Influences of ecological compensation areas on earthworm populations

A case study at the trial area Rutzendorf in the Marchfeld region of Lower Austria

SS 2014

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Abstract

The influences of ecological compensation areas on the above-ground fauna and flora are well established. However, there is a lack of studies focusing on the below-ground organisms. Therefore this study examines the influences of ecological compensation areas on the earthworm population of the trial area Rutzendorf in the Marchfeld region, Lower Austria. This was done by taking earthworm samples with a combination of hand-sorting and chemical expulsion with a mustard suspension. For analyzing the earthworm population the abundance, the biomass and the species composition was used for indication. As covariate the depth in which soil texture changes as well as the depth of the A-horizon was determined for every sampling point. As further influencing aspect the vegetation of both flower strip mixture was recorded.

The vegetation survey showed slight differences between the flower strip mixtures whereby the earthworm abundance and biomass were higher for the “beneficial mixture”. The earthworm abundance in the flower strip was significantly higher than in the hedgerow. The species diversity was compared to other studies relatively low, in major just two endogeic species were found – *Aporrectodea rosea* and *Aporrectodea caliginosa*. Important anecic species like *Lumbricus terrestris* were not found.

In the end this study tries to set a focus on ecological compensation areas as supporting measure for earthworm populations in the agro-ecosystem.

Acknowledgement

At this point I would like to thank all those who were involved in the development of this thesis and in achieving my educational goals.

First of all I would like to thank Prof. Dr. Jürgen K. Friedel for his open communication and supervision. He has given me the possibility of a free working frame, which I needed to create this thesis. He was always there for giving critical feedback and for supporting me by handling with difficult questions of my work.

Furthermore, I would like to thank Dr. Pascal Querner for the many suggestions and hints in terms of earthworms themes and the general structure of the thesis.
Moreover, I express my gratitude to Dr. Gabriele Gollner for initiating the development of this thesis.
Another very important person of this thesis was Doris Meisinger who supported the practical part of this study not only with her forces but also with her cheerful personality which gave a lot of motivation.

Likewise, I would like to thank my fellow student Ramona who helped me by the earthworm sampling as well as for being there as mental support.
A great thanks goes to my boyfriend Sven for his help by sampling earthworms and soil samples, for reviewing my thesis and for his motivating and inspiring support.

Thanks also to my parents who always support me and who were giving me the meaningful possibility of graduating a master study.
For further support I express my gratitude to Andreas Surböck, Markus Heinzinger, Prof. Dr. Susanne Papaja-Hülsbergen and last but not least to the earthworms who sacrificed their lives for this study.

Table of content

1. Introduction and problem description

Agriculture is the main land-use type in Europe. In 2010, Austria had 7.347.535 ha of agricultural land (STATISTIK AUSTRIA 2013) which represents around 35 % of the total land of this country (THE WORLD BANK 2014) which shows the important status of agriculture.

Since mankind has started with farming, a typical type of landscape has been developed and a special diversity of plant and animal species had begun to adapt to this type of land-use. However, with the intensification of agriculture after the Second World War the habitats of these species started to decline. The major reason for this was the loss of structural elements within the agricultural landscape. With the enlargement and specialization of farms, the management of broader areas started to get common. Structural elements like hedgerows and field margins had to create space for arable land. Also in Austria there is a trend of expanding the farm size where intensification of the farm management often comes along (STATISTIK AUSTRIA 2013). In addition to the loss of landscape diversity also the intensive use of pesticides is relevant for the species loss (ROBINSON and SUTHERLAND 2002).

Besides the aspect of biodiversity loss, modern agriculture including cropping of monocultures, usage of heavy machinery as well as intensive use of pesticide and fertilizer which leads to other serious environmental issues like soil erosion, soil compaction and leaching of nitrate. To counteract these destructions there are agro-environmental schemes subsidizing farmers managing their farm in an extensive, environmental-friendly way. One aspect, especially in terms of maintaining the biodiversity in the agro-ecosystem, is to build up ecological compensation areas (ECAs) to ensure a high diversity of the landscape. Of special importance is the construction of flower strips and special strips for beneficials. With building up such ECAs not only the nature in general but the farmer in particular can benefit amongst others by enhancing the abundance of antagonist of pests and by improving the number of pollinators close to the field (MARSHALL 1993 p. 99, SCHEPER et al. 2013).

There are many studies analyzing the influence of ECAs on certain animal species (CARVELL et al. 2004, MUCHOW et al. 2007, KORPELA et al. 2013) where the focus is mainly on the above-ground fauna. Nevertheless there could be also an influence on the soil fauna. According to BARDGETT and WARDLE (2010 p. 165-166) the loss or gain of species in a community will have important consequences not only for the above-ground but also the below-ground subsystem, which is in special the case when species with key functions are concerned. Recently the importance of soils in agriculture is becoming a focus point of interest. Especially earthworms are often seen as the key soil organism which is based on their essential contribution to soil fertility and soil stability (PALM et al. 2013, PFIFFNER 2014). Beyond that different earthworm species affect the soil food web at several levels and thereby also the construction and functioning of the above-ground food web (UVAROV 2009).

Therefore this study is trying to find out:

- How ECAs of the trail area in the Marchfeld region differ in terms of the earthworm population
- How the earthworm population of the ECAs differs compared to the adjacent field
- Whether the type of vegetation cover of the flower strip has an influence on the earthworm population

The following chapter will give a state of the art of the concerned topics. So that in the end specific hypotheses can be derived for this study.

2. Theoretical background

In this part important aspects for the study will be explored as a theoretical background of this thesis. It will start with definitions and a broad overview of biodiversity and ECAs, coming then to specific themes about earthworms, where in the end both subjects will be brought together.

2.1. Biodiversity in agro-ecosystems

Biodiversity is a widely used term which has gained more and more importance in the recent past. The United Nations defined this term as

> *"[…] the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems." (UNITED NATIONS 1992)*

However, it is seen that not only wild landscapes have a high natural value but also some agricultural regions. There are parts of Austria, where traditional land-use is still practiced, which belong to hot-spots of biodiversity in this Austria (WRBKA et al. 2005). SCHMITZBERGER et al. (2005) stated that almost one fifth of the country is covered by agricultural landscapes which can be considered as national hot-spots of biodiversity. In **Figure 1** important components and their functions influencing the agro-ecosystem biodiversity are illustrated.

Components		**Function**
Pollinators		Pollination Genetic introgression
Predators and Parasites		Population regulation Biological control
Herbivores		Biomass consumption Nutrient cycling
Non-crop Vegetation	**Agro-ecosystem Biodiversity**	Competition Allelopathy Sources of natural enemies Crop wild relatives
Earthworms		Soil structure Nutrient cycling
Soil Mesofauna		Decomposition Predation Nutrient cycling
Soil Microfauna		Nutrient cycling Disease suppression

Figure 1: The components and functions of biodiversity in the agro-ecosystem (Modified from ALTIERI 1999)

All these components are essential for the biological balance of the agro-ecosystem, since they function together as a holistic system. For instance, earthworms influence the soil condition in a way that many other soil organisms are supported. This in fact leads to a greater supply of resources in terms of biodiversity and biomass for higher trophic levels above-ground, which again improves the biodiversity of the whole agro-ecosystem (WOLFRUM et al. 2010).

However, nowadays the intensification in agriculture threatens the biodiversity of these systems. The focus of agro-ecosystems in the end lies on gaining services in form of products for the human consumption (NEHER and BARBERCHECK 1999 p. 27) where maintenance and enhancement of biodiversity often has no significant place. Hence, different agro-environmental schemes exist to promote the conservation of biodiversity in the agro-ecosystem (KLEIJN et al. 2006, MERCKX et al. 2009). This is of great importance since the destruction of biodiversity can hardly be compensated. With increasing biodiversity in the agricultural ecosystem, especially when focusing on features for important processes in the system, the functioning of it will also increase. Furthermore the resilience of the whole agro-ecosystem will be enhanced at the same time. “Enhancing functional biodiversity in agro-ecosystems is a key ecological strategy to bring sustainability to production” (ALTIERI 1999). This means that the system can more easily come back to its equilibrium after some sort of disturbance, than this is the case in an agro-ecosystem with less biodiversity.

There are several approaches to maintain and enhance the biodiversity in the agricultural ecosystem which can be divided into two levels (VANDERMEER and PERFECTO 1995 quoted in ALTIERI 1999):

- **Planned biodiversity** at field level which is influenced by the agricultural management, for instance by the spatial and temporal arrangement of the crops and of diversity of used crop varieties.
- **Associated biodiversity** represents the flora and fauna of the surrounding structures, for example ECAs colonizing the agro-ecosystem

Since ECAs are an essential measure for improving the biodiversity in agro-ecosystems, the following chapter will further define the term and explore several values of it.

2.2. Ecological compensation areas in agriculture

Since 1958 the today called European Union (EU) has taken over the first attempts for a European Agricultural Policy and nowadays has also taken the task for most environmental issues. Hence, for European states most regulations in the agricultural field are set by the European Union.

This policy driven by the research results about the importance of the conservation of biodiversity, leads to a growing importance of this theme in policy. The maintenance and enhancement of biodiversity in agricultural ecosystems is especially supported by the regulations of ecological compensation areas (also called ecological focus areas) which are set by the European States (MOUYSSET et al. 2012). In the regulation No. 2013/1307 (Art. 46) of the European Commission it is appointed that

> "*[e]cological focus areas should be established, in particular, in order <u>to safeguard and improve biodiversity</u> on farms. The ecological focus area should therefore consist of areas <u>directly affecting biodiversity</u> such as land lying fallow, landscape features, terraces, buffer strips, afforested areas and agroforestry areas, or <u>indirectly affecting biodiversity</u> through a reduced use of inputs on the farm, such as areas covered by catch crops and winter green cover*." (EUROPEAN PARLIAMENT AND OF THE COUNCIL 2013; modified by author)

This part clearly emphasizes the aims of the agri-environmental schemes by the European agricultural policy. Therefore a clear definition of ECAs is mostly based on the guidelines of public authorities. The aim of the expanding use of ECAs is the support of biodiversity, in order to regain the biological balance. That is why the implementation of ECAs is such an important goal of these regulations. Based on the non-economical use of ECAs, they are often seen as competition for land in the perspective of farmers (MOONEN and BÁRBERI 2008). This is the reason why the establishment and maintenance of ECAs is financially supported. For Austria a farmer who is implementing a compensation measure gets financially supported by the so called „ÖPUL“ (Österreichische Programm zur Förderung einer umweltgerechten, extensiven und den natürlichen Lebensraum schützenden Landwirtschaft). The here mentioned ECAs are characterized by several conditions, like no-use of pesticides, fungicides and fertilizer.

Semi-natural subsystems can improve the biodiversity on the level of species as well as on the level of the whole ecosystem. In former times they were a common part of the agro-ecosystem for instance for indicating the edges of the fields and for many more issues (**Table 1**). Today these structures are built up to compensate the destruction of (semi-)natural habitats and at the same time to counteract the threat of biodiversity, when using the land for agriculture. Due to changed purposes of semi-natural subsystems they can have potential new functions (**Table 1**). Besides the positive effects of these subsystems for the environment, like enhancing landscape diversity and buffering pesticide drift, many benefits directly concern the farmer. The enhancement of the pollinator population, the promotion of ecological stability in crops as well as the reduction of soil erosion support the agro-ecosystem and help to make it more stable.

Table 1: Original and potential functions of semi-natural habitats in the agro-ecosystem (MARSHALL 1993, p. 97-99).

Original roles and requirements	1. To define the field edge 2. To be stock- or trespasser-proof, to keep animals in or out 3. To provide shelter for stock 4. To provide shelter for crops, particularly as windbreaks 5. To reduce soil erosion by wind or water 6. Not to compete with the crop for light, moisture or nutrients 7. Not to harbour weeds, pests and diseases 8. To harbour beneficial plants and animals 9. To act as a refuge or corridor for wildlife 10. To provide a source of fruits and wood
Current and potential functions of field margins	a. Promotion of ecological stability in crops b. Reducing pesticide use: exploiting pest predators and parasitoids c. Enhancing crop pollinator populations d. Reducing weed ingress and herbicide use e. Buffering pesticide drift f. Reducing fertiliser and other pollutant movement, especially in run-off g. Reducing soil erosion h. Promotion of biodiversity and farm wildlife conservation i. Maintaining landscape diversity j. Promotion of game species

There are several studies which approve the potential functions of semi-natural habitats in the agricultural landscape. For example, SCHEPER et al. (2013) and CARVELL et al. (2004) confirmed the supporting effect of ECAs on pollinators. The enhancement of natural antagonist of pests by enclosed hedges (EWALD and LOBSIGER 1997) and wildflower strip (REISNER et al. 1997) is well known. Additionally environmental services of semi-natural subsystems like the compensation of water, soil and air pollution are declared by MOONEN and BÁRBERI (2008).

In these first chapters we have seen the first attempts of this work. For the support of the biodiversity, ECAs can give a decisive contribution. Since this study focuses on the belowground influences of ECAs, earthworms will be regarded as representative organisms of the soil. Certain issues of earthworms will be explored in the following chapters for a better understanding of this important soil organism.

2.3. Earthworms

From a taxonomic perspective earthworms belong to the family lumbricidae. They are distributed nearly all over the world and live thereby in many different soils. Around 400 earthworm species can be found in Europe (PFIFFNER 2014) whereof more than 60 species can be found in Austria (CHRISTIAN and ZICSI 1999).
In former times earthworms were said to be a pest because they feed on plant roots. Darwin was the first one who changed the view on these animals by writing the book "The formation of vegetable mould, through the action of worms with observations on their habits" in 1881 and thereby stating about their great contribution to the soil.
Nowadays the knowledge about the important role of the earthworms for the soil is consolidated. In terrestrial ecosystems earthworms are important helpers in terms of decomposition and reconstruction of the soil (PFIFFNER and LUKA 2007). They have many positive influences on the soil which are especially desired in agriculture.

In the following chapters certain topics about earthworms will be discussed so that in the end some hypotheses can be derived therefrom.

2.3.1. Ecological background of earthworms and their importance in agroecosystems

Earthworms are in general the key soil organisms contributing to the composting and recycling processes of nutrients in the soil. By observing the way of living of earthworms it has been found that they have different habits for instance in terms of feeding. These characteristics can be used to divide the earthworm species into so called ecophysiological groups. BOUCHÉ (1972) firstly described these three ecophysiological categories of earthworms. In particular they are divided in

a. epigeics
b. endogeics
c. anecics

The **epigeic** species are also called leaf litter or compost-dwelling earthworms. These expressions already point out that worms belonging to this group mainly stay in the litter section of the soil. They do not burrow tubes but live just in the soil litter and feed on decomposing organic matter (see **Figure 2**). They are significantly involved in the formation of the humus horizon. Because of their natural habit they mostly appear in forest and grassland soils, since they are permanently covered with organic material. In contrast to this, epigeic species can rarely be found in agricultural soils (KARIGER et al. 1993, PFIFFNER 2014). They are from small size, around 2-6 cm long and based on their environment, pigmented as protection against the sunlight (PFIFFNER 2014).

Endogeic species live in the top- and subsoil and are therefore also called topsoil- or subsoil-dwelling earthworms. Earthworms of this category can be either small or can reach a size up to 18 cm. As they live mostly in the soil but do not have much contact with light, they are hardly pigmented. They feed on mineral soil with incorporated organic material and burrow thereby horizontal burrows in the upper part of the soil (STÄHLI et al. 1997, PFIFFNER 2014). These non-permanent burrows are often filled with the excreta of the earthworms, which are important for a good soil structure. As these earthworms do not depend on a permanent litter layer, they can more often be found in agricultural soils than epigeic species (KARIGER et al. 1993).

Species belonging to the group of **anecic** living earthworms are said to be from special importance for the soil functions. This is based on their deep-burrowing activity through which vertical burrows are created. These burrows are very stable and can exist over a long time (PFIFFNER 2014). This is the reason why the infiltration and aeration of the soil and therefore also the root growth of the plants is greatly supported by anecic species. Furthermore they support the maintenance and improvement of the soil structure (WOLFRUM et al. 2010). The deep burrows are the appearance of the feeding habit of the anecic earthworms. They come to the soil surface for collecting plant material, for instance leaves, and then they pull it into their burrows for feeding on it. Thereby material from the litter layer of the soil is incorporated into deeper parts of the soil. The so called earthworm droppings can often be found around the exit of the burrow (STÄHLI et al. 1997). Anecic earthworms have in general a great size ranging from 15-46 cm. The head of the earthworms is darker as the rest of the body (PIFFNER 2014) since this is the part mainly emerging out of the soil surface while collecting the litter. A well-known species belonging to this category is *Lumbricus terrestris*, also called nightcrawler or common earthworm.

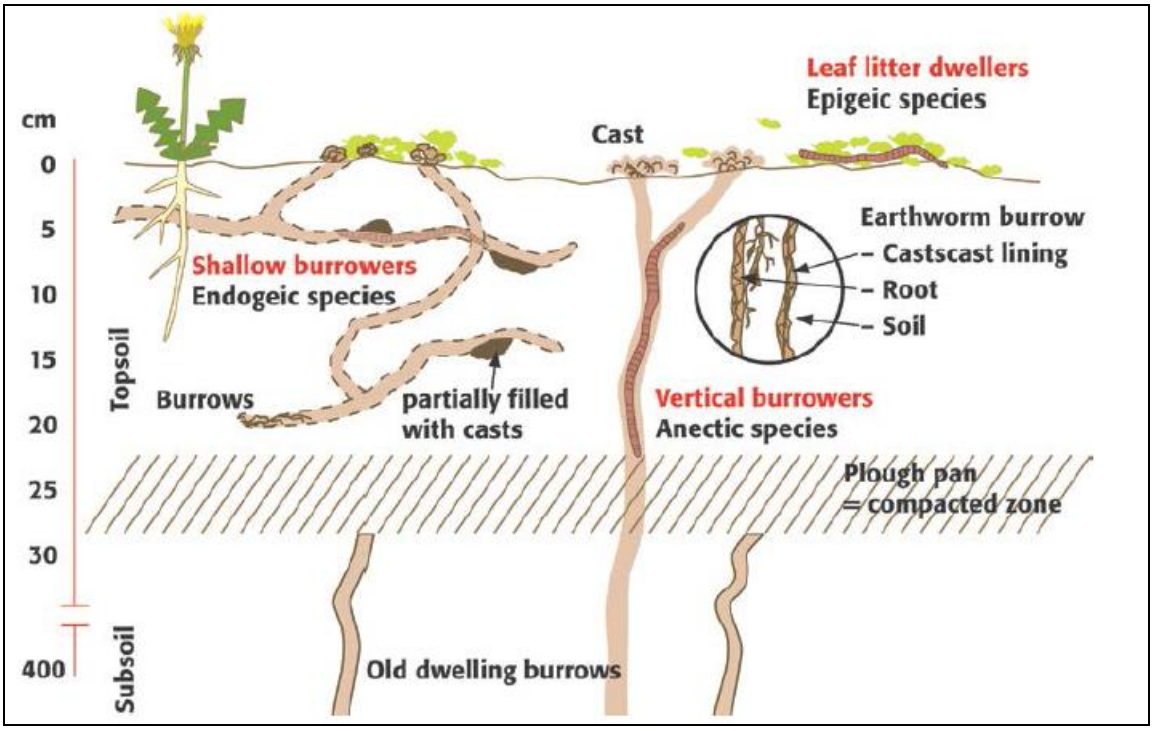

Figure 2: Eco-physiological categories of earthworms and their spatial distribution in the soil (PFIFFNER 2014).

Earthworms in general promote the soil fertility on several levels. Not only by the before mentioned support of infiltration and aeration of the soil but in many more aspects. They

incorporate for instance enormous amounts of dead organic matter into the soil and have therefore also the function of a “natural plough”. Furthermore they produce so called clay-humus complexes which represent an important source of directly available nutrients which are deposited in form of the worm droppings (PFIFFNER 2014). These clay-humus complexes have a higher amount of microorganisms than the initial soil. Mucilage produced by these microorganism lead to a soil structure of high stability (KARIGER et al. 1993).
Another important point is the reduction of the disease pressure by pulling infected plant parts, for instance leaves infected by apple scab, into their burrows. Since earthworms positively affect beneficial bacteria and fungi in the soil, the diseases of the infected plant parts are then degraded biologically (PFIFFNER 2014).

Summing up at the end of this chapter, we know that there are three different categories in which earthworms can be divided, based on their habits. All together they have many important traits which can positively influence the agro-ecosystem, some of them being special for one of the three categories.
Having already started to talk about the feeding habits of the different eco-physiological categories, the next chapter will further discuss how vegetation and soil influence earthworm populations.

2.3.2. Influence of soils and vegetation cover on earthworms

The living habits of earthworms are generally determined by several facts. Regarding the vegetation and the soil following points should be mentioned. Even if this figure does not claim to be complete it illustrates some important linkages (**Figure 3**).

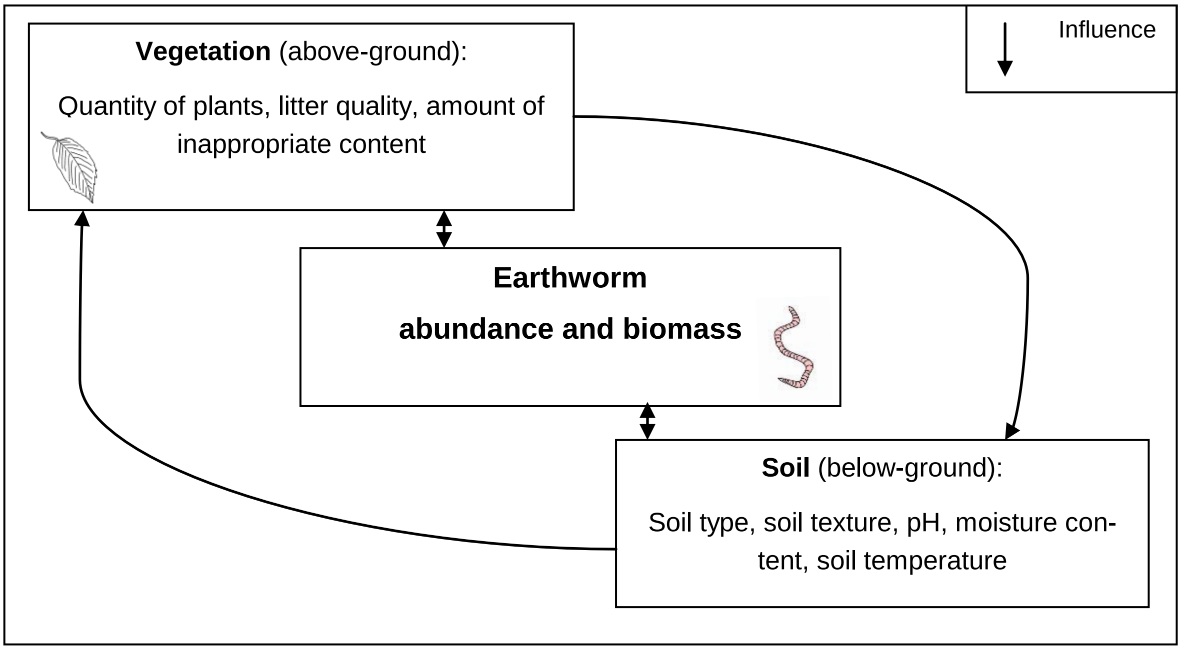

Figure 3: Important linkages between vegetation, soil and earthworms.

The characteristics of the soils are, amongst others, defined by the texture, soil type, pH value, soil temperature and the soil structure. According to PFIFFNER (2014) medium-heavy loam to loamy sand soils are preferred by earthworms compared to heavy clay and dry sand soils which are said not to be positive for the development of the earthworms. Similar observations were done by HÖVELMANN (1989 quoted in KARIGER 1993) and STÄHLI et al. (1997).
Sandy soils are in general problematic in terms of their lacking soil structure and the large considerable fluctuation of the moisture content (STÄHLI et al. 1997). Commonly, soils with values around 20 % of moisture turned out to be a particular good habitat for earthworms (RÖMBKE 1997). For different species also certain preferences of moisture content are known (KNÜSTING 1992).
Also the pH-value influences the appearance of earthworms which is specifically different for certain species. Thus, for instance only some epigeic species can live in soils with a pH-value below 4 (STÄHLI et al. 1997). Furthermore, especially when comparing sandy soils with soils with a high lime content, they have only a poor buffer capacity in terms of the pH-value (STAHR et al. 2008 p. 54-56).

Besides the soil characteristics also the vegetation cover influences the earthworm population. This is not only done by offering food but also by interactions of vegetation and soil due to the above- and below-ground linkages. As we are looking at the feeding preference of earthworms, we obtain a very heterogeneous image (CURRY and SCHMIDT 2007). The individual species are specialized in different feeding types and thereby fulfilling their special task in the utilization of available food resources (see chapter **2.3.1**). Several studies have examined the feeding habits of earthworms and showed differences between the nutritional behaviour and also differences between various plants in their food composition of individual species (RÖMBKE 1997).
A categorization of feeding types can be done in different systems. Based on the already above used classification (see chapter **2.3.1**) following feeding behaviour is seen:

- Epigeic living earthworms feed mostly on scattered parts or occurring micro flora. Also specialized species on decomposed wood and compost occur among the epigeic living earthworms (RÖMBKE 1997).
- Anecic earthworms feed on leaves which they have drawn from the soil surface. Here they prefer leaves with high nitrogen content and a small amount of tannins. These can be found increasingly in the litter of lime, ash and sycamore maple. Leaves with a high amount of tannin are eaten only at very advanced decay (RÖMBKE 1997).
- Endogeic worms feed on mineral soil containing organic compounds (RÖMBKE 1997). In addition dead plant roots with a certain amount of living micro-organisms are of greater importance for endogeic earthworms (CURRY and SCHMIDT 2007).

Some species also have specialized in various food sources. For instance *Aporrectodea caliginosa* showed a specialization on soil algae and *Aporrectodea* rosea differs active in his diet behaviour between mineral and organic soil constituents (RÖMBKE 1997, CURRY and SCHMIDT 2007). Additionally PONGE et al. (1999) figured out that the amount of calcium in the feed is important for some species, especially for endogeic ones. Roots as mentioned before are used only in a very late state of decay. Some other authors even deny the usage of living roots as diet in earthworm feed (CURRY and SCHMIDT 2007). Several studies have also shown the negative influence of phenolic compounds and tannins in the food of earthworms (HENDRIKSEN 1990 quoted in NEILSON and BOAG 2002), same is true for high acid concentrations like citric acid (MANGOLD 1953 quoted in NEILSON and BOAG 2002). Different other substances like allylisothiocyanat, carvon and allicin were also shown as inappropriate both in food and by irritating the earthworms' skin (WESTERNACHER-DOTZLER 1988).

To sum up, vegetation cover and soil characteristics are important influencing factors of earthworm populations by serving as food source and habitat. In the next chapter it will be explored how ECAs can support earthworm populations in the agro-ecosystem and if these structures can serve as immigration pool for adjacent fields.

2.3.3. Support of earthworm populations in ecological compensation areas

As stated in chapter 2.3.1 earthworms have many supporting features which are desired for the agro-ecosystem. Hence the question is how to promote earthworms besides changing the agricultural practise itself? Here ECAs can be one solution because of several aspects:

1. Undisturbed soils
2. Permanent soil cover
3. No pesticide-use

Starting with the firstly mentioned point there are several studies showing the negative influence of soil cropping. NEHER and BARBERCHECK (1999 p. 36) stated that tilled soils are influenced in their physical status so that the moisture content is widely changing and the connection of the pore space is destroyed. This leads to the fact that soil fauna becomes scarce in the top soil layers. FRASER et al. (1996) showed that the conversion from a pasture soil to an intensively cropped soil let the number of earthworms drop down from initially 800 individuals per m² to less than 100 within 6 years. Especially in conventional tillage earthworm burrows are destroyed and earthworms themselves are either directly harmed or devoured by enemies e.g. birds after they have been brought to the soil surface (KARIGER 1993). Also the distribution of crop residues into deeper soil regions makes them inaccessible for some species, especially for anecic ones and reduces therefore the amount of food being available (KARIGER 1993, CHAN 2001).

This leads us to the second aspect of why ECAs can support earthworm populations – the permanent soil cover. As we know from previous chapters especially anecic species feed on plant material from the soil surface. This means that a permanent soil cover delivers a great amount of food throughout the year. This also holds true for endogeic species feeding on dead roots and for epigeic species depending on the sort of litter. Besides the permanent soil cover also indirectly influences the earthworm populations by regulating the microclimate of the area. A permanent soil cover offers shade thus the evaporation of the soil is lowered so that the water supply is positively influenced.

The non-use of plant protection agents on ECAs is a further issue that benefits earthworm populations. “It is well known that pesticides, particularly insecticides, nematicides, certain fungicides (e.g., benomyl), and herbicides (e.g., dinoseb), reduce earthworm populations” (PFIFFNER and LUKA 2007). The harming effect of the fungicide benomyl is also affirmed by ANDRÈN and LAGERLÖF (1983 quoted in NEHER and BARBERCHECK 1999 p. 40). Since ECAs are not treated with pesticides, as they are not used in an economical perspective or it is even forbidden by policy, earthworms in these areas are save in terms of the effect of pesticide-usage.

Finally it can be said that ECAs can support earthworm populations in the agro-ecosystem. Now the further question is: can ECAs serve as potential source for immigration into the arable field where all the benefits of earthworms are wanted?

According to EHRMANN (1996) the distribution of earthworms can either be done in a passive or active way. The passive transport is done by animals, for instance birds, by streams or by soil adhering at machinery. If the migration is done actively earthworms can cover wide distances. PIFFNER (2014) stated that earthworms can immigrate into the field from undisturbed surroundings like field margins, at which *Lumbricus terrestris* for example can migrate up to 20 m per year. Decreasing numbers of earthworms from a field margin going to the center of an adjacent field are recorded in the study of GNAN (2002). It was concluded that field margins can serve as a source of immigration and emigration of the surrounded fields. EHRMANN (1996) concluded that field margins and other similar structures in the agro-ecosystem are very suitable habitats compared to the arable land and further can serve as source of immigration of earthworms to the field. Thus we can say that earthworm populations are promoted by ECAs in the agro-ecosystem and that these areas at the same time can function as immigration source for the adjacent fields.
Taking the theoretical information from this chapter as a starting point of the study, we can derive certain hypotheses from it. Below there are three Null hypotheses stated:

H_{01}: There is no difference in the species diversity of earthworms between a hedgerow, a flower strip, the field margin and the center of an adjacent field

H_{02}: There is no difference in the abundance of earthworms between a hedgerow, a flower strip, the field margin and the center of an adjacent field

H_{03}: There is no difference in the biomass of earthworms between a hedgerow, a flower strip, the field margin and the center of an adjacent field

Before we will come to the actual material and method part of this study a short excursus of current literature about the earthworm sampling will be introduced so that it is clear why certain materials and methods have been used for this study.

2.3.4. Excursus: Earthworm sampling

Time of sampling

Figure 4: Dormant earthworm curled together into a clew.

The time at which the sampling of earthworms should take place depends strongly on the activity time of the earthworms. This again is determined by soil temperature and water content of the soil. According to (PFIFFNER 2014) the highest activity of burrowing and reproduction of earthworms in the temperate climate zone occurs in spring time from March to April and in autumn from September to October. The soil temperatures when earthworms are active are ranging from 5 to 15 °C (GNAN 2002). When it is too cold, too hot or too dry the earthworms migrate to deeper soil levels or curl together into a clew (see **Figure 4**) and stay in this dormancy until the environmental conditions are acceptable again (EHRMANN 1996).
Therefore the time during the year at which the earthworm sampling should take place, has to be considered.

Sampling methods

In literature several different approaches how to sample earthworms can be found. There are three main strategies which can partly be implemented in numerous ways.

One strategy is the **octet method** according to THIELEMANN (1986). Here eight electrodes are arranged in a circle so that an electric field is built up which drives the earthworms to the soil surface. Disadvantageous is that the soil needs to be humid enough for conduction of the electricity and also the soil type influences the outcome of this sampling method. Furthermore the fairly expensive equipment is required. Nevertheless, the non-destructive way is a positive aspect of the octet method (ĈOJA et al. 2008).

Another strategy is the **sorting of earthworms** out of a soil sample. A soil monolith is thereby dug out of the ground and subsequently analysed of containing earthworms. This can be undertaken in different ways. One common approach is the **hand-sorting** of earthworms. Here a specific volume of soil is dug out and the earthworms subsequently sorted out of this soil by hand. Nevertheless JIMÉNEZ et al. (2006) found out that hand sorting alone can lead to an underestimation of smaller earthworms. Likewise it is not the best strategy for sampling large anecic earthworms as they can hide in their deep-burrow system (ĈOJA et al. 2008). Instead of sorting only by hand a sieve can be used for sorting also. Moreover the sorting can be done by using a Kempson apparatus where heat leads to the extraction of earthworms of the soil sample (ĈOJA et al. 2008).

A further strategy is the **chemical expulsion** by pouring a so called vermifuge on a defined soil area. In different studies diverse vermifuge agents are used for sampling earthworms (listed in appendix II). However, the effect on the earthworms is all the same: irritation of the earthworm which leads to an upward movement to the soil surface so that they can be collected.

Formerly, formalin was a commonly used chemical for earthworm sampling. However, nowadays the application of formalin is seen critically. This is based on the negative impacts on flora and fauna but also on the health-risk aspect for humans when working with it. It could be shown that the application of formalin leads to a strong decline of soil organisms as well as a decreasing of vegetative cover (EICHINGER 2004). Consequently, the usage of other vermifuge agents is becoming increasingly meaningful.

Allyl-isothiocyanate (AITC), also called mustard oil, is one chemical which is currently used. AITC is first diluted with Isopropanol as it is hardly soluble in water. This solution is mixed with water immediately before the application on the field. The concentration of mustard oil and the amount of water which is added later on varies between different studies. ZABORSKI (2003) recommends a concentration of 100 mg/l AITC which should be irrigated in 10 l applications in 10 min intervals on a 0.5 m² quadrat. Comparable concentrations of 49.6 mg/l and 99.2 mg/l AITC respectively are seen as optimal according to (ĈOJA et al. 2008). Although the stock solution of AITC was diluted in a higher amount of water (30l instead of 2x10l), both studies suggest a rather low concentration of AITC for earthworm sampling. VALCKX et al. (2011), testing the efficacy of different chemical expulsions, found out that different concentrations of AITC lead to a sampling of either higher biomasses or individuals of earthworms. 150 mg/l AITC seems to be optimal for sampling biomass of earthworms and a lower concentration of 100 mg/l AITC is said to be optimal when focusing on amount of individuals. They “indicate [it as] a [possible] trade-off between the recovery of smaller and more numerous individuals (juveniles and epigeics) on the one hand and heavier and less numerous individuals on the other (adults/subadults and anecics)” (VALCKX et al. 2011, modified by author).

A low-cost alternative is the usage of a common mustard suspension or a mustard powder solution. As both materials contain only a small amount of the irritant AITC, a higher concentration is necessary than applying the pure agent. However, the trade-off between biomass and numbers of individuals seem to be the same also for this alternative application with mustard (VALCKX et al. 2011). For the monitoring of the introduction of *Lumbricus terrestris* into a field NUUTINEN et al. (2011) were applying a mustard powder solution with a concentration of 60 g/10 l water. A solution of commercial hot mustard with a concentration of 15 g/l water was used by PELOSI et al. (2009) which was, however, not that effective than AITC and formalin also used in this study.

Another recent study of STEFFEN et al. (2013) was testing the efficacy of onion solution for earthworm collection. With a concentration of 175 g onion extract per litre the results of the earthworm extraction is comparable with the ones from extracting with the commonly used formalin. Even though onion solution seems to be a good alternative, this was the first study indicating this, so that further research is needed to confirm this knowledge also for other side conditions.

In the before mentioned study of PELOSI et al. (2009) they also made a comparison of chemical expulsion with and without combination of hand-sorting of earthworms and they are stating that "earthworm populations are greatly under-estimated without hand-sorting". Anyhow, in the end a combination of hand-sorting and chemical expulsion seem to enable a good overview of the earthworm population of a sample side as this combination accounts for small and large individuals as well as for the different ecological groups of earthworms.

Inactivation and storage

In literature different instructions about killing and storing of earthworms can be found. There are two main chemicals which are commonly used. KOVÁCS-HOSTYÁNSZKI et al. (2013) were using 70 % ethanol for killing and conserving earthworms. Same was used by KARIGER (1993). FRÜND and JORDAN (2003) killed and stored in 3.5 % formalin. The earthworm expert Susanne Papaja-Hülsbergen, from the University of Weihenstephan, recommended the usage of 5 % formalin for killing and 70 % ethanol for storing the earthworms, to avoid the toxic vapor of formalin during the species identification work (Phone call, March 2014).

2.3.5. Species identification of earthworms

The identification of earthworms can be done by the help of existing taxonomic keys for example with the synoptic key of CHRISTIAN and ZICSI (1999) and is either done with dead or alive earthworms. Different characteristics, mostly external characters, have to be regarded to come up with the correct earthworm species. Important features for instance are the body length, the head (in German: Kopflappen), the arrangement of the bristles (**Figure 5**), the number of segments until the Clitellum starts as well as the number of segments the Clitellum includes.

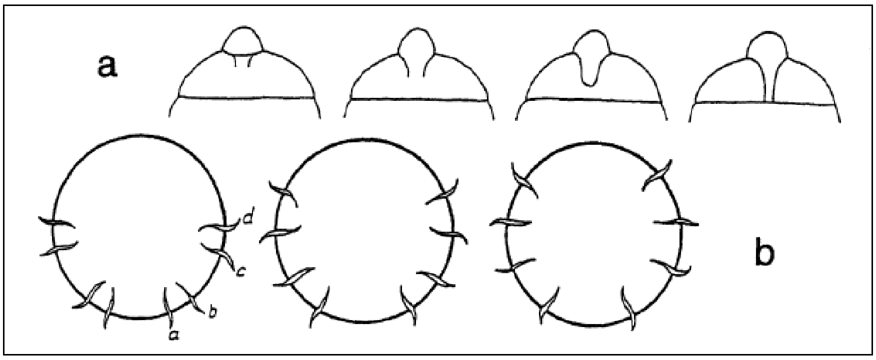

Figure 5: Characteristics of earthworms used for species identification, (a) head: proepilob, epilob 1/2 open, epilob 1/2 closed, tanylob; (b) arrangement of bristles: closely paired, broadly paired, unpaired (CHRISTIAN and ZICSI 1999).

Now that we have a short background of important issues that have to be considered when undertaking a field study with earthworm, the following part will describe the study side and will clearly show which materials and methods were actually used for this study.

3. Material and Methods

This chapter will start with a description of the study side including environmental conditions. After this the experimental design and the used material and methods will specified, ending up with the statistical methods used to analyze the results of the study.

3.1. Site description

The study has taken place at a trial area of the “Landwirtschaftlichen Bundesversuchswirtschaften (BVW) GmbH” in Rutzendorf. It is located in the so called Marchfeld region east from Vienna, in Lower Austria. The Marchfeld is one of the largest plains in Austria and has a size of about 1000 km². Since 2003 a long-time monitoring of the implication of conversion to organic farming, called MUBIL, is done at the trial area, which is scientifically coordinated by the Division of Organic Farming, University of Natural Resources and Life Sciences Vienna (FREYER et al. 2011a).

3.1.1. Climate

The Marchfeld region is influenced by the pannonian climate. This leads to hot and dry summers and cold winters. The strong wind affects the unprotected fields (EGLE 2010). According to ZAMG (2014) the average annual temperature of the close-by research farm Groß-Enzersdorf is 9.8°C. In **Figure 6** the average monthly temperatures of the trial area Rutzendorf are demonstrated for the time span of 2003 to 2013.

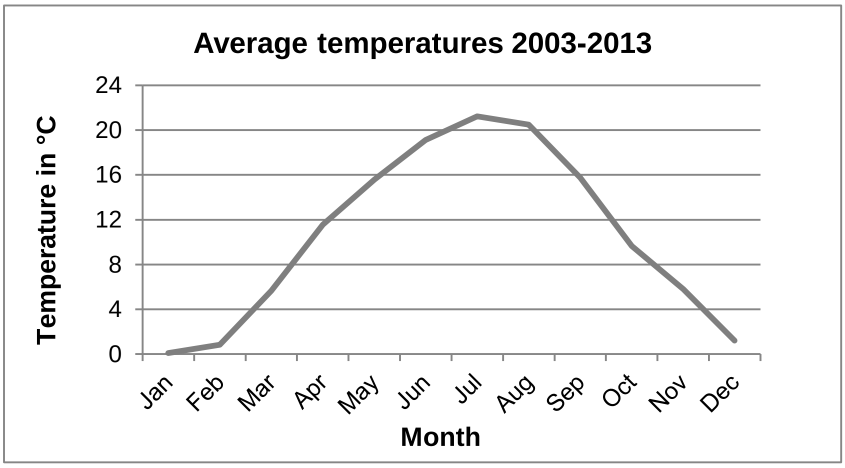

Figure 6: Average temperatures from 2003 to 2013 at the trial area Rutzendorf.

The temperatures at the trial area throughout the year show the typical bow-shaped curve of the temperate climate zone. Highest temperatures are reached in July, whereas the lowest temperatures occur in January.

The average precipitations of the years from 2003 to 2013 at the trial area are shown in **Figure 7** below. The year 2007 has had the highest precipitation in the last years. In comparison to that 2011 was an extremely dry year with an average annual precipitation of around 300 mm. The previous year 2013 showed a quite average amount of precipitation.

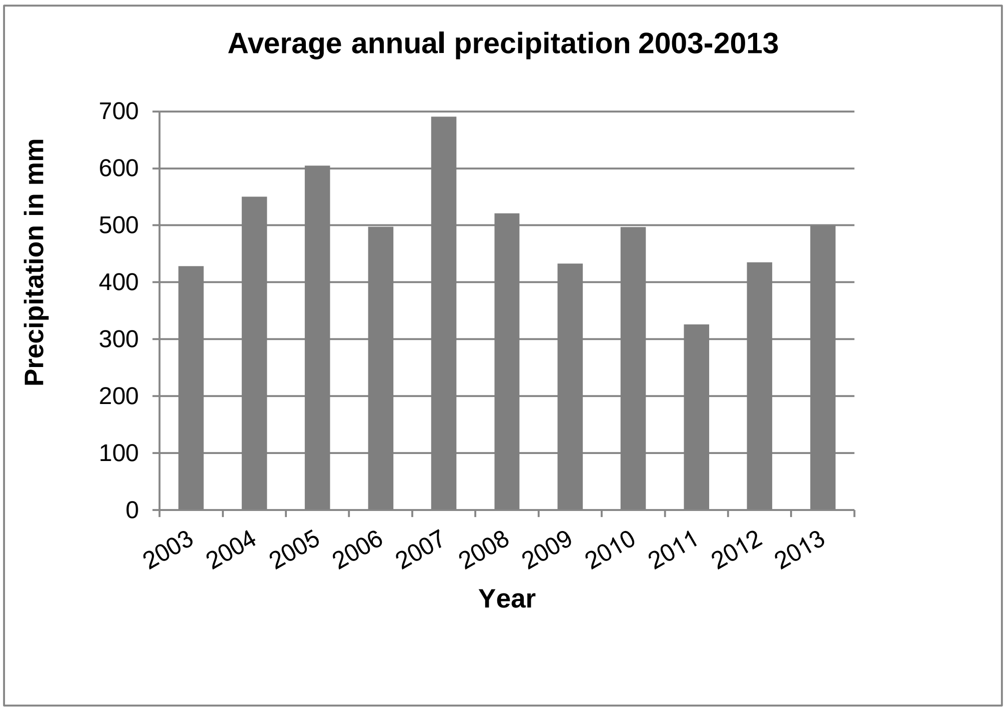

Figure 7: Average annual precipitation from 2003 to 2013 at the trial area Rutzendorf.

3.1.2. Soils

The geological history of the area is still visible today. In the postglacial time the melt water of glaciers broadened the river base of the Danube so that the study area was covered with several river arms of the Danube trough a certain time. Hence the soils of this area are built upon calcareous fine sediments which were deposited there from the Danube River (Excursion to Rutzendorf FRIEDEL 2014).

According to the digital soil map of Austria (eBOD 2014) the soil type of the trial area is a Tschernosem. The soil texture ranges from loamy sand to loamy silt and has high silt content in general. The soil in the study area has a medium high humus content with a C_{org} of 1.89 % and a N_{tot} of 0.16 % in the topsoil (FREYER 2011b). The depth of the soils is classified as deep. From an agricultural perspective the soil is categorized as middle to high-class (eBOD 2014)

3.1.3. Weather

The winter 2013/2014 was, compared to the average of the past years, dominated by relatively warm (**Figure 8**) and dry (**Figure 9**) weather conditions.

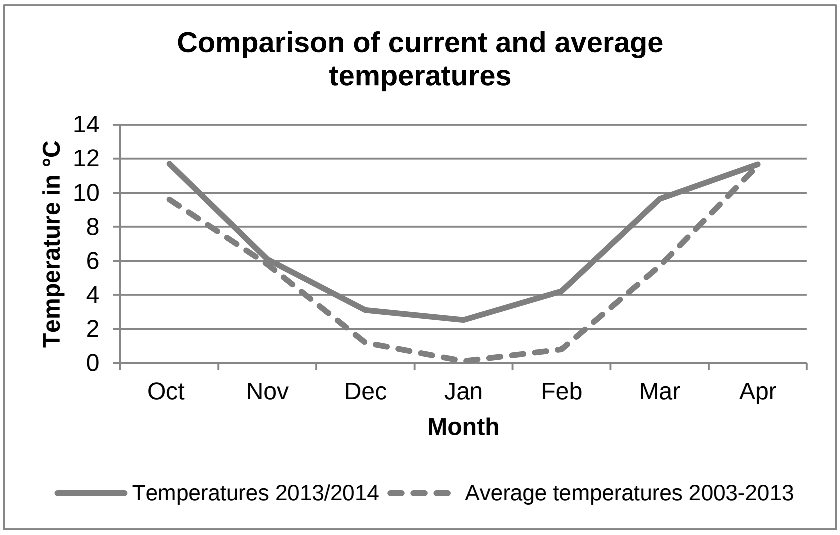

Figure 8: Comparison of the current temperatures 2013/2014 with the average temperatures from 2003 to 2013 at the trial area Rutzendorf.

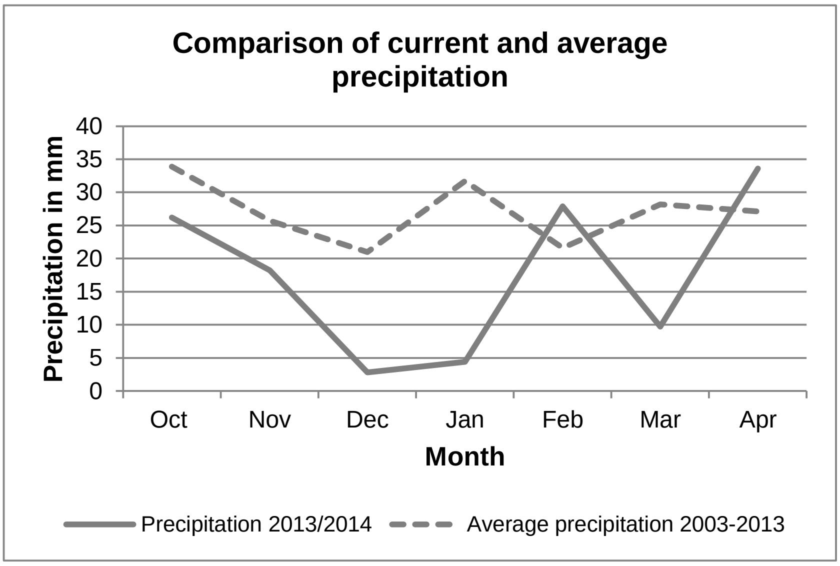

Figure 9: Comparison of the current precipitation values 2013/2014 with the average precipitation from 2003 to 2013 at the trial area Rutzendorf.

3.1.4. Soil use, cropping, soil management

The organic farm Rutzendorf is certified organic since 2003. A total area of 143 ha is cultivated by the farm. The conversion time lasted two years from 2001 to 2002. Since then a crop rotation consisting of eight parts was tried to be achieved (FREYER et al. 2011a):

1. Lucerne
2. Lucerne
3. Winter Wheat + Cover crop mixture
4. Grain Maize
5. Spring Barley + Cover crop mixture
6. Pea + Intercrop
7. Winter Wheat
8. Winter Rye

However, the real crop rotation of the field, where the samples were taken, from was arranged since 2003 in the following way:

Table 2: Crop rotation including soil tillage of the field from 2003-2014

Year	Crop	Soil tillage
2003	Lucerne	Plough
2004	Lucerne	-
2005	Winter Wheat + Intercrop	Plough
2006	Sunflower	Plough
2007	Winter Rye + Intercrop	Disc Harrow
2008	Peas + Intercrop	Plough
2009	Winter Rye	Plough
2010	Lucerne	Stubble Cultivator
2011	Lucerne	-
2012	Winter Wheat + Intercrop	Plough
2013	Grain Maize	Plough
2014	Spring Barley	Plough

3.1.5. Ecological compensation areas

Within the area of the farm tree- and hedgerows, with a total length of 6034 m, are bordering the fields. Furthermore, flower strips are covering a total area of 3.8 ha, which represents 2.6 % of the farm area (FREYER et al. 2011a).

Figure 10: Hedgerow, flower strip and the adjacent field of the study in May.

After regarding the map of the trial area it was decided to take one field with an adjacent hedgerow and flower strip as selected area for this study (see **Figure 11**).

Hedgerow of the study

The hedgerow where the earthworm sampling took place was originally planted in the 1980ies for wind protection. It is orientated in north-south direction and has a total length of around 600 m. It is a broad mixture of different shrubs and trees like *Ligustrum vulgare, Sambucus nigra, Robina pseudoacacia* and *Acer pseudoplatanus* (FREYER et al. 2011a).

Flower strip of the study

The flower strip for this study is located on the eastern side of the hedgerow, directly next to it. Therefore the flower strip has in total the same length as the hedgerow but is divided into three different seed mixtures, whereof the two northern mixtures were part of the study (see **Figure 11**). The most northern flower strip mixture is called “Beneficial mixture”. The name is based on its flower composition which is said to function as support of natural beneficial organisms in the agro-ecosystem. For detail information about the original seed composition see appendix I. The length of this flower strip part is around 170 m. The flower strip mixture located in the south of the just mentioned “Beneficial mixture” is called “Spontaneous succession” has here no active seeding has taken place. Instead, only the bare soil was left and the seeds from the local environment had started to develop there. This part of the flower strip covers a length of around 235 m. The total flower strip has width of 6 m and was arranged in 2003.

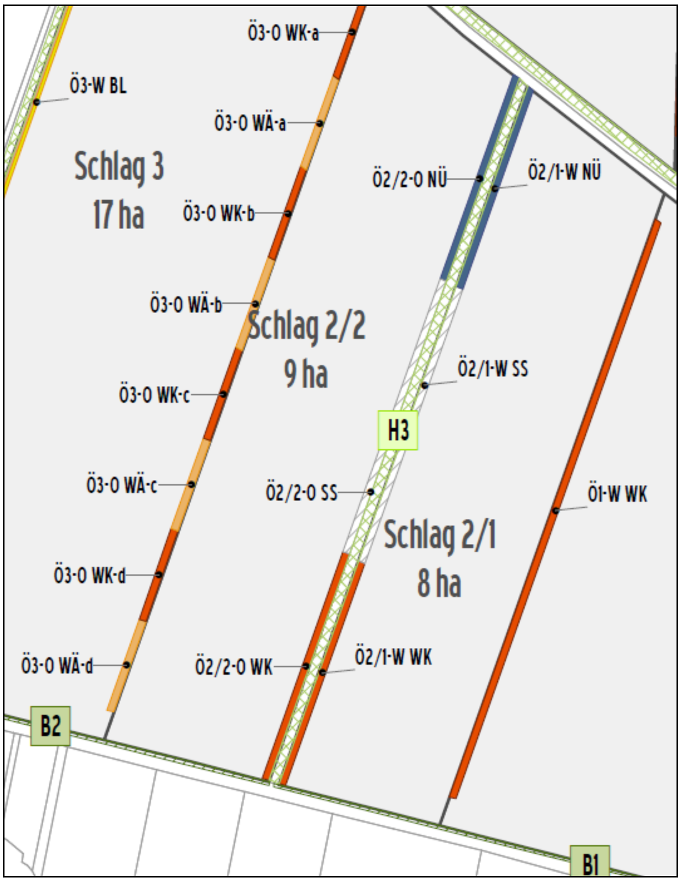

Figure 11: Section of a map of the trial area Rutzendorf; Ö2/1-W NÜ = flower strip "beneficial mixture", Ö2/1-W SS= flower strip "spontaneous succession", Schlag 2/1= field, H3 = hedgerow.

3.2. Experimental design

For each treatment (land-use type) of this study eight replicates were arranged (see **Table 3**). The distribution of the replicates was done by walking along the flower strip and marking randomly the points where the earthworm and soil sampling should take place. The only criteria were that the replicates should be arranged in the middle of the strip and that the distance of the replicates in the row should be at least 20 m. This was done to guarantee independency of the replicates. Also the location of the margin replicate to the close-by track and to the adjacent flower strip mixture was arranged with a distance of minimum 20 m to minimize the influences.

Based on the distribution of the replicates in the flower strip all the other replicates of the further land-use types were arranged on the same height. This means that the first sampling point in the hedgerow was defined by using a tape measure which was placed between the first replicate in the flower strip to the middle of the hedgerow. This was done for each of the eight replicates. In terms of the field it was done in the same way accept for the defined distance. For the land-use type "field (2 m)" the tape measure was placed on the edge of the flower strip and then the replicate was arranged two meters straight in the field. For the treatment "field (50 m)" the same was done with 50 m straight into the field. Finally eight transects consisting of four sampling points each, one for each land-use type, were established.

Table 3: Experimental design for taking the earthworm and soil samples.

Sample/ Replicate	**Land-use type /Treatment**			
	Hedgerow	Flower strip Spontaneous succession & Beneficial mixture	Field (2m)	Field (50m)
1	■	■	■	■
2	■	■	■	■
3	■	■	■	■
4	■	■	■	■
5	■	■	■	■
6	■	■	■	■
7	■	■	■	■
8	■	■	■	■

3.2.1. Sampling of earthworms

The sampling of earthworms was done on two successional days with similar weather conditions, on the 1st and 2nd of April 2014.

Figure 12: Soil monolith dug out of the ground.

As described in chapter **2.3.4** there are several possibilities how to sample earthworms. I decided to make a combination of hand-sorting and expulsion with a mustard powder solution. After consulting my Co-supervisor Pascal Querner I concluded to start with the hand-sorting with subsequent chemical expulsion.

The process of sampling was undertaken in the following manner: A metal frame of 25 cm x 25 cm (0.0625 m²) was pressed into the soil and then dug out with the help of a spade. Afterwards the soil monolith was given into a plastic box for hand-sorting. The sorting was done for 30 minutes. For the chemical expulsion a mustard powder solution was prepared in a way that 30 g mustard powder (from Eder Gewürze KG) was mixed with 100 ml water approximately 3 hours before the first application. Immediately before the application at each sample point, the stock solution was diluted with 5 l of water. For 15 minutes this solution was filled into the hole in the ground which was left after digging out the soil monolith. Then the hole was scanned for new occurring worms.

All earthworms detected by hand-sorting and by collecting them from the top of the hole were washed in water and then stored in 70% ethanol.

Figure 13: Metal frame and infiltrating mustard solution in the hole of the ground (left); Hand-sorting of the soil monolith (right).

Determination of earthworm abundance and biomass

Abundance and biomass are amongst others suitable measures for describing communities of soil invertebrates (NEHER and BARBERCHECK 1999, p. 34, VALCKX 2011). Therefore these two indicators were used in this study to describe the earthworm populations at the trial area. For determining the abundance, the individuals of every sample were counted and thereby divided into adults and juveniles. For measuring the biomass of the earthworms, a precision scale (Sartorius Mechatronics Austria GmbH) was used. The earthworms of each sample were dapped with a paper towel and then weighed (with gut content), adults and juveniles separately.

Figure 14: Identification of earthworm species with a binocular.

Species identification

In the beginning the identification of species was planned to be done at the living earthworm, as this is more ethical justifiable. But for practical reasons this was not possible so that it was done with dead individuals. For the identification a binocular was used (see **Figure 14**). The species determination was done according to CHRISTIAN und ZICSI (1999). The classification of ecological categories was done according to BOUCHÉ (1972).

3.2.2. Soil sampling

As mentioned in chapter **2.3.2** the soil influences the earthworm population. Since the soil at the trial area is very heterogeneous in terms of depth and texture, soils samples were taken for every sample point at which earthworms were collated. This was done for being able to use the soil as a possible influencing factor for the statistical analyses later on.

Figure 15: Soil sample in boring rot, with a folding rule for measuring the depth of soil horizons.

The soil sampling was done in April and May. With a boring rod next to each earthworm sample point a 1 m deep soil sample was taken. The depth of the soil horizons was measured directly on the field with the help of a folding rule. Afterwards material from each horizon was filled into a plastic bag for further analysis of the soil texture.

Finger test (In German: Finger Probe)

The finger test is a method to identify the soil texture without using special machinery for it. The only tools needed are water, an identification key for this test and your hands. By kneading and forming the moistened soil sample the soil texture class can be determined. This was done for every horizon of each soil sample point, using the identification key of SPONAGEL et al. (2005). In the end the soil depth was defined in which the texture changes from silt/loam to sand occured.

3.2.3. Vegetation survey

The vegetation survey was done in May 2014, according to BRAUN-BLANQUET (1964). For each of the two flower strip mixtures one plot (5m x 5m) was randomly assigned and the vegetation cover recorded.

3.3. Statistical methods

For the statistical analyses several tests were performed with SPSS 20. All parameters were checked with the Levene's test for the homogeneity of variance.

For the comparison of the earthworm abundance and the biomass between the different land-use types, an analysis of variances (ANOVA) was done for both variables.
As the different soil characteristics on the sampling points can have an influence on the earthworm population, additional analysis of covariance (ANCOVA) were performed with the same models used before. On the one hand the depth of the A-horizon was used as covariate and on the other hand the depth of texture change, meaning the soil depth in which the texture is changing from silt/loam to sand, was defined as covariate. For the post-hoc comparison a t-test with Bonferroni correction was used.
In the following chapter, only the results of the ANCOVA of earthworm abundance and of earthworm biomass using the soil texture change as covariate are demonstrated. All other results of the statistical tests will not further be discussed but can be regarded in appendix IV.

The analysis of earthworm species between the land-use types was undertaken with descriptive methods because of the lack of species diversity.

Based on range of this study the vegetation survey was not done for each sampling point. As the question of the influence of the vegetation cover on the earthworm population was nevertheless interesting the analyses of the vegetation cover as well as the comparison of earthworm abundance and biomass of the two flower strip mixtures were done in a descriptive way.

4. Results

In this chapter the results of the study will be described and illustrated by using graphs and tables. It is divided it certain issues of the study, starting with broader subjects like the vegetation survey, ending up with analyses of the earthworm abundance and biomass.

Vegetation and earthworms of the flower strip mixtures

The plant species of the two flower strip mixtures which were recorded during the survey are shown in **Table 4**. The ground cover of the flower strip mixture "spontaneous succession" is represented by 95 % and the one of the "beneficial mixture" by 98 %.

Table 4: Vegetation survey of the flower strips according to BRAUN-BLANQUET (1964).

Flower strip	Species	Abundance	Ground cover (%)
Spontaneous succession	*Bromus erectus*	3	**95**
	Syringa vulgaris	3	
	Leonurus cardiaca	2	
	Galium aparine	2	
	Silene latifolia	1	
	Dipsacus sylvestris	1	
	Lolium repens	1	
	Cornus sanguinea	1	
	Acer pseudoplatanus	+	
	Urtica dioica	+	
	Stellaria media	+	
	Ligustrum vulgare	+	
	Echinops sphaerocephalus	+	
	Rosa sp.	r	
	Antrhiscus sylvestris	r	
Beneficial mixture	*Syringa vulgaris*	2	**98**
	Arrhenatherum elatius	2	
	Daucus carota	2	
	Echinops sphaerocephalus	1	
	Leonurus cardiaca	1	
	Verbascum sp.	+	
	Dipsacus sylvestris	+	
	Acer pseudoplatanus	+	
	Prunus mahaleb	+	
	Galium aparine	+	
	Cirsium arvense	+	
	Robinia pseudoacacia	r	
	Ligustrum vulgare	r	
	Sambucus nigra	r	
	Cornus sanguinea	r	

Categories in portion of ground cover/number of individuals:
r (individual/shoot), + (2-5 individuals),1 (<5%), 2 (5-25%), 3 (>25-50%), 4 (>50-75%).

For each flower strip mixture 15 plant species could be found. In the flower strip part "spontaneous succession" *Bromus erectus* is dominating the plant cover, followed by *Syringia vulgaris, Leonurus cariaca* and *Galium aparine*. The flower strip mixture "beneficial mixture" is dominated by *Syringia vulgaris* and *Arrhenatherum elatius.*

The following two figures show boxplots of the abundance (**Figure 16**) and the biomass (**Figure 17**) of earthworms separated for each flower strip mixture. The median of the abundance in the "spontaneous succession" mixture is with 208 individuals per m² clearly lower than the abundance of the "beneficial mixture" with a median of 400 individuals per m². Also the scattering of the abundance is much higher for the "beneficial mixture" with a minimum value of 176 and a maximum value of 480 individuals per m² compared to the "spontaneous succession" with 192 and 272 individuals per m² as minimum and maximum value, respectively.

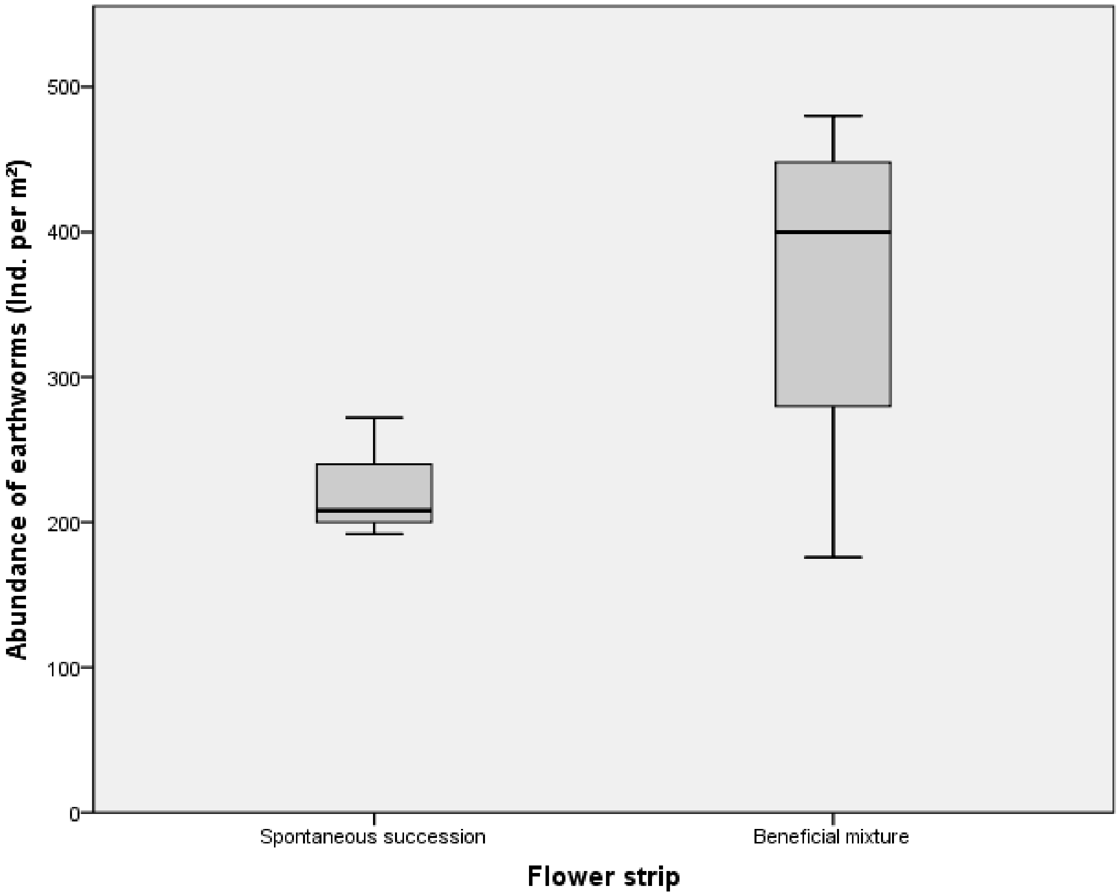

Figure 16: Box-plots for the abundance of earthworms per m² of the flower strip mixtures „Spontaneous succession" and „Beneficial mixture".

The boxplots for the earthworm biomass look a bit different than those regarded before. Here the distinction between the earthworm biomass found for each flower strip mixture is not that distinct. The quartile boarders are partly overlapping so that some values of both flower strip mixtures lay within the same range. Nevertheless also here the "beneficial mixture” is the one showing higher values in total compared to the mixture “spontaneous succession”. This can be seen for instance by the median value of the beneficial mixture with 49.4 g per m² being nearly twice as high as the median of the mixture spontaneous succession with 92.1 g per m².

Figure 17: Box-plots for the earthworm biomasses per m² of the flower strip mixtures „Spontaneous succession“ and „Beneficial mixture“.

Earthworm species

The identification of earthworms revealed that only two different species were collected. This is the reason why the results are only analyzed descriptively. In **Table 5** the mean and standard deviation of the abundance of the two species are shown for the different treatments. It is important to mention that here only the adults are considered since the juveniles could be only identified up to the genus level. Having a closer look at the table below, it shows that the abundance of the two species is distributed differently between the treatments. In the hedgerow mainly *A. rosea* could be found whereas the number of *A. caliginosa* is much lower. In the flower strip and the field (2 m) the abundance of both species is nearly the same. For the field (50 m) the number of *A. caliginosa* is lower than half of the number of *A. rosea*. The standard deviation of some results has partly quite high values and has therefore to be regarded carefully.

Table 5: Mean and standard deviation of the abundance of the collected earthworm species (adults/m²) for the different treatments.

Treatment	Mean *A. rosea*	Standard deviation	Mean *A. caliginosa*	Standard deviation
Hedgerow	70.0	± 87.2	6.0	± 11.9
Flower strip	58.0	± 39.1	54.0	± 39.1
Field (2m)	16.0	± 22.6	22.0	± 17.0
Field (50)	12.0	± 16.6	4.0	± 7.4
Total	156.0		86.0	

Both species belong to the eco-physiological category of endogeic earthworms.

Nearly all juveniles found in this study belong to the genus Aporrectodea and where distributed throughout all treatments. The only acceptance represents the findings of juveniles of the genus Lumbricus. Here 2 individuals per m² were found in the hedgerow and 4 individuals per m² in the flower strip.

Since only two earthworm species could be collected and both of them were represented in all treatments the

- **Hypothesis H_{01}** can be rejected. Finally it can be said that there is no difference in the species diversity between the treatment groups.

Earthworm abundance

The statistical differentiation of the abundance between the treatment groups is illustrated in **Table 6** due to the results of the ANCOVA. With an p-value of 0.011 the influence of the covariate soil texture is highly significant. Also the differences between the groups are significant with a p-value of 0.036.

Table 6: ANCOVA of earthworm abundance with depth of texture change as covariate.

Source	df	Sum of squares	Mean squares	F-ratio	P-value
Corrected Model	4	836.6	209.2	3.095	0.032*
Texture (Covariate)	1	498.9	498.5	7.381	0.011*
Treatment	3	664.5	221.5	3.277	0.036*
Error	27	1824.9	67.6		
Corrected Total	31	9506.0			

*Significant at the level of 0.05

With a mean abundance of 152 individuals per m² the hedgerow shows the lowest value of all treatments. This is followed by the treatment field (50 m). For the field (2 m) an average number of 256 individuals per m² could be collected. The highest abundance could be detected in the flower strip.

Table 7: Mean and standard deviation of earthworm abundance (Ind./m²) for each treatment.

Treatment	Mean	Standard deviation
Hedgerow	152.0[a]	± 141.3
Flower strip	292.0[b]	± 117.8
Field (2m)	256.0[ab]	± 84.7
Field (50)	242.0[ab]	± 209.2

Different letters indicate significant differences ($p<0.05$)

For knowing exactly which of the treatment groups are significantly different from each other, the output of the post-hoc comparison has to be regarded. As shown in **Table 6** the hedgerow and the flower strip are significantly different from each other ($p=0.045$), while there is no difference between the other groups (see appendix IV). Finally we can partly reject

- **Hypothesis H_{02}**. Thus, it can be said that there is a difference in the abundance of earthworms between the hedgerow and the flower strip.

For a more detailed insight, beside the statistical analysis, **Figure 18** shows how the total abundance is composed of adult and juvenile earthworms with the portions of that for every treatment.

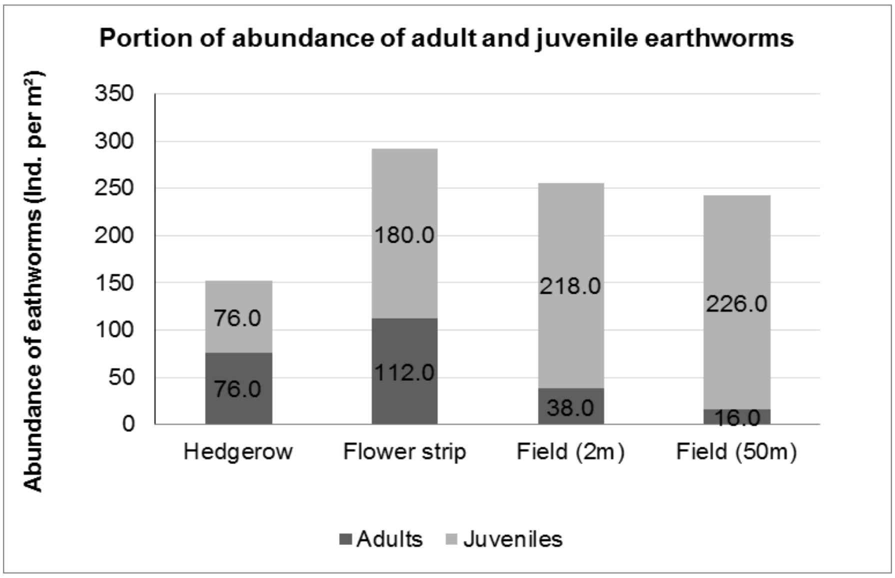

Figure 18: Portion of adult and juvenile earthworm abundance for each treatment.

Starting with the hedgerow it has exactly the same values for the abundance of adults and juveniles. In the flower strip the proportion of abundance is slightly shifted to the side of the juveniles. For the treatment of the fields both distances - 2 m and 50 m - show a distinct dominance of the juveniles in terms of the abundance.

Earthworm biomass

After the examination of the results of the earthworm abundance we will now come to the comparison of the biomass of the different treatments. The ANCOVA shown in **Table 8** illustrates the statistical results of the treatment differences for the earthworm biomass. Again as covariate the soil texture was used, which has a significant influence (p=0.046) on the treatment differences. However, the differences between the treatment groups for the earthworm biomass are not significant (p=0.085)

Table 8: ANCOVA of earthworm biomass with depth of texture change as covariate.

Source	df	Sum of squares	Mean squares	F-ratio	P-value
Corrected Model	4	68.0	17.0	3.095	0.113
Texture (Covariate)	1	35.9	35.9	7.381	0.046*
Treatment	3	60.549	20.183	2.452	0.085
Error	27	222.2	8.2		
Corrected Total	31	290.231			

*Significant at the level of 0.05

Likewise the abundance, also here the hedgerow is the treatment showing the lowest value with an earthworm biomass of 41.2 g per m². The flower strip, being the treatment actually having the highest abundance, shows here the second lowest biomass of 74.7 g per m². This is followed by the treatment field (50 m) and closely after that by the treatment field (2 m) with the highest value of 80.1 g per m².

Table 9: Mean and standard deviation of earthworm biomass (g/m²) for each treatment.

Treatment	Mean	Standard deviation
Hedgerow	41.2^a	± 43.4
Flower strip	74.7^a	± 32.6
Field (2m)	80.1^a	± 21.6
Field (50)	78.8^a	± 77.7

Different letters indicate significant differences (p<0.05)

In the end we can accept

- **Hypothesis H_{03}**: There is no difference in the biomass of earthworms between a hedgerow, a flower strip, the field margin and the center of an adjacent field

Also for the biomass the division into adults and juveniles was done for every treatment (**Figure 19**). Since abundance and biomass are strongly correlated (FRASER et al. 1996), the earthworm biomass of the juveniles is like the abundance before highest for the treatments field (50 m) with 72.8 g per m² and field (2 m) with 58.0 g per m². Comparing these values with the total biomass of the two treatments, the proportion of juveniles is by far the highest for field (50 m) followed by field (2 m). For the flower strip a biomass of 24.6 out of 74.7 g per m² can be allocated to juveniles, so that this represents the third highest portion of juveniles. In the end, the lowest biomass of juvenile earthworms was found in the hedgerow with 10.6 g per m² which represents around 26 % of the total earthworm biomass collected in the hedgerow.

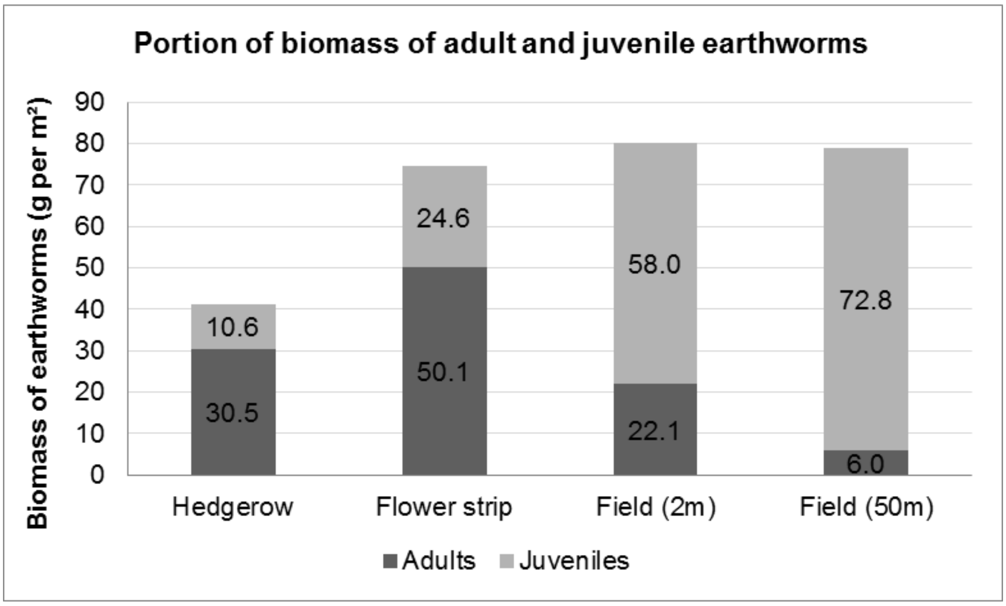

Figure 19: Portion of adult and juvenile earthworm biomasses for each treatment.

5. Discussion

Vegetation and earthworms of the flower strip mixtures

In principle the results of the influence of the vegetation on the earthworm population are promising. Even though there was no extensive statistical analysis, the difference between the two flower strip parts is interesting. For the beneficial mixture a higher amount in abundance and in the biomass were found. Even if the difference in biomass was not as clear as the difference in abundance, further study on this could be from interest.

The ground cover of both flower strip mixtures is nearly the same and with values close to 100 %, quite high. On the one hand the plant composition of both flower strip types is similar, like this is the case for *Syringia vulgaris* which was found in both mixtures but with higher abundance in the spontaneous succession. On the other hand there are also some clear differences, especially for the species *Bromus erectus,* which is mostly dominating in the mixture “spontaneous succession” but which was not found in the “beneficial mixture”. The occurrence of same species in both mixtures can be explained by the distribution of the seeds. Based on the nearby location the seeds of both flower strip types can easily be exchanged. The hedgerow next to the flower strip further inserts plant species into the flowers strip mixtures for instance *Acer pseudoplatanus* and *Ligustrum vulgare.* The plant composition is also influenced by other surrounding flower strips. *Bromus erectus* for example was part of the initial seed mixtures of another flower strip mixture in the region of the trial area.
Since litter quality is a major influencing factor of the vegetation cover, the plant composition will be discussed in connection with their effects on earthworms
One of the main influencing points of the vegetation cover on earthworms is due to the usage as food. To make a distinction the plant species with the highest abundance should be considered in detail. As we know from the theoretical part the ingredients of plants are of great importance for the usage as food for earthworms. There it was shown that a high amount of tannin, phenolic compounds or certain acids in the litter are refused by earthworms. Moreover regarding the plant composition of the two flower strip mixtures there are some plant species where such substances are contained.

Since there are only few studies about the feeding preferences of earthworms, which are not going too much into detail about the decisive compounds, it has to be said <u>that the following remarks has to be seen as a slight approach for an explanation.</u>
For the “spontaneous succession” *Bromus erectus* is the most occurring plant. Even if there is no detailed research of *Bromus erectus* as food of earthworms, there is no evidence for inappropriate substances (KLAPP and VON BOBERFELD 2013 p. 184) which would lead to a conclusion that *Bromus erectus* could be rejected as food. There are neither tannins nor a higher amount of phenolic compounds or acids proven. For many of the other frequently occurring plants in the “spontaneous succession” a different picture can be drawn. *Syringa vulgaris* in general contains Syringin, from which many inappropriate chemicals like acids and phenolic-compounds are derived (KARRER 1976 p.110). There

are also other plants containing tannin, acids and essential oils in greater amount which is true for *Leonurus cardiac* (SCHAUBERGER and PARIS 1975 p. 29), *Galium aparine* (KARRER 1976 p. 454, FLEISCHHAUER et al. 2009) *and Cornus sanguinea* (FLEISCHHAUER et al. 2009).
For the "beneficial mixture" following situation was found. Although the number is lower than for the "spontaneous succession" mixture, there are also some plant species in the "beneficial mixture" containing inappropriate substances. There are *Syringa vulgaris and Leonurus cardiaca* which are commonly known to contain high amounts of such substances. In contrast, *Arrhenatherum elatius* (KLAPP and VON BOBERFELD 2013 p. 175)*, Daucus carota* (SCHAUBERGER and PARIS 1975 p. 42) imply only a low amount of inappropriate substances.
By consolidating the results of the earthworm populations in both flower strip mixtures and the results of the vegetation survey following tendencies can be assumed: The "benefical mixture" showed a higher abundance and biomass of the earthworm population compared to the "spontaneous succession" mixture. The composition of plants in the strips may lead to a migration into the "beneficial mixture". This could be based on the lower abundance of plants containing unsuitable substances in the "beneficial mixture". But not only the impact of plant substances in terms of food, but also the irritating impact on the earthworms´ skin could lead to this migration (WESTERNACHER-DOTZLER 1988).
Furthermore there are other points which could lead to the differences between the flower strip mixtures. For example the "spontaneous succession" laid fellow at the first time, while the "beneficial mixture" was seeded. This could have led to a faster vegetation cover on the "beneficial mixture" and therefore to a faster colonization by the earthworms.

While this descriptive analysis does not lead to any strong results, it can nevertheless be seen as a first attempt for further studies. In different studies there was a correlation between plants and the appearance of earthworms, so it can be possible for the distinction between the seed mixtures on this trial area. Further studies with a stronger database should confirm these first attempts later on.

Earthworm species

The appearance of earthworm populations mainly depends on several abiotic factors such as soil structure, temperature and humidity where in arable soils also the management factors play an important role (EHRMANN 1996). Equally, the biotic factors like the interaction between and within species has an influence on the population (PALM et al. 2013). Based on the different living habits, these factors determine the presence or absence of certain species in a defined area. In ecosystems of temperate climatic regions up to 10 to 15 species can occur, but more often they consist of only 2 to 6 species (LEE 1985 quoted in UVAROV 2009). PFIFFNER (2014) stated that on average 4 to 11 species can be found in arable land. In contrast, EDWARDS and BOHLEN (1996 quoted in VALCKX 2011) mention that earthworm communities are in general species-poor so that in arable land for example only 4 to 6 species occur usually.

In the study of QUERNER et al. (unpublished), earthworms were sampled and analyzed at the same trial area (Rutzendorf) in the year 2004 and 2012. By using formalin for extracting earthworms in hedgerows and flower strips the following species could be found:

- **Hedgerow 2004:** *A. caliginosa*, *A. rosea*, with dormancy of the latter
- **Hedgerow 2012:** *A. caliginosa*, *A. rosea, L. terrestris*, with dormancy of the first
- **Flower strip 2004:** *A. caliginosa*, *A. rosea,* with dormancy of the latter
- **Flower strip 2012**: *A. caliginosa*, *A. rosea, L. rubellus, Dendrobena sp.,* with dormancy of the second

Although another method of sampling earthworms was used the species found are comparable with the present study especially with the results of 2004. The additionally found species missing in the present study can maybe explained by the mentioned differences in the used methods.
However, in this study only two species were found in all treatment groups – namely *Aporrectodea rosea* (Savigny 1826) and *Aporrectodea caliginosa* (Savigny 1826). Therefore different reasons can be stated. Besides the method also the remarkably low precipitation in the Marchfeld region in winter and spring time prior to sampling could be a reason for the fact that only two species could be found in the present study.
Besides the usage of formalin QUERNER et al. (unpublished) also used hand-sorting for sampling earthworms in a flower strip and on several points in the adjacent field. Here the dominant species which could be found in all points of this study were again *A. rosea and A. caliginosa.* Additional species which were only found at some points were *L. rubellus, O. cyaneum* and *O. tyrtaeum*. However, these species were only found in a very low amount so that this tends to go along with the outcome of the present study were only A. *rosea and A. caliginosa* could be found but both with high numbers of individuals.
Comparing the occurrence of these two species with the results of other studies it gets clear that based on their living habits both of them can handle with soil disturbances by agricultural measures. The incorporation of crop residues in the upper part of the soil by ploughing can even lead to a positive effect of the abundance of endogeic species (CHAN 2001, PELOSI et al. 2009). This is confirmed by KNÜSTING (1992) where *A. caliginosa* was mostly found in intensively cropped soils. Here it is further mentioned that this species was often found in the rhizosphere area of cereals which was interpreted as the ability of searching for areas with a high content of organic matter selectively. A high production rate and the capability to stay dormant in times of unfavorable environmental conditions are two characteristics being responsible for the high abundance of *A. caliginosa.* This species builds burrows up to a depth of 60 cm; hence it is able to escape from the upper soil part. In contrast to this *A. rosea* mostly occurs in the upper 30 cm so it is more exposed to soil disturbances. Nevertheless, also *A. rosea* is a species occurring at many different sides. In a study of STÄHLI et al. (1997) this species showed in some plots a very high abundance, where it dominated the biomass of endogeics in 16 % of the investigated grassland soils.

In the present study both species were found with quite similar abundance in the field as well as in the flower strip. The only wide difference was found in the hedgerow were *A. rosea* was represented with nearly the twelvefold number of individuals as *A. caliginosa.* According to BOSTRÖM and LOFS-HOLMIN (1986) *A. caliginosa* prefers food with low physical size. They found that particles being smaller than 0.2 mm lead to a weight gain of earthworms which was twice as high as food particles between 0.2 to 1.0 mm. This could mean that the food sources in the hedgerow – mainly wooden parts of trees and shrubs and their litter – is not that appropriate for this species.

As already mentioned in the results, juveniles of the genus Lumbricus were only found in the hedgerow and the flower strip. Since the genus Lumbricus includes also anecic species these findings are from great importance, because of the well-known positive influence of anecic species like *Lumbricus terrestris*. The fact that Lumbricus sp. was only found in the ECAs could show the trend of preferring the ECAs as habitat compared to the arable field. The living conditions in undisturbed arable land are in general better for anecic species like this is the case in ECAs. There are different reasons for this assumption. On the one hand the feeding behavior of *L. terrestris,* as one example of anecic species, depends on the food of the soil surface. Here they move to the surface and take the litter into their burrows. The soil tillage removes these food sources (KNÜSTING 1992). On the other hand the soil tillage destroys the burrows itself, so that the food pathway of these earthworms is demolished (KNÜSTING 1992).
Moreover, it is seen that especially *L. terrestris* migrates to deeper soil regions under inappropriate conditions (inappropriate temperature and moisture conditions like mentioned above) (KNÜSTING 1992), while other species like *A. caliginosa* and *A. rosea* are not migrating but stay at their soil levels in dormancy (RÖMBKE 1997). This could be the reason why only such a low amount of *Lumbricus sp.* was found in this study where the possibility exists that among these also anecic species were included. In comparison the results of other studies on this trail site are in line with previous findings. FRIEDEL et al. (2000) stated that there was no evidence of anecic earthworms for the trail area Groß-Enzersdorf, as the studies of QUERNER (unpublished) showed only few anecic earthworms.

Earthworm abundance and biomass

The earthworm abundance can explicitly differ among certain land-use types (PFIFFNER 2014). The abundance in agricultural soils tends to be lower than the abundance in forest or grassland soils (DUNGER 1983 quoted in KNÜSTING 1992). So that at first glance one could expect higher numbers of earthworms in the EACs than in the field, since they can be relatively similar to the conditions in forests and grassland soils. Especially the hedgerow being much older than the flower strip is offering an undisturbed habitat. Nevertheless the lowest number of individuals was found in the hedgerow. The reason for this could be the lower ground cover compared to the flower strip. The hedgerow is a dense mixture of different shrubs and trees but only a small amount of herbaceous plants are part of it. This leads to a poorly covered soil so that the evaporation and therefore the

water content of the soil are worse than that of the flower strip. Additionally the rhizosphere is nerved with largely wooden roots which are both not useable as food in non-decomposed status so that they lower the potentially habitable area.
Comparing the abundance in the hedgerow of 152 individuals per m² with the stated range of PFIFFNER (2014) for a hardwood forest of 150 to 250 earthworms per m² the finding of this study tend to be affirmed, but they are stated at the lower boarder. Even if hardwood forests differ in some aspects, these values are the most comparable for the results of the hedgerow.

Same sort of comparison can be done for the flower strip. Here we take the results of two studies of permanent grassland and field margins. For the permanent grassland the study of KRÜCK (1999 p. 51) showed 312 individuals per m², which is slightly higher than the 292.0 earthworms per m² found in the flower strip. In a study of EHRMANN (1996) the earthworm abundance of field margins in different regions of South Germany were compared. Here the number of individuals ranged from below 50 to more than 700 individuals per m², depending on soil moisture and texture.

Regarding the abundance of the two field points (2 m and 50 m) 256 and 242 earthworms per m² were found here. This is in line with the 120 to 250 earthworms per m² which were stated by PFIFFNER 2014 to be the average in a low-input arable field. In a study of PFIFFNER and LUKA (2007) on some of their investigated sites earthworm abundance and biomass was even higher in arable fields than in the close-by perennial grassland strips, which was mostly the case in organically managed fields. The organically managed field of the presented study may offers enough food resources through application of organic fertilizer and especially through the establishment of two year lucerne, so that the harm of the conventional tillage practiced on this field may be partly compensated by this (PALM et al. 2013).
Furthermore there can be a positive influence of the attached flower strip which can serve as immigration pool for the field, thus a faster recolonization is possible. This could may be showed by the slight trend of a decreasing abundance and biomass from field (2 m) to field (50 m) meaning that earthworms immigrate from the adjacent flower strip whereof only some reach more afar parts in the field (even though the statistical analysis does not show a difference yet). These findings are in line with the observations of PALM et al. (2013) who found a higher density of edogeics and epigeics in field plots close to a meadow and with the observations of GNAN (2002) who found decreasing numbers of earthworms going from a field margin to the center of an adjacent field.
Although the field contains quite high numbers of earthworms, conventional tillage significantly decreases abundance and biomass and additionally influences the species structure (CHAN 2001). Thus, changing the cropping system towards reduced tillage could positively alter the species composition on this site to a more diverse population (KUNTZ 2013).

Comparing the values for the abundance with the values of the study of QUERNER et al. (unpublished) the abundance in the flower strip and the different field points (by hand-sorting) were much lower than that for the present study. In the flowers strip they only found 200 individuals per m². The values in the field ranged from 86.4 to 180.1 earthworms per m² which is below the 242.0 and 256.0 earthworms per m² which were found in the present study. This indicates that the abundance of earthworms at the trial area further increased from 2012 to 2014. It has to be said that on this point possible effects of different sampling methods can be neglected since the chemical expulsion of the present study was not efficient. In the end nearly all of the earthworms it were collected by hand-sorting like in 2012.

The abundance can also be separately regarded for juveniles and adults. In this study the number of juvenile earthworms is higher than that for adults in each of the treatments. In the study of QUERNER et al. (unpublished) the number of juveniles found was mostly lower than the number of adults. The portion of juveniles in the field for instance was only 4 % and 7.5 % respectively. In the present study it is the other way around which is in special the case for the points in the field were a portion of juveniles of around 85 % and 90 % (field 2 m and field 50 m) was found.
The high portion of juveniles, in particular for the field, stays in contrast to the observations of KUNTZ et al. (2013). They compared the influence of different tillage methods where the reduced tillage showed a significant increase in juvenile earthworms compared to the conventional tillage. This was explained by the enhanced food supply and the reduced disturbance of the soil. In terms of the high number of juveniles they further illuminated the allocation of energy whereby the regular destruction of the burrows in conventional tillage leads to reconstructing activities of them which means less energy is invested in reproduction by the earthworms. The high number of juveniles in the field of the present study might be explained by a migration from the flower strip as result of a reached carrying capacity there (Mathieu et al. 2010). Intraspecific competition influences the earthworm populations at favorable sites in a way that quality of food determines the density of earthworms – irrespective of the ecological category they belong to (UVAROV 2009).

Analyzing the earthworm biomass can bring additional information about the ecological impact of an earthworm population, since the biomass gives a better illustration of factors like loosening and bioturbation of the soil. This means that earthworms of different sizes show also a different degree of ecological impact on the soil (STÄHLI et al. 1997). Although the field does not show the highest earthworm abundance both - field (2 m) and field (50 m) have the highest values for the earthworm biomass with 80.1 and 78.8 g per m². This is also remarkable since the portion of juveniles is much higher than in the flower strip. This means that even though the abundance in the field is lower than in the flower strip, the positive effect of bioturbation and soil loosening does not necessarily have to be much lower than in the flower strip, due to the high biomass in the fields.

In the study of KRÜCK (1999) an earthworm biomass of 109 g per m² was found in permanent grasslands. This value is close to the ones of STÄHLI et al. (1997) where the earthworm biomass ranged from 130 to 515 g per m² were found at several permanent grassland locations in Switzerland. The results of the present study are below these values. With 74.7 g per m² the flower strip had a lower value than the biomass ranges in the mentioned permanent grassland studies. For the field KRÜCK (1999 p. 57-60) mentioned values ranging from 16 to 75 g per m², hence the 78 and 80 g per m² were higher in in this study.

The dry climatic conditions of the trial area could be responsible for a general lower abundance and biomass in comparison to more suitable conditions on other sides. Nevertheless the discussion showed that compared to other studies the earthworm population in this study showed average values. This is also remarkable, since the weather conditions in the winter and spring time were explicitly dry this year and may have decreased the earthworm populations, while actually may higher numbers could be expected.

6. Conclusion

In conclusion this study tried to show main differences between the earthworm populations in a hedgerow, a flower strip and the adjacent field of the trial area Rutzendorf in the Marchfield region.

For the vegetation cover, due to the restriction of workload for this thesis, only a descriptive analysis was done. To get appropriate results for broader analysis, more points should have been taken. Hence, this study can only find results which are careful to interpret. The comparison of the earthworm populations of the two flower strip mixtures showed the trend of the "beneficial mixture" having a higher earthworm abundance and biomass than the mixture "spontaneous succession". This was mainly tried to be explained by the specific vegetation cover of each flower strip mixture.

The analysis of earthworm species showed only two species: *Aporrectodea caliginosa* and *Aporrectodea rosea.* For the general distinction between the treatments no differences could be found for the earthworm species. Even if other studies observed more species, these two were frequently the ones with the highest abundance, since both *A. caliginosa* and *A. rosea* are well adapted to the ecosystem. Anecic species like *L. terrestris* were not found, but at least some juveniles of the genus Lumbricus.

Further it could be shown that there is a trend over time of increasing earthworm populations for the hedgerow, the flower strip and the adjacent field at the trial area. This fact can be attributed to the existence of the flower strip, which can partly be based on the function as recovery and immigration area for earthworms. This is may also supported by the favorable conditions in the organically managed fields in terms of food resources, e.g. two-year lucerne. However, the only statistical significant difference has been found for the earthworm abundance between the hedgerow and the flower strip. The abundance was highest in the flower strip and lowest in the hedgerow. Different results were found for the biomass whereof the lowest values were found in the hedgerow but highest in the field - without significant statistical difference. The values for the abundance and biomass of this study were mostly confirmed by several other studies. The main variations to other studies were the high portions of juveniles and the high total abundance of earthworms found in the field, proportional to the attached flower strip.

Finally, the results of this thesis lead to some assumptions and suggestions. The organic cultivation of the field in combination with an ecological compensation area starts to show a slow development towards rising population of earthworms. Here the aspect of long-term persistence of the ECAs is very important for the positive development of earthworm populations. Furthermore, detailed studies for the influence of the vegetation cover on the earthworm population are recommended. For the development of higher species diversity the reduction of tillage, towards conservation tillage could be useful. Generally spoken, highly structured agro-ecosystems meaning small fields crossed by ecological compensation areas can serve as support for a resilient and sustainable system. This promotes a good development of all organisms being part of the agricultural ecosystem, the earthworm amongst others as key soil species.

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Tables and Figures

Appendix

Appendix I: Seeding mixture of the flower strip „beneficial mixture“

- *Anchusa officinalis*
- *Anthemis austriaca*
- *Astragalus onobrychis*
- *Ballota nigra*
- *Camelina microcarpa*
- *Camelina sativa*
- *Centaurea cyanus*
- *Centaurea scabiosa*
- *Centaurea stoebe*
- *Crepis rhoeadifolia*
- *Crepis setosa*
- *Daucus carota*
- *Diplotaxis tenuifolia*
- *Linaria vulgaris*
- *Medicago falcata*
- *Melilotus officinalis*
- *Onobrychis viciifolia*
- *Papaverrhoeas*
- *Picris hieracioides*
- *Rapistrum perenne*
- *Reseda luteola*
- *Saponariaofficinalis*
- *Senecio jacobaea*
- *Sisymbrium altissimum*
- *Sisymbrium loiselii*
- *Sisymbrium orientale*
- *Vaccaria hispanica*
- *Verbascum speciosum*

Appendix II: Overview of studies using different earthworm sampling methods

Study	Soil	Methods	Area	Time
FRÜND and JORDAN (2003)		– Löwensenf extra scharf 1/2 glass / 10 l – Mustard seed (Löwen-Senf milled) 100 g / 10 l – Mustard seed (Kanadischer Senf milled) 100 g / 10 l – Mustard flour (Gelbsenf milled) 60 g/10 l →60g in 500ml Plastic bottle, at least 1 hour before sampling filing up with H_2O→diluted in 9.5 l H_2O → in 3 portions → 1. vermifuge 2. hand-sorting after 15min – conserved in 3,5 % Formalin	– 50 x 50 cm (0.25 m^2) – 25 x 25 cm, spade deep (hand-sorting)	– 8./9. May2001 – 23. April 2002 – 2. June 2003
BUTT and CHAMBERLAIN (2007)	sand dune	– mustard powder 50 g/10 l – 1. hand-sorting 2. vermifuge	– 0.1 m^2, 40 cm deep (distance in transect: 50 m)	– April 2005
PELOSI et al. (2009)		– Common mustard solution (15 g/l water concentration) – AITC: first diluted with isopropanol 5 g/l, than diluted with water 0.1 g/l – prepared shortly before experiment – 3 l for each sample in 2 portions (10 min interval) – 20 min after first application →hand-sorting – conserved in 4 % formalin	– 40 x 40 cm vermifuge – 40 x 40 cm, 30 cm deep hand-sorting – 4 replicates	– May 2006 → maximum period of activity under temperate climatic conditions
LAWRENCE and BOWERS (2002)		– 50 g hot mustard powder mixed with 100 ml water – before sampling this paste is mixed in a sprinkling can with 7 l water → 1. vermifuge, sampling for 20 min 2. hand-sorting	– 42 x 42 cm (0.18 m^2) – 42 x 42 cm (0.18 m^2), 25 cm deep hand-sorting	– 24. March to 18. April
LEROY et al. (2008)		– 6 g of mustard powder mixed with 15 ml to produce a paste – before sampling mixed with 0.8 l water – Interval of 10 min (2 applications) → followed by hand-sorting	– 20 x 20 cm – 20 x 20 cm, 20 cm deep hand-sorting	– 16-17. May – 4-5. June
NUUTINEN et al. (2011)	vertic cambisol	– 60 g/10 l of water for ½ hour – no hand-sorting (because only L. terrestris was collected and wanted)	– 0.5 m^2 (diam. 0.8 m^2), 0.27 m^2 (diam. 0.6 m^2) respectively	– autumn

PFIFFNER and LUKA (2007)		– **0.33 % mustard flour solution (=100 g powder in 1 l; thereof 165 ml filed up to 5 l)** – **15 l in 40 min**	– **50 x 50 cm, 15 cm deep hand-sorting** – **distance between samples min. 15 m** – **6 samples per field**	– **after WW harvest**
EMMERLING (1995)	„schwach pseudover-gleyte Parabrauner-den“	– 1.5 % mustard solution – 10 l in 2 intervals each 20min – no hand-sorting	– 50 x 50cm (0.25m2) – 4 replicates	– May after a period of rainfall
VALCKX J., et al. (n.y.)	sandy loam soil	– Indasia™ mustard powder suspensions (0.75, 1.5, 3 and 4.5 g/l →powder mixed with 20 l water – AITC solutions (50, 100, 150 and 200 mg/l) →first diluted with isopropanol (5 g/l) than diluted with water to volume of 20l – 2 intervals each 15min – recommended: 1.application 3 g/l mustard solution (powder), 2. application 6 g/l – conserved in 5 % formalin	– (0.707 x 0.707 m^2) – 5 replicate plots	– May
ZABORSKI (2013)	drummer silty clay loam	– 5g/l AITC in Isopropanol – 4 concentrations of AITC (100, 150,200, 250 mg /l) – mixing the appropriate volume of stock solution into 10 l of water in plastic watering cans – 20 l for each sample, 10 min interval – Earthworms stored at 4 °C and then sorted, identified, counted and weighed within the next 2 days	– 76 cm×66 cm, 5 cm deep – 76 cm x 66 cm, 20 cm deep hand sort-ing	– 5-7 Novem-ber 2001
ČOJA et al.(2008)	non-calceric cambisol on fluvio-glacial sands	– 0.25, 0.5, 1.0, 2.0, and 4.0 ml of AITC dissolved in 8 ml acetone, added to 10 l water – 30 l water per sample – sampling 15min after application, in total 25 min per sample	– (50 cm x 50 cm x 20 cm)	– 2-3 May 2005

Appendix III: Basic dataset

See CD attached

Appendix IV: Statistical results

See CD attached

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die Abschlussarbeit selbstständig verfasst habe und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Alle Ausführungen die anderen Schriften wörtlich oder sinngemäß entnommen wurden sind kenntlich gemacht worden. Die Arbeit war in gleicher oder ähnlicher Fassung noch nicht Bestandteil einer Studien- oder Prüfungsleistung.

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