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Earthworm communities in arable fields and restored field margins, as related to management practices and surrounding landscape diversity

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Running headline: Earthworm responses to arable land management and landscape diversity

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Abstract

Agricultural intensification has negative impacts on biodiversity at spatial scales from field to landscape. Earthworms are important for soil functioning, so it is crucial to understand the responses of earthworm communities to agricultural management and land use. We aimed to: 1) investigate whether earthworm communities differed between relatively undisturbed field margins, and highly disturbed arable fields; and 2) quantify how earthworm communities of arable fields and field margins are affected by three environmental filters, i.e. soil properties, management practices, and composition of the surrounding landscape. Earthworms were sampled in 26 arable fields and 15 field margins, across a polder area in The Netherlands. While earthworm density, total biomass and species richness did not differ significantly among arable fields and field margins, rarefied earthworm species richness and community composition did. The three environmental filters affected earthworm communities of arable fields and field margins differently. In arable fields, earthworm communities were explained by arable management only (26%). In contrast, all three filters contributed significantly to the variation in earthworm communities of field margins, where management practices explained a larger part of the variation (18%) than the surrounding landscape (11%) and soil properties (10%). Our results suggest that soil properties and surrounding landscape can affect earthworm communities of field margins. However, in the arable fields, where more diverse lumbricid communities are desirable to improve soil functions, such influences are negated by the impact of management at field scale. We demonstrated that field margins enhance earthworm biodiversity in arable landscapes, but surrounding landscape and field margins had limited impact on earthworm communities in arable fields . Decision-making and research should focus on less intensive management options for arable fields to stimulate earthworms and earthworm-mediated soil functions.

1. Introduction

Earthworms play important roles in arable cropping systems, contributing to nutrient cycling, organic matter formation and decomposition, soil structure formation, and water infiltration (Edwards, 2004; Keith and Robinson, 2012). Their presence in agroecosystems can increase crop yields by 25% (van Groenigen *et al.*, 2014). It is well known that earthworms are affected by several environmental filters, which constrain the earthworm species pool found in particular habitats (Decaëns *et al.*, 2008). Examples of environmental filters acting on earthworm communities are soil properties (e.g., soil moisture, organic matter, texture and pH (Curry, 2004)) and agricultural management practices (e.g., tillage (Chan, 2001), pesticide application (Pelosi *et al.*, 2014) and organic matter management (Curry and Schmidt, 2007)).

In general, agricultural intensification negatively affects earthworm communities (Postma-Blaauw *et al.*, 2010). Although agricultural intensification occurs across spatial scales from the field to the landscape (Ettema and Wardle, 2002), landscape effects on earthworm communities have hardly been studied. Landscape-scale agro-intensification refers to the ongoing loss of (semi-) natural area, the increasing surface area for agricultural production, and consequently the homogenization of landscapes. In an attempt to reverse the effects of intensification, agro-environment measures are being implemented in Europe (EU-Commission, 2005). These measures are partly focussed on enhancing biodiversity in agricultural landscapes, and partly on promoting alternative management practices at the field and farm scale, e.g., crop diversification and restoration of non-productive landscape elements on farm, such as field margins (EU-Commission, 2005). To better understand the effects of (de)intensification of agriculture, both farm management practices and landscape characteristics need to be considered (e.g., Tscharntke *et al.*, 2005). Most studies that considered landscape effects on earthworm communities in arable fields focussed on the relevance of (semi-)permanent field margins as potential sources for earthworm colonization of arable fields (e.g., Smith *et al.*, 2008; Roarty and Schmidt, 2013; Crittenden *et al.*, 2015, but see Flohre *et al.*, 2011 and Lüscher *et al.*, 2014 for larger scale effects). Semi-permanent field margins are edges of arable fields that have been converted and restored to non-crop area, e.g. strips sown with grass(-herb) mixtures. They are subject to a lower frequency and intensity of soil disturbance. To our knowledge, environmental filters, such as soil properties, management practices and surrounding landscape, affecting earthworm communities of arable fields and field margins have scarcely been studied collectively. Given the fact that fields and margins neighbour each other spatially, but strongly differ in frequency, type and intensity of disturbance, quantifying effects of environmental filters on earthworm communities of these habitats may help to support management and spatial planning at farm and landscape scales to enhance soil biodiversity (Bianchi *et al.*, 2013).

The objectives of this study were two-fold. First, earthworm communities were compared between arable fields (hereafter named “fields”) and semi-permanent field margins (hereafter named “margins”) with different spatial configurations (fields had margins present or not). Second, the relative contribution of the environmental filters, soil properties (hereafter named “soil”), management practices (hereafter named “management”) and composition of the surrounding landscape up to 500 m radius (hereafter named “landscape”), on earthworm communities of fields and margins was quantified. We hypothesized that earthworm density, species richness, and biomass would be lower in fields than margins, but not between fields with and without a margin. Furthermore, we hypothesized that earthworm communities would differ between margins and fields, but not between fields with and without a margin. We did not expect differences between fields with and without margins, because previous studies only showed limited spill-over effects of earthworms from margins to fields (e.g. Smith *et al.*, 2008; Roarty and Schmidt, 2013; Crittenden *et al.*, 2015). Our third hypothesis was that a higher proportion of nearby non-arable surface area would contribute to more diverse earthworm communities in margins, and not in fields. It was thus hypothesized that for fields, landscape effects would be overshadowed by management practices, because of an expected large effect of management-associated periodic disturbance (physical, chemical and biological) on earthworms.

2. Materials and Methods

2.1 Study area

Our study was carried out in the Hoeksche Waard, in the southwestern part of The Netherlands. The region, with a surface area of about 324 km^2 comprises a set of polders, progressively reclaimed from the sea since the 15th century, and is dominated by prime agricultural soils for arable cropping, mostly potato, sugar beet and wheat (Crittenden *et al.*, 2015). Soils are hydromorphic calcareous sandy loam to clay formed in marine sediments (de Bakker and Schelling, 1966). Daily average temperature is 10.8 °C and annual precipitation is 883 mm (Royal Netherlands Meteorological Institute). The region is also characterized by a large network of margins (> 400 km) including annual flower strips and semi-permanent grass or grass-herb mixtures.

2.2 Sampling design and methods

Farm selection was aimed at an even geographic representation over the Hoeksche Waard, and was dependent on farmers' willingness to participate in the project. Twenty-six fields and 15 margins were sampled across a total of 15 farms. All fields had been under crop production for at least 25 years, and had been cultivated to winter wheat in the year of sampling. Thirteen of the 26 fields had margins, in which sampling was conducted. In addition, there were two margins sampled where the associated field was not sampled because they did not have winter wheat at the time. Sampling was done in September and October

2012, after harvest and before tillage in the arable fields. At the time of sampling, fields were covered with either wheat stubble and residue, or with a green manure of *Lolium* grasses or radish (*Raphanus sativus* subsp. *oleiferus*). Sampled margins had been sown with perennial grasses or mixtures of herbs and grasses between 2000 and 2010 and did not undergo soil disturbance since then. Grass(-herb) margins established later than 2010 were excluded from this study, as the time between the last ploughing event and our sampling campaign was considered too short; additionally, margins sown with annual flowers were also excluded from this study because they are ploughed and re-sown every year.

In each field, six earthworm samples were taken within a 10 m radius. The center of the circle was at about 40 m from the edge of the field or the margin, when present. In the margins, four earthworm samples were taken along the margin, 20 m apart. The center of the sampling areas was georeferenced to allow for further spatial analyses.

Earthworm sampling was done using the methodology described by van Vliet and de Goede (2006): a soil monolith of 20 x 20 x 20 cm was dug out and hand-sorted for earthworms, followed by the application of 0.5 l of 0.2 % formaldehyde solution onto the bottom of the pit, to expel burrowing anecic earthworms. Each sample of earthworms was weighed the same day upon extraction, and subsequently stored in 70% alcohol until identification. Biomass was measured taking into account not only whole individuals, but also pieces, heads and tails. However, only intact individuals or heads were considered for identification, and consequent quantifications of species richness, density and composition. Adult and juvenile individuals were identified using Sims and Gerard (1999) and Stöp-Bowitz (1969), respectively; 0.2% of the intact individuals could not be identified and were therefore excluded from data analysis.

Around each earthworm sampling pit, five soil cores were taken to a depth of 20 cm and pooled into one composite soil sample per sampling location. Samples were analysed for pH-

H_2O with a volume ratio soil:water of 1:5, and texture using laser diffraction (Buurman *et al.*, 2001). Total nitrogen and carbon were analysed by the Stable Isotope Facility of UC Davis with a PDZ Europa ANCA-GLS elemental analyser (Sercon Ltd, Crewe, Cheshire, UK) after removal of inorganic C using the acid fumigation method (Harris *et al.*, 2001). Soil moisture content at the time of sampling was measured gravimetrically after 24h at 105˚C. For details regarding soil properties, see Tables A1 (with detailed explanations), A2 and A3 (with summary statistics of the explanatory variables of fields and margins, respectively) of Appendix A in Supplementary material.

2.3 Management

Farmers were interviewed using standardized questionnaires about the management of the sampled fields and margins, with focus on the last rotation cycle from 2009 to 2012. Farmers were asked about the main and cover crops that were cultivated, tillage operations, crop residue management, pesticide types and number of applications, as well as types and amounts of mineral fertilizers and manure applications. A detailed description of the management-related variables of arable fields is provided in Table A1 of Appendix A in Supplementary material, and summary statistics in Table A2 of Appendix A in Supplementary material.

Regarding the margins, farmers were asked to provide information about the year of establishment, the sown mixture type (grass vs. grass-herb mixtures), the mowing frequency and whether the mown material was removed from the soil surface or not (Table A3 of Appendix A in Supplementary material).

2.4 Landscape

The surrounding landscape of the sampling locations in fields and margins was examined for the area corresponding to circles of four radii (50, 100, 250, and 500 m). Our main focus was on land-use types where earthworms can potentially occur (hereafter named “inhabitable land-uses”): arable land, deciduous forests, productive and semi-natural grasslands, orchards, unpaved infrastructures, cemeteries, grass and flower field margins. Landscape was characterized in terms of relative surface area and diversity of land use types. Relative surface area was calculated based on the proportion of arable land within each radius, whereas diversity was quantified using the Shannon diversity index of the inhabitable land-uses excluding arable land surface (Tables A1 to A3 of Appendix A in Supplementary material). Arable land was excluded when computing the Shannon diversity index to eliminate the high correlations between the surface area and landscape diversity metrics (Fischer *et al.*, 2011).

Official PDOK-TOP10 topographic maps (scale of 1:10000), were complemented by GIS maps of grass and flower margins, provided by the Waterboard “Hollandse Delta”. After transforming linear elements of the TOP10 maps to polygons, each land-use surface area was quantified for the four considered radii. Analysis was done using the BUFFER tool in ArcGIS 10.2.1 (ESRI Inc. Redlands, California). Margins were manually transformed to polygons *a posteriori* by multiplying their length by 3 m, which is the usual width of margins in the region. Subsequently, the estimated surface area of margins was subtracted from the surface of arable land.

2.5 Data analysis

2.5.1 Univariate analysis

To compare species richness among margins and fields with and without margins, sample- and individual-based rarefaction curves (Figure B1 of Appendix B in Supplementary material) were computed. Species richness among different habitats is only meaningfully comparable when a clear asymptote for each curve is reached (Gotelli and Colwell, 2001). Furthermore, because species richness increases with sample size, it can only be compared when the sample size among the habitats is equal. Rarefying species richness removes the effects of varying sample size by standardizing richness through interpolation of a sample to a smaller number of individuals, usually the total abundance of the least abundant site (Gotelli and Colwell, 2001). We rarefied earthworms to 25 individuals, which was the lowest total number of individuals collected in any of the habitats considered in this dataset. Differences in earthworm density, biomass and rarefied species richness (RFSR) among margins and fields with and without margins were analysed with linear models. Observed species richness (SR), based on true counts, was analysed with generalized linear models (GLM), with a Poisson distribution and a log link function. Density and biomass were expressed as number of individuals or biomass per meter square, while SR was calculated on a margin or field basis (i.e., the four or six subsamples taken in margins or fields, respectively, were pooled per site). Differences between margins and fields with and without margins were assessed with F-tests for the linear models and X^2-tests for the GLM. Pairwise comparisons were computed when the overall models were statistically significant, but due to the low number of comparisons (three, in total), p-value adjustments to avoid inflation of type I error were considered unnecessary. Model residuals were inspected visually to validate distribution and variance assumptions (Zuur *et al.*, 2009), and when the assumption of variance homogeneity was violated among treatments, a variance structure was used to allow different variance in each habitat type (Zuur *et al.*, 2009).

2.5.2 Multivariate analysis

Earthworm community composition differences between fields with and without margins, and between fields and margins were tested by redundancy analysis (RDA), after log(x+1) transformation of the abundance data per unit of area (m^2) (ter Braak and Šmilauer, 2014) where margins and fields with and without margins were used as nominal explanatory variables. Pairwise comparisons among fields with and without margins, and margins were computed and model significance was assessed by Monte Carlo permutation tests (999 permutations, $p < 0.05$).

Further statistical analysis considering the relationships between environmental filters (soil, management and landscape) and earthworm community composition was conducted separately for fields and margins, because their management-related explanatory variables were different (Tables A2 and A3 of Appendix A in Supplementary material). Furthermore, fields with and without margins were pooled, since no significant differences were found in earthworm community composition between the differently configured fields (see section 3.1). The effects of the three environmental filters on earthworm community composition were tested using a 2-step approach. First, we estimated the most parsimonious model explaining earthworm community composition for each individual filter resulting in three models per habitat, hereafter named "separate RDA's". Second, we constructed an RDA model combining the "separate RDA's" resulting in one overall model per habitat, hereafter named "combined RDA". Explanatory variables showing strong collinearity in each of the separate RDA's were identified by calculating variance inflation factors (VIF). One by one, variables with VIF> 10 were withdrawn from the model, starting with the variable with the highest VIF (Zuur *et al.*, 2009; Borcard *et al.*, 2011) (Tables A2 and A3 of Appendix A in Supplementary material). Forward selection was then used to obtain the most parsimonious separate RDA's for each filter. Parsimony was achieved by applying the double-stopping criterion (Blanchet *et al.*, 2008), i.e. alpha significance level and adjusted r^2 of the separate RDA's. In the second step of the approach, to obtain the combined RDA for each habitat, the forward selection procedure was applied on all explanatory variables that were included in the parsimonious separate RDA's, which were subsequently tested for significance with 999 Monte Carlo permutation tests ($p < 0.05$). To quantify the relative contribution of each filter to earthworm community composition of fields and margins, variation partitioning was computed. The proportion of variation of earthworm community composition due to each of the filters was quantified with adjusted r^2 and tested for statistical significance using Monte

Carlo permutation tests (999 permutations, $p < 0.05$) (Borcard *et al.*, 2011). All analyses were performed with R 3.2.2 (R Core Team, 2014), using packages nlme 3.1-128, vegan 2.3-2, biodiversityR 2.7.1 and packfor 0.0-8.

3. Results

3.1 Earthworm community metrics in fields and margins

In total eleven species of earthworms were found. Fields hosted a total of nine and margins harboured ten species (Table 1). Neither earthworm total density ($F= 1.172$, $p= 0.193$), biomass ($F= 1.172$, $p= 0.321$), nor SR-species richness ($X^2= 2.607$, $p= 0.272$) showed statistically significant differences between fields and margins, irrespective of the presence of a margin. The RFSR-species richness overall model, on the other hand, revealed significant differences ($F= 4.8685$, $p= 0.013$), where RFSR was higher in margins than in fields both with and without margins ($p < 0.05$) (Table 1). RDA of earthworm composition constrained by habitat (i.e. margins and fields with and without margins) separated margins from fields along the first RDA axis (overall model: adjusted $r^2= 9.5\%$, $p= 0.001$, Fig. 1). The presence of margins adjacent to the fields did not affect earthworm species composition in fields ($p= 0.104$), whereas there was a significant difference in earthworm composition between margins and fields ($p < 0.05$). In fields, the most abundant species were *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), *Lumbricus rubellus* (Hoffmeister, 1843) and *Allolobophora chlorotica* (Savigny, 1826). In margins *A. caliginosa*, *Lumbricus castaneus* (Savigny, 1826) and *A. rosea* were dominant, whereas *Aporrectodea limicola* (Michaelsen, 1900), *L. rubellus*, *A. chlorotica* and *Lumbricus terrestris* (Linné, 1758) occurred relatively frequently. The least abundant species in fields were *Murchieona minuscula* (Rosa, 1906), *L. terrestris*, *Eiseniella tetraedra* (Savigny, 1826), *A. limicola* and *L. castaneus* (all less than 10 individuals m^{-2}), and *Aporrectodea longa* (Ude, 1885) and

Satchellius mammalis (Savigny, 1826) were not found in this habitat. In margins, the least abundant species were *M. minuscula*, *S. mammalis* and *A. longa* (all less than 10 individuals m^{-2}), and from the pool of sampled species only *E. tetraedra* was not detected in this habitat (Table 1).

3.2 Effects of environmental filters on earthworm communities in arable fields

In fields, only the variables representing the environmental filters management and soil explained a statistically significant part of the variation in community composition when considering RDA models for each filter separately (separate RDA models; Table 2). The management related variables, i.e. applications of herbicides (adjusted r^2= 12%, p= 0.001), fungicides (adjusted r^2= 8%, p= 0.002), and insecticides (adjusted r^2= 6%, p= 0.012), cumulatively explained 26% (p= 0.001) of the variation in species composition. For soil (cumulative adjusted r^2= 4%, p= 0.042) only clay content was selected (Table 2). In the subsequent RDA model that combined the separate models of all previously selected variables (combined RDA model), the variation explained by clay content became negligible (Table 2). Most earthworm species were at least weakly negatively associated with the number of applications of insecticides and/or herbicides in 2012 (Fig. 2). The only positive association found was an increase in density of *L. rubellus* with fungicide application rate. In particular, *A. chlorotica*, *E. tetraedra* and *L. castaneus* showed strong negative correlations with the number of herbicide applications, and *A. limicola* and *L. castaneus* with the number of insecticide applications.

3.3 Effects of environmental filters on earthworm communities in field margins

The variables representing the three environmental filters (landscape, management and soil) significantly explained part of the variation in community composition of the margins (Table 3). Within the separate RDA model for management, age of margin (adjusted r^2= 14%, p= 0.004) and mowing frequency (adjusted r^2= 10%, p= 0.017) were selected (cumulative adjusted r^2= 24%, p= 0.001). The separate RDA model for the filter soil included pH (adjusted r^2= 17%, p= 0.002). In contrast to the fields, variables representing the filter landscape were selected in the separate RDA model: the proportion of arable area within a radius of 500 m explained 17% (p= 0.003) of the variation in earthworm community composition (Table 3). All the variables in the separate RDA models for the three filters appeared also in the RDA model that combined all filters (Table 3). This combined RDA model explained 45% (p= 0.001) of the variation in the earthworm community composition. The earthworm species *A. limicola, L. terrestris, A. chorotica, A. longa* and *S. mammalis* were positively associated with the age of the field margin (Fig. 3) and negatively correlated with pH and surface area occupied by arable fields within a radius of 500 m. The species *L. castaneus, L. rubellus, A. rosea, A. caliginosa*, and to a smaller extent *M. minuscula*, correlated negatively to mowing frequency of the margins.

3.4 Variation partitioning of environmental filters

Since the combined RDA model for the fields only comprised variables related to management (Table 2), variation partitioning among environmental filters was not necessary. In the case of margins, all three environmental filters were included in the combined RDA model (Table 3). Variation partitioning for the three environmental filters disclosed that the earthworm community variation in margins that could be uniquely attributed to the filter management (18%, p= 0.001) was almost twice as large as the variation attributed to the filters soil or landscape (10% and 11%, respectively, $p < 0.05$) (Table 4). Only about 6% of the variation in earthworm community composition was shared between the three filters.

4. Discussion

4.1 Density, biomass, species richness and composition of earthworms

The earthworm species pool found during this study was comparable to that of other studies in Dutch polders (Crittenden *et al.*, 2014; 2015), as well as in other countries of north-western Europe (Ernst and Emmerling, 2009; Ernst *et al.*, 2009; Nieminen *et al.*, 2011). However, earthworm density, biomass and species richness were generally higher than reported in those studies, both in fields and in margins. In accordance with Nieminen *et al.* (2011); de Oliveira *et al.* (2012); Crittenden *et al.* (2015), the endogeic species *A. caliginosa* was the dominant species, accounting for 46% of the total density in margins and 57% and 65% in fields with and without margins, respectively. Anecic species (*L. terrestris* and *A. longa*) were mostly found in the margins. Their densities in fields were considerably lower, probably due to mechanical soil disturbance and limited food availability (Chan, 2001; van Capelle *et al.*, 2012). Also epigeic species were mostly found in margins, with the exception of *L. rubellus*, which occurred at comparable densities in margins and fields. Anecic and epigeic earthworms feed on organic matter at the soil surface (Bouché, 1977), which is likely more available in margins than in fields (Chan, 2001; van Capelle *et al.*, 2012), and additionally, soil disturbance is lower in margins than in the annually ploughed fields. Of all encountered epigeic species, *L. rubellus* has the highest fecundity (up to 106 cocoons produced per year (Edwards and Bohlen, 1996)), and the resulting potential for population recovery may account for its similar densities across fields and margins. Despite dissimilarities in species distribution among the different habitats, we could not detect significant differences in earthworm total density, biomass and SR-species richness, which partially contradicted our first hypothesis. However, earthworm RFSR-species richness was higher in margins than in fields, and no differences were found between fields with and without margins, which is in partial agreement with our first hypothesis. This indicates that fields have a lower richness than margins. On the other hand, the steeper rarefaction curves of the fields compared to the ones of the margins indicate that earthworm communities of the fields are more even than those of the margins (Olszewski, 2004). Considering this, it is very relevant, though unfortunately rare in earthworm ecology studies, to include rarefaction computations when evaluating land-use and management effects on earthworm communities. Our finding of differences in earthworm community composition between margins and fields, but not between fields with and without margins, agrees with previous findings. Smith *et al.* (2008) also studied earthworm densities in margins and fields with and without margins and concluded that the presence of margins, whilst harbouring higher densities, had no spill-over effect to the adjacent field. Likewise, Crittenden *et al.* (2015) and Roarty and Schmidt (2013) observed no increase in earthworm density in conventionally tilled fields with decreasing distance to the margin. The latter study, however, did show that the establishment of new, uncultivated margins in between the arable fields and the already existing permanent margins resulted in similar earthworm populations in old and new margins within three years. This indicates either a spill-over effect from the permanent to the newly created margins, or that the local earthworm populations did have the chance to develop to abundances comparable to the ones in the existing permanent margins. Evidence so far suggests that margins contribute to increased earthworm biodiversity in arable landscapes, but have little influence on earthworm communities in the fields as long as these are intensively cultivated.

4.2 Effects of environmental filters

Variation partitioning allowed testing for the relative contribution of the three environmental filters, i.e. soil, management and landscape, on earthworm communities in margins and fields. Overall our results suggested that earthworm communities were affected by environmental filters operating at different spatial scales and that the effects depended on habitat disturbance. These findings are in line with those of Decaëns *et al.* (2008), who acknowledged that the earthworm species pool found in a particular habitat is constrained by a set of abiotic factors inherent to the land-use under focus (broad habitat and land-use constraints as referred by Decaëns *et al.*, 2008). For fields, management was the most important filter, and neither soil nor the landscape at any radius played a substantial role in earthworm species sorting (Table 4). Our results are partly in line with those of Lüscher *et al.* (2014) who did not find any effects of the surrounding landscape on earthworm composition of fields. However, in contrast to our findings, those authors could not demonstrate any relationship between earthworm community composition and management-related variables, either.

With respect to the margins, earthworm community composition was influenced by all three filters, where management-related variables were the most important in constraining earthworm species assemblages, followed *ex aequo* by the composition of the landscape within a radius of 500 m, and soil properties.

4.2.1. Soil

Although soil texture, organic matter, moisture and pH are well known to affect earthworms (Curry, 2004), these soil properties did not contribute to the explained variation between earthworm communities in the fields of this study area. Our research area is rather homogeneous in terms of soil texture, has dominantly been managed for crop production, and the sampled arable fields differed little in soil properties. The limited variation in soil properties was therefore likely to have only a small influence on earthworm community variation. However, in margins, pH contributed significantly to explaining variation in earthworm community composition (Table 3), even though variation in pH was relatively small. All species abundances in the margins decreased with increasing pH, which in turn decreased with margin age (Fig. 3). The effect on earthworms was not necessarily caused by differences in pH per se, but rather by margin ageing, since time without disturbance would allow the establishment and development of earthworm communities.

4.2.2 Management

With respect to management of fields, we found that variables associated with the use of pesticides (fungicides, herbicides and insecticides) explained a large part of the variation (26%) in earthworm community composition (Tables 2, 4). Not unexpectedly, increased application frequencies had a negative effect on the abundance of most species in fields (Fig. 2) (Baveco and de Roos, 1996). Pelosi *et al.* (2013; 2014) found these three groups of pesticides to negatively affect earthworms, particularly for species living at the soil surface. For most of the species in the current research the results are in line with the observations of those authors. Only *L. rubellus* showed a positive correlation to the number of fungicide applications, possibly due to a competitive advantage for example for available food, combined with its relatively high population recovery rate (Edwards and Bohlen, 1996). It is also well known that earthworms are hampered by soil disturbance like tillage (Chan, 2001), or decreased food availability due to crop residue removal (Edwards and Bohlen, 1996), but that this effect is species dependent. Furthermore, the use of tuber crops within the crop rotation has been identified as negatively affecting earthworms, again due to the intensive soil disturbances during soil preparation and harvesting (Marinissen, 1994; Curry *et al.*, 2002). However, in a study aiming at understanding how fast earthworm populations would recover from autumn ploughing, Crittenden *et al.* (2014) found populations to be similar to before ploughing by the following Spring. In our study, neither tillage, removal of crop residues, nor the use of tuber crops in the past were found to play a role in explaining the variation of earthworm composition in our data. In fact, the variation in crop rotations and crop management practices was relatively small across the farms in our research area, posing some limitations in terms of testing which crops or management practices could favour earthworm diversity in arable fields.

Among the management-related variables that explained variation in earthworm community composition in margins, age of margins (Fig. 3) positively affected long-lived species with low fecundity. For example *L. terrestris*, a species highly associated to older margins, can only produce up to 38 cocoons per year, reaching maturity after as much as 50 weeks (de Lange *et al.*, 2013). Mowing frequency negatively affected the epigeic species found in margins (*L. rubellus* and *L. castaneus*), but had little influence on anecics (*L. terrestris* and *A. longa*). Both groups feed at the soil surface (Bouché, 1977), but the burrower *L. terrestris* is apparently less sensitive than the topsoil-dwelling *L. rubellus* and *L. castaneus*.

4.2.3 Landscape

In our study area, the landscape within a radius of 500 m proved to be the second most important filter in explaining earthworm community variation in margins (Table 4). So far, most studies have focussed on margins as a source for earthworm colonization into the fields (e.g., Roarty and Schmidt, 2013; Crittenden *et al.*, 2015). The current study is, to our knowledge, the first attempt to quantify the relationships between the earthworm community composition of margins and soil properties, management practices and surrounding landscape together. The increase in proportion of arable area within a radius of 500 m revealed a negative effect on earthworm community composition in margins (Fig. 3), suggesting that inhabitable land-uses other than arable land could provide a source for more diverse earthworm communities, particularly for species like *A. limicola, L. terrestris, A. chorotica, A. longa* and *S. mammalis*. Earthworm mobility and dispersal ability is considered to be limited. In earthworm-free arable land of young polders in the Netherlands, after introduction, *L. rubellus* and *A. caliginosa* dispersed only at rates of 14 and 7 m yr^{-1}, respectively (Marinissen and van den Bosch, 1992). Although slow, dispersal and therefore colonization can take place over the years (Eijsackers, 2011). Furthermore, passive dispersal by, e.g., tires of (agricultural) vehicles (Marinissen and van den Bosch, 1992; Cameron and Bayne, 2014), waterways and animals (e.g. birds) (Schwert, 1980) plays a role in earthworm movement. Although we can only speculate whether earthworm populations in the margins are a product of facilitated population development after the restoration of margins, colonization from inhabitable land-uses, or both, our data suggests that dispersal from inhabitable land-uses plays a role to some extent. As we do not have information about species composition in the surrounding habitats, their role as potential sources of earthworms into the margins remains to be investigated.

In accordance with our second hypothesis, the landscape did not explain variation in earthworm community composition in the fields at any of the studied radii. This is in agreement with the findings of Lüscher *et al.* (2014) who found no significant relationships between earthworm community composition of fields and characteristics of the surrounding landscape, although these authors only considered a radius of 250 m. A plausible explanation for the lack of such effects of the surrounding landscape on earthworm communities in the case of fields could be the dominance of harsh management practices, e.g. disruption of earthworm burrows, soil compaction and water logging, pesticide application and removal or displacement of food through tillage, hampering the development of earthworm populations.

5. Conclusions

Our study clearly illustrated that although arable fields and field margins neighbour each other spatially, earthworm community composition of the two habitats was affected differently by the considered environmental filters (soil properties, management practices, and surrounding landscape). Regarding earthworm composition of arable fields, only management-related variables played a significant role, whereas for earthworm communities of field margins, all three filters were relevant. This suggests that management practices of arable fields overrule potential positive effects of the surrounding landscape and of soil properties on earthworm community diversity. The current growing awareness and policy-support for recovering a mosaic-like structure of arable landscapes includes restoration of semi-natural landscape elements, such as field margins. Although such elements could help promoting earthworm (re)colonization of arable fields, their re-establishment in arable landscapes will not be sufficient for restoring earthworm communities of arable fields, unless the impact of arable management practices is reduced.

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Table 1 – Earthworm species density (ind. m^{-2}), total earthworm density (ind. m^{-2}) and biomass (g m^{-2}), and actual (SR) and rarefied (RFSR) species richness in fields with and without margins and in margins. Mean, standard errors (SE) and frequency of occurrence (Freq) are given.

Species	**Fields**						**Margins**		
	with margins			**without margins**					
	(n=13)			**(n=13)**			**(n=15)**		
	Mean	**SE**	**Freq**	**Mean**	**SE**	**Freq**	**Mean**	**SE**	**Freq**
A. caliginosa	238.1	31.7	13	220.5	29.0	13	246.7	76.5	15
A. chlorotica	45.2	19.7	8	3.8	3.5	2	35.8	13.8	8
A. rosea	64.7	22.1	12	43.9	10.5	12	52.5	16.9	13
A. limicola	7.7	7.7	1	1.0	0.5	3	43.3	23.0	6
M. minuscula	0.3	0.3	1	0.6	0.6	1	0.4	0.4	1
A. longa	0.0	0.0	0	0.0	0.0	0	9.2	6.5	3
L. terrestris	3.5	1.8	4	1.0	0.7	2	31.7	12.3	9
E. tetraedra	3.2	1.4	6	1.6	1.3	2	0.0	0.0	0
L. rubellus	42.0	11.3	10	57.4	18.2	11	37.5	11.8	12
L. castaneus	8.0	3.5	9	7.1	2.3	7	77.9	28.4	13
S. mammalis	0.0	0.0	0	0.0	0.0	0	5.0	5.0	1
Total density	414.1 NS	51.2	-	336.9 NS	42.2	-	541.3 NS	115.3	-
Total biomass	62.3 NS	8.8	-	60.8 NS	7.6	-	96.3 NS	22.1	-
SR	4.9 NS	0.3	-	4.1 NS	0.4	-	5.4 NS	0.4	-
RFSR	3.7 B	0.3	-	3.4 B	0.2	-	4.5 A	0.3	-

SR= actual number of observed species; RFSR= species richness based on rarefaction (rarefied to 25 individuals).

Letters indicate significant habitat type differences at $p < 0.05$, NS: not significant.

Table 2 – Percentage of variance explained (adjusted r^2) and p-values from Monte Carlo permutations in earthworm species abundance data from fields for separate RDA models per environmental filter and the combined RDA model combining all statistically significant relationships within the three filters.

Environmental filter	**Separate RDA**		**Combined RDA**	
	Adj. r^2	**p-value**	**Adj. r^2**	**p-value**
Soil				
Clay	4.3%	0.042	-	NS
Management				
Insecticide	6.0%	0.012	6.0%	0.012
Herbicide	11.8%	0.001	11.8%	0.001
Fungicide	8.5%	0.002	8.5%	0.002
Landscape	-	NS	-	NS

NS: not significant.

Table 3 – Percentage of variance explained (adjusted r^2) and p-values from Monte Carlo permutations in earthworm species abundance data from margins for separate RDA models per environmental filter and the combined RDA model combining all statistically significant relationships within the three filters.

Environmental filter	**Separate RDA**		**Combined RDA**	
	Adj. r^2	**p-value**	**Adj. r^2**	**p-value**
Soil				
pH	16.8%	0.002	16.8%	0.002
Management				
Age of margin in 2012	14.0%	0.004	7.6%	0.011
Mowing frequency	10.0%	0.017	10.7%	0.006
Landscape				
Arable area within a radius of 500 m	16.7%	0.003	10.1%	0.004

Table 4 – Partitioning (partial RDA) of the variation in earthworm density data by the environmental filters, soil properties, management practices and surrounding landscape for earthworm communities in fields and margins.

	Variation partitioning			
	Total contribution		**Unique contribution**	
	Adj. r^2	**p-value**	**Adj. r^2**	**p-value**
FIELDS				
Soil	-	NS	-	NS
Management	26.2%	0.001	26.2%	0.001
Landscape	-	NS	-	NS
MARGINS				
Soil	16.8%	0.003	9.6%	0.015
Management	23.8%	0.002	18.3%	0.001
Landscape (500 m)	16.7%	0.003	11.0%	0.003

NS: not significant

Figure captions

Figure 1 – Biplot of RDA of total earthworm species density using the sampled habitats as constraints (p= 0.001, 999 Monte Carlo permutations). Adjusted r^2 is 9.5%, the first RDA axis explains 11.6% of the constrained variance (p= 0.001) and the second axis 2.5% (p= 0.364). The first PCA axis explains 22.8% of the variance. Species whose variation explained by the constraints was smaller than 10% were excluded from the plot. Scaling based on species correlations.

Figure 2 – Biplot of the combined RDA model explaining 26% (adjusted r^2) of the variance in earthworm species abundance in fields using explanatory variables selected by forward selection as constraints. Open circles represent fields. The first and second RDA axes explain 20% and 14% of the constrained variance (p= 0.001, 999 permutations), respectively. Species whose variation explained by the constraints was smaller than 10% were excluded from the plot. Scaling based on species correlations.

Figure 3 – Biplot of the combined RDA model explaining 45% (adjusted r^2) of the variance in earthworm species abundance in margins using explanatory variables selected by forward selection as constraints. Open squares represent margins. The first and second RDA axes explain 32% and 16% of the constrained variance (p= 0.001, 999 permutations), respectively. Species whose variation explained by the constraints was smaller than 10% were excluded from the plot. Scaling based on species correlations.

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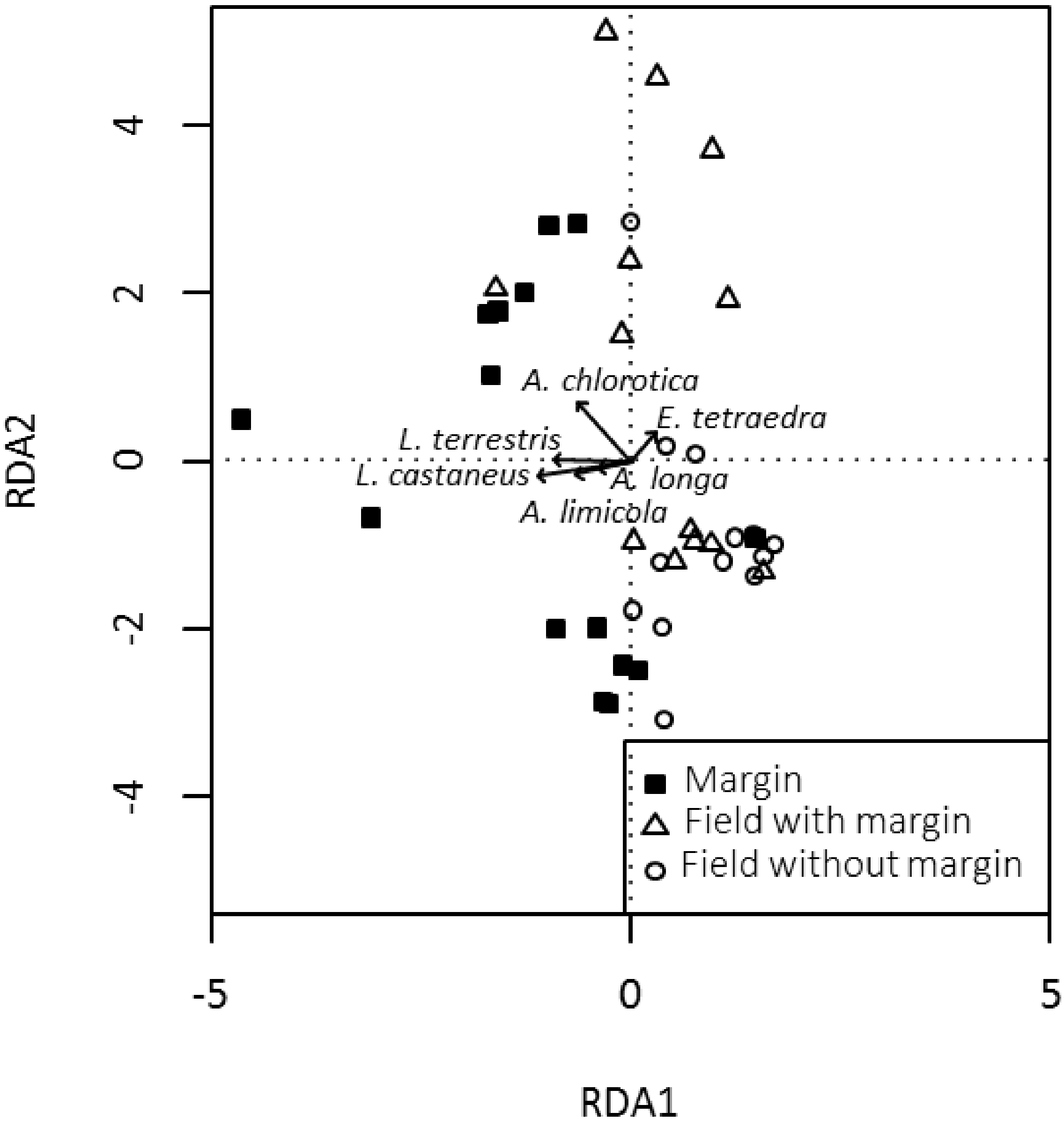

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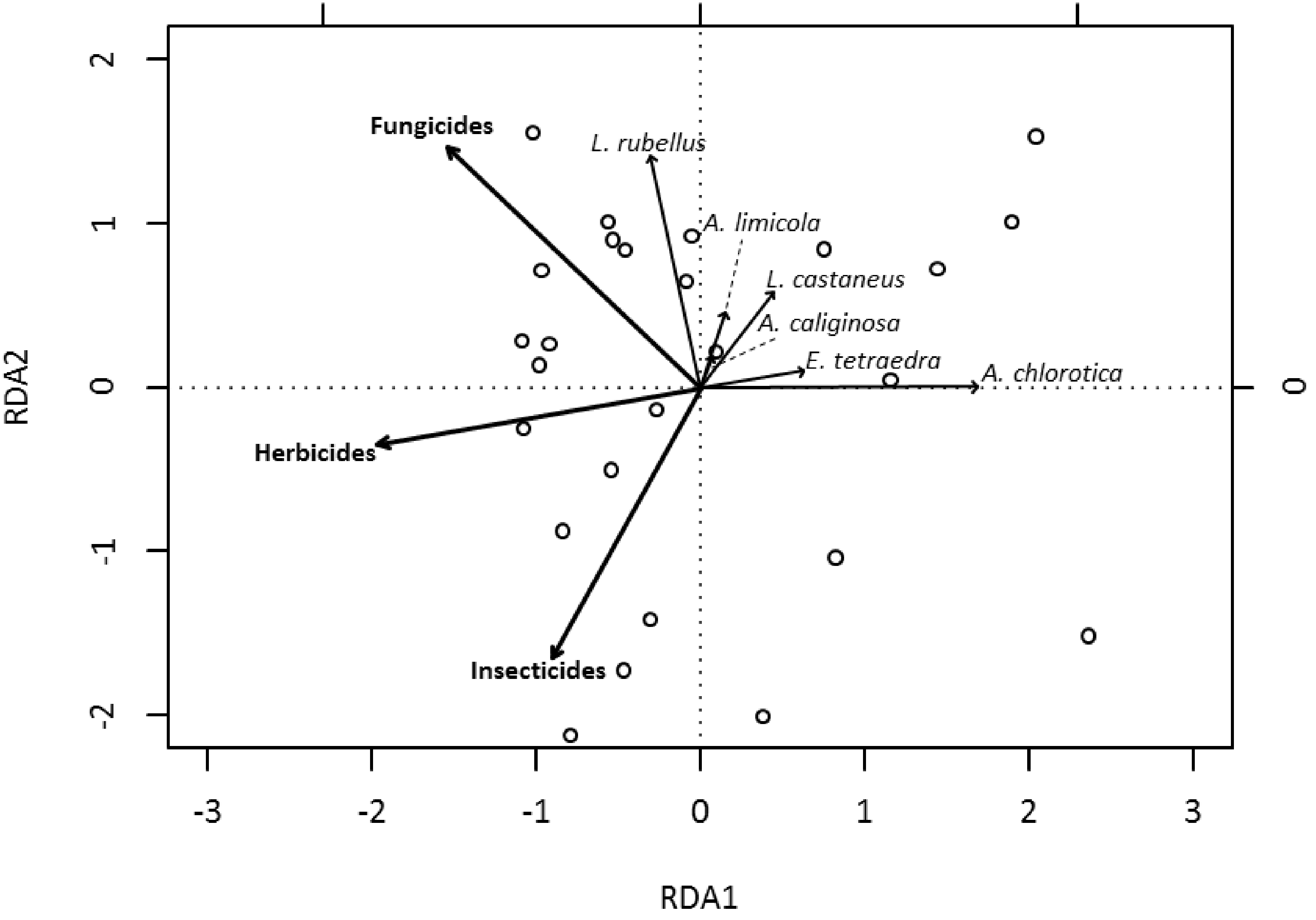
656 Figure 1



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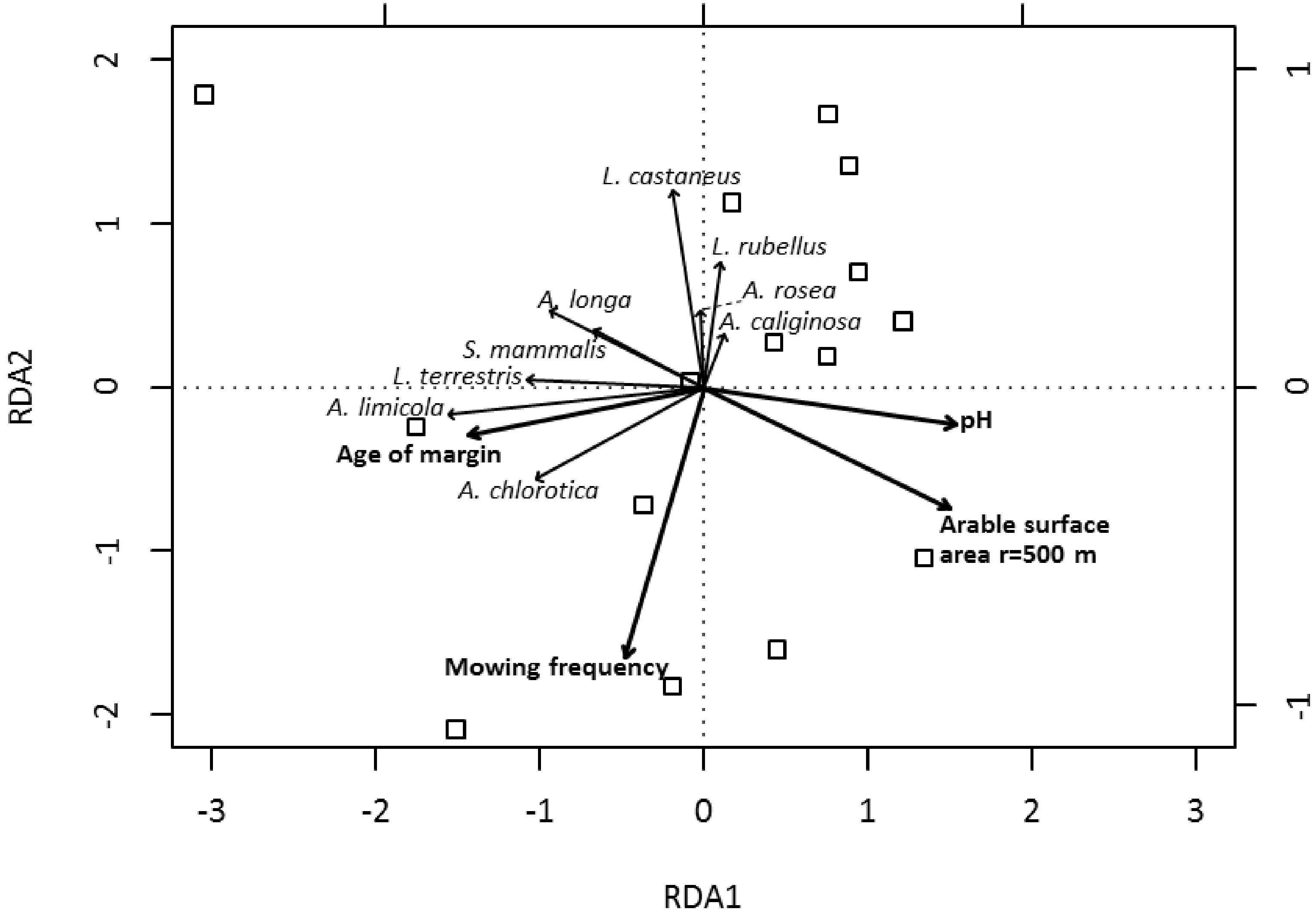
658 Figure 2



L. castaneus
L. rubellus
A. rosea
A. caliginosa
A. longa
S. mammalis
L. terrestris
A. limicola
Age of margin
A. chlorotica
pH
Arable surface area r=500 m
Mowing frequency
RDA2
RDA1
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
1
0
-1

Figure 3