

**Linking earthworm activity and physical soil quality
under conventional and conservation agriculture in Central Mexico**

Antonio Castellanos Navarrete
MSc thesis Soil Quality, Wageningen University

Dr. Mirjam Pulleman

Dr. Maja Kooistra

Dr. Ron DeGoede

Prof. Lijbert Brussaard

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ABSTRACT

It is the triad of relationships between crop management, soil properties (particularly soil structure and soil organic matter content) and the activity, abundance and composition of soil biota, which determines to a great extent the functioning of agricultural soils. Inadequate management can result in physical degradation of the soil. Consequences at farm level can range from low rainwater use efficiency to crop failure. Therefore, a good physical soil quality, particularly with respect to water infiltration and retention, is essential to ensure sustainable production in the subtropical Central Highlands of Mexico. A long term trial at “El Batán” Research Station at CIMMYT (International Maize and Wheat Improvement Center) was used to test: (1) the effect of tillage (+/-) and residue (+/-) management on earthworm populations (abundance and size class) and on physical soil attributes (aggregation size and stability, time-to-pond and soil bulk density); (2) the effect of crop rotation (maize-maize or maize-wheat) in conservation agriculture (CA) systems (no-till with residues left on the surface) on earthworm populations (abundance and size class) and on physical soil attributes (aggregation size and stability, time-to-pond and soil bulk density). This was supported by qualitative micromorphological analyses of thin sections to better understand the link between earthworm activity and soil structure. Main results were: earthworms proliferate especially under no-till plots with a residue cover (189 earthworms/m²) while the other combinations of tillage and residue treatments counted on an average of 53 earthworms/m². Aggregate size and stability (measured as mean weight diameter of dry and wet-sieved aggregates, respectively) were significantly influenced by residue management, but not by tillage, and were strongly correlated with soil organic matter content. Infiltration of water (time-to-pond) was favoured by absence of tillage and presence of residues, while tillage led to an increase that was shown to be short-term only. Soil bulk density values were higher in no-till plots, and as learnt from the thin sections this is not necessarily related to detrimental forms of soil compaction, with respect to root development and activity of other soil organisms. Crop rotations did not lead to significant effects on the physical soil attributes measured.

The best management combination in terms of physical soil quality was found to be no-till with residues left on the surface. This was also the treatment that resulted in highest yields. From this study it became clear that earthworms greatly contributed to the build-up of a better physical soil status when conditions are conducive to their proliferation.

Keywords: No-tillage, Residue management, Earthworms, Soil organic matter, Soil aggregation, Aggregate stability, Water infiltration.

TABLE OF CONTENTS

Chapter 1 – General Introduction	7
1.1 The importance of soil structure in agroecosystems	7
1.2 Soil degradation	7
1.3 Physical soil quality	8
1.4 Soil structure and its link to soil fauna	9
1.5 Conservation agriculture (CA)	9
1.6 Conservation agriculture in the Central Mexican Highlands	10
1.7 Objective of the research	11
Chapter 2 – Theoretical/Scientific background	13
2.1 Soil aggregation: size distribution and stability	13
2.2 Soil porosity	15
2.3 The impact of earthworms in the soil matrix	16
2.4 Research questions and hypothesis	18
Chapter 3 – Materials and methods	21
3.1 Characterization of the “El Batán” Research Station	21
3.2 Description of the long-term trial	21
3.3 Soil texture and chemical soil properties	22
3.4 Earthworm sampling	22
3.5 Soil aggregation	23
3.6 Time-to-pond measurements	24
3.7 Soil moisture and bulk density	24
3.8 Soil micromorphology	25
3.9 Data analysis	27
Chapter 4 – Results	29
4.1 Chemical properties of soils	29
4.2 Influence of tillage and residue management on earthworm abundance and size class	30
4.3 Influence of crop rotation on earthworm abundance and size class under ZT+RES	31
4.4 Influence of tillage and residue management on physical soil quality	31
4.5 Influence of crop rotation on physical soil quality under ZT+RES	34
4.6 Soil micromorphology: qualitative observation of thin sections	36

Chapter 5 – Discussion	41
5.1 Tillage and residue management	41
5.2 Crop rotation	47
Chapter 6 – Conclusions	49
Acknowledgements	51
References	53

1.1 The importance of soil structure in agroecosystems

As Margalef (1968) elegantly stated an ecosystem can be considered as the material path followed by energy that generally enters in the form of photons and goes out into an energy sink. Margalef's concept of ecosystem can readily be applied to agroecosystems, where crop photosynthesis constitutes the entrance door of energy into the system, energy that is partially stored in the form of carbohydrates and other organic compounds in the plant biomass. A fraction of the input energy travels "downwards" to the soil system and through a diverse array of trophic levels (where generally detritus-based soil biota is considered beneficial for agricultural production while herbivorous soil organisms can imply potential damage to yields) to be finally stored in organic matter in the soil (an energy sink). Regarding physical matter, structure (e.g. soil structure) not only poses limits to the ecosystem development, but it is also a device that stores information on past and current processes (Margalef, 1968). In other words, soil structure provides a playing field for biotic factors (e.g. earthworms), a playing field of which the plasticity is not unlimited, and that on the other hand is influenced and rebuilt by soil biological activity. Soil physical properties are thus key to reach a better understanding of soil processes, the role of soil biota and thus provide information for contributing to the design of more sustainable soil management practices.

The goal of the farmer in sustainable agriculture is to maximize the entrance of solar energy, while minimizing energy losses and directing as much as possible of the energy in the system to biomass production without compromising its long-term sustainability (i.e. maintaining adequate levels of soil organic matter as well as an optimum soil structure).

1.2 Soil degradation

Physical degradation of agricultural soils is one of the major agronomic factors leading to low yields or, in extreme cases, even to crop failure, especially so in (semi)arid regions under rainfed conditions. The global assessment of human-induced degradation (GLASOD) established that damage has occurred on 15 percent of the world's total land area (13 percent light and moderate, 2 percent severe and very severe) mainly resulting from erosion, nutrient decline, salinization and physical compaction (<http://www.fao.org/wfs/final/WFSmaps/Map12-e.pdf>). In Mexico, 64 percent of the national territory presents some degree of soil degradation (SEMARNAP, 1999; cited by Fuentes, 2005).

At farm level, soil deterioration is frequently related to soil compaction where an increase in soil bulk density, a decline in the percentage and stability of aggregates and a decrease in macroporosity and pore continuity, or both, are verified. The main problems associated with the deterioration of the physical soil quality are: poor crop establishment and root development, decreased aeration and water infiltration and an increase of surface runoff and erosion (Lal and Shukla, 2004). Preventing the loss of available water or increasing the use efficiency of available water for crop production is particularly important in the case of agriculture in the subtropical Central Highlands of Mexico where rainfed agriculture is the predominant system (Lorenzo-Bautista, 1980). Moreover, rainfall is unevenly distributed over the year, with 87% of total rainfall in summer and often in the form of heavy showers (Govaerts *et al.*, 2005). Deterioration in physical soil quality, and soil structure in particular, can lead to inefficient use of rainfall and thus increase the risk of crop failure by water stress.

1.3 Physical soil quality

Soil quality can be seen as a conceptual translation of the sustainability concept towards soil. Soil quality, as sustainability, can be defined in a pragmatic way to make the concept operational and measurable. This is known as the “Hard System” (Rhoades, 2001; cited by Govaerts, 2003) where scientific research requires moving away from vague statements to more rigorous boundaries, defined by precise empirical measurements. However, it is important not to confuse such approach to soil quality with what local farmers actually see as problems, potentialities, or long-term goals (Rhoades, 2001; cited by Govaerts, 2003). Soil quality was defined, following the simple definition by Gregorich *et al.* (1994), as: “The degree of fitness of a soil for a specific use.” In addition, I followed the comparative assessment approach, frequently used in the evaluation of sustainable management systems. A comparative approach is one in which the performance of the system is determined in relation to alternatives, where the characteristics and outputs of alternative systems are compared at some time, with respect to biotic and abiotic soil system attributes (Larson and Pierce, 1994; cited by Govaerts, 2003). Regarding physical soil quality, the concept was operationalized as the fitness of physical soil attributes (size distribution of dry and wet-sieved soil aggregates expressed as mean weight diameter (MWD), rain water infiltration measured by time-to-pond, soil bulk density and micromorphological observations of thin sections) to perform functions of interest determined by land use, in this case sustainable crop production.

1.4 Soil structure and its link to soil fauna

Soil structure along with soil organic matter (SOM) is considered an important indicator of soil quality in agricultural soils (Six *et al.*, 2000; Pulleman, 2002). Soil structure formation depends on abiotic forces such as shrinking and swelling due to changes in the water status of soils and freezing and thawing, as well as biotic forces such as the activity of large biota, microbes and anthropogenic forces including tillage and traffic by humans. Soil management practices are a major force in modifying the soil structure in agricultural lands, in both formation and destruction, while biological influences have been proved to be more important with regard to soil stabilization (Oades, 1993). However, soil management does not only influence physical and chemical processes but also soil biology due to changes in soil organism communities and their habitats. It is this triad of relationships between crop management, soil properties (particularly soil structure and soil organic matter content) and the activity and composition of the soil biota community that determines to a great extent the functioning of agricultural soils. Earthworms can be particularly important since they mediate the formation of stable macroaggregates and soil porosity (Oades, 1993; Kladivko, 2001; Pulleman, 2002; Six *et al.*, 2004). Stable aggregates play an essential role in SOM protection (Six *et al.*, 2004) and resistance to soil erosion (Barthes and Roose, 2002), and soil porosity is directly related to rain water infiltration processes (Lal and Shukla, 2004).

While the importance of soil structure has received more attention when considering the effects of farm management on soil quality, soil biota and their activity have been largely overlooked. Only in the second half of the twentieth century, the role of soil biota and, particularly, of macrofauna became gradually more valued and understood. But not all farmers and scientists have ignored the importance of soil biota. Darwin dedicated a whole book to their role in 1881, called “The formation of vegetable mould, through the action of worms with observations on their habits.” An example of a traditional farming system, currently on use, that specifically considers the beneficial role of earthworms is the Totonac management in the State of Veracruz (Mexico) (Ortiz-Espejel, 1997; cited by Ortiz *et al.*, 1999) where earthworm communities are maintained systematically.

1.5 Conservation agriculture (CA)

Tillage, continuous removal of residues and monoculture have been identified as causes of soil degradation through soil structure deterioration and losses of soil organic matter and nutrients (Salinas-Garcias *et al.*, 2001; Fischer *et al.*, 2002; FAO, 2003; Lal and Shukla, 2004). Tilling the soil has been done over centuries to achieve a soil status that would favour crop growth. The mechanical disturbance is mainly done for the purpose of weed and disease control, but the

loosening of the soil structure also facilitates seed germination and short-term N mineralization. However, on the long-term, tillage can result in loss of soil organic matter (SOM) and degradation of soil structure (Lal and Shukla, 2004). Regarding residue management, the absence of a residue cover can lead to high evaporation and soil structure degradation problems at the surface resulting in impeded infiltration (due to soil crusts) and soil erosion. Besides, when the residue is incorporated into the soil profile through tillage it is readily accessible to soil biota and thus rapidly decomposed, without relevant build-up of SOM levels (FAO, 2003). Finally, monocultures have been often coupled with soil physical degradation, fertility decline as well as build-up of pests and diseases (Lal, 1997; Fischer *et al.*, 2002).

Zero tillage with residue cover has been a traditional practice in prehispanic America, including Mexico, where the *coa* (a wood stick of about half meter) was used to plant, and residues were generally left on the surface (Hernandez-Xolocotzi, 1987). In the last decades such cropping systems have been promoted under the label of conservation agriculture as an alternative management system to combat soil erosion and degradation. The two principal characteristics of CA systems are minimal soil movement and continuous soil cover, either in the form of crop residues or cover crops. Ideally, the system should include crop rotation to minimize the build-up of certain weeds, pests and diseases. Some of the benefits that CA theoretically has are: (1) increased water infiltration and reduced water run-off and soil erosion; (2) increased soil organic matter content resulting in better soil structure, higher cation exchange capacity and nutrient availability, as well as greater water-holding capacity; (3) increased biological activity in both the soil and the aerial environment; (4) reduced traction and labour, and thus savings in fuel and labour costs (Wall, 2005). However, some of the restrictions for adoption by farmers are: (1) the greater requirement for herbicides in the initial stages of CA, compared to tilled systems; (2) lack of availability or access to direct seeding technology by small farmers; and, (3) mindset and lack of knowledge and information on the specific management of CA systems (Derpsch, 2005; cited by Wall, 2005).

1.6 Conservation agriculture in the Central Mexican Highlands

Rainfed cropping predominates in the temperate sub-humid high valleys of Central Mexico. A 4-6 month wet season is followed by a dry and often frosty winter resulting in a single crop cycle per year during summer. Maize (*Zea mays L.*) monoculture is favoured in the wetter parts and wheat (*Triticum aestivum L.*) monocultures are favoured in the drier parts, although many other crops are found in smaller amounts. Maize stover is often removed from the field for animal feed as well as for ornamental uses (Fischer *et al.*, 2002; Bram Govaerts, personal communication, 2006). Nowadays there is a predominance of continuous maize monoculture wherever moisture permits, and the widespread use of mechanical tillage, at least for land preparation prior to

seeding (Fischer *et al.*, 2002). Regarding CA, promoters and users, and also researchers (Lal and Shukla, 2004; Pulleman *et al.*, 2003; Govaerts *et al.*, 2005), state that it improves soil physical conditions (i.e. soil aggregation and porosity), maintain soil organic matter levels and favours the build-up of beneficial soil biota populations, particularly earthworms, leading to a system that makes better use of rainfall water, decreases erosion, and increases productivity. CA has been recently promoted in different regions of Mexico, however two questions arise: To what extent can CA management be considered the cause of improvements in physical soil quality under the soil and climate conditions of the Central Highlands of Mexico? And what is the type and extent of link between earthworms and soil structure in this region as affected by different tillage and residue management and crop rotation?

1.7 Objective of the research

The objective of this study was to analyze the relationship between soil physical properties and earthworm abundance, size class and activity under two management systems: conventional tillage (without residues or with residues incorporated into the soil, under maize monoculture), as practiced extensively in the Highlands of Central Mexico (Salinas-Garcia *et al.*, 2001), and zero tillage (zero tillage without and with residue retention, under maize-maize or maize-wheat). The effects of crop rotations (maize monoculture vs. maize-wheat rotation) were only tested under conservation agriculture (no-till with a cover of residues). The research was done in a long-term trial at the International Maize and Wheat Improvement Center (CIMMYT, by its Spanish acronym) Research Station: “El Batán” (Central Highlands of Mexico).

2.1 Soil aggregation: size distribution and stability

From a physical point of view, the soil matrix is constituted by soil aggregates and soil pores. Such structure can be roughly described as constituted by empty spaces (soil pores) and filled spaces (aggregates), although, of course, the boundary between both dominions is diffuse in many cases (e.g. aggregates present micropores). Soil structure then refers to three aspects: (i) size distribution of aggregates and aggregate stability, (ii) porosity, pore size distribution, shape, tortuosity, continuity and stability, and (iii) spatial and temporal alteration in aggregates and pores in relation to natural (pedogenesis) and anthropogenic (management) factors (Lal and Shukla, 2004).

A precise definition of what is an aggregate is lacking due to the complex and multiple processes leading to its formation. For example, Lal and Shukla (2004) defined an aggregate as a distinct physical entity with quantifiable attributes, and exterior and interior properties. Aggregates are a subject of interest to agricultural research because, along with soil pores, they are a meaningful unit affecting many soil processes. Soil aggregates pose boundaries and conditions to physical (e.g. water infiltration, water-holding capacity, water flow in the soil, protection of soil organic matter), chemical (e.g. adsorption/desorption of chemical compounds and nutrient cycles) and biological processes (e.g. decomposition of organic matter, constitute a hierarchical array of workable habitats for soil biota in space). The complexity rest on the fact that these processes also modify soil aggregates, determining a highly dynamic and complex interaction between aggregates and soil processes. To take into account their complex nature, aggregates are divided by size into macroaggregates ($> 250 \mu\text{m}$) and microaggregates ($53\text{-}250 \mu\text{m}$). Different studies have shown that size determine different roles in soil processes (Pulleman, 2002; Six *et al.*, 2004) and relate to the dominant agents by which they are formed (Tisdall and Oades, 1982; Oades, 1984; Six *et al.*, 1999).

The aggregate hierarchy concept proposed by Tisdall and Oades (1982) contributed to a much better understanding of aggregate dynamics. This theory postulates that free primary particles and silt-sized aggregates ($< 20 \mu\text{m}$) are bound together into microaggregates by persistent binding agents (i.e. humified organic matter and polyvalent metal cation complexes), oxides and highly disordered aluminosilicates. These microaggregates, in turn, constitute macroaggregates when bound together by temporary¹ (i.e. fungal hyphae and roots) and transient² (i.e. microbial- and

¹ Binding agents that are built-up in the soil within a few weeks or months as the root system and associated hyphae grow. They persist for months or perhaps years, and are affected by management of the soil (Lal and Shukla, 2004).

² Materials that decompose very rapidly by microorganisms (Lal and Shukla, 2004).

plant-derived polysaccharides) binding agents. More recently, a modification of this concept was proposed by Oades (1984), Beare *et al.* (1992) and Six *et al.* (1999, 2000) stating that new microaggregates are formed within macroaggregates. Roots and hyphae growing inside and at the surface of aggregates hold together such structures and provide nuclei for microaggregate formation in the interior of the macroaggregate. Since roots and hyphae are temporary binding agents, they do not persist and decompose into fragments. These fragments coated with mucilages produced during decomposition become encrusted with clays resulting in the inception of a microaggregate within a macroaggregete (Oades, 1984; Six *et al.*, 2004). In most soils, soil organic matter (SOM) has found to be an essential binding agent leading to soil aggregation. Soil structure and SOM, two indicators of soil quality, are thus directly related. In addition, soil aggregation strongly determines SOM dynamics: according to most recent understanding of these interactions (1) microaggregates, rather than macroaggregates, are considered to protect SOM in the long-term (due to low accessibility to decomposers); (2) However, due to their role in facilitating microaggregate formation, macroaggregate turnover is a crucial process influencing the stabilization of SOM (Six *et al.*, 2004).

The main factors influencing soil aggregation are: (1) inorganic binding agents; (2) soil microorganisms; (3) roots; (4) soil fauna; (5) environmental variables (cited by Six *et al.*, 2004); and (6) soil management (Lal and Shukla, 2004). In this research the focus is on soil management and soil macrofauna.

Tillage produces important changes in soil aggregation and breaks up compaction but only locally and temporarily. On the long-term, tillage leads to plough pan formation and soil crusting and thus further tillage is necessary. In addition, SOM is distributed more uniformly in the whole soil profile facilitating its decomposition by microbial activity and diminishing the chances of build-up (FAO, 2003). In comparison with ploughing, no-till management systems supposedly count on more stable aggregates and greater soil organic carbon contents (Filho *et al.*, 2002; cited by Bronick and Lal, 2005).

Regarding mulches, in general, the proportion of macroaggregates could be increased by addition of decomposable organic materials (Oades, 1984). The presence of mulches on the soil surface might protect surface aggregates from raindrop impact and thus increase aggregate stability (Layton *et al.*, 1993; cited by Bronick and Lal, 2005).

Crop rotations could also be found beneficial to soil aggregation if they provide greater biomass levels for permanent residue cover (FAO, 2003) and in the form of roots when compared to monocultures. Diversity of organic matter inputs might also favour aggregation agents. The effect of different crops could then reflect the crop chemical composition (Martens, 2000; cited by

Bronick and Lal, 2005), rooting structure and ability to alter the chemical and biological properties of the soils (Chan and Heenan, 1996).

Understanding aggregate dynamics through aggregate size alone (obtained through dry sieving) could be equated to predict the winner of a race just through looking at his/her picture. Because of this, aggregate size distribution is complemented with a measure of aggregate stability (obtained through wet sieving), which refers to the ability of aggregates to withstand the disruptive forces of rapid wetting (Lal and Shukla, 2004). This helps to understand if management practices are contributing to the build-up and slow turnover or collapse and fast turnover of soil aggregates and associated soil organic matter (Six *et al.*, 1999).

2.2 Soil porosity

Water is an essential resource in rainfed agriculture, especially when its amount or temporal distribution is limiting, like in the Central Highlands of Mexico, lowering yields in many cases. Soil degradation processes are directly linked to soil porosity and reduce the fraction of water reaching the crops' roots. Increasing, (i) the efficiency of rain water infiltration into the soil (and thus reducing loss of water by runoff), (ii) the capacity of a soil to retain water, and, (iii), diminishing the physical barriers to root development, are key in mitigating low yields caused by water stress (FAO, 2003).

Physical degradation of the soil can occur at two levels, at the surface through the formation of soil crusts as well as within the soil profile due to soil compaction by heavy traffic or mechanical soil disturbance. Soil crusts are formed by: (i) the direct impact of raindrops that break surface soil aggregates (FAO, 2003); and (ii) the spontaneous slaking³ or breakdown of soil aggregates during rapid wetting (Lal and Shukla, 2004). In both cases, the break-down of aggregates into small particles leads to clogging of the surface pores and the formation of surface seals, reducing the hydraulic conductivity of the crust layer (Lal and Shukla, 2004). This problem is further exacerbated in arid and semi-arid regions due to the rapid drying of soil (Carnes, 1934; Isiumov, 1938; Hillel, 1959, 1960; Taylor, 1962; Williams, 1963; Heinonen, 1965; Cary and Evans, 1974; Miller and Gifford, 1974; Prihar, 1974; Gupta and Yadav, 1978; Hoogmoed, 1980; cited by Awadhwal and Thierstein, 1985).

Soil compaction is the mechanical compression of a mass of soil into a smaller volume and deformation resulting in decrease in total porosity (and macroporosity) (Lal and Shukla, 2004). Consequences of soil compaction are a reduction in water transmission. Yield reductions can also

³ Slaking is a term used to describe the initial fragmentation of soil aggregates several millimeters in diameter, which may disintegrate further to become microaggregates (Lal and Shukla, 2004).

be directly explained by restricted germination or root development due to soil compaction. In fact, in many occasions compaction is occurring only at a certain depth of the soil profile (e.g. plough pan) but this can be severe enough to cause poor crop development (FAO, 2003).

Reduced tillage can result in greater volumes of macropores and biochannels that influence water movement and availability (Logan *et al.*, 1991; Warkenin, 2001; cited by Bronick and Lal, 2005). Pores in no-till systems are theoretically more interconnected due to their biological origin, leading to adequate infiltration rates during the whole growing season (FAO, 2003).

A residue cover might absorb most of the energy of the raindrops impact. Its ability to disintegrate soil aggregates and detach fine particles would be hence greatly reduced. Consequently, there would be little or no clogging of surface soil pores by detached particles, and thus soil crust formation would be impeded (FAO, 2003). In addition, residues can increase surface roughness enhancing water ponding and thus water infiltration (Gilley and Kottwitz, 1994; cited by Findeling *et al.*, 2003), as well as reduce evaporation of water from the surface (Lal and Shukla, 2004). The organic matter input is also key in enhancing water infiltration and retention in the soil (Rao *et al.*, 1998; Scopel *et al.*, 1998; Valentin and Bresson, 1992; Zachmann and Linden, 1989; cited by Findeling *et al.*, 2003). Moreover, residue cover is expected to have a beneficial impact on soil porosity due to enhanced soil faunal activity.

The effects of crop rotation on surface soil structure could be dependent on organic matter inputs above the soil. If crop rotations ensure greater biomass availability for permanent residue cover (FAO, 2003) compared to monocultures, they will have a beneficial impact compared on soil porosity due to increased soil aggregation and protection of the surface structure. The canopy closure may also play a role on surface porosity (Farres and Muchena, 1996).

2.3 The impact of earthworms in the soil matrix

Extensive research has been done on the impact of earthworms on soil structure. These organisms burrow through the soil, producing large pores that are important for water flow and retention, aeration, and root development. They help mix organic materials into the soil and aid in aggregate formation (Kladivko, 2001).

Generally, studies have shown that earthworm populations are almost always higher under no-till than under conventional practices (Wardle, 1995; cited by Kladivko, 2001). Numerous examples from research plots (Barnes and Ellis, 1979; Edwards and Lofty, 1982; Mackay and Kladivko, 1985; House and Parmelee, 1985; cited by Kladivko, 2001) and producers' fields support the conclusion that reduction of tillage intensity encourages earthworm populations (Kladivko,

2001). Exceptions, where earthworms proliferate better under tilled soils when compared to no-till treatments, have been documented in a review by Chan (2001).

Earthworms, and thus their impact, are altered by management practices. Tillage is supposed to negatively affect earthworm populations by direct abrasion and by destroying the porous networks. Residue management also has an impact of primary order on earthworms since the presence of residue mulch is not only a source of food supply but can flatten temperature and moisture fluctuations daily and seasonally. This could lengthen the active periods of earthworms (Kladivko, 2001). Regarding crop rotations, information is limited and scattered (Edwards, 1983; Lavelle *et al.*, 1992; cited by Bhadauria *et al.*, 1997). Crop rotations could favour worm populations due to greater diversity of residue quality and if crop rotations lead to greater organic inputs to the soil (both above in the form of a residue cover and below in the form of roots).

Earthworms are classified into three functional groups (Bouche, 1977; cited by Chan, 2001), depending on their habitats and their feeding habits:

1. Epigeic earthworms: species that live above the mineral soil surface, typically in the litter layers. They tend to have relatively high reproductive rates and grow rapidly.
2. Anecic earthworms: species that live in burrows in mineral soil layers but come to the surface to feed on dead leaves which they drag into their burrows; some make burrows that extend deep into the subsoil.
3. Endogeic earthworms: species that inhabit mineral soil horizons, feeding on soil more or less enriched with organic matters.

In addition, Blanchart *et al.* (1999) classified tropical earthworms in two main groups based on their effect on physical properties: (1) compacting species, and (2) decompacting species. In their research, they found compacting species and decompacting species to be generally large and small species, respectively. However, their effects were also dependent on soil organic matter content.

These two types of earthworms have opposite effects on physical soil properties and their simultaneous presence permits the conservation of a dynamic structure. Large sized species can egest very large casts, aggregates, that are relatively compact and can lead to the formation of a surface crust when organic matter is absent. In addition, these worms tend to decrease total soil porosity, decreasing the infiltration rate and improving the water retention capacity of soil. Small sized earthworms could have generally an opposite effect. They produce smaller casts (0.5 – 2 mm), increasing the turn-over of large sized aggregates and limiting the development of a crusted surface horizon (Blanchart *et al.*, 1999).

2.4 Research questions and hypotheses

Research questions followed by hypotheses are the following:

1. What is the influence of tillage and residue management on earthworm abundance and size class?
 - i. Hypothesis: tillage decreases earthworms populations due to direct physical disruption, lack of food on the soil surface and greater variations in soil temperature and moisture (Kladivko, 2001). Greatest number of earthworms is expected in no-till plots with a residue cover. Regarding size classes, Kladivko (2001) states that the impact of tillage is scale dependent with larger organisms being more sensitive. It is expected then that the percentage of large earthworms is greatest in no-till plots with a cover of residues present.
2. What is the influence of maize-maize vs. maize-wheat rotation on earthworm abundance and size class under conservation agriculture (no-till with a cover of residues)?
 - i. Hypothesis: plots under maize-wheat favour earthworm numbers due to a different input of residue (i.e. differing food quality and may be greater biomass returns to the soil). Crop rotation increases diversity of residue inputs but does not necessarily lead to changes on size classes percentage, since size classes do not divide earthworms based on feeding characteristics.
3. What is the influence of tillage and residue management on physical soil quality (size distribution of aggregates, stability of aggregates, time-to-pond and soil bulk density)?
 - i. Hypothesis: soil aggregate size and stability as well as infiltration decrease with tillage (due to the disruption of existing aggregates and loss of connected bioporosity) and increase when residues are present on the surface (their presence avoids the collapse of aggregates at the soil surface and residues are a source of stabilizing organic matter). Soil bulk density values are greater in no-till plots due to lack of mechanical disruption, and when no residue cover is present due to lower presence of organic matter. The density of organic matter is lower than the mineral soil and this implies that lower organic matter content results in greater soil density.
4. What is the influence of maize-maize vs. maize-wheat rotation on physical soil quality (size distribution of aggregates, stability of aggregates, time-to-pond and soil bulk density) under conservation agriculture (no-till with residues)?
 - i. Hypothesis: soil aggregate size and stability as well as time-to-pond increase with maize-wheat rotation compared to maize monoculture due to the greater earthworm populations. Similarly, soil bulk density values are

hypothesized to decrease under maize-wheat systems due to higher earthworm activity.

5. What is the qualitative impact of earthworm activity on soil structure micromorphology under different tillage and residue management?
 - i. Hypothesis: greater proportion of biogenic structure is found in no-till with residues due to the positive and complementary effect of no-tillage and residue cover on earthworm populations as well as on physical soil attributes.

3.1 Characterization of the “El Batán” Research Station

The research station is situated in the semi-arid, subtropical highlands of Central Mexico. The station has a mean annual temperature of 14 °C and an average annual rainfall of 600 mm per year, with about 520 mm falling between May and October (calculated over 1990-2001). Short, intense rain showers followed by dry spells typify the rainy season and evapotranspiration exceeds rainfall throughout the year (total amount of yearly potential evapotranspiration is 1900 mm). The El Batán experimental station has an average growing period (FAO, 1978; cited by Goaverts *et al.*, 2005) of 152 days. The research station is located in the area of the former lake of Texcoco. Soil is a *fine, mixed, thermic, Cumulic Haplustoll* in the USDA soil taxonomy system (Soil Survey Staff, 1998; cited by Govaerts *et al.*, 2005) with a clayey loam texture. The major limitations are periodical drought, periodical water excess and wind- and water- erosion.

3.2 Description of the long-term trial

The trial consists of two separate experiments with a split strip design: (1) an experiment to compare the effects of different tillage (+/-) and residue (+/-) management practices; and, (2) to compare the effects of crop rotation (maize-maize = MM and maize-wheat = MW) within zero tilled plots with residue cover. Three internal replicates have been laid out randomly for the first experiment, and seven for the second experiment, although only a random selection of three plot replicates was sampled for this study (**Figure 1**).

The treatments were: (1) conventional tillage, residues removed and maize (*Zea mays* L.) monoculture (MM/CT-RES); (2) conventional tillage, residues incorporated and maize monoculture (MM/CT+RES); (3) zero tillage, residues removed and maize monoculture (MM/ZT-RES); (4) zero tillage, residues left as a cover, and maize monoculture (MM/ZT+RES); and, (5) zero tillage, residues left as a cover, and maize-wheat (*Triticum aestivum* L.) rotation (MW/ZT+RES). The 5th treatment includes both D and E strips. Maize and wheat were parallel in their stage of growth. Tillage was done with a disc plough until a depth of approximately 15 cm. The plots of the B strip (MM/ZT-RES and partially MM/ZT+RES) were managed with minimum tillage until 2003. In 2004 and 2005 the strip has been under ZT.

3.3 Soil texture and chemical soil properties

Composite soil samples consisting of five cores per plot were taken to soil depths of 0-5 cm, 5-15 cm, and 15-30 cm, on the harvest rows. Sampling was done in October, two weeks before harvest. Samples were air-dried and crushed to pass through a 2 mm sieve. Analysis for soil texture, pH, soil organic matter (SOM) and nutrients, Ca²⁺ and Mg²⁺ was done.

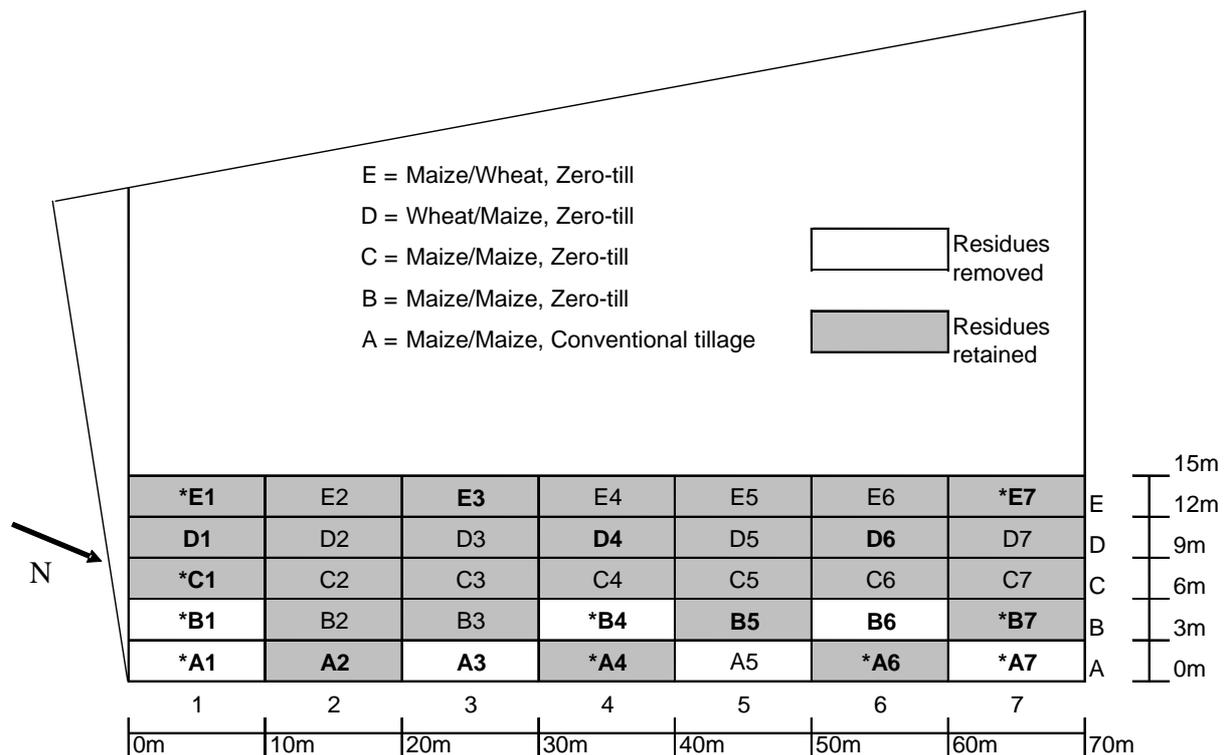


Figure 1. Trial map within “El Batán” Research Station. Bold letters indicate the plots used for earthworm sampling and an asterix indicates plots used for thin sections sampling.

3.4 Earthworm sampling

Earthworm sampling was based on the TSBF methodology (Anderson and Ingram, 1993): six replicate monoliths (30 x 30 wide and 30 cm deep) were extracted per management treatment (three replicate plots and two internal replicates per plot, one within the harvest rows and another outside the harvest area). Monoliths were taken at least one meter from the plot border. The monolith was then subdivided in two depths layers (0-15 and 15-30 cm). The soil was hand sorted and earthworms collected, killed in 70 percent ethanol and preserved in diluted formol (10

percent). After 48 hours, earthworms were then transferred to flasks containing 50 percent ethanol (Lijbert Brussaard, personal communication, 2005).

Earthworm length was measured to divide individuals into two size classes: ≤ 7 mm and > 7 mm. Earthworms were divided into size classes to find a relationship between size and compacting/decompacting behavior. Blanchart *et al.* (1999) did not mention an exact size distribution, they were more focused on a set of worm species. This tentative division in size classes was undertaken as an attempt to simplify a complex scenario, and hopefully provide a tool for rapid assessment of possible relationships between earthworm populations and physical features.

3.5 Soil aggregation

Monoliths extracted, as described in the previous section, were subdivided in two depth layers (0-15 and 15-30 cm). Coarse plant residues and roots were removed and soil samples were gently broken up along natural planes of weakness into natural aggregates, passed through an 8 mm sieve, air-dried at room temperature and thoroughly mixed. The two internal replicate soil samples were mixed taking into account depth (0-15 and 15-30 cm) constituting a composite sample that was used for dry and wet sieving. Dry sieving is a methodology developed to simulate aggregate resistance to wind erosion, while wet sieving is done to simulate erosion by water and assess stability to quick wetting/slaking (Lal and Shukla, 2004). To determine dry aggregate distribution, a sub-sample of 500 g was separated by shaking the samples through a stack of six sieves with openings of 6.3, 4, 2, 1 mm, and then 500 and 250 μm . They were shaken for 5 minutes at a frequency of 210 cycles/min. Soil remaining on each sieve was collected and weighed (Kemper and Chepil, 1965; Limon-Ortega *et al.*, 2002; cited by Govaerts *et al.*, 2006).

To determine water-stable aggregate size distribution, 50 g of air-dried soil was separated into water-stable aggregate fractions. The samples were submerged in demineralised water and immediately sieved over a sieve with a mesh opening of 2 mm according to the method of Elliot (1986). The sieves were gently moved up and down 50 times for two minutes. Then the suspension with the soil material that passed the 2 mm sieve was transferred to a basin holding the 250 μm sieve and the procedure was repeated. Similarly the procedure was repeated for the 53 μm sieves. All size fractions were oven dried at 75 °C and weighed. The < 53 μm fraction was calculated by difference.

The aggregate size distribution after dry and wet sieving was expressed as the mean weight diameter (MWD_{ds} and MWD_{ws} , respectively) (van Bavel, 1949; cited by Pulleman, 2002). The

MWD of soil samples was calculated as the sum of the proportion of each size fraction's weight multiplied by the mean diameter of that fraction:

$$\text{MWD} = \sum_{i=1}^n \langle d \rangle_i w_i$$

where $\langle d \rangle$ is the mean diameter of each size fraction (i), w is the proportion of total sample weight (w) occurring in the size fraction (i) and n is the number of size fractions.

Macroaggregate stability after wet sieving was also presented as percentage of large macroaggregates (2-8 mm) and small macroaggregates (250 μm – 2 mm) because earthworms were expected to be dominant agents producing aggregates larger than 2 mm.

3.6 Time-to-pond measurements

Direct surface infiltration (time-to-pond) was determined with a metallic ring made out of wire placed on the ground (296 cm²), but not impeding water to flow out of this area. The area was watered using a watering pot with outflow of 0.15 l/s from a height of 50 cm. The shower was stopped when water began to run out of the area marked by the ring. The time-to-pond and the amount of water added onto the soil were measured twice, once during the crop cycle (early September), and once just after tillage (mid January). Six points per plot within the harvest rows and three replicate plots were measured. This methodology was developed by Govaerts *et al.* (2006).

3.7 Soil moisture and bulk density

Two samples per stratum of each plot at three depths (5 cm, 15 cm and 25 cm) were collected using a metal ring of known volume (100 cm³) and a diameter of 5 cm. Soil was oven-dried for 48h at 105 °C, weighed and moisture was calculated. Bulk density was calculated according to Blake and Hartge (1986; cited by Govaerts *et al.*, 2006). Moisture was measured at the same time monoliths for earthworm sampling were extracted to relate it to the depth distribution of earthworms to the moisture profile at the time of sampling.

3.8 Soil micromorphology

Two samples per treatment (tillage, residue and rotation) were collected. In each of the selected plots (**Table 1**) undisturbed samples were taken from the wall of one of the 30 cm deep pits that remained after extraction of the monolith for earthworm sampling. Three 8 x 6 cm metal tins were cut vertically into the soil at a depth of 0-8, 7-15 and 18-26 cm depth. The sample location was 10-20 cm from the stem of the maize plant, in the direction of the furrow (row spacing = 75 cm). Samples were taken during the growing season between 20 and 29 September 2005. Additional samples were taken on November 1st in the plots B7 and C1.

The selected samples were carefully air-dried at stable room temperature and impregnated with a colourless unsaturated polyester resin. After evaporation of the largest part of the acetone from this solution, samples were hardened by gamma radiation. The thin sections, with a thickness of 25 µm, were made from the core of the hardened block, to avoid disturbances as much as possible. The thin sections were prepared after the procedure developed by Jongerius and Heintzberger (1975; Murphy 1986; cited by Kooistra, 1991). The method of hardening with gamma radiation is mentioned in Bisdom and Schoonderbeek (1983; cited by Kooistra, 1991). In Kooistra (1990, 1991) overviews are given of the procedures followed to reconstruct the processes required for the study of the geogenesis, soil development and human impact using micromorphology.

Microscopic observations of thin sections were made using polarized light and normal light at a magnification of 25-100x and the microstructure was described qualitatively following the terminology of Bullock *et al.* (1985; cited by Pulleman *et al.*, 2005).

Features were divided in groundmass, organic particles and voids (**Figure 2**). Groundmass was divided then into biogenic and physicogenic. Biogenic structures are characterized by a stable, granular to subangular blocky macroaggregation (Pulleman *et al.*, 2005). Physicogenic structure on the other hand consists of mainly relatively large, angular blocky peds (Pulleman *et al.*, 2005). Biogenic structures were further divided into macrofauna and mesofauna-worked groundmass, based on their size. Worm-worked groundmass could be identified to a certain extent due to its microstructural aspect, such as spherical and ellipsoidal shaped faecal pellets, often consisting of a core of coarse silt and clay mixed with organic matter. As the groundmass was identical for all the profiles, one description was given of this material. Physicogenic features were divided into pure and slaked material. Organic particles were classified as intact roots or residues. Finally, voids were divided into physicogenic and biogenic. Physicogenic voids were divided into pure (e.g. cracks) or modified (by biotic and abiotic parameters) and biogenic voids with further division in macro and mesovoids. Macrovoids were then classified as clay-coated voids or voids without clay coating present on their walls. To conclude, infillings were also studied. Infillings

Table 1. Sampling details for the thin section study. The sample code refers to the sampled plot as well as the soil layer. Samples were archived at the International Soil Reference and Information Center (ISRIC).

	Sample code	ISRIC Archive nr	Tillage treatments	Residue treatment	Rotation	Depth (cm)	Replicate
1	A1-1 (0-8 cm)	05080	Conventional	-	Maize-Maize	0-8	1
2	A1-1 (7-15 cm)	05081				7-15	
3	A1-1 (18-26 cm)	05082				18-26	
4	A7-1 (0-8 cm)	05089	Conventional	-	Maize-Maize	0-8	2
5	A7-1 (7-15 cm)	05090				7-15	
6	A7-1 (18-26 cm)	05091				18-26	
7	A4-1 (0-8 cm)	05083	Conventional	+	Maize-Maize	0-8	1
8	A4-1 (7-15 cm)	05084				7-15	
9	A4-1 (18-26 cm)	05085				18-26	
10	A6-1 (0-8 cm)	05086	Conventional	+	Maize-Maize	0-8	2
11	A6-1 (7-15 cm)	05087				7-15	
12	A6-1 (18-26 cm)	05088				18-26	
13	B1-2 (0-8 cm)	05092	Zero-till	-	Maize-Maize	0-8	1
14	B1-2 (7-15 cm)	05093				7-15	
15	B1-2 (18-26 cm)	05094				18-26	
16	B4-1 (0-8 cm)	05095	Zero-till	-	Maize-Maize	0-8	2
17	B4-1 (7-15 cm)	05096				7-15	
18	B4-1 (18-26 cm)	05097				18-26	
28	B7-1 (0-8 cm)	05104	Zero-till	+	Maize-Maize	0-8	2
29	B7-1 (7-15 cm)	05105				7-15	
30	B7-1 (18-26 cm)	05106				18-26	
25	C1-1 (0-8 cm)	05107	Zero-till	+	Maize-Maize	0-8	1
26	C1-1 (7-15 cm)	05108				7-15	
27	C1-1 (18-26 cm)	05109				18-26	
19	E1-1 (0-8 cm)	05098	Zero-till	+	Maize-Wheat	0-8	1
20	E1-1 (7-15 cm)	05099				7-15	
21	E1-1 (18-26 cm)	05100				18-26	
22	E7-1 (0-8 cm)	05101	Zero-till	+	Maize-Wheat	0-8	2
23	E7-1 (7-15 cm)	05102				7-15	
24	E7-1 (18-26 cm)	05103				18-26	

are structures that correspond to any type of void produced by the soil fauna, including modified ones that have been partially infilled with shaped units. In some cases they can be infilled by soil material, shapeless excreta or directly during animal passage (Kooistra and Brussaard, 1995). In this study, an infilling was classified as having less than 15 percent of empty space (otherwise it was considered as a pore) and less than 85 percent of solid material (in that case it would be classified as groundmass).

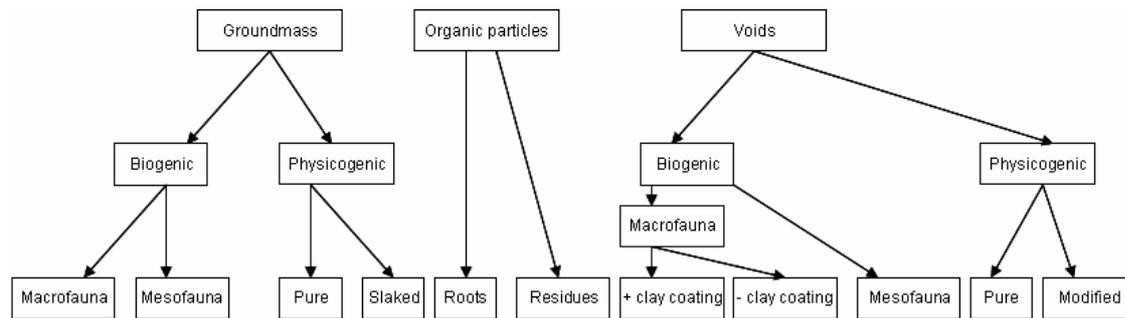


Figure 2. Classification of physical features used for the micromorphology observation of thin sections.

3.9 Data analysis

Data were analysed using the SPSS package. Mean comparisons were performed with ANOVAs and with Mann-Whitney test for non-parametric data. Non-parametric data were: earthworm total abundance (number/ m²) and time-to-pond during the crop cycle (s). Attempts to normalize non-parametric data were unsuccessful. Significance of differences between treatment means was examined by an LSD range test procedure at the 5 percent level of significance. Correlations were used to evaluate relationships between different soil parameters using both Pearson's (for parametric data) and Spearman's (for non parametric data).

4.1 Chemical properties of soils

Soil texture analysis confirmed that no significant differences exist across the area of the two trials (35.1 % clay, 38.6 % sand and 26.3 % silt). Most of the soil chemical parameters measured did not differ significantly by treatments either, except for soil organic matter (SOM) and N, which was closely related to SOM content (data not shown). Zero till with residue cover (ZT+RES) had 2.1 percent of SOM in the 0-15 cm depth and that constituted the greatest percentage (**Table 2**). This treatment was also characterized by a marked decrease of SOM by depth. In the case of crop rotations (**Table 3**), SOM was similar under maize-maize and maize-wheat, and both values were significantly greater in the top 15 cm.

Table 2. Results of chemical analyses by depth and treatment. Values followed by same letters are not significantly different between the fields; an asterisk indicates a significant difference with depth ($p < 0.05$). The standard error is given between parentheses. Treatments are: conventional tillage without residue incorporation (CT-RES), conventional tillage with residue incorporation (CT+RES), zero tillage without residue cover (ZT-RES), and zero tillage with residue cover.

Depth	Treatment	Soil Organic Matter	pH (1:2)	Ca ²⁺	Mg ²⁺
Cm		%	H ₂ O	ppm	Ppm
0-15	CT-RES	1.6 (0.14) ^a	6.98 (0.08) ^a	1650 (0.05) ^a	639 (0.03) ^a
	CT+RES	1.7 (0.04) ^a	6.47 (0.13) ^a	1602 (0.05) ^a	636 (0.02) ^a
	ZT-RES	1.8 (0.13) ^a	6.80 (0.28) ^a	1549 (0.05) ^a	640 (0.02) ^a
	ZT+RES	2.1 (0.06) ^{b*}	6.44 (0.10) ^{a*}	1739 (0.04) ^{a*}	650 (0.01) ^a
15-30	CT-RES	1.5 (0.12) ^a	7.27 (0.09) ^a	1899 (0.06) ^a	728 (0.03) ^a
	CT+RES	1.5 (0.08) ^a	7.00 (0.12) ^a	1891 (0.11) ^a	729 (0.04) ^a
	ZT-RES	1.5 (0.14) ^a	7.20 (0.15) ^a	1805 (0.04) ^a	729 (0.02) ^a
	ZT+RES	1.4 (0.06) ^a	7.00 (0.10) ^a	2095 (0.08) ^a	766 (0.03) ^a

Table 3. Results of chemical analyses by depth and treatment. Values followed by same letters are not significantly different between the fields; an asterisk indicates a significant difference with depth ($p < 0.05$). The standard error is given between parentheses. Treatments are: continuous maize (MM) and maize-wheat rotation (MW).

Depth	Treatment	Soil Organic Matter	pH (1:2)	Ca ²⁺	Mg ²⁺
Cm		%	H ₂ O	ppm	Ppm
0-15	MM	2.0 (0.10) ^{a*}	6.3 (0.00) ^a	1707 (0.02) ^a	637 (0.02) ^a
	MW	2.2 (0.05) ^{a*}	6.5 (0.11) ^{a*}	1755 (0.05) ^{a*}	657 (0.02) ^a
15-30	MM	1.3 (0.10) ^a	6.8 (0.20) ^a	2301 (0.14) ^a	735 (0.05) ^a
	MW	1.5 (0.06) ^a	7.1 (0.10) ^a	2127 (0.11) ^a	782 (0.05) ^a

4.2 Influence of tillage and residue management on earthworm abundance and size class

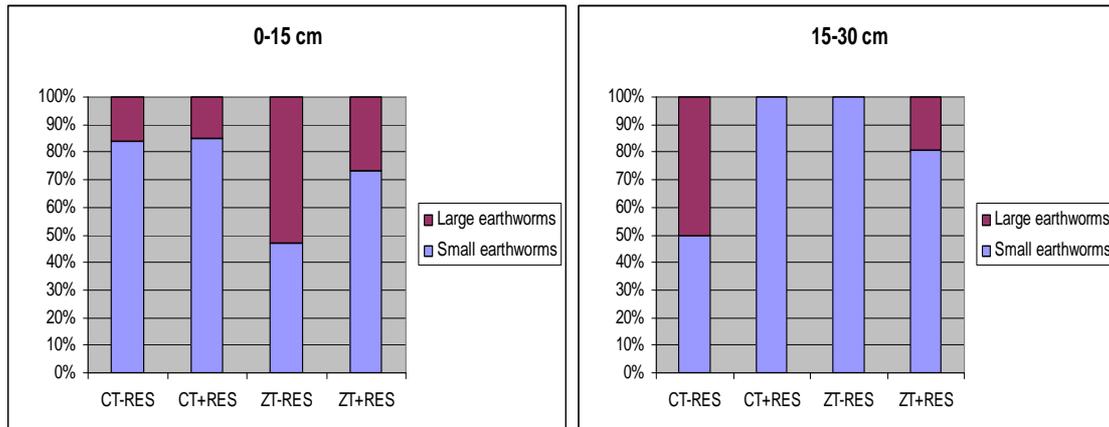
Regarding total values by treatment, total earthworm abundance was not significantly different between CT-RES (49 earthworms/m²), CT+RES (55 earthworms/m²) and ZT-RES (56 earthworms/m²). These three treatments constitute a cluster clearly different from the ZT+RES treatment that showed an average of 189 earthworms/m². As hypothesized, ZT+RES was the treatment more conducive to earthworm proliferation. In **Table 4**, abundance of earthworm by treatment and depth are detailed.

Table 4. Earthworm abundance (no./m²) per depth layer and treatment. Values followed by same letters are not significantly different between the fields; an asterix indicates a significant difference with depth ($p < 0.05$). The standard error is given between parentheses. Treatments are: conventional tillage without residue (CT-RES); conventional tillage with residue (CT+RES); zero tillage without residue cover (ZT-RES); and, zero tillage with residue cover (ZT+RES).

Depth	Treatment	Abundance
Cm		No./m ²
0-15	CT-RES	45 (11.60) ^{a*}
	CT+RES	52(4.06) ^{a*}
	ZT-RES	53 (47.07) ^{a*}
	ZT+RES	165 (24.06) ^{b*}
15-30	CT-RES	4 (4.17) ^a
	CT+RES	2 (2.17) ^a
	ZT-RES	3 (3.03) ^a
	ZT+RES	25 (5.26) ^b

Regarding percentages of earthworms in each size class, CT-RES and CT+RES had 82 and 87 percent small earthworms. Small earthworms constituted 69 and 74 percent of total earthworms in ZT-RES and ZT+RES respectively. Large-sized earthworms were comparatively more numerous (not statistically significant) in no-till plots (31 percent and 26 percent, respectively). Differences in percentages by depth and treatment are detailed in **Figures 3 and 4**.

For the whole sampled soil (0- 30 cm), an average of 52 earthworms per square meter were found in conventionally tilled plots. No-till plots had a significantly ($p < 0.05$) greater amount of earthworms, an average of 156 earthworms per square meter were found in such treatment. Tillage effects also proved to be significant both for the 0-15 and the 15-30 cm soil layer. Differences in size were not statistically significant, although there was a trend in which greater percentage of large earthworms was found in no-till plots.



Figures 3 and 4. Percentage of earthworms by size class (small, ≤ 7 mm, and large, > 7 mm) against the different treatments at the 0-15 cm (left) and at 15-30 cm depth (right). Treatments are: conventional tillage without residue (CT-RES); conventional tillage with residue (CT+RES); zero tillage without residue cover (ZT-RES); and, zero tillage with residue cover (ZT+RES).

Residue management was also found to lead to significant differences in total number of earthworms: 53 earthworms/m² in plots without residue and 155 earthworms/m² in plots where residue was incorporated or present as a cover. Significant differences were maintained in the topsoil but not in the 15-30 cm soil layer. For the whole soil profile, identical percentages were found in size classes (77 percent of small earthworms and 23 percent of large earthworms).

4.3 Influence of crop rotation on earthworm abundance and size class under ZT+RES

Earthworm abundance (no./m²) was found to be 155 for maize monoculture (MM) and 206 for maize-wheat (MW). 84 percent were small worms in maize monoculture and the remaining 16 percent were big-sized worms. In the case of MW, 69 percent were small and 31 were greater than 7 mm. However, crop rotation did not lead to significant differences in any case.

4.4 Influence of tillage and residue management on physical soil quality

Results of factorial ANOVA analysis of dry sieving data (expressed as MWD) showed that for the whole soil profile, there was a significant main effect of residue management on the mean weight diameter ($p < 0.05$) where plots with incorporation or presence of a residue cover had higher MWD_{ds} values (i.e. the mean size of aggregates was higher). This is only evident in the 0-15 cm soil layer (**Table 5**). Tillage management did not significantly affect MWD_{ds}.

Table 5. Mean weight diameter (MWD) after dry and wet sieving followed by percent of reduction under the different treatments. Values followed by same letters are not significantly different between the fields; an asterix indicates a significant difference with depth ($p < 0.05$). The standard error is given between parentheses. Treatments are: conventional tillage without residue incorporation (CT-RES), conventional tillage with residue incorporation (CT+RES), zero tillage without residue cover (ZT-RES), and zero tillage with residue cover.

Depth	Treatment	MWD		Reduction of MWD
		Dry sieving	Wet sieving	
cm		mm	mm	%
0-15	CT-RES	1.6 (0.18) ^a	0.6 (0.05) ^a	62 (7.24) ^a
	CT+RES	2.2 (0.18) ^{ab}	0.7 (0.07) ^{ab}	69 (2.36) ^a
	ZT-RES	1.6 (0.13) ^a	0.6 (0.02) ^a	57 (6.61) ^a
	ZT+RES	2.4 (0.12) ^b	1.0 (0.05) ^{b*}	60 (1.67) ^a
15-30	CT-RES	2.1 (0.40) ^a	0.6 (0.05) ^a	67 (6.46) ^a
	CT+RES	2.0 (0.08) ^a	0.6 (0.06) ^a	69 (2.49) ^a
	ZT-RES	2.3 (0.11) ^a	0.6 (0.07) ^a	75 (3.53) ^a
	ZT+RES	2.3 (0.09) ^a	0.7 (0.06) ^a	69 (2.50) ^a

However, there was a significant interaction effect between residue management and depth ($F(1,28) = 8.64, p < 0.05$). MWD_{ds} was lower in the top 15 cm in plots without residue incorporated (1.6 mm) when compared to the same plots at a deeper layer (15-30) (2.2 mm). Differences were not consistent in the case of residue present.

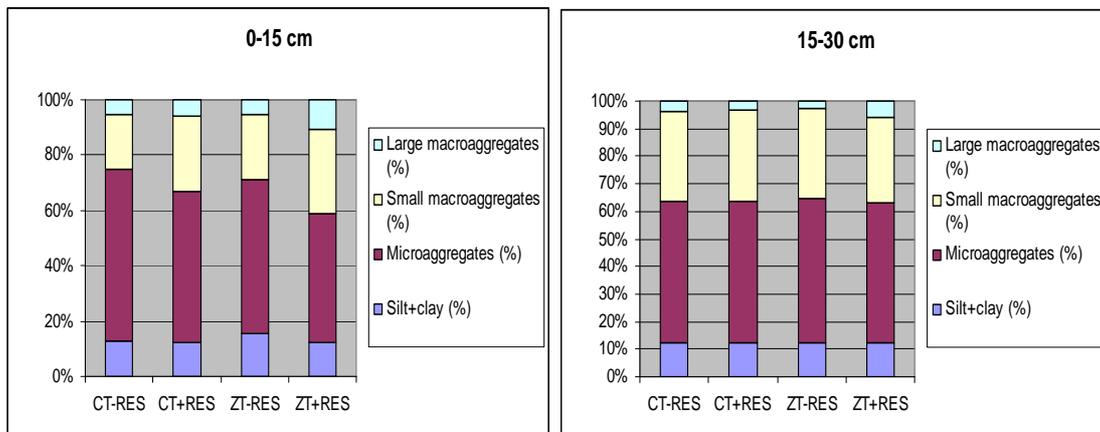
The mean weight diameter of wet-sieved samples (MWD_{ws}) has been used in research as a measure of soil stability. For the whole soil profile, MWD_{ws} was 0.8 mm in plots with residue (composed of both CT+RES and ZT+RES) and differed significantly from plots without residue (MWD_{ws} was 0.6 mm) (composed of both CT-RES and ZT-RES). Tillage management effect was not significant in this case. There were significant differences by depth only in the case of ZT+RES.

While wet sieving is useful to understand aggregate stability, the reduction of MWD (%) between dry and wet sieving serves to understand the actual extent of management impact under field conditions. Depth was the only factor that led to significant differences ($F(1, 28) = 7.84, p < 0.05$). The fact that there were no differences by management factor indicates that aggregates were not significantly different in their stability with regard to residue and tillage management.

The percentage of soil material present as large macroaggregates (2-8 mm) differed when we consider the interaction of both residue and tillage management. ZT+RES plots had significantly greater percentage of these large aggregates when compared to ZT-RES plots, 8.4 and 3.9 percent respectively (**Figures 5 and 6**). Under conventional tillage large macroaggregates amounted for 4.7 percent for CT-RES and 4.6 percent for CT+RES, although significant differences with

ZT+RES nor ZT-RES were found. The proportion of large macroaggregates was found to decrease with depth ($F(1, 28) = 8.69, p < 0.05$) in all cases.

Contrary to large macroaggregates, the proportion of small macroaggregates increased with depth. For this type of aggregates, interaction of residue management and depth implied significant differences ($F(1, 28) = 4.99, p < 0.05$), where less macroaggregates were found in samples obtained from the top layer of plots without residue compared to samples from plots with and without residue from the 15-30 cm depth.

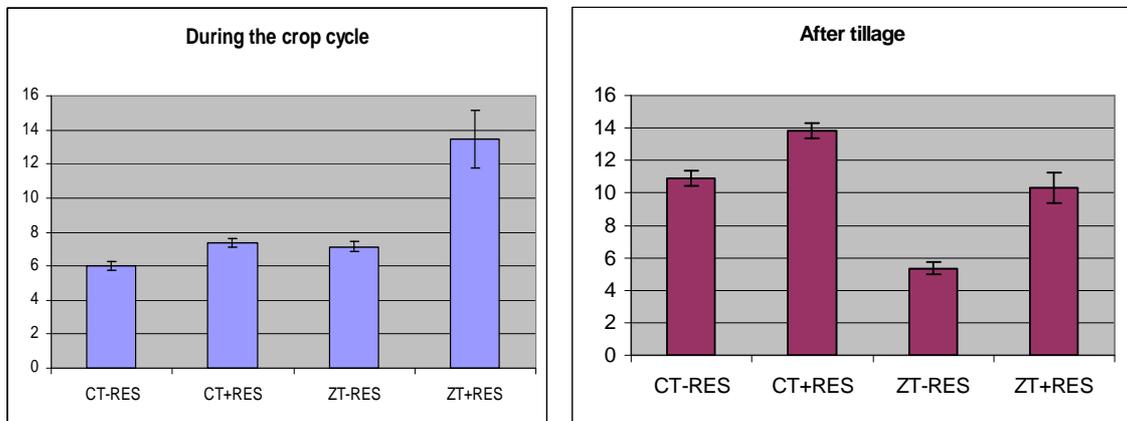


Figures 5 and 6. Aggregate size distribution against the different treatments at the 0-15 cm (left) and at 15-30 cm depth (right). Aggregates sizes are: silt + clay ($<53 \mu\text{m}$), microaggregates ($53\text{-}250 \mu\text{m}$), small macroaggregates ($250 \mu\text{m} - 2 \text{mm}$) and large macroaggregates ($2 - 8 \text{mm}$). Treatments are: conventional tillage without residue (CT-RES); conventional tillage with residue (CT+RES); zero tillage without residue cover (ZT-RES); and, zero tillage with residue cover (ZT+RES).

Microaggregates ($53\text{-}250 \mu\text{m}$) differed depending on the type of residue management ($F(1, 28) = 6.69, p < 0.05$). The proportion increased from 49.8 percent in plots with retained residue to 55.6 percent in residue-free plots. Tillage management was close to being significant ($p = 0.07$) with considerably greater proportion of microaggregates in tilled plots.

For the time-to-pond (s) measurements taken during the crop cycle, both the effects of tillage and residue management were found to be significant. Conventional tilled plots had an average time-to-pond of 6.7 s while no-till plots had 11.9 s. In the case of residue, average infiltration rate was found to be 6.6 s in plots without residue and 12.0 s in plots with residue. Similar to the previous values, time-to-pond after tillage was significantly different under the different tillage and residue practices. Time-to-pond in conventional tilled plots was on average 12.4 seconds while zero tilled

plots had a mean of 9.1 s. Residue free plots had a time-to-pond rate on average of 8.2 s compared to the 11.2 s of plots with residue (**Figures 7 and 8**).



Figures 7 and 8. Time-to-pond (s) measured during the crop cycle (left) and after tillage (right) under the different treatments. Treatments are: conventional tillage without residue (CT-RES); conventional tillage with residue (CT+RES); zero tillage without residue cover (ZT-RES); and, zero tillage with residue cover (ZT+RES).

A Post Hoc Test showed that soil bulk density values (g/cm^3) differed significantly by depth ($p < 0.001$) with the highest value in the intermediate soil layer (1.45 g/cm^3 ; 15 cm depth) and no significant differences between topsoil (1.38 g/cm^3 ; 5 cm depth) and the deepest layer (both 1.38 g/cm^3 ; 25 cm depth). For the whole soil profile (0-30 cm), the interaction of residue and tillage management was found to be significant ($F(1, 42) = 4.26, p < 0.05$). Both CT-RES and ZT-RES treatments were significantly lower in bulk density than ZT+RES ($p < 0.05$). Irrespective of tillage, bulk density was also significantly higher when residue was present ($F(1, 42) = 6.89, p < 0.05$). For results by depth layer, see **Table 6**.

4.5 Influence of crop rotation on physical soil quality under ZT+RES

MWD of dry aggregates in the top 15 cm was significantly greater under MW, compared to MM (2.1 mm under maize monoculture and 2.5 mm under maize-wheat rotation) (**Table 7**). In the 15-30 cm soil layer, MWD_{ds} in MM and MW were equivalent (2.3 mm). No depth or interaction effects were found.

Mean weight diameter of wet-sieved samples under MW was found to be 0.9 mm, while the value under MM was 0.8 mm. Significant differences in MWD_{ws} were only found with depth: aggregate stability decreased significantly with depth in the case of MW.

Table 6. Soil bulk density (g/cm^3) measured at three different depths (5, 15 and 25 cm). Values followed by same letters are not significantly different between the fields; an asterisk indicates a significant difference with the following soil layer ($p < 0.05$). The standard error is given between parentheses. Treatments are: conventional tillage without residue incorporation (CT-RES), conventional tillage with residue incorporation (CT+RES), zero tillage without residue cover (ZT-RES), and zero tillage with residue cover.

Depth	Treatment	Soil bulk density
cm		g/cm^3
5	CT-RES	1.38 (0.04) ^{ab}
	CT+RES	1.33 (0.04) ^{ab}
	ZT-RES	1.31 (0.04) ^a
	ZT+RES	1.42 (0.01) ^b
15	CT-RES	1.43 (0.01) ^{a*}
	CT+RES	1.46 (0.05) ^a
	ZT-RES	1.41 (0.05) ^a
	ZT+RES	1.48 (0.04) ^a
25	CT-RES	1.31 (0.01) ^a
	CT+RES	1.36 (0.00) ^{ab}
	ZT-RES	1.36 (0.03) ^{ab}
	ZT+RES	1.41 (0.02) ^b

No differences were found between maize monoculture and maize-wheat rotation with regard to percentage of MWD reduction.

For aggregate size distribution of wet-sieved samples, the only difference found in the case of small aggregates was found in MM plots with lower percentage in the topsoil (28 percent) than in the 15-30 cm soil layer (35 percent) (data not shown). Macroaggregates (2-8 mm) differed by depth when MM and MW plots were clustered together. The topsoil had a percentage of 11 against a 6 percent deeper in the soil.

Table 7. Mean weight diameter (MWD) after dry and wet sieving followed by percent of reduction under the different treatments. Values followed by same letters are not significantly different between the fields; an asterisk indicates a significant difference with depth ($p < 0.05$). The standard error is given between parentheses. Treatments are: maize-maize (MM) and maize-wheat rotation (MW).

Depth	Treatment	MWD		Reduction of MWD
		Dry sieving	Wet sieving	
cm		mm	Mm	%
0-15	MM	2.1 (0.22) ^a	0.9 (0.13) ^a	59 (0.04) ^a
	MW	2.5 (0.09) ^b	1.0 (0.05) ^{a*}	61 (0.02) ^a
15-30	MM	2.3 (0.16) ^a	0.7 (0.04) ^a	68 (0.01) ^a
	MW	2.3 (0.12) ^a	0.7 (0.09) ^a	69 (0.04) ^a

During the crop cycle, the rate of direct surface infiltration in seconds was 8.3 s in plots under maize monoculture, while in plots under maize-wheat rotation was 16.1 s ($p < 0.05$). Data measured in mid-January (after plants were harvested) were not different between MM (8.3 s) and MW (11.4 s).

For soil bulk density values, no differences between MM and MW rotation were found.

4.6 Soil micromorphology: qualitative observation of thin sections

The groundmass material of all plots was rather uniform. It has a clayey loam texture and contains fine silt (which is partly weathered), large minerals (angular, fresh to slightly altered) and small amounts of rocks (up to 10 mm in diameter). Many minerals and most rock fragments are of volcanic origin. There is no sedimentary layering visible. A general overview on physical features follows, while for more detailed information, **Table 8** can be consulted.

Soil surfaces were slaked in both conventional treatments (**Picture 1**), although one of the conventional plots with residue incorporated had a loose and open structure with increased soil faunal activity. The topsoil in zero till treatments was generally much more biogenic and loose, except for plots where no residue cover was present. In that case, a slaked layer was present but alleviated by some meso- and macrofauna activity. Up to 14 cm depth, conventional plots had a less structured profile characterized by tilled groundmass consisting of mechanically reworked soil and with remnants of compacted material (generally up to 10 mm in diameter). Zero tilled plots without residue (B strip of the trial) had some degree of soil compaction diminished to a certain extent by burrowing and casting by soil biota. The rest of the zero tilled treatments had a more diverse and complex structure with a greater biogenic character and, as in the previous case, some degree of soil compaction modified by biological activity.

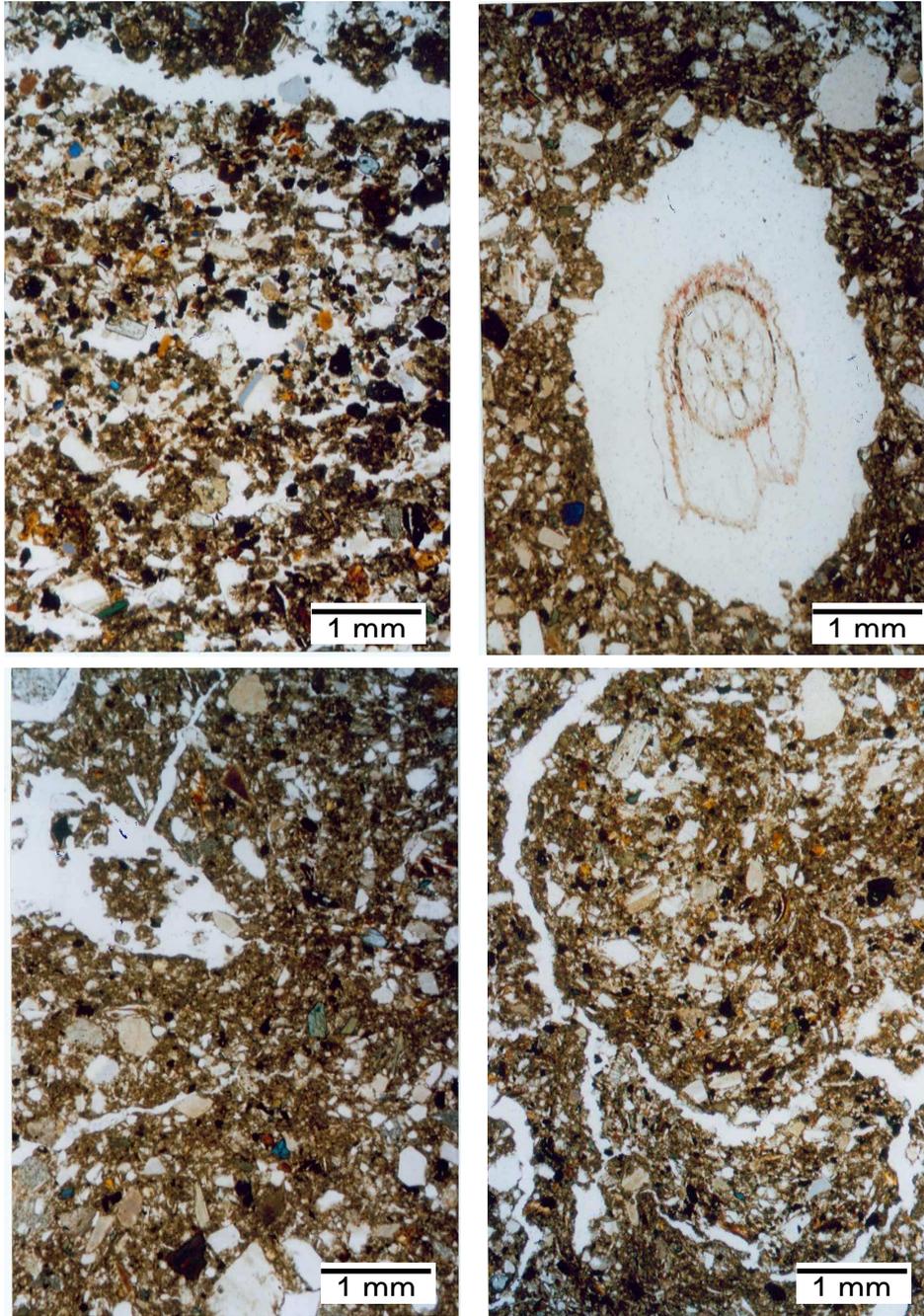
At 15 cm depth, a clear compacted layer with pressure-induced cracks (plough pan) was present in conventionally tilled treatments, partially reworked by soil fauna when residue was incorporated. In zero tilled plots and under maize monoculture, a discontinuous plough pan was present (penetrated by soil macrofauna and loosened, with voids infilled by worm casts and soil aggregates). In the MW/ZT+RES treatment, there was not any trace of a compacted layer. The deepest section (18-26 cm) was found to have a subangular blocky structure, generally weak, with casts of 1-2 mm in diameter, in conventional plots. No-till plots were characterized by an extremely biogenic structure (except for weak and loose structure found in MM/ZT-RES). In conventional plots, faunal activity was found to be more visible in deeper parts (below 10 cm), both through pores and casts. In conventional plots without residue incorporation most of the pores were root voids or irregular voids (planar voids/cracks), and some small worm channels (up

Table 8. Description of the qualitative analysis of the soil thin sections. On the first column, treatments are found with: conventional tillage without residue incorporation and continuous maize (MM/CT-RES), conventional tillage with residue incorporation and continuous maize (MM/CT+RES), zero tillage without residue cover and continuous maize (MM/ZT-RES), zero tillage with residue cover and continuous maize (MM/ZT+RES), and, zero tillage with residue cover and maize-wheat rotation (MW/ZT+RES). Plots are included between parentheses. In the second column, the depth in cm of the described physical features is indicated. Ø stands for diameter. (NB. Descriptions provided by M. Kooistra and M. Pulleman).

	Depth	Qualitative description
MM/CT-RES (A1-1/A1-7)	0-2 cm	Slaked material, probably caused by traffic, reworked by soil fauna (A1-1).
	2-14 cm	2-6 cm. Tilled groundmass consisting of mechanically reworked soil and with remnants of compacted material (up to 10 mm Ø). 7-14 cm. Few visible organic fragments and soil material somewhat looser with infillings containing worm casts. Porosity mainly based on irregular voids (tillage and root voids).
	15 cm	Compact layer with pressure-induced cracks (plough pan). In A7-1 particularly reworked by soil meso- and macrofauna.
	18-26 cm	Subangular blocky structure (weakly developed in A1-1 and well developed in A7-1 with cracks and 2-3 cm Ø peds). Macrofauna casts and fauna channels partly filled with mesofauna excrements and used by roots.
MM/CT+RES (A4-1/A6-1)	0-2 cm	A4-1 presents a surface crust while A6-1 has a loose and open structure due to decomposing roots and residues that attract soil biota.
	2-14 cm	Tilled groundmass constituted by mechanically reworked soil with fragments of slaked material (up to 10 mm Ø). More worm channels than in MM/CT-RES, but many burrows disturbed by tillage.
	15 cm	Plough pan but less distinct than in MM/CT-RES and modified by meso-, macrofauna and roots.
	18-26 cm	Weak and loose subangular blocky structure with casts of 1-2 mm Ø. Many pores of earthworms (<i>circa</i> 1 mm Ø).
MM/ZT-RES (B1-2/B4-1)	0-2 cm	Slaked layer on the surface but less developed than in MM/CT-RES. Some washed excrements of earthworms and mesofauna.
	2-14 cm	2-6 cm. In B1-2, a compact layer with some pressure-induced cracks. In B4-1, a loose mixture of biogenic soil and mechanically reworked groundmass with remnants of compacted material. Many modified and infilled small voids by mesofauna, around rounded cores (worm casts and tillage aggregates) of compacted soil material, varying between 5 and 15 mm Ø. 7-14 cm. Areas between rounded cores of compacted soil material are more open packed. More meso- and macrofauna activity in B1-2.
	15 cm	Plough pan reworked by soil biota. Some large tillage induced voids have been loosely infilled by worm casts and soil aggregates.
	18-26 cm	Mesofauna activity became more common, as well as earthworms' channels (some with a clay coating) and casts. Voids are often infilled, generally with excrements.

MM/ZT+RES (B7-1/C1-1)	0-2 cm	B7-1 counts on a completely biogenic crumb structure consisting of many worm casts, enchytraeid cast and organic excrements of larvae. C1-1 has a layered surface crust constituted by degraded worm casts, slaked soil material and enriched with organic residues and microaggregates. In C1-1, surface casts are of greater size, reaching up to 3 mm Ø and containing many microaggregates of <i>circa</i> 200 µm Ø. Besides between 1 and 2 cm depth, there is a visible layer made of worm casts and elongated worm channels (either open or filled with cast material).
	2-14 cm	Loose and highly biogenic structure. Big worm channels sized 2-5 mm Ø that in B7-1 are mainly filled with excrements. In C1-1 some isolated remnants of compacted soil and after 11 cm depth, both abundant welded and fresh excrements.
	15 cm	Compacted layer, possibly remnant of a plough pan, but less densely compacted and discontinuous due to soil biological activity.
	18-26 cm	Extremely biogenic structure with larger macrofauna activity. Casts of 1-2 mm Ø, with low quantity of organic matter, and wide worm channels (up to 4 mm Ø) are prevalent. In C1-1, channels are filled with welded and compacted worm casts. Organic rich parts of casts are reingested by mesofauna, including enchytraeids, and their excrements from loosely infilled areas between compacted worm cast zones.
	0-2 cm	Topsoil constituted mainly by excrements, fragmented organic residues, biological channels and packing voids between the casts.
MW/ZT+RES (E1--1/E7-1)	2-14 cm	2-5 cm. Pore system with vertical and horizontal pores up to 4 mm Ø.
		5-9 cm. Compacted layer with pressure-induced cracks reworked by both earthworms and roots. Worm casts with diameter of 2 to 4 mm are found. Many voids loosely infilled with mesofauna excrements.
		9-13 cm. Less compacted soil. Earthworm channels (some with a clay coating) and casts are common, with casts reingested by mesofauna and voids partially filled by their excrements.
	13-26 cm	Extremely biogenic structure with many earthworm channels (up to 4 mm wide) and chambers (up to 2 cm wide). Voids are commonly modified by mesofauna and infilled with loosely accumulated small excrements and some rare soil aggregates.

to 500 µm in diameter). In conventional plots where residue was incorporated, more irregular voids were modified by macrofauna activity (up to 1 mm in diameter). Although meso- and macrofauna were evident along the whole soil profile, in MM/ZT-RES as in the previous case, biological activity increased by depth. In MM/ZT+RES as well as MW/ZT+RES, earthworm channels and worm casts were evident along the whole soil profile. In MM/ZT+RES, channels and casts of greater diameter were found in deeper parts. However, in MW/ZT+RES big sized casts and channels were found even close to the surface, and there was a clear pore system, both vertical and horizontal. More worm channels were found to have a clay coating when compared to conventional and MM/ZT-RES plots.



Picture 1. Examples of physical features found: layer of slaked soil due to traffic under wet conditions at 1.5-2 cm depth in MM/CT-RES (top left); root in worm channel at 25 cm depth in MM/CT-RES (top right); plough pan being reworked by faunal activity at 14 cm depth in MM/ZT+RES (bottom left); worm worked soil at 3 cm depth in MM/ZT+RES (bottom right). Treatments are conventional tillage without residue incorporation and continuous maize (MM/CT-RES), conventional tillage with residue incorporation and continuous maize (MM/CT+RES), zero tillage without residue cover and continuous maize (MM/ZT-RES), zero tillage with residue cover and continuous maize (MM/ZT+RES), and, zero tillage with residue cover and maize-wheat rotation (MW/ZT+RES).

5.1 Tillage and residue management

Results confirmed the hypothesis that tillage significantly reduces earthworm abundance (no./m²). This was verified for the whole soil profile as well as for each soil layer. This reduction in number of earthworms in tilled plots is probably explained by the direct physical damage caused by the tillage implement and due to drastic changes in their habitats (Kladivko, 2001). Lee (1985) indicates that the reconstruction of pore networks could reduce the energy available for reproductive activities and thus further reduce earthworm populations. The absence of tillage below 15 cm depth explains why in the 18-26 cm soil layer a subangular blocky structure, typically present when biotic influence predominates, and more traces of earthworm activity were found in conventional tilled treatments without and with residue (CT-RES and CT+RES). In no-till plots with residue cover, earthworm casts and pores were clearly evident in the 0-15 cm soil layer. However, in zero-till treatments with residue cover and maize monoculture the traces of earthworm activity were less evident than under maize-wheat rotations. This is probably related to the fact minimum tillage was performed at least six seasons (until 2003 inclusive) and illustrates the extent and the legacy of the impact that even minimum tillage can have on earthworm activity and soil structure. The previous disturbance of the B strip due to minimum tillage was not known when the research was started but became clear when studying the thin sections. Artificial fragments of slaked or compacted and reworked soil material were clearly visible.

The effect of residue management on earthworm numbers also proved to be significant, both for the whole soil profile (0-30 cm) and for the topsoil (0-15 cm). Residues have often been identified as a key factor for earthworm proliferation. They do not only constitute a source of food supply for these organisms but also reduce temperature and moisture fluctuations, both daily and seasonally, expanding their active periods (Kladivko, 2001). In fact, adequate moisture conditions are essential since these organisms do not have the ability to maintain constant internal water content, and temperature ranges also determine their viability (Chan, 2001).

When the interaction of tillage and residue was considered, earthworms were significantly more numerous in zero till plots with residue cover (ZT+RES) than in the rest of treatments, as hypothesized. It was thus the combination of both absence of tillage and presence of a residue cover that constituted the most beneficial environment for earthworm proliferation. When one of these two conditions was missing, earthworm abundance was greatly reduced. In fact, no differences were found between conventional tillage without or with residue (CT-RES and CT+RES), and zero till without residue (ZT-RES).

The differences in earthworm abundance found by depth were not necessarily expected for the whole active period. Different species of earthworms respond differently to changing regimes of temperature and soil water content and migrate up and down the soil profile throughout the season (Gerard, 1967; cited by Chan, 2001). There was no clear correlation of moisture data (not shown) and earthworm distribution on the soil profile but this is probably explained by the fact that their distribution depends also highly on soil temperature (not measured), as well as on management.

There were no significant differences between earthworm populations based on size classes under the different treatments, but a greater percentage of large earthworms was found in no-till treatments. Large earthworms (Blanchart *et al.*, 1999) have been found to contribute to the formation and stability of large aggregates through large casts. This could be related to the greater percentage of large macroaggregates (2-8 mm) in no-till treatments in case residues were retained. The micromorphological observation of thin sections further showed that more biopores and pores with a clay coating were present in no-till plots. Pores with a clay coating are typically made by large anecic worms (Maja Kooistra, personal communication, 2006). This could indicate that tillage impact is scale dependent. Wardle (1995; cited by Kladivko, 2001) summarized impact of tillage on different soil organisms and found that larger organisms were more negatively affected. In the specific case of earthworms, Wyss *et al.* (1992) found that ploughing reduced the number of large-bodied earthworms but small-bodied species survived well. In this case, no distinction between juveniles and adults was taken into account. More research on earthworms' size distribution and other morphological and ecological characteristics is needed to ascertain if ploughing is scale dependent.

Aggregate size was measured by dry sieving while water stable aggregate size distribution was measured by wet sieving. The fact that average mean weight diameters of both dry-sieved (MWD_{ds}) and wet-sieved samples (MWD_{ws}) were found to be greater in plots with residue retention, as hypothesized, is not surprising. It has been frequently reported that residue retention as well as organic matter inputs increase the size and stability of aggregates (Kushwaha *et al.*, 2001). Residues are a source of soil organic matter (SOM) to the soil and of primary importance for aggregate formation and stability. In this study this is supported by two facts: (1) SOM levels were significantly greater in topsoils of plots where residue was left on the surface; (2) a trend was observable in which greater SOM levels led to greater MWD_{ds} values (Pearson's; $p = 0.05$); and (3) a highly significant (Pearson's; $p < 0.01$) and positive correlation ($r^2 = 0.39$) was found between MWD_{ws} and SOM. Soil organic matter accounted for 38.6 percent of the variability in mean weight diameter of wet-sieved soil samples. Part of the aggregate size variation and stability, and therefore, the dry and wet sieving aggregation indices can thus be attributed to

variations in SOM. When fresh residues enter the soil, they become sites for microbial activity and nuclei for aggregation (Puget *et al.*, 1995; Jastrow, 1996; cited by Six *et al.*, 1999).

Parallel to MWD_{ds} and MWD_{ws} , average earthworm abundance was greater (66 percent) in plots with residue when compared to plots without residue retention. Earthworms have been recognized as a key factor in the formation and stability of soil aggregates. In this case, they were found to play a role in aggregate stability: a significant positive correlation (Spearman's; $r^2 = 0.19$; $p < 0.01$) was found between earthworm abundance and MWD_{ws} and earthworms explained 19.2 percent of the unexplained variance of MWD_{ws} . However, both this and SOM percentage (substrate that provide energy for soil biota) indicates that other organisms are probably playing a role in aggregation. Earthworms increase aggregate size and stability by burrowing and cast formation. During burrowing a pressure is exerted on the surrounding soil and external mucus is deposited on the burrow walls (Edwards and Bohlen, 1996; cited by Six *et al.*, 2004). Regarding casts, earthworms ingest organic matter, mix it with inorganic soil material, pass the mixture through their gut and excrete it as a cast (Six *et al.*, 2004). Although at first those casts are highly dispersible, when dried and aged they become more stable than physical soil aggregates (Blanchart *et al.*, 1999; Six *et al.*, 2004).

Contrary to expectations, tillage did not significantly affect aggregate size and its stability. However, in the 0-15 cm soil layer of ZT+RES plots, both MWD_{ds} and MWD_{ws} were greater than in CT-RES. Under conservation agriculture (ZT+RES), SOM tends to be concentrated on the surface due to the presence of a residue cover and enters gradually into the soil through the action of soil biota, while in conventional systems SOM is more uniformly distributed along the soil profile (Castro Filho *et al.*, 2002). In fact, in the thin sections of ZT+RES fragments of organic residues were more frequent between the depths 0 to 6 cm than in the rest of the treatments. In addition, SOM levels were greater in the 0-15 cm soil layer of plots under the treatment ZT+RES when compared to the rest. Lack of tillage also avoids the disruption of existing soil aggregates, exposing less organic matter to microbial attack (Beare *et al.*, 1994; cited by Kushwaha *et al.*, 2001; Six *et al.*, 1999; Six *et al.*, 2000). The consequence is higher SOM available as a cementing agent and this further enhances soil aggregation. This explains the predominantly biogenic and stable structure in soils under conservation agriculture (ZT+RES). CT-RES constituted a highly unfavourable situation due to lack of organic inputs and thus of SOM build-up, due to the disruption of aggregates with the consequent mineralization of available SOM. At micromorphological level, these are low-structured soils with a partially reworked tilled groundmass and remnants of compacted material. Besides, earthworm abundance was highest in ZT+RES due to the lack of tillage and presence of a residue cover. Kladivko (2001) states that when these organisms are present in sufficient numbers, the impact on soil properties and processes becomes significant. Their presence and activity partially explained the higher aggregate size and stability found in conservation agriculture plots.

Differences in SOM levels in the 15-30 cm soil layer were not found to be significant between treatments. The lower level of SOM input in the 15-30 cm soil layer, plus the low impact of tillage and other disruptive forces below 15 cm depth, probably explain why there were no significant differences in aggregation between the treatments at this depth level.

Regarding aggregate size distribution data of wet-sieved samples, a positive and significant correlation was found between 2-8 mm macroaggregates and SOM (Spearman's; $r^2 = 0.35$; $p < 0.001$), while no correlation was found for smaller macroaggregates (250 μm – 2mm) and a significant negative correlation was found for microaggregates (Pearson's; $r^2 = 0.14$; $p < 0.05$). Increasing cultivation leads to a loss of SOM-rich macroaggregates and an increase of SOM-depleted microaggregates (Six *et al.*, 2000). In fact, macroaggregate size and stability decreased with tillage and absence of residues. Similar results have been found by Kushwaha *et al.* (2001).

The 0-15 cm soil layer under ZT+RES resulted in the highest macroaggregate size and stability whereas those parameters were strongly reduced in ZT-RES to a level comparable to CT soil (+/- residues). This indicates again how important residues are, especially under ZT, to attain higher soil organic matter levels and stable aggregation. The proportion of large macroaggregates was also lower with depth, where SOM levels are lower. Levels of large macroaggregation for CT+RES and CT-RES were intermediate and not significantly different from no-till plots, although closer to ZT-RES. The increase in macroaggregation was parallel to the increase in earthworm abundance (Spearman's; $r^2 = 0.40$; $p < 0.01$). Earthworms probably contributed to increase the proportion of large macroaggregates (> 2 mm).

The percentage of MWD reduction between dry and wet-sieved samples was found to differ by depth. This can be explained by the fact that aggregates in 15-30 cm soil layer are less exposed to aggregate disrupting forces, although they are less stable (due to lower SOM levels). However, it is important to stress that the percentage of MWD reduction did not differ by management system indicating that overall stability was not significantly different by treatment. In other words, when aggregates are disrupted by wet sieving, the reduction in aggregate mean diameter is similar in all cases. However, the mean diameter of stable aggregates was found to be higher in the top 15 cm of ZT+RES plots when compared to the other treatments.

Results on time-to-pond denoted significantly greater water infiltration in no-till plots when compared to conventionally tilled plots, as long as residue is present. In fact, Bronick and Lal (2005) state that reducing tillage increases the volume of macropores and biochannels and thus influence water infiltration. Macropores have been found to conduct as much as 80 percent of water percolating through the soil profile (Lal and Shukla, 2004). But pore connectivity is essential. No-till systems generally result in greater pore connectivity (FAO, 2003). The potential

positive impact of earthworms on soil porosity due to their burrowing activities and thus on water infiltration is supported by the significant positive correlation found between time-to-pond values during the crop cycle and total earthworm abundance (Spearman's; $r^2 = 0.35$; $p < 0.001$). However, results also confirm that residue retention in ZT plots is an absolute requirement to increase the infiltration capacity of the soil, whereas ZT-RES seriously impedes infiltration. Residue presence prevents runoff, absorbs most of the energy of the raindrops (McGarry *et al.*, 2000; FAO, 2003), ensures a more gradual wetting of soil aggregates, and enhances soil aggregation, diminishing the risk of soil crust formation. In fact, Awadhwai and Thierstein (1985) state that enhancing the stability of soil aggregates is key to the development of better techniques for reducing soil crusting and thus favouring water infiltration. In addition, residues increase surface roughness enhancing water ponding and thus water infiltration (Gilley and Kottwitz, 1994; cited by Findeling *et al.*, 2003). Residue protection of the surface is particularly important in the Central Highlands of Mexico due to the commonly high-energy storm rainfalls that can cause rapid surface sealing, runoff and soil erosion.

The previous explanations for increased water infiltration in no-till plots or when residue is present are supported by the soil thin section analysis. CT-RES and CT+RES were characterized by the presence of slaked material on their surfaces as well as a soil crust, although one of the two samples in CT+RES had a loose and open structure due to decomposing roots and residues. ZT+RES resulted in a completely biogenic crumb structure and its surface was characterized by high levels of macrofauna activity (enhanced porosity and high casting). Differing from the stated hypothesis, ZT-RES had an intermediate status between CT+RES and ZT+RES, but results for the ZT-RES were not conclusive due to serious legacy effects of past minimum tillage operations.

The pattern of time-to-pond as affected by management was greatly modified right after tillage. As in the previous case, infiltration increased when residue was present, but after tillage surface water infiltration significantly increased in conventional tillage plots compared to the zero-till plots. Tillage is known to increase macroporosity, water retention and thus water infiltration (Franzluebbers, 2002; Lal and Shukla, 2004). However, the differences in time-to-pond during the crop cycle and after tillage, suggest that this effect is reversed during the growing season. A study by Mapa *et al.* (1986; cited by Osunbitan *et al.*, 2005) showed that increased total porosity in conventionally tilled plots is usually temporary as persistent actions of rainfall on the soil result in compaction. Further and more frequent measurements during the season are needed to determine when changes in infiltration on the different treatments take place in relation to the rainfall pattern.

Besides porosity, water passage through a bed of stable aggregates is higher due to lower levels of fine material occluding pores (Mbagwu and Auerswald, 1999). MWD of wet-sieved

aggregates was found to be highly positively correlated ($p < 0.01$) with time-to-pond during the crop cycle. But it was the percentage of large macroaggregates that was particularly positively correlated with time-to-pond (Spearman's; $r^2 = 0.32$; $p < 0.01$) where plots with greater macroaggregate proportion had greater infiltration. Higher levels of aggregation, and particular of macroaggregation, improve water infiltration. Although the direct effects of residue cover on water infiltration on one hand and earthworm activity and aggregation on the other may explain the fore mentioned correlation without necessarily indicating a casual relationship. Such comparison was not made with time-to-pond after tillage since soil aggregation was measured before the tillage event.

As expected, bulk density values were greater in no-till plots, but contrary as hypothesized, values were greater when residue was present. Research by Genter and Blake (1978; cited by Kushwaha *et al.* (2001), Kushawaha *et al.* (2001) and Osunbitan *et al.* (2005) arrived to similar results and reached the conclusion that elimination of soil mechanical loosening caused by tillage operations is responsible for the increase in soil bulk density. Regarding the effect of residue and in the case of ZT+RES, increased soil moisture levels under conservation agriculture might result in a soil status with greater risks of compaction when traffic occurs under wet conditions (Pulleman *et al.*, 2003). However, more research is needed on this aspect.

Increased "hardness" has been identified as a key factor for farmers to abandon of no-till systems (Ken Sayre, personal communication, 2006). Soil bulk density, or weight (mass) of soil per unit volume, is generally used to measure soil porosity and detect soil compaction. The problem lies in the fact that soil bulk density is an indicator of total pore volume, a rather limited information taking into account that the size and distribution of pores have been proved to be important to retention and conduction of fluids in and through the soil (Lal and Shukla, 2004; Osunbitan *et al.*, 2005). In fact, the increase at 15 cm depth in bulk density of both conventionally managed plots and zero-till plots had different meanings as became clear from the micromorphological study of thin sections. In the case of conventionally managed plots, such increase was related to the existence of a plough pan that could restrict both root development and water infiltration (less developed in CT-RES). In ZT-RES a compacted layer reworked by soil biota was visible, while in ZT+RES such layer was more discontinuous. Plots under ZT+RES and maize-wheat rotation were characterized by greater biogenic structure and some degree of compaction. Thus in the case of no-till, the increase in soil bulk density was more related to packed biogenic structures without necessarily harmful soil compaction. Osunbitan *et al.* (2005) found a weak relationship between water conductivity and bulk density and concluded that total pore volume was not the major determinant of saturated hydraulic conductivity in soils. Soil bulk density measures are therefore a rather limited measure of soil compaction since two very similar values can constitute very different situations from a structural point of view. This indicator thus seems to loose some of its

predictive power to indicate the existence of harmful levels of soil compaction under conservation agriculture.

5.2 Crop rotation

Crop rotation of maize and wheat versus maize monoculture under ZT+RES did not have a significant effect on the numbers and size class of earthworms. Differences, if any, in amount and quality of organic matter inputs, canopy closure, water dynamics and rooting patterns did not affect worm proliferation.

The effect of crop rotations on soil structural stability is less understood than that of tillage (Madari *et al.*, 2005) and little information is available (Chan and Heenan, 1996). No significant differences in aggregate stability were found between crop rotations, in accordance with the few recent studies on the subject (e.g. Pinheiro *et al.*, 2004; Madari *et al.*, 2005), aggregate size distribution of wet-sieved samples, and soil bulk density.

Time-to-pond was greater in MW plots than in maize monoculture plots. Theoretically, this could be due to more favourable rooting patterns or a more dense soil cover when crops are rotated. However, the previous minimum tillage in MM/ZT+RES plots (B strip) might have affected the results and may thus explain the differences in time-to-pond.

No significant differences in time-to-pond were found depending of which crop was present (wheat from MW or maize from MW). It seems that more short-term effects such as type of canopy or differences in root systems are not leading to visible differences in water infiltration.

Although crop rotation did not lead to significant differences in earthworm populations and physical soil quality, this practices is advisable for pest management, since it avoids the build-up of pests and diseases (Wall, 1995), and is a risk aversion strategy from an economic perspective.

The simultaneous implementation of no-till and the presence of a residue cover on the soil surface, that characterizes conservation agriculture (CA), led to the greatest earthworm populations. As soon as any of those management components was absent, earthworm abundance was drastically reduced. The division of earthworms by size into small earthworms or “decompacting species” (≤ 7 mm) and large worms or “compacting species” was not meaningful since differences by treatment were not significant. Crop rotation of maize-wheat versus maize monoculture under CA did not lead to significant differences in earthworm numbers nor in any of the physical soil quality parameters measured.

Both dry aggregate size and water stability were highly influenced by residue management although not by the type of tillage practices. Aggregate stability was found to be greatly correlated to soil organic matter content (SOM). In fact, SOM is considered as a main cementing agent for the build-up of stable soil aggregates. SOM constitutes the link between the biological and the physical domain in soils since it is a source of energy for organisms (Franzluebbers, 2002) that increase soil aggregation through their activity (e.g. earthworms, termites, fungi, bacteria; cited by Six *et al.*, 2004). The fact that SOM explained 38.6 percent of the variability of mean weight diameter of wet-sieved samples, while earthworms 19.2 percent, suggests that the role of other soil organisms could also be probably important in explaining aggregate formation and stabilization processes. The percentage of MWD reduction did not differ by management systems indicating that overall stability was similar for all treatments. However, the mean diameter of stable aggregates was found to be higher in the topsoil of plots under conservation agriculture when compared to the other treatments and this means better physical soil conditions.

The soil surface is a critical zone that can either impedes (e.g. soil crusting) or facilitates (e.g. optimum soil structure with water-stable aggregates) the movement of water into the soil (Franzluebbers, 2002). Absence of tillage and residue presence was found not only to provide a protective cover to the soil surface, increase SOM levels, but also to favour earthworm abundance and activity and thus lead to a better surface structure. This led to higher rates of time-to-pond (direct surface infiltration), which are essential in the rainfed cropping systems found in the Central Highlands of Mexico where strongly seasonality can lead to water stress. Although tillage increased water infiltration, its effect seems to be temporary, since during the growing season, a critical period for water use efficiency, conservation agriculture was characterized by greater infiltration rates. Direct surface infiltration was found to be positively correlated with both soil aggregation and earthworm abundance and activity.

Soil bulk density measurements were found to be poor indicators of detrimental levels of soil compaction since high values, as proved by the micromorphological study, did have different meanings in conventional tillage systems (where soils were compacted) and in conservation agriculture (characterized by a highly dense biogenic structure at 15 cm depth).

Zero tillage without residues did not differ significantly from conventional tillage with or without residues regarding earthworm abundance, activity and physical soil quality. However, it should be kept in mind that ZT-RES was partly measured in plots with a history of minimum tillage until 2003. In accordance with trends in yield data, conservation agriculture, when compared to the other three tillage and residue management systems, was characterized by optimum physical soil quality: greater aggregate size and stability, greater percentage of large macroaggregates, an extremely biogenic structure with high porosity along the whole soil profile and high levels of water infiltration at the surface levels. As stated by Franzluebbers (2002) an optimum physical quality at the topsoil is key in sustainable agriculture. Earthworms, as it is clear in this study, are able to contribute greatly to the build-up of a better physical status of soils when the conditions are conducive to their proliferation.

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