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## Effect of tillage on earthworms over short- and medium-term in conventional and organic farming

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### ABSTRACT

Earthworms play an important role in many soil functions and are affected by soil tillage in agricultural soils. However, effects of tillage on earthworms are often studied without considering species and their interactions with soil properties. Furthermore, many field studies are based on one-time samplings that do not allow for characterisation of temporal variation. The current study monitored the short (up to 53 days) and medium term (up to 4 years) effects of soil tillage on earthworms in conventional and organic farming. Earthworm abundances decreased one and three weeks after mouldboard ploughing in both conventional and organic farming, suggesting direct and indirect mechanisms. However, the medium-term study revealed that earthworm populations in mouldboard ploughing systems recovered by spring. The endogeic species *Aporrectodea caliginosa* strongly dominated the earthworm community (76%), whereas anecic species remained <1% of all earthworms in all tillage and farming systems over the entire study. In conventional farming, mean total earthworm abundance was not significantly different in reduced tillage ( $153 \text{ m}^{-2}$ ) than mouldboard ploughing (MP;  $130 \text{ m}^{-2}$ ). However, reduced tillage in conventional farming significantly increased the epigeic species *Lumbricus rubellus* from  $0.1 \text{ m}^{-2}$  in mouldboard ploughing to  $9 \text{ m}^{-2}$  averaged over 4 years. Contrastingly, in organic farming mean total earthworm abundance was 45% lower in reduced tillage ( $297 \text{ m}^{-2}$ ) than MP ( $430 \text{ m}^{-2}$ ), across all sampling dates over the medium-term study (significant at 3 of 6 sampling dates). Reduced tillage in organic farming decreased *A. caliginosa* from  $304 \text{ m}^{-2}$  in mouldboard ploughing to  $169 \text{ m}^{-2}$  averaged over 4 years (significant at all sampling dates). Multivariate analysis revealed clear separation between farming and tillage systems. Earthworm species abundances, soil moisture, and soil organic matter were positively correlated, whereas earthworm abundances and penetration resistance were negatively correlated. Variability demonstrated between sampling dates highlights the importance of multiple samplings in time to ascertain management effects on earthworms. Findings indicate that a reduction in tillage intensity in conventional farming affects earthworms differently than in organic farming. Differing earthworm species or ecological group response to interactions between soil tillage, crop, and organic matter management in conventional and organic farming has implications for management to maximise soil ecosystem functions.

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## 1. Introduction

Earthworms affect many soil properties in agricultural land including nutrient availability, soil structure, and organic matter dynamics (Edwards, 2004). Earthworms in turn are influenced by soil moisture, organic matter, texture, pH, and soil management (Curry, 2004).

Tillage systems can affect soil biota through changes in habitat (Van Capelle et al., 2012), loss of organic matter (Hendrix et al., 1992), moisture and temperature dynamics (Curry, 2004) and mechanical damage (Lee, 1985). Earthworm population change

due to soil tillage depends on tillage intensity (Chan, 2001; Curry, 2004) and may be higher under root than cereal crops (Curry et al., 2002). Moreover, tillage may differentially affect earthworm species, depending on their feeding and burrowing behaviour. Earthworm species classified into ecological groups, defined by Bouché (1977), are epigeic that live on or near the soil surface, endogeic that live and feed in mineral soil, and anecic that are deep burrowing but feed at the soil surface (Sims and Gerard, 1999). Earthworm ecological groups affect soil processes to differing degrees and therefore have varying importance for ecosystem services (Keith and Robinson, 2012).

Conflicting tillage effects on earthworms have been presented in literature (Chan, 2001). On one hand, Van Capelle et al. (2012), in a review of studies conducted in Germany, concluded that reduced tillage intensity increased earthworm abundances and

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species diversity. On the other hand, ploughing can positively influence endogeic species by increasing organic matter availability to them (Ernst and Emmerling, 2009), while it has the opposite affect on anecics (Capowiez et al., 2009). Many studies have focused on earthworms in no-tillage versus conventional ploughing systems in cereal crops, and have often not quantified earthworm species or their functional roles. Therefore, clarification is needed on tillage and arable soil management effects on earthworm species in a wider range of crop rotations.

Intermediate reduced tillage systems that de-compact, yet do not invert soil, are being implemented in arable systems where there is high soil compaction risk (e.g., root crops, high soil moisture). Non-inversion tillage systems, like other reduced tillage systems, are aimed at enhancing soil physical properties (e.g., structural stability, water retention) and soil organic matter (Morris et al., 2010), increasing soil biodiversity (El Titi, 2003), and reducing production costs (Soane et al., 2012). Soil compaction from tillage and field traffic can be detrimental to earthworms when it limits their burrowing activity (Langmaack et al., 1999; Capowiez et al., 2012). In particular, crops such as potatoes and sugar beets require the use of heavy machinery for land preparation and harvesting (Marinissen, 1992) which results in considerable soil disturbance (Buckerfield and Wiseman, 1997), especially under wet soil conditions. There is a lack of research that examines earthworms in reduced tillage systems that include potato or sugar beet, particularly where soils are susceptible to compaction during harvest with heavy machinery.

Additionally, farming system can have a large influence on earthworms. Organic farming, where synthetic pesticides and fertilisers are prohibited, makes greater use of animal and green manures, diverse crop rotations, and mechanical weeding (Gomiero et al., 2011). Hole et al. (2005) reviews studies where earthworms are both positively and negatively affected by organic farming. Most studies of earthworms in organic arable farming have been limited to short duration experiments that compared fields without proper experimental design to account for spatial variability in soil properties (Irmler, 2010).

Recent studies have investigated arable soil tillage effects on earthworms (Capowiez et al., 2009; Ernst and Emmerling, 2009; Peigné et al., 2009; De Oliveira et al., 2012). However, an extensive literature search revealed few studies that have assessed the effects of tillage systems on earthworms over short- and medium-timescales simultaneously in both conventional and organic farming systems.

The objective of this study was to quantify the effects of tillage systems on earthworm populations in conventional and organic farming. It was hypothesised that mouldboard ploughing reduces earthworm populations immediately following ploughing (epigeic and anecic species in particular) in both conventional and organic farming and that this decrease would continue for several weeks relative to the reduced tillage treatment. Over the medium term (4 years), it was hypothesised that reduced tillage intensity systems increase earthworm populations relative to mouldboard ploughing in both conventional and organic farming (epigeic and anecic species in particular). Furthermore, earthworm species abundances were expected to be positively correlated with soil organic matter content and soil moisture but negatively correlated to soil compaction.

## 2. Materials and methods

### 2.1. Site characteristics

The study was conducted at the PPO Lelystad experimental farm of Applied Plant Research Wageningen UR, in the Netherlands, in a polder reclaimed in 1957 ( $52^{\circ} 31'N$ ,  $5^{\circ} 29'E$ ). The daily mean

temperature ranged from  $2^{\circ}C$  in winter to  $17^{\circ}C$  in summer months, and mean rainfall was 794 mm per year during the study (Royal Netherlands Meteorological Institute, 2013). The soil type is a calcareous marine clay loam with 23% clay, 12% silt, and 66% sand. Soil pH is 7.9, and soil organic matter is 3.2% averaged across fields at the experimental farm.

### 2.2. Experimental design

Soil tillage treatments were sampled in two parallel field experiments (conventional and organic farming) in this study (Fig. 1). Conventional and organic farming systems had unique crop rotations with individual fields at a different phase of their rotation (Table 1). Rotations contained mainly root and cereal crops, although grass and cabbage were also included in organic farming. Cover crops were grown during fallow periods when feasible. Conventional fields received yearly synthetic fertiliser applications and were treated bi-weekly with herbicides during the growing season. Organic fields received yearly cow manure (solid or slurry) applications of  $20\text{--}40\text{ t ha}^{-1}\text{ yr}^{-1}$ . Organic field A in autumn 2010 did not receive manure because of the reduced nitrogen required by the following leguminous crop (wheat/faba). Tillage treatments received the same amounts of fertilisers and herbicides in conventional fields, or manure in organic fields. Organic fields received certification in 2004 and no synthetic fertilisers or pesticides have been used since 2002.

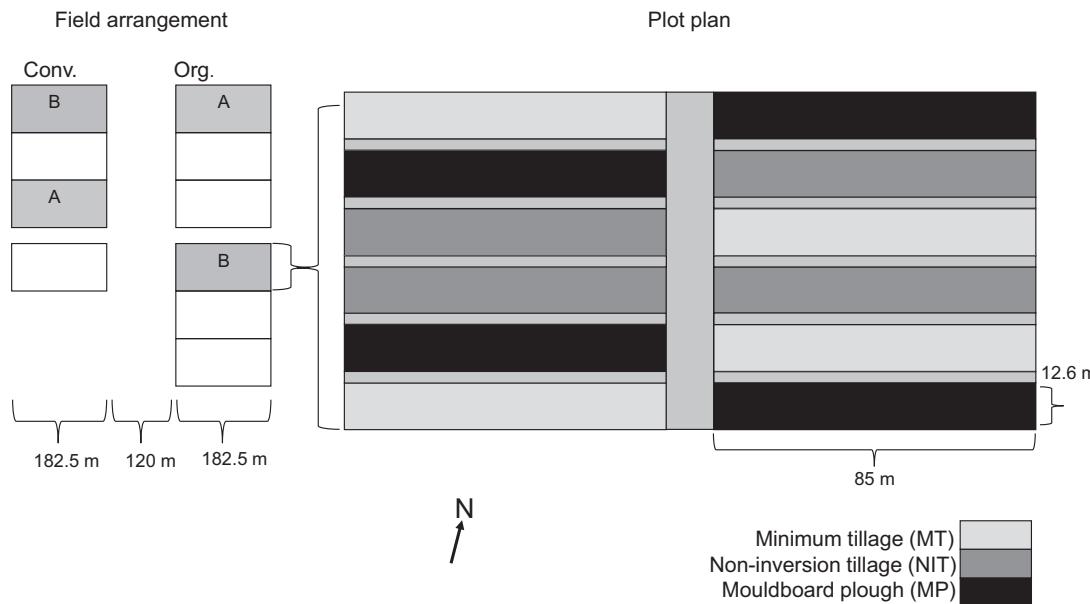
Sampling was conducted in two fields under conventional and two fields under organic farming. Each field contained 12 plots (3 tillage systems by 4 blocks) of 85 m by 12.6 m each, arranged in randomised complete blocks (Fig. 1). Each plot contained 4 beds of 3.15 m along controlled traffic lanes where all field operations, except harvest, were done. All plots were mouldboard ploughed annually previous to tillage system initiation in autumn 2008. Tillage systems were: (i) minimum tillage (MT) with optional sub-soiling to 20 cm in autumn if soil compaction was high (based on visual assessment of soil pit and/or penetrometer readings) with cultivation to 8 cm for seedbed preparation, (ii) non-inversion tillage (NIT) with yearly sub-soiling to 20 cm in autumn and cultivation to 8 cm for seedbed preparation, (iii) mouldboard ploughing (MP) to 25 cm in autumn and cultivation to 8 cm for seedbed preparation. Sub-soiling in MT (done only in 2009 and 2010) and NIT plots was done using a Kongskilde Paragrubber Eco 3000.

A short-term study was conducted in conventional field B (Conv B) and organic field B (Org B), and medium-term earthworm monitoring was done in conventional field A (Conv A) and organic field A (Org A) (Fig. 1 and Table 1). Separate fields were used for the short- and medium-term studies to reduce disturbance due to sampling.

### 2.3. Data collection and analyses

#### 2.3.1. Short-term study

A sampling campaign was conducted during autumn 2011 to investigate the short-term effects of mouldboard ploughing on earthworm populations. Earthworms were sampled 15 days (d) before ploughing in MP and NIT plots of Conv B, then 5 d, 16 d, and 35 d after ploughing to assess effects over time. MP and NIT plots of Org B were sampled 3 d before ploughing then 2 d, 20 d, and 53 d, and 191 d (after seeding of spring wheat) after ploughing. NIT plots were sampled, as a reference, on the same dates as MP plots, to account for changes in earthworm populations resulting from changing environmental conditions with time. Conv B was non-inversion tilled on 28-Oct-2011, before initiation of the short-term study. Org B was not non-inversion tilled during autumn 2011. Three 20 cm × 20 cm × 20 cm monoliths were handsorted for earthworms from each plot according to Van Vliet and De Goede (2006). To extract anecic earthworms from below 20 cm, 500 ml of 0.185%



**Fig. 1.** Arrangement of experimental fields (left) and plot plan (right).

formaldehyde solution was applied to the bottom of the pit. Since MT had been sub-soiled in 2009 and 2010 to reduce soil compaction, it was not sampled for the short-term study to avoid the redundancy of including two treatments (NIT + MT) that had been treated equally.

### 2.3.2. Medium-term study

To monitor medium-term effects of tillage systems on earthworm populations Conv A and Org A were sampled between 2009 and 2012 during spring and autumn seasons (excluding spring 2010). Earthworms were sampled as described in Section 2.3.1, after seeding in spring and again before ploughing in autumn. Soil moisture was measured at each earthworm sampling by taking composite soil samples ( $n=5$ , 20 mm diameter) to 20 cm depth immediately adjacent to each excavated monolith (data not shown). In addition, soil organic matter (SOM) content and soil penetration resistance (as a proxy for soil compaction) were measured during the autumn 2011 sampling. A randomised subsample of each soil monolith that had been handsorted for earthworms was taken for SOM analysis. Penetration resistance profiles ( $n=4$ ) were taken in undisturbed soil within 20 cm of each monolith using a penetrometer (Eijkelkamp Agrisearch 2011, 1 cm<sup>2</sup> cone diameter 60°).

### 2.3.3. Laboratory analyses

Earthworm samples were stored, with a small amount of soil so earthworms would not dry out, at 4 °C for a maximum of two days. Earthworms were cleaned with water and patted dry with tissue paper, after which they were counted, weighed (including gut contents) and fixed in 70% ethanol. Biomass was not measured in

spring 2009. Adults were identified according to Sims and Gerard (1999) and juveniles with Stöp-Bowitz (1969) to species level. Where species level identification of juveniles was not possible individuals were grouped as either *Aporrectodea/Allolobophora* or *Lumbricus* juveniles. Soil moisture was determined gravimetrically by drying subsamples at 105 °C for 24 h. Soil organic matter content was determined by loss-on-ignition at 550 °C (Normalisatie-Instituut, 1992).

### 2.3.4. Statistical analyses

Tillage system effects on earthworm species abundances, total earthworm abundance (adults + juveniles), total earthworm biomass, adult/juvenile ratio, species richness, and Shannon diversity index were investigated using linear mixed effects models with repeated measures. Fixed effects were tillage system and sampling date, and random effects were block and plot. Earthworm species abundances and total biomass were averaged per plot before statistical analysis. Species richness and Shannon diversity were calculated using species abundances averaged per plot. Farming systems (conventional or organic) were analysed separately. Fields under conventional and organic farming were separated spatially (Fig. 1), and not inside of the randomised complete block design and could not be statistically tested. A squared-root transformation of earthworm species abundances, total earthworm abundance, and total earthworm biomass was used to fit model assumptions. Autoregressive correlation was used for repeated measures. Relations between earthworm and soil parameters across tillage and farming systems were explored by redundancy analysis (RDA) of autumn 2011 data. Earthworm species abundances, soil organic matter, soil moisture, and penetration resistance (averaged per

**Table 1**

Crop rotation per field (cover crop/green manure).<sup>a</sup>

Year	Field			
	Org A	Org B	Conv A	Conv B
2009	Spring wheat (white mustard)	Potato (grass clover)	Spring barley (rye grass)	Sugar beet
2010	Carrot (white clover)	Grass clover	Onion	Winter wheat
2011	Wheat/faba (white mustard)	Cabbage	Potato (rye grass)	Onion (yellow mustard in MT and NIT only)
2012	Potato	Spring wheat	Sugar beet	Potato

<sup>a</sup> Organic farming has a 6 year crop rotation. Only 4 years of the rotation are shown here.

plot ( $n=4$ ) per 5–30 cm depth) were used as response variables to the explanatory variables farming systems (Conv and Org) and tillage systems (MP and NIT). Computations for linear mixed effects models (Pinheiro et al., 2012), multiple means comparisons (Lenth, 2012), RDA, and Shannon diversity (Oksanen et al., 2012; Kindt and Coe, 2005) were performed using R (R Core Team, 2012). The type I error rate ( $\alpha$ ) was set at 0.05 for all statistical tests, unless otherwise stated.

### 3. Results

#### 3.1. Short-term study: effect of mouldboard ploughing on earthworm populations

Total earthworm abundance in conventional field B (Conv B) prior to ploughing in autumn 2011 was  $512 \text{ m}^{-2}$  in mouldboard ploughing (MP), about 20% higher than non-inversion tillage (NIT) (Table 2). Following ploughing, earthworm abundance was reduced by 66% after 5 days and a further 74% after 2 weeks, whereas in NIT earthworm abundance did not change with time. Earthworm biomass responded similarly. Mean adult/juvenile (A/J) ratio was 0.17. A total of 6 earthworm species were found in Conv B (Table 2). *Aporrectodea caliginosa* was 83%, *Aporrectodea rosea* was 10%, *Eiseniella tetraedra* was 5%, and *Lumbricus rubellus* 2% of earthworms. *Lumbricus castaneus* and *Lumbricus terrestris* were also found but were less than 1% of earthworms. *A. caliginosa* and *A. rosea* abundances were significantly reduced more than 6 fold after ploughing relative to pre-ploughing. Mean species richness was significantly reduced from 4 to about 2 after ploughing. Mean Shannon diversity was not significantly affected by tillage system or sampling date, but on average was 0.49 in NIT and 0.46 in MP (data not shown).

Total earthworm abundance in organic field B (Org B) prior to ploughing was  $585 \text{ m}^{-2}$  in MP, about 50% lower than NIT (Table 3). Following ploughing, total earthworm abundance was reduced by 85% after 3 weeks. Total earthworm biomass however declined by more than 50% in both MP and NIT during the short-term experiment. Earthworm abundance and biomass recovered to pre-ploughing levels by spring 2012. A/J ratio was 0.17 in NIT before ploughing, significantly higher than MP, and declined slightly with time to levels similar to MP by the 3rd sampling date. A total of 7 earthworm species were found in Org B (Table 3) in autumn 2011. *A. caliginosa* was 82%, *L. rubellus* was 13%, *E. tetraedra* was 2%, and *A. rosea* and *Allolobophora chlorotica* were 1% of earthworms. *L. terrestris* and *Lumbricus castaneus* were also present but less than 1% of earthworms. Mean species richness did not decrease following ploughing, and was significantly lower in MP (2.7) than in NIT (4.0). Mean Shannon diversity was not significantly affected by tillage system or sampling date, but on average was 0.64 in NIT and 0.42 in MP (data not shown).

#### 3.2. Medium-term study: effect of reduced tillage systems on earthworm populations

In Conv A, total earthworm abundance was not significantly affected by tillage system at any sampling date. Mean total earthworm abundance was  $153 \text{ m}^{-2}$  and total earthworm biomass was  $32 \text{ g m}^{-2}$  for reduced tillage (minimum (MT) and NIT averaged), 15% higher than MP over the medium-term study across all sampling dates (Table 4). One or both reduced tillage systems had higher total earthworm biomass than MP at autumn 2009 ( $P=0.05$ ), spring 2011 ( $P=0.05$ ), and spring 2012 ( $P=0.07$ ). Mean A/J ratio was 0.64 in reduced tillage and 0.36 for MP. A total of 8 earthworm species were found in Conv A (Table 4). *A. caliginosa* was 86%, *A. rosea* was 7%, and *L. rubellus* 5% of earthworms. *L. castaneus*, *E. tetraedra*, *A. chlorotica*, *L. terrestris*, and *Aporrectodea limicola* were also found but were less

than 1% of earthworms. Mean species richness in reduced tillage systems was 2.3, significantly higher than 1.7 in MP, and was significantly higher in one or both reduced tillage system than MP at 3 sampling dates. No significant effects of tillage system on Shannon diversity were found, however mean Shannon diversity was 0.4 in reduced tillage and 0.2 in MP (data not shown). *L. rubellus* was not present in MP at 5 out of 6 sampling dates and had significantly higher abundance in MT and/or NIT than MP at 3 sampling dates.

In Org A, at 3 of 6 sampling dates total earthworm abundance in MP was significantly higher than reduced tillage (M or NIT). Mean total earthworm abundance was  $297 \text{ m}^{-2}$  and total earthworm biomass was  $52 \text{ g m}^{-2}$  for reduced tillage, 45% and 15% lower than MP respectively across all sampling dates (Table 5). No significant tillage system effects were found for A/J ratio, however mean A/J ratio was 0.56 in reduced tillage and 0.45 in MP. A total of 9 earthworm species were found in Org A (Table 5). *A. caliginosa* was about 63%, *L. rubellus* 16%, *E. tetraedra* 16%, and *A. rosea* was 4% of earthworms. *A. chlorotica*, *L. castaneus*, *L. terrestris*, *Aporrectodea longa*, and *Murchieona minuscula* were also present but were less than 1% of earthworms. No significant tillage system effects on species richness or Shannon diversity were found. Mean species richness was 3.4 in reduced tillage and 3.5 in MP and mean Shannon diversity was 0.8 in reduced tillage and 0.7 in MP (data not shown). *A. caliginosa* had significantly higher abundance in MP than at least one of MT or NIT at all sampling dates.

#### 3.3. Relations between earthworms, management, and soil properties

Soil property data used in the RDA are presented in Table 6. RDA eigenvalues indicated that 44% of total variance within earthworm and soil parameters measured in autumn 2011 were explained by the 1st and 2nd axes of the ordination diagram (Table 7 and Fig. 2). Both tillage and farming system explained significant proportions of total variance (Permutation test,  $P<0.01$ ). The 1st axis separates farming systems (Org A on left and Conv A on right) (Fig. 2). Furthermore, the 2nd axis separates tillage within the Conv system, whereas there is overlap in Org. *L. rubellus* and *E. tetraedra* were more abundant in Org NIT than Conv MP and Conv NIT. Org NIT had higher soil organic matter, and *L. rubellus* and *E. tetraedra* abundances, which were positively correlated. Penetration resistance, at all depths, was highest in Conv NIT.

### 4. Discussion

#### 4.1. Short-term effects of ploughing on earthworms

Mouldboard ploughing was shown to consistently reduce total earthworm abundance in the short term (up to 53 days). As hypothesised, earthworm abundance decreased immediately after ploughing and continued to decrease at subsequent samplings in both conventional and organic farming. This decrease may indicate that both direct (e.g., physical damage, predation) and indirect (e.g., food re-distribution) mechanisms may play a role (Curry, 2004). Ploughing and intensive tillage have been found to reduce earthworm populations over the short term (Boström, 1995; Curry et al., 2002; De Oliveira et al., 2012). *Lumbricus rubellus* populations were affected by mouldboard ploughing similarly to other species. Anecic species abundances in the short-term study were too low (<1% of earthworms) to ascertain mouldboard ploughing effects.

In Org B, *A. caliginosa* abundance began to recover by 53 d after ploughing. Similarly, De Oliveira et al. (2012) found a mean increase of *A. caliginosa* of  $141 \text{ m}^{-2}$  in a 7 day period, after ploughing. Schmidt and Curry (2001) reported an increase of  $125 \text{ m}^{-2}$  following an initial decrease after ploughing in November and December

**Table 2**Earthworm abundances and biomass before and after ploughing in Conv B.<sup>1</sup>

Tillage system	Sampling date (2011)	<i>Aporrectodea caliginosa</i> (m <sup>-2</sup> )	<i>Aporrectodea rosea</i> (m <sup>-2</sup> )	<i>Eiseniella tetraedra</i> (m <sup>-2</sup> )	<i>Lumbricus rubellus</i> (m <sup>-2</sup> )	Total abundance (m <sup>-2</sup> )	Biomass (g m <sup>-2</sup> )	Adult/juvenile ratio <sup>2</sup>	Species richness
MP	1-Nov (MP – 15 d)	425a	38a	21	13a	512a	68a	0.22	4.0a
	21-Nov (MP + 5 d)	150b	11ab	3	0b	175b	25b	0.36	2.0b
	2-Dec (MP + 16 d)	36c	1b	1	2ab	45c	7c	0.13	2.2b
	21-Dec (MP + 35 d)	65c	1b	18	0b	97cb	8c	0.25	1.7b
NIT	1-Nov (MP – 15 d)	375a	29ab	0	3*	420	41*	0.11	2.2*
	21-Nov (MP + 5 d)	345ab*	47ab*	3	2	406*	46*	0.13*	2.7*
	2-Dec (MP + 16 d)	227b*	56a* <sup>4</sup>	4	1	301*	28*	0.08	3.0*
	21-Dec (MP + 35 d)	295ab*	17b*	9	2	344*	36*	0.06*	3.0*

<sup>1</sup> Species abundances, total abundance, and total biomass are back-transformed means. Tillage systems: non-inversion tillage (NIT) and mouldboard plough (MP). Species with > 1% of overall abundance are included, other species present were *Lumbricus terrestris* and *Lumbricus castaneus*. Species abundance columns are ordered from left to right by decreasing overall abundance. Letters indicate significant treatment differences within tillage system between sampling dates and \* indicates significant differences between tillage systems within sampling date ( $P < 0.05$ ).

<sup>2</sup>  $P = 0.05$ .<sup>3</sup>  $P = 0.08$ .<sup>4</sup>  $P = 0.06$ .<sup>5</sup>  $P = 0.08$ .**Table 3**Earthworm abundances and biomass before and after ploughing in Org B.<sup>1</sup>

Tillage system	Sampling date (2011)	<i>Aporrectodea caliginosa</i> (m <sup>-2</sup> )	<i>Lumbricus rubellus</i> (m <sup>-2</sup> )	<i>Eiseniella tetraedra</i> (m <sup>-2</sup> )	<i>Aporrectodea rosea</i> (m <sup>-2</sup> )	Total abundance (m <sup>-2</sup> )	Biomass (g m <sup>-2</sup> )	Adult/juvenile ratio	Species richness
MP	26-Oct (MP – 3 d)	510a	67a	0b	0b	585a	71a	0.09	2.0c
	31-Oct (MP + 2 d)	381a	51a	1ab	0b	446a	35b	0.04	2.5bc
	18-Nov (MP + 20 d)	80c	9b	0b	1ab	94c	16b	0.04	2.0c
	21-Dec (MP + 53 d)	204b	7c	1b	4ab	242b	26b	0.05	3.0ab <sup>2</sup>
	7-May-2012 (MP + 191 d)	519a	85a	19a	6a	639a	81a	0.08	3.8a
NIT	26-Oct (MP – 3 d)	1023*	178*	14ab*	5b*	1243*	120ab*	0.17a*	3.8b*
	31-Oct (MP + 2 d)	939*	142*	41a*	1b	1156*	82bc*	0.10ab*	3.8ab*
	18-Nov (MP + 20 d)	737*	125*	15ab*	31a*	963*	77c*	0.09b	4.8a*
	21-Dec (MP + 53 d)	720*	93*	6b	20a*	876*	64c*	0.06b	4.0ab*
	7-May-2012 (MP + 191 d)	844*	164*	25ab	10ab	1065*	119a*	0.10b <sup>4</sup>	3.8ab

<sup>1</sup> Species abundances, total abundance, and total biomass are back-transformed means. Tillage systems: non-inversion tillage (NIT) and mouldboard plough (MP). Species with > 1% of overall abundance are included, other species present were *Allolobophora chlorotica*, *Lumbricus terrestris*, and *Lumbricus castaneus*. Species abundance columns are ordered from left to right by decreasing overall abundance. Letters indicate significant treatment differences within tillage system between sampling dates and \* indicates significant differences between tillage systems within sampling date ( $P < 0.05$ ).

<sup>2</sup>  $P = 0.09$ .<sup>3</sup>  $P = 0.06$ .<sup>4</sup>  $P = 0.06$ .**Table 4**Earthworm abundances and biomass in Conv A.<sup>1</sup>

Sampling date	Tillage system	<i>Aporrectodea caliginosa</i> (m <sup>-2</sup> )	<i>Aporrectodea rosea</i> (m <sup>-2</sup> )	<i>Lumbricus rubellus</i> (m <sup>-2</sup> )	Total abundance (m <sup>-2</sup> )	Biomass (g m <sup>-2</sup> )	Adult/juvenile ratio	Species richness
Spring 2009	MT	50	7	1	68	–	1.01b	2.2a
	NIT	26	2	2	38	–	2.37a	1.7ab
	MP	40	1	0	41	–	0.30b	1.2b
Autumn 2009	MT	101	4	0	110	15ab	0.41	1.7ab
	NIT	153	7	2	169	26a <sup>2</sup>	0.14	2.5a
	MP	87	4	0	95	11b	0.14	1.5b
Autumn 2010	MT	170b	7	17a	208	56	0.79	2.5
	NIT	277a	38	22a	358	77	0.37	3.0
	MP	240ab	30	0b	279	79	0.89	2.5
Spring 2011	MT	49	3	2	60	12a <sup>3</sup>	0.63	2.0
	NIT	52	2	0	61	8ab	0.50	2.0
	MP	23	3	0	29	3b	0.25	1.5
Autumn 2011	MT	218a	2	12a	245	44a <sup>4</sup>	0.48	2.8
	NIT	113b	3	0b	127	25b	0.49	2.0
	MP	181ab	10	0b	192	26ab	0.16	2.0
Spring 2012	MT	154	2	29a	188	24ab	0.24	2.5a
	NIT	176	7	17a	204	35a <sup>5</sup>	0.19	2.8a
	MP	136	1	1b	143	18b	0.36	1.5b

<sup>1</sup> Species abundances, total abundance, and total biomass are back-transformed means. Tillage systems: minimum (MT), mouldboard plough (MP), and non-inversion tillage (NIT). Species with > 1% of overall abundance are included, other species present were *Lumbricus terrestris*, *Lumbricus castaneus*, and *Eiseniella tetraedra*. Species abundance columns are ordered from left to right by decreasing overall abundance. Letters indicate significant differences between tillage systems within sampling date ( $P < 0.05$ ).

<sup>2</sup>  $P = 0.05$ .<sup>3</sup>  $P = 0.05$ .<sup>4</sup>  $P = 0.07$ .<sup>5</sup>  $P = 0.07$ .

**Table 5**Earthworm abundances and biomass in Org A.<sup>1</sup>

Sampling date	Tillage system	<i>Aporrectodea caliginosa</i> (m <sup>-2</sup> )	<i>Lumbricus rubellus</i> (m <sup>-2</sup> )	<i>Eiseniella tetraedra</i> (m <sup>-2</sup> )	<i>Aporrectodea rosea</i> (m <sup>-2</sup> )	Total abundance (m <sup>-2</sup> )	Biomass (g m <sup>-2</sup> )	Adult/juvenile ratio	Species richness
Spring 2009	MT	125b	45	6	8	195b	–	0.41	4.3
	NIT	147b	31	15	17	236ab	–	0.26	3.8
	MP	273a	45	21	5	375a	–	0.42	3.3
Autumn 2009	MT	227b <sup>2</sup>	168	0	9	415	129a	0.46	3.0
	NIT	151b	116	1	5	289	82b	0.36	3.5
	MP	271a	80	2	16	389	78b	0.24	4.0
Autumn 2010	MT	89b	52	1	10	159b	40b	0.61	3.0
	NIT	64b	15	1	5	104b	34b	0.57	2.8
	MP	271a	44	7	23	357a	75a	0.35	3.8
Spring 2011	MT	18ab	4	1	1	28	11	2.00	2.0
	NIT	8b	5	1	1	21	6	0.25	2.5
	MP	58a	2	1	2	75	16	1.22	2.2
Autumn 2011	MT	365b	50	21	10	560b	97	0.38	3.8
	NIT	293b	51	85	6	555b	84	0.42	3.5
	MP	566a	44	88	31	841a	93	0.19	4.0
Spring 2012	MT	309ab	84	20	12	557	74a	0.50	4.2
	NIT	230b	80	11	9	446	58ab	0.51	4.0
	MP	383a	38	8	5	543	35b	0.26	3.8

<sup>1</sup> Species abundances, total abundance, and total biomass are back-transformed means. Tillage systems: minimum (MT), mouldboard plough (MP), and non-inversion tillage (NIT). Species with >1% of overall abundance are included, other species present were *Lumbricus terrestris*, *Lumbricus castaneus*, *Aporrectodea longa*, *Allolobophora chlorotica*, *Murcheonea minuscula* and *Aporrectodea/Allolobophora* or *Lumbricus* juveniles. Species abundance columns are ordered from left to right by decreasing overall abundance. Letters indicate significant differences between tillage systems within sampling date ( $P<0.05$ ).

<sup>2</sup>  $P=0.07$ .

**Table 6**Soil property data used in RDA.<sup>1</sup>

Farming system	Tillage system	Soil organic matter (g kg <sup>-2</sup> )	Soil moisture (g kg <sup>-2</sup> )	Penetration resistance (MPa)					
				0–5 cm	5–10 cm	10–15 cm	15–20 cm	20–25 cm	25–30 cm
Conv	NIT	31.3a	203b	0.5	1.2a	1.9a	2.2a	1.9a	1.9a
	MP	29.4b	219a	0.4	0.8b	1.1b	1.2b	1.0b	1.2b
Org	NIT	33.4a	235	0.4	0.5	0.6b	0.9	1.1	1.1
	MP	32.2b <sup>2</sup>	217	0.4	0.5	0.7a <sup>3</sup>	0.9	1.0	1.2

<sup>1</sup> Soil property data were measured simultaneously with earthworms in autumn 2011. Tillage systems: non-inversion tillage (NIT) and mouldboard plough (MP). Letters indicate significant treatment differences within farming system ( $P<0.05$ ). Soil organic matter and soil moisture were measured to 20 cm.

<sup>2</sup>  $P=0.06$ .

<sup>3</sup>  $P=0.07$ .

1995. There was a significant increase of 124 m<sup>-2</sup> *A. caliginosa* and 8.47 g m<sup>-2</sup> (assuming 84.7% of total biomass is *A. caliginosa* since *A. caliginosa* is 84.7% of total abundance) in 33 days (Table 3, Org B, MP 18-Nov to 21-Dec). This increase can be justified by earthworm population growth, assuming sufficient cocoon presence and a Q<sub>10</sub> of 2, according to Boyle (1990) cited in Curry (2004). However, it should be noted that the decrease and subsequent increase in earthworm populations following mouldboard ploughing cannot be attributed to the ploughing effect itself with absolute certainty and may partly be an artefact of the sampling method. Ploughing may have caused displacement of earthworms below 20 cm depth, and the formaldehyde extraction below 20 cm depth may be less effective in recently ploughed, unconsolidated soil.

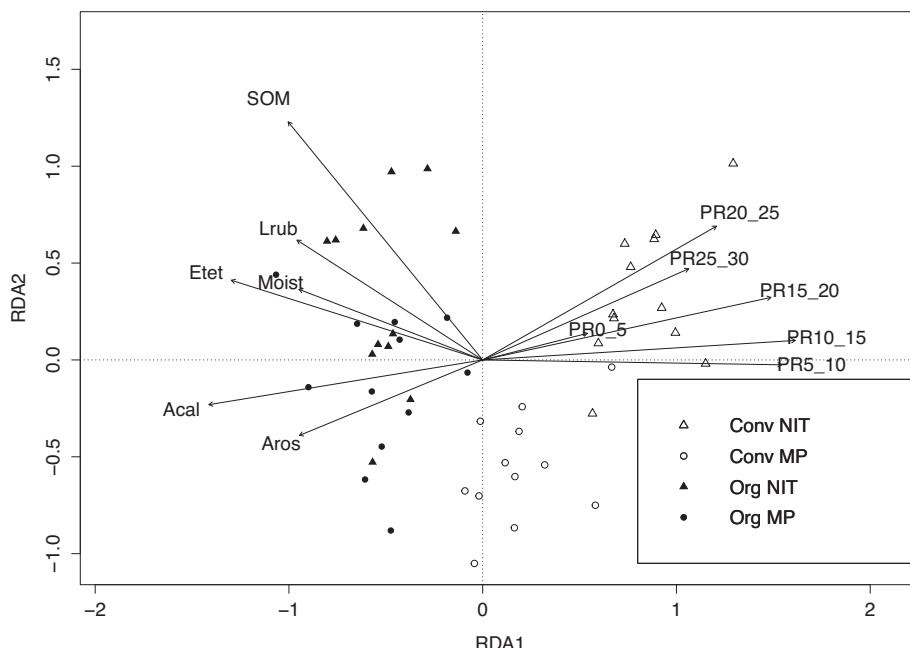
**Table 7**

Results of redundancy analysis and permutation test for autumn 2011 earthworm and soil data.

Ordination axis	Axis 1	Axis 2
Eigenvalues	5.92	1.11
Cumulative proportion of total variance explained	0.37	0.44
Species-environment correlations	0.91	0.69
Permutation significance test	F-ratio	P-value
Farming system	28.36	0.005
Tillage system	6.91	0.005

Earthworms can recover by the following season, as seen in both the short- and medium-term studies shown in Tables 3 and 5. Boström (1995) attributed earthworm recovery to cocoon production and redistribution of organic matter through the plough layer making the food source more available for endogeics. Curry (2004) also suggests that soil inversion may increase organic matter availability to endogeic earthworms and that short-term factors such as predation may not play a role over the medium term.

In Org B pre-ploughing earthworm total abundance was two times higher in non-inversion tillage (NIT) than mouldboard ploughing (MP), in contrast to Conv B. Mouldboard ploughing clearly affected total earthworm abundance in Conv B and Org B. Conv B and Org B differed in that *Aporrectodea rosea* was second-most abundant and *L. rubellus* least abundant in Conv B, whereas the reverse was true in Org B. Manure additions (Peigné et al., 2009) and more diverse crop rotations (including legumes as ley) (Metzke et al., 2007; Riley et al., 2008; Peigné et al., 2009) may account for the differences in earthworm assemblages between farming systems. Therefore, as hypothesised, ploughing reduced total earthworm abundance and total earthworm biomass over the short term in Conv B. However, in Org B the hypothesis is confirmed for total earthworm abundance but not total earthworm biomass. In Org B ploughing reduced total earthworm abundance, however total earthworm biomass decreased in both MP and NIT. Contrary to expectations, Shannon diversity was not affected by ploughing



**Fig. 2.** RDA triplot of earthworm and soil properties from Org A and Conv A from autumn 2011. Symmetric scaling was used. Explanatory variables were tillage (mouldboard ploughing (MP) or non-inversion tillage (NIT)) and farming system (conventional (Conv) or organic (Org)). Response variables were earthworm species abundances (*Aporrectodea caliginosa* (Acal), *Lumbricus rubellus* (Lrub), *Eiseniella tetraedra* (Etet), and *Aporrectodea rosea* (Aros)), soil organic matter (SOM), penetration resistance (PR) by depth (cm), and soil moisture (Moist) measured at time of earthworm sampling.

over the short term. Species richness did decrease after ploughing in Conv B, although this could be due to rarefaction (Sanders, 1968).

#### 4.2. Medium-term effects of reduced tillage on earthworms

Reduced tillage in the conventional farming system resulted in higher earthworm total biomass and *L. rubellus* (epigaeic) in the medium-term study (4 years) as hypothesised. Contrastingly, reduced tillage in the organic farming system decreased total earthworm abundance driven by consistently lower endogeic *A. caliginosa* abundances. However, total earthworm biomass in MT was higher than MP at sampling dates with no total earthworm abundance effect (autumn 2009 and spring 2012), which may have been caused by a higher, if not significant, percentage of adults. *L. rubellus* abundances did not increase from reduced tillage in organic farming. Anecic species abundances were too low throughout the experiment, in all cases, to be able to draw meaningful conclusions on tillage effects.

Endogeics, in particular *A. caliginosa*, dominated all farming and tillage systems, as was also noted by Marinissen (1992) at a nearby location. *A. caliginosa* was also the most abundant species in the arable soils studied by Nuutinen (1992), Emmerling (2001), Bithell et al. (2005), and De Oliveira et al. (2012). *A. caliginosa* was the most abundant earthworm species in the medium-term study in both Conv A and Org A, as was also noted in Conv B and Org B in the short-term study. *A. rosea* was second most abundant in Conv A and last in Org A, and *L. rubellus* was second in Org A and third in Conv A. Hence, the relative abundances of the more numerous species show a consistent pattern.

It has been suggested that incorporation of organic matter during ploughing gives an advantage to endogeic species by increasing food availability (Chan, 2001; Ernst and Emmerling, 2009; Van Capelle et al., 2012). Cropping and tillage systems that compact soils have a negative impact on earthworms (Wyss and Glassetter, 1992; Capowicz et al., 2009). Marinissen (1992) noted that sugar beet harvest, a crop also in the current study (Table 1), under wet conditions resulted in high adult *L. rubellus* mortality, at one of their

sampling dates. Soil organic matter was likely increased by the application of manure to organic farming fields. Incorporation of manure and crop residues by mouldboard ploughing likely resulted in the higher total earthworm abundances in MP than in one or both of the reduced tillage systems at 3 sampling dates. Higher total earthworm abundances in MP were driven by higher *A. caliginosa* in MP. Lacking this organic matter addition, reduced tillage in Conv A increased earthworm total biomass and benefited the epigaeic species *L. rubellus* by leaving crop residues on the soil surface. Therefore, interactions between tillage system and organic matter management are important in explaining earthworm ecological group responses.

The earthworm community found in this study was similar to others in north western Europe (Ernst and Emmerling, 2009; Valckx et al., 2009; Nieminen et al., 2011; De Oliveira et al., 2012). *E. tetraedra* was more abundant than in other studies perhaps because of its affinity for moist conditions (Sims and Gerard, 1999), however *E. tetraedra* has been found previously in this polder (Van Der Werff et al., 1998; Faber and Hout, 2009) and so its presence is not surprising. Earthworm communities in arable land are often dominated by endogeic species with low amounts of anecics, especially when under intensive tillage systems (Ernst and Emmerling, 2009; De Oliveira et al., 2012). In the current study, anecic earthworm abundances were negligible, which may be caused by a history of continuous mouldboard ploughing (Chan, 2001; Ernst and Emmerling, 2009; Van Capelle et al., 2012) or from soil disturbance during potato harvesting (Curry et al., 2002). Neither earthworm abundance, biomass, species richness, nor Shannon diversity showed clear increase over the course of the medium-term study, indicating a lack of cumulative tillage system effect after 4 years.

Differences in earthworm dominance, abundance, and biomass were consistent between farming systems and between tillage systems over 4 years despite crop rotation and climatic factors having strong effects on absolute earthworm abundance and biomass at individual sampling dates. Position in the crop rotation may explain differing tillage system effects between Org B and Org A. In Org

A MP had consistently higher earthworm abundances, whereas in Org B NIT had higher earthworm abundances. Higher earthworm abundances in Org B NIT may be due to organic matter inputs from grass clover clippings left on the soil surface during 2011 (Table 1), indicating that crop rotation plays an important role in earthworm population change.

#### 4.3. Relations between earthworms and soil properties

Redundancy analysis (RDA) showed clear distinctions between tillage systems and farming systems. Pulleman et al. (2003) also found higher soil organic matter (SOM) and earthworm activity under organic farming in a similar soil type in the south west of the Netherlands. The review by Hole et al. (2005) suggests organic amendments in organic farming systems improve soil organic matter and increase earthworm abundance. Organic farming has been reported to have higher earthworm abundance (Pfiffner and Mäder, 1997; Hole et al., 2005; Kragten et al., 2010) and species richness (Pfiffner and Mäder, 1997; Flohre et al., 2011) than conventional farming. Contrastingly, Pelosi et al. (2009) found earthworm abundance, biomass and diversity to be the same in conventional and organic farming over their 3 year study on arable soils in France.

Hypotheses regarding earthworms and soil properties are partially confirmed in the current study. Redundancy analysis showed positive correlations of SOM and soil moisture at the time of sampling with *L. rubellus* and *E. tetraedra*, but only weak correlation with *A. caliginosa* and *A. rosea*. Ernst and Emmerling (2009), also using RDA, found that endogeics benefit from SOM in ploughed systems, which agrees with current findings for *A. caliginosa* and *A. rosea*. Soil compaction, represented by penetration resistance, was negatively correlated with *A. caliginosa* and *A. rosea* but not correlated to *L. rubellus*, *E. tetraedra* or soil moisture. Other studies have also found that reduced tillage compacts soil and negatively impact earthworms, particularly endogeics (Wyss and Glasstetter, 1992; Langmaack et al., 1999; Capowiez et al., 2012).

## 5. Conclusions

In the short term, mouldboard ploughing (MP) negatively affected earthworm abundances (up to 53 days), however they recovered to pre-ploughing levels by the following spring. This fast earthworm population recovery was also reflected in the medium-term study as shown by the general lack of negative MP effects on earthworm abundances. Total earthworm abundances in organic farming tended to be lower in reduced tillage than MP systems driven by the predominant species *Aporrectodea caliginosa*, whereas, reduced tillage positively affected the epigeic *Lumbricus rubellus* in conventional farming. Interactions between tillage and organic matter management probably explain differing responses of earthworm ecological groups in the two farming systems. In general, organic farming had higher earthworm abundances, biomass, and Shannon diversity than conventional farming. Variation between sampling dates was large, likely due to effects of crop and climatic conditions. Despite this variation consistent tillage system effects were observed on certain species.

Future work should clarify the interaction of tillage systems, crop rotation, and organic matter management on earthworm populations, in particular anecic species. Long-term studies should monitor earthworm diversity in relation to biophysical properties and how these affect the development of soil functioning.

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