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Original article

Earthworm assemblages as affected by field margin strips and tillage intensity: An on-farm approach

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ABSTRACT

Earthworm species contribute to soil ecosystem functions in varying ways. Important soil functions like structural maintenance and nutrient cycling are affected by earthworms, thus it is essential to understand how arable farm management influences earthworm species. One aim of arable field margin strips and non-inversion tillage is to enhance agrobiodiversity, however their influence on earthworm species assemblages remains unclear. In particular, on-farm studies conducted over multiple years that capture variability across the landscape are rare. The current study monitored earthworm species assemblages on 4 farms in Hoeksche Waard, The Netherlands, from 2010 to 2012. It was hypothesised that arable field margin strips (FM) and non-inversion tillage (NIT; a reduced tillage system that loosens subsoil at 30–35 cm depth) would have higher earthworm species abundances (epigeics and anecics in particular), soil organic matter, and soil moisture than adjacent mouldboard ploughing (MP) fields, and that earthworm numbers would decrease with distance away from FM into arable fields (MP only). FM contained a mean total earthworm abundance of 284 m^{-2} and biomass of 84 g m^{-2} whereas adjacent MP arable fields had only 164 earthworms m^{-2} and 31 g m^{-2}. *Aporrectodea rosea*, *Lumbricus rubellus*, *Lumbricus terrestris*, and *Lumbricus castaneus* were significantly more abundant in FM than adjacent arable soil under MP. However, no decreasing trend with distance from FM was observed in earthworm species abundances. A tillage experiment initiated on the farms with FM showed that relative to MP, NIT significantly increased mean total earthworm abundance by 34% to 275 m^{-2} and mean total earthworm biomass by 15% to 51 g m^{-2} overall sampling dates and farms. *L. rubellus*, *A. rosea*, and *L. terrestris* were significantly more abundant overall in NIT than MP. FM and NIT positively affected earthworm species richness and abundances and it is noteworthy that these effects could be observed despite variation in environmental conditions and soil properties between samplings, farms, and crops. Higher top-soil organic matter and less physical disturbance in FM and NIT likely contributed to higher earthworm species richness and abundances. The anecic species *L. terrestris* (linked to water infiltration and organic matter incorporation) was more abundant in FM, but densities remained very low in arable soil, irrespective of tillage system.

1. Introduction

Functional agrobiodiversity (FAB) programs are being implemented to reverse negative impacts of agricultural land-use intensification. Practises such as non-crop areas (i.e., field margin strips), reduced tillage, and crop diversification aim to promote above and/or below-ground biodiversity and function [1]. Earthworms play important roles in soil nutrient and organic matter dynamics, and soil structure formation [2] and are strongly affected by soil pH, organic matter, and soil moisture [3]. Arable cropping and soil tillage affect earthworms through mechanical damage, reduction and vertical redistribution of organic matter, changes in soil water regime, and habitat disruption [3–6]. Ecological groups of earthworms [7] play important roles in determining certain soil functions [8]. Epigeic earthworms live and feed at the soil surface and contribute to organic matter incorporation and decomposition, anecic earthworms also feed at the soil surface but create deep vertical burrows and are considered most important for continuous soil pore formation and water infiltration [8,9]. Endogeic

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earthworms affect soil porosity and aggregate stability by feeding in the upper mineral soil layers [8,9]. However, farm management effects on total earthworm numbers have often been studied without acknowledgement of changes in species composition [10].

Field margin strips are border areas of arable fields that can contain grass/herb mixtures with flowering species to encourage above-ground biodiversity and natural enemies of crop pests [11,12] and may be implemented as part of FAB programs [1] and agri-environmental schemes [13]. Field margin strips have also been created as buffer strips to reduce surface water contamination and enhance landscape aesthetics [1]. It has been proposed that grassy field margin strips along arable fields can contribute to higher soil macrofaunal diversity and provide source populations for species, including earthworms, that can colonise arable fields [14,15]. Studies have shown higher earthworm numbers and diversity in grassy field margin strips compared to adjacent arable soil [15,16], however field margin strips have also been shown to contain lower earthworm numbers than adjacent arable fields [14]. Therefore, effects of grassy field margins on earthworm species assemblages require clarification.

Reduced tillage systems that improve soil structure (e.g., aggregate stability, friability and shear strength) [17,18] and reduce farming costs [19] continue to gain attention in The Netherlands and other parts of Europe. Contrasting results have been reported for effects of tillage systems on earthworms, probably due to large variation in reduced tillage practises and implements, and due to lack of attention for differing responses among earthworm species [20]. In particular, non-inversion tillage, a reduced tillage system without soil inversion by ploughing but still a relatively intense cultivation, may benefit earthworms, especially epigeics and anecics, by decreasing the intensity of soil disturbance while leaving an increased proportion of crop residues at the soil surface [3,19–21]. On the other hand, ploughing may give advantage to endogeic species (e.g., *Aporrectodea caliginosa*) because of increased access to food after incorporation of crop residues [6,20].

Influences of field margin strips and non-inversion tillage on earthworm assemblages in field studies should be conducted at multiple on-farm locations to capture spatial heterogeneity across the landscape and to verify patterns observed at single field research stations (e.g., [21–23]). Moreover, it is important that earthworm samplings take place over multiple seasons and years to encompass temporal variability [24,25]. The objective of the current study was to quantify the effects of field margin strips and reduced tillage on earthworms species assemblages for multiple farms and cropping seasons. Arable field margin strips were expected to contain higher earthworm numbers than adjacent arable land (i.e., total abundance and total biomass, epigeic and anecic species abundances, and adult/juvenile ratio). These earthworm parameters were expected to decrease with distance from field margin strips. In addition, non-inversion tillage would result in higher earthworm parameters compared to mouldboard ploughing. Lastly, higher earthworm species abundances (epigeic and anecic species in particular) in FM and NIT were expected to coincide with increased topsoil soil organic matter and soil moisture at the time of sampling compared to MP (due to crop residues left at the soil surface to a greater extent, longer cover crop presence, and less soil disturbance compared to MP).

2. Materials and methods

2.1. Study area

The study was conducted in Hoeksche Waard, The Netherlands. The region is a 325 km^{-2} island consisting of polders that were gradually reclaimed from the sea starting in the 15th century. Currently, Hoeksche Waard is mainly under arable land use with crop rotations that include potato, sugar beet, and winter wheat among other cereal and horticultural crops [26,27]. A functional agrobiodiversity (FAB) program began in 2004 on farms where field margin strips were created to promote natural crop pest enemies [1,26]. Daily mean temperature is 10 °C and annual precipitation is 900 mm [28]. Soils are hydromorphic calcareous sandy loam to clay [29], formed in marine deposits that, in general, overlay more sandy layers (below 45–60 cm) [30]. Mean high groundwater depths are 45–60 cm and mean low depths are 140–170 cm [30].

2.2. Experimental design

Earthworms were sampled on 3 private farms in the eastern part of Hoeksche Waard and at PPO Westmaas research farm of Wageningen University and Research Centre, all within a 10 km radius of each other. Sampling was done during spring 2010, autumn 2010, autumn 2011, and spring 2012.

Transects ($n = 4$) were set up within fields neighbouring field margin strips (FM) to test the effects of distance from FM on earthworm species abundances. Sampling along the transects consisted of 4 sample locations in grassy field margin strips, and 4 at 0.5 m, 30 m, and 60 m from field margin edges in each mouldboard ploughed field. Earthworm samples at each distance were spaced 8 m apart laterally (Fig. 1).

An additional aspect of land management was investigated at each farm by using a Tillage Experiment set up in 2008 that consisted of non-inversion tillage (NIT) plots within pre-existing conventional mouldboard ploughing fields ($n = 4$). Sampling locations in NIT plots were paired with adjacent locations in MP fields. At least a 2 m buffer was maintained between the outermost sampling locations and plot edges. In each tillage pair ($n = 4$) a total of 8 earthworm samples were taken per plot per sampling date. The sampling scheme consisted of 4 sample locations spaced 8 m apart at 30 m and 4 sample locations at 60 m from field edges (Fig. 1). Only 3 of 4 farms had complete tillage system pairs at the autumn 2011 and spring 2012 samplings.

Simultaneous sampling of earthworms and soil properties in the FM and Tillage Experiments allowed for data to be combined and inferences to be drawn on the influences of land management on soil properties and correlations with earthworm species abundances.

2.3. Farm management

In the FM Experiment permanent FM were established between 2001 and 2005. Strips (3–4 m wide) located between ditches and arable fields were seeded with grass or grass/herb mixtures. FM were mown 1–2 times per year. Cuttings from FM were left in the strips on two farms, and were removed on the other two. FM were driven upon incidentally during ditch cleaning and other occasions. Neither fertilisers nor agrochemicals were applied to the strips.

In the Tillage Experiment both tillage systems contained a set of distinct practises which were uniform across farms. The principle difference between tillage systems is the primary tillage instrument (mouldboard ploughing (MP) or non-inversion tillage (NIT)). MP was done every autumn to 25–30 cm depth (in the FM and Tillage experiment). NIT was characterised by use of the Kongskilde Paragrubber Eco 3000 (or chisel plough in some cases) to 30–35 cm to replace the mouldboard plough as primary tillage instrument so that soil was loosened at depth (about 50% of subsoil volume directly affected by tines) and not inverted during tillage. Cover crops and crop residues were managed differently in NIT and MP due to the difference in primary tillage. Cover crops and crop residues are left at the soil surface and not incorporated into the soil in

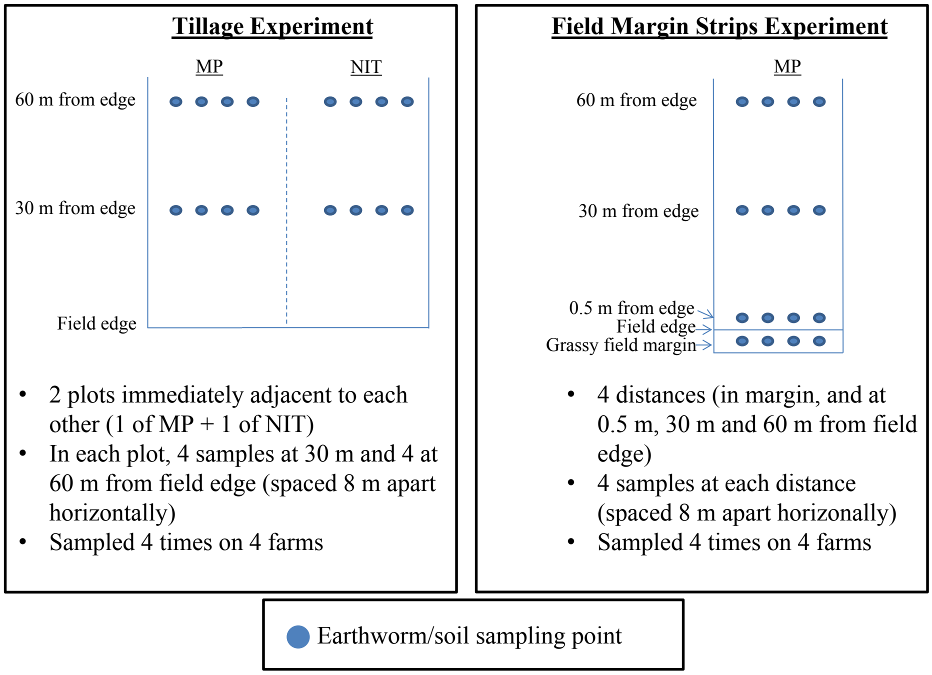

Fig. 1. Experimental design. Arrangement of earthworm and soil sampling points used in the Tillage Experiment (left) and in the arable Field Margin Strips Experiment (right).

NIT due to the absence of mouldboard ploughing. In the MP system cover crops and crop residues are ploughed under in autumn and soil is left bare until spring. Cover crops are therefore maintained as a live mulch at the soil surface for longer in NIT than in the standard MP practise in The Netherlands. Crop residues were retained (except for wheat straw in some cases) and superficially incorporated in NIT before seeding of the next crop and ploughed under in autumn in MP.

All farms used synthetic fertilisers and chemical pesticides according to normal practises in the area, which were applied in equal quantities to MP and NIT. Pig or cow slurry was applied in/after cereal crops and sometimes sugar beet at 15–50 $t ha^{-1}$ on all farms except Westmaas.

Crop rotations included cereals and tuber crops (crop rotations are given in Tables 1 and 2 of supplementary material). Crop residues were left on the soil surface after harvest in general, but wheat straw (not stubble) was sometimes removed on some farms. Cover crops were used in most years (Tables 1 and 2 of supplementary material). A superficial tillage operation using a harrow (7–10 cm depth) to prepare the seed bed is done in spring for the main crop and following harvest in autumn for the cover crop.

2.4. Sampling and laboratory analyses

Earthworms were sampled following Van Vliet and De Goede [31]. Soil monoliths of 20 × 20 × 20 cm were dug out and hand-sorted for earthworms. To extract anecic earthworms 500 ml of 0.185% formaldehyde solution was applied to the bottom of the monolith pits. Earthworms were counted, weighed fresh, and species were identified using Sims and Gerard [9] and Stöp-Bowitz [32] for juveniles. Earthworm samplings were conducted during spring and autumn seasons when conditions are cool and moist and so favourable to earthworm activity.

Soil properties were measured to assess the influence of land management on soil and to then infer resultant impacts on earthworm species abundances using multivariate analysis. Composite soil samples were taken to 20 cm depth around each earthworm sampling location. Gravimetric soil moisture was measured at each sampling date by drying representative subsamples at 105 °C for 24 h. Soil moisture conditions were on average 220 g kg^{-2}. Additionally, soil samples pooled by distance from field margin strips, taken during the autumn 2010 earthworm sampling, were used to measure soil pH, texture [33], total nitrogen [34], and soil organic matter by loss-on-ignition [35].

2.5. Statistical analyses

Earthworm species abundance data, less abundant species in particular, did not meet the ANOVA normality assumption even after data transformation, therefore generalised linear models were used. Effects of distance (in the FM Experiment) and tillage (in the Tillage Experiment) on earthworm total abundance, biomass, species abundances, species richness, and adult/juvenile ratio were analysed using generalised linear mixed effects models (negative binomial error distribution) with repeated measures. Earthworm population structure can be used as an indication of disturbance or stress where stressed individuals fail to reach adulthood and reduce the adult/juvenile ratio [36,37]. Species richness was calculated on a per monolith basis. Farms were considered as replicates. Both farm and sampling date were considered random variables in the overall models whereas only farm was considered random in the models investigating effects per sampling date (included as supplementary data). A continuous first order autocorrelation was used for repeated measures. Standard diagnostic plots were used to check model assumptions. In the FM data analysis, lack of model convergence for *Allolobophora chlorotica* and *Aporrectodea limicola* for per sampling date models (farm and sampling date as fixed effects, Tables 3 and 4 of supplementary data) necessitated a change in error distribution family to 'quasi', one of only two (Gaussian was the other) families that did not produce errors for both models.

Relations between management (i.e., field margin strips (FM), mouldboard ploughing (MP), and non-inversion tillage (NIT)) and soil properties, and between management and earthworm species

Table 1
Earthworm abundances and biomass along transects from field margin strips.[a]

	Distance	Total abundance (m^{-2})	Total biomass ($g\ m^{-2}$)	Adult/ juvenile ratio	*Aporrectodea caliginosa* (m^{-2})	*Aporrectodea rosea* (m^{-2})	*Lumbricus rubellus* (m^{-2})	*Aporrectodea limicola* (m^{-2})	*Lumbricus terrestris* (m^{-2})	*Lumbricus castaneus* (m^{-2})	*Allolobophora chlorotica* (m^{-2})	Species richness
Overall	FM	268 (51) a	73 (15) a	0.91 (0.16) a	127 (40) a	50 (13) a	22 (6) a	7 (5) a	10 (10) a	4 (4) a	1 (1) ab	3.29 (0.27) a
means	0.5 m	137 (26) c	27 (6) b	0.69 (0.13) b	84 (27) b	18 (5) b	2 (1) c	4 (3) ab	1 (1) b	0 (1) b	1 (1) a	1.84 (0.16) b
	30 m	133 (25) c	24 (5) b	0.38 (0.09) c	96 (31) ab	14 (4) b	7 (2) b	1 (1) c	1 (1) b	0 (0) b	0 (0) b	1.70 (0.15) b
	60 m	182 (35) b	31 (6) b	0.44 (0.09) c	125 (40) a	12 (3) b	13 (3) ab	2 (2) b	1 (1) b	0 (0) b	0 (0) ab	1.75 (0.15) b

[a] Mean total abundance, total biomass and species abundances are given with standard errors in parentheses. Samples were taken in the grassy field margin strips (FM) and 0.5 m, 30 m, and 60 m from field edge. Species with >1% of overall abundance are included, other species present were *Satchellius mamalis*, *Aporrectodea longa*, *Murchieona minuscula*, *Eiseniella tetraedra*, and *Dendrodrilus rubidus*. Species abundance columns are ordered from left to right by decreasing overall abundance. Letters indicate significant treatment differences at $P \leq 0.1$ between sampling distances. Adults and juveniles are combined, except for adult/juvenile ratio.

abundances were explored using redundancy analysis (RDA). Data from the Tillage system and FM Experiments were combined in two separate redundancy analyses. The first RDA explored relations between management and soil properties measured during the autumn 2010 earthworm sampling (soil moisture, total soil nitrogen, soil organic matter, pH, and soil texture). Farm was included as a covariable. The second RDA explored relations between management and earthworm species abundances overall 4 sampling dates. Farm and sampling date were included as covariables. Only observations with no missing earthworm or soil property values could be included in RDA. Permutation tests were used to detect statistical significance of explanatory variables. All statistical computations were performed using 'MASS' (*glmmPQL*), 'lsmeans', and 'vegan' packages of R [38–41]. The type I error rate (α) was set at $\leq$0.1 for all statistical tests.

3. Results

3.1. Distance from field margin strips

In the Field Margin Strips (FM) Experiment a total of 11 earthworm species were found inside the FM, 12 species at 0.5 m, 9 at 30 m, and 9 at 60 m from field edges. Mean earthworm total abundance was 284 m^{-2} in field margin strips (FM), 154 m^{-2} at 0.5 m, 146 m^{-2} at 30 m, and 192 m^{-2} at 60 m (Tables 1 and 2). Mean earthworm total biomass was 84 gm^{-2} in field margin strips (FM), 30 gm^{-2} at 0.5 m, 28 gm^{-2} at 30 m, and 34 gm^{-2} at 60 m (Tables 1 and 2). When averaged overall samplings, *Aporrectodea rosea*, *Lumbricus rubellus*, *Lumbricus castaneus*, and *Lumbricus terrestris* abundances were significantly higher in FM than samples in MP fields (i.e., at least two of: 0.5 m, 30 m, or 60 m from field edges). *A. caliginosa* was the dominant species in FM (54% of all individuals) and in arable fields (75–80% of all individuals) and their abundances did not vary significantly between FM and arable fields (30 m, or 60 m from field edges). *L. rubellus* abundance was lowest at 0.5 m from field edges overall samplings, significantly lower than in FM and 60 m from field edges. Mean species richness (calculated on a per monolith basis) overall samplings was 3.36 in FM, significantly higher than all other locations where species richness ranged between 1.70 and 1.86. Mean adult/juvenile ratio in field margin strips overall samplings was 0.96, however the value is skewed by the high ratio found in spring 2010. Median adult/juvenile ratio in field margin strips overall samplings was 0.41, and was significantly higher in FM than 30 m and/or 60 m from field edges at 3 of 4 samplings (Table 3 of supplemental material).

3.2. Tillage comparison

In the Tillage Experiment a total of 9 earthworm species were found in non-inversion tillage (NIT) whereas 7 were found in mouldboard ploughing (MP). In NIT, mean earthworm total abundance was 275 m^{-2} and mean earthworm total biomass was 51 gm^{-2} overall sampling dates and farms, significantly higher by 15% and 33% than MP plots respectively (Tables 3 and 4). NIT had significantly higher *L. rubellus* (165% higher) and *A. rosea* (79% higher) than MP overall. *L. terrestris* was also significantly higher in NIT (2.1 m^{-2}) than MP (0.2 m^{-2}), though numbers remained low throughout the study. *A. caliginosa* was the dominant species with 81% of all earthworms in MP and 71% in NIT. Mean species richness (calculated per monolith) overall samplings was 2.2 in NIT, significantly higher than 1.8 in MP. No difference in mean adult/juvenile ratio was found.

Table 2
Summary of GLMM output for Field Margin Strips Experiment.

	Total abundance	Total biomass	A/J ratio[a]	*Aporrectodea caliginosa*	*Aporrectodea rosea*	*Lumbricus rubellus*	*Aporrectodea limicola*	*Lumbricus terrestris*	*Lumbricus castaneus*	*Allolobophora chlorotica*	Species richness
Fixed effects (*p*-values)											
(Intercept)	0.00	0.00	0.57	0.00	0.00	0.00	0.01	0.03	0.23	0.89	0.00
Distance 0.5 m	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.46	0.00
Distance 30 m	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.99	0.00
Distance 60 m	0.00	0.00	0.00	0.91	0.00	0.15	0.01	0.00	1.00	0.03	0.00

[a] Adult/juvenile ratio.

3.3. Soil properties and earthworms

Variation in soil properties used in redundancy analysis (RDA) of earthworm species abundances from FM and Tillage Experiments (combined data from Tables 1 and 3) are presented in Fig. 2. Redundancy analysis of soil properties constrained by management (field margin strips (FM), mouldboard ploughing (MP), and non-inversion tillage (NIT)) are presented in Fig. 3. Management explained 6% of total variance ($P < 0.01$, 100 permutations) in the partial RDA model with soil properties from autumn 2010. Farm (covariable) explained 59% of total variance. Fig. 3 shows that FM contained higher soil organic matter (SOM) and total nitrogen (N_{tot}) but less clay than both MP and NIT. Soil moisture at the time of sampling was higher in FM and NIT than MP. Soil pH, N_{tot}, SOM, and moisture were negatively correlated with clay content.

A second RDA where earthworm species abundances from all sampling dates were constrained by management and distance from FM (in FM, 0.5 m, 30 m, and 60 m) is given in Fig. 4. Soil moisture measured simultaneously with earthworm samplings did not explain a significant amount of variance in the RDA of earthworm species abundances and was therefore dropped from the model. Explanatory variables management and distance explained 7% of total variance (both $P < 0.01$, 100 permutations), and covariables farm and sampling date explained 5% of total variance. *A. caliginosa* and *L. rubellus* were positively correlated, had higher abundance in NIT than FM and MP, and were negatively correlated with Distance-0.5 m. *A. caliginosa* was not correlated, and *Eiseniella tetraedra* was negatively correlated with FM whereas all other earthworm species abundances were positively correlated and higher in FM.

4. Discussion

4.1. Earthworms in field margin strips

In general, field margin strips (FM) had higher total earthworm abundance and biomass, as well as individual species abundances, than adjacent arable fields. However this did not result in a gradient of earthworm abundance into adjacent arable fields. *L. terrestris*, a species considered important for soil water infiltration [42] and crop residue incorporation [43], remained at negligible levels in arable fields even though abundances were significantly higher in adjacent FM. Little is known about the distribution of *L. terrestris* in The Netherlands. In a survey of 42 grassland and horticultural sites across The Netherlands Didden [44] only found *L. terrestris* on 2.4% of sites. The epigeic species *L. rubellus* and *L. castaneus* were most abundant in FM relative to NIT and MP. In a study conducted in England, fields with grassy field margin strips contained higher earthworm species abundances than fields without strips, however abundances in adjacent arable fields were not affected by presences of strips [15], in accordance with the current study. In a recent study, Roarty and Schmidt [25] studied earthworms in permanent and new field margin strips, and in adjacent arable fields over a 3 year period. Earthworm abundance and biomass was 3-fold higher in field margin strips than adjacent conventionally ploughed fields on average [25]. However, as in the current study, these margins did not enhance earthworm populations in near-by arable fields.

Anecic species were almost non-existent outside of field margin strips. Burrow destruction by tillage operations, use of heavy machinery at harvest, insufficient food quantity or inaccessible food may account for low anecic abundances [6,20]. Arable fields under reduced tillage in combination with adjacent field margin strips may provide greater opportunity for earthworm species that require less disturbed soil and greater food availability at the soil surface to migrate (e.g., *L. terrestris*). However, this is not supported by Roarty and Schmidt [25], who conclude that field margin strips support *L. terrestris*, but that reduced tillage does not benefit earthworm dispersal from field margins strips relative to ploughing. It may be that more time is needed for *L. terrestris* to establish in arable fields under reduced tillage. Nuutinen et al. [45] reported that inoculated *L. terrestris* did not spread from field margins and inoculation points in significant numbers after 5 years, however after 13 years a clear gradient with distance from field margin strips and inoculation points had established into arable fields under no-tillage. *L. castaneus*, was found to be more abundant in field margin strips than in adjacent arable fields in the current study, which is corroborated by Nieminen et al. [46]. *L. rubellus*, also epigeic, was similarly more abundant in FM than in arable fields at all distances from field edge. Furthermore, *L. rubellus* abundances were lowest at 0.5 m from field edge compared to other distances along transects in the current study. *L. rubellus* may have preferred FM over 0.5 m from field edge because FM contains more food resources. As an epigeic species *L. rubellus* spends more time at or near the soil surface relative to other species and thus has greater opportunity for mobility. *L. rubellus* dispersal rates in Dutch polders have been estimated at 11 my^{-1} and experimentally found to be 5 my^{-1} [47,48]. Also, since the field edge is the headland in some cases it may receive a greater number of tractor passes and have higher soil compaction which can limit *L. rubellus*.

4.2. Earthworms as affected by tillage systems

Non-inversion tillage (NIT) significantly increased earthworm total abundance, total biomass, and species abundances relative to mouldboard ploughing (MP) overall samplings in the Tillage Experiment. This confirms the hypothesis that NIT increases earthworm numbers relative to MP. An increase in species abundances with time cannot conclusively be attributed to a cumulative tillage system effect since it could not be disentangled from the influence of crop and climatic conditions. NIT consists of a less intensive soil manipulation than MP, though it is still more disruptive than strict no-till systems [19,49]. In NIT crop residues

Table 3
Earthworm abundances and biomass in non-inversion tillage and mouldboard ploughing plots.[a]

	Tillage system	Total abundance (m^{-2})	Total biomass (gm^{-2})	Adult/juvenile ratio	*Aporrectodea caliginosa* (m^{-2})	*Lumbricus rubellus* (m^{-2})	*Aporrectodea rosea* (m^{-2})	*Aporrectodea limicola* (m^{-2})	Species richness
Overall average	MP	185 (28)	36 (5)	0.42 (0.09)	144 (21)	13 (5)	14 (2)	2 (1)	1.85 (0.15)
	NIT	225 (33)*	44 (6)∘	0.43 (0.09)	165 (24)	22 (8)*	25 (4)*	2 (1)	2.16 (0.16)*

[a] Mean total abundance, total biomass, and species abundances are given with standard errors in parentheses. Tillage systems are non-inversion tillage (NIT) and mouldboard ploughing (MP). Species with >1% of overall abundance are included, other species present were *Lumbricus terrestris*, *Lumbricus castaneus*, *Allolobophora chlorotica*, *Murchieona minuscula*, *Aporrectodea longa*, and *Satchellius mamalis*. Species abundance columns are ordered from left to right by decreasing overall abundance. Significant differences between tillage systems within sampling dates are indicated by ∘ $P \leq 0.1$, *$P \leq 0.05$. Adults and juveniles are combined, except for adult/juvenile ratio.

Table 4
Summary of GLMM output for Tillage Experiment.

	Total abundance	Total biomass	Adult/juvenile ratio	*Aporrectodea caliginosa*	*Lumbricus rubellus*	*Aporrectodea rosea*	*Aporrectodea limicola*	Species richness
Fixed effects (*p*-values)								
(Intercept)	0.00	0.00	0.00	0.00	0.00	0.00	0.51	0.00
Tillage (NIT)	0.05	0.07	0.90	0.19	0.01	0.02	0.88	0.04

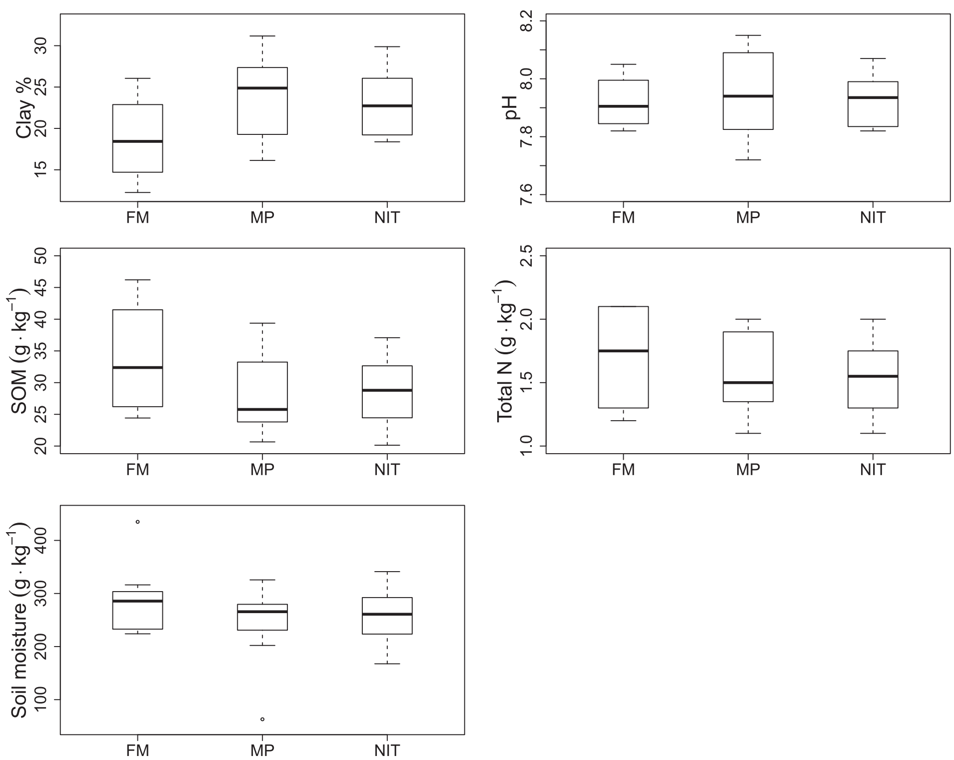

Fig. 2. Soil properties from autumn 2010 used in redundancy analysis. Data from the tillage comparison and field margin transects were combined. Non-inversion tillage (NIT, $n = 8$; $n = 30$ for soil moisture), mouldboard ploughing (MP, $n = 15$) ($n = 63$ for soil moisture), and field margin strips (FM, $n = 4$; $n = 16$ for soil moisture) are displayed.

are left at the soil surface and more opportunity for cover crops exist in autumn, both of which may contribute to higher earthworm numbers relative to MP systems [19,50,51]. Even though individual earthworm species abundances were higher in NIT than MP, the anecic species abundances (*L. terrestris* and *Aporrectodea longa*) remained very low in arable fields. Anecic species may not benefit from NIT systems because tillage operations (e.g., seed bed preparation, weed control) in NIT may still be too disruptive to burrows, insufficient organic matter may be retained, crop rotations that include tuber crops (e.g., potato, sugar beet) that require intensive ridge building operations and heavy machinery for harvest are too damaging, or insufficient time has passed for population increase to have occurred [3,22,23,45].

Reduced tillage in the current study had a positive effect on *L. rubellus*, likely due to retention of crop residues at the soil surface. On the other hand, Ernst and Emmerling [21] found no significant tillage effect on epigeic earthworms. The endogeic species *A. caliginosa* made up 70% of all earthworms in the current study and was more dominant in MP (75–81%) than NIT (71%) and FM (54%). This dominance by endogeic species, *A. caliginosa* in particular, in arable systems is congruent with previous findings [22,46,52].

4.3. Earthworm, soil property, and management relations

Earthworm species assemblages were similar to those of other studies conducted in Dutch polder soils [53–55]. Studies conducted

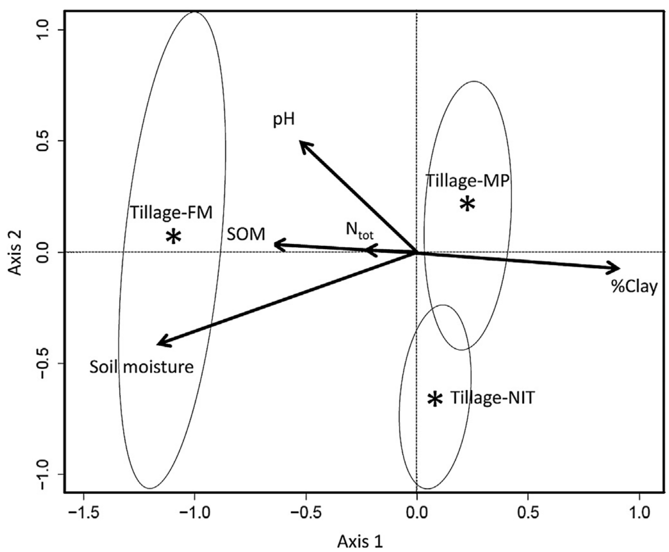

Fig. 3. Redundancy analysis biplot of soil properties from autumn 2010 ($P < 0.01$) constrained by management (field margin (TillageFM), mouldboard ploughing (TillageMP), and non-inversion tillage (TillageNIT) ($P = 0.01$)) with farm as covariable. The first RDA axis explains 14% of variance, the second RDA axis 0.4%, the first PCA axis 33%, and the second PCA axis 27% after variance due farm was removed (59% of total variance). Confidence intervals (95%) are indicated by ellipses around class centroids.

in arable and grassland sites in north-western Europe also had similar earthworm assemblages to the current study [46,52,56,57].

Integration of earthworm species abundance data from the FM and Tillage Experiments by multivariate analysis (Fig. 4) confirmed relations revealed by generalised linear models (Tables 1 and 3). Fig. 4 confirms that *L. rubellus* and *A. caliginosa* were more abundant

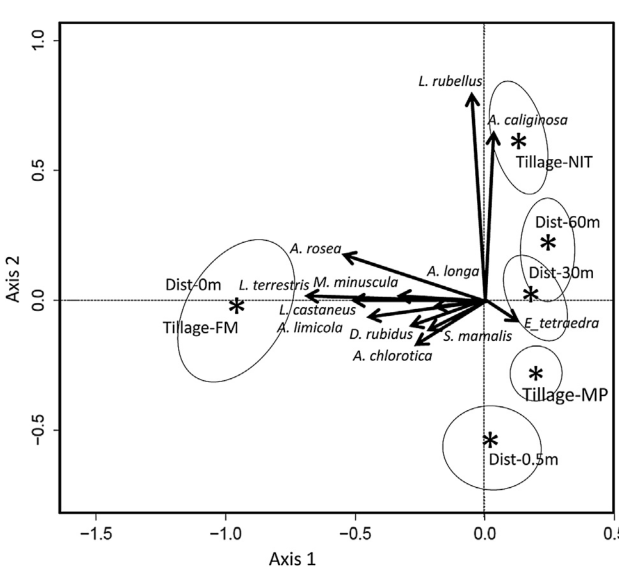

Fig. 4. Redundancy analysis (RDA) biplot of earthworm species abundances from all samplings ($P < 0.01$) in Field Margin Strips and Tillage system Experiments. RDA constraints were management (field margin strips (TillageFM), mouldboard ploughing (TillageMP), non-inversion tillage (TillageNIT) ($P = 0.01$)) and distance from field edge (Dist0 (TillageFM), Dist0.5 m, Dist30 m, or Dist60 m ($P = 0.01$)), farm and sampling date as covariables. Partitioning of correlation: 5.3% covariables, 6.7% constraints. The first RDA axis explains 4.6% of variance, the second RDA axis 2.0%, the first PCA axis 12%, and the second PCA axis 10% after variance due to farm and sampling date were removed. Species displayed are: *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Lumbricus rubellus*, *Aporrectodea limicola*, *Lumbricus castaneus*, *Lumbricus terrestris*, *Allolobophora chlorotica*, *Satchellius mamalis*, *Aporrectodea longa*, *Murchieona minuscula*, *Eiseniella tetraedra*, and *Dendrodrilus rubidus*. Confidence intervals (95%) are indicated by ellipses around class centroids.

in NIT than MP, and that many of the less common earthworm species (i.e., *L. terrestris*, *L. castaneus*) were more abundant in FM than adjacent arable soil. Redundancy analysis, in addition, showed that FM contained higher earthworm species abundances than MP or NIT for most species, indicating that FM can support more diverse earthworm communities than adjacent arable soil [15,46]. Earthworms in FM likely benefited from higher SOM and soil moisture relative to adjacent arable soil (Fig. 3) and from reduced soil disturbance and a permanent food source [15,25]. FM also contained less clay than the arable soils (NIT, MP; see Fig. 2) probably as a result of deposition of ditch dredging material [58].

Soil properties measured in the current study are known to affect earthworm species abundances [3], however soil properties varied relatively little across farms located within the same landscape (Fig. 2), and therefore likely had small influence on variation in earthworm species abundances. Additional soil properties (e.g., bulk density) could help explain variation in earthworm species abundances. Even though farm accounted for a large part of the variance in soil property data (59%, Fig. 3) it contributed, together with sampling date, only 5% of variance in earthworm species abundances (Fig. 4). Management (MP, NIT, FM) and differences in environmental conditions between sampling dates likely had a greater influence than farm due to the small variation in soil properties between farms. Significant management effects on earthworm species abundances across farms were detected despite large temporal variation (see Tables 3 and 4 in supplemental material).

5. Conclusions

Field margin strips and non-inversion tilled soil harboured higher earthworm numbers and more species than adjacent arable fields under mouldboard ploughing. However, anecic earthworm species (i.e., *L. terrestris*), considered important contributors to soil functioning, were virtually absent in mouldboard ploughed soil regardless of their presence in nearby field margin strips or in soil under non-inversion tillage. Soil disturbance and compaction resulting from crop rotations including sugar beets and potatoes and lack of crop residues (food for earthworms) left at the soil surface likely played a role. Field margin strips and tillage system effects on earthworm numbers were apparent in this on-farm study, conducted at multiple locations, even with variation due to changes in climatic conditions between samplings and heterogeneity between farms. The combination of decreased soil disturbance associated with tuber crops and increased duration of reduced tillage and non-crop areas may entice anecic species from adjacent non-crop areas, but further (longer term) studies are needed to confirm this. Functional agrobiodiversity programs that promote non-crop areas and reduced tillage can benefit earthworm abundance and diversity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2014.11.007.

References

[1] F. Bianchi, V. Mikos, L. Brussaard, B. Delbaere, M. Pulleman, Opportunities and limitations for functional agrobiodiversity in the European context, Environ. Sci. Policy 27 (2013) 223–231.

[2] C.A. Edwards, The importance of earthworms as key representatives of the soil fauna, in: C. Edwards (Ed.), Earthworm Ecology, second ed., CRC Press, Boca Raton, 2004.

[3] J.P. Curry, Factors affecting the abundance of earthworms in soils, in: C. Edwards (Ed.), Earthworm Ecology, second ed., CRC Press, Boca Raton, 2004.

[4] K. Lee, Earthworms: Their Ecology and Relationship with Soils and Land Use, Academic Press, Sydney, 1985.

[5] P. Hendrix, B. Mueller, R. Bruce, G. Langdale, R. Parmelee, Abundance and distribution of earthworms in relation to landscape factors on the Georgia Piedmont, U.S.A, Soil Biol. Biochem. 24 (12) (1992) 1357–1361.

[6] C. van Capelle, S. Schrader, J. Brunotte, Tillage-induced changes in the functional diversity of soil biota: a review with a focus on German data, Eur. J. Soil Biol. 50 (2012) 165–181.

[7] M. Bouché, Stratégies lombriciènnes, Ecol. Bull. 25 (1977) 122–132.

[8] A.M. Keith, D.A. Robinson, Earthworms as natural Capital: ecosystem service providers in agricultural soils, Econ. J. II (January 2012) 91–99.

[9] R. Sims, B. Gerard, Earthworms: notes for the identification of British species, in: J. Crothers, R.S.K. Barnes (Eds.), Synopses of the British Fauna, FSC Publications, 1999 no. 31 (revised) edn.

[10] D.J. Spurgeon, A.M. Keith, O. Schmidt, D.R. Lammertsma, J.H. Faber, Land-use and land-management change: relationships with earthworm and fungi communities and soil structural properties, BMC Ecol. 13 (2013) 46.

[11] P. Dennis, G.L. Fry, Field margins: can they enhance natural enemy population densities and general arthropod diversity on farmland? Agric. Ecol. Environ. 40 (1–4) (1992) 95–115.

[12] E.J.P. Marshall, Agricultural landscapes: field margin habitats and their interaction with crop production, J. Crop Improv. 12 (1–2) (2004) 365–404.

[13] C. Musters, F. van Alebeek, R. Geers, H. Korevaar, A. Visser, G. de Snoo, Development of biodiversity in field margins recently taken out of production and adjacent ditch banks in arable areas, Agric. Ecol. Environ. 129 (1–3) (2009) 131–139.

[14] J. Lagerlöf, B. Goffre, C. Vincent, The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape, Agric. Ecol. Environ. 89 (2002) 91–103.

[15] J. Smith, S. Potts, P. Eggleton, The value of sown grass margins for enhancing soil macrofaunal biodiversity in arable systems, Agric. Ecol. Environ. 127 (1–2) (2008) 119–125.

[16] A.R. Hof, P.W. Bright, The impact of grassy field margins on macro-invertebrate abundance in adjacent arable fields, Agric. Ecol. Environ. 139 (1–2) (2010) 280–283.

[17] M.R. Carter, Influence of reduced tillage systems on organic matter, microbial biomass, macro-aggregate distribution and structural stability of the surface soil in a humid climate, Soil Tillage Res. 23 (1992) 361–372.

[18] L. J. Munkholm, P. Schjùnning, K. J. Rasmussen, Non-inversion tillage effects on soil mechanical properties of a humid sandy loam, Soil and Tillage Res. 62.

[19] N. Morris, P. Miller, R. Froud-Williams, The adoption of non-inversion tillage systems in the United Kingdom and the agronomic impact on soil, crops and the environment: a review, Soil Tillage Res. 108 (1–2) (2010) 1–15.

[20] K.Y. Chan, An overview of some tillage impacts on earthworm population abundance and diversity: implications for functioning in soils, Soil Tillage Res. 57 (2001) 179–191.

[21] G. Ernst, C. Emmerling, Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period, Eur. J. Soil Biol. 45 (3) (2009) 247–251.

[22] J. Marinissen, Population dynamics of earthworms in a silt loam soil under conventional and "integrated" arable farming during two years with different weather patterns, Soil Biol. Biochem. 24 (1992) 1647–1654.

[23] J.P. Curry, D. Byrne, O. Schmidt, Intensive cultivation can drastically reduce earthworm populations in arable land, Eur. J. Soil Biol. 38 (2) (2002) 127–130.

[24] M. Pulleman, R. Creamer, U. Hamer, J. Helder, C. Pelosi, G. Pérès, M. Rutgers, Soil biodiversity, biological indicators and soil ecosystem services — an overview of European approaches, Curr. Opin. Environ. Sustain. 4 (5) (2012) 529–538.

[25] S. Roarty, O. Schmidt, Permanent and new arable field margins support large earthworm communities but do not increase in-field populations, Agric. Ecol. Environ. 170 (2013) 45–55.

[26] E.G. Steingröver, W. Geertsema, W.K. Wingerden, Designing agricultural landscapes for natural pest control: a transdisciplinary approach in the Hoeksche Waard (The Netherlands), Landsc. Ecol. 25 (6) (2010) 825–838.

[27] M. Rutgers, H. van Wijnen, A.J. Schouten, C. Mulder, A. Kuiten, L. Brussaard, A. Breure, A method to assess ecosystem services developed from soil attributes with stakeholders and data of four arable farms, Sci. Total Environ. 415 (2012) 39–48.

[28] Royal Netherlands Meteorological Institute, Daily Weather Data of the Netherlands, 2013. URL, http://www.knmi.nl/climatology/dail_data/download.html.

[29] H. de Bakker, J. Schelling, D.J. Brus, C. van Wallenburg, Systeem van bodem-classificatie voor Nederland: de hogere niveaus, Pudoc, Wageningen, 1989.

[30] Alterra, BIS Nederland De Bron Voor Bodeminformatie, 2013. URL, http://www.bodemdata.nl/.

[31] P.C. Van Vliet, R.G. De Goede, Effects of slurry application methods on soil faunal communities in permanent grassland, Eur. J. Soil Biol. 42 (2006) S348–S353.

[32] C. Stöp-Bowitz, A contribution to our knowledge of the systematics and zoogeography of Norwegian earthworms (Annelida Oligochaeta: Lumbricidae), Nytt Mag. Zool. 17 (1969) 169–280.

[33] D. Kroetsch, C. Wang, Particle size distribution, in: M. Carter, E. Gregorich (Eds.), Soil Sampling and Methods of Analysis, CRC, 2008, pp. 713–726.

[34] I. Novozamsky, V. Houba, E. Temminghoff, J. van der Lee, Determination of "total" N and "total" P in a single soil digest, Neth. J. Agric. Sci. 32 (1984) 322–324.

[35] N. Normalisatie-Instituut, Bodem: bepaling van het gehalte aan organische stof in grond volgens de gloeiverliesmethode = Soil: determination of organic matter content in soil as loss-on-ignition, in: Nederlandse Norm (NEN 5754), Nederlands Normalisatie-Instituut, Delft, 1992.

[36] C. Klok, A.M. De Roos, Population level consequences of toxicological influences on individual growth and reproduction in *Lumbricus rubellus* (Lumbricidae, Oligochaeta), Ecotoxicol. Environ. Saf. 33 (2) (1996) 118–127.

[37] C. Klok, A.M. De Roos, J.C.Y. Marinissen, Assessing the effects of abiotic environmental stress on population growth in *Lumbricus rubellus* (Lubricidae, Oligochaeta), Soil Biol. Biochem. 29 (314) (1997) 287–293.

[38] W.N. Venables, B.D. Ripley, Modern Applied Statistics with S, fourth ed., Springer, New York, 2002, ISBN 0-387-95457-0. URL, http://www.stats.ox.ac.uk/pub/MASS4.

[39] R.V. Lenth, Lsmeans: Least-squares Means, 2012. URL, http://CRAN.R-project.org/package=lsmeans. r package version 1.05-00.

[40] J. Oksanen, F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, H. Wagner, Vegan: Community Ecology Package, 2012. URL, http://CRAN.R-project.org/package=vegan. r package version 2.0-5.

[41] R Core Team, R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2012. URL, http://www.R-project.org/.

[42] M.J. Shipitalo, R.-C. Le Bayon, Quantifying the effects of earthworms on soil aggregation and porosity, in: C. Edwards (Ed.), Earthworm Ecology, second ed., CRC Press, Boca Raton, 2004.

[43] J.P. Curry, T. Bolger, Growth, reproduction and litter and soil consumption by *Lumbricus terrestris* in reclaimed peat, Soil Biol. Biochem. 16 (3) (1984) 253–257.

[44] W.A.M. Didden, Earthworm communities in grasslands and horticultural soils, Biol. Fert. Soils 33 (2) (2001) 111–117. ISSN: 0178–2762.

[45] V. Nuutinen, K.R. Butt, L. Jauhiainen, Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population, Pedobiologia 54 (2011) S167–S172.

[46] M. Nieminen, E. Ketoja, J. Mikola, J. Terhivuo, T. Sirén, V. Nuutinen, Local land use effects and regional environmental limits on earthworm communities in Finnish arable landscapes, Ecol. Appl. 21 (8) (2011) 3162–3177.

[47] J.C.Y. Marinissen, Colonization of arable fields by earthworms in a newly reclaimed polder in The Netherlands, in: Advances in Management and Conservation of Soil Fauna: [10th International Soil Zoology Colloquium Held during August 7–13, 1988, at Bangalore, India], 1991, pp. 341–348.

[48] J.C.Y. Marinissen, F. Van den Bosch, Colonization of new habitats by earthworms, Oecologia 91 (1992) 371–376.

[49] F. Tebrügge, R.-A. Düring, Reducing tillage intensity: a review of results from a long-term study in Germany, Soil Tillage Res. 53 (1999) 15–28.

[50] J. Holland, The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence, Agric. Ecol. Environ. 103 (1) (2004) 1–25.

[51] J. Peigné, B.C. Ball, J. Roger-Estrade, C. David, Is conservation tillage suitable for organic farming? A review, Soil Use Manag. 23 (2) (2007) 129–144.

[52] T. De Oliveira, M. Bertrand, J. Roger-Estrade, Short-term effects of ploughing on the abundance and dynamics of two endogeic earthworm species in organic cropping systems in northern France, Soil Tillage Res. 119 (2012) 76–84.

[53] P.A. Van der Werff, R. Noordhuis, T.B.M. Dekkers, Introduction of earthworms into an organic arable farming system, Appl. Soil Ecol. 9 (1998) 311–317 (February 1997).

[54] J.H. Faber, A.V. Hout, Introductie Van Regenwormen Ter Verbetering Van Bodemkwaliteit, Tech. Rep., Alterra, Alterra-rapport 1905, Wageningen, 2009.

[55] S. Crittenden, T. Eswaramurthy, R. de Goede, L. Brussaard, M. Pulleman, Effect of tillage on earthworms over short- and medium-term in conventional and organic farming, Appl. Soil Ecol. 83 (2014) 140–148.

[56] G. Ernst, D. Felten, M. Vohland, C. Emmerling, Impact of ecologically different earthworm species on soil water characteristics, Eur. J. Soil Biol. 45 (3) (2009) 207–213.

[57] J. Valckx, L. Cockx, J. Wauters, M. Van Meirvenne, G. Govers, M. Hermy, B. Muys, Within-field spatial distribution of earthworm populations related to species interactions and soil apparent electrical conductivity, Appl. Soil Ecol. 41 (3) (2009) 315–328.

[58] A.A.J.V. Strien, J.V.D. Linden, T.C.P. Melman, M.A.W. Noordervliet, Factors affecting the vegetation of ditch banks in peat areas in the western Netherlands, J. Appl. Ecol. 26 (3) (1989) 989–1004.