). X-axis labels showing assessment times, growth stages (BBCH- scale) of winter wheat and the experimental years. Different letters indicate significant differences at P<0.05 (LSD-test with square-root transformed data) for each date (df= 57).

2.4.2.2 Crops and Weeds Affected by Tillage

In general, the perennial grasses, *S. media,* and *Veronica* spp. were dominant under RT whereas *Matricaria* spp. and *V. hederifolia* were predominantly observed under CT. Initial weed cover in CT in November was higher in Expt 2 than in Expt 1, while for RT the values were similar (Figure 2.2B). During the long and cold winter in Expt 1, plant growth was slow, resulting in weed cover of 15% in RT and less than 5% in CT in April 2013 (Figure 2.2B). Weed cover had been similar in CT and RT in fall 2013 in Expt 2 and remained similar throughout Expt 2 despite the higher overall growth rates. An attempt at resowing the clovers in Expt 1 was accompanied by mechanical disturbance in early May 2013 (Table 2.1). In the RT plots, this disturbance did not kill the already larger weeds, such as volunteer grasses, but it greatly affected the small weeds, such as *Veronica* spp. and *S. media* in the CT plots. This is consistent with a study from the UK (Davies & Welsh, 2002) and thus, considered to contribute to the large differences in final weed soil cover between years under CT.

Differences in weed biomass at wheat flowering (Figure 2.3A) were higher than differences in weed cover, especially in Expt 1 (Figure 2.2B). In Expt 2, weed DM was highly variable in both tillage systems (Figure 2.3A). Unsatisfactory termination of the clover-grass pre-crop in Expt 2 resulted in the survival and reestablishment of perennial grasses, such as *Lolium perenne*, *Dactylis glomerata*, and *Festuca pratensis*, throughout the winter wheat cropping period. These volunteer crops were responsible for 24% of the weed soil cover at wheat booting in Expt 2

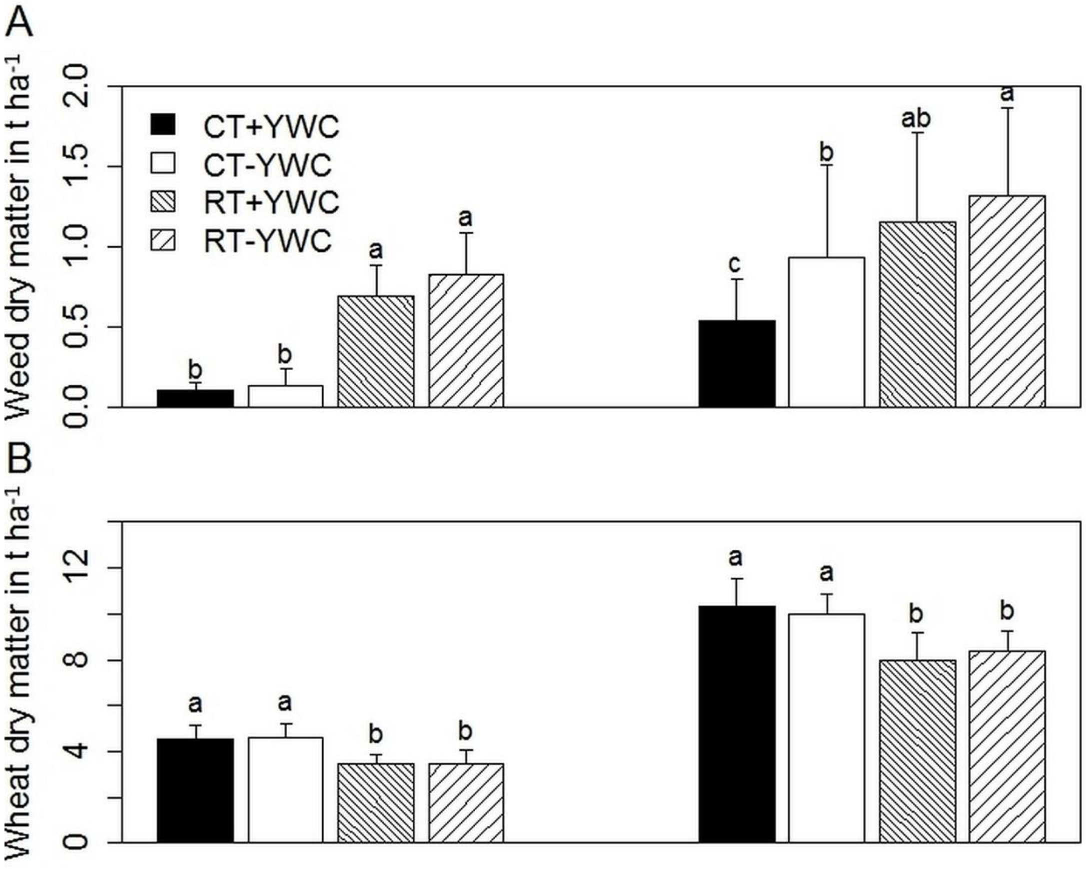

Figure 2.3 (A) Average and standard deviations of total weed dry mass yield at wheat flowering under conventional (CT) and reduced (RT) tillage with (+YWC) and without (-YWC) yard waste compost in Expt 1 and Expt 2; different letters indicate significant differences between treatment within each experiment at *P*<0.05 (LSD-test with square-root transformed data; df=57). (B) Averages and standard deviations of total wheat DM yield at flowering under conventional (CT) and reduced (RT) tillage with (+YWC) and without (-YWC) yard waste compost in Expt 1 and Expt 2; different letters indicate significant differences between treatments within each experiment at *P*<0.05 (LSD-test; df=57).

under RT compared with 0.5% under CT (data not shown). In addition, rhizomes of *E. repens* were not sufficiently damaged under RT. This is consistent with other studies that consider perennial grasses and volunteer crops to be especially problematic under RT (El Titi, 2003; Menalled et al., 2001; Pekrun et al., 2003; Ruisi et al., 2015). In contrast, *V. hederifolia* produced high soil cover under CT but little biomass at wheat flowering. Therefore, weed biomass was always higher under RT with several extreme values above 2 t ha^{-1}. RT resulted in significantly reduced wheat biomass at flowering of 25 and 20 % in the first and second experiment, respectively, compared to CT (Figure 2.3B).

The species richness reflected the results of soil cover and biomass. The index was significantly higher under RT than CT (Table 2.3), but the difference between tillage systems was lower in Expt 2. Overall, our results are similar to many studies on initial effects of RT on crop and weed performance under temperate climates: Crop biomass and yields are generally lower within the first years after transition to RT (Pittelkow et al., 2015), and weed soil cover and biomass under RT usually exceeds that observed under CT (Vakali et al., 2011).

Nevertheless, weed response to field experiments may differ according to the farming system, e.g. conventional versus organic management. For example, in a conventional system despite of lower weed diversity under moldboard ploughing, species richness and weed biomass remained unaffected compared to no-till in the U.S.A (Davis et al., 2005).

2.4.2.3 Weeds but not Wheat are Affected by Compost in the Field

Soil cover by weeds was often lower in the plots that had received compost (Figure 2.2B) but there was no interaction between tillage and compost application. Depending on sampling time, compost reduced the weed cover up to 30%. This was also reflected in the lower weed biomass at wheat flowering (Figure 2.3A). In Expt 2, weed biomass in compost amended plots was 14 and 33% lower in RT and CT, respectively, compared with plots that did not receive compost. At species level, the dominance of *Veronica* spp. was consistently lowered by compost in both experiments (data not shown). In comparison, wheat emergence (data not shown) and biomass (Figure 2.3B) were not affected by compost.

The trend towards lower biomass and sometimes lower soil cover of weeds in the field in treatments that were fertilized with 5 t DM ha^{-1} of compost was unexpected and suggests that even small amounts of compost may be able to suppress weed growth. In a study conducted in Washington, U.S.A, significantly lower grass and broadleaved weeds were observed when compost had been applied previously (Carpenter-Boggs et al., 2000). This may be due to higher rates of weed-suppressive bacteria often associated with organic management systems that apply organic fertilizer (Kremer & Li, 2003). Salt content, pH and C/N ratio of composts may also have influenced germination of weed species near the soil surface. For example, numbers of *C. bursa-pastoris* seedlings were lower in soils amended with compost with a C/N ratio of 15 compared with the unfertilized control (Fennimore & Jackson, 2003). In another study, the numbers of *Bromus tectorum, T. arvense,* and *Descurainia sophia* seedlings were generally higher if a cattle manure compost with a C/N ratio of 10-12 was applied compared to manure with a C/N ratio of 15-18 (Blackshaw et al., 2005). Weed germination may be further affected by high concentrations of trace metals (cadmium, copper, lead, nickel, and zinc), volatile fatty acids, and other phytotoxins commonly occurring in immature composts (Ozores-Hampton et al., 2001).

2.4.3 Comparison of Weeds in the Field and in the Seed Bank

Information about the soil weed seed bank can be useful in predicting infestations of annual weed species in the field. The abundance of weed seeds occurring at specific depths and the number of weeds that establish in the field usually correlate well up to depths of 15 cm (Benvenuti et al., 2001; Dessaint et al., 1997; Rahman et al., 2006; Zhang et al., 1998).

In the constrained correspondence analysis (CCA; Figure 2.4) the seed species that emerged from the top 15 cm of soil in the seed bank versus in the field based on dominance indices (DI) in both experiments accounted for 28%, and conditional variables (compost treatment and replicates) for 1.4% of the total variance. The permutation test of the CCA was significant at $P<0.01$ for the experimental year (Expt 1, Expt 2), seed bank versus field, and tillage. The first and the second CCA axes of the ordination explained 62% and 31% of the constrained variance, respectively.

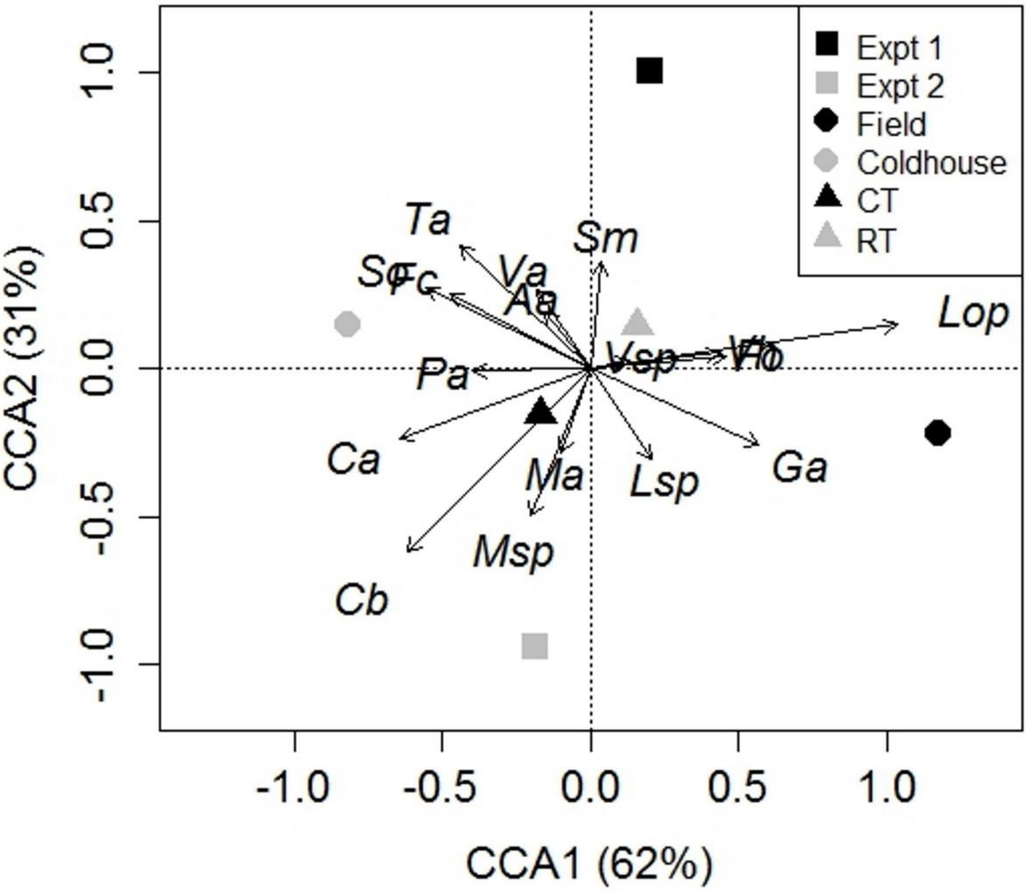

Figure 2.4 Partial constrained correspondence ordination with treatment centroids of combined weed data sets of both experimental years (Expt 1, Expt 2) as well as field and coldhouse (0-15 cm depth) experiments, including conventional (CT) and reduced tillage (RT) treatments. Compost application and replicates were included as conditional variables. The 17 most abundant species (Aa=*Aphanes arvensis*, Cb=*Capsella bursa-pastoris*, Ca=*Chenopodium album*, Fo=*Fumaria officinalis*, Ga=*Galium aparine*, Lsp=*Lamium* spp., Lop=*Lolium perenne*, Msp=*Matricaria* spp., Ma=*Myosotis arvensis*, Pa=*Poa annua*, Fc=*Fallopia convolvulus*, Sm=*Stellaria media*, So=*Sonchus oleraceus*, Ta=*Thlaspi arvense*, Vh=*Veronica hederifolia*, Vp=*Veronica* spp., Va=*Viola arvensis*) in the field and soil seed bank are represented.

Our results therefore sugguest that the environmental conditions (experiment, field versus seed bank) greatly affected dominances of certain weeds while fixed factors, such as tillage and compost, could only explain minor parts of the weed dominace in first year after application.

Locations (field vs. seed bank) contributed 16.8% constrained variance to the CCA and had the largest effect on the dominance of certain weeds. *Veronica hederifolia, G. aparine* and *F. officinalis* were more dominant in the field than in the coldhouse in both years, while *C. album, C. bursa-pastoris, S. oleraceus, F. convolvulus* and *T. arvense* dominated in the seed bank. The experimental year was reflected on the second axis, accounting for 8.6% of the total variance due to differences in occurrence of several species. Tillage accounted for 2.2% of the constrained variance in both experiments and was connected to the third CCA axis (not shown) while compost did not affect species significantly and was therefore used as conditional variable.

Previous results have shown a 3- to 5-fold higher seedling emergence for the seed bank compared with field conditions under CT, and an even greater effect under no-till management (Cardina & Sparrow, 1996). Our results matched closely to those of a study comparing seed bank and field emergence of weeds over a nine year period (Dessaint et al., 1997), in which an average weed seed bank to field emergence ratio of 8.5 and 6.5 was observed across all species at 0-15 and 25-30 cm deep ploughed soil, respectively. Dominant species under field conditions in our study, *F. officinalis, G. aparine,* and *V. hederifolia* (Figure 2.4), had a seed bank to field ratio below 4, independent of the tillage depth. Moreover, species that were dominant under coldhouse conditions in our study, *S. oleraceus, C. album, and C. bursa-pastoris,* and *T. arvense* (Figure 2.4), had seed bank to field emergence ratios ranging from 6-7 to >15. One exception was *F. convolvulus* which was observed at seed bank to field ratio averaging 3, not corresponding to the species dominance in the coldhouse in our study. The fact that this species commonly germinates in summer and was suppressed by wheat in our study could explain its dominance in the seed bank. Pedoclimatic conditions and cropping systems including rotations, tillage, and individual management factors, such as sowing time and weeding, further affect weed infestation forecasts and may explain large differences in weed composition between field and coldhouse conditions in our study (Colbach et al., 2014).

Overall, the difference in the species composition between tillage treatments was low. Nevertheless, *L. perenne* was positively related to the first and negatively to the third (not shown) axis of the CCA, indicating a strong dominance in the RT treatment under field conditions as discussed above. *Matricaria* spp. were more common under CT conditions, particularly in Expt 2 (Figure 2.1, Figure 2.4). Likewise, *C. bursa-pastoris* was dominant in Expt 2 while *S. media* and *T. arvense* dominated in Expt 1. The strong dominance of *Matricaria* spp. under CT in both the field and coldhouse in Expt 2, could be explained by the tillage intensity of CT. Compared to undercutting under RT, ploughing may have activated the *Matricaria* spp. seed bank much more, probably due to light induction and increased nitrogen mineralization. Furthermore, RT can rapidly increase soil aggregate size in the top soil (Liang et al., 2011), so *Matricaria* spp., usually present on poorly structured loam soils (Dord et al., 1978), can be favored by intensive soil tillage, such as under CT in our study. This contradicts the review of Froud-Williams et al. (1981) who cited studies that found *Matricaria* spp. increased under reduced tillage, although many other annual dicotyledonous species decreased.

2.4.4 Management Implications and Future Considerations

The long-term organic management and diverse crop rotations in the two experimental fields used for this study probably contributed to high numbers of weeds and the high species richness. The predominance of weeds in the field was highly influenced by the crop species winter wheat, and resulted in winter annual weeds dominating in this study. The data clearly support our first hypothesis that total seed numbers in the weed seed banks did not differ substantially among tillage treatments and RT reduced weed seeds in the deeper soil layer while CT mixed non-dormant seeds homogenously within the two soil layers. The fact that some small seeded species, such as *Matricaria* spp., *A. arvensis* and *M. arvensis*, were more frequent under CT in the seed bank may be due to effects of CT on dormancy as seeds were exposed to more light and potentially a nitrogen mineralization flash in the field before transfer to the cold house. Variable effects of RT on the weed seed bank were most likely due to differences in long-term crop rotation histories and the relatively uniform vertical distribution of seeds due to earlier deep and intensive tillage.

As expected, the weed dominance and soil cover in the field was already affected in the initial phase of transition to RT. As always, the weed occurrence was highly influenced by weather conditions. Under the organic conditions in our fields, however, crop competitive ability was likely reduced compared to conventional conditions due to reduced nutrient availability. This combined with large amounts of volunteering grasses due to insufficient pre-crop termination resulted in greatly increased weed vegetation under RT in the initial phase studied here.

Compost effects on weeds in the field were promising, although not always statistically significant. Soil amendments need to be studied over a longer period with repeated applications to determine if there will be cumulative effects over time.

In summary, weed communities and weed pressure cannot be well predicted based on weed data from the field or soil seed bank without considering the major impacts of crop management and weather conditions. The long-term effects of RT on weeds will require repeated field and seed bank analyses. We conclude that the ability to predict weed infestations from weed seed banks under organic management largely depends on the success of the reduced tillage operation in terminating previous crops in the absence of herbicides. Here it appears that undercutting to a depth of 4 cm in Expt 2 in contrast to the 12 cm in Expt 1 was a promising approach for primary tillage as it reduced the non-dormant seed bank compared to ploughing.

Early Effects of Reduced Tillage and Compost on Weeds

Infestation by volunteers was high, and so further development of the undercutting techniques are needed. If performed successfully, annual weed infestation could potentially be pushed below the level observed under conventional tillage. However, the development of perennial weeds, which was not part of this study, has to be monitored in future as these are expected to increase under reduced tillage.

3 Cover Crops and Compost Prevent Weed Seed Bank Build-up in Organic Wheat-potato Rotations under Conservation Tillage

3.1 Abstract

Weeds are a major constraint affecting crop yields in organic farming and weed seed bank analysis can be an important tool for predicting weed infestation and assessing farming system sustainability. We compared the weed seed banks two and four years after transition from conventional to reduced tillage in organically managed winter wheat-potato cropping sequences in two replicated field trials. Experimental factors were either conventional (CT) or reduced tillage (RT) systems based on moldboard (30 cm) and chisel ploughing (5-15 cm). Dead mulch (8-10 cm), consisting of rye-pea or triticale-vetch mixtures, were additionally applied to potatoes in the RT system. In both systems, one half of the plots received 5 t (ha year)$^{-1}$ dry matter of a commercially sold yard waste compost as an organic amendment. Furthermore, subsidiary crops were grown in both systems, either as legume living mulches undersown in wheat or as cover crops sown after wheat. Prior to sowing the wheat and after potatoes, the soil seed bank from 0-15 and from 15-30 cm was sampled and assessed in an unheated glasshouse over nine months. The initial weed seed bank size in the topsoil was uniform (4,420 seedlings m^{-2}). Two years later, wheat associated weeds, such as *Galium aparine, Lamium* spp., and *Myosotis arvensis,* were 61% higher on average in RT than in CT. This was independent of subsidiary crops used. In contrast, *Chenopodium album,* a potato associated weed that depends on intensive tillage was reduced by 15% in the mulched RT system compared to CT. When RT was combined with cover crops and compost application the seed bank was maintained similar to the CT system. We therefore conclude that subsidiary crops, mulches, and potentially compost are important management tools that contribute to the success of RT in organic cereal based systems in temperate climates.

Keywords: Seed bank; weed dynamic; conservation agriculture; cover crop.

3.2 Introduction

The adoption and promotion of conservation agriculture is an approach that can greatly reduce agricultural pollution caused by nitrogen leaching, soil erosion, and excessive diesel consumption (Köller, 2003). While conservation agriculture is broadly applied in North and South America, its use is limited in Europe (Kassam et al., 2010), especially in organic systems due to the generally higher weed pressure (Peigné et al., 2007).

The basis of weed and soil fertility management in organic farming are appropriate crop rotations (Leoni et al., 2015). Balancing summer- and winter-annual crops in combination with perennial forage crops can reduce the weed seed bank to a minimum. Furthermore, cover crops and longer-term grass-clover leys play important roles in the suppression of annual and perennial weeds (Sjursen, 2001). For example, weed biomass could be reduced by half in no-tillage winter wheat when cropped with perennial forage living mulches (*Trifolium* spp., *Medicago sativa)* compared to pure wheat (White & Scott, 1991). Furthermore, cover crops of *Sinapis alba*, *Avena strigosa,* and *Phacelia tanacetifolia* reduced weeds and volunteer wheat up to 97% compared to a system without cover crops (Brust & Gerhards, 2012).

Typically, organic rotations in Europe are combined with deep soil-inversion tillage, which effectively terminate pre-crops as well as annual and perennial weeds, and “stale seedbed” techniques that allow weeds to emerge after the first tillage operation. Thereafter, weeds are killed with a second cultivation, thus reducing the overall weed pressure (Finckh & van Bruggen, 2015).

However, deep inversion tillage and frequent cultivation generally undermine the sustainability of agricultural systems: Soils are often exposed to higher erosion risks and increased organic matter decay, soil communities adapted to specific soil depths are disturbed, arbuscular mycorrhizal networks disrupted, and earthworm populations decimated (Tebrügge & Düring, 1999; Gosling et al., 2006; Carr et al., 2013). Therefore, organic farmers have to learn to manage their systems with a minimum of tillage if they truly aim for long-term sustainability. Weed suppression is still the major challenge for organic minimum tillage systems under temperate climates. An important component in non-chemical weed suppression are high biomass producing (> 8 t ha^{-1} dry matter) cover crops in the rotation. These can suppress weeds either physically by building barriers for germinated seeds and altering light and temperature conditions or by releasing toxic substances to the soil (biofumigation) that prevent weed seed emergence (Mirsky et al., 2012).

To determine long-term effects of management on weeds, not only the weed vegetation in the field but also weed seed banks need to be considered. Although both parameters generally correlate, weed seeds can buffer short- and long-term cropping system effects due to their longevity and are therefore better indicators for long term system effects than the aboveground vegetation (Mayor & Dessaint, 1998). This is particularly important if cropping systems are compared that affect weed seed dispersal, composition, and dormancy, as well as depth distribution such as conventional versus conservation agriculture based on minimum soil disturbance, a maximum of soil cover by crops, residuals and mulch, as well as the use of crop rotations (Hobbs, 2007). In addition, weather conditions and weed management largely affect the short term weed dynamics in the field resulting in weak correlations between field observations and the seed bank (see Chapter 2 (Figure A 2).

Minimum tillage keeps weed seeds near the soil surface (Cardina et al., 2002) fostering those species that can germinate at the soil surface or at shallow depths. Thus, the abundance of monocotyledonous weed seeds, such as *Lolium* spp., *Setaria faberi* and *Phalaris* spp. is particularly high in no-tilled soils (Bàrberi & Lo Cascio, 2001; Cardina et al., 2002; Ruisi et al., 2015). In contrast, the abundance of *Chenopodium album* can be higher in moldboard and chisel ploughed than in in ridge- and no-tilled soils (Clements et al., 1996). This was likely due to different crop management and shading by pre-crop residues left on the surface as dead mulch under ridge- and no-tillage. Appropriate crop management may therefore prevent weed seed bank build-up in minimum tillage systems compared to plough systems and thus, avoid severe weed infestations in the long term in organic farming systems.

While several studies have documented the effects of crop rotation on weed pressure in organic farming in the short or medium term (Menalled et al., 2001; Sjursen, 2001; Teasdale et al., 2004; Albrecht, 2005), information about the effects of non-inversion tillage on the soil weed seed bank dynamics over time is scant. A German study showed that chisel ploughing resulted in two-fold higher weed seed banks compared with deep ploughing five years after differential tillage was started (Gruber & Claupein, 2009). However, initial seed bank densities and seed banks of single annual weed species five years after differential tillage were not examined, thus preventing any conclusions about seed bank dynamics. To obtain such information, there is a need to follow the transition process to non-inversion tillage from the beginning preferably in comparison to a conventionally tilled system.

In two adjacent fields, managed organically since 1989, experiments were set up in 2010 and 2011 to study the transition and longer term effects of conservation agriculture in an organic farming system. A typical plough based system is compared with a non-inversion tillage system that includes applications of transfer mulch. The differential tillage started in 2012 and 2013 with the termination of the grass-clover pre-crop. Winter wheat was sown subsequently, either together with clover living mulches that were left to grow after wheat harvest, or with cover crops that were sown after wheat harvest. Cover crops and living mulches were terminated in spring 2014 and 2015 with differential tillage and followed by potatoes. Three weeks after planting, potatoes in the non-inversion tillage system were mulched with rye-pea (2014) and triticale-vetch (2015) mixtures. An additional experimental factor was the application of 5 t ha^{-1} dry matter of yard waste composts in half of the plots to the wheat and 10 t ha^{-1} to the potatoes, respectively.

The seed banks after the first differential tillage following the grass-clover leys in 2012 and 2013 have been reported (Chapter 2). They consisted of < 10,000 seedlings m^{-1} and were evenly composed of summer, winter-, and non-seasonal weeds. Although only minor effects of tillage on weed seedling emergence were observed, there was a trend for higher seedling numbers in the ploughed system. The application of high quality yard waste composts reduced weed biomass during the vegetation period of wheat but did not affect initial weed seed banks (Chapter 2).

In this study, the development of the weed seed banks in two tillage systems four years after the start of the experiments is reported. Specifically, the effects of the mulch based non-inversion tillage potato system and the application of a total of 15 t ha^{-1} compost over three years as affected by tillage system were in focus. Using the same methodology that had been applied initially after the first differential tillage in 2012 and 2013 before sowing wheat (Chapter 2), after potato harvest in 2014 and 2015 the viable weed seed bank of each plot was assessed covering the minimally tilled horizon (0-15 cm) and the horizon reached by ploughing (15-30 cm). The specific aims of the study were to determine (i) quantitative and compositional changes in the weed seed bank over the course of the wheat-potato cropping sequence under mulch based non-inversion tillage compared to ploughing; (ii) the effect of specific agricultural management options (non-inversion vs. plough tillage, living mulches vs. cover crops, compost vs. no compost) adapted for organic farming on the viable weed seed bank; and (iii) interactions among these systems with respect to the weed seed bank. Data on the initial weed seed bank were reported in Chapter 2. Only where needed, these data are included in this chapter.

3.3 Material and Methods

The two field experiments were set up in 2010 and in 2011 in adjacent fields located on the organic experimental farm of the University of Kassel in Neu-Eichenberg (51°22'51"N, 9°54'44"E, 231 m ASL with an eastern incline of 3%). The soil type is a Haplic Luvisol with 3.3% sand, 83.4% silt and 13.3% clay (USDA classification Zc). Both experiments started with two years of grass-clover, which was mulched repeatedly, followed by winter wheat and potato. In the years preceding the grass-clover, the soil had been regularly ploughed 20-25 cm deep. The experiments consisted of a split- plot design with four replicates. The main split factor (12 x 90 m) was non-inversion tillage by chisel ploughing including the application of dead mulch to potatoes (RT) versus conventional tillage based on moldboard ploughing (CT) to terminate the grass-clover and subsidiary crops. Tillage treatments contained two times four 6 x 15 m² plots per replicate. As second factor, two clover species were undersown in the winter wheat as "living mulch" (*Trifolium repens* L. and *T. subterraneum* L.) in half of the plots while either summer vetch (*Vicia sativa* L.) or an oilseed radish/ black oat mixture (*Raphanus sativus* L., *Avena strigosa* L.) were sown as cover crops after wheat harvest in the remaining plots. The direct drilling of cover crops was accompanied by shallow undercutting (4 cm) with 36 cm overlapping duck-foot shares. The third factor was 5 t and 10 t dry matter ha^{-1} yard waste compost (Table 3.1) applied manually after soil tillage before sowing wheat and planting potatoes, respectively, versus no compost. Before planting potatoes, potassium (K_2SO_4) and phosphorus (rock phosphate) fertilizer were applied approximately matching the concentration of the composts used in 2014 and 2015 (Table 3.1). Potatoes were planted in late April and received an 8-10 cm layer of rye-pea (2014; 12 t ha^{-1} dry matter; C:N = 27) and triticale-vetch (2015; 26.5 t ha^{-1} dry matter; C:N = 23) transfer mulch three weeks after planting in the RT treatments after the first hilling. No further tillage was applied to the RT plots while potatoes in CT plots were harrowed and hilled once more in June. If not already killed by late blight (*Phytophthora infestans*), potatoes and weeds were mulched in early August and harvested in early September.

Table 3.1 (Expansion of Table 2.2) Chemical characteristics, including dry matter (DM), bulk density, pH, electrical conductivity (EC), potassium (K), phosphorous (P), total nitrogen (N), carbon (C), and C/N ratio of yard waste composts (≤ 20 mm sieved) from municipal trees and shrubbery from the composting plant at Dransfeld, Germany (three-month-old) used in 2012 and 2013 before wheat, and from a composting plant near Hannover (Aha, nine-month-old) used before potatoes in 2014 and 2015.

Year	DM (%)	Bulk density (g L^{-1})	pH	EC (µS cm^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Total N (%)	Total C (%)	C/N ratio
2012	85	389	7.5	498	3104	541	1.8	29.0	16.0
2013	81	282	6.4	778	NA	807	1.5	37.4	25.5
2014	75	604	7.3	915	5276	547	1.3	20.8	16.2
2015	60	731	8.1	1011	4858	616	1.3	16.9	13.0

3.3.1 Weed Seed Bank Analysis

Twenty evenly-distributed soil cores were taken from the central 10 m x 3 m section of each plot after seedbed preparation and compost application, but before sowing of winter wheat (start of the experiment in 2012 and 2013) and after potato harvest (end of the experiment in 2014 and 2015) as described in Chapter 2. In brief, soil cores with a diameter of 2.4 cm were taken and divided into 0-15 cm and 15-30 cm layers. Plastic trays (200 cm²) were filled with 600 ml soil and placed in an unheated greenhouse with a plastic roof (coldhouse). There were 128 trays representing the trial plus 4 pure compost controls. The soil in the trays was kept moist, but watering was suspended when frost occurred. From October until June emerged seedlings were periodically identified, counted, and removed from plastic trays. Vegetative reproductive parts of perennial weeds were not assessed as they were very rare after two years of grass-clover ley. In December 2014 and 2015 (assessment after potatoes only), all trays were placed in a heated greenhouse for two weeks to assess all seedlings before start of the frost period. This reduced the number of unknown species that had been high during the initial assessments in 2012 and 2013 (Chapter 2). In May, when the emergence rate was decreasing, the soil in the plastic trays was mixed to simulate field disturbance and to break compacted soil clods.

3.3.2 Data Processing and Statistical Analysis

Both living mulch species in wheat largely failed in both experiments due to winter kill and were treated as weedy fallow in the analysis. The two cover crops performed poorly due to late sowing in the first and unfavourable sowing conditions paired with a common vole (*Microtus arvalis* Pallas) epidemic in the second experiment and were combined into one cover crop treatment, thus resulting in eigth weedy fallow – cover crop comparisons per experiment. Emerged seedling densities from plastic trays were extrapolated to 1 m² prior to analysis:

$$Seeds\ m^{-2} = seeds\ (tray) \times \frac{10000\ cm^2 \times volume\ (core)}{volume\ (tray) \times area(core)}$$

Statistical analyses were performed with R version 3.2.2 (R Core Team, 2013) following instructions of Dormann and Kühn (2012) and Hedderich and Sachs (2012), using the package Vegan (Oksanen et al., 2015) for multivariate analysis of data. Three-way analyses of variance (ANOVA) with factors tillage, subsidiary crops, and compost including field experiments and replicates as blocks, were performed for the numbers of the 15 major weed species and total number of seedlings in the seed bank (0-30 cm). The same analysis was applied to the number of weed species (species richness) at the end of the experiment for each soil depth separately. Normal distribution of residuals and variance homoscedasticity were visually assessed using QQ-plots and comparing residuals versus fitted values, respectively. The species richness did not need transformation, while the number of the 15 major weed species and total number of seedlings were square-root (x+1)- transformed prior to the ANOVA to improve normal distribution and homogeneity of variances. In the text, untransformed numbers are reported. The seed bank sizes before and after the wheat-potato cropping sequence were compared with a paired t-test for each factor combination after assessing for equality of variances with F-tests for comparison of two variances.

For multivariate data analyses Relative Abundance Indices (RAI) were computed of the 15 major species or genera (>1% relative frequency in the seed bank at the end of the experiment) for each soil layer according to Bàrberi and Lo Cascio (2001) and Derkson et al. (1993) as (relative density + relative frequency)/2. The relative density is defined as the number of seedlings of a species relative to the total number of weed seedlings in percent. The relative frequency is obtained by dividing the absolute frequency of a species (number of samples where the species occurred/ total number of samples) by the sum of absolute frequencies of all identified species in percent. The RAI corrects for patchy occurrences of weeds (Derksen et al., 1993) and thus, generally improves the explanatory power of ordination techniques. Data were arcsine-transformed to increase the variance homogeneity and improve the normal distribution prior to analysis.

The detrended correspondence analysis on RAI values revealed short gradients (<2) on the first axis suggesting the application of a redundancy analysis (RDA, (Dormann & Kühn, 2009)). The RDA was performed for each depth separately with all experimental factors and the factor "time" (start vs. end of the experimentas well as the co-variates "experiment" (2012-2014, 2013-2015) and the four replicates per experiment. Co-variates explained about 20% of the variance which was removed from the total variance in the further analysis, thus increasing the overall power. In contrast to the factors time and tillage, subsidiary crops and compost application had no statistically significant effect on the weed species composition dynamic according to permutation test (999 permutations) and were removed from the analysis. The permutation test further revealed significant interactions between tillage and time, which are therefore shown in detail.

3.4 Results

The experimental factors rarely interacted with each other with respect to the species observed, and if, no general pattern was observed. For this reason, only main effects are shown. The weed seed bank community and size before and after the wheat-potato rotation was mainly affected by tillage. Overall, only few monocotyledonous weeds were identified. Dicotyledonous species with generally higher seed longevities (> 3 years) dominated the seed banks (Table 3.2). Weed seedlings germinated independently of their emergence seasons and those with annual life cycles dominated in this study

In general, weed species that were frequent after the termination of grass-clover (Start, Table 3.2), e.g. *Aphanes arvensis, Chenopodium album, Matricaria* spp., *Veronica* spp., also occurred at high densities after potatoes (End, Table 3.2). The relative frequency of *Veronica* spp., *Matricaria* spp., *Lamium* spp., and *Myosotis arvensis* increased throughout the wheat-potato rotation while *A. arvensis, Fallopia convolvulus, Stellaria media,* and *Thlaspi arvense* declined. New upcoming species were rarely identified of which only *Chenopodium polyspermum* appeared at higher densities (Table 3.2).

Table 3.2 Emergence season, germination depth (Depth), seed longevity, and relative frequency (in %) of weed species in the total seed bank (0-30 cm) after grass-clover and before wheat (Start) and two years later after the wheat-potato cropping sequence (End) across all experimental variables according to Clarke et al. (2009) and Klaassen and Freitag (2004).

Species	Emergence season	Depth (cm)	Seed longevity (years)	Relative frequency	
				Start	End
Elymus repens (L.) Gould	Spring	n.a.	1-5	0.7	0.1
Alopecurus myosuroides Huds.	Autumn	6	1-5	0.5	0.4
Aphanes arvensis L.	Autumn	n.a.	>5	10.7	6.5
Arabidopsis thaliana (L.) Heynh.	Spring/Autumn	n.a.	>5	0.1	<0.1
Capsella bursa-pastoris (L.) Medicus	Non-seasonal	0.5	>5	6.4	6.2
Chenopodium album L.	Spring	5	>5	8.5	8.1
Chenopodium polyspermum L.	Spring	5	>5	0.0	0.7
Cirsium arvense (L.) Scop.	Spring	5	>5	0.0	<0.1
Euphorbia helioscopia L.	non-seasonal	n.a.	n.a.	0.1	<0.1
Fallopia convolvulus (L.) A. Loeve	Spring	n.a.	>5	8.3	2.3
Festuca pratensis Huds.	Autumn	n.a.	1-5	0.0	0.1
Fumaria officinalis L.	Spring	n.a.	>5	1.6	0.8
Galinsoga spp.[1]	Spring	n.a.	>5	0.2	0.1
Galium aparine L.	Autumn	7	1-5	1.4	1.4
Geranium dissectum L.	Non-seasonal	6	1-5	<0.1	<0.1
Lamium spp.[2]	Non-seasonal	1	>5	4.5	7.3
Matricaria spp.[3]	Non-seasonal	0-5	>5	7.7	11.4
Myosotis arvensis (l.) Hill.	Spring/Autumn	5	>5	2.7	6.6
Papaver rhoeas L.	Autumn	0.5	100	0.0	0.1
Poa annua L.	Non-seasonal	n.a.	>5	1.1	1.8
Polygonum spp.[4]	Spring	5	>5	1.6	2.7
Ranunculus repens L.	Spring	n.a.	n.a.	0.0	<0.1
Rumex spp.[5]	Non-seasonal	3-5	>5	0.1	0.1
Sinapis arvensis L.	Spring	4	>5	0.1	0.1
Stellaria media (L.) Vill.	Non-seasonal	4	>5	8.9	1.6
Sonchus oleraceus L.	Spring	2	>5	3.9	0.8
Thlaspi arvense L.	Spring	n.a.	>5	8.9	2.5
Veronica hederifolia L.	Autumn	0.5	>5	4.7	5.3
Veronica spp.[6]	Spring/Autumn	n.a.	>5	9.2	31.2
Viola arvensis Murray	Spring/Autumn	0.5	>5	1.1	1.1
Unidentified species	-	n.a.	-	7.2	0.9

[1] *Galinsoga ciliata* (Rafin) S.F. Blake, *G. parviflora* Cav.

[2] *Lamium amplexicaule* L., *L. purpureum* L.

[3] *Matricaria recutita* L., *M. inodora* L.

[4] *Polygonum aviculare* L., *Persicaria lapathifolia* L., *P. persicaria* L.

[5] *Rumex obtusifolius* L., *R. crispus* L.

[6] *Veronica agrestis* L., *V. arvensis* L., *V. persica* L.

Cover Crops and Compost Prevent Weed Seed Bank Build-up

Seedling densities and Relative Abundance Indices (RAI) for the 15 major weed species were similar, although differences between factor levels were generally reduced for RAI (Table 3.3). An exception was the RAI for *C. album* that was significantly ($P < 0.05$) higher if compost was applied than without compost while there were no significant effects on seedling densities.

The 15 most common species that occurred at > 1% relative frequency after potatoes were little affected by the applied treatments in 0-30 cm soil layer (Table 3.3). Numbers of seedlings and the RAI of *Galium aparine* and *Lamium* spp., and numbers of seedlings of *M. arvensis* were higher under RT than under CT ($P < 0.05$). Both, *C. album* and *Matricaria* spp. seedlings tended to be lower under RT than CT ($P < 0.1$). At 0-30 cm depth, autumn emerging seedlings tended to be higher under RT while spring emerging seedlings were slightly higher under CT, however, these effects were statistically not significant (Table 3.3, Table 3.4).

Overall, subsidiary crops and compost did not substantially affect the weed species composition and the total number of viable seedlings in the seed bank (Table 3.3, Table 3.4). However, *Capsella bursa-pastoris* seedlings were significantly higher after the weedy fallow (638 seedlings m^{-2}, untransformed data) than after cover crops (537 seedlings m^{-2}) (Table 3.3). Likewise, *Poa annua* seedlings tended to be less frequent in cover crop (234 seedlings m^{-2}) than in weedy fallow plots (348 seedlings m^{-2}, $P < 0.1$).

While seedlings of *M. arvensis* were significantly less frequent in plots that had received compost (469 seedlings m^{-2}) than in plots without compost application (628 seedlings m^{-2}), the difference was not evident for the RAI. In contrast, the RAI of *C. album* was significantly higher in compost amended than in unamended plots.

Table 3.3 Seedling densities (seedlings m^{-2}) from seed bank tests and Relative Abundance Indices (italic letters) of 15 major weed species and the total number of seedlings with non-seasonal emerging and main emerging season in spring, autumn, and spring/autumn in 0-30 cm depth. Effects of conventional (CT) versus non-inversion tillage (RT), two crop rotations consisting of wheat and potato with a weedy fallow (WF) versus cover crops sown after wheat (CC), and compost (+YWC) versus no compost (-YWC) application are shown[1].

	CT	RT	sign.	WF	CC	sign.	+YWC	-YWC	sign.
Aphanes arvensis	20.7	23.8	[2]	22.2	22.2		21.9	22.5	
	25.2	*26.5*		*25.8*	*25.9*		*25.7*	*25.9*	
Capsella bursa-pastoris	20.2	22.5		22.8	19.9	*	20.2	22.5	
	25.6	*26.2*		*26.5*	*25.3*		*25.2*	*26.6*	
Chenopodium album	34.1	29.0		30.7	32.3		31.8	31.2	
	36.7	*32.2*	+	*33.9*	*35.0*		*35.5*	*33.3*	*
Fallopia convolvulus	11.9	14.6		14.2	12.3		14.0	12.5	
	20.5	*21.0*		*20.9*	*20.7*		*21.3*	*20.2*	
Galium aparine	5.8	11.9	*	8.3	9.4		8.4	9.3	
	15.3	*17.8*	*	*16.2*	*16.9*		*16.3*	*16.8*	
Lamium spp.	15.4	22.5	*	18.0	19.9		19.3	18.6	
	21.2	*23.7*	*	*22.0*	*23.0*		*22.4*	*22.5*	
Matricaria spp.	23.8	17.0		20.7	20.1		19.9	20.9	
	24.6	*21.0*		*22.8*	*22.7*		*22.7*	*22.8*	
Myosotis arveis	15.1	19.9	*	16.9	18.1		15.9	19.1	*
	20.6	*22.4*	+	*21.0*	*22.0*		*20.9*	*22.1*	+
Poa annua	9.7	13.9		13.3	10.4	+	12.7	10.9	
	18.0	*20.6*		*20.2*	*18.4*	+	*19.6*	*19.1*	
Polygonum spp.	12.6	12.8		13.0	12.4		14.1	11.3	
	20.3	*19.9*		*20.1*	*20.1*		*20.7*	*19.5*	
Stellaria media	11.5	9.8		10.7	10.7		9.8	11.5	
	19.6	*18.5*		*19.3*	*18.7*		*18.8*	*19.2*	
Thlaspi arvee	14.7	14.6		14.4	14.9		14.2	15.1	
	22.1	*21.8*		*21.7*	*22.3*		*21.8*	*22.1*	
Veronica hederifolia	16.7	17.6		18.4	15.8		17.4	16.8	
	22.5	*22.4*		*23.0*	*21.9*		*22.8*	*22.1*	
Veronica spp.	40.4	45.6		43.4	42.6		41.8	44.2	
	37.5	*38.5*		*37.9*	*38.1*		*37.4*	*38.6*	
Viola arvensis	4.8	4.9		5.4	4.2		4.6	5.1	
	7.3	*7.4*		*7.5*	*7.3*		*7.3*	*7.5*	
Spring	53.9	51.2		52.1	52.9		53.0	52.1	
Autumn	23.4	29.2		25.8	26.8		25.6	27.0	
Spring/Autumn	45.0	51.9		48.5	48.4		46.5	50.0	
Non-seasonal	45.1	47.3		47.9	44.5		45.4	47.0	

[1] Seedling densities and RAI are square-root [$\sqrt{(x + 1)}$]- and arcsine-transformed, respectively;

[2] Results of a three-way ANOVA; df = 7, 14, and 92 for tillage, crop rotation, and compost, respectively.

+,* = significant at $P < 0.1$ and $P < 0.05$, respectively.

The weed species richness depended on soil depth and was influenced by tillage, though not by cover crops, weedy fallow, or compost applications (Table 3.4). Significantly more weed species and total number of seedlings were identified in the top soil layer (0-15 cm) under reduced tillage while for the weed species the reverse effect occurred in the sub soil layer (15-30 cm). No significant differences were observed when considering the 0-30 cm depth for both parameters (Table 3.4).

Table 3.4 Number of weed species (Species richness) and total number of seedlings m^{-2} (untransformed) in each soil layer after the 4-yr crop rotation consisting of two years grass-clover, winter wheat, cover crops, and potato averaged over both field experiments. Applied treatments were non-inversion (RT) vs. conventional (CT) tillage (df =7) after grass-clover, weedy fallow (LM) vs. cover crops (CC) sown after wheat (df = 14), and compost (+YWC) vs. no compost (-YWC) application (df = 92).

	Soil layer (cm)	CT	RT	Sign.	WF	CC	Sign.	+YWC	-YWC	Sign.
Species richness	0-15	8.4	9.9	*	9.2	9.0	NS	8.9	9.3	NS
	15-30	7.8	6.7	*	7.3	7.1	NS	7.3	7.1	NS
	0-30	11.3	12.1	NS	11.9	11.5	NS	11.7	11.7	NS
Number of seedlings	0-15	4,857	6,097	*	5,514	5,439	NS	5,329	5,625	NS
	15-30	4,482	4,274	NS	4,521	4,235	NS	4,251	4,505	NS
	0-30	9,339	10,371	NS	10,036	9,674	NS	9,580	10,130	NS

* = significant at $P < 0.05$. NS = not significant.

The redundancy analysis (RDA) shows a strong influence of the sampling time (start vs. end of the wheat-potato rotation) and confirmed some of the overall effects of tillage from Table 3.3 on the weed seed bank community. However, it also gives a clearly differential picture for the two soil layers as effects shown in Table 3.3 were enlarged between tillage systems in the 0-15 cm layer (Figure 3.1) while there were almost no differences between CT and RT at 15-30 cm soil depth (data not shown).

In the top soil layer (0-15 cm, Figure 3.1), the first (RDA 1) and second (RDA 2) axis were significant at $P < 0.01$ with 18.1 and 1.5% explained eigenvalues, respectively. *Polygonum* spp., *C. album*, *Veronica* spp., and *P. annua* were plotted with the end of the crop rotation, indicating an increase over time. In contrast, S. *media*, *F. convolvulus*, and *T. arvense* were plotted with the beginning of the crop rotation, indicating a decline over time. Most species were neither correlated with the positive nor negative side of the second RDA axis and thus, unaffected by tillage. However, *C. album* was plotted together with CT at the end of the rotation, indicating that the increase of this species was higher under CT than RT. In contrast, *P. annua*, *M. arvensis*, and *G. aparine* were plotted with RT at the end of the rotation, indicating that these species increased more under RT than CT over time. The strong interactions of tillage treatments with sampling time are illustrated by the closeness of RT and CT at the start of the crop rotation, while both parameters were clearly separated on the RDA 2 at the end of the crop rotation.

The time effects were also observed in the sub soil (15-30) and in both soil layers combined (0-30 cm, data not shown). However, no statistically significant second axes emerged. Thus, tillage did not affect the weed seedbank community at 15-30 cm depth as much as in the top soil layer.

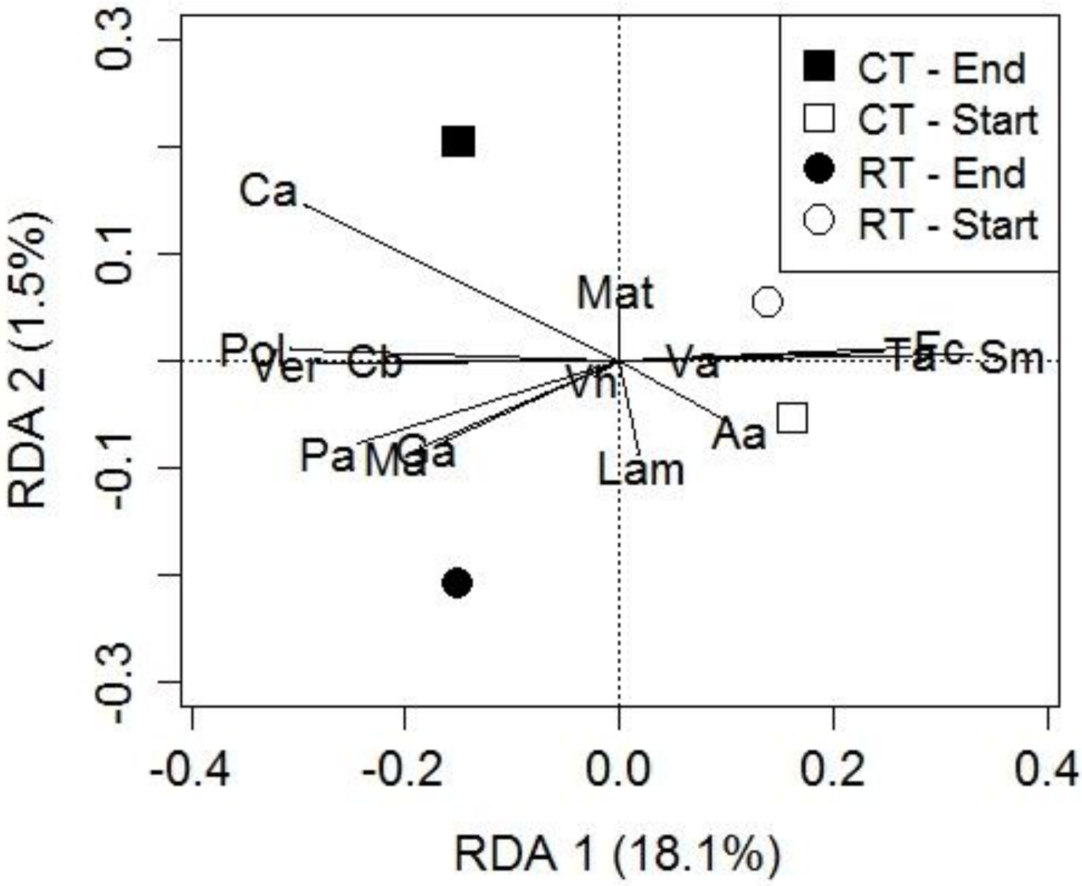

Figure 3.1 Redundancy analysis biplot comparing the weed seed bank community under non-inversion (RT, circles) and conventional (CT, squares) tillage before wheat (Start, unfilled symbols) and after the wheat-potatoes cropping sequence (End, filled symbols) in 0-15 cm depth. The first and second RDA axes are showing the proportion of explained eigenvalues by the factors after removing the variance caused by conditional variables (experiment, replicates). The 15 species (Aa=*Aphanes arvensis*, Cb=*Capsella bursa-pastoris*, Ca=*Chenopodium album*, Fc=*Fallopia convolvulus*, Ga=*Galium aparine*, Lam=*Lamium* spp., Mat=*Matricaria* spp., Ma=*Myosotis arvensis*, Pa=*Poa annua*, Pol=*Polygonum* spp., Sm=*Stellaria media*, Ta=*Thlaspi arvense*, Vh=*Veronica hederifolia*, Ver=*Veronica* spp., Va=*Viola arvensis*) with more than 1% frequency in the soil seed bank after potatoes are represented.

Cover Crops and Compost Prevent Weed Seed Bank Build-up

In the top soil layer, before start of the wheat-potato cropping sequence, the seed bank size was on average 4,422 seedlings m^{-2} and there were no singificant differences among all factor combinations (*P* > *0.*05, Figure 3.2A). The seed bank in the top soil did not increase significantly over the course of the two years except for the RT treatment with the weedy fallow (Figure 3.2A). In CT, weedy fallow and cover crops did not differ both in the presence and

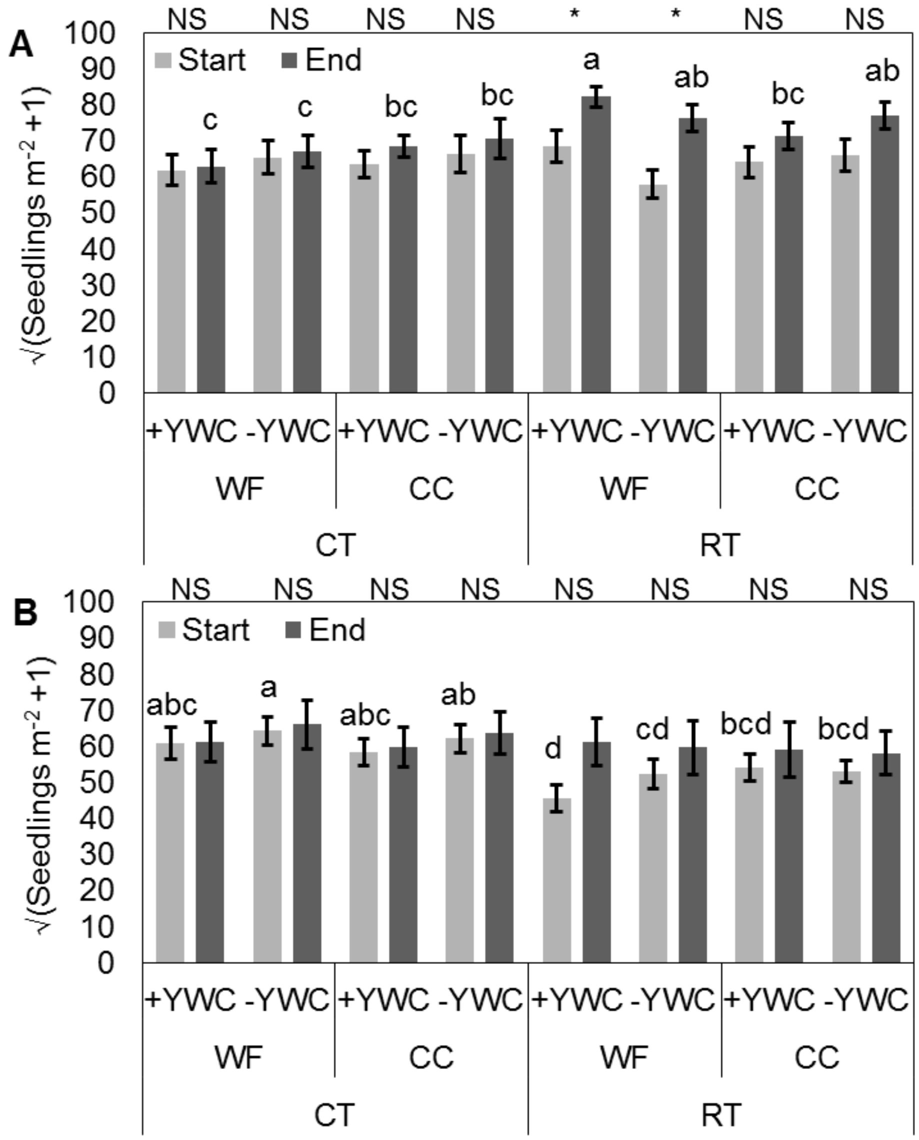

Figure 3.2 Total number (square-root-transformed) of weed seedlings m^{-2} (mean + SE) in the top soil (A, 0-15 cm) and subsoil (B, 15-30 cm) layer before (light grey bars) and after (dark grey bars) the wheat-potato cropping sequence for each factor combination averaged over both experimental fields. Factor levels were conventional (CT) versus non-inversion (RT) tillage, weedy fallow (WF) versus cover crops (CC), and compost application (+YWC) versus no compost (-YWC). Same lower-case letters indicate not statistically different treatment combinations (LSD-test; $P < 0.05$; df = 113). * indicate statistically significant differences in seed bank size before (Start) and after (End) the wheat-potato cropping sequence at *P*<0.05 (paired t-test).

absence of YWC. In contrast, under RT, combining cover crops and yard waste compost led to the lowest number of seedlings m^{-2} (5286) which was significantly lower than after the weedy fallow with yard waste compost (6888 seedlings m^{-2}). Furthermore, combination of RT, cover crops, and compost kept the seed bank size at the same level than all CT treatments (Figure 3.2A).

In the sub soil layer, initial seed bank sizes after the first differential tillage had been applied were overall lower under RT (2,849 seedlings m^{-2}) than under CT (4,028 seedlings m^{-2}, Figure 3.2B and Figure 2.1). In contrast, all treatments were similar at the end of the wheat-potato cropping sequence. Thus, apparently, weed seed banks increased under RT but not under CT with the strongest increase in the RT and weedy fallow treatments.

3.5 Discussion

3.5.1 Crop Rotation largely Influences Weed Seed Bank Communities

Crop rotations that include perennial leys as well as balanced spring and winter crops are effective weed control strategies in low input and organic farming systems. In such systems, weed communities are generally diverse and dominances of few weeds that are adapted to specific crop vegetation periods rarely occur (Nichols et al., 2015). Furthermore, perennial leys, such as grass-clover, can efficiently reduce weed seeds in soil especially those with low persistence (Sjursen, 2001). This is consistent with the weed seed bank composition at the start of the experiments in this study that contained mostly weed species capable to survive more than five years in the soil. In addition, a uniform relative frequency of the present weed species was observed here and although the total number of seedlings was low, the number of different weed species was high (Table 3.2).

Winter wheat and potatoes grown in rotation generally affect weed seed banks differently. In a German study, winter wheat increased the presence of *G. aparine*, *Matricaria recutita, M. arvensis, P. annua, S. media,* and *Veronica* spp., while potato increased the presence of *C. album, Polygonum lapathifolia,* and *Sonchus asper* (Albrecht, 2005). Except for *S. asper* and *S. media,* all these weed species were more frequent before than after the wheat-potato cropping sequence in our study. Our results were consistent with the results of Albrecht (2005), who found that winter wheat and potatoes reduced *T. arvense* and *A. arvensis,* respectively. The large increase in frequency of *Veronica* spp. may be explained by the generally high persistence of the seeds and the non-seasonal emerging behaviour of *Veronica persica* as well as the autumn and spring/autumn emerging season of *V. arvensis* and *V. agrestis*, respectively (Clarke et al., 2009). Thus, the *Veronica* complex is rather unaffected by rotations of spring and autumn sown crops. This also explains the generally higher frequency of non-seasonally or spring and autumn emerging weeds than weeds that germinate either in spring or autumn and is consistent with Albrecht (2005).

3.5.2 Tillage Determines Weed Seed Bank Size and Community

The effect of RT on the weed seed bank can be variable (Nichols et al., 2015), though many authors reported increases in seed banks and species richness compared to CT (Moyer et al., 1994; Cardina et al., 2002; Sosnoskie et al., 2006; Carter & Ivany, 2006). Field histories, climate, and duration of the experiments, which generally result in different weed (seed bank) communities, are important factors influencing the weed seed bank responses of tillage experiments (Nichols et al., 2015). Our results confirm the results of others (Bàrberi & Lo Cascio, 2001; Cardina et al., 2002; Nichols et al., 2015) that reduced tillage is concentrating weed seeds at the top soil layer resulting in higher species richness and total numbers of seedlings in 0-15 cm and opposite effects in 15-30 cm depth under RT compared with CT. After four years, no clear trends for an overall increased seed bank at 0-30 cm could be confirmed in contrast to the longer term study of Carter and Ivany (2006). The authors always observed greater weed seed banks in 0-10 and 10-20 cm after rotary harrowing (10 cm) and direct drilling than after moldboard ploughing (20 cm). In addition, the dominant weed species in the subsoil was *Gnaphalium uliginosum*, which is a small-seeded species with very low seed weigth but long persistence in soil (Thompson et al., 1993). Carter and Ivany (2006) stated that the fine loamy sand in their fields probably contributed to higher seed movement to deeper soil layers compared to clay textured soils, which may be supported by the low seed size and weigth of the dominant weed. These results suggest that the silt loam texture in fields used in our study should have reduced seed movement to deeper soil layers. The overall increase of the seed bank at 15-30 cm under RT appears to be counterintuitive in our study. One possibility is that the high incidence of common voles producing numerous large subterranean networks may have helped seed movement to deeper layers under RT. Furthermore, the potato harvester used in the experiments can reach working depths of 25 cm. The resulting homogenization of weed seeds in this layer at least partially camouflaged all previous tillage effects on the weed seed bank.

Although the total weed seedbank under RT was higher than under CT, varying effects were observed on the species level. This is not unusual as germination requirements are generally different among weed species. For instance, grass weeds germinate from shallow depths and are therefore likely to increase in less disturbed environments such as present in RT systems (Davis et al., 2005; Nichols et al., 2015). In wheat production systems, *P. annua* was frequently found at higher densities in minimum and no-tilled than mouldboard ploughed fields (Froud-Williams et al., 1981; Wilson et al., 1986). This may explain why *P. annua* was more frequent when a weedy fallow with no soil disturbance was allowed after wheat harvest compared to the cover crops that were accompanied by shallow (4 cm) tillage which may have reduced the aboveground biomass of this species and thus, its re-seeding rate. Furthermore, we observed significantly higher numbers of *G. aparine, Lamium* spp., and *M. arvensis* seedlings under RT compared with CT in 0-30 cm (Table 3.4) and 0-15 cm (Figure 3.1). This is in line with the results of a study from France in which seed banks in shallow (0-10 cm) and deep (25-30 cm) ploughed soils were assessed in a spring pea- spring barley- winter wheat rotation (Dessaint et al., 1997). The authors found 5- and 25-fold higher seed densities of *G. aparine* and *M. arvensis,* respectively, in the shallow than the deep ploughed topsoil (0-10 cm).

Deep ploughing can increase the number of viable weed seedlings by transferring seeds to the soil surface and thus, breaking weed dormancy (Peigné et al., 2007). In addition, field histories can affect the study outcome (Chapter 2) but are rarely discussed due to lack of information about initial weed seed banks (Mohler, 2001; Nichols et al., 2015). For example, Albrecht (2005) found that *M. recutita* increased during winter wheat. This leads to an expected increase for *Matricaria* seed banks after a wheat-potato rotation rather than the maintenance as observed in our study. High densities of viable *Matricaria* spp. seeds were observed initially under CT in one of the two fields (Chapter 2). Their generally high seed longevity (Table 3.2) may therefore explain higher densities of *Matricaria* spp. in CT than in RT also two years after the initial assessments (Table 3.3) and its low response to sampling dates in the redundancy analysis (Figure 3.1).

In contrast, the trend for a higher RAI of C. *album* under CT than RT after potatoes (Table 3.3) was not due to field history effects. Initial seed banks were similar among both tillage systems (Chapter 2.4.1: Figure 2.1) and the redundancy analysis showed a clear correlation between *C. album* and CT after the wheat-potato cropping sequence (Figure 3.2). The reproduction of *C. album* was likely directly affected by the application the 8-10 cm of dead mulch after emergence of potatoes in RT plots. The mulch was decaying slowly due to its high C:N ratio (25:1) and thus, providing almost 100% soil cover from mid-May until mid-July covering the main germination period of *C. album* (Clarke et al., 2009). In contrast, potatoes under CT were hilled until mid-June, thus enabling seeds of *C. album* to germinate and reproduce. Already 9 t ha^{-1} of rye mulch (half the amount used in this study) can provide excellent weed control in summer crops (Reberg-Horton et al., 2012). Thus, mulch effects can explain generally lower seedling densities of spring germinating species under RT here. Also, *C. album* is more adapted to intensive tillage due to its strong dormancy and thus, tends to be more prevalent under CT (Clements et al., 1996; Peigné et al., 2007).

3.5.3 Low Effects of Cover Crops and Compost on the Weed Seed Bank

Subsidiary crops are grown predominantly for their ecosystem services, such as nitrogen uptake and supply, weed control, reduction of soil erosion and nitrogen leaching, increase of soil fertility (Ilnicki & Enache, 1992; Hartwig & Ammon, 2002; Baresel & Reents, 2008). In maize, subterranean clover grown as living mulch (LM) suppressed weeds by up to 50% in minimum and no-tilled soils compared to the non-mulched control (Ilnicki & Enache, 1992). Likewise in Switzerland, weed seed bank sizes in maize intercropped with white clover/ orchard grass (*Dactylis glomerata* L.) mixtures did not exceed those in pure maize with herbicide applications (Ammon et al., 1995). Unlike spring crops, the establishment of LM in fall crops, such as winter wheat, can be critical. Winter hardy white clover species are generally very competitive if sown too early and may reduce winter wheat yields (Baresel & Reents, 2008). In contrast, subterranean clover, which is generally less competitive than white clover and susceptible to severe frost, may develop sufficiently if sown from mid-August to mid-September (Baresel & Reents, 2008). The establishment of LM in our study failed for several reasons. Both LM did not develop sufficiently due to late sowing together with winter wheat. The small plants either died over winter or were suppressed by the strong growth of wheat and weeds in spring. For experimental reasons, these plots were continued as weedy fallows after wheat in contrast to the cover crop plots. This further explains low differences for wheat associated weeds, e.g. *G. aparine, Lamium* spp., *M. arvensis,* and *Veronica* spp., in the seed bank of weedy fallow and cover crop treatments as there was no suppression by LM. As discussed above, only *P. annua* and *C. bursa-pastoris* seedlings tended to be higher in the weedy fallow than in cover crop plots. Both species flower all over the year (Clarke et al., 2009) and could have produced seeds in the weedy fallow until termination prior to planting potatoes. This is particularly important for *P. annua* which can live for at least two seasons in undisturbed soils and generally produces more seeds in the second year (Law et al., 1977). In contrast, both species were disturbed after wheat harvest by the cover crop sowing that was accompanied by shallow tillage, probably resulting in lower seed shed.

Composts can have varying effects on weeds depending on their initial composition (Blackshaw et al., 2005), composting duration and methods (Cayuela et al., 2008), and probably the composting plants. Mechanisms behind weed suppression may be chemical and biological compounds of the composts, such as high C:N ratios resulting in the unavailability of free nitrogen, volatile fatty acids, phytotoxins, higher seed predation by compost inhabiting microorganisms, and high concentrations of trace metals (cadmium, copper, lead, nickel, zinc) in immature compost (Ozores-Hampton et al., 2001; Kremer & Li, 2003; Blackshaw et al., 2005). On the species level, we only found *M. arvensis* seedlings at significantly lower numbers in compost amended plots, while spring crops showed opposite trends. The compost applied in spring to the potatoes was composted six months longer and was derived from a different composting plant than the compost applied in fall to the wheat. The older compost may have had a lower potential for weed suppression due to lower concentrations of volatile fatty acids and C:N ratio (Table 3.1). In one study, composts with a C:N ratio of 10-12 resulted in higher weed concentrations than compost with a C:N ratio of 15-18 (Blackshaw et al., 2005). This may explain low or even beneficial effects of compost on potato associated spring weeds, such as *C. album* and *Polygonum* spp., in our study while the opposite effect was observed for winter wheat associated weeds, such as *Veronica* spp. and *M. arvensis*. This is consistent with the weed biomass production in wheat in the first year of the rotation that tended to be lower in compost amended plots (Chapter 2.4.1.1).

3.5.4 RT with Cover Crops and Compost did not Build-up Weed Seed Banks

The weed seed banks in up to 15 cm depth generally correlate with the aboveground weed community (Benvenuti et al., 2001; Dessaint et al., 1997; Rahman et al., 2006; Zhang et al., 1998) and can therefore be used to assess the efficiency of weed management systems. However, pedo-climatic conditions may affect these correlations, such as observed in Chapter 2.4.3, where weed biomass production was higher in RT than in CT despite of similar initial seed banks. In contrast in Italy, subterranean clover cover crops grown from fall to spring in maize monocultures resulted in lower weed biomass under no-till compared to maize monocultures without cover crops in a ploughed system, although weed seed banks were initially larger under no-till (Moonen & Bàrberi, 2004). In addition, weed seed banks were always lower if subterranean clover cover crops were grown compared to the control without cover crops. Similarly here, the weed seedling density was lower in the cover crop treatment than in the weedy fallow under RT, although the numbers of seedlings were generally higher under RT than under CT. The fact that LM treatments failed and served as a weedy fallow after wheat harvest while the soil was shallowly tilled for cover crop sowing contributed to differences between both treatments. Therefore, the cover crop sowing was similar to a “stale seedbed" technique as weeds and volunteer wheat could germinate after harvest and were killed subsequently by tillage (Finckh & van Bruggen, 2015). Hence, our results are consistent with a German study in which the weed density was overall lower among different tillage intensities if stubble tillage was applied after wheat than without stubble tillage (Gruber et al., 2012). Furthermore, long-term organic management with frequent and deep soil tillage before start of the experiments may have resulted in weed seed banks whose seeds primarily require light for germination (Buhler et al., 1997). Direct drilling of cover crops after wheat via shallow undercutting could have minimized the weed seed exposure to light probably maintaining weed seed dormancies. Thus, cropping of a weedy fallow after wheat due to failures of the LM is not an option for organic farming systems but should be mended by the use of cover crops. However, the weed suppressive potential of succesful LM has been clearly demonstrated (den Hollander et al., 2007; Bergkvist et al., 2010; Campiglia et al., 2014).

With respect to the complex treatment combinations in this study, interactions among single treatments generally occur. For example under monoculture, no-tillage resulted in highest numbers of *C. album* seeds compared with chisel and mouldboard ploughing while seed densities were similar among tillage systems in a three year crop rotation (Cardina et al., 2002). This implies that low weed seed banks can be maintained if management factors that increase (e.g. RT here) as well as decrease (e.g. cover crops and compost here) weed infestations are combined. This may explain why weed seed bank densities under RT in combination with cover crops and compost were not significantly different from CT treatments.

3.5.5 Future Management Implications

A recent meta-analysis underlined that no-tillage practices should be accompanied by crop rotation and residue retention to maintain crop yields compared with conventional tillage systems (Pittelkow et al., 2015). Our results clearly support this in relation to weed seed banks, i.e. RT with dead mulch application to potatoes only resulted in similar weed seed banks compared to CT if cover crops and compost were used in addition in a wheat-potato cropping sequence. Thus, after two years differential tillage we found no evidence for increased pressure of annual weeds in well balanced RT systems. Although simultaneous sowing of legume LM and winter wheat proved inappropriate for the local conditions here and thus, failed to maintain weed seed banks as observed for cover crops, their great potential for weed suppression should be further considered. This is crucial as the total weed seed bank was particularly increased by winter wheat associated weeds that were not controlled by cover crops. For example, winter wheat sown in existing LM swards via strip-tillage could be an alternative which deserves future attention. Important aspects here are earlier suppression of pre-crops as well as different widths of tillage strips at wheat sowing to balance inter crop as well as crop-weed competition.

4 Oilseed Radish/Black Oat Subsidiary Crops Can Help Regulate Plant-parasitic Nematodes under non-inversion Tillage in an Organic Wheat-Potato Rotation

4.1 Abstract

Soil conservation is one of the major challenges for agriculture in the 21st century. For this reason, non-inversion tillage systems including subsidiary crops have become popular over the last three decades in Europe. However, the adoption of new agricultural practices may change the diversity and abundance of certain pests and diseases. For example, plant-parasitic nematodes which are major threats towards cultivated plants may be promoted if good hosts, such as certain subsidiary crops and weeds, occur more frequently. The indigenous plant-parasitic nematode fauna under organic farming systems is already adapted to diverse crop rotations and usually dominated by broad host range nematodes. These may be further enhanced in organic farming systems if non-inversion tillage is introduced which generally increases the abundance and biomass of certain weeds. We evaluated the early effects of non-inversion tillage and subsidiary crops in an organic wheat-potato rotation on plant-parasitic nematodes in two field experiments in two successive years. The total densities of plant-parasitic nematodes increased from an initial 1260 nematodes 100 ml soil-1 at experimental start to 1850 and 1700 nematodes 100 ml soil-1 after wheat under non-inversion and conventional tillage, respectively. Plant-parasitic nematode densities then decreased on average to 1100 and 560 nematodes 100 ml soil-1 after subsidiary crops and potatoes, respectively. Parasitic nematode densities tended to be higher under non-inversion than conventional tillage except where oilseed radish and black oats had been used as cover crops no differences between tillage treatments occurred. In the second experiment, about 1700 free-living nematodes ml soil-1 were found under conventional tillage without mulch while under reduced tillage with mulch their numbers were significantly higher at 3100 nematodes ml soil-1. We conclude that an appropriate choice of subsidiary crops can be an important management factor for the long term sustainability of non-inversion tillage systems.

Keywords: conservation agriculture, crop rotation, nematode dynamic, compost.

4.2 Introduction

The three principles of conservation agriculture are defined as permanent soil cover, crop rotation and minimum tillage (Hobbs, 2007). Within this concept, permanent soil cover can be achieved by subsidiary crops, i.e. cover crops and living mulches used for their ecosystem services. If done correctly, conservation agriculture will provide the nutrients required for the subsequent cash crop and at the same time control pests and diseases. Although no-tillage, minimum tillage, and reduced tillage systems are increasingly promoted, organic farming in Europe is mostly based on intensive soil tillage. Main reasons for conventional tillage in organic farming are concerns regarding yield losses due to reduced nutrient mineralization in non-inversion tillage systems as well as excessive weed infestations (Peigné et al., 2007; Carr et al., 2013).

On the positive side, conservation agriculture increases soil organic matter, microbial activity and soil biodiversity, which in the long term might lead to soil suppressiveness (van Bruggen & Semenov, 2015). Among others, free-living nematodes are especially stimulated providing a range of ecosystem services such as nutrient mineralization and disease control (Barker & Koenning, 1998; Briar et al., 2007; Ferris, 2010). Contrary to free-living nematodes, the role of plant-parasitic nematodes in conservation agriculture is still unclear. So far it can only be speculated that they might become more important, especially if good hosts, such as certain subsidiary crops and weeds, occur more frequently within rotations.

Both, nutrient deficiency and weeds affect plant-parasitic nematodes. While nutrient-deficient plants might be more susceptible to nematode attack, weeds within a non-host crop may serve as alternative hosts allowing nematode reproduction. Similarly, subsidiary crops meant to provide continuous soil cover can serve as a food source for plant-parasitic nematodes in times when otherwise no host plants would be grown (Hallmann & Kiewnick, 2015; Thomas et al., 2005). Subsidiary crops and weeds especially promote plant-parasitic nematodes with a broad host spectrum, such as *Meloidogyne, Pratylenchus* and several ectoparasitic species (Thomas, 1978; Thomas et al., 2005) that can build up high population densities in otherwise apparently sustainable systems (Barker & Koenning, 1998).

To avoid build-up of plant-parasitic nematodes, certain non-host subsidiary crops might also be used in conservation agriculture for nematode control. For example, certain cultivars of *Raphanus sativus* and *Sinapis alba* are resistant to *Heterodera schachtii*. *Crotalaria*, and *Tagetes* spp., have been shown to suppress *Meloidogyne* spp. and *Pratylenchus* spp. (Barker & Koenning, 1998; Hallmann & Kiewnick, 2015; Hirling, 1977). Furthermore, black oat *(Avena* *strigosa)* is a non-host for *Pratylenchus penetrans* and *Meloidogyne hapla* (Visser & Molendijk, 2015).

Besides subsidiary crops, conservation agriculture often employs compost and organic mulches for nutrient supply and enhanced soil health (Watson et al., 2002). However under temperate climatic conditions, organic amendments do not lead to a general suppressiveness of plant-parasitic nematodes (McSorley, 2011). In cases where nematode suppression was observed composts had often been applied in unpractically high dosages (McSorley & Gallaher, 1996) or contained toxic rates of nitrate or ammonia (McSorley, 2011). Nonetheless, crop yields generally increased after organic amendments despite of limited nematode control (McSorley, 2011).

Within this study, we evaluated the effects of 1) a standard organic crop rotation (2 years grass-clover, wheat, and potato), 2) subsidiary crops following wheat, and 3) minimum tillage on the dynamics of plant-parasitic nematodes. We hypothesized that under minimum tillage leguminous subsidiary crops and weeds will increase plant-parasitic nematode densities while opposite effects are expected from oilseed radish/black oat as subsidiary crops.

Specific objectives of this study were to evaluate (i) the spectrum of plant-parasitic nematodes after two years of grass-clover and subsequently during the wheat-potato cropping sequence, (ii) the effect of non-inversion tillage and organic mulch on plant-parasitic and free-living nematodes, and (iii) the role of subsidiary crops and weeds on plant-parasitic nematode dynamics.

4.3 Materials and Methods

The experiments were conducted at the organically managed experimental farm of the University of Kassel near Witzenhausen, Germany (51°22'N, 9°54'E) and were established in successive years, i.e. experiment 1 from 2010 to 2014 and experiment 2 from 2011 to 2015. The soil was a Typic Hapludalf with 13% clay, 84% silt, and 3% sand with 2% organic matter and a pH of 6.0. Throughout the experimental period, the mean temperature was 9.8°C and the annual rainfall 636 mm.

Both experiments were designed as randomized complete blocks with split-plot arrangements with three factors (two tillage systems (main factor as split plot), four subsidiary crops, and two fertilization regimes) and four replicates, i.e. 64 plots in total. Each experiment started with two years of regularly mulched grass-clover as pre-crop and was followed by winter wheat cv. Achat. The wheat was either undersown with subterranean clover or white clover that continued to grow after the wheat was harvested or the wheat was followed by a legume or non-legume subsidiary crop. The following spring, all plots were planted with potato cv. Marabel. Factor I (split) was either 1) one and two times chisel ploughing at about 10 cm depth followed by moldboard ploughing at about 25 cm soil depth (hereafter called CT) before wheat and potato, respectively or 2) two to three times chisel ploughing at about 10 cm soil depth (hereafter called RT) prior to wheat and at about 15 cm soil depth prior to potato. Furthermore, potatoes in RT received an 8-10 cm mulch layer of a winter pea-rye (2014) or triticale-vetch mixture (2015) of about 12 and 26 t DM ha^{-1}, respectively. Factor II was: (1) winter wheat undersown with subterranean clover (*Trifolium subterraneum* cv. Dalkeith), (2) winter wheat undersown with white clover (*T. repens* cv. Huia), (3) wheat followed by a legume subsidiary crop (*Vicia sativa* cv. Berninova) and (4) wheat followed by a non-legume subsidiary crop (*Raphanus sativus* var. *oleiformis* cv. Kompass/*Avena strigosa* cv. Pratex mixed 1:4). Due to complete failure of both undersown clover species in both experimental years, living mulches were considered as green fallow. This reduced the number of levels for subsidiary crops to one in wheat and to three for the time thereafter. Factor III was either 1) application of 5 and 10 t ha^{-1} DM of a yard waste compost to wheat and potato, respectively, applied manually (YWC); and 2) mineral fertilization consisting of potassium (K_2SO_4) and phosphorus (rock phosphate) approximately matching the potassium and phosphorus concentration of the composts that were applied to potatoes in 2014 and 2015 (Table 3.1). Total nitrogen in the compost was below 2% and the C/N ratio ranged from 13-25 (Table 3.1). The pH and electrical conductivity in composts ranged from 6.4 to 8.0 and 500-1000 µS cm^{-1}, respectively (Table 4.1).

Table 4.1 (Expansion of Table 2.2) Chemical characteristics, including dry matter (DM), bulk density, pH, electrical conductivity (EC), potassium (K), phosphorous (P), total nitrogen (N), carbon (C), and C/N ratio of yard waste composts (≤ 20 mm sieved) from municipal trees and shrubbery from the composting plant near Dransfeld (three-month-old) used in 2012 and 2013 before wheat, and from the composting plant near Hannover (Aha, nine-month-old) used before potatoes in 2014 and 2015.

Year	DM (%)	Bulk density (g L^{-1})	pH	EC (µS cm^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Total N (%)	Total C (%)	C/N ratio
2012	85	389	7.5	498	3104	541	1.8	29.0	16.0
2013	81	282	6.4	778	NA	807	1.5	37.4	25.5
2014	75	604	7.3	915	5276	547	1.3	20.8	16.2
2015	60	731	8.1	1011	4858	616	1.3	16.9	13.0

4.3.1 Nematode Sampling and Assessments

Soil samples for nematode extractions were collected four times throughout each experiment: (1) in August of year 2 from the clover grass just before it was terminated, (2) in August of year 3 after wheat, (3) in March of year 4 prior to termination of subsidiary crops, and (4) in September/October of year 4 after potato. Soil samples were collected from the top 25 cm soil using an auger with 2.4 cm diameter. To maintain a representative sample compensating for the heterogeneous distribution of plant-parasitic nematodes within the soil, a total of 20 cores per experimental plot were taken always from the same 4 m² sampling area of each plot. The soil was collected in a bucket, thoroughly mixed and an aliquot of 500 ml soil was filled in plastic bags, labeled and shipped within one week to the Julius Kühn-Institut in Münster, Germany (JKI). At JKI, soil samples were stored at 6°C until evaluation.

Aboveground crop and weed biomass were determined at wheat flowering (BBCH 65) and at fruit development of potatoes (BBCH 75). Subsidiary crops and fallow plots were assessed for soil cover 10 weeks after subsidiary crop sowing. In addition, weeds and subsidiary crops were separated and quantified in the subsidiary crop plots. Biomass samples were oven dried for 24 h at 105°C. Weeds in the fallow and subsidiary crops were similar, mainly consisting of grasses (*Lolium perenne,* volunteer wheat, and *Poa annua*) with similar correlations of soil cover and biomass. Therefore, weed biomass in the fallow treatment was estimated from weed soil cover using the formula:

$$Weed\ biomass\ (fallow) = weed\ soil\ cover\ (fallow) x \frac{weed\ biomass\ (subsidiary\ crops)}{weed\ soil\ cover\ (subsidiary\ crops)}$$

4.3.2 Nematode Evaluation

Soil samples were passed through a 1 cm sieve to remove root debris and stones, thoroughly mixed, and 250 ml aliquots were taken for nematode extraction following the centrifugal flotation method as described in Hooper et al. (2005) using $MgSO_4$ at 1.15 specific density. Nematodes collected after the final centrifugation step on a 20 µm sieve were transferred into a measuring cylinder and concentrated in 10 ml tap water. The suspension was thoroughly mixed by agitating with air and 1 ml was transferred into a nematode counting slide. Plant-parasitic nematodes were identified and counted at genus level using a Leitz Labovert FS (Wetzlar, Germany) inverse microscope at 63x magnification. Free-living nematodes were counted in extracts from soil after potatoes in the second experiment but not classified into functional groups. For plant-parasitic nematode species identification, a composite sample out of all plots was prepared for each experimental site and sampling date. Nematodes were killed with gentile heat, fixed in a triethanolamine formalin (TAF) solution containing 7 ml formalin (40% formaldehyde), 2 ml triethanolamine and 91 ml distilled water (Courtney et al., 1955). Fixed nematode specimen were then processed to anhydrous glycerol over a period of 12 days using the slow evaporation technique at 39 ± 1°C (Hooper et al., 2005). Species identification was done after transferring female nematodes into anhydrous glycerol on permanent slide mounts following the method described by Hooper *et al.* (2005). Specimen were examined under a Leitz Diaplan compound microscope equipped with differential interference contrast at 630-1000x magnification.

4.3.3 Statistical Analysis and Data Processing

The statistical analysis was performed with R version 3.2.2 (R Core Team, 2013). Total and individual species densities as well as crop and weed biomass were pooled over both field experiments for statistical analysis. Likewise, green fallow plots with identical treatment combinations (with and without compost) were pooled per split to achieve balanced designs resulting in a total number of plots of 48. To improve variance homogeneity, all data were ln (x + 1) - transformed prior to analysis of variance (ANOVA). The ANOVA that accounted for the split-plot arrangement, where the first main factor (split) is tillage, the second main factor is subsidiary crops, and the third is compost, with experiment and replicates as co-variates was performed on crop and weed biomass, the total density of nematodes, and the density of each nematode genus after subsidiary crops and potatoes. The same ANOVA design was used for data after wheat but with exclusion of the subsidiary crops which were sown subsequently and therefore could not have an impact on plant-parasitic nematode composition. Protected Fisher-LSD tests ($P< 0.05$) were used for multiple comparisons of treatments using the R-package *agricolae* (Mendiburu, 2010).

To achieve requirements for multivariate data analysis, nematode data observed at each date were ln (x + 1) - transformed. To assess crop effects on nematode dynamics, final minus initial population densities (pf-pi values) of transformed data were calculated for wheat, subsidiary crops including green fallow, and potato. A redundancy analysis (RDA) using the R-package *vegan* (Oksanen et al., 2015) was performed after averaging all crop-tillage combinations per replicate. These combinations were used as constraining and experimental years/fields as conditional variables in the RDA following instructions of Dormann and Kühn (2009). Significances of the applied model, factors, and axes were analysed via permutation test with 999 permutations.

A three-factorial ANOVA accounting for the split-plot arrangement with replicates as covariates was performed to analyse tillage, subsidiary crop, and compost effects on free-living nematodes after potatoes in the second experiment (free-living nematodes were not counted in the first experiment).

4.4 Results

Both field experiments were run successfully and results were similar, although climatic conditions during the potato season were wet and cool in the first and dry and warm in the second experiment. Therefore, data of both experiments were pooled except for the redundancy analysis. Besides the living mulches that failed in both experiments, solely the vetch in the second experiment was sparse due to damage by common voles (*Microtus arvalis*).

The aboveground biomass of all crop species varied considerably ranging from 0.2 t ha^{-1} (vetch) to 6.5 t ha^{-1} (wheat) (Table 4.2).

Table 4.2 Ln (x + 1) - transformed aboveground biomass of main and subsidiary crops including the green fallow and their corresponding weeds (untransformed data in brackets) in t ha^{-1} under conventional (CT) and non-inversion (RT) tillage (df = 7) across the crop rotation. Means over both experiments are shown.

	Wheat	Weeds	Potato	Weeds	Fallow	Weeds[1]	Vetch	Weeds	OR/BO[2]	Weeds
CT	1.943 (7.38)	0.229 (0.42)	0.712 (1.08)	0.052 (0.06)	-	0.334 (0.41)	0.183 (0.21)	0.23 (0.29)	0.342 (0.43)	0.16 (0.18)
RT	1.729 (5.83)	0.615 (0.99)	0.784 (1.23)	0.081 (0.09)	-	0.445 (0.58)	0.195 (0.24)	0.293 (0.35)	0.253 (0.37)	0.23 (0.27)
MSE[3]	0.008	0.068	0.047	0.003		0.088	0.012	0.027	0.002	0.015
LSD[4]	0.043	0.126	0.09	0.024		0.175	0.091	0.138	0.04	0.103

[1] estimated from soil cover values and biomass of weeds under Vetch and OR/BO
[2] oilseed radish/ black oat mixed 1:4
[3] mean square errors are results from ANOVA after ln (x +1) - transformation
[4] Least significant difference of ln (x + 1) transformed means at $P < 0.05$

For all crops, weed biomass was highest under non-inversion tillage, in particular under wheat. In contrast, wheat and oilseed radish/black oat biomasses were highest under conventional tillage while potato and vetch biomasses were higher under non-inversion tillage.

Wheat yields under conventional tillage were 6.6 t ha^{-1} and 5.5 t ha^{-1} in experiment 1 and 2, respectively. Yields were 20% lower under non-inversion tillage. Potato yields under conventional tillage were 32.4 t ha^{-1} and 25 t ha^{-1} in experiment 1 and 2, respectively. Under non-inversion tillage, yields were 12% lower in experiment 1 but 20% higher in experiment 2 (OSCAR, 2016). These varying results for potatoes were caused by different weather conditions, i.e. a wet and relatively cool late spring 2014 overall favouring potato growth in experiment 1, compared to an extremely dry and warm spring in 2015 that especially suppressed potato growth in the unmulched ploughed plots in experiment 2. As indicated above, the undersown clover species in wheat failed in both experiments and thus, were referred to as green fallow that consisted of annual and perennial grass weeds as well as volunteer wheat. Furthermore, the spring vetch largely failed in experiment 2 because of common voles that multiplied to high densities due to mild weather conditions. Compost had no effects on the plant-parasitic nematode dynamics and therefore is not shown in detail.

4.4.1 Initial Nematode Densities

Nematode genera frequently found at the first sampling after grass-clover were *Helicotylenchus* (408 individuals 100 ml soil^{-1}, averaged over both experiments), *Paratylenchus* (446 individuals 100 ml soil^{-1}), *Pratylenchus* (250 individuals 100 ml soil^{-1}), and *Tylenchorhynchus* (148 individuals 100 ml soil^{-1}). *Criconematidae* and *Meloidogyne* occurred at densities below 20 nematodes 100 ml soil^{-1} and were summarized as “*other*” in the detailed analysis.

Regarding the spectrum of plant-parasitic nematodes, the most prevalent species were *Pratylenchus neglectus, P. flakkensis,* and *P. penetrans* that occurred in both years. Other commonly detected species were *Helicotylenchus vulgaris*, *Paratylenchus projectus,* and *Tylenchorhynchus dubius.* Several additional species occurred at low densities or only in one experiment, such as *Helicotylenchus digonicus, Meloidogyne naasi*, *Paratylenchus bukowiniensis, Rotylenchulus borealis*, and some members of the family *Criconematidae* (for details see Table 5.2).

4.4.2 Plant-parasitic Nematode Dynamics

On average, a total of 1250 plant-parasitic nematodes 100 ml soil^{-1} were detected in the grass-clover pre-crop immediately before incorporation (Figure 4.1). Main effects are shown as no significant interactions were observed ($P > 0.05$). Averaged over both experiments nematode densities increased to 1771 nematodes 100 ml soil^{-1} in the wheat following grass-clover. The slightly higher densities under non-inversion than conventional tillage were statistically not significant ($F_{1,7} = 0.915$, Figure 4.1 A).

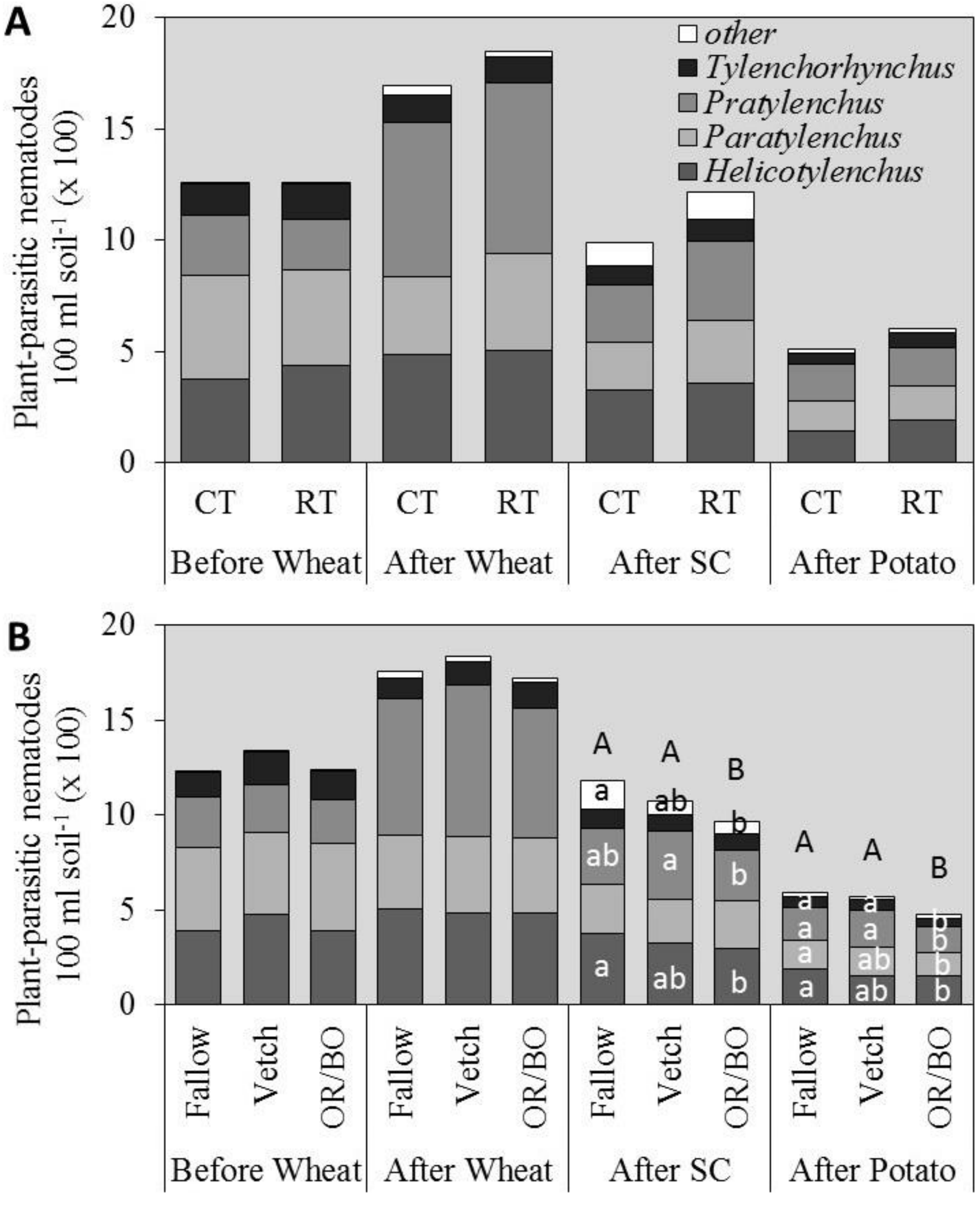

Figure 4.1 Nematode dynamics over time (before wheat, after wheat, after subsidiary crops (SC), and after potatoes) for the four most common genera (*Helicotylenchus, Paratylenchus, Pratylenchus*, and *Tylenchorhynchus*) and others (*Criconematidae, Meloidogyne*) affected by (A) conventional (CT) and non-inversion (RT) tillage and (B) spring vetch (Vetch) and oilseed radish/ black oat (OR/BO) cover crops sown after wheat compared to a green fallow (Fallow). Data are averaged across both experiments. Different capital and lower letters indicate statistically different treatments for total nematode densities and single nematode genera, respectively, after respective crops ($P<0.05$, protected LSD-test).

Oilseed Radish/Black Oat Regulates Plant-parasitic Nematodes

Total densities of plant-parasitic nematodes declined after the subsidiary crops ($F_{1,7}$ = 2.085) and after the potato crops ($F_{1,7}$ = 2.025). The differences between both tillage treatments remained albeit not statistically significant. However, plant-parasitic nematode densities were similar in both tillage treatments with oilseed radish/black oat subsidiary crops (about 967 and 473 nematodes 100 ml soil^{-1} after subsidiary crops and potatoes, respectively, data not shown). The oilseed radish/black oat subsidiary crop mixture (OR) reduced the total density of plant-parasitic nematodes significantly more than the green fallow and the vetch ($F_{2,70}$ = 3.967, Figure 4.1 B). This effect persisted until after potatoes ($F_{2,70}$ = 5.769, Figure 4.1 B).

Besides those general trends some more specific differences were observed at the genus level. Populations of *Helicotylenchus* and *Pratylenchus* increased under wheat (Figure 4.1) resulting in densities of 500 and 730 nematodes 100 ml soil^{-1}, respectively, compared to initial densities of 408 and 250 individuals 100 ml soil^{-1}, respectively. In the following subsidiary crops and potatoes, densities of both genera decreased to 300 and 170 nematodes 100 ml soil^{-1}, respectively. In contrast, densities of *Tylenchorhynchus* and *Paratylenchus* continuously decreased from an initial density of 148 and 446 nematodes 100 ml soil^{-1} before wheat to 50 and 150 nematodes 100 ml soil^{-1} after potatoes, respectively (Figure 4.1). Abundance of *Meloidogyne* was low at the beginning of the experiment (< 20 individuals 100 ml soil^{-1}), increased under wheat and subsidiary crops reaching a peak of 145 nematodes 100 ml soil^{-1} in the green fallow, and finally dropped under potato to densities comparable with the initial densities (Figure 4.1, “*other*”).

4.4.3 Effects of Tillage and Subsidiary Crops

Overall, tillage had no statistically significant effects on single nematode genera. While the four most dominant plant-parasitic nematode genera were uniformly distributed before wheat (Figure 4.1), their densities were always slightly higher under non-inversion than inversion tillage after wheat, subsidiary crops, and potatoes (Figure 4.1A). For example, *Paratylenchus* occurred at 430 individuals 100 ml soil^{-1} before and after wheat under non-inversion tillage, compared to 465 before to 353 individuals 100 ml soil^{-1} after wheat under conventional tillage ($F_{1,7}$ = 2.219). Subsidiary crops significantly affected *Helicotylenchus* ($F_{2,70}$ = 4.319), *Pratylenchus* ($F_{2,70}$ = 5.466), and other plant parasitic nematodes, predominantly *Meloidogyne naasi* ($F_{2,70}$ = 5.067, Figure 4.1B). Accordingly, the highest total densities of plant-parasitic nematodes occurred in the vetch and green fallow treatments again carrying through until after potatoes. Here, the densities of *Helicotylenchus* ($F_{2,70}$ = 3.253) and *Paratylenchus* ($F_{2,70}$ = 3.614) were higher in the green fallow than in the oilseed radish/black oat treatment, while the densities of *Pratylenchus* ($F_{2,70} = 5.324$) and *Tylenchorhynchus* ($F_{2,70} = 6.536$) were significantly higher in the fallow and vetch than in the oilseed radish/black oat treatment.

The models of the redundancy analysis of nematode dynamics and factors for each experiment (Figure 4.2) were significant at $P<0.01$. Constraining (crop, tillage) and conditional (replicates) variables explained 68.7% and 2.5% of the variance in the first experiment, respectively, and 65.7% and 3.8% of the variance in the second experiment, respectively. Furthermore, the first and second RDA axes were significant at $P<0.01$ in both experiments. The factors tillage and crop (wheat, subsidiary crops, and potato) interacted strongly and are therefore shown separately (Figure 4.2).

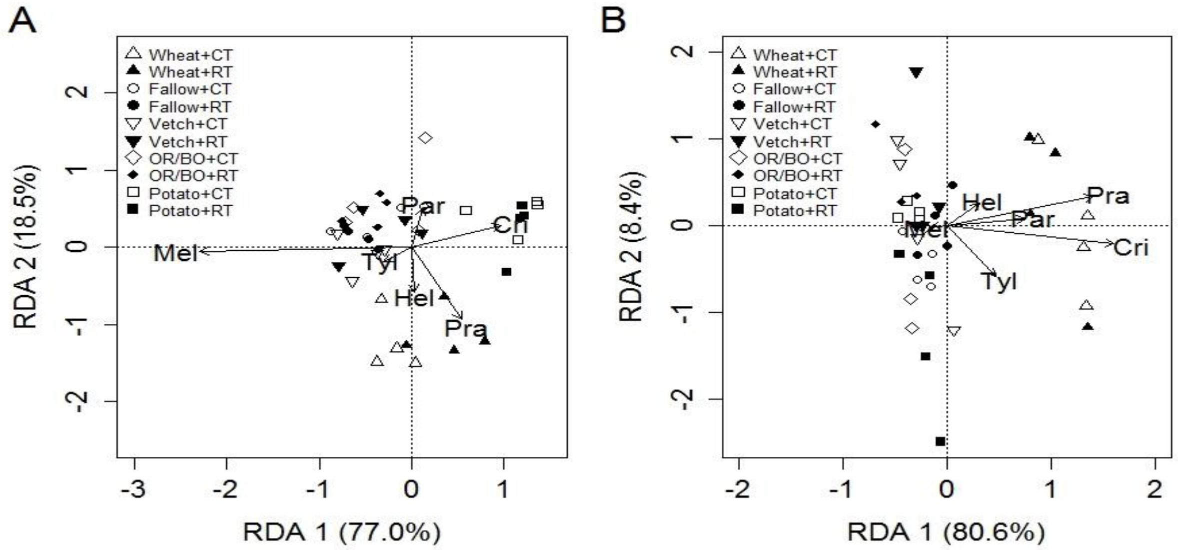

Figure 4.2 Redundancy analysis biplots for (A) experiment 1 and (B) experiment 2 of plant-parasitic nematode species dynamics (ln(Pf+1)-ln(Pi+1)) averaged across compost and replicates. Responses to the interactions of main crops (wheat, triangles point up; potato, squares) and subsidiary crops (green fallow, cirles; vetch, triangles point down; oilseed radish/black oat (OR/BO), diamonds) with tillage (CT, conventional tillage, unfilled symbols; RT, non-inversion tillage, filled sysmbols) are shown including replicates as co-variables. Axis labels indicate percentage of explained variance. Arrows are showing directions of increasing nematode species abundance. Abbreviations: Cri = *Criconematidae*, Hel = *Helicotylenchus*, Mel = *Meloidogyne*, Par = *Paratylenchus*, Pra = *Pratylenchus*, Tyl = *Tylenchorhynchus*.

In the first experiment (Figure 4.2A), *Helicotylenchus* and *Pratylenchus* were more associated with wheat and *Pratylenchus* was strongly correlated to RT, which is in line with the dynamics shown in Figure 4.1A. Subsidiary crops were negatively related to the first axis (RDA 1) and thus, negatively correlated with *Pratylenchus* and *Criconematidae*. *Meloidogyne* was also negatively correlated with the first axis, indicating positive correlations with subsidiary crops.

Potatoes were clustered on the positive site of RDA 1 indicating high correlations with the family of *Criconematidae* in experiment 1 (Figure 4.2A) but not in experiment 2 (Figure 4.2B). In experiment 2 (Figure 4.2B), the first axis was positively correlated with all plant-parasitic nematode genera and wheat while it was negatively correlated with subsidiary crops and potatoes, indicating highest densities of all genera after wheat followed by a decline in the subsequent crops.

4.4.4 Free-living Nematodes

After potato harvest in experiment 2, free-living nematodes were significantly lower under conventional tillage (1681 nematodes 100 ml soil^{-1}) compared with non-inversion tillage (3115 nematodes 100 ml soil^{-1}) ($F_{1,3} = 36.5$, Figure 4.3). There were no significant effects of subsidiary crops and compost application, although densities of free-living nematodes were lower in the oilseed radish/ black oat mixture (2140 nematodes 100 ml soil^{-1}) than in the green fallow (2500) or vetch (2450). Interactions between main effects were not significant.

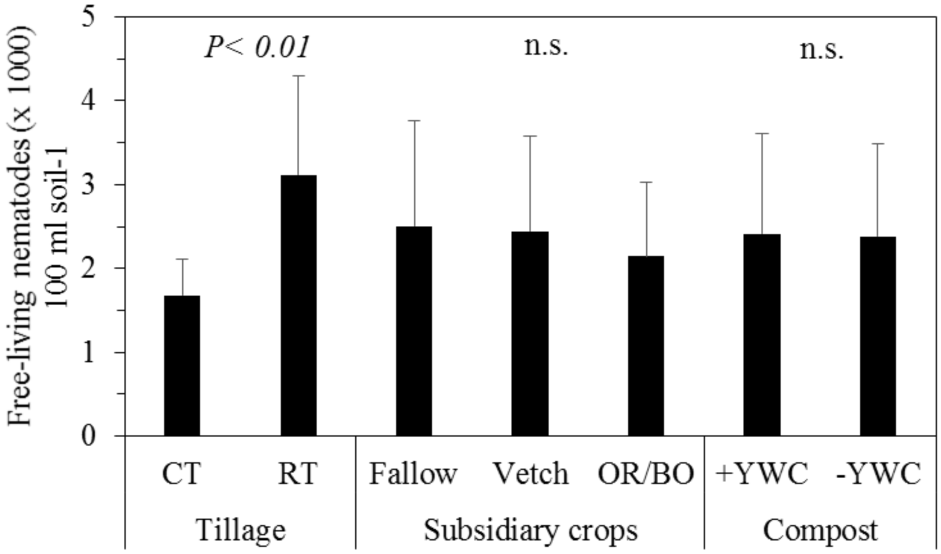

Figure 4.3 Densities of free-living nematodes (untransformed means + SDs) after harvest of potatoes in the second experiment as affected by conventional (CT) and non-inversion (RT) tillage, subsidiary crops (summer vetch, oilseed radish/ black oat (OR/BO), and green fallow), and yard waste compost (+YWC, with; -YWC, without); P-values and not significant (n.s.) factors are results of the 3-factorial ANOVA with ln (x +1) – transformed data including replicates as conditional variables; df are 3 and 30 for tillage and subsidiary crops and compost, respectively.

4.5 Discussion

The most dominant plant-parasitic nematode genera at the experimental site were *Helicotylenhus, Paratylenchus, Pratylenchus,* and *Tylenchorhynchus*. Nematode dynamics were affected by the main crops, types of subsidiary crops versus weeds and tillage intensity, but not by compost and mulch application. The highest population densities of *Paratylenchus* and *Tylenchorhynchus* were recorded at the first sampling before grass-clover was incorporated while the highest densities of *Pratylenchus* and *Helicotylenchus* were reached after winter wheat.

The extraction technique, i.e. soil (centrifugal flotation using $MgSO_4$) versus plant (e.g. Oostenbrink elutriation) extraction, does not affect the relative proportion of plant-parasitic nematodes between the applied treatment levels. Earlier results from Germany confirm this (Hirling, 1977) as for *Pratylenchus* the relative differences between applied treatment levels were similar between a root (modified Baermann funnel method) and soil extraction (Löcher technique) method. This was despite of greater *Pratylenchus* densities obtained from the root extraction technique. Although the organic fraction was not investigated in this study for nematode populations, consistent results were obtained from the applied method throughout both experiments. The precision and usefulness of the centrifugal flotation technique for nematode evaluations at field scale is therefore clearly demonstrated, even though population densities of endo- and semi-endoparasitic species, such as *Pratylenchus* and *Helicotylenchus* may be underestimated. Furthermore, this technique allows not only to extract plant-parasitic nematodes in the absence of plants in the field but also free-living nematodes besides plant-parasitic species.

4.5.1 Effects of Crop Rotation

The pre-crop grass-clover is a good host for several plant-parasitic nematodes and thus, explains the high initial densities of *Helicotylenchus, Paratylenchus, Pratylenchus* and *Tylenchorhynchus* (Sharma, 1971; Wouts & Yeates, 1994; Knight et al., 1997). The subsequently grown winter wheat is a good host for the endoparasitic root-lesion nematode *Pratylenchus* as well as the semi-endoparasitic spiral nematode *Helicotylenchus*, as confirmed by Esmenjaud et al. (1990), Florini & Loria (1990), and O'Bannon & Inserra (1989). As a result, population densities of *Pratylenchus* and *Helicotylenchus* increased in wheat. In contrast, population densities of *Paratylenchus* and *Tylenchorhynchus* decreased indicating that wheat is a less preferable host.

Oilseed Radish/Black Oat Regulates Plant-parasitic Nematodes

Subsidiary crops can in principle serve as a food source for plant-parasitic nematodes providing a "green bridge" between two main crops, e.g. between wheat and potato (Gruver et al., 2010). Under such conditions, the often reported decline of nematode populations over winter (Hirling, 1977) may be reduced or even turned into an increase. However, in our studies, plant-parasitic nematode densities declined under all subsidiary crops tested. Probable reasons could be a poor host status or weak establishment of those crops and overall low nematode activity over winter. For example, plant biomass for subsidiary crops was 10-30 fold lower than the biomass of the previous wheat thus indicating poor conditions for nematode reproduction.

Among the subsidiary crops, the oilseed radish/black oat mixture tended to reduce plant-parasitic nematodes more than the vetch or the green fallow. Both, oilseed radish and black oat are poor hosts or non-hosts for *Pratylenchus,* the dominating genus after winter wheat (Hirling, 1977; Visser & Molendijk, 2015).

The higher biomass of the oilseed radish/black oat mixture compared to vetch or green fallow probably contributed to the greater reduction of *Pratylenchus* in this treatment. Main *Pratylenchus* species in our study were *P. neglectus* and to a lower extent *P. penetrans*, both known to poorly propagate on oilseed radish. As shown by Hirling (1977), *P. neglectus* was reduced by 45% when using an oilseed radish subsidiary crop after wheat compared with the green fallow control (with weeds and winter wheat volunteers). Likewise, oilseed radish grown as a biofumigation crop resulted in comparable reductions of *P. penetrans* (Korthals et al., 2010). In this study, the remarkable effect of the oilseed radish also remained after potatoes confirming earlier results in Germany, where *P. neglectus* was still reduced by 25% in the oilseed radish treatment compared to the green fallow control one year after growing the cover crop (Hirling, 1977). Therefore, *P. neglectus* densities declined after oilseed radish followed by a delayed population build-up after subsequent maize and wheat.

Regarding potatoes, plant-parasitic nematodes generally declined from the beginning to the end of the cropping season. It appears that potatoes were poor hosts for those plant-parasitic nematodes occurring in the field experiment. This is somewhat surprising as potato is known to be a good host for *P. penetrans*. Threshold levels of *P. penetrans* for potatoes in loamy soil are reported to be 70-200 individuals 100 ml soil^{-1} (Hallmann & Kiewnick, 2015); however, in our study even 300 *Pratylenchus* 100 ml soil^{-1} before potatoes did not cause any yield loss (data not shown). This may in part be explained by the dominance of *P. neglectus* compared with *P. penetrans* and the often uncertain host status of potatoes for *P. neglectus*. According to the breeder (Europlant), nothing is known about the resistance of the cultivar "Marabel" used in our experiments to root-lesion nematodes. Whether the damage by *Pratylenchus* spp. was affected by the different environmental conditions in both experimental years, can only be speculated.

4.5.2 Effects of Tillage and Weeds

Non-inversion tillage was accompanied by a continuously higher weed pressure (Table 4.2) and resulted in consistently, but not significantly, higher plant-parasitic nematode densities throughout the rotation compared to conventional tillage. Under these conditions, *Pratylenchus* benefited most among the locally observed plant-parasitic nematode community (Figure 4.1A, Figure 4.2A). Overall, the effect of non-inversion tillage on plant-parasitic nematodes is discussed controversially. Non-inversion tillage either increased (Thomas, 1978; Okada & Harada, 2007), decreased (Alby et al., 1983; Minton, 1986; Govaerts et al., 2006) or did not substantially affect (Gallaher et al., 1988; McSorley & Gallaher, 1993) nematode densities. Effects of tillage on plant-parasitic nematodes are generally related to crop and weed performance. Furthermore, it has to be considered that most of these studies were conducted under conventional management in long-term trials. Hence, we need to consider that early effects of non-inversion tillage on plant-parasitic nematodes under organic management might be different.

In general, fields under non-inversion tillage are more infested with volunteer crops and grass weeds than conventionally tilled fields (Moyer et al., 1994; Nichols et al., 2015). Monocotyledonous plant species are in particular good hosts for *Pratylenchus* and *Paratylenchus* (Sarathchandra et al., 2001; Townshend & Potter, 1976; Vanstone & Russ, 2001b; Wood, 1973). Therefore, in the presented study perennial grass weeds in non-inversion tilled winter wheat could have fostered reproduction of *Pratylenchus* and *Paratylenchus* resulting in overall higher densities of plant-parasitic nematodes under non-inversion than under conventional tillage.

Furthermore, wheat plants under non-inversion tillage were weaker and produced less biomass, most likely caused by nitrogen deficiency due to lower mineralisation rates in less disturbed soils (Donald et al. 2009). Nutrient-deficient plants are in general more susceptible to plant-parasitic nematodes (Melakeberhan et al., 1997) and can result in higher nematode densities as observed here. However, Thompson (1992) and Okada and Harada (2007) observed contrary effects with higher *Pratylenchus* densities in plants that received nitrogen fertilizer compared to no fertilization. Nevertheless, regardless of plant available nitrogen in soil, zero tillage with or without high weed infestation resulted in significantly higher *Pratylenchus* densities compared with conventional tillage (Thompson, 1992; Pankaj et al., 2006).

Besides the above mentioned effects, tillage intensity can also affect the survival strategies of plant-parasitic nematodes in soil. For example, significantly more eggs of the soybean cyst nematode *Heterodera glycines* were obtained from a chisel ploughed compared to a ploughed soil over a two year period (Donald et al., 2009) suggesting higher egg survival rates in chisel ploughed soils. Furthermore, organic matter generally decays faster under conventional than under non-inversion tillage while the latter generally increases stubble and root fragments near the soil surface (Morris et al., 2010). Likewise, more fragments of stubble and roots fragments were noticed under non-inversion than conventional tillage in the present study. These fragments can harbour large densities of plant-parasitic nematodes, such as *Pratylenchus,* which can survive periods of food scarcity and drought in these stubble and root fragments in an anhydrobiotic resting stage (Glazer & Orion, 1983; Talavera & Vanstone, 2001). Altogether, these factors explain the maintenance of higher total plant-parasitic nematode densities under non-inversion than conventional tillage after subsidiary crops and potato in this study.

4.5.3 Free-living nematodes as affected by tillage, subsidiary crops, and compost

In our study, non-inversion tillage with mulch application had the strongest positive effect on free-living nematodes whereas the effect of subsidiary crops and compost was negligible. This appears in contrast to other published results. For example, it is well known that subsidiary crops, such as oilseed radish, can increase bacterivorous nematodes (Gruver et al., 2010; Hallmann & Kiewnick, 2015). Vetch and lupine winter cover crops in a long-term experiment in Brazil resulted in higher microbial biomass than grassy fallow and thus are expected to increase bacteriovorous nematodes (Balota et al., 2014). Similar, densities of free-living nematodes in a wheat-lupine rotation were higher than in a wheat monoculture (Rahman et al., 2007). According to the authors, the higher root dry matter, lower C/N ratio, and different canopy structure of the lupine compared to the wheat explained those differences in free-living nematode densities. The overall very low subsidiary crop biomass in our study is probably the main reason for the lack of effects on the free-living nematode abundance. In this study, the application of 12 and 26 t ha^{-1} mulch to plots under non-inversion tillage in experiment 1 and 2, respectively, might also have concealed any previous soil treatments, such as subsidiary crops and compost application. Overall, free-living nematodes may not be strongly affected by a single application of subsidiary crops while their long-term use may change the abundance of free-living nematodes, such as observed for the microbial biomass in Brazil (Balota et al., 2014).

In contrast to plant-parasitic nematodes, the positive effect of non-inversion tillage on the build-up of free-living nematode populations is desired due to their beneficial effects on the soil food web and nutrient mineralization (Fu et al. 2000, Pankaj et al. 2006). However, nematode trophic groups may react differently to soil organic matter accumulation in such systems. For example, fast growing bacterivorous nematodes dominate previously tilled soils as a result of stimulated bacterial degradation of organic matter. This also explains why inconsistent or lower densities of bacterivorous nematodes are generally found in long-term systems with reduced tillage compared to systems with intensive soil movement (Hendrix et al., 1986; Carter et al., 2009a). If soil disturbance is reduced and organic compounds are more stable, decomposition will be dominated by fungi and thus, fungal feeding nematodes will increase (Briar et al., 2007; Freckman & Caswell, 1985; Neher, 2010). Nevertheless, all trophic groups of free-living nematodes are important contributors to the soil food web and suitable indicators for soil health and sustainability of farming systems (Freckman, 1988; Neher, 2010).

4.6 Conclusion

Our results suggest that crop diversity in a rotation can be a sustainable option to keep plant-parasitic nematodes below the economic threshold level. Rapid increases of plant-parasitic nematodes, such as observed for *Pratylenchus* under wheat, can be tackled if subsequent crops are poor hosts, non-hosts, or even antagonistic. Thus, oilseed radish mixed with black oat used as subsidiary crop can reduce plant-parasitic nematodes, particularly species of the genus *Pratylenchus* and *Helicotylenchus,* and will probably replace fallows if nematode control is desired. In contrast, transition to non-inversion tillage can limit nematode control by non-host crops if weeds are not sufficiently controlled. Although plant-parasitic nematodes did not reduce crop yields under non-inversion tillage in our study, this cannot be generalized for subsequent crops. Cereals potentially harbour high numbers of weeds, particularly in non-inversion tillage systems without adequate weed control, and can contribute to a continuous increase of plant-parasitic nematodes over time. Thus, investigation of all crops in a rotation under non-inversion tillage is needed to evaluate farming systems for their nematode control potential. Free-living nematodes increased under non-inversion tillage in combination with mulch application and may therefore contribute to a higher agricultural sustainability due to their importance for the soil food web.

5 Effect of Tillage, Subsidiary Crops, and Fertilization on Plant-parasitic Nematodes in a Range of Agro-environmental Conditions within Europe

5.1 Abstract

The overall goal in nematode management is to develop sustainable systems where nematode populations are kept under the economic damage threshold. Conservation tillage and subsidiary crops, applied as cover crops and living mulches, generally improve soil health by increasing soil organic matter content and stimulating soil microbial activity. However, more permanent crop and weed cover associated with subsidiary crops and non-inversion tillage, respectively, may benefit plant-parasitic nematodes with broad host spectra such as *Meloidogyne* and *Pratylenchus*. These genera are major constraints to many field crops throughout Europe and there is a need to identify effective and reliable management options that can be applied to avoid excessive infestations. The dynamics of the indigenous fauna of plant-parasitic nematodes were studied in eight coordinated Multi-Environment field Experiments (MEEs) under four agro-environmental conditions in Europe (Continental, Nemoral, Atlantic North and Mediterranean North). The MEEs consisted of a two-year sequence of wheat combined with a living mulch or subsequent cover crops and second main crops maize, potatoes, or tomatoes depending on site. Additionally, the effects of inversion tillage using the plough were compared with various forms of conservation tillage (no-tillage, shallow and deep non-inversion tillage).

Overall, *Helicotylenchus, Paratylenchus, Pratylenchus,* and *Tylenchorhynchus* were the most frequent genera across sites while *Meloidogyne* occurred only in Germany at very low densities. During the wheat-maize sequences in Switzerland, the populations of *Pratylenchus* increased from 63 to 146 nematodes 100 ml soil^{-1} and *Helicotylenchus* from 233 to 632 nematodes 100 ml soil^{-1}. The effects of tillage on plant-parasitic nematodes were generally minor, although no tillage in Italy supported higher densities of *Pratylenchus* (184 nematodes 100 ml soil^{-1}) than inversion tillage (59 nematodes 100 ml soil^{-1}). Furthermore, *Pratylenchus* densities were 160 nematodes 100 ml soil^{-1} when leguminous subsidiary crops were grown, 122 nematodes 100 ml soil^{-1} in the green fallow, and 84 nematodes 100 ml soil^{-1} after growing black oat (*Avena strigosa*) or oilseed radish (*Raphanus sativus*). The differences were greatest in Italy, in a sandy soil with low organic matter. Application of compost or nitrogen fertilizer had no consistent effects on plant-parasitic nematodes. We conclude that crop rotations including specific subsidiary crops are prominent factors affecting the indigenous nematode community, while tillage and fertilizer are of lower importance.

Keywords: Plant-parasitic nematodes; conservation tillage; cover crops; living mulches; fertilizer; compost

5.2 Introduction

Non-inversion and no-tillage systems, permanent soil cover, and diversified crop rotations are the three principles of conservation agriculture for improving soil fertility and soil health (Hobbs et al., 2008). However, permanent soil cover by subsidiary crops, which are included in the rotation for their agro-environmental benefits, such as living mulches (undersown in main crops) or cover crops (growing in between main crops), as well as higher weed infestations, might form a “green bridge” for certain pests or diseases that can accumulate over time.

Obligate plant pests such as plant-parasitic nematodes react to changes in crop rotation and tillage intensity and are therefore good indicators to use when evaluating farming systems (Neher, 1999; Fu et al., 2000; Berkelmans et al., 2003; van Capelle et al., 2012). For example, the generally more diverse crop rotations used in organic farming systems often support a high diversity of plant-parasitic nematodes with a broad host spectrum such as *Meloidogyne, Pratylenchus,* and *Tylenchorhynchus* (van Diepeningen et al., 2006; Hallmann & Kiewnick, 2015). In contrast, conventional management systems with narrower rotations generally cause a reduction in the diversity of plant-parasitic nematode species and host specific taxa such as *Heterodera* and *Globodera* might increase (Briar et al., 2007; Hallmann & Kiewnick, 2015). Furthermore, increasing densities of plant-parasitic nematodes were frequently reported to occur in non-inversion and no-tillage systems with or without subsidiary crops (Govaerts et al., 2007; Okada & Harada, 2007; Carter et al., 2009b; Smiley et al., 2014a). This is especially the case when those subsidiary crops are good hosts for certain plant-parasitic nematodes, such as legumes that are commonly good hosts for root-knot (*Meloidogyne* spp.) and root-lesion (*Pratylenchus* spp.) nematodes (Riggs & Niblack, 1993).

If subsidiary crops are used in non-inversion and no-tillage systems, care needs to be taken that those crops do not build up nematode densities to levels causing economic damage in the main crop. This can be achieved by either resistant cultivars or poor and non-host crops. Several fodder radish (*Raphanus sativus*) cultivars are widely used to control certain species of *Meloidogyne* and *Heterodera schachtii* in temperate climates, while sun hemp (*Crotalaria juncea*) is used to control *Meloidogyne* spp., *Rotylenchus reniformis* and *Helicotylenchus multicinctus* in warmer regions (Hallmann & Kiewnick, 2015). However, if poor or non-host subsidiary crops are grown, sufficient weed control is important as most of the common weeds are good hosts for plant-parasitic nematodes (Viaene et al., 2013; Visser & Molendijk, 2015).

In the long term, non-inversion and no-tillage combined with subsidiary crops should increase the organic matter content in the soil (Tebrügge & Düring, 1999) and thus, the antagonistic potential, which might increase soil suppressiveness towards plant-parasitic nematodes. Although the general mechanisms leading to nematode suppressive soils are still unclear, natural enemies, such as nematode trapping fungi, predatory nematodes, and other non-specific organisms, are likely to increase under such conditions. Furthermore, the mortality rates of plant-parasitic nematodes may be increased throughout the decomposition process of organic matter, for example due to toxic metabolites released by organic matter degrading microbes (Viaene et al., 2013; Stirling, 2014; Hallmann & Kiewnick, 2015). Additional application of compost might enhance these processes due to maintenance of an abundant and active soil microbial community (Pocasangre et al., 2015).

We hypothesized that the adoption of agricultural practices, such as cover cropping as well as non-inversion and no-tillage, could lead to an increase of plant-parasitic nematodes. However, pests and diseases may be diversely affected by agricultural management according to site specific climate, soil conditions, and rotational crops (Strand, 2000). To address such questions, European scientific networks have been established aiming for a holistic examination of cropping system functions (Lechenet et al., 2017) whose “complexity is more than the sum of its parts” (Drinkwater, 2002). With such holistic approaches, which are also addressed in our study, impacts of cropping systems can be generalized while taking into account year and environmental effects as well as site specific system adaptations (Lechenet et al., 2017).

Therefore, the overall objective of this study was to investigate the effect of non-inversion and no-tillage combined with subsidiary crops on the short term population dynamics of plant-parasitic nematodes in coordinated multi environment experiments (hereafter called MEEs) in four agro-environmental zones in Europe ranging from Nemoral (Sweden) to Mediterranean North (Italy). The specific objectives of this study were to evaluate: (i) the initial effects of non-inversion and no-tillage practices compared to inversion tillage on plant parasitic nematodes as affected by pedo-climatic conditions; (ii) the effect of leguminous and non-leguminous subsidiary crops in crop rotations versus green fallow on locally occurring plant parasitic nematodes; and (iii) the effect of nitrogen fertilizer or yard waste compost application on plant-parasitic nematodes.

5.3 Materials and Methods

The MEEs were conducted at four sites within Europe representing the following agro-environmental zones (Jongman et al., 2006): Continental: Agroscope, Tänikon, Switzerland (47°30'N, 8°55'E); Nemoral: Swedish University of Agricultural Science, Uppsala, Sweden (59º49’N, 17º42’E); Atlantic North: Kassel University, Witzenhausen, Germany (51°22'N, 9°54'E), and Mediterranean North: Tuscia University, Viterbo, Italy (42°25'N, 12°05'E). At each experimental site, two MEE’s were established in successive years, MEE 1 from 2012 to 2014, MEE 2 from 2013 to 2015.

The MEEs all started with wheat, i.e. winter wheat (Switzerland, Sweden, Germany) or durum wheat (Italy) in autumn of the first year followed by the summer main crops maize (Sweden, Switzerland), potato (Germany), or tomato (Italy). Wheat was either cultivated as pure crop or undersown with subterranean clover (Switzerland, Italy, and Germany) or white clover (Sweden, Germany). The clovers were intended to continue after the wheat was harvested as cover crop. Where no clovers were present, wheat was followed by a cover crop that was established shortly after the harvest of wheat. A green fallow was used as control. The subsidiary crops were terminated before the second main crop was sown in the following spring. For practical and economic reasons, such as precise tillage and sowing operations, experiments at all sites were arranged as split-blocks (see descriptions below). Site-specific climatic conditions and soil characteristics are summarized in Table 5.1.

Table 5.1 Soil characteristics and climate of the four agro-environmental sites

Site	% clay	% silt	% sand	soil type (USDA)	soil pH	% organic matter	Temp[1] (°C)	Ppt.[1] (mm)
Switzerland	21	35	44	Hapludalf	7.0	2.1	10.0	1,185
Sweden	16	64	20	Inceptisol	5.9	4.4	7.7	562
Germany	13	84	3	Typic Hapludalf	6.0	2.0	9.8	636
Italy	19	21	60	Typic Xerofluvent	6.7	1.2	11.4	729

[1] annual means during the experiment: Temp.: Temperature, Ppt.: Precipitation

5.3.1 Wheat-maize MEEs

The MEEs in Switzerland were conducted at the Agroscope experimental station in Tänikon (537 m a.s.l.). In the years preceding the experiment, the soil was annually ploughed 20 cm deep and the crops were managed conventionally. Forage pea (*Pisum sativum* subsp. *arvense)* was grown prior to the start of the experiments. The two MEEs representing a wheat (*Triticum aestivum* cv. CH Claro)-maize (*Zea mays* cv. LG 30.222) crop sequence were arranged in a strip-split-block design with four replicates. Factor I (main plots) was tillage intensity: 1) inversion tillage by mouldboard ploughing at about 20 cm soil depth (hereafter called CT), 2) non-inversion tillage at about 5 cm soil depth (hereafter called RT) and 3) no-tillage (hereafter called NT), all applied before maize in the second year of each experiment. Factor II (first split) were subsidiary crops either as: 1) undersown subterranean clover (*Trifolium subterraneum* cv. Campeda) in winter wheat and re-sown after wheat harvest (LM), 2) leguminous cover crop (L CC), *Vicia villosa* cv. Hungvillosa, 3) non-leguminous cover crop (NL CC), *Raphanus sativus* cv. Pegletta, and 4) green fallow as control (C). Both L CC and NL CC were sown after wheat harvest. Factor III (second split) was two levels of ammonium nitrate application to the wheat (70 and 140 kg N ha^{-1}) and the maize (45 and 90 kg N ha^{-1}). The combination of the three factors resulted in 96 plots per MEE (6 x 8 m plot size). Weed control in wheat was performed by herbicide application (8.25 g ha^{-1} active ingredient (a.i.) iodosulfuron and mesosulfuron each + 180 g ha^{-1} a.i. fluroxypyr) in the pure wheat treatments (control and both cover crop treatments), whereas no weed control was performed in the LM treatment. Weeds in maize were controlled by herbicides in the CT and NT treatments with a mixture of 105 g ha^{-1} a.i. mesotrione and 495 g ha^{-1} a.i. terbuthylazine + 1.2 kg ha^{-1} a.i. S-metolachlor + 40 g ha^{-1} a.i. nicosulfuron in MEE 1 and 105 g ha^{-1} a.i. mesotrione and 495 g ha^{-1} a.i. terbuthylazine + 36 g ha^{-1} a.i. nicosulfuron + 96 g ha^{-1} a.i. dicamba in MEE 2. The RT treatment was mechanically hoed. Differential tillage was applied to kill subsidiary crops and for seedbed preparation to the maize. This was done by, ploughing (20 cm) and rotary harrowing in CT, three times chisel ploughing about 5 cm deep in RT, and 1.44 kg ha^{-1} a.i. glyphosate application and direct sowing in NT, respectively. Maize was sown at the end of May in both MEE's.

The two MEEs in Sweden were conventionally managed and set up as strip-blocks with four replicates. Pre-crops were winter wheat in MEE 1 and summer oilseed rape in MEE 2. Factor I (main plots) was tillage intensity: 1) CT at about 20 cm soil depth applied late in autumn; and 2) an RT non-inversion tillage system where weeds and subsidiary crops were killed with 1.2 kg ha^{-1} a.i. glyphosate applied in early spring and maize (*Z. mays* cv. Activate) was direct drilled in MEE 1 and sown after stubble cultivation in MEE 2. Direct drilling was not considered feasible in MEE 2 due to large amounts of crop residues. NT was not applied. Factor II (split plots) were subsidiary crops either applied as: 1) LM of white clover (*T. repens* cv. Klondike) undersown in wheat (*T. aestivum* cv. Olivin), 2) L CC, *V. villosa* cv. Minnie, 3) NL CC, *R. sativus* cv. Doublet, and 4) green fallow (weeds) as control (C). Both L CC and NL CC were sown after wheat harvest. In total, the experiments consisted of 32 plots (9 x 12 m plot size). Weeds in wheat were not controlled in MEE 1, but controlled in MEE 2 by 1 kg ha^{-1} a.i. bentazone. A mixture of 45 g ha^{-1} a.i. mesotrione, 7.5 g ha^{-1} a.i. foramsulfuron + 0.025 g ha^{-1} a.i. iodosulfuron-methyl-sodium + 7.5 g ha^{-1} a.i. isoxadifen-ethyl (safener), and 0.67 l ha^{-1} maize oil was applied twice yearly to the maize.

5.3.2 Wheat-potato MEEs

The organically managed MEEs in Germany were also set up as strip-split-blocks with four replicates. The MEEs were started after two years of regularly mulched grass-clover as pre-crop. Factor I (main plots) was either: 1) CT at about 25 cm soil depth after one and two times chisel ploughing at about 10 cm depth before wheat and potatoes, respectively or 2) RT with two to three times chisel ploughing at about 10 cm depth prior to wheat and at about 15 cm prior to potato cultivation. In addition, potatoes in RT were mulched with an 8-10 cm layer of a winter pea-rye (C/N ratio = 27.5; ~30% legumes) and triticale-vetch mixtures (C/N ratios = 22.5; ~60% legumes) four weeks after planting. This translates into about 150 and 360 kg nitrogen ha^{-1} (in MEE1 and MEE 2, respectively) that were applied in the non-inversion tillage system. Based on the C/N ratio of the mulch, the model of Laber (2002) suggests that 0 and 54 kg of this N became plant available during the potato season in MEE1 and MEE2, respectively. Although this potentially affected potato yields between both MEEs, the effects on plant-parasitic nematodes were assumed low due to the relatively low difference in nitrogen available to the plants. The mulch application was part of the RT treatment as recommended by the manufacturer (Friedrich Wenz GmbH) of the RT equipment. NT was not applied. Factor II (split-plots) was subsidiary crops applied as: 1) a LM of either subterranean clover cv. Dalkeith or white clover cv. Huia undersown in wheat (cv. Achat), 2) L CC, *V. sativa* cv. Berninova grown after wheat, and 3) NL CC, *R. sativus* var. *oleiformis* cv. Kompass/ *Avena strigosa* cv. Pratex mixed 1:4, grown after wheat. Due to complete failure of both undersown clover species in both experimental years, the LM treatments are considered as green fallow in the data analysis (i.e. control C). Factor III (split-split plots) was either: 1) manual application of 5 and 10 t ha^{-1} dry matter of a yard waste compost before sowing wheat or planting potatoes, respectively; or 2) mineral fertilization consisting of potassium (~167 kg ha^{-1} K_2SO_4) and phosphorus (~33 kg ha^{-1} rock phosphate) fertilizer matching the concentration of the composts. Total nitrogen in the composts was below 2% with a maximum of 200 mg kg^{-1} plant available (mineral) nitrogen and C/N ratios of 16-25. The pH and electrical conductivity in the composts ranged from 6.4 to 8.0 and 500-930 µS cm^{-1}, respectively. The total number of plots was 64 (6 x 15 m plot size). Weeds were only controlled in wheat in MEE1 via hoeing and harrowing in spring.

5.3.3 Durum Wheat-tomato MEEs

In Italy, the two MEEs were carried out at the conventionally managed, experimental farm of Tuscia University (Viterbo). The experimental fields were kept bare via periodically tilling during 14 months before start of the MEEs with a durum wheat (*T. durum* cv. Claudio) - tomato (*Solanum lycopersicum* cv. San Marzano Kero) crop sequence. The experimental design was a strip-split-block with three factors and four replicates. Factor I (main plots) was subsidiary crops applied either as: 1) LM, i.e. undersown subterranean clover (*T. subterraneum* cv. Campeda) in durum wheat, 2) L CC, *V. villosa* cv. Capello and 3) NL CC, *Avena strigosa* cv. Pratex, and 4) green fallow control (C). Both L CC and NL CC were sown after wheat harvest. Factor II (first split) was soil tillage, managing cover crop aboveground biomass with: 1) CT to a depth of 25 cm and 2) NT with residues left on the soil surface in strips as dead organic mulch. RT was not applied. Factor III (second split) were two levels of nitrogen fertilization in wheat (60 and 120 kg N ha^{-1}) and tomato (75 and 150 kg N ha^{-1}). Total number of plots was 64 (12 x 4 m plot size). Weeds in tomatoes were controlled with 0.5 kg ha^{-1} a.i. flufenacet + 17 g ha^{-1} a.i. metribuzine applied 30 days after transplanting in May.

5.3.4 Nematode Sampling and Assessments

Soil samples for initial and final nematode extraction were collected from the end of August until early November depending on site specific sowing and harvesting dates. Thus, soil samples for the assessments of initial plant-parasitic nematode densities were taken in late August/early September (Germany), late September (Switzerland), and October (Sweden, Italy). Soil samples for the assessments of final nematode densities were taken in September/October (all sites), except of MEE1 in Switzerland (early November). In Switzerland, initial plant-parasitic nematode densities were assessed from 48 plots that were thereafter divided in two sub-plots for the two nitrogen fertilization levels resulting in 96 plots for the assessment of final population densities. In Germany in MEE2, 32 plots before the application of tillage and compost were sampled for the assessment of initial plant-parasitic nematode densities. In all other cases, all plots were sampled for the assessment. Soil samples were collected from the upper 20 cm soil using an auger with 2-3 cm diameter. To maintain a representative sample, compensating for the heterogeneous distribution of plant-parasitic nematodes within the soil, a total of 30 cores per experimental plot were taken following a zigzag pattern. The soil was collected in a bucket, thoroughly mixed and an aliquot of 500 ml soil was filled in plastic bags, labelled, and shipped within one week to the Julius Kühn Institute in Münster, Germany (JKI). At JKI, soil samples were stored at 6°C until evaluation.

5.3.5 Nematode Evaluation

Soil samples were passed through a 1 cm sieve to remove root debris and stones, thoroughly mixed, and 250 ml aliquots were taken for nematode extraction following the centrifugal flotation method as described in Hooper et al. (2005) using $MgSO_4$ at 1.15 specific density. Nematodes collected after the final centrifugation step on a 20 µm sieve were transferred into a glass beaker and filled up to 10 ml with tap water. The suspension was thoroughly mixed by agitating with air and 1 ml was transferred into a nematode counting slide. Plant-parasitic nematodes were identified and counted at genus level using an inverse microscope at 63x magnification. For better comparison with similar studies, nematode densities were finally expressed as number of specimen 100 ml soil^{-1}. For species identification, a composite sample was prepared for each experimental site and sampling date. Nematodes were killed with gentile heat, fixed in a triethanolamine formalin (TAF) solution containing 7 ml formalin (40% formaldehyde), 2 ml TAF and 91 ml distilled water (Courtney et al., 1955). Fixed nematode specimen were then processed to anhydrous glycerol over a period of 12 days using the slow evaporation technique at 39 ± 1°C (Hooper et al., 2005). According to this method, species identification was done after transferring female nematodes into anhydrous glycerol on permanent slide mounts. Specimen were examined under a Leitz Diaplan compound microscope (Leitz, Wetzlar, Germany) equipped with differential interference contrast at 630-1000x magnification.

5.3.6 Statistical Analysis and Data Processing

The statistical analyses were performed with R, version 3.2.2 (R Core Team, 2013). Prior to analysis, residuals of final nematode data were visually tested with qq-plots for homogeneity of variances and normal distribution. Data were square root-transformed to improve the homogeneity of variances. The frequent nematode genera were analysed per MEE based on 2-factorial (tillage and subsidiary crops; Sweden) and three-factorial (tillage, subsidiary crops, and fertilizer/ compost; Switzerland, Germany, Italy) designs. Frequent genera were defined by densities above 100 nematodes 100 ml soil^{-1} for at least one site at the beginning of both MEEs. The R package 'nlme' (Pinheiro et al., 2016) was used to build linear mixed effect models fitted by restricted maximum likelihood ratios taking into account block designs and site specific split-plot arrangements. F- and *P*-values were obtained from Wald- tests, which appeared to be equally conservative than Kenward-Roger and Satterthwaite approximations for degrees of freedom (Luke, 2016), probably due to the large sample size.

A detrended correspondence analysis (DCA) of the nematode community revealed long gradients on the first axis. According to Dormann and Kühn (2009), long gradients (>4) on the first DCA axis reveal that the unimodal reaction of species is fully covered, while short gradients (<2) solely cover a part (ascending, descending) of the species reaction (linear reactions). For this reason, a constrained correspondence analysis (CCA, unimodal model) was performed with the R package *vegan* (Oksanen et al., 2015). The CCA was performed to identify site specific differences in the nematode composition at the beginning (Pi) and the end (Pf) of the cropping sequence. Therefore, sites (Switzerland, Germany, Sweden, and Italy) and sampling dates (Pi, Pf) were constraining and MEEs and replicates were conditional variables. Tillage, subsidiary crops, and fertilizer were not considered in this analysis. Prior to analysis, data were scaled to meet requirements of the CCA and permutation tests. Significances of the CCA model, factors, and axes were calculated using a permutation test with 999 permutations.

5.4 Results

All main crops in the MEEs were grown successfully, although in Germany, the undersown clover species did not establish in both MEEs and thus, were referred to as green fallow (C). Furthermore, the summer vetch failed in MEE 2 due to a common vole (*Microtus arvalis*) epidemic since 2014. In Switzerland, subterranean clover (LM) was re-sown after wheat harvest to ensure a satisfactory establishment during the fallow period. The winter wheat in Sweden in MEE 1 yielded poorly and was infested with *Matricaria inodora* in all treatments. The oilseed radish in MEE 1 was particularly sparse and one cannot expect it to have much impact on the soil microbial community.

5.4.1 Initial Nematode Densities

Initially, the most prevalent species was *Pratylenchus neglectus* that occurred at all sites in both years (Table 5.2). The second most common species occurring at three out of four sites were *Helicotylenchus vulgaris* (Switzerland, Germany, and Italy) and *Tylenchorhynchus dubius*

(Switzerland, Sweden, and Germany). Several species were site specific, such as *Geocenamus quadrifer* (syn. *Scutylenchus quadrifer*), a species known to be associated with wheat growth inhibition in Poland (Winiszewska et al., 2012) and Spain (Talavera & Jiménez, 1997), *Heterodera avenae* and *Paratylenchus similis* for Sweden, *Amplimerlinius icarus*, *Meloidogyne naasi*, *Paratylenchus projectus*, *Pratylenchus flakkensis*, *P. penetrans* and *Rotylenchulus borealis* for Germany, and *Xiphinema* sp. for Italy (Table 5.2).

Within sites, species within the genera of *Helicotylenchus*, *Paratylenchus*, and *Pratylenchus* found in MEE 1 were also found in MEE 2 with few exceptions. For example, *P. crenatus* was the dominant species in MEE 1 in Sweden, but was not detected in MEE 2. Variability in species spectrum between both MEEs at the same site was also observed for members of the *Tylenchorhynchus*-group in Switzerland and Sweden (Table 5.2).

Table 5.2 Plant-parasitic nematode species occurring at each site separated by taxa.

Site	Helicotylenchus	Paratylenchus	Pratylenchus	Tylenchorhynchus-Group	Others
Switzerland					
MEE 1	*H. vulgaris*		*P. neglectus*	*T. dubius*	
MEE 2	*H. vulgaris*	*Paratylenchus* sp.	*P. neglectus*	*Nagelus obscurus* *Amplimerlinius macrurus*	
Sweden					
MEE 1	*H. canadensis* *H. variocaudatus*	*P. similis*	*P. crenatus** *P. neglectus*	*Geocenamus quadrifer*	*Heterodera avenae* *Rotylenchus* sp.
MEE 2	*H. canadensis*	*Paratylenchus* sp.	*P. neglectus*	*T. dubius*	*H. avenae*
Germany					
MEE 1	*H. digonicus* *H. vulgaris*	*P. projectus*	*P. flakkensis* *P. neglectus* *P. penetrans*	*T. dubius*	*Rotylenchulus borealis* *Meloidogyne naasi* *Criconematidae*
MEE 2	*H. vulgaris*	*P. bukowinensis* *P. projectus*	*P. flakkensis* *P. neglectus* *P. penetrans*	*Amplimerlinius icarus* *Amplimerlinius macrurus* *T. dubius*	*M. naasi*
Italy					
MEE 1	*H. vulgaris*		*P. neglectus*		
MEE 2	*H. vulgaris*		*P. neglectus*		*Xiphinema* sp.

*****dominant species accounting for > 80% of the specimen within this genus

The most frequent nematode genera found at the beginning of the experiments were *Helicotylenchus, Pratylenchus,* and *Tylenchorhynchus* in all sites, and *Paratylenchus* in all sites but Switzerland (Figure 5.1). All other genera occurred in densities below 20 nematodes 100 ml soil^{-1} and were not further included in the detailed analysis. Initial species densities in Switzerland and Sweden were generally below 100 nematodes 100 ml soil^{-1} (median) in both MEEs except for *Helicotylenchus* in Switzerland that occurred in densities of 100-300 nematodes 100 ml soil^{-1} and *Tylenchorhynchus* in Sweden that was present in densities above 200 nematodes 100 ml soil^{-1} (Figure 5.1). However, some extreme values were observed in Sweden for *Paratylenchus* and *Pratylenchus* causing deviations between medians and means. At the German site, initial plant-parasitic nematode densities were higher than at the other sites. In particular, *Helicotylenchus, Paratylenchus,* and *Pratylenchus* reached densities of 100-400 nematodes 100 ml soil^{-1} (medians). In Italy, high initial densities of *Helicotylenchus* and *Pratylenchus* occurred in MEE 1 (~200 and ~300 nematodes 100 ml soil^{-1}, respectively), whereas in MEE 2, nematode densities were low except for *Pratylenchus* with 133 nematodes 100 ml soil^{-1} (Figure 5.1).

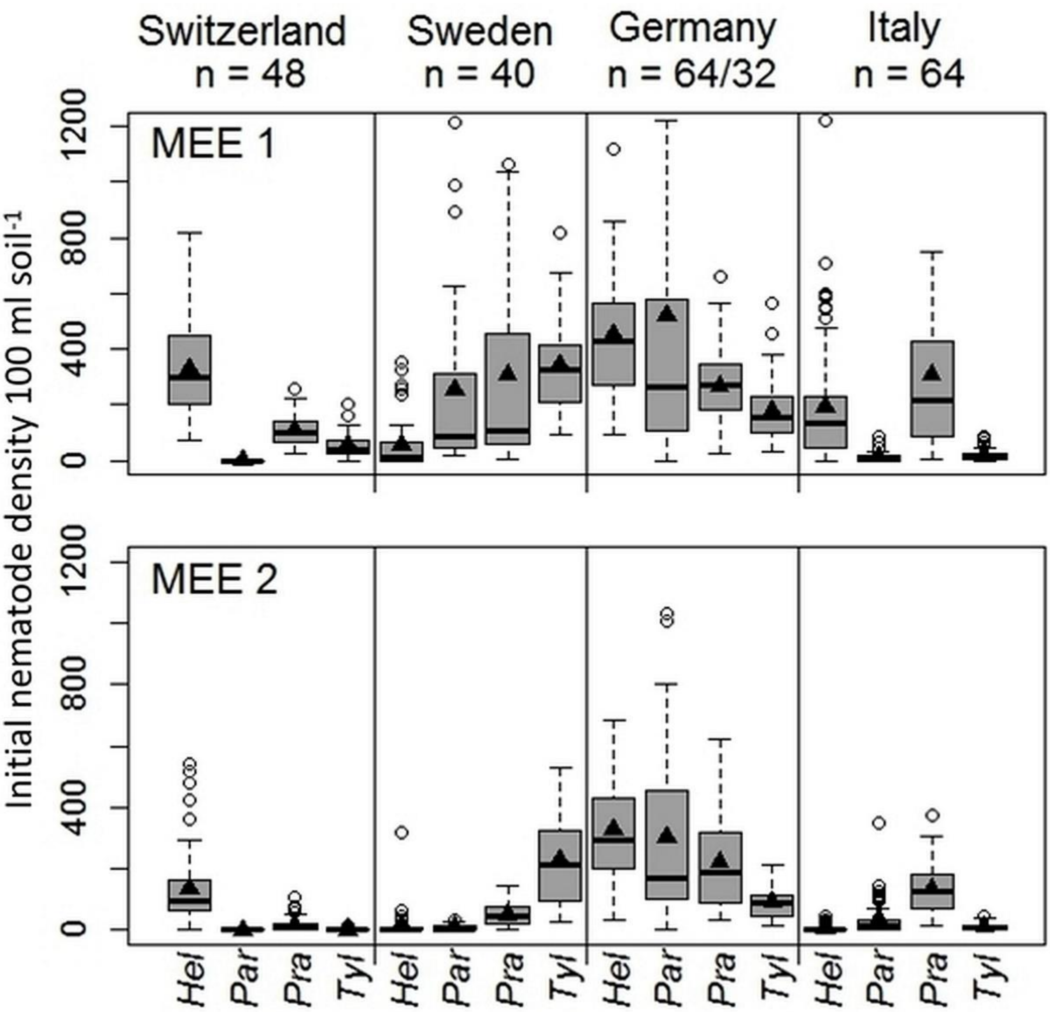

Figure 5.1 Medians, means (triangles), upper and lower quartiles (whiskers), and outliers (circles) of initial (Pi) population densities of *Helicotylenchus* (*Hel*), *Paratylenchus* (*Par*), *Pratylenchus* (*Pra*), and *Tylenchorhynchus* spp. (*Tyl*) in the two multi environment experiments (MEEs) at each site. Very high values (above 1200 nematodes 100 ml soil^{-1}) and sporadically occurring *Heterodera, Meloidogyne,* and *Criconematidae* are not shown. N was 64 and 32 for MEE 1 and MEE 2, respectively for Germany.

5.4.2 Nematode Dynamics

The total density of plant-parasitic nematodes increased 2.7 and 1.6 fold in MEE1 and MEE 2, respectively, in Switzerland and 1.1-fold in MEE 2 in Sweden. In contrast, populations decreased by 26-28% in Italy, 38-63% in Germany and 69% in MEE 1 in Sweden (data not shown). The dynamics of *Helicotylenchus, Paratylenchus, Pratylenchus,* and *Tylenchorhynchus* were generally low within sites, resulting in similar initial (Pi) and final (Pf) nematode compositions (Figure 5.2). However, treatments and crops grown in the crop sequence studied in the experiments had major impacts on final densities of *Helicotylenchus* and *Pratylenchus* (Figure 5.3,Figure 5.4). There were no statistically significant interactions

(*P*> *0.05)* between tillage system and subsidiary crops in any of the variables measured in any of the experiments and therefore, only main effects are presented.

Final densities of other nematode genera, i.e. *Amplimerlinius, Criconematidae, Meloidogyne, Heterodera, and Xiphinema* were generally below 20 nematodes 100 ml soil^{-1} and were therefore considered less important for the detection of biologically interesting patterns of treatments. However, the site specific occurrence of these nematode genera explained a large proportion of variation between sites in the constrained correspondence analysis (CCA, Figure 5.2).

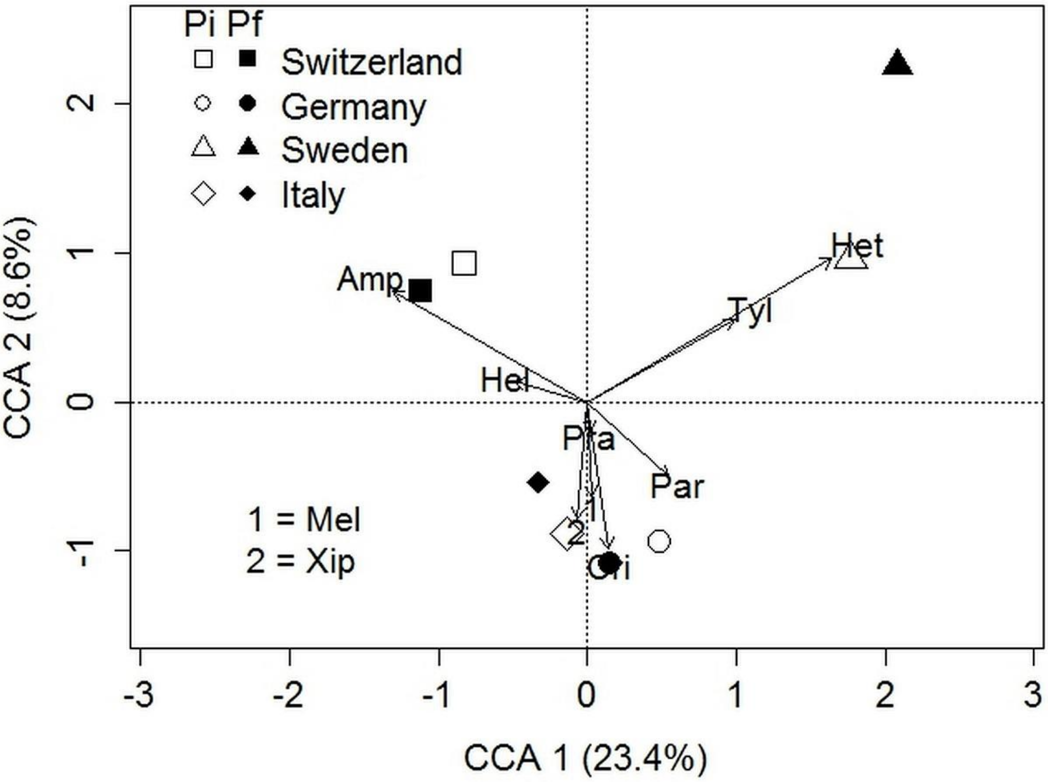

Figure 5.2 Constrained correspondence analysis (CCA) biplot showing site effects on nematode composition at the beginning (Pi) and the end (Pf) of the cropping sequence with both experimental years (MEE 1, MEE 2) and replicates as conditional variables. Axis labels showing proportion of constrained total variance. Nematode genera scores were shown with abbreviations and arrows in direction of increasing density (Amp = Amplimerlinius, Cri = Criconematidae, Hel = Helicotylenchus, Het = Heterodera, Mel = Meloidogyne, Par = Paratylenchus, Pra = Pratylenchus, Tyl = Tylenchorhynchus, Xip = Xiphinema).

The first three ordination axes of the CCA biplot were significant at $P<0.01$ (permutation test) explaining 96% of constrained eigenvalues. The first and second axes accounted for 23.4% and 8.6% of the total variance, respectively. The sites and sampling time (Pi, Pf) explained 38.8% of the total variance in nematode genera composition ($P<0.01$, permutation test).

Overall, the CCA discriminated strongest between Switzerland and Sweden on the first axis (Figure 5.2). The experimental site in Switzerland was characterized by the highest densities of *Amplimerlinius* and *Helicotylenchus*, which increased during the cropping sequence, regardless of the treatments. *Heterodera* were only found in Sweden, predominantly at the beginning of the experiments (20 and 4 nematodes 100 ml soil^{-1} in MEE1 and MEE2, respectively). Therefore, the CCA clearly put Sweden apart from the other sites in Figure 5.2. The CCA discriminated strongly between final and initial population densities in Sweden and to a lesser degree in the other sites. Germany and Italy fell close to each other in the CCA. *Xiphinema* correlated with the site Italy at the beginning of the cropping sequence. The German site was correlated with *Meloidogyne* and *Criconematidae* at the end of the cropping sequence suggesting an increase of these taxa during the experiment. In contrast, *Paratylenchus* correlated with the beginning of the cropping sequence in Germany, suggesting a decrease throughout the experiment. *Pratylenchus* was not correlated to any site, suggesting an equal occurrence (Figure 5.2).

5.4.3 Effect of Tillage on Plant-parasitic Nematodes

In general, non-inversion (RT) and no-tillage (NT) had inconsistent effects on plant-parasitic nematodes compared to conventional tillage (CT). Statistically significant results ($F_{df1,df2}$, $P< 0.05$) were only obtained for the genera *Helicotylenchus* and *Pratylenchus,* which occurred at higher densities under NT than under CT.

In Switzerland, plant-parasitic nematode densities were highly variable and no statistically significant effect of tillage treatment ($F_{2,6} < 3.5$, $P> 0.05$) was observed for any of the genera in both MEEs. In MEE 1, very high densities of *Helicotylenchus* with up to 1241 nematodes 100 ml soil^{-1} under RT (untransformed densities are shown here in contrast to Figure 5.3A) were observed. In MEE 2, nematode densities were much lower than in MEE1.

In Sweden, tillage effects on plant-parasitic nematode densities in both MEEs were low (Figure 5.3) and not statistically significant for all genera ($F_{1,3}< 4.3$, $P> 0.1$). The largest average difference was observed for the densities of *Pratylenchus* that were higher in MEE2 under RT (67 nematodes 100 ml soil^{-1}) than under CT (27 nematodes 100 ml soil^{-1}). The dominating genus, *Tylenchorhynchus*, with 260 nematodes 100 ml soil^{-1}) was not significantly affected by tillage.

Similar to Switzerland and Sweden, no significant effects of any of the nematode genera was observed in Germany. However, densities of *Helicotylenchus* tended to be higher under RT than under CT in MEE1 (Figure 5.3A, $F_{1,3}$= 7.9, *P= 0.067*). In MEE 2, no differences among tillage systems occurred.

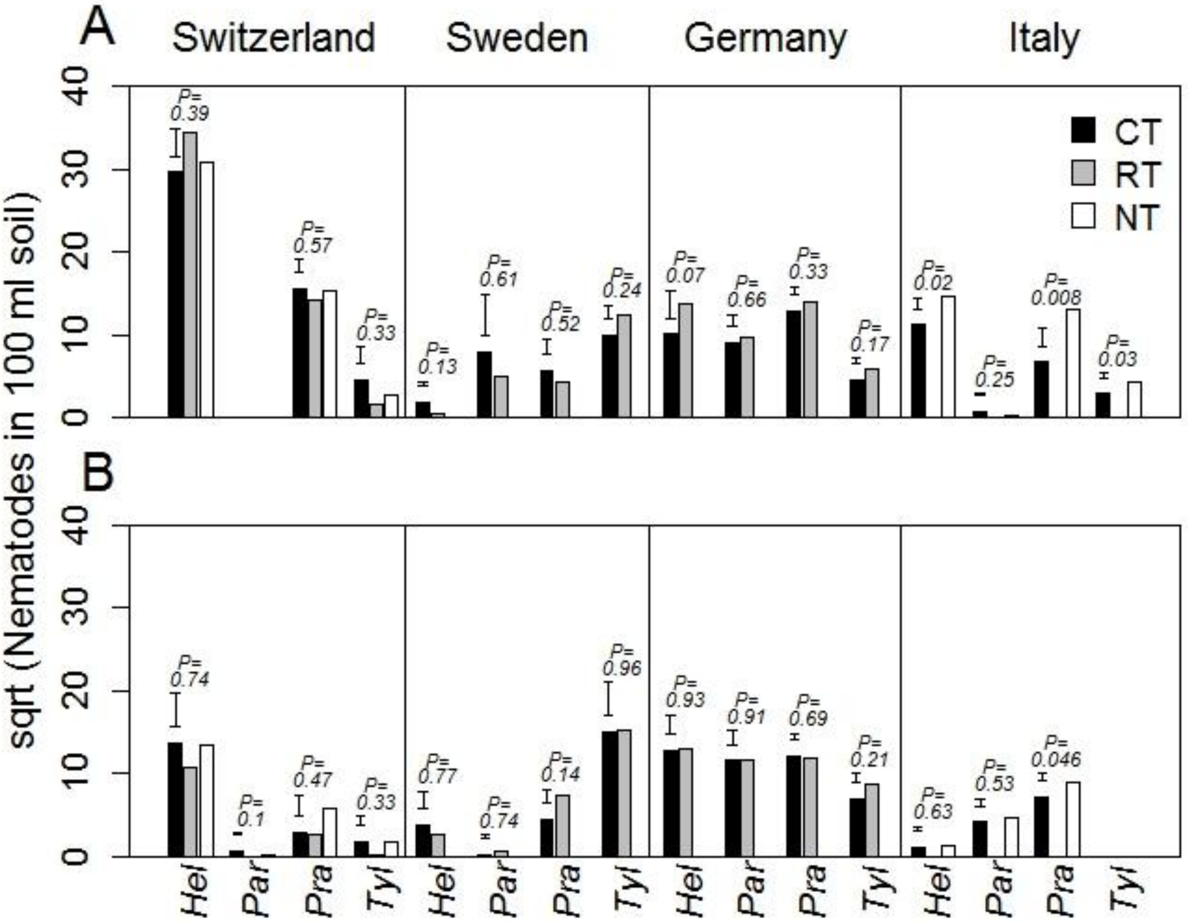

Figure 5.3 Effects of tillage (CT: inversion tillage, RT: non-inversion tillage, NT: no-tillage) on square root-transformed final densities (means + SEDs) of *Helicotylenchus (Hel)*, *Paratylenchus (Par)*, *Pratylenchus (Pra)* and *Tylenchorhynchus (Tyl)* 100 ml soil^{-1} in the first (A) and second (B) multi environment experiment (MEE) in Switzerland (degrees of freedom denominator (dfd)=6), Sweden (dfd=3), Germany (dfd=3), and Italy (dfd=15), all separated by vertical lines; actual *P*-values are shown according to Wald-tests.

In Italy, NT in combination with herbicide application significantly increased densities of *Helicotylenchus* ($F_{1,15}$=7.1, *P= 0.02), Pratylenchus* ($F_{1,15}$= 9.3, *P= 0.008),* and *Tylenchorhynchus* ($F_{1,15}$= 6.0, *P= 0.03)* in MEE 1 (Figure 5.3A). In particular, densities of *Pratylenchus* were more than four times higher (270 nematodes 100 ml soil^{-1}) under NT compared with CT (60 nematodes 100 ml soil^{-1}). In MEE 2, the nematode densities were generally lower than in MEE 1 and only *Pratylenchus* densities were significantly higher ($F_{1,15}$= 4.7, *P= 0.047)* in the NT system (94 nematodes 100 ml soil^{-1}) compared with CT (61 nematodes 100 ml soil^{-1}, Figure 5.3B). Both, *Helicotylenchus* and *Paratylenchus* densities were not affected by NT in combination with herbicide application.

5.4.4 Effects of Subsidiary Crops on Plant-parasitic Nematodes

Overall, leguminous subsidiary crops, either applied as living mulches (LM) or cover crops (CC), increased the densities of *Pratylenchus* and *Helicotylenchus* by the end of the experiment compared to the non-leguminous CC. In both MEEs in Switzerland, subsidiary crops significantly affected densities of *Pratylenchus (*$F_{3,33}$> 8.0, *P< 0.001)* and *Helicotylenchus (*$F_{3,33}$> 2.9, *P< 0.051)*. Hence, leguminous CCs and LM supported population build-up of *Pratylenchus* and *Helicotylenchus* compared with the non-leguminous CC but not necessarily compared to the green fallow control (C) (Figure 5.4). Other plant-parasitic nematode genera were not affected by cover crops.

In Sweden, subsidiary crops did not affect plant-parasitic nematode densities significantly in neither of the MEEs (**Fehler! Ungültiger Eigenverweis auf Textmarke.**, $F_{3,21}$< 1.5, *P> 0.2*).

Similarly, in Germany effects of subsidiary crops on plant-parasitic nematodes were low. Solely in MEE1, the densities of *Pratylenchus* were somewhat lower in the non-leguminous CC (152 nematodes 100 ml soil^{-1}) than in the leguminous CC (196 nematodes 100 ml soil^{-1}) or the green fallow (205 nematodes 100 ml soil^{-1}) (Figure 5.4A, $F_{2,14}$= 3.0, *P= 0.08*). In MEE 2, no significant differences were found between treatments (Figure 5.4B, $F_{2,14}$< 2.2, *P> 0.1*).

In the MEE 1 in Italy, *Helicotylenchus (*$F_{3,9}$=4.1, *P= 0.04)* and *Pratylenchus (*$F_{3,9}$= 5.0, *P= 0.03)* were significantly affected by subsidiary crops. Highest densities of *Helicotylenchus* were found in the leguminous CC (296 nematodes 100 ml soil^{-1}) and LM (261 nematodes 100 ml soil^{-1}) treatments compared with green fallow (116 nematodes 100 ml soil^{-1}) or non-leguminous CC (159 nematodes 100 ml soil^{-1}, Figure 5.4A). Likewise, higher densities of *Pratylenchus* occurred in leguminous CC (170 nematodes 100 ml soil^{-1}) and LM (385 nematodes 100 ml soil^{-1}) than in non-leguminous or green fallow treatments (54 nematodes 100 ml soil^{-1}). In contrast, total nematode densities in MEE 2 were much lower than in MEE 1 and did not exceed 130 nematodes 100 ml soil^{-1}. Nevertheless, in MEE2, subsidiary crops showed tentatively similar patterns for *Pratylenchus* ($F_{3,9}$= 3.8, *P= 0.052)* than observed in MEE1. Accordingly, leguminous CC (81 nematodes 100 ml soil^{-1}) as well as LM (121 nematodes 100 ml soil^{-1})

treatments resulted in higher densities of *Pratylenchus* than green fallow (45 nematodes 100 ml soil^{-1}) and non-leguminous CC (64 nematodes 100 ml soil^{-1}, Figure 5.4B).

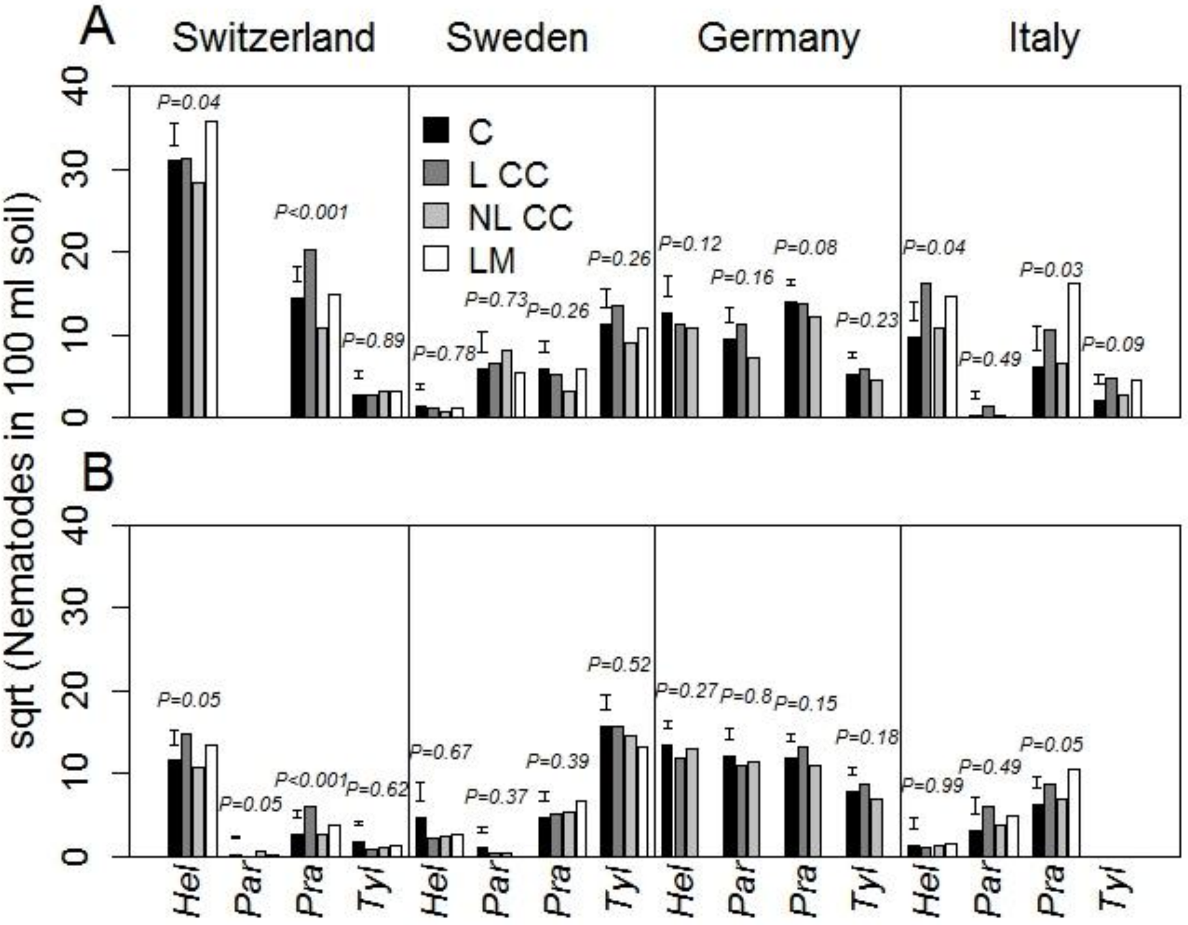

Figure 5.4 Effects of subsidiary crops (C: green fallow, L CC: leguminous cover crop, NL CC: non-leguminous cover crop, LM: living mulch) on square root-transformed final densities (means + SEDs) of *Helicotylenchus (Hel)*, *Paratylenchus (Par)*, *Pratylenchus (Pra)* and *Tylenchorhynchus (Tyl)* 100 ml soil^{-1} in the first (A) and second (B) multi environment experiment (MEE) in Switzerland (degrees of freedom denominator (dfd)=33), Sweden (dfd=21), Germany (dfd=14), and Italy (dfd=9), all separated by vertical lines; actual *P*-values are shown according to Wald-tests.

5.4.5 Effects of Nitrogen Fertilizer and Compost on Plant-parasitic Nematodes

Different nitrogen application rates in Switzerland and Italy (full or half dose; $P > 0.25$) and the use of compost in Germany ($F_{1,39} < 1.6$, $P > 0.2$) had no statistically significant effects on plant-parasitic nematode dynamics in any of the MEEs. Only in MEE1 in Switzerland, densities of *Pratylenchus* were significantly higher (300 nematodes 100 ml soil^{-1}) at full nitrogen dose compared to half dose (220 nematodes 100 ml soil^{-1}, $F_{1,47} = 11.7$, $P = 0.001$).

5.5 Discussion

The densities of plant-parasitic nematodes by the end of the experiments was not higher with non-inversion tillage than inversion tillage at three of the four sites. However in Italy, the densities of plant-parasitic nematodes with a broad host spectrum, such as *Pratylenchus* and *Helicotylenchus* were greater under no-tillage than inversion tillage. Furthermore, both genera were more abundant in the vetch (*Vicia* spp.) and clover (*Trifolium* spp.) treatments. Thus, the use of non-leguminous subsidiary crops could be a useful strategy to reduce the density of some species of *Pratylenchus* and *Helicotylenchus*. However, compost and different application rates of nitrogen did not affect the plant-parasitic nematode community. The effects of tillage, subsidiary crops, and compost/fertilizer were evident despite of highly site and year specific effects in the eight multi-environment experiments (MEEs), conducted in four countries over two successive years.

Site specific climatic and soil conditions, such as organic matter contents that ranged from 1.2% (Italy) to 4.4% (Sweden) and soil texture (sandy in Italy to loamy in Germany), may have contributed to varying initial plant-parasitic nematode populations as well as to the nematode dynamics during the crop rotations.

5.5.1 Initial Infestation

Initial densities of plant-parasitic nematodes were generally below the economic threshold level reported for each of the species. The highest nematode densities at experimental start was found in Germany, a site managed organically. This can probably be attributed to the two years of grass-clover mixtures (*Trifolium* spp., *Medicago sativa, Lolium perenne, Dactylis glomerata, Festuca pratensis*) in the preceding years. These are excellent hosts for migratory species with a broad host spectrum such as *Helicotylenchus, Paratylenchus, Pratylenchus,* and *Tylenchorhynchus* (Sharma, 1971; Wouts & Yeates, 1994; Knight et al., 1997).

For Sweden, the differences in initial nematode densities were most likely caused by the varying host status of the pre-crops, i.e. winter wheat in MEE 1 and summer oilseed rape in MEE 2. Low initial densities of plant-parasitic nematodes in Switzerland and Italy are explained by growing pea (*Pisum sativum* L.) as preceding crop or applying 14 months of bare fallow before the start of the experiment, respectively. Peas are known to be poor hosts for the plant-parasitic nematodes occurring in Switzerland (Ferris et al., 1993; Taylor et al., 2000; Kruse, 2006; Smiley et al., 2014a). In Italy, the bare fallow fostered natural decline of plant-parasitic nematodes due to the absence of any food source (Viaene et al., 2013).

The number of plant-parasitic nematode genera found at each site varied between three (Switzerland, Italy) and six (Sweden, Germany). Among all identified genera, *Pratylenchus* was the only one recorded from all sites, independent of the pre-crops. This can be explained by the broad host range of this genus (Castillo & Vovlas, 2007). However, the high densities of *Pratylenchus* after 14 months of bare fallow in summer-arid Italy were unexpected. Barley that was grown before the bare fallow period is a good host for *P. neglectus* (Taylor et al., 2000). It apparently left over high densities of *P. neglectus* that could have survived the bare fallow under arid conditions in an anhydrobiotic resting stage (Glazer & Orion, 1983; Talavera & Vanstone, 2001). Another explanation could be that weeds have contributed to *Pratylenchus* spp. survival. Although measures were applied to keep the fallow bare, ryegrass (*Lolium* spp.) survived in the fallow before MEE 1 in Italy and could have served as host for *Helicotylenchus* and *Pratylenchus*.

5.5.2 Final Infestation

In general, plant-parasitic nematode genera that were found initially were also detected at the end of the experiment. Final population densities of plant-parasitic nematodes were higher than initial densities in Switzerland, but were equal or lower in Sweden, Germany, and Italy. The increase in nematode densities in Switzerland can be best explained by low initial densities followed by good hosts (wheat-maize). The reductions in Germany (MEE 1 + 2) and Sweden (MEE 1) were most likely due to high initial densities followed by less suitable hosts to the initially dominant species within the crop rotation. Somewhat unexpected were the low final infestations in Italy (MEE 1 + 2) following good host plants (legumes, tomato).

5.5.3 Tillage Effects

The effect of tillage intensity on plant-parasitic nematode dynamics is discussed quite ambiguous in the literature. For example, an increase of *Pratylenchus* under long term no-tillage conditions compared with 15 cm deep rotary tilling is reported from Japan (Okada & Harada, 2007). Similarly, in a study conducted in Iowa State, U.S.A., *Helicotylenchus* and *Pratylenchus* tended to increase under no-tillage (Thomas, 1978). Furthermore, Thompson *et al.* (2008) reported from Australia that the root lesion nematode *Pratylenchus thornei* was more abundant under non-inversion and no-tillage compared to frequently tilled soils. In contrast, *Pratylenchus* and *Helicotylenchus* densities were not different among conventional ploughing (0-20 cm), minimum tillage (7 cm) and no-tillage, in a study conducted in the U.K. (Griffiths et al., 2012) and Westphal *et al.* (2009) even reported that reducing tillage intensity reduced population densities of the specialized soybean cyst nematode *Heterodera glycines*. An explanation for those controversial observations could be variable weed infestation levels. In general, conservation tillage results in higher weed pressure than conventional tillage (OSCAR, 2016) supporting a higher density of plant-parasitic nematodes. However, if weeds are thoroughly controlled in a minimum tillage system by herbicides or other means, plant-parasitic nematode densities can be lower than in a ploughed system without weed control as shown by Corbett & Webb (1970) for *Pratylenchus* in wheat. This underlines the importance of proper weed management for preventing plant-parasitic nematode build-up (Hallmann & Kiewnick, 2015).

5.5.4 Crop Rotation Effects

Plant-parasitic nematodes require a suitable host plant to feed on and propagate. Thus, the host status of a crop is the most important determinant of seasonal and annual changes in their population structure, density and distribution (Nusbaum & Ferris, 1973). As crops differ in their host status for certain nematode species, crop rotation plays a key role in managing plant-parasitic nematodes. However, the use of crop rotation for nematode management might be of limited value when several damaging species of nematodes are present or for species with broad host ranges (Barker & Koenning, 1998).

In the wheat-maize rotations, plant-parasitic nematode densities tended to increase. However, the constrained correspondence analysis showed that nematode spectra and dynamics were quite different between Switzerland and Sweden. For example, in Switzerland populations of *P. neglectus* and *H. vulgaris* increased over time, whereas in Sweden populations of *P. crenatus* and *H. variocaudatus* declined. On the other hand, *T. dubius* decreased in Switzerland, but increased in Sweden in MEE 2. Most likely this can be explained by site- and species-specific effects, such as different pre-crops resulting in different nematode spectra and therefore different initial infestation levels, climatic distinctions, soil discrepancies (e.g. organic matter) and differences in cultivars grown (Nusbaum & Ferris, 1973). The host status is species and cultivar specific and thus, contributes a great deal to the often observed variability in nematode reactions towards a certain crop species (Nicol & Rivoal, 2008).

This may further explain the higher densities of *Helicotylenchus* and *Pratylenchus* after leguminous than after non-leguminous subsidiary crops, especially in Switzerland and Italy. Both genera have a broad host range (Castillo & Vovlas, 2007) and are enhanced by the leguminous subsidiary crops used in this study (Townshend & Potter, 1976; Taylor et al., 2000; Berry et al., 2011; Visser & Molendijk, 2015). In contrast, non-leguminous subsidiary crops, such as *Raphanus sativus* and *Avena strigosa,* are non-hosts of *Pratylenchus* (Hirling, 1977;

Visser & Molendijk, 2015). This could explain the lower densities of this genus in the non-leguminous CC treatments compared to the leguminous CC in Switzerland and Italy in both MEEs (Figure 5.4).

Many nematode species reproduce best on sand and sandy-loam soils (Norton, 1979) most likely due to optimal particle sizes for nematode movement and water:oxygen ratio (Hallmann & Kiewnick, 2015). In addition, low soil organic matter contents are generally associated with higher densities of plant-parasitic nematodes due to the lack of antagonists (Sikora, 1992). For these reasons, the effects of subsidiary crops (and also tillage) were most apparent and consistent in Italy and Switzerland, sites with the highest sand and low organic matter contents.

5.5.5 Compost and Fertilizer Effects

The lack of effects of 5 t ha^{-1} and year compost application in Germany on the observed plant-parasitic nematode species could be due to the low dosage of application. However, the application of 269 t ha^{-1} fresh yard waste compost (>10-fold the amount used in this study) also did not affect *Meloidogyne* and *Pratylenchus* spp. (McSorley & Gallaher, 1997). Although immediate effects of compost products appeared to be generally too low for nematode suppression (Akhtar & Malik, 2000), the long term use of such organic amendments may increase the soil microflora (Griffiths et al., 2010) and probably microbial antagonists of nematodes over time and therefore, need further investigation.

Similarly, nitrogen dose did not affect plant-parasitic nematode species, except for higher densities of *P. neglectus* that were found after application of the full nitrogen dose in MEE1 in Switzerland. Similarly, five years repeated application of full and half nitrogen dose in field experiments did not affect *Helicotylenchus*, *Pratylenchus,* and *Tylenchorhynchus* densities in the UK (Boag et al., 1998). However, nitrogen effects on plant-parasitic nematodes may also be source and dose dependent. For example, nitrogen applied in high doses as ammonia suppressed nematode populations in soil (Akhtar & Malik, 2000), while Elmer & LaMondia (1999) found higher root-lesion nematodes densities in strawberry roots after ammonia than nitrate fertilization. Higher nematode densities following nitrogen fertilization were also observed by Okada & Harada (2007) in field experiments with soybean in Japan where the authors compared the effect of fertilizer application with no fertilizer application. Thus, plants not receiving fertilizer probably failed to provide sufficient nutrients for nematode propagation, which could explain the lower densities of plant-parasitic nematodes without fertilizer in their study. In our study, the low nitrogen level still provided enough nitrogen for adequate crop growth, while the nitrogen concentration in the high nitrogen level was probably too low to suppress plant-parasitic nematodes.

5.6 Conclusion

We conclude that the combination of non-inversion or no-tillage with specific subsidiary crops can be used to influence plant-parasitic nematode densities and the species composition to a certain extent. However, site specific effects need to be taken into account. Thus, the effects were most evident for light soils with low organic matter contents. Nitrogen rates and compost application did not affect plant-parasitic nematode compositions under our circumstances.

In general, effects of tillage intensity on plant-parasitic nematodes were marginal, although the genera *Helicotylenchus* and *Pratylenchus* were promoted by no-tillage on one site with sandy soils and low humus contents. Likewise, leguminous subsidiary crops supported higher densities of both species than the non-leguminous subsidiary crops *Raphanus sativus* and *Avena strigosa*. Thus, it is possible to counteract potential problems occurring with no-tillage with respect to nematode infestations by making use of the right cover crops However, longer-term results with a variety of subsidiary crops are needed to provide farmers with appropriate and widely applicable recommendations suitable to their particular site specific conditions.

6 General Discussion

Two of the principles of CA, rotations and subsidiary crops, are not new to organic agriculture in Europe. Diversified crop rotations are the basis of organic farming to provide nutrients for subsequent crops and to suppress weeds, pests and diseases (Leoni et al., 2015). In addition, subsidiary crops applied as cover crops and living mulches are frequently used to suppress weeds and to take up remaining nutrients from soil after harvest of the main crop (Campiglia et al., 2015, 2014; Brust & Gerhards, 2012). However, the success of subsidiary crops can be limited (i) if they are sown too late, thus, reducing their overall competetivness to weeds; (ii) if they are too competitive to the main crop when grown as living mulch, thus, reducing crop yields; (iii) if the wrong varieties are chosen that are not adapted to the local conditions; and (iv) if they host pests and diseases of subsequent main crops in the rotation (Doltra & Olesen, 2013).

In contrast to crop rotation and subsidiary crops, conservation tillage being the 3rd principle of CA is still rare in European organic agriculture. This is especially the case in Northern Europe where cool spring conditions limit nutrient availability during the early season and where the weed pressure is high (Peigné et al., 2007, 2015).

However, the correct combination of all three principles of CA is essential to gain maximum benefits in terms of total yield and yield stability (Dimassi et al., 2013; Pittelkow et al., 2015). This work documents the first four years of transition to CA, applying the three principles in different ways in organic agriculture compared to a plough based system. The focus of this study is on weeds and plant-parasitic nematodes. While effects may change with time after transition, general trends could already be seen. Those trends might be helpful in identifying the main factors involved that could be used to better adapt the farming system.

6.1 Phytomedicinal Benefits Arising with CA

The concept of CA, i.e. use of crop rotations, reduction of soil tillage, and increase of soil cover by subsidiary crops neither increased weed seed banks significantly (Chapter 3) nor resulted in yield losses due to higher numbers of plant-parasitic nematodes (Chapter 4) compared with the conventional system. Moreover, CA significantly increased the number of free-living nematodes already two years after transition. Free-living nematodes are a significant part of the soil food web where they play an important role in nutrient cycling (Griffiths, 1994). High numbers of free-living nematodes help to overcome nutrient limitations that generally occur in first years after transition to CA (Chapter 1.1).

This study further highlights the great importance of crop rotations for nematode and weed control (Leoni et al., 2015). High nematode populations occurring after grass-clover and wheat were considerably reduced by subsequent non-host subsidiary crops and potatoes (Chapter 4 and 5). Furthermore, crop rotations resulted in homogeneous weed seed banks, i.e. high weed diversity without dominating species, in both fields used for this study (Chapter 2).

Although effects of composts were generally minor, its surface application to the wheat significantly affected weed cover and biomass (Chapter 2). The high C:N ratio of the compost may have induced nitrogen deficiency in the top soil and thus, limited weed seed germination although not the growth of volunteers (grass species), which do not depend on seeds, and crops. This is evidenced by the occurrence of these effects under both tillage regimes (Chapter 2, Figure 2.3)

Although single weeds and plant-parasitic nematodes were overall little affected, some important species could be controlled with different CA measures during the transition phase. For example, the *Chenopodium album* seed bank was reduced under conservation tillage with dead mulch application to potatoes (Chapter 3). This summer annual weed species frequently occurs in organic potato systems at high densities and is a major factor limiting potato yields (Gallandt et al., 1998) and hindering harvest operations. In addition, oilseed radish and black oat cover crops reduced the root-lesion nematode *Pratylenchus* compared to vetch cover crops or legume living mulches across various climate and soil conditions (Chapter 4 and 5).

In Germany, shallow tillage applied for cover crop sowing reduced the weed biomass compared with green fallow plots which were infested with perennial ryegrass (*Lolium perenne*) and annual bluegrass (*Poa annua*) (Chapter 4.4). *Poa annua* can shed seeds also later in the season and may also act as biennial species. This explains its lower seed banks if cover crops were grown instead of green fallow after unsuccessful living mulch in the wheat (Chapter 3). Clearly, in practical agriculture, it is not an option to use a green fallow if the living mulch fails and was here only done for experimental reasons (see Chapter 6.3). In addition, the lower weed infestation under non-inversion tillage using cover crops could have reduced the number of plant-parasitic nematodes with a broad host spectrum. These taxa are generally difficult to target by crop rotations (Thomas et al., 2005), and black fallows, which can effectively reduce nematode populations (Hallmann & Kiewnick, 2015), are discouraged in the recent consensus of conservation agriculture as sustainable farming systems (Hobbs, 2007). In particular, the root-lesion nematode *Pratylenchus*, which parasitizes among several crop plants a broad range of different grass weeds commonly occurring in cultivated soils (Smiley et al., 2014b; Vanstone

& Russ, 2001a), was likely affected not only by the cropping of non-host plants (see above) but also by the absence of weed hosts.

6.2 Predicting Future Threats for Agricultural Sustainability in CA Systems

In contrast to the multiple benefits of CA regarding weeds and plant-parasitic nematodes observed in the first four years after transition, we also identified probable future limitations that should be targeted by system adaptations to reach a sustainable agricultural equilibrium (Chapter 1, Figure 1.1). High infestations with volunteer weeds are frequently arising with tillage reductions, particularly in organically managed CA systems (Peigné et al., 2015; Pekrun et al., 2003). In the present study, the grass-clover pre-crops were not sufficiently terminated by chisel ploughing or undercutting and thus, resulted in increased weed pressure in the subsequent winter wheat (Chapter 2). Furthermore, clover undersown in winter wheat as living mulches failed due to late sowing in autumn, low winter hardiness, and high competition by the main crop. The resulting high percentage of bare soil probably increased seed banks of wheat associated annual weed species (Chapter 3). Out of these species, the *Veronica* complex was rather unaffected by rotating spring and fall sown crops and dominated the weed seed bank after four years of conservation agriculture. Although those species are generally considered to be minor competitors (Welbank, 1963), their high presence in the seed bank can probably lead to yield losses due to nitrogen competition. Nitrogen deficiency is considered to be one of the most yield limiting factors during the transition to conservation tillage (Mäder & Berner, 2012). In contrast to *Veronica* spp., *Galium aparine* is considered a strong competitor in fall sown crops and can cause yield losses up to 50% depending on wheat sowing densities and cultivars (Mennan & Zandstra, 2005). Inthis study, the seed bank of this particular weed was already two-fold higher after potato under conservation than conventional tillage (Chapter 3). This makes it a major threat for any subsequent fall sown crop. Likewise, perennial weeds, such as *Cirsium arvense, Elymus repens,* and *Rumex* spp., are generally problematic in CA as they are not only difficult to control in organic but also in conventional agriculture that includes the use of herbicides (Peigné et al., 2007; Trichard et al., 2013). Although not shown here, these species did become more abundant in the fields studied (Junge et al., 2016; Junge, 2016). Thus, further monitoring of perennial weeds, *Veronica*, and *G. aparine* seed banks as well as field infestations needs to be done and necessary sanitation measures (Chapter 6.3) that can maintain crop yields and farming system sustainability need to be integrated into the system.

Similarly as observed for weeds, nematodes respond differently to CA practices depending on their feeding habit. For instance, van Capelle et al. (2012) reviewed tillage induced changes of nematode communities and found numbers of bacterivorous and fungivorous nematodes increased under conservation tillage while plant-parasitic nematodes remained unaffected. Although data of this study generally support these results, I agree with earlier conclusions that tillage effects should not be generalized among plant-parasitic nematodes without knowledge of present weed flora as alternative hosts, soil conditions, and feeding habits of the plant-parasitic nematode community (Corbett & Webb, 1970; Thomas et al., 2005). Thus, frequent high weed infestations under conservation tillage (see above) can directly benefit plant-parasitic nematodes with a broad host spectrum and increase population densities (Thomas et al., 2005). These effects may be more severe in sandy soils that allow better nematode movement (Chirchir et al., 2008) and therefore increase host-pathogen contacts. Among the plant-parasitic nematodes observed in this study, *Pratylenchus* spp. were generally more frequent under conservation tillage and legume subsidiary crops than under conventional tillage and non-legume cover crops (Chapter 4 and 5). Although their presence was overall too low to cause yield losses in wheat and potato (Chapter 4), these species may build up high populations over time if good hosts, such as perennial fodder crops, leguminous (subsidiary) crops, wheat, and maize, are frequently grown in CA systems (Chapter 5). Furthermore, “synergistic” interactions of weeds and *Pratylenchus* spp. were observed throughout the wheat cropping period under conservation tillage (Chapter 4) indicating the need for adapted weed control.

6.3 Approaches to Increase Sustainability in Weed and Nematode Management in CA Systems

As described in Chapter 6.2, the termination of grass-clover and the sowing of living mulches and wheat are crucial management steps that need further improvement in organic farming systems. Hence, grass-clover should be terminated at least one month earlier via shallow undercutting (3 cm) followed by shallow rotary hoeing (3-5 cm) two weeks later aiming for better suppression of perennial grasses. Consequently, living mulches can be sown in early September followed by direct drilling of winter wheat in October. If successful, establishment of living mulches may reduce seedbanks of *Veronica* spp., *G. aparine,* and *Lamium* spp. and therefore the overall competition by weeds. Another approach is the use of cover crops after grass-clover. Therefore, grass-lover ley should be terminated in June/July followed by the sowing of a highly competitive and productive cover crop, such as the mixture of oilseed radish radish and black oat or rye-vetch mixtures. One of the pioneers of organic zero tillage in temperate agro-ecosystems, the Rodale Institute in Pennsylvania, U.S.A. (http://rodaleinstitute.org), suggests that cover crops should yield a minimum of 8 t ha^{-1} dry matter to obtain adequate weed control. These cover crops should be terminated with a roller crimper followed by direct drilling of wheat with inter-row distances of 15 cm in contrast to 30 cm used in this study. If sufficient biomass produced by cover crops, adequate weed control may be obtained until spring, when the winter wheat is competitive enough to control weeds by itself. In addition, Mennan and Zandstra (2005) showed that specific wheat varieties, such as ‘Bezostaja’, were more efficient in control of *G. aparine* than others, indicating that the choice of farming system adapted varieties, species mixtures, or composite crosses may further contribute to weed control.

In case of living mulch failures, cover crops should be sown rather than keeping the green fallow. Here, leguminous cover crops should be preferred if nitrogen supply to the subsequent cash crop is important. In contrast, oilseed radish and black oat cover crops (or mixtures of both) should be grown if the overall goal is soil nitrogen uptake and nematode suppression. Both species helped reduce the root-lesion nematode *Pratylenchus* which may become a significant pest in wheat-maize rotations (Chapter 5). However, independent of the choice, cover crops should be sown directly after wheat harvest to maximize their effects before winter. This will also guarantee a maximum of weed suppression, improve soil properties, such as structure, organic matter contents, and microbial activity, and reduce the risk of soil erosion. Although all these benefits were also achieved by mulch application to the potatoes, the large amount of material (19 t ha^{-1} dry matter) needed for the 8-10 cm deep layer in this study appears impractical. (i) It is difficult to get enough mulch material in early May under mid-European temperate conditions and therefore, about 3 ha grown with mulch crops are needed to mulch 1 ha potatoes. The situation may be even worse in agricultural production systems based on animal husbandry in which these crops are rather used as animal fodder than as mulch. (ii) The total nitrogen input can be extremely high (up to 350 kg total N ha^{-1}) and will be available to plants as nitrate also after potato harvest; nitrate leaching is therefore very likely throughout fall and winter periods.

Possible solutions to overcome these limitations are: (i) both, mulch amounts and shredder lengths should be reduced to provide 100% soil cover after application for sufficient weed control; (ii) application of silage or hay mulch which can be prepared the year before in sufficient amounts (iii) the mulch compounds should have a rather large C:N ratio to avoid rapid decay while a legume winter cover crop should be grown before potatoes for sufficient nitrogen supply.

7 General Conclusion

Most long-term experiments usually lack initial data, complicating comparisons of future recorded data with the status quo. While the transition process from conventional to organic agriculture has been sufficiently researched, biological, chemical, and physical processes during the transition to CA in long-term organic fields are largely unknown. For this reason, weeds and (plant-parasitc) nematodes were selected, not only as they are major threats in organic and conventional agriculture, but also as typical indicators that (i) can be easily monitored, also in the long-term, without the need of specific host crops in the rotation; (ii) appeared to react rapidly on farming sytem adaptations; and (iii) are standard organisms in the CA research community, simplifying comparisons with other studies which is important when a new research field is studied.

Although the present work solely provided detailed information about initial weed and plant-parasitic nematode populations, many assessments have been conducted that were not included in this thesis. For example, the status quo of many soil physical, chemical and biological parameters, crop yields, pest and diseases, and perennial weeds have been recorded creating a playground for future soil scientists, phytopathologists, agronomists and economists.

Furthermore, the results of this study provide excellent future research perspectives as the number of long-term experiments dealing with CA in Europe is low, in particular those managed organically (Peigné et al., 2015). In addition, CA in organic agriculture is a particularly new research field in Europe, established since 1990 (Mäder & Berner, 2012) with many open questions that need to be adressed. For example, according to Figure 1.1 (Chapter 1), the CA transition phase ends 6-7 years after adoption with generally higher outputs than before transition. However, can this be generalized for temperate European agriculture and under organic management? If not, how long will the transition phase last and will an agricultural equilibrium be reached at all? Which limitations will occur in the long-term?

This thesis clearly showed that on the one hand, the root-lesion nematode *Pratylenchus* and the annual weed species *Galium aparine* may become important threats to organic CA systems based on legume subsidiary crops. On the other hand, this thesis also revealed sufficient sanitation measures, such as oilseed radish and black oat cover crops for *Pratylenchus* control. Furthermore, *G. aparine,* which primarily sprouts in fall, may be controlled by higher rates of spring crops in the rotation, productive cover crops before sowing of fall crops, and successful establishment of living mulches in fall sown crops.

General Conclusion

In conclusion, organic matter management that includes frequent application of organic fertilizer, green manures, living and dead mulches, and fertility building leys, plays a key role for the successful transition to CA in organic agriculture. However, soils and climates determine site specific cropping systems and therefore, organic matter management in CA systems should be adjusted to the local conditions.

8 Summary

Soil conservation is one of the major challenges for agriculture in the 21st century. For this reason, non-inversion tillage systems including subsidiary crops have become popular over the last three decades in Europe. However, the adoption of new agricultural practices may change the diversity and abundance of certain pests and diseases. For example, one of the major obstacles to conservation agriculture (CA), particularly in organic farming, is a large increase in weed populations and seed banks following transition to non-inversion tillage. Although subsidiary crops, either undersown in main crops or sown subsequently may help to suppress weeds, they may also benefit plant-parasitic nematodes with a broad host spectrum.

In this study, the focus is on the first 2 to 4 years after transitioning to CA in long term organically managed fields in a typical organic wheat-potato cropping sequence following grass-clover. Weed and plant-parasitic nematode communities were studied as they are good indicators of the degree of farming system sustainability. In Chapter 5, the effects of CA during the transition on plant-parasitic nematodes were compared among four agri-environments throughout Europe in organically and conventionally farming systems.

Experimental factors were either conventional or conservation agriculture systems based on moldboard (30 cm) and chisel ploughing (5-15 cm) with dead mulch application (8-10 cm) to potatoes, respectively. In both systems, 5 t of high quality yard waste compost (ha year)$^{-1}$ were applied as an organic amendment. Furthermore, subsidiary crops were grown in both systems, either as legume living mulches undersown in wheat or as cover crops sown after wheat.

Prior to sowing the wheat and after potatoes, the soil weed seed bank from 0-15 and from 15-30 cm was sampled and assessed in an unheated glasshouse over nine months. Weed biomass and soil cover were assessed several times during the wheat-subsidiary crop-potato cropping sequence. Plant-parasitic nematodes were assessed and counted from soil samples taken before wheat, after wheat, after subsidiary crops, and after potatoes.

The initial weed seed bank size in the topsoil after grass-clover was uniform (4,420 seedlings m^{-2}) and never increased statistically significant in CA systems based on cover crops (5,723 seedlings m^{-2}). However, high weed infestations, also due to volunteer crops, were observed in wheat under non-inversion tillage. This resulted in large seed banks of winter wheat associated weeds, such as *Galium aparine, Lamium* spp., and *Myosotis arvensis*, after potatoes under non-inversion tillage. In contrast, non-inversion tillage with mulch application to potatoes reduced seed banks of *Chenopodium album.*

Summary

It is likely that weeds under non-inversion tillage were also responsible for the higher densities of plant-parasitic nematodes with a broad host spectrum, such as *Pratylenchus spp.*, in this system compared with conventional tillage. Although subsidiary crops differently affected plant-parasitic nematodes, the results were consistent among the four agri-environments studied. In general, leguminous subsidiary crops and wheat-maize rotations increased plant-parasitic nematodes with a broad host spectrum (*Pratylenchus*). In contrast, *Pratylenchus* densities were lowest after growing oilseed radish and black oat subsidiary crops which may therefore provide an alternative nematode control option.

The present study therefore highlights the need for adapted weed control in organic CA systems. This can be achieved by the use of productive and competitive subsidiary crops in rotations.

In conclusion, organic matter management, that includes frequent application of organic fertilizer, green manures, living and dead mulches, and fertility building leys, plays a key role for the successful transition to CA in organic agriculture. However, soils and climates determine site specific cropping systems and therefore, organic matter management in CA systems should be adjusted to the local conditions.

9 Zusammenfassung

Der Bodenschutz ist eine der wichtigsten Anforderungen an die Landwirtschaft im 21. Jahrhundert. Daher sind nicht-wendende Bodenbearbeitungssysteme, basierend auf dem Anbau von Untersaaten und Zwischenfrüchten, verstärkt in den Fokus der europäischen Agrarwirtschaft geraten. Allerdings können diese neuartigen Systeme das Spektrum und die Abundanz gewisser Krankheiten, Schädlinge und Unkräuter grundlegend verändern. Ein Beispiel ist die Zunahme von Unkräutern und ihren Samenbanken während der Übergangsphase von gepflügten zu pfluglosen Anbausystemen, die vor allem die ökologische Landwirtschaft vor große Probleme stellt. Hier können Untersaaten, etabliert zum Beispiel in Winterweizen oder Mais, und Zwischenfrüchte nachhaltig zur Unkrautreduktion beitragen. Die somit erhöhte Pflanzenproduktion kann allerdings pflanzenparasitäre Nematoden mit breiten Wirtsspektren fördern und einen Populationsanstieg über die Zeit bedeuten.

Die vorliegende Arbeit beschreibt die Auswirkungen von pfluglosem Anbau in den ersten 2-4 Jahren nach der Umstellung von einem gepflügten System. Das Hauptaugenmerk liegt dabei auf Unkräutern und pflanzenparasitären Nematoden in zwei langfristig ökologisch bewirtschafteten Feldern. In diesem Zeitraum wird eine Winterweizen-Kartoffel Fruchtsequenz nach zweijährigem Kleegras ausgewertet. In Kapitel 5 wird die Auswirkung der Umstellung auf pfluglose Anbausysteme auf pflanzenparasitäre Nematoden in vier europäischen Agrarumwelten unter ökologischem oder konventionellem Management evaluiert.

Experimentelle Faktoren waren gepflügte (30 cm) versus gegrubberte (5-15) Anbausysteme. Die Grubbervariante sah zusätzlich eine Totmulchauflage (Transfermulch) zu Kartoffeln vor. Auf die Hälfte der Parzellen der jeweiligen Anbausysteme wurden 5 t (ha Jahr)$^{-1}$ eines Grüngutkompostes appliziert. Zusätzlich wurden in beiden Bodenbearbeitungssystemen entweder Untersaaten im Winterweizen oder Zwischenfrüchte nach Weizen etabliert.

Zur Bestimmung der Unkrautsamenbank wurde der Boden in 0-15 und 15-30 cm Tiefe beprobt und die auflaufenden Unkräuter über 9 Monate in einem Kalthaus bestimmt. Die Unkrautbiomassen und -deckungsgrade wurden mehrmals in der Fruchtsequenz bestimmt. Pflanzenparasitäre Nematoden wurden aus Bodenproben vor Weizen, nach Weizen, nach Untersaaten/ Zwischenfrüchten und nach Kartoffeln extrahiert, bestimmt und gezählt.

In pfluglosen Systemen basierend auf Zwischenfrüchten konnte kein statistisch signifikanter Anstieg der Unkrautsamenbank im Oberboden festgestellt werden. Bedingt durch den Durchwuchs der Vorfrucht war der Unkrautbefall im Winterweizen in pfluglosen Systemen allerdings sehr viel höher als nach der Bearbeitung mit dem Pflug. Dieses führte zu einem

verstärkten Auftreten von mit Winterweizen assoziierten Unkräutern, z.B. *Galium aparine, Lamium* spp. und *Myosotis arvensis,* in der Samenbank nach Kartoffeln. Dagegen führte die Mulchauflage zu Kartoffeln in pfluglosen Systemen zu einer geringeren Samenbank von *Chenopodium album* im Vergleich zur gepflügten Variante.

Das erhöhte Unkrautvorkommen in pfluglosen Systemen förderte möglicherweise den Befall mit pflanzenparasitären Nematoden mit breiten Wirtsspektren, z. B. *Pratylenchus* spp., verglichen mit den gepflügten Systemen. Die Auswirkungen der Untersaaten und Zwischenfrüchte auf pflanzenparasitäre Nematoden waren konsistent in allen untersuchten Agrarumwelten. Leguminosen und Weizen-Mais Fruchtsequenzen führten zu erhöhten Dichten von *Pratylenchus,* während dieser Erreger durch die Zwischenfrüchte Ölrettich und Rauhafer reduziert werden konnte.

Der Bedarf nach angepassten Unkrautkontrollstrategien für den ökologischen Anbau in pfluglosen Systemen geht aus dieser Arbeit eindeutig hervor. Der Anbau von produktiven und konkurrenzstarken Zwischenfrüchten/ Untersaaten steht dabei im Vordergrund.

Zusammenfassend kann dem Humusmanagement, das organische Dünger, Gründüngungen, Lebend- und Totmulche sowie mehrjährige Graslandwirtschaft einschließt, eine Schlüsselrolle zur erfolgreichen Umstellung auf pfluglose Bearbeitungssysteme in der ökologischen Landwirtschaft zugeordnet werden. Dieses muss allerdings den lokalen Boden- und Klimabedingungen angepasst werden, da diese das jeweilige Anbausystem bestimmen.

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11 Acknowledgements

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With all my gratitude,
Jan
.

12 Appendix

Chapter 2:

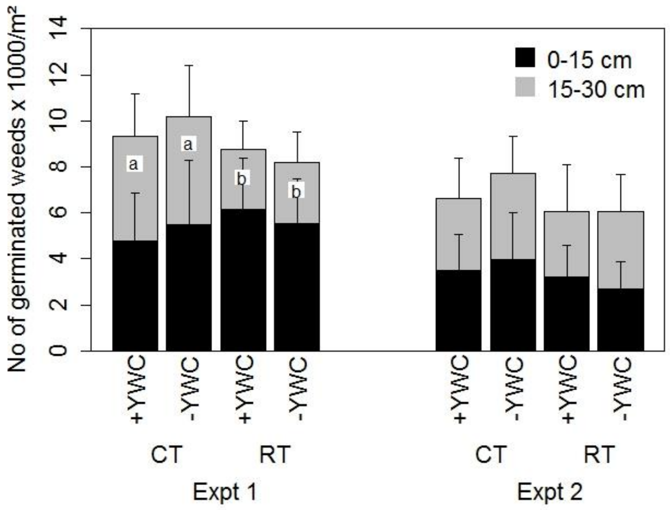


Figure A 1: Mean (SD) number of weed seedlings m^{-2} emerging from the seed bank as affected by soil depth, tillage, and compost. Different letters indicate statistical differences per depth at P<0.05 (HSD-Test, df=59).

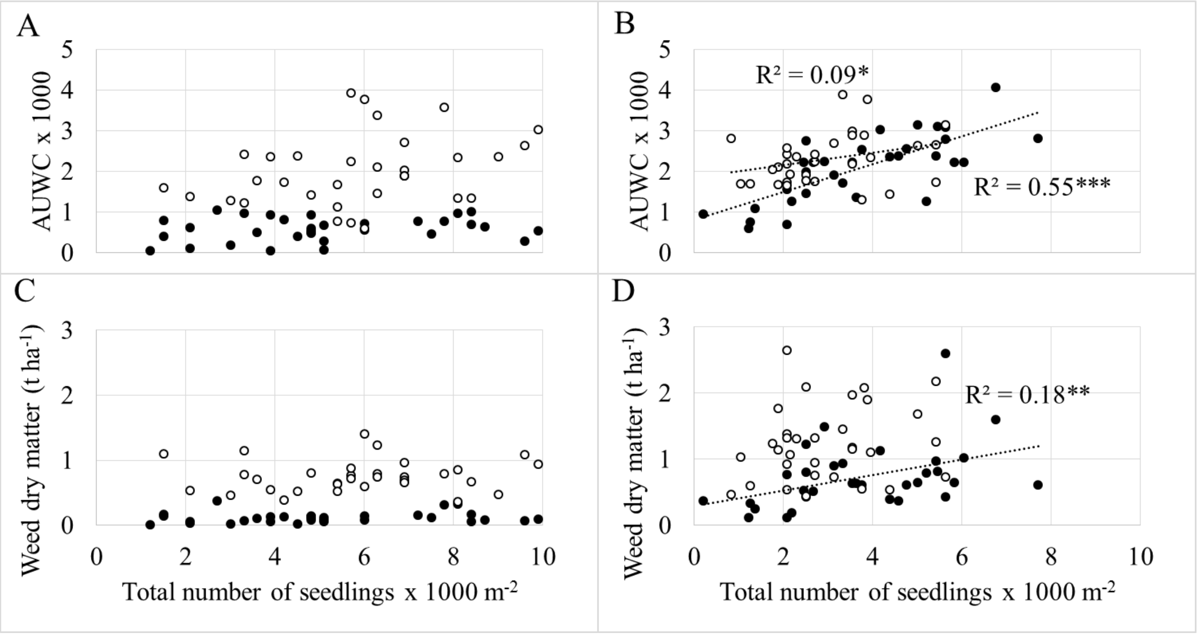


Figure A 2: Seedling density in the top 15 cm in the cold house versus area under the weed cover curve (AUWC; A, B) and weed dry matter in the field (C, D) for Expt1 (A,C) and Expt2 (B,D). Filled points: CT, empty points: RT management. Significant correlations are shown with dotted lines. *, **, *: correlations significant at *P*<0.05, <0.01, and <0.001, respectively. Kendals tau rank correlation (df=30).**

Appendix

Chapter 3:

Statistical outputs were explained in bold letters.

a) Redundancy analysis with Relative abundance data after arc-sin transformation:

```
> #top15 cm
> par(mar= c(5,4.5,2,1.5))
> par(mfrow=c(1,1))
> nema.cca <- rda(top15asin1[,9:23] ~ Condition(Exp) +Condition
(Rep)+factorcombi, data = top15asin1)
```

The model is saved under nema.cca and with a permutation test it can be checked whether used factors have an influence on weeds. Factorcombi means the combination of Tillage and samoling time. Conditional variables were experimental field/year (Exp) and repetitions (Rep).

```
> anova.cca(nema.cca, step = 999)
Permutation test for rda under reduced model
Permutation: free
Number of permutations: 999

Model: rda(formula = top15asin1[, 9:23] ~ Condition(Exp) + Condition(Rep) +
factorcombi, data = top15asin1)
          Df Variance      F Pr(>F)
Model      3 0.021035 20.543  0.001 ***
Residual 248 0.084646
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

We can see that the factor combination significantly influence weeds

```
> anova.cca(nema.cca, step = 999, by="terms")
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Permutation: free
Number of permutations: 999

Model: rda(formula = top15asin1[, 9:23] ~ Condition(Exp) + Condition(Rep) +
factorcombi, data = top15asin1)
             Df Variance      F Pr(>F)
factorcombi   3 0.021035 20.543  0.001 ***
```

Appendix

```
Residual    248 0.084646
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

If there are more than one factor applied to the formula, this Anova output will show the significant factors. However, as there is only one factor, it simply shows the same results as for the analysis above.

```
> anova.cca(nema.cca, step = 999, by="axis")
Permutation test for rda under reduced model
Marginal tests for axes
Permutation: free
Number of permutations: 999

Model: rda(formula = top15asin1[, 9:23] ~ Condition(Exp) + Condition(Rep) + factorcombi, data = top15asin1)
          Df Variance       F Pr(>F)
RDA1       1 0.019119 56.0144  0.001 ***
RDA2       1 0.001533  4.4925  0.005 **
RDA3       1 0.000383  1.1219  0.506
Residual 248 0.084646
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

With this formula, we can see which RDA axis explain a significant part. Both, RDA1 and 2 are significant and should therefore be shown in the figure (Figure 3.1)

```
> #nema.cca <- rda(top15cm[,7:21] ~ Condition(Exp) +factorcombi+Condition(compost), data = top15cm)
> head(summary(nema.cca), tail=2)
The function”head” gives the RDA output in a shorter version (see below)

Call:
rda(formula = top15asin1[, 9:23] ~ Condition(Exp) + Condition(Rep) + factorcombi, data = top15asin1)
```

This table below is one of the most important outputs: It shows that the conditional variables (Exp, Rep) and our factor (factorcombi) explain 20 % and 15.9 % of the total variance, respectively.

```
Partitioning of variance:
              Inertia Proportion
```

Appendix

```
Total         0.13224     1.0000
Conditioned   0.02656     0.2008
Constrained   0.02103     0.1591
Unconstrained 0.08465     0.6401
```

Below, Eigenvalues are shown and their contribution to the variance after removing the contribution of conditiniong variables: Here we can see that the proportion explained by the first (RDA1) and second axes (RDA2) are 18.1 and 1.5% of the total variance. The third axis is explaining 0.36 % of the total variance which further explains why this axis was not significant according to the permutation test (see above).

```
Importance of components:
                        RDA1      RDA2       RDA3      PC1       PC2      PC3
PC4      PC5      PC6      PC7     PC8      PC9     PC10     PC11
Eigenvalue             0.01912 0.001533 0.0003829 0.02776 0.009397 0.00695
0.005792 0.004972 0.004627 0.004322 0.00408 0.003734 0.003059 0.002863
Proportion Explained  0.18091 0.014510 0.0036200 0.26266 0.088910 0.06576
0.054810 0.047050 0.043780 0.040890 0.03861 0.035330 0.028950 0.027090
Cumulative Proportion 0.18091 0.195420 0.1990400 0.46170 0.550620 0.61638
0.671190 0.718230 0.762010 0.802910 0.84152 0.876850 0.905800 0.932890
                         PC12     PC13     PC14      PC15
Eigenvalue            0.002738 0.002283 0.001383 0.0006888
Proportion Explained  0.025910 0.021600 0.013090 0.0065200
Cumulative Proportion 0.958800 0.980400 0.993480 1.0000000

Accumulated constrained eigenvalues
Importance of components:
                        RDA1     RDA2      RDA3
Eigenvalue            0.01912 0.001533 0.0003829
Proportion Explained  0.90890 0.072900 0.0182000
Cumulative Proportion 0.90890 0.981800 1.0000000
```

The table above shows how much of the constrained variance is explained by the first three axes.

```
Scaling 2 for species and site scores
* Species are scaled proportional to eigenvalues
* Sites are unscaled: weighted dispersion equal on all dimensions
* General scaling constant of scores:  2.40976
```

Appendix

The Table below shows the species scores which indicate how each weed species is plotted in a more dimensional room with RDA1, RDA2, and RDA3 meaning the x,y, and z-axes in a coordinate system. This is pictured in Figure 3.1

```
Species scores

                    RDA1      RDA2       RDA3      PC1      PC2      PC3
APHAR           0.114185 -0.064898  0.0347644  0.21825  0.29087 -0.09607
CAPBU          -0.228205  0.001474 -0.0004677 -0.49075  0.13968  0.16845
CHEAL          -0.326402  0.162282  0.0121540 -0.63223 -0.25881 -0.20818
GALAP          -0.180748 -0.081886  0.0095224 -0.08967  0.02844  0.08453
LAMIUM.spp.     0.019648 -0.097681 -0.0173083  0.14593  0.08345 -0.09858
MAT            -0.001841  0.066718  0.0849299 -0.06912  0.28525  0.12111
....
Veronica.spp. -0.312483 -0.001454 -0.0473071  0.36819 -0.35407  0.29977
VIOAR           0.069851  0.003481  0.0115574  0.35220 -0.01606 -0.06751
```

Likewise, site scores show coordinates for each experimental plot which is analysed with the permutation test and which can all be shown in a figure (however as there are so many plots involved, 256 for the 0-15 cm depth, this makes no sense to show them in a graph). The same accounts for Site constraints which are differently calculated.

```
Site scores (weighted sums of species scores)

          RDA1    RDA2     RDA3      PC1      PC2      PC3
1     -0.24714  0.4819  0.23240 -0.14826 0.053504 -0.11420
2     -0.21582 -0.5679  0.97241  0.04004 0.366602  0.33095
3     -0.15206 -0.1205  0.05489  0.07504 0.046666  0.31211
4     -0.26552  0.9927  0.90008 -0.21962 0.005808 -0.02691
5     -0.20577  0.2500 -0.08274 -0.20231 0.094907  0.04603
6     -0.24496 -0.1664  0.67314 -0.06723 0.208783  0.34722
....
575    0.15470  0.7400  0.21636 -0.17900 0.012573  0.02189
576    0.07378 -0.1975  0.18680 -0.12662 0.209982  0.09373
```

```
Site constraints (linear combinations of constraining variables)

         RDA1    RDA2     RDA3      PC1      PC2      PC3
```

Appendix

```
1    -0.1497 0.20467  0.06115 -0.14826 0.053504 -0.11420
2    -0.1497 0.20467  0.06115  0.04004 0.366602  0.33095
3    -0.1497 0.20467  0.06115  0.07504 0.046666  0.31211
4    -0.1497 0.20467  0.06115 -0.21962 0.005808 -0.02691
5    -0.1497 0.20467  0.06115 -0.20231 0.094907  0.04603
6    -0.1497 0.20467  0.06115 -0.06723 0.208783  0.34722
....
575   0.1396 0.05505 -0.21339 -0.17900 0.012573  0.02189
576   0.1396 0.05505 -0.21339 -0.12662 0.209982  0.09373
```

Biplot scores (below) are only for metric variables, they are indicated in the coordinate system by arrows that start from point (0,0,0).

```
Biplot scores for constraining variables

                                         RDA1    RDA2    RDA3 PC1 PC2 PC3
factorcombiconventional tillage Start  0.6181 -0.1998  0.7603   0   0   0
factorcombireduced tillage End        -0.5792 -0.7958 -0.1767   0   0   0
factorcombireduced tillage Start       0.5351  0.2110 -0.8180   0   0   0
```

This is the most important table (below); it shows where each of our factor levels is arranged in the coordinate system. Here we can already see that the most imporatant factor was the sampling time (separated on the RDA1 which explains the largest part of variance). See Figure 3.1.

Centroids for factor constraints

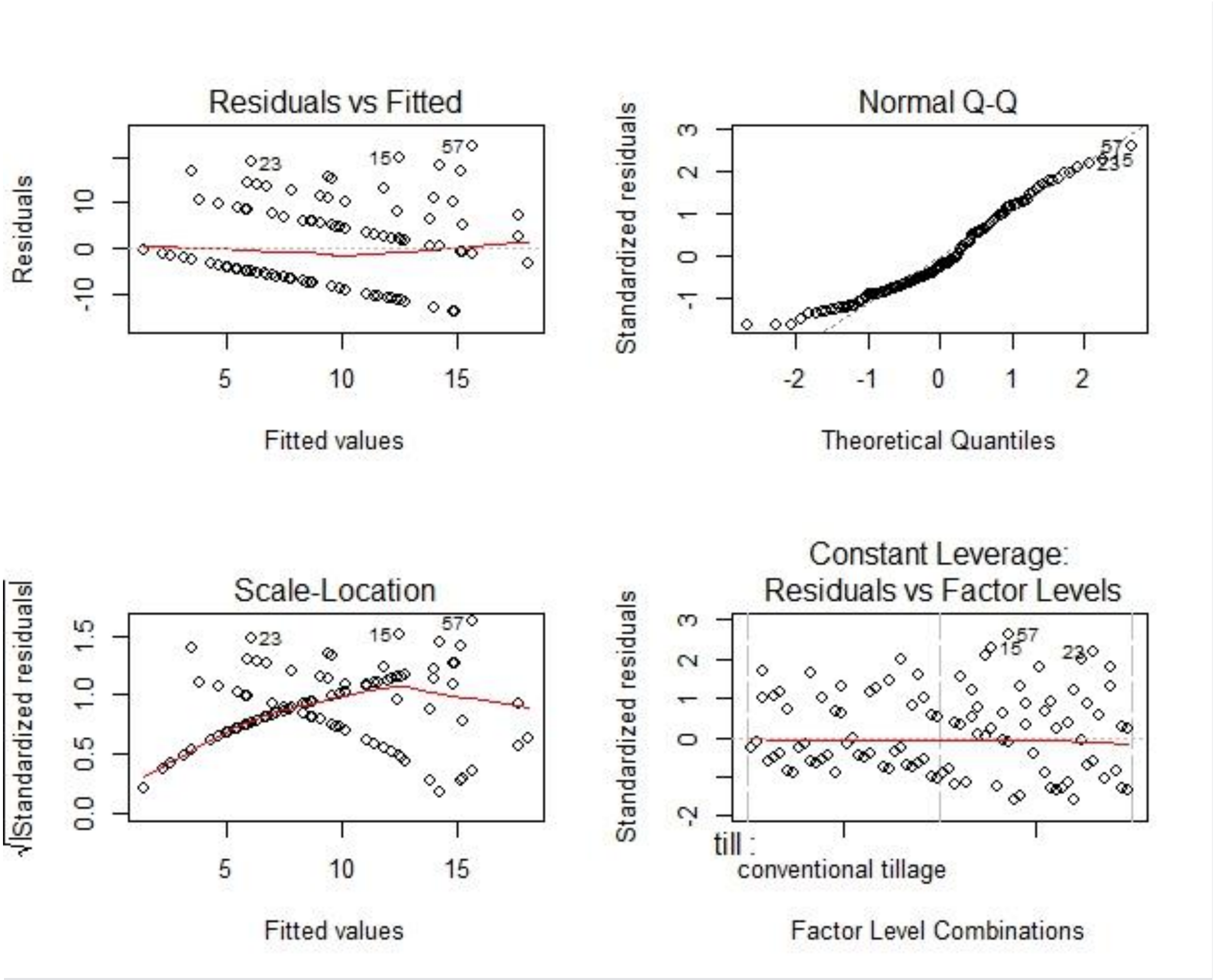
```
                                         RDA1     RDA2     RDA3 PC1 PC2 PC3
factorcombiconventional tillage End   -0.1497  0.20467  0.06115   0   0   0
factorcombiconventional tillage Start  0.1612 -0.05213  0.19833   0   0   0
factorcombireduced tillage End        -0.1511 -0.20759 -0.04609   0   0   0
factorcombireduced tillage Start       0.1396  0.05505 -0.21339   0   0   0
```

Appendix

`b) 3-factorial Anova Script`

First we build a linear model to investigate the requirements for ANOVA:

```
lm_GALAP <- lm(GALAP~ till*sc.1*compost+Exp+Rep)
> par(mfrow=c(2,2))
> plot(lm_GALAP)
```



Variances are not very homogenous and normal distribution can be neglected. However, according to recent publications normal distribution is not required for ANOVA any more if a balanced design is used (which we have: each factor combination has the same number of repetitions). Thus, there is a need to look closer at variances. Variances between treatment levels are allowed to vary maximum by 10-fold:

```
> tapply(lm(GALAP~ till*sc.1*compost+Exp+Rep)$residuals,list(till,sc.1,compost),var)
```

Appendix

```
, , compost

                       CC    Fallow
conventional tillage 51.43159  46.86112
reduced tillage      80.30389 123.23853

, , without compost

                        CC   Fallow
conventional tillage  46.01908 66.58297
reduced tillage      128.09102 95.57815
```

Thus, the lowest variance is at conventional tillage, with cover crops, and without compost (46.02), while the highest is at reduced tillage, with covercrops, and without compost (128.09) which is not 10-fold as high as the lowest variance. Therefore, ANOVA can be used....

```
> summary(aov(GALAP~ till*sc.1*compost+Error(Exp/Rep/till/sc.1)))

Error: Exp
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1  185.7   185.7

Error: Exp:Rep
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals  6  552.9   92.14

Error: Exp:Rep:till
          Df Sum Sq Mean Sq F value Pr(>F)
till       1   1192  1191.8   11.12 0.0125 *
Residuals  7    750   107.1
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix

```
Error: Exp:Rep:till:sc.1
          Df Sum Sq Mean Sq F value Pr(>F)
sc.1       1   41.4    41.4   0.284  0.602
till:sc.1  1   41.4    41.4   0.284  0.602
Residuals 14 2039.1   145.7

Error: Within
                  Df Sum Sq Mean Sq F value Pr(>F)
compost            1     27   26.51   0.368  0.545
till:compost       1      3    2.97   0.041  0.839
sc.1:compost       1     45   45.28   0.629  0.430
till:sc.1:compost  1    132  132.03   1.835  0.179
Residuals         92   6620   71.96
```

The experimental field/year (Exp) and the repetition (Rep) are inserted as co-variables which take some variance out of the data set. The factor tillage (till) is significant at *P=0.0125,* while subsidiary crops (sc.1) and composts are not. No interactions between treatments were observed.

Experiment 4:

Effect of *Pratylenchus* on Grain and Tuber Yields

Pratylenchus densities before wheat varied between 24 and 660 nematodes 100 ml soil^{-1} while between 20 and 900 individuals 100 ml soil^{-1} were observed before planting of potatoes (Figure A 3). Wheat grain yields were considerably lower under non-inversion tillage while on average no yield differences occurred for potatoes. Negative correlations between *Pratylenchus* densities and yields of both crops did not occur, although initial numbers were partially high, in particular before potatoes. Tillage had no effect on correlations. Likewise, no negative correlations were found between other nematode genera and crop yields (data not shown).

Appendix

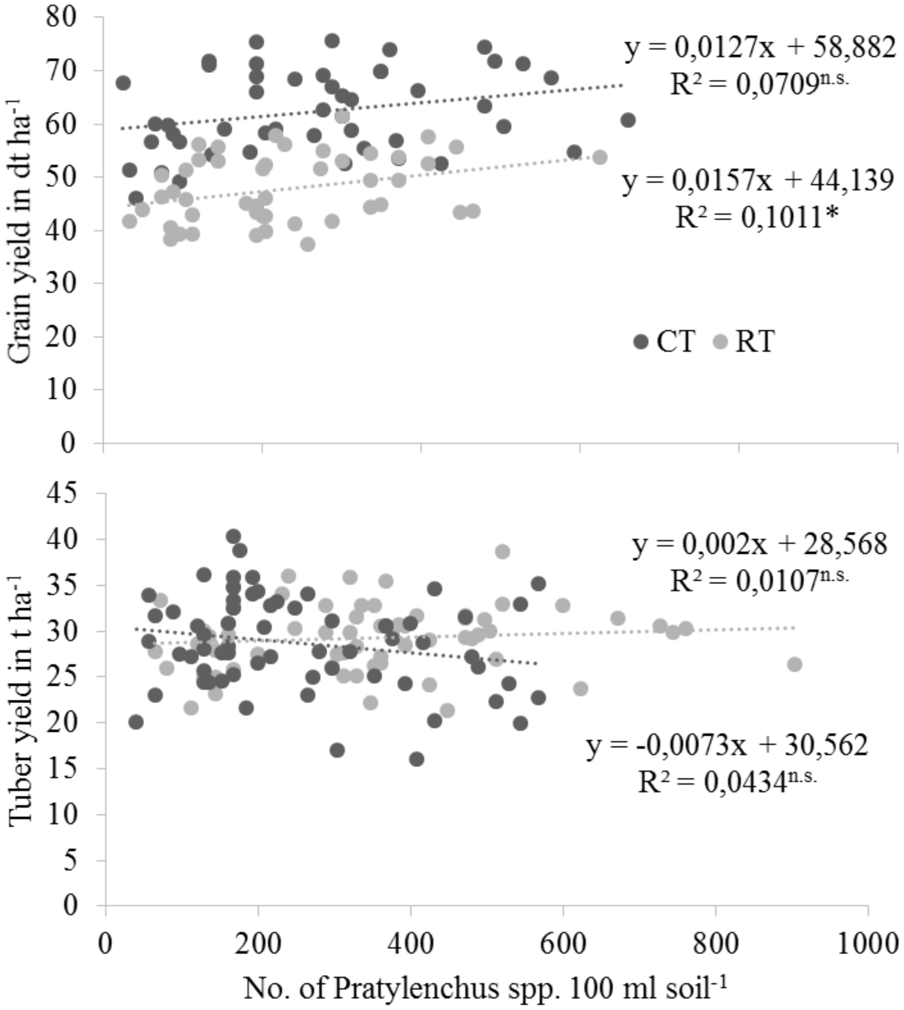


Figure A 3 Correlation of *Pratylenchus* densities prior to crop sowing and wheat grain or potato tuber yields under conventional (dark grey cirlces) non-inversion (light grey circles) tillage across both experimental years; *, **, and * indicate whether the correlation is significant at *P*<0.05, *P*< 0.01, *P*<0.001 or not (n.s.) via Kendall's-Tau rank correlation**

Statistics:

PAIRWISE COMPARISON OF TREATMENTS AFTER KRUSKAL-WALLIS

The Kruskal-Wallis test was used to compare different tillage and subsidiary crop treatments according to the total nematode density initially, after wheat, after subsidiary crops, and after potatoes.

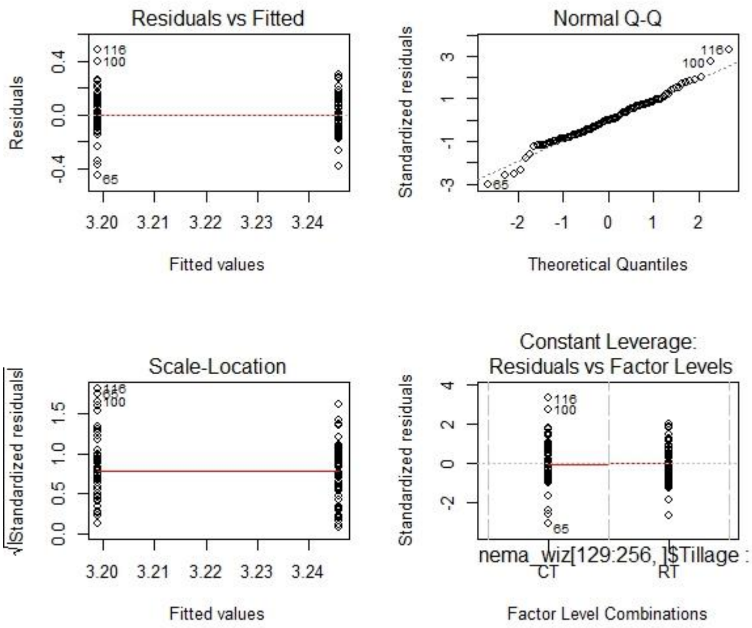
AFTER wheat:

first we check for standard deviation and variance homogeneity

```
par(oldpar)
```

Appendix

```
par(mfrow= c(2,2))
wheat_till<-lm(nema_wiz[129:256,]$ALLlog ~ nema_wiz[129:256,]$Tillage)
plot(Wheat.aov)
```



we can see that both anova requirements are not given: Kruskal-Wallis test:

```
> library(agricolae)
> kruskal.test(nema_wiz[129:256,]$ALL, nema_wiz[129:256,]$Tillage)

        Kruskal-Wallis rank sum test

data:  nema_wiz[129:256, ]$ALL and nema_wiz[129:256, ]$Tillage
Kruskal-Wallis chi-squared = 3.865, df = 1, p-value = 0.0493
```

Pairwise comparison:

```
> x <-kruskal(nema_wiz[129:256,]$ALL, nema_wiz[129:256,]$Tillage)
> x
$statistics
    Chisq   p.chisq      LSD
  3.864952 0.0493046 12.82764
```

Appendix

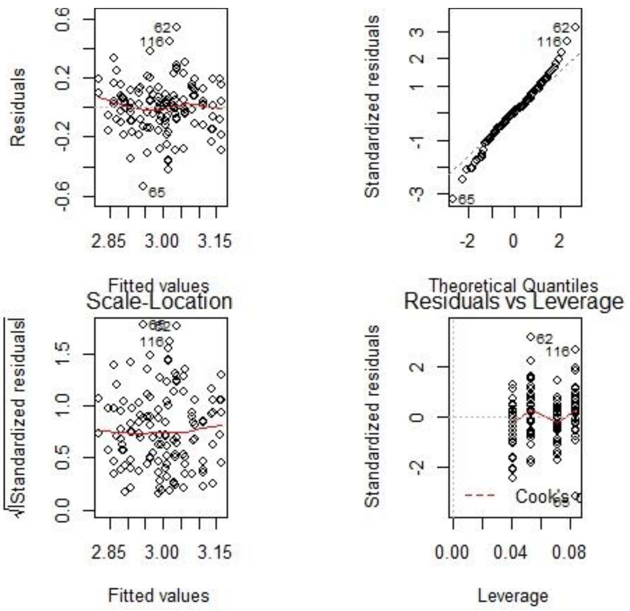
```
$parameters
  Df ntr  t.value alpha           test                    name.t
   1   2 1.978971  0.05 Kruskal-Wallis nema_wiz[129:256, ]$Tillage

$means
       rank nema_wiz.129.256....ALL      std  r Min  Max
CT 58.05469                1694.812 703.6310 64 560 4860
RT 70.94531                1846.125 579.2089 64 720 3516

$comparison
NULL

$groups
  trt    means M
1  RT 70.94531 a
2  CT 58.05469 b
```

RT and CT are different at P<0.049 (p. chisq). The LSD is 12.82 between both treatments. The Kruskal wallis test is not working with conditional variables (repetition) so we cannot include our strip-strip-plot design.

After Subsidiary Crops:

```
> SC_till<-lm(nema_wiz[257:384,]$ALLlog ~
nema_wiz[257:384,]$Tillage*nema_wiz[257:384,]$SC+nema_wiz[257:384,]$Exp+nema_wi
z[257:384,]$Rep)
> plot(SC_till)
```

Appendix



Anova requirements not given:

```
> kruskal.test(nema_wiz[257:384,]$ALLlog, nema_wiz[257:384,]$Tillage)


        Kruskal-Wallis rank sum test


data:  nema_wiz[257:384, ]$ALLlog and nema_wiz[257:384, ]$Tillage
Kruskal-Wallis chi-squared = 4.8901, df = 1, p-value = 0.02701


> x <-kruskal(nema_wiz[257:384,]$ALLlog, nema_wiz[257:384,]$Tillage)
> x
$statistics
     Chisq    p.chisq      LSD
  4.890081 0.02701143 12.77437


$parameters
  Df ntr  t.value alpha           test                    name.t
   1   2 1.978971  0.05 Kruskal-Wallis nema_wiz[257:384, ]$Tillage


$means
    rank nema_wiz.257.384....ALLlog       std  r      Min      Max
CT 57.25                   2.961362 0.1743026 64 2.409933 3.466719
```

```
RT 71.75             3.038961 0.2011397 64 2.594393 3.580355

$comparison
NULL

$groups
  trt means M
1  RT 71.75 a
2  CT 57.25 b
```

Building subsets to evaluate effects of cover crops and fallows in each tillage system

```
> wheat_CT_SC <-subset(nema_wiz[257:384,],nema_wiz[257:384,]$Tillage=="CT")
> wheat_RT_SC <-subset(nema_wiz[257:384,],nema_wiz[257:384,]$Tillage=="RT")
> x <-kruskal(wheat_CT_SC$ALLlog, wheat_CT_SC$SC)
> x

$statistics
     Chisq   p.chisq
  2.055231 0.3578593

$parameters
  Df ntr  t.value alpha           test         name.t
   2   3 1.999624  0.05 Kruskal-Wallis wheat_CT_SC$SC

$means
       rank wheat_CT_SC.ALLlog        std  r      Min      Max
C  35.20312           2.982106 0.17945690 32 2.557507 3.281715
L  32.56250           2.960912 0.09344767 16 2.742725 3.073718
NL 27.03125           2.920325 0.22270689 16 2.409933 3.466719

$comparison
NULL

$groups
```

Appendix

```
  trt    means M
1   C 35.20312 a
2   L 32.56250 a
3  NL 27.03125 a

> x <-kruskal(wheat_RT_SC$ALLlog, wheat_RT_SC$SC)
> x
$statistics
     Chisq   p.chisq
  4.300785 0.1164384

$parameters
  Df ntr  t.value alpha           test          name.t
   2   3 1.999624  0.05 Kruskal-Wallis wheat_RT_SC$SC

$means
       rank wheat_RT_SC.ALLlog       std  r      Min      Max
C  35.04688           3.065464 0.2341170 32 2.594393 3.580355
L  35.75000           3.061912 0.1471938 16 2.755112 3.348889
NL 24.15625           2.963005 0.1635979 16 2.703291 3.370143

$comparison
NULL

$groups
  trt    means M
1   L 35.75000 a
2   C 35.04688 a
3  NL 24.15625 a
```

After Potatoes:

qq.plot:

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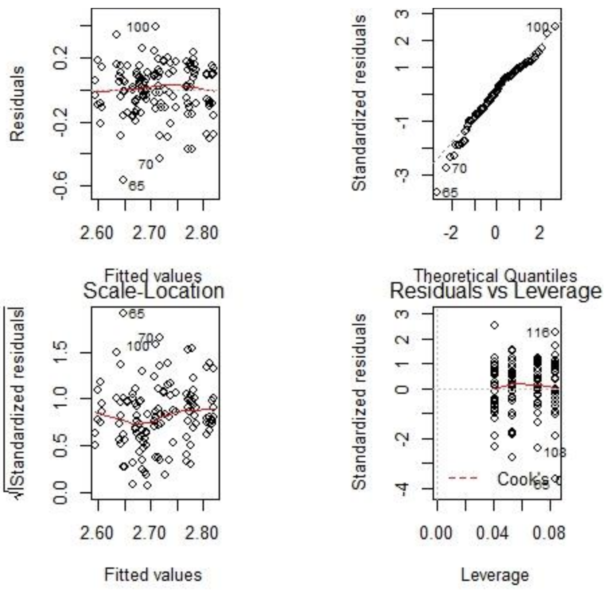

Anova requirements not given: Kruskal-Wallis test

$P<0.02$ for the factor tillage:

```
x <-kruskal(nema_wiz[385:512,]$ALLlog, nema_wiz[385:512,]$Tillage)
> x
$statistics
    Chisq    p.chisq      LSD
  6.165742 0.01302478 12.70703


$parameters
  Df ntr  t.value alpha           test                    name.t
   1   2 1.978971  0.05 Kruskal-Wallis nema_wiz[385:512, ]$Tillage


$means
       rank nema_wiz.385.512....ALLlog       std  r       Min       Max
CT 56.35938                   2.678449 0.1715891 64 2.082785 3.104828
RT 72.64062                   2.754534 0.1569968 64 2.352183 3.012415


$comparison
NULL


$groups
```

Appendix

```
  trt    means M
1  RT 72.64062 a
2  CT 56.35938 b
```

Subsetting for evaluation of subsidiary crops:

```
>potato_CT_SC <-subset(nema_wiz[385:512,],nema_wiz[385:512,]$Tillage=="CT")
>potato_RT_SC <-subset(nema_wiz[385:512,],nema_wiz[385:512,]$Tillage=="RT")

> x <-kruskal(potato_CT_SC$ALLlog, potato_CT_SC$SC)
> x
$statistics
    Chisq   p.chisq
  1.32937 0.5144357

$parameters
  Df ntr  t.value alpha           test          name.t
   2   3 1.999624  0.05 Kruskal-Wallis potato_CT_SC$SC

$means
       rank potato_CT_SC.ALLlog       std  r      Min      Max
C  33.82812            2.692359 0.1653435 32 2.285557 3.104828
L  34.46875            2.706814 0.1338147 16 2.436163 2.937016
NL 27.87500            2.622263 0.2112242 16 2.082785 2.982723

$comparison
NULL

$groups
  trt    means M
1   L 34.46875 a
2   C 33.82812 a
3  NL 27.87500 a

> x <-kruskal(potato_RT_SC$ALLlog, potato_RT_SC$SC)
```

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```
> x
```

NL= Non-legume cover crops are significantly different from C (control)

```
$statistics
    Chisq    p.chisq
  8.370141 0.01522113

$parameters
  Df ntr  t.value alpha           test          name.t
   2   3 1.999624  0.05 Kruskal-Wallis potato_RT_SC$SC

$means
       rank potato_RT_SC.ALLlog       std  r      Min      Max
C  37.89062            2.793808 0.1552241 32 2.403121 3.012415
L  32.81250            2.760956 0.1620483 16 2.409933 2.941014
NL 21.40625            2.669565 0.1281842 16 2.352183 2.943000

$comparison
NULL

$groups
  trt    means  M
1   C 37.89062  a
2   L 32.81250 ab
3  NL 21.40625  b
```

ORDINATION OF Pf-Pi-DYNAMIC FOR NEMATODE GENERA

Prior to analysis Pf and Pi values were log(x+1)-tranformed followed by subtractionof Pi from Pf. That means, nematode numbers in grass-clover were subtracted from nematode numbers after wheat, nematode numbers after wheat from nematode numbers after

subsidiary crops, nematode numbers after subsidiary crops from numbers after potato. This was done for each plot separately.

Replication as factor:

```
factor(Log_nema_dynamic$Rep)
```

treatments extracted from table and saved on separate name

```
trt<- subset(Log_nema_dynamic[,1:8])
```

nematode genera response values extracted from table and saved on separate name

```
species <- subset(Log_nema_dynamic[,9:14])
```

factors tillage and crop rotation combined and saved under a separate column in “trt”

```
trt <-within(trt, Tillage_CR <- paste(trt$CR, trt$Tillage, sep='+'))
```

loading the package for community analysis (Oksanen)

```
library(vegan)
```

According to ANOVA compost had no effects on the nematode community and was therefore introduced as conditional variable as well as the experimental year (Exp) and field replicates (Rep).

The redundancy analysis (rda) was performed over all occurring nematode genera (Log_nema_dynamic[,9:14]) with the single factor tillage in combination with crop rotation:

```
> nema.cca <- rda(Log_nema_dynamic[,9:14] ~ Tillage_CR+ Condition(compost)+
Condition(Exp)+ Condition(Rep),data = trt)
> head(summary(nema.cca), tail=2)

Call:
rda(formula = Log_nema_dynamic[, 9:14] ~ Tillage_CR + Condition(compost) +
Condition(Exp) + Condition(Rep), data = trt)

Partitioning of variance:
              Inertia Proportion
Total          1.87163    1.00000
Conditioned    0.03396    0.01815
Constrained    0.38775    0.20717
Unconstrained  1.44992    0.77468
```

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Explained variance (constrained) by the rda formula which is 20.7%, a rather good value for such community analysis. Conditioned variance is the variance explained by conditional variables (compost, experimental year, repetition) and very low (1.8%)

```
Eigenvalues, and their contribution to the variance
after removing the contribution of conditiniong variables

Importance of components:
                    RDA1   RDA2   RDA3    RDA4   RDA5    RDA6   PC1   PC2   PC3   PC4
PC5   PC6
Eigenvalue         0.2028 0.15242 0.02365 0.007284 0.00128 0.0003082 0.5758 0.3414 0.2565
0.17485 0.06793 0.03341
Proportion Explained  0.1104 0.08294 0.01287 0.003960 0.00070 0.0001700 0.3134 0.1858
0.1396 0.09515 0.03696 0.01818
Cumulative Proportion 0.1104 0.19330 0.20617 0.210140 0.21083 0.2110000 0.5244 0.7101
0.8497 0.94485 0.98182 1.00000

Accumulated constrained eigenvalues
Importance of components:
                    RDA1   RDA2   RDA3    RDA4   RDA5    RDA6
Eigenvalue          0.2028 0.1524 0.02365 0.007284 0.00128 0.0003082
Proportion Explained  0.5230 0.3931 0.06100 0.018790 0.00330 0.0007900
Cumulative Proportion 0.5230 0.9161 0.97712 0.995900 0.99921 1.0000000

Scaling 2 for species and site scores
* Species are scaled proportional to eigenvalues
* Sites are unscaled: weighted dispersion equal on all dimensions
* General scaling constant of scores:  5.062695



Species scores
```

Species scores show where each species is arranged in a coordinate system. As for us only two dimensions are rational in a coordinated system, only RDA1 and RDA2 are displayed, whereas more axis can be important if significant (see anova below). We can see large

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influences of Meloidogyne and Pratylenchus on the first and second RDA axes, respectively.

```
                              RDA1     RDA2     RDA3    RDA4     RDA5     RDA6
Helicotylenchus..Rotylenchus -0.3496429  0.53857 -0.05540  0.1107 -0.05772 -0.04569
Meloidogyne                  -1.5044160  0.11258 -0.06656 -0.1074 -0.01072  0.01348
Paratylenchus                -0.0005082 -0.01789  0.22937  0.1066 -0.08887  0.03393
Pratylenchus                  0.1719016  1.26546  0.19352 -0.0466  0.03189  0.01063
Tylenchorhynchus             -0.1514505  0.28855 -0.32574  0.2040  0.03511  0.02402
Criconematida                 0.5824445  0.31563 -0.34680 -0.1442 -0.06269  0.01052


Site scores (weighted sums of species scores)

          RDA1     RDA2    RDA3    RDA4    RDA5    RDA6
row1   -0.96147  0.34729  0.5305 -1.7631  0.1678  7.3062
row2   -1.00940  0.46952 -0.3706 -1.6160  3.8814  0.7921
row3   -0.61561  1.02188  0.6771 -0.7958  2.6876  1.0827
row4   -0.33861  0.43481  0.5923  0.4129  0.5532  4.3490
row5   -0.62322  0.30535  0.5483 -1.0017  0.3416  1.8949
row6   -0.51054  0.43199 -1.1477  0.2858  7.0774 -6.2091
....
row351  0.18510 -0.05878 -1.5843 -0.8119 -2.3733  1.8937
row352  0.04182 -0.24673  0.0208 -0.4365  0.6429  0.7892


Site constraints (linear combinations of constraining variables)

         RDA1     RDA2    RDA3     RDA4    RDA5    RDA6
row1   -0.1891  0.40060 -0.2041 -0.36325 0.07154  0.1540
row2   -0.1950  0.40613 -0.2117 -0.37581 0.07388  0.1589
row3   -0.1950  0.40613 -0.2117 -0.37581 0.07388  0.1589
row4   -0.1891  0.40060 -0.2041 -0.36325 0.07154  0.1540
row5   -0.1891  0.40060 -0.2041 -0.36325 0.07154  0.1540
```

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```
row6   -0.1950  0.40613 -0.2117 -0.37581 0.07388  0.1589
....
row351  0.3094 -0.06574 -0.4208  0.09413 0.01183 -0.1409
row352  0.3094 -0.06574 -0.4208  0.09413 0.01183 -0.1409


Biplot scores for constraining variables

                                     RDA1    RDA2     RDA3     RDA4     RDA5      RDA6
Tillage_CRPotato+reduced tillage       0.5518 -0.1899 -0.73302  0.17573  0.01747 -0.254201
Tillage_CRW-F+conventional tillage   -0.3012 -0.2425  0.03369  0.52890  0.26134  0.508631
Tillage_CRW-F+reduced tillage        -0.4222 -0.1440 -0.15366  0.12458 -0.51950 -0.007292
Tillage_CRW-OR+conventional tillage  -0.1533 -0.2640  0.01255 -0.15027 -0.36083  0.280882
Tillage_CRW-OR+reduced tillage       -0.2272 -0.1804  0.24976 -0.03575  0.33620 -0.328475
Tillage_CRW-V+conventional tillage   -0.2399 -0.1531 -0.05536 -0.10339  0.61095 -0.219059
Tillage_CRW-V+reduced tillage        -0.2165 -0.1299  0.20045 -0.14921 -0.30232 -0.604506
Tillage_CRWheat+conventional tillage -0.2860  0.6331 -0.30306 -0.54355  0.10757  0.232196
Tillage_CRWheat+reduced tillage       0.1735  0.6294  0.38626  0.53892 -0.08721 -0.164103


Centroids for factor constraints

                                      RDA1    RDA2     RDA3     RDA4      RDA5      RDA6
Tillage_CRPotato+conventional tillage  0.3088 -0.1446  0.30246 -0.24145  0.001979  0.166241
Tillage_CRPotato+reduced tillage       0.3159 -0.1087 -0.41960  0.10059  0.010002 -0.145510
Tillage_CRW-F+conventional tillage    -0.2571 -0.2070  0.02875  0.45132  0.223010  0.434024
Tillage_CRW-F+reduced tillage         -0.3603 -0.1228 -0.13112  0.10631 -0.443301 -0.006223
Tillage_CRW-OR+conventional  tillage      -0.1895  -0.3265    0.01552   -0.18582   -0.446198
0.347332
Tillage_CRW-OR+reduced tillage        -0.2809 -0.2231  0.30885 -0.04421  0.415736 -0.406184
Tillage_CRW-V+conventional  tillage      -0.2966  -0.1893  -0.06846  -0.12785    0.755488  -
0.270883
Tillage_CRW-V+reduced tillage         -0.2677 -0.1606  0.24788 -0.18450 -0.373838 -0.747516
```

```
Tillage_CRWheat+conventional tillage   -0.1942   0.4299  -0.20580  -0.36912    0.073048
0.157682
Tillage_CRWheat+reduced tillage       0.1178  0.4274  0.26231  0.36597 -0.059224 -0.111440
```

In the period from termination of cover crops until potato harvest, the 3 different subsidiary crops did not affect the nematode abundance significantly via permutation tests, so they were merged.

Now a permutation test for rda under reduced model is performed with 999 permutations. First the model per se is analysed which showed that the factor Tillage_CR significantly affected the nematode community response (Pf-pi)

```
> anova.cca(nema.cca, step = 999)
Permutation test for rda under reduced model
Permutation: free
Number of permutations: 999

Model: rda(formula = Log_nema_dynamic[, 9:14] ~ Tillage_CR + Condition(compost) +
Condition(Exp) + Condition(Rep), data = trt)
          Df Variance      F Pr(>F)
Model      9  0.38775 10.073  0.001 ***
Residual 339  1.44992
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> anova.cca(nema.cca, step = 999, by="terms")
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Permutation: free
Number of permutations: 999

Model: rda(formula = Log_nema_dynamic[, 9:14] ~ Tillage_CR + Condition(compost) +
Condition(Exp) + Condition(Rep), data = trt)
            Df Variance      F Pr(>F)
Tillage_CR   9  0.38775 10.073  0.001 ***
```

```
Residual   339  1.44992
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Factor tillage in combination with crops ist significant at *P<0.001.*

```
> anova.cca(nema.cca, step = 999, by="axis")
Permutation test for rda under reduced model
Marginal tests for axes
Permutation: free
Number of permutations: 999
```

Now each axis is analysed for significance: We can see that that the first three axes are significant which means that also the third axis may be discussed:

```
Model: rda(formula = Log_nema_dynamic[, 9:14] ~ Tillage_CR + Condition(compost) + Condition(Exp) + Condition(Rep), data = trt)
         Df Variance       F Pr(>F)
RDA1      1  0.20280 47.8359  0.001 ***
RDA2      1  0.15242 35.9523  0.001 ***
RDA3      1  0.02365  5.5795  0.001 ***
RDA4      1  0.00728  1.7181  0.127
RDA5      1  0.00128  0.3018  0.912
RDA6      1  0.00031  0.0727  0.997
Residual 342  1.44992
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Now, we want to create a coordinate system with all important varibales. Therefore we simply use the function plot() and insert our redundancy model called “nema.cca”. Other parameter in this function are simply elegancy of the plot. On centr we save the centroids of the factors (see above) and insert these into the coordinate system with the function text(). On spe we save scores of each nematode genus (see above) and plot these again with

the function text(). As the rda is a linear ordination, species have to be displayed with arrows which is done with the arrow() function, starting from (0,0).

```
> par(mfrow= c(1,1))
> par(mar= c(5,5,2,2))
> plot(nema.cca,type="n", xlim=c(-1.5,1),ylim=c(-0.5,1.5), cex.axis=1.4, cex.lab=1.4, xlab="RDA 1 (52.3%)",ylab="RDA 2 (39.3%)")
> centr <- scores(nema.cca)$centroids
> text(centr, labels=c("P+CT","P+RT","F+CT","F+RT","OR+CT","OR+RT","V+CT","V+RT","W+CT","W+RT"), cex=1)
> spe <- scores(nema.cca)$species
> text(spe, labels = (c("Hel","Mel","Par","Pra","Tyl","Cri")), col = "black", cex = 1.3)
> arrows(0,0,spe[,1]*0.9,spe[,2]*0.9, length = 0.1, col="black")
```

Erklärung

„Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig, ohne unerlaubte Hilfe Dritter angefertigt und andere als die in der Dissertation angegebenen Hilfsmittel nicht benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Dritte waren an der inhaltlichen Erstellung der Dissertation nicht beteiligt; insbesondere habe ich nicht die Hilfe eines kommerziellen Promotionsberaters in Anspruch genommen. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren durch mich verwendet worden.“

Witzenhausen, August 2017

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