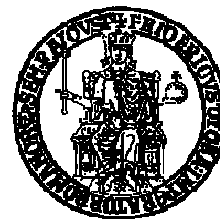**UNIVERSITÁ DEGLI STUDI DI NAPOLI FEDERICO II**

UNIVERSITE DE LORRAINE – ENSAIA – VANDOEUVRE LES NANCY

PhD Course in Biologia Applicata:
Ecologia

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EFFECTS OF LAND USE TRANFORMATION ON MIRCOARTHROPOD COMMUNITY STRUCTURE IN MEDITERRANEAN AREA

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Rerum Natura nusquam magis
quam in minimis tota est

PLINIO

PREFACE

At the end of the 19th century, humanity started to become an urban species. Large cities, not villages and towns anymore, became the main human habitat. Urban agglomerations became the dominant feature of the human presence on earth, profoundly changing humanity's relationship to the planet and the ecosystems. Unfortunately, urbanised humanity began to draw upon resources from ever more distant hinterlands, to use the biosphere, the oceans and the atmosphere as a sink for its wastes at such accelerating rate that it is unlikely that the planet will be able to accommodate much longer the current human life style.

Resources on Earth are not inexhaustible, so their degradation could be irreversible. Among the principal non-renewable resources of which man disposes, there are terrestrial ecosystems. All along, the soil represented the substrate on which many human activities take place, being also one of the strengths for several civilisations. Unfortunately, for long time the soil was considered as an inactive and unlived surface, and for that subjected to an excessive exploitation that has caused a substantial alteration of its characteristics. Notwithstanding soil have a natural ability to mitigate most of the degradation processes, the alteration of physical, chemical and biological properties can be drastic and irreversible, leading to a severe decline of its quality and fertility. Only lately, with the growing concern about soil degradation and non-renewability of its resource, changing in soil employment by humans occurs. In fact, maintenance and management of soil quality and fertility, as well as reduction of fragmentation, deforestation, erosion and pollution, are central factor for soil sustainable use.

In order to preserve a good soil quality and to ensure its fertility is necessary to preserve soil physical, chemical and biological characteristics unaltered. In fact, the safeguard of abiotic soil properties is a fundamental step to assure the functionality of soil biotic processes. In ecosystem functioning, in fact, soil biotic component plays a central role in many ecosystem services, since it is implicated in nutrient cycling processes, degradation of many pollutants, regulation of plant communities, global climate change. Obviously, life of soil dwelling organisms is strictly dependent to the abiotic soil properties, which make the soil a favourable habitat to life and reproduction of organisms. So, it is simpe to understand that a little perturbation occurring in such complex system, can lead to a series of reaction

reflecting on soil biotic communities. Soil biological structure can be severely impacted by different alteration, manifesting changing in species assemblages or, in the worst cases, a drastic loss of biodiversity, with severe consequences on many ecosystem functioning. In fact, the net result of biodiversity simplification is an artificial ecosystem that requires constant human intervention with a substantial increase of economic and environmental costs.

Therefore, understanding soil system dynamics is a fundamental step to a sustainable management of this resource. Unfortunately, soil remains a "poor relation" among the natural resources indispensable to life and development. It does not in general enjoy the attention given to rivers, seas and atmosphere, and its sustainable management has not yet become a subject of debate and concern as it deserves to be. In addition, its ecological and productive values are still today not sufficiently recognised. Improving information about soil functionality and responses to different stress and enhance its sustainable management is vital for the life of all the living being. If the present trends about soil employment and exploiting were confirmed, serious and long-term consequences are to be waited.

CHAPTER I

Introduction

1.1 Soil ecosystem

1.1.1 Abiotic properties

The thin layer of material that covers much of the Earth's surface is known as soil. This fragile skin is sometimes less than a meter thick and is absolutely vital for the life on Earth. Composed, by inorganic and organic components, by water and air, soil is often described as the most complicated biomaterial on the planet (Pepper et al., 2009). As the resulting of the action of climate and living organism upon the parent material along the time, soil system reveals high complexity issuing from the interaction of all its components (De Bartolo et al., 2011). In fact, soil can be described as a system "*composed of interconnected parts that as a whole exhibit one or more properties, not obvious from the properties of the individual parts*" (Joslyn and Rocha, 2000).

Physically, soil consists of three different phases: solid, liquid and gaseous. Solid phase is dominant and is composed by inorganic, organic components (plant, animal, microbial residues and humic acids) and living organisms, present as independent entities or mixed conglomerates (Pietramellara et al., 2002).

Soil inorganic component is composed by different sized particles: sand, silt and clay particles. Variations in percentage composition of these classes of particles define texture and, in turn, porosity, permeability and water movement and retention along the soils. Soil organic matter is composed decaying materials, which also comprises small amorphous or granular particles and humic substances. The composition and proportion of organic material is central in soil as it forms a reserve of energy and nutrients, links mineral components within the soil matrix through their colloidal and charge properties and retain cations on their predominantly negatively charged surfaces.

Soil particles are surrounded by aqueous and gaseous phase, which the amount and composition fluctuate markedly in time and space. Water generally available by plants and soil organisms is the water surrounding colloid particles, which thanks the

charges presents on their surface, are able to retain water molecules. Then, thickness of the water film depends on the surface charge value of the colloid (Pietramellara et al, 2002).

Soil atmosphere represent the air occupying empty soil spaces. Soil atmosphere differs from the atmospheric one only for the higher concentration of CO_2.

Chemistry in soil is depending from physical properties, and it is mainly controlled by little particles, such as colloidal sized particles (≤ 2 μm). These particles include inorganic fine clays and organic material. Due to their chemical structure and large surface area, colloids have charged surfaces that are able to sorb, or attract, ions within the soil solution. In addition, as colloids have on their surface both negative than positive charges, allow the exchange of anions and cations useful for the plants. Cation and anion exchange capacity is also affected by several soil properties. pH, for example, affect this properties, as variations in its values alterations of colloid surface charge. Variations in soil pH, ion exchange capacity and organic matter content and quality are involved in soil element mobility, fundamental for organism nutrition. However, soil nutrient content depends also on pedogenetic substrate, as it is composed by silicate minerals of iron, sodium, potassium, calcium and magnesium, and variable and much smaller amounts of other elements. Smaller amounts of elements may also derive from atmospheric sources. On the basis of biota requirements, soil elements can be classified in macro-nutrients (N, Ca, Mg, K, P, S), essential in high amount for organisms growth; micro-nutrients (Cl, Na, Cu, Fe, Mn, Mo, Ni, Zn, Co, F, I, Se) which may stimulate organism growth if present in small amount, but can exert a toxicity if present in higher quantities; non-essential elements (Al, Pb, Hg, Cd) that can show toxicity on organisms even at low concentrations (Lavelle and Spain, 2001; Kabata-Pendias and Mukherjee, 2007). The uptake of nutrients and other elements by organisms is of prime importance since it affects the entire biological community.

1.1.2   Biotic component

Soil physical and chemical complexity, together with climatic conditions, makes the soil system a heterogeneous matrix with a wide variety of ecological niches, hosting a wide and diversified biological community (Ettema and Wardle, 2002).

Along the years, the studying of soil system highlighted soils as the richest systems in term of biodiversity. In fact, soil and litter dweller organisms represent approximately one fourth of all living species present on earth (Decaëns et al., 2006). Although soil organisms occupying less than 5% of the total soil volume (Ingham et al., 1985), mature soils appear to have a phylogenetic diversity greater than any habitat, with the possible exception of coral reefs (Hågvar, 1998). Soil organisms are not just inhabitants of the soil, but they are part of it. In fact, they are essential for primary production and the decomposition of organic residues and waste materials, heavily influencing soil hydrology, aeration and gaseous composition,.

Because of the great number of soil inhabitants, several classifications have been proposed. Surely, body dimension is the first distinctive feature (Swift et al., 1979), which allows categorizing soil biota (Fig. 1.1) in:

- *micro-flora* (1-100 μm);
- *micro-fauna* (5-120 μm);
- *meso-fauna* (0.08-2 mm);
- *macro-fauna* (500 μm-50 mm);
- *mega-fauna* (>20 mm).

Soil organisms can be also classified (Turbé et al., 2010) on the basis of the functions they perform in the soil (Fig. 1.1), in:

- *Chemical engineers* (transformers and decomposers): organisms responsible for carbon transformation through the decomposition of plant residues and other organic matter, and for the recycling of nutrients;
- *Biological regulators*: organisms responsible for the regulation of populations of other soil organisms, through grazing, predation or parasitism, including soil-borne pests and diseases;
- *Ecosystem engineers*: organisms responsible for maintaining the structure of soil by the formation of pore networks and bio-structures, and aggregation, or particle transport.

The two classifications are interconnected, as functions of soil organisms are partially influenced by body size. Thus chemical engineers are essentially composed by microorganisms. Biological regulators tend to be largely composed by meso-fauna, while ecosystem engineers tend to belong mostly to macro-fauna.
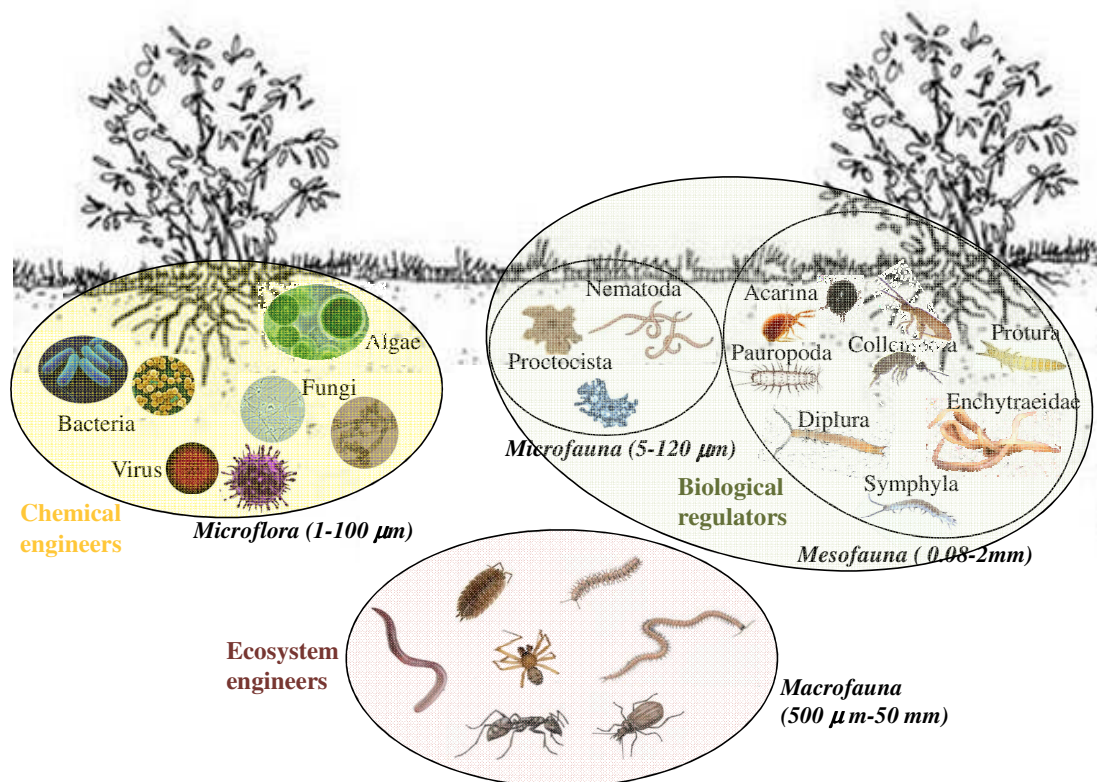
Figure 1.1. Main groups of organisms living in the soils. Organisms are grouped on the basis of body size and functional role.

1.2 Soil biota and their functions

Benefits supplied by natural ecosystems from a multitude of resources and processes are defined ecosystem services. They can be classified in (Barrios, 2007) those associated to the provision of goods, to the life on the planet, to the regulation of ecosystem processes and to the cultural services that are not associated with material benefits. Thanks to its abiotic and biotic components, soil can contribute to all four different dimensions of ecosystem services.

In particular, soil biota is involved in processes associated with the provisions of goods, life on the planet and regulation of ecosystem processes (Fig. 1.2).
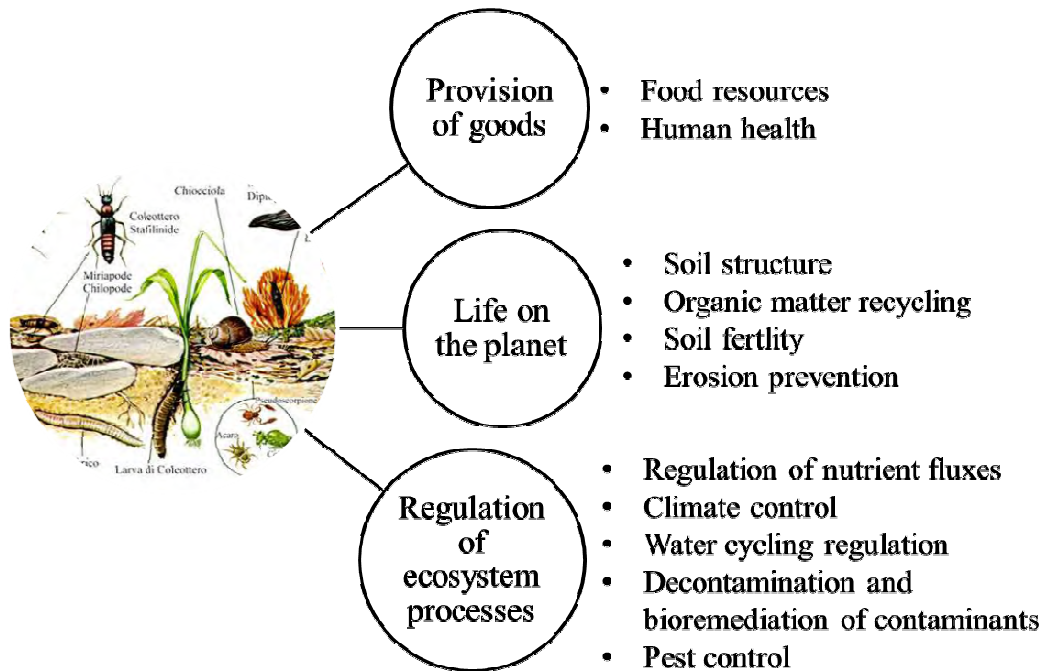
Figure 1.2. Ecosystem services performed by soil biota.

In fact, besides to constitute a food resource for many animals, soil organisms are involved in a number of benefits for human health, such as be a resource for the development of new pharmaceuticals.

Moreover, soil organisms, with the movements through the soil, contribute to soil structure formation (Lavelle, 1997; Hedde et al., 2005; Mora et al., 2005). They also alter ecosystem dynamics directly, by modifying or creating habitats, or indirectly, by regulating the availability of resources for other species (Jones et al., 1994).

The decomposition of organic materials and the recycling of nutrients are among the most important ecosystem services performed by soil organisms as they represent the catabolic complement of photosynthesis. Decomposition and biogeochemical cycles involve the majority of soil organisms, as each is an actor of different steps (Swift et al., 1979; Coleman et al., 2004). These processes release nutrients in forms usable by plants and other organisms. The residual soil organic matter forms humus, which serves as the main driver of soil quality and fertility (Turbé et al., 2010). As a result, soil organisms support the quality and abundance of plant primary production, contributing, indirectly, to the food production.

Cycle of carbon is also regulating by soil biological processes driven by soil biota. The organic fraction represents the part of carbon involved into biological processes. In fact, the speed of decomposition regulates the fluxes of CO_2 in atmosphere,

contributing to the global warming. In addition to CO_2, soil biota can also control fluxes of other greenhouse gases, such as methane (CH_4), much more powerful greenhouse gas than CO_2. Thus, through their capacity to stock carbon, soils can act as a buffer compartment in a context of climate change (Turbé et al., 2010).

Soil organisms involved in soil structuration also affect the infiltration and distribution of water in the soil, by creating soil aggregates and pore spaces. Soil biodiversity may also indirectly affect water infiltration, by influencing the composition and structure of the vegetation (Turbé et al., 2010).

Soil microorganisms play a key role in bioremediation, by accumulating pollutants in their bodies, degrading pollutants into smaller, non-toxic molecules, or modifying those pollutants into useful metabolic molecules.

Finally, some soil organisms can influence interactions between plants and aboveground pests and diseases (Scheu, 2001; Van der Putten et al., 2001). Ecosystems presenting a high diversity of soil organisms typically present a higher natural control potential, since they have a higher probability of hosting a natural enemy of the pest (Altieri and Nichols, 2003). Efficient pest control is essential to the production of healthy crops, and the impairment of this service can have important economic costs, as well as food-safety costs.

Soil system is able to perform all processes presented above, only thanks to the great diversity of ecological from and roles of organisms living in it. Therefore, conservation of soils biodiversity represents a necessary action, as in the long-term, species which seem redundant or which are very rare today may achieve important ecological functions or represent valuable genetic material for future evolution (Hågvar, 1998).

1.3 Soil mesofauna

Meso-fauna is composed by microarthropods and enchytraieds, which acts as regulators of microbial activities. Microarthropods belong to the branch of arthropods and are all characterized for a small covered by a hard external skeleton. As mainly epigeic decomposers, microarthopos are constant inhabitants of leaf litter and soil interstices in which they perform all the life cycle. Acari and Collembola are generally dominant by far, both numerically and in terms of biomass. However, other

Apterygota (Protura, Diplura and Thysanura), Micromyriapoda (Symphyla and Pauropoda) and small Diptera larvae and Coleoptera may be locally important.

Soil mesofauna play a central role in soil functioning. These organisms contribute directly to decomposition and nutrient cycling. In addition, microbial grazing mesofauna, affect growth and metabolic activities of microbes and alter the microbial community, regulating decomposition rate and nitrogen mineralization. Microarthropods, in particular, fragment detritus and increase surface area for microbial attack. They accelerate the decomposition, breaking and moistening the organic detritus, making it available for microorganisms. Plants, also, benefit from increased mineralization of nitrogen by soil mesofauna. In addition, soil mesofauna transport bacteria, fungi and protozoa across regions in soils, enhancing microbial colonization of organic matter (Neher and Barbercheck, 1998).

1.3.1 Collembola

Collembola are currently considered to be a class of the phylum Arthropoda although their exact taxonomic position is still the subject of some debate (Hopkin, 1997). Current knowledge classifies Collembola as a branch of Hexapoda, Entognata class, distinguishing them from insects. On systematic point of view Collembola comprise 3 orders (Fig. 1.3):
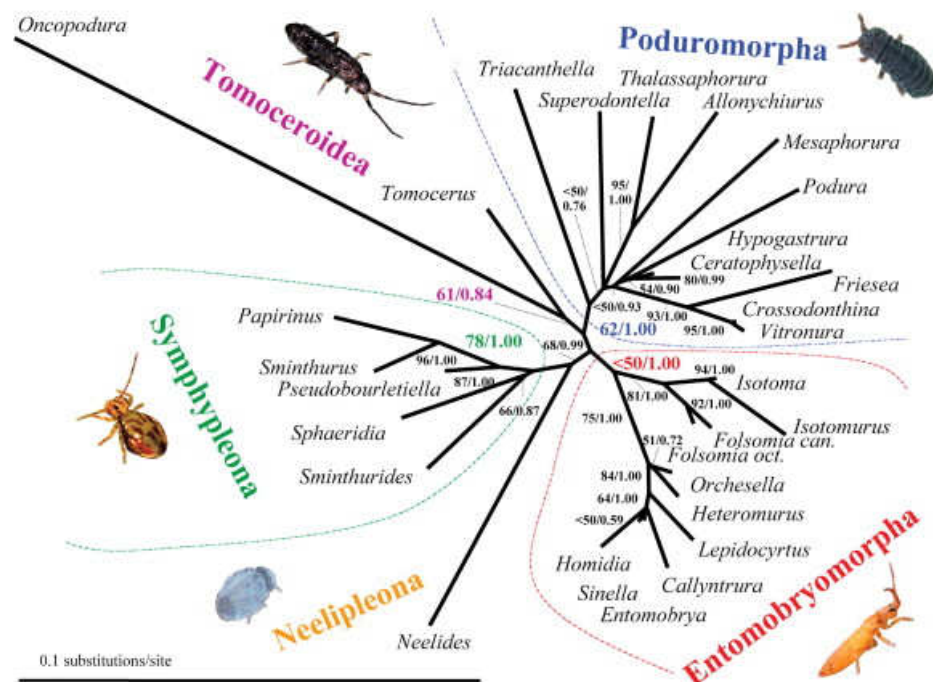
Figure 1.3. Systematic classification of Collembola orders.

Neelipleona, Symphypleona (Sminthuridae family), Arthropleona, subdivided in two suborders, Poduromorpha, Entomobryomorpha, with two bigger families Entomobrydae and Isotomidae (Fig. 1.3).

Morphologically, Collembola are apterous hexapods, smalls and elongates with a characteristic salutatory organ (*furca*) which allows rapid jumping movements. Collembola have also a ventral tube, which consists in an eversible sac, extremely important in fluid balance.

Collembola have a very wide global distribution, being abundant on every continent, including Antarctica. Collembola distribution is strictly depending on temperature, moist condition and presence of oxygen (Kardol et al. 2011). Collembola populations mostly live in holorganic horizon of soils, in the pore space of the upper 10 to 15 cm of soil, even though others can live on trees and are abundant in rain forest canopies.

Collembola feeding habit is considered as generalist, even though the majority mainly feed on fungal hyphae, bacteria or decaying plant material. They may influence the growth of mycorrhizae and control fungal diseases of some plants. Some species also feed on pollen; others are predators, feeding on protozoa, nematodes and enchytraeids (Hopkin, 1997). In turn, Collembola are also the food for many predator organisms.

Reproduction is bisexual or parthenogenetic, but in the latter case is mainly controlled by Bacteria belonging to Wolbachia group (Czarnetzki and Tebbe, 2004a, b; Merçot and Poinsot, 2009).

1.4 Land anthropization and urban soils

1.4.1 Generalities

Due to the pervasive influence that humans are having on the Earth's atmosphere, lithosphere, hydrosphere and biosphere, some scientists (Crutzen, 2002; Steffen et al., 2005) refer to the current geological period as the "Anthropocene Era". In the latter 300 years, in fact, Earth's population grew about 10-fold, passing from 600 million people to 6.3 billion and is expected to grow by 47 %, reaching to 8.9 billion in 2050 (Cohen, 2003; United Nations, 2004). Parallel to a demographic growth, the urbanized areas increase as well. Worldwide urbanization began at least two

centuries ago and accelerated greatly in the 20th century. Between 1800 and 2000, in fact, the number of city dwellers rose more than about 25-fold, from 18 million to 2.9 billion (Cohen, 2003; United Nations, 2004). Urban growth is changing the face of the earth and the condition of humanity. Nowadays, more than half of the human population worldwide lives in cities (United Nations, 2008) on less than 3% of the global terrestrial area (Grimm et al., 2008). As a result of human population growth and urbanization increasing, the Earth is more and more subjected to human-mediated modifications (Sanderson et al., 2002, Steffen et al., 2005). Assessments of the percentage of ice-free land affected by human action vary from 20% to 100%. More than 75% of Earth's ice-free land area could no longer be considered wild, whereas 83% is likely directly influenced by human beings. As a result, Earth's land surface result occupied for 46.6% by agricultural and forestry lands, for 6.9% by human infrastructures, and 46.5% by natural lands (Hooke et al., 2012). Land transformation encompasses a wide variety of activities that vary substantially in their intensity and consequences. Anthropized ecosystems, in fact, influence important ecosystem services at local, regional and even global levels (Endlicher et al., 2011), but despite the growing interest in, and research about, very little is known about the effects of anthropization on ecosystem transformations, pollution, changing in biodiversity and ecosystem services.

Land use practices change according to the ever-evolving human needs, and although soil usage greatly varies across the world, its ultimate outcome is generally the acquisitionof natural resourcesfor human populations. In this way, natural forests are converted in managed forests regularly cut for construction materials, paper or fuel; grasslands, and forests as well, are converted in managed crop fields, some of which are gradually consumed by growing urbanisation. Any land transformations imply characteristics modifications on soil system with a consequent switch of soil biota community. The kind and the degree of modifications are linked to type of transformation to which soil is subjected. Natural habitat can be transformed in crops or in urban and industrial areas, which cause alteration in soil abiotic and biotic properties.

Humans are likely the premier geomorphic agent currently sculpting Earth's surface (Hooke et al., 2012). Although land-use practices vary greatly across the world, their ultimate outcome is generally the same: the acquisition of natural resources for immediate human needs, often at the expense of degrading

environmental conditions. Several decades of studies have revealed that land-use practices play a role in affecting processes at global scale, such as carbon cycle and global climate alteration (35% of anthropogenic CO_2 emissions resulted directly from land use), modification of hydrologic cycle (irrigation, industry, domestic consumption), changings in soil chemical composition (fertilisation and pollution) and water eutrophication (Foley et al., 2009). In addition, land transformation represents the primary driving force in the loss of biological diversity worldwide, as the fast loss, modification, and fragmentation of habitats, not allow the species to adapt or to move away to other areas.

1.4.2 Characteristics of urban soils

Urban areas consist of highly modified habitats, with over 80% of most central urban areas covered by pavement and buildings. As urban soils are inserted in urban area, they are usually defined as all soils influenced extensively by human activities in the urban landscape (De Kimpe and Morel, 2000). Because of their nearness with human presence, urban soils are generally thought of as highly disturbed and heterogeneous, with little systematic pattern in their characteristics (Pouyat et al., 2010). On the basis of their degree of disturbance, indeed, urban soils are often distinguished in three categories (Lehmann and Stahr, 2007):

-*Man-influenced soils* contain little or no artefacts (artefacts are bricks, pottery, glass, crushed or dressed stone, industrial waste, garbage, processed oil products, mine spoil and crude oil) and are built from soil material, which was mixed by man through excavation and transportation as well as by deposition. Such soils mainly show inherited soil properties and only weak *in-situ* development.

-*Man-changed soils* characterized by peculiar characteristics such as alkalinity, high contents of coarse fragments and organic matter. The age of such soil layers often increases with depth. Such soils show predominately inherited soil properties. Numerous man-changed soils are covered with more or less impervious layers by sealing.

-*Man-made soils* from artefacts comprise artefacts solely or mainly from anthropogenic material, such as rubble, ashes, slugs, sludges, waste and spoil. These soils develop only slightly *in situ* and their properties are mainly derived from the artificial parent material. Man-made soils commonly occur below sealing.

Despite the detected differences, human pressure is the main driving force in lithogenesis and pedogenesis processes of urban soils (Lorenz et al., 2005), which share similar characteristics which can be resumed in the Table 1.1 (Lehmann and Stahr, 2007).

Characteristics	Common in urban soils	Rare in urban soils
Artefacts/fragments	**Many** -in soils containing construction residues and other large artefacts causing high water permeability - in soils with surface or underground sealing	**None** -in soils from sludges and ashes
pH	**Alkaline** -in soils containing construction residues like plaster or concrete	**Acidic** -in sois containing sulphur from cola or technically produced sulphuric acid
Technical organic carbon and nutrients	**High** -in soils affected by accumulation of organic waste, dust and combustion residues -in soils formerly used in horticulture -in soils with subsoils containing former topsoil material	**Low in organic carbon** -in soils with regularly swept topsoil to keep it free from vegetation **Low in nutrients** -in soils from parent material poor in nutrients
Contaminants	**High** -in soils containing combustion residues and other residues from production processes in highly industrialized cities	**Low** -in soils only affected by input of contaminants via dust deposition and rain caused by urban environment
Bulk density	**High** -in the topsoil: soils affected by mechanical forces on the surface -in the subsoil: soil affected by compaction through construction activities	**Low** -in soils affected by mechanical loosening -in soils high in organic matter content -in soils containing much ash
Soil temperature	**High** -in city with increased air temperature this is crucial for permafrost regions -in soils influenced by technically increased soils -in soil affected by heating facilities, or warmed technical activities	**Low** -in soils affected by technical induced cooling and by cold water -in wet soils
Soil moisture	**Low** -in soils affected by drainage, mostly for construction purposes	**High** -in soils affected by irrigation, by leakages, by drainage from sealed surfaces and by other fluxes of water
Age	**Young** -in soils affected by frequent relocations due to construction activities	**Old** -soils situated in long-term undisturbed niches in old city quarters, also the cultural layers of urban soils
Development	**Strong ex-situ** -soils which are from relocated soil material from strong developed soils, which were often deposited in layers while multiple construction activities	**Diverse strong in-situ** -soils free of relocated strongly developed soil material (numerous soils from an age of 50 years or older show quite

	proceeded over longer period of times	strong development, especially if they contain material of amorphic structure and material with large relative surface, such as dust and ashes)

1.4.3 Effects of urbanization on soil system

When a land is converted to urban uses, the first occurring effect is the fragmentation. This process results in the creation of distinct soil parcels, having characteristic disturbance and management regimes that will affect soils through space and time, depending on human population density, development patterns and transportation networks, among other factors. Indirect effects of urban pressure on soils involve changes in the abiotic and biotic environment. Due to the activities of construction and traffic, urban soils are subject to sealing, compaction, metal and PAH pollution, increasing of temperatures in the urban cores (Turbé et al., 2010). Sealing is a process through which soils are covered by an impermeable layer that impedes exchanges between aboveground and belowground worlds, whereas and compaction is physical degradation due to the reorganisation of soil micro and macro aggregates, which are deformed or even destroyed under pressure. Sealing and compaction lead to an increase of bulk density and a decrease of water drainage in urban environment. However water drainage and moisture content in urban soils is rather variable, being depending on several factors, such as organic matter content, frequencies of irrigation, temperature (Pouyat et al., 2010).

For urban soils, elevated heavy metal and polycyclic aromatic hydrocarbons (PAHs) concentrations are almost universally reported. PAHs are components of most fossil fuels and are ubiquitous in the natural environment, as they can be released in forest fires and from volcanic eruptions. Likely, metals are cations widespread in soil system, and except for iron and aluminium, they occur in the Earth's crust in amounts less than 0.1% (KabataPendias and Mukherjee, 2007). In urban environment, most of the heavy metal and PAH sources have been associated with roadside environments. Urban soils are the main PAH pollution concerned systems (Banger et al., 2010), being more exposed by both stationary (power plants, industries, and residential heating) and diffused sources (traffic emissions, and road by products such as wearing of they are mainly emitted by automobile and truck emissions, hazardous waste sites (Wcisło, 1998) tires and asphalt constituents). In the

same way, residential heating and vehicular traffic are the main metal pollution causes in urban soils. Because of their characteristics of duration, enrichment, toxicity and concealment (Dube et al., 2001), metals pose serious threats to soils system.

Due to all these alterations, usually the conversion of native habitats to urban land uses greatly contributes to local extinction rates of plant and animal species (Pouyat et al., 2010). However the faunal composition of urban soils is highly variable. When urbanization process leads to strong soil alteration, a decline of soil biodiversity can occur. The loss of biodiversity results in the loss of almost all services provided by soil biodiversity. Litter decomposition is made almost redundant, given the reduced litter quantity and man-made management practices. As a result, carbon storage and climate control services are impaired (Turbé et al., 2010). Therefore, the transformation to urban soils causes a significant disruption of whole biota community. Urban soil communities are dominated by bacteria and microarthropods, whereas earthworms are mostly absent. However, often the occurrence novel habitats, such as built structures, greenhouses, and green roofs, add to the species richness of urban landscapes (Pouyat et al., 2010). These species are peculiar of urban environment, as they are tolerant to several kind of disturbance. As a result, urban soils have fundamentally different soil faunal communities, with a higher proportion of introduced species when compared with their native soil counterparts.

1.4.4 Benefits provided by urban soils

As shown above, urban environment pose serious threat on urban soils. The management of urban soils is thus a topic of strongly growing significance, especially considering that the functioning of cities is dependent on its soil. Urban soils provide an essential service to inhabitants living in an overwhelmingly artificial environment. They are indispensable for a liveable city and visiting them has become a social institution (Jim, 2003). In an urban environment soils can reduce the risk of flood damage considering their capability for water infiltration. In addition, they provide a convenient water resource for drinking, cleaning and cooling. Yet, soils anthropogenically developed, accumulate high organic matter content, providing immense water retention and can produce very much biomass. Urban soil may serve as habitat, rooting and leaching space. Urban soils are also sinks of uncontaminated

and contaminated dust, carbon and other solids or dissolved compounds. Therefore, polluting materials are diluted and retained in soils and may be immobilized, or decomposed or leached (Lehmann and Stahr, 2007). Benefits provided by urban soils can be grouped in:

-beneficial functions of urban soils:

- providing groundwater recharge for water supply
- providing plant products for food supply

-functions of urban soils contributing to infrastructure

- providing a medium for alternative storm-water management
- providing sites for recreational activities

-functions of urban soils for disaster control

- infiltration of (rainstorm) water to prevent flooding
- retention, decomposition and immobilization of contaminants

-functions of urban soils to ensure environmental quality and cultural heritage

- dust entrapment to reduce the dust content in the breathing air
- carbon sequestration to reduce the concentration of carbon dioxide in the atmosphere
- buffering of temperature and humidity, mainly through cooling by evaporation
- prehistorical and historical archives

1.5 Land anthropization in the Mediterranean environment

Mediterranean soils are soils which, by definition, form under Mediterranean climatic conditions. The main characteristic of the Mediterranean climate is that it has two well defined seasons in the year, with the rain period coinciding with low temperatures (winter) while summers are hot and almost completely dry. In the world as a whole, Mediterranean soils are not very extensive. FAO (1991) estimates their extension at approximately 420 million ha. The main area is around the Mediterranean Sea, with smaller areas in California, Chile, The Western Cape Province of South Africa, and West and South Australia. Another distinctive feature of Mediterranenan areas is the frequency of fire. In this area, fire is natural phenomenon greatly depending on climate, even though lately is mainly human-driven. Due to all these features typical vegetation of Mediterranean area is mainly

composed by sclerophyllous plants, with hard leaves and several adaptations to face long periods of drought. However, thanks to climate Mediterranean areas represent one the world's major centre of biodiversity, with high degree of endemisms (Heywood, 1998).

For these peculiar characteristics, all along the centuries, the Mediterranean basin has represented one the most interesting socio-economic area. It was an important location for many ancient civilizations, and also an important route for merchants and travellers (Abulafia, 2011). Unfortunately, in the Mediterranean area the increasing of the population and its concentrations in certain areas gradually led a rising of ecosystem degradation with serious interferences in natural biological balance. In addition, strong is also the tourism pressure, as Mediterranean lands are surrounded by sea, occurring in scenic areas. Mediterranean citizens is concentring especially near coasts, due to the destructuring of traditional inland economies and societies, the development of economic activities near urban areas and coastlines, and the very rapid development of national and international tourism (De Franchis and Ibanez, 2003). These processes make possible that some area are more degraded than others. In addition, Mediterranean soils are already naturally fragile, for several reasons. Firstly, the irregular and often violent precipitations that cause erosion; the steepness of the slope in numerous hilly and mountainous sectors worsens this phenomenon; high temperatures accelerate the mineralisation of organic matter; the reduction of plant cover because of the climate's severity, the wind erosion and salinization of soils (De Franchis and Ibanez, 2003).

The fauna of Mediterranean soils mainly account hygrophilous organisms (Radea and Arianoutsou, 2002). In these ecosystems, soil organic matter and water availability are the two key environmental factors which control the dynamics of soil invertebrate populations. In fact, in Mediterranean-type ecosystems the amount of soil organic matter is rather low and, therefore, its role in the structure of soil fauna is of great importance (Radea and Arianoutsou, 2002).

Despite the recognized peculiarity of the Mediterranean environment in terms of productivity and biodiversity and studies about (Cortet and Poinsot-Balaguer, 1998; Radea and Arianoutsou, 2002; Doblas-Miranda et al. 2007; Gergócs et al., 2011), many studies investigate on forest soils. Nowadays, little is known about the effects of anthropization on Mediterranean soils and the dynamics of soil arthropod in urban soils with Mediterranean characteristics.

1.6 Urban soils of volcanic regions

Soils of volcanic regions are unique natural resources. When volcanic material is exposed to weathering small sized minerals and humic substances are formed. These colloidal particles give the soil distinctive features called andic properties. These properties include high organic carbon content, variable charge characteristics, high phosphorous retention, low bulk density and great water retention. Volcanic soils cover 1-2% of Earth's surface (Olafur et al., 2007). They are among the most fertile soils, even though for their high pollutant binding capability can accumulate high amount of toxic substances. However, they are the foundations for some of the most densely populated areas of the world, and as they often occur in the scenic areas, are subjected to extreme pressures from tourism.

Anthropized soils with andic properties strongly differ from common urban soils. Volcanic soils, in fact, usually present lower values of pH, higher organic matter content and lower bulk density than the soils present in the most of the cities. However, they naturally show higher amounts of pollutants, especially trace elements, than other soils. Thanks to the binding capability, pollutants in urban volcanic soils can reach dangerous thresholds. The whole soil system would result heavily affected, whit serious risks to the whole soil system. Many studies (Adamo et al., 2002; Imperato et al., 2003; Maisto et al., 2004, 2006; De Nicola et al., 2005, 2011) are performed to monitor the amount of trace elements and PAHs in andic anthropised soils. However, there is a wide lack of studies focusing on biological activities of andic anthropised soils, and even less if considering the responses of soil arthropods in volcanic urban soils in Mediterranean area.

1.7 Soil quality

1.7.1 Definitions and assessment

Soil transformation and the related alterations even at ecosystem scale have contributed to grow the interest about the definition and assessment of soil quality. Although several definitions (Doran, 1996; Karlen et al., 1997; Knoepp et al., 2000; Schoenholtz et al., 2000; Filip, 2002) of soil quality exist, they share relevant elements summarized as the sustainability of the soil as a resource for food

production, to support human life and to preserve or improve the soil for future generations. Soil quality is understood as an integral value of compositional structures and natural functions of soil in relation to soil use and environmental conditions on site (Filip, 2002). In this context, soil quality acquires an important dimension related to the strategies for productivity and its conservation and health. The concept of soil quality includes assessment of soil properties and processes as they relate to ability of soil to function effectively as a component of a healthy ecosystem.

Monitoring of function and long-term sustainability of soil relies on use of indicators. In the case of soil quality, an indicator is a measurable surrogate of a soil attribute that determines how well a soil functions (Schoenholtz et al., 2000). However, as the soil quality is a combination of the physical, chemical and biological properties, it is often difficult to clearly separate them because of the dynamic, interactive nature of these properties. In alternative, it may be useful to describe a set of identifiable properties that such soil must possess in order to perform some functions, but there is seldom a one-to-one relationship between function and indicator; more likely, a given function is supported by a number of soil properties. Then, to describe soil quality it is recommendable to consider together physical, chemical and biological indicators.

A good soil quality indicator must be responsive to management practices, integrate ecosystem processes, and be components of existing, accessible data bases. Physical soil quality indicators should indicate the soil capability to: promote root growth, accept, hold, and supply water, hold, supply, and cycle mineral nutrients, promote optimum gas exchange and biological activity and accept, hold, and release carbon (Schoenholtz et al., 2000). Therefore, the most widespread physical indicators in soil quality assessment are: texture, depth, bulk density, water holding capacity, hydraulic conductivity, porosity, potential erosion. Some of these soil physical properties are static in time, and some are dynamic over varying time scales.

Chemical indicators are required to indicate soil carbon status, soil acidity, measures of element concentrations and availability. Among the chemical indicators pH, cation exchange capacity, organic matter content, carbon content, element concentrations and compounds (nutrients, metals, PAHs…) are the widely used in soil quality evaluation.

To assess the total sustainability of soil natural functions and different uses, key indicators should include biological and biochemical soil parameters. Those are capable to characterize soil as a dynamic part of the biosphere. Biological investigations can be conducted on various levels of biological integration. The measurement of biochemical and physiological variables in individuals or their excretion products, providing information on exposure or damage, usually goes under the name of *biomarkers*. A *bioassay* is an ecotoxicological test system usually of short duration and with a defined protocol in which the activity of a chemical is a measured as an adverse effect on some test species at individual or population level (Van Straalen, 1998). These tools have the advantage based on simplicity in use, in an easy standardization of the individual methods involved and can be repeated in the time. However, they quite oversimplify both biological and abiotic structural complexity and heterogeneity of soil and mainly disregard soil ecological functions. A whole ecological approach, through field investigations, is required. They can include investigations on the soil biota populations and their linkage with main ecosystem functions. Nutrient availability, such as carbon and nitrogen, is a common indicator of soils quality as it is an index of biological activity within the soils. This process is also regulated by several properties, such as quality of organic matter, temperature and moisture. This index, however, is very responsive to site disturbance although it can show wide natural variation depending on spatial and temporal variability (Knoepp et al., 2000). Litter decomposition is a fundamental biological indicator, involving the interaction of vegetation, soil nutrient availability and soil biota (Virzo de Santo et al., 2009). Increasing rates of litter decomposition accelerate nutrient cycling rates within the site and indicates increased soil quality. The study of faunal populations in soils immediately inform about the capability of soil to host fauna, detecting the presence or absence of some organisms. In addition the presence of some functional groups would indicate also on nutrient availability, decomposition rate and soil structure modifications. However, results coming from field investigations are often difficult to explain, as the presence or lack of some organisms can be functions of several factors.

Setting and monitoring soil quality indicators is important to ensure that soil function is maintained not only for the current land use, but also for potential future uses. The difficulty in determining appropriate indicators and their values for

multiple use sites increases in complexity as it is required to combine soil chemical, physical and biological variables.

1.7.2 Soil arthropods as biondicators of soil quality

Assessment of soil quality implies the study of the soil as a system apt to function as living system. In order to achieve this purpose, it is necessary to identify indicators capable of expressing this soil ability (Parisi et al., 2005).

Recently the study of soil quality focused on the evaluation of soil arthropod community (Nahmani and Lavelle, 2002; Eitminaviciute, 2006; Gongalsky et al., 2010). Arthropods, in fact, are convenient in soil quality assessment, as they are abundant, easy to sample, have relatively short generation times, and quickly respond to soil disturbance (McIntyre et al., 2001). Among them, Collembola is a focal taxon, as they have various feeding strategies and functional roles within the soil system (Fiera, 2009), influencing nutrient availability through their interactions with soil microorganisms (Cassagne et al., 2004), readily responding to microenvironment changes and human disturbance. For all these reasons, the study of soil arthropods and Collembola gives the possibility to better understand the linkage between soil abiotic alterations and soil biological processes. Unfortunately, because of contrasting results, little is known about how arthropods respond to soil abiotic and anthropic alterations. In fact, soil anthropic modifications has shown to exert, on soil biodiversity, a wide range of effects, which vary with soil use typology and can lead both to a decline and an enhancement of biodiversity (Battigelli and Marshall, 1993; Salminen et al., 2001; Eitminaviciute, 2006; Gongalsky et al., 2010).

Until few years ago, the study of soil fauna has mainly pointed on species richness, using a simple index, such as the number of species (Levrel et al., 2007), or other indices based on the taxonomical composition of communities under study, such as the diversity indices (i.e. Simpson, Shannon indices…) and evenness indices (i.e. Pielou, Menhinick indices…). Other measurements of soil quality took into account the ratios between peculiar taxon among soil community as the ratio Acarina/Collembola. High values of this ratio indicate high soil quality, as it has been established that in degraded soils the number of Acarina species decreases (Jacomini et al., 2000). These indices have shown to be not reliable for all ecosystems, as they are affected by highly abundant or very rare species. In many

cases, high values of these indices could derive from the presence of invasive species rather than from the presence of species that are well structured in the community (Jacomini et al., 2000; Parisi et al., 2005). Nevertheless, these indices are quantitative indices, expecting to assess soil quality on the basis of soil community taxonomical composition. They completely overlook the ecological role of the species and the interactions among the organisms and the environment (Vandewalle et al., 2010).

To overcome these hurdles, an attempt to build a soil biological quality index (QBS) based on ecological characteristics has been proposed (Parisi et al., 2001). The QBS consider a measure of soil quality, the adaption of organisms to live in the soil layer than in surface, separating them according to the morphology. QBS states that more groups of arthropods adapted to soil life, are present in a soil, higher will be its quality (Parisi et al., 2005). Notwithstanding QBS index can be considered as an ecological approach, it takes into account only the adaptation of organisms to soil life, without considering other parameters (diet, reproductive habits, life cycle), fundamental in estimating possible alterations of soil properties.

Therefore, the need to produce a suitable tool integrating more ecological characteristics of the arthropods is desirable to assess soil quality, as it has been suggested that ecosystem processes depend more on functional diversity than on species richness per se (Diaz and Cabido, 2001). One of these new tools, for the soil organisms, is the trait-based approach. This approach analyses morphological, physiological or phenological features of organisms, called traits (Violle et al., 2007). Traits are not directly linked with the environment, as intrinsic characteristics of organisms and, therefore, are not linked to the organism fitness. The link between traits and environment is assured because traits influence activities of organisms, called performances (Violle et al., 2007). When a trait influences a performance can be defined "functional" and can be an expression of environmental changes. Functional traits, capturing the environment selective pressure on the organisms, allow comparison among soils of different biogeography (Statzner et al., 2001; Hodgson et al., 2005). Traits such as body size, pigmentation, reproduction, mouthpart type can indicate specific sensitivity, adaptation or activities of organisms. Body size, in fact, can inform on the vulnerability of organisms (Makkonen et al., 2011), because smaller organisms have higher surface/volume ratio and thus higher susceptibility to exposure to soma contaminants. Pigmentation, on the other hand, indicate the adaptation of organisms to surface life, as pigmented organisms are more

difficult to predate than apigmented ones (Salmon and Ponge, 2012). The analysis of organism mouthparts is informative about diet (Bernays, 1998), and thus on the trophic role of each organisms within the community.

Trait-based approach was and is currently used in different fields of environmental studies, like ecology of plants (Lavorel and Garnier, 2002; Cornelissen, 2003; McKinney, 2002; Violle et al., 2007), stream invertebrates (Archaimbault et al., 2005) or fishes (Mims et al., 2010). Conversely, this approach remained little applied on soil ecology, although functional trait profile has been stressed to be an important parameter for revealing invertebrate responses to soil alteration (Ribera et al., 2001; Skalski et al., 2010). Only in the last decades this topic grew its interest (Ribera et al.; 1999; Weiser and Kaspari, 2006; Hedde et al., 2012), even though the investigations on soil arthropods in urban environment are still missing. Some studies (Blair, 1999; Clergeau et al., 2006; Posa and Sodhi, 2006; Sadler et al., 2006) have described changes in composition and structure of ecological communities associated with urban land use. Urbanization tends to act as an environmental filter selecting species on the basis of their biological traits (Lizée et al., 2011). Therefore, anthropized soils can show a different assemblage of species, able to perform different functions than natural soils. Likely, soils differently anthropized (i.e. urban, agricultural, industrial) can also vary among them for species composition and related activities. As a result, each human pressure could generate soils capable to ensure different functions in the ecosystem. Responses to these questions are still unknown.

CHAPTER II

Objective of the work

The goal of this research work was to analyse the quality and functionality of Mediterranean volcanic soils undergone to different kind of anthropization, through physical, chemical and soil mesofauna analyses.

Firstly, the present work evaluated the responses of soil organisms to soil metal pollution in urban environment. To this purpose, laboratory bioassays were performed on standard organism exposed to the collected urban soils. Then, responses of organism in controlled conditions were compared to the responses of *in situ* arthropod community. In addition, analysis of arthropod community aimed at assessing the presence of sensitive/tolerant taxa to soil different soil properties. As Mediterranean area is subjected to strong differences in climatic conditions along the year, the effects of seasonal variations on *in situ* arthropod community were also evaluated.

On the basis of anthropic impact the environment is differently modified. Therefore, the further aim of the project focused on the evaluation of physical, chemical and biological properties in soils subjected to different land uses. Soil physical and chemical characteristics were related to different soil typologies, in order to highlight if soils with similar land uses shared similar abiotic properties. Then, the effects of the abiotic properties on Collembola community were evaluated. Collembola community structure was studied following the taxonomical approach, studying specie assemblage and composition, and the functional approach, through the analysis of organism functional traits.

Laboratory assessments, as well as, field taxonomic and functional investigations are useful tools in defining the quality of a soil.

CHAPTER III

Materials and Methods

3.1 Studied area

The work was realized in Naples city and surroundings, in Campania region, Southern Italy. Surfaces of 17 soils were sampled and the different soils were grouped on the basis of land use typology in natural, urban, industrial and agricultural. Vesuvius National Park (VES N 40° 49' 23'' E 14° 23' 47.5'') and Astroni National Park (AST N 40° 50' 38.8'' E 14° 9' 34.4'') were natural soils. *Quercus ilex* L. forest was the main vegetation cover in both the soils. Urban soils undergone to different traffic flows were sampled in Naples city near two different roadsides (ACT N 40° 50' 7.4'' E 14° 15' 8.3''and MIA N 40° 52' 17'' E 14° 14' 59.7''), near two motorways (MAD N 40° 51' 17.1'' E 14° 16' 59.1'' and IOL N 40° 51' 59'' E 14° 15' 4.7''), and in an urban park (CAP N 40° 52' 19.2'' E 14° 15' 15''). All the sites are filling soils about 200 years old. These soils were chosen because they were not fertilised, and the only anthropogenic impact is traffic-related pollution. Also the urban soils were mainly covered by *Quercus ilex* L. trees. Two soils of dismissed metallurgical industries (BAGN and BQi N 40° 48' 12.7'' E 14° 10' 37.9'') and two soils near metallurgical industries (POMI N 40° 54' 54.2'' E 14° 23' 49''and TQ N 40° 59' 2.6'' E 14° 23' 49'') were sampled as soils representative of industrial activities. Vegetation cover was scarce in all the soils, but it was composed by shrubs and few *Quercus ilex* L. trees. Agricultural soils were sampled under different vegetation covers: two under orchards (TP N 40° 59' 2.6'' E 14° 23' 49'' and COMI N 40° 56' 32.4'' E 14° 32' 41''), three under vegetables (TV N 40° 59' 2.6'' E 14° 23' 49'', CIST N 40° 54' 20.5'' E 14° 24' 57.6'' and VC N 40° 53' 46'' E 14° 21' 2.2'') and one untilled field with no vegetation coverture (VU N 40° 53' 46'' E 14° 21' 2.2'').

For its geographical position, studied area was inserted in a typical Mediterranean climatic condition, characterized by dry-warm summer and cold-wet winter.

3.2 Plan of the analyses

The experimental work was made in two steps. In the first step the effects of urban soils (CAP, MAD, ACT, MIA and IOL), undergone to different traffic flows on soil organisms were assessed. Soil corers of 0-10 cm deep were sampled following the random sampling methods twice a year in autumn (September 2010). The soils were characterized for physical and chemical properties and for total and water-extractable Cu, Pb and Zn concentrations. In addition, in order to test the toxicity of urban soils, laboratory bioassays exposing *Eisenia andrei*, *Enchytrius crypticus* and *Folsomia candida* were performed. In the same soils arthropod community was extracted through Berlese-Tullgren extractor and the results of *in situ* arthropod community were compared with responses of laboratory exposed organisms. In order to assess the effects of seasonality on *in situ* arthropod community soil sampling was repeated in spring (April 2011).

In the second step the effects of different land use typologies (natural, urban, industrial and agricultural) on taxonomic and functional structure of Collembola (Arthropod: Exapoda) community were assessed and compared among them. Soil corers of 0-5 cm deep were sampled following the random sampling methods twice a year, in autumn (October 2011) and in spring (March 2012). The soils were characterized for physical and chemical properties, for total and water-extractable Cd, Cr, Cu, Ni, Pb and Zn concentrations and for total content of Polycyclic Aromatic Hydrocarbons (PAH). Collembola individuals were extracted by MacFadyen extractor (MacFadyen, 1961). In order to assess the effects of seasonality on Collembola community soil sampling was repeated in spring (March 2012).

A schematic plan of the analyses performed during the experimental work is reported in Figure 3.1.
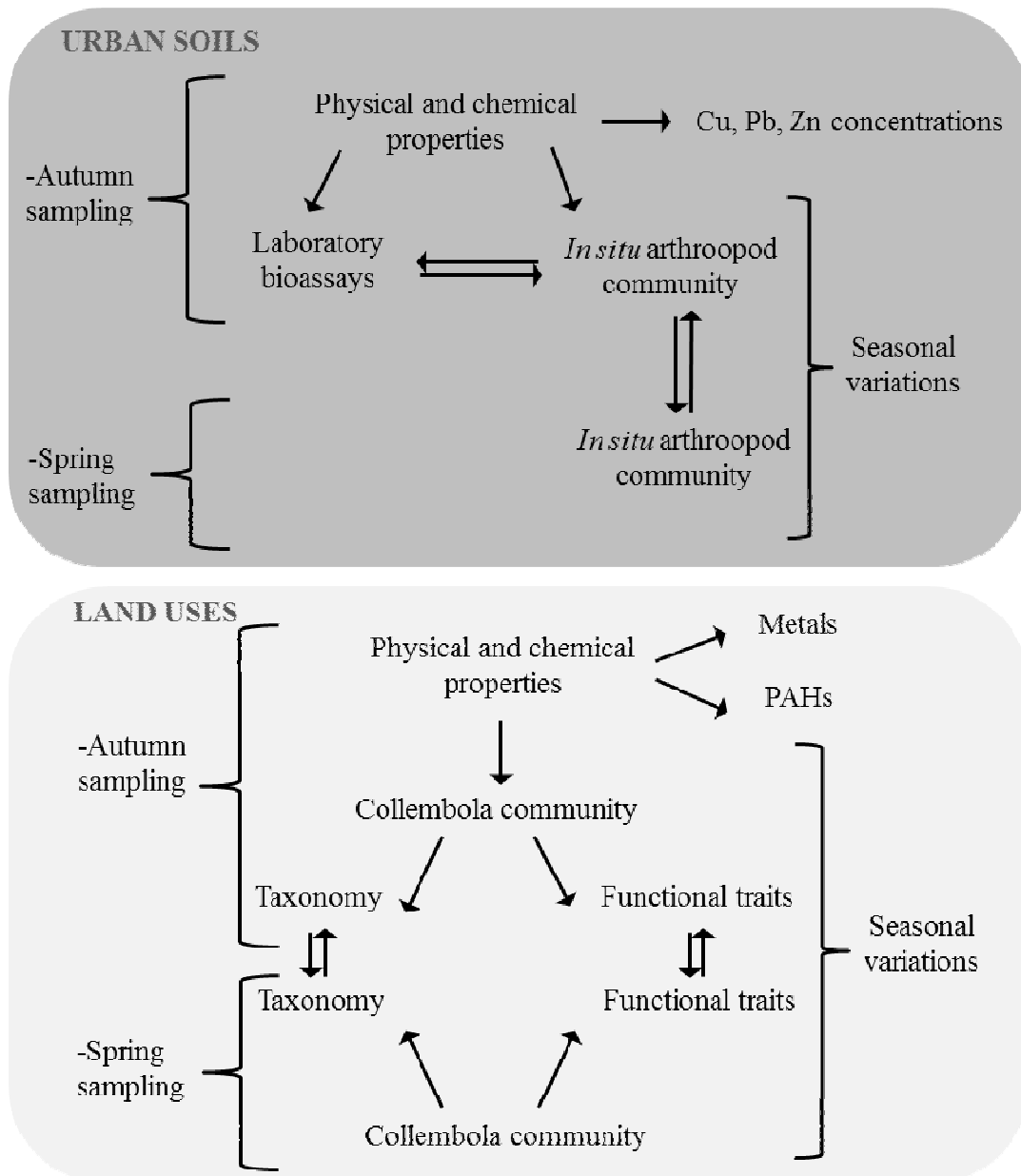
Figure 3.1. Schematic plan of the experimental work performed during the PhD

3.3 Soil physical and chemical properties

3.3.1 Soil physical and chemical properties

The following chemical and physical analyses were performed in triplicate, after mixing the soil samples collected at each site. in the result section only the mean values of soil physic chemical characterization were reported, as the standard error (s.e.) was always lower than 0.05. The sieved (2 mm) and oven dried (75°C over night) soil samples were characterized for bulk density (BD) calculated as the ratio of dry soil weight and soil volume (Baize, 1988), water holding capacity (WHC)

determined by gravimetric method after oven-drying to constant weight at 105 °C. In addition, pH (NF ISO 10390), total N content and organic matter content (OM) calculated on the basis of soil organic carbon content (ISO 10694), cation exchange capacity (CEC) total and exchangeable Al, Ca, Fe, Mg, Na (NF ISO 31-130), and 3 texture fractions (sand, silt and clay) were determined by the Laboratoire d'Analyse des Sols (INRA, Arras). The results of all the analyses were reported as a mean for each soil and the mean (s. e.) for each soil typology.

3.3.2 Soil metal concentration analyses

3.3.2.1 Soil metal concentration analyses in urban soils

In order to measure Cu, Pb and Zn concentrations, the soils were sieved (2 mm) and oven-dried (75° C over night). To measure total metal concentrations, 0.1 g oven-dried soil samples were digested with 2 ml of a mixture (4:1 = v:v) of HNO_3 (65%, p.a., Riedel-deHaën, Seelze, Germany) and HCl (37%, p.a. Baker Philipsburg, NJ, USA) at 140° C for 7 h in amacro destruction oven. The quality of the analysis was checked using ISE sample 989 (International Soil-Analytical Exchange) certified by Wageningen Evaluating Programs for Analytical Laboratories as reference material. Recoveries of Cu, Pb and Zn were always within 10-15% of the certified concentrations. To measure water-extractable metal concentrations, an oven-dried soil:distilled water suspension (1:2.5 = v:v)was prepared, shaken for 2 h at 200 rpm and filtered over a 0.45 mm filter. The total and water-extractable metal concentrations were measured by atomic absorption spectrometry equipped with a graphite furnace (PerkineElmer 5100; Cu and Pb) or flame (PerkineElmer AAnalyst 100; Zn) unit.

The Cu, Pb and Zn concentrations measured in urban soils by atomic absorption spectrometry were not reported in result section, as the values and trend in the soils were similar to those measured with the method described below. However for comparison, Cu, Pb and Zn concentrations were reported in the Table 3.1.

Table 3.1. Mean (s.e.) of total (tot) and water-extractable (w.e.) concentrations of Cu, Pb and Zn detected in urban soils form Naples collected in September 2010, and measured with atomic absorption,. Different letters indicate statistically significant differences (P < 0.05) among the soils (one-way analysis of variance with Holm-Sidak posthoc test).

	CAP	MAD	ACT	MIA	IOL
Cu$_{tot}$ (µg g^{-1} d.w.)	26.6 a (2.18)	33.7 a (1.62)	30.6 a (3.63)	114 b (8.65)	58.7 c (6.36)
Cu$_{w.e.}$ (µg g^{-1} d.w.)	0.35 a (0.08)	0.51 b (0.03)	0.58 b (0.11)	0.62 b (0.03)	0.40 ab (0.049
Pb$_{tot}$ (µg g^{-1} d.w.)	237 d (80.6)	126 c (6.56)	76.0 a (8.45)	94.9 b (7.41)	684 e (114)
Pb$_{w.e.}$ (µg g^{-1} d.w.)	0.09 a (0.03)	0.14 b (0.01)	0.14 b (0.04)	0.07 a (0.01)	0.10 a (0.03)
Zn$_{tot}$ (µg g^{-1} d.w.)	141 a (4.11)	270 b (1.24)	203 c (12.7)	330 d (8.32	999 e (28.7)
Zn$_{w.e.}$ (µg g^{-1} d.w.)	0.07 a (0.00)	0.21 b (0.00)	0.27 b (0.03)	0.39 c (0.00)	0.33 c (0.00)

3.3.2.2 Soil metal concentration analyses among different land uses typologies

Total and water-extractable Cd, Cr, Cu, Ni, Pb and Zn content were measured in each soil. To measure total metal concentrations, 0.5 g of soil samples were digested with 10 ml HNO3 (65% Sigma-Aldrich- Germany), 5,5 ml of H_2O_2 (AnalaR Normapur - France), and 5 ml of HCl (37%, Carlo Erba, Italy) at 95°C for 4 hours. After, the solutions were filtered over a 0.45 µm Whatman filter (US-EPA 3050b). To measure water-extractable metal concentrations, an oven-dried soil:distilled water suspension (1:2.5=v:v) was prepared, shaken for 2 h at 200 rpm and filtered over a 0.45 µm filter. The total and water-extractable metal concentrations were measured at ICP spectrometer (iCAP duo 6000 Series, Thermo Scientific). The quality of the analysis was checked using Certified Reference Material BCR-143R. Recoveries of Cd, Cr, Cu, Ni, Pb and Zn were always within 10-15% of the certified concentrations. The results of metal concentrations were reported as a mean (s. e.) for each soil and the mean (s. e.) for each soil typology.

3.3.3 Soil PAH concentration analyses

In order to measure PAH concentrations, the collected soils were pulverized and sieved to 500 µm. PAH extractions were done with an automated extractor Dionex® ASE 350. Approximately, 1 g of the each sample and 1 g of soil for the control were

placing in ASE Corers (10 ml). The extractions were performed at 100°C and 130 bars with Dichloromethane. Addition of copper powder and Na_2SO_4 in order to remove respectively the molecular sulfur and the remaining water has been realized before the extractions.
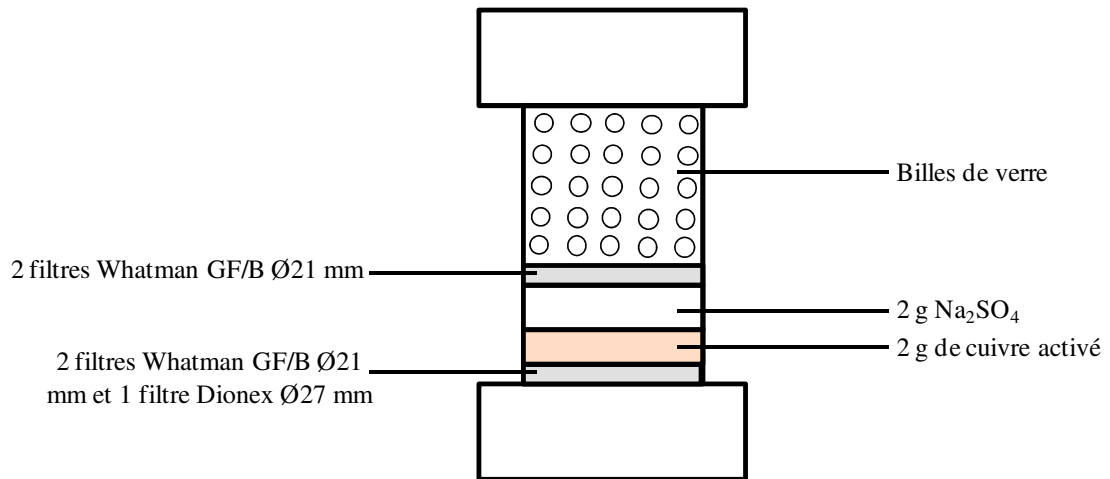
Figure 3.2. Schematic representation of PAH extraction by using Corers ASE.

The recovered extracts were, then, concentrated on nitrogen fluxes (Turbovap automatic evaporator) in order to evaporate the Dichloromethane, until 2-3 mm of volume. Then, the extracts have been diluted with Dichloromethane at 20 ml. Finally, an aliquot of 3 ml has been sampled and dried under a Sorbonne to quantify the extractible organic matter. The organic extracts have been analyzed by a gas chromatograph coupled to a mass spectrometer (GC-MS). For the PAH quantification, an internal PAH standard mix (naphthalene-d8, acenaphtene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12) has been added to the extract before the injection in the GC-MS. The PAH concentrations were expressed as ng g^{-1}, and as the mean of PAH concentration sum for all soil typology. The percentage PAH profile composition in each soil was also reported.

3.4 Laboratory exposition of standard organisms to urban soils

Urban soils collected in September 2010 were analyzed for their toxicity to the *Eisenia andrei*, *Enchytraeius crypticus* and *Folsomia candida*. Body metal accumulation, were related to survival, reproduction and growth of organisms. The results were compared with Lufa 2.2 used as a control soil (K).

3.4.1 Metal bioaccumulation analyses

In order to measure the metal uptake from the soil by the test organisms, the body metal concentrations in surviving adults of each species were measured. At the end of the bioassays, the animals were collected. To ensure no soil particles remained attached to the surface of the animals, the earthworms and enchytraeids were washed and placed on wet filter paper while the collembolans were scooped from the water surface and placed on moist plaster of Paris for some minutes. The earthworms were placed for 24 h on moist filter paper to empty their guts. Afterwards all animals were frozen and subsequently dried by freeze-drying (24 h). *F. candida* and *E. crypticus* were digested using a mini-digestion method. The animals were placed in thoroughly cleaned Pyrex tubes, and 0.5 mL of a mixture (7:1 = v:v) of HNO3 (Ultrex II) and $HClO_4$ (Ultrex) was added. Digestion was performed by heating the tubes, steadily increasing temperature. After evaporation of all acid, residues were taken up in 300 ml 0.1 N of HNO_3 (Utrex II). *E. andrei* individuals were digested in Teflon bombs, with 2 mL of a mixture (4:1 = v:v) of HNO3 (65%, p.a., Riedel-deHaën, Seelze, Germany) and HCl (37%, p.a., Baker Philipsburg, NJ, USA), by heating at 140° C for 7 h in a destruction oven. The digest was diluted to a volume of 10 mL, adding 8 mL of deionized water. The body metal concentrations were measured by atomic absorption spectrometry equipped with a graphite furnace (Perkin-Elmer 5100) for *E. crypticus* and *F. candida* or flame (Perkin-Elmer AAnalyst 100) for *E. andrei*. The quality of the analysis was checked using DOLT 2 certified by the National Research Council of Canada as reference material. Recoveries of Cu, Pb and Zn were always within 10–15% of the certified concentrations.

3.4.2 Bioassays

E. andrei were cultured in a substrate of potting soil and peat, and fed abundantly with manure from healthy horses not treated with any pharmaceuticals for at least 6 weeks. The tests used adult earthworms, with fully developed clitellum, and followed OECD guideline 222 (OECD, 2004b). Each toxicity test had four replicate 850 mL glass test containers covered with aluminum caps containing approximately 300 g soil (dry weight), with 5 earthworms randomly assigned to each container. At the start of the test, each batch of 5 animals was weighed to determine the starting

weights (1.96-2.33 mg; average ± s.e. 2.2 ± 0.04 mg; n = 5). After introduction of the earthworms, 5 g (dry weight) finely ground and moistened horse dung was introduced as a food source in a hole in the middle of the test soil. Once a week all test containers were opened to aerate the soils, correct for water losses and add additional food was provided if no food was visible anymore. After 4 weeks, surviving adults were collected, counted and weighed, and the soils were incubated for another 4 weeks to allow for hatching of the cocoons. After the second 4-week period, juveniles were extracted by placing the test containers in a water bath at 60 °C. All emerging juveniles were collected from the soil surface and counted. *E. crypticus* was cultured in aqueous agar prepared from a Lufa 2.2 soil extract (1 l of soil mixed with 3 l of tap water), and fed with oat meal. Tests followed OECD guideline 220 (OECD, 2004a). Ten adult animals, with clearly visible clitellum, were introduced into 100 mL glass test containers containing approx. 30 g moist soil. Five replicate test containers were used for each soil. Test containers were closed with perforated aluminum foil, and a small amount of crushed oat meal was added for food on top of the soil. Once a week, water losses were compensated by weighing all test containers and additional food was added if no food was visible anymore. After 4 weeks, the enchytraeids were fixed by adding 10 ml ethanol to each test container. After 1 min the suspension was transferred to a plastic jar using 100 ml of distilled water. The enchytraeids were stained by adding 300 ml of a 1% Bengal rose solution. The samples were again shaken rigorously and incubated for 24 h at approximately 4° C to achieve an optimal dying effect. Then the bright pink coloured adult and juvenile enchytraeids were counted. *F. candida* Willem 1902 were cultured in containers with a bottom of plaster of Paris and active charcoal (7:1 = w:w). Granulated dry baker's yeast was given for food. To age-synchronise animals, adults from the culture were allowed to produce eggs for 48 h in freshly prepared culture containers. After removing the adults, the eggs were allowed to hatch and the juveniles were used in the experiments when they were 10–12 d old. Tests followed ISO guideline 11267 (ISO, 1999), using 10 replicate 100 mL glass test containers covered with a plastic caps. Test containers were opened twice a week to aerate the test soils, and once a week to correct water losses and add additional food if no food was visible anymore. After 4 weeks, the content of a test container was flushed with 100 mL water into a 300 ml glass beaker. Upon gently stirring, all animals came to float to the water surface, and by making a photograph the number of juveniles

produced was counted. The number of surviving adults was counted by eye. All toxicity tests were incubated in a climate room at 20° C, and constant illumination. The results of survival and growth were reported as percentages, whereas those of reproduction as number of juveniles produced. Bioassays were also performed in uncontaminated natural Lufa 2.2 standard soil (loamy sand soil; 1.93% organic carbon, pH-CaCl$_2$ 5.5) that was used as a control.

3.5. Arthropod community analyses in urban soils

The analyses of soil arthropod communities collected in September 2010 and April 2011 were performed on each sub-sample collected at each site. To extract the arthropods, a core of 0-10 cm of soil samples were placed in a Tullgren apparatus (VU University, Amsterdam, The Netherlands) for four weeks (Van Straalen and Rijninks, 1982).

Figure 3.3. Tullgren extractor used for arthropod extraction in urban soils collected in September 2010 and April 2011, in Naples and surroundings.

The air temperature above the samples was 30° C while that at the bottom of the sample was kept at 5° C. The arthropods were collected in jars containing a 70% ethanol solution and saved at 4°C until the identification. The animals were counted and identified according the major taxonomic groups. The results of the arthropod community analyses are reported, for each soil, as density (i.e. individual number/m^2 soil), taxa richness (sum of taxa in each soil) and relative abundance (i.e. percentage of the individuals represented by each species on the total number of organisms).

For each site, data on the abundance and richness of arthropod taxa were integrated to calculate the (H) Shannon (1948) and (E) Pielou (1969) indices, Acarina/Collembola ratio (A/C) and soil biological quality index (QBS).

Shannon index is a measure of arthropod community diversity in each soil and was calculated as reported:

$$\textbf{Shannon index: H} = - \Sigma \ Pi*ln(Pi)$$

where Pi is the percentage of the individuals represented by species i on the total number of individuals. High diversity is indicated by high values of H index.

Pielou index is a measure of taxa distribution in the soils (evenness) and was evaluated as the following formula:

$$\textbf{Pielou index: E} = H/ln \ (total \ number \ of \ taxa)$$

High evenness is indicated by high values of the Pielou index.

The ratio between the total numbers of Acarina and Collembola (A/C) was calculated for each soil.

The soil biological quality index (QBS) was evaluated as reported by Parisi (2001). This QBS index classifies soil microarthropods on the basis of morphological characteristics, assigning to each microarthropod group a different weight, represented by a different score, thereby defining the Ecomorphological indices (EMI) shown in Parisi (2001). The QBS is calculated as the sum of EMI values in each soil.

3.6. Collembola community analyses in different land uses

The analyses of the soil Collembola communities collected in October 2011 and March 2012 were performed on each sub-sample collected at each site. The organisms were extracted using the MacFadyen extraction (Fig. 3.4) method (University of Lorraine, Nancy, France) trough dry extraction for 1 week, according to MacFadyen (1961).

Figure 3.4. MacFadyen extractor used for Collembola extraction in soils with different land uses collected in October 2011 and March 2012, in Naples and surroundings.

The organisms were saved in boxes with a 70% ethanol solution, counted, and identified to species level, at phase-contrast microscope, according to the dichotomic keys Hopkin (2007), Bretfeld (1999), Potapow (2001), Thibaud et al. (2004).

In order to evaluate taxonomic structure of Collembola community of each soil, the results of the Collembola community analyses were reported as density (i.e. number of Collembola m^{-2}) and species richness (i.e. sum of species found in each soil), and species composition relative abundances, reported for the four soil typologies. In addition, density and species richness results were integrated to calculate Shannon index (H) and Pielou index (E), according to formula reported above for the arthropod community analysis. The results of all the analyses were reported as a mean (s. e.) for each soil and the mean (s. e.) for each soil typology.

3.6.1. Functional traits calculation

Functional traits were analysed in order to evaluate differences in functional structure of community among land use typologies.

Five traits (Body lenght, Motion strategy, Mouthpart type, Pigmentation and Reproduction) describing morphology and physiology of Collembola, were collected from numerous identification keys or synopses. These traits were chosen because

considered responsive characteristics of environmental changes, as land use transformation is supposed to be. Attributes of each trait (Table 3.2) were considered as variables, resulting in a list of 14 attributes.

Table 3.2. Traits and attributes considered for the analysis of Funtional structure of Collembola community

Trait	Attribute
Body lenght	Inferior 0.35 mm
	0.35-1 mm
	1-2 mm
	2-4 mm
	4-7.5 mm
Motion strategy	Walk
	Jump
Mouthpart type	Without
	Normal
	Strong
Pigmentation	Without
	With
Reproduction	Asexual
	Sexual

Trait attributes were coded for each species following the fuzzy method developed by Chevenet et al. (1994) and applied for the BETSI project (Biological and Ecological Traits of Soil Invertebrates.). This method attempts to synthetize and code the diverse information available on functional traits. Because of the heterogeneity of organisms within and between species and the missing data, traits were coded by reducing continuous variables into a limited number of subsets with specific characteristics.

The information obtained from each source was coded as showed in the table 3.3, by an affinity score ranging from 0 to 3 (from no to very high affinity of the species to a trait category, respectively). Subsequently, affinities were summed to build the trait profile (e.g. the distribution of affinity within category of a trait).

Table 3.3. example of trait information coding for Body length (BLR) in Folsomia candida (FOL_CAN)

Species	Code	Class of lenght	Code attribute	Description	References
FOL_CAN	1	BLR_0.35_1	BLR	Total body length varies, adults from 0,9 to 2,5mm	Potapow 2001
FOL_CAN	2	BLR_1_2	BLR	1,5-3mm	Gisin 1960
FOL_CAN	3	BLR_1_2	BLR	Total body length varies, adults from 0,9 to 2,5mm	Potapow 2001
FOL_CAN	1	BLR_2_4	BLR	Size up to 2.5mm or larger	Fjellberg 2007
FOL_CAN	2	BLR_2_4	BLR	1,5-3mm	Gisin 1960
FOL_CAN	1	BLR_2_4	BLR	3mm	Hopkin 2007

If the extension of the trait is between [0.66 – 1.00] the value of attribute will be «3»

If the extension of the trait is between [0.33 – 0.66] the value of attribute will be «2»

If the extension of the trait is between [0.00 – 0.33] the value of attribute will be «1»

Trait profiles were standardized so that their sum for a given species and a given trait equalled 100%. Scores 'zero' for all categories of a trait signify that information is not currently available. In that case, taxa take the mean trait profile of all other taxa in subsequent trait analyses (i.e. such a species does not contribute to patterns). Finally, we calculated the mean trait category affinity per community (CMW), as the average of category affinity values weighted by the relative abundance of species carrying each affinity (Garnier et al., 2004) with the following equation:

$$\mathbf{CMW} = \Sigma \ PiXi$$

where Pi was the relative abundance of each species and xi was the trait category affinity of the i species.

3.7. Statistical analyses

The Kolmogorov Smirnov test was applied to assess the normality of the distribution of the data sets. Pearson's regression test was performed to evaluate the relationships between the assessed variables. One-way Analysis of Variance (ANOVA), with Holme Sidak posthoc test was performed to highlight differences among the sites with respect to the tested variables. The package Sigma-Plot 11.0 (Jandel Scientific, USA) was used for all these analyses.

A Principal component analyses was performed on all the physical and chemical properties of collected soils, in order to highlight the Euclidean distance among the

soils and classify the soils in different groups. PCA aims at representing the major features of the data along a reduced number of axes (hence, the expression "ordination in reduced space"). This analysis, therefore, gave the positions of the soils in a system of coordinates. Two plots are represented in the result section. One plot showed the objects (soils) as points and the second plot showed the variables, which define the objects, as arrows. The eigenvalues reported on each axe displayed the amount of variance is explained by that axe.

Nonmetric multidimensional scaling (NMDS) was performed on the responses of survival, reproduction and growth of standard organisms exposed to urban soils, on the responses to seasonal variations of arthropod community in urban metal polluted soils and on the responses to seasonal variations of Collembola functional traits in soils with different land uses. NMDS is not an eigenvector-based method, but it tries to represent as well as possible the ordering relationships among objects in a small and specified number of axes. It is performed to represent the relationships among the variables describing the objects.

Redundancy analysis (RDA) was carried out in order to assess the relationships between soil physical and chemical properties and Collembola functional traits. RDA is a canonical multivariate ordination which explores the relationships between two matrices. RDA is a method combining regression and principal component analysis (PCA). It is a direct extension of regression analysis to model multivariate response data. The RDA was carried out considering independent variables represent by abiotic properties of collected soils and dependent variables, represented Collembola functional traits, in order to define the direct effect of soil physical and chemical properties on collembolan functional structure. The results give the eigenvalues themselves, as well as the cumulative proportion of variance explained (for the RDA axes) or represented (for the residual axes). The last cumulative value is therefore 1. The cumulative contribution to the variance obtained by the independent variables is the proportion of the total variance of the response data explained by the RDA.

PCA, NMDS and RDA were carried out with the package R-2.15.2.

CHAPTER IV

Results and Discussion

4.1 Soil physical and chemical characterization of investigated soils

4.1.1 Physical and chemical properties

The textural composition highlighted high content of clay and silt in all the investigated soils (Fig. 4.1), that belonged to clay, silty clay, silty clay loam and silt loam textural classes (Fig. 4.1).
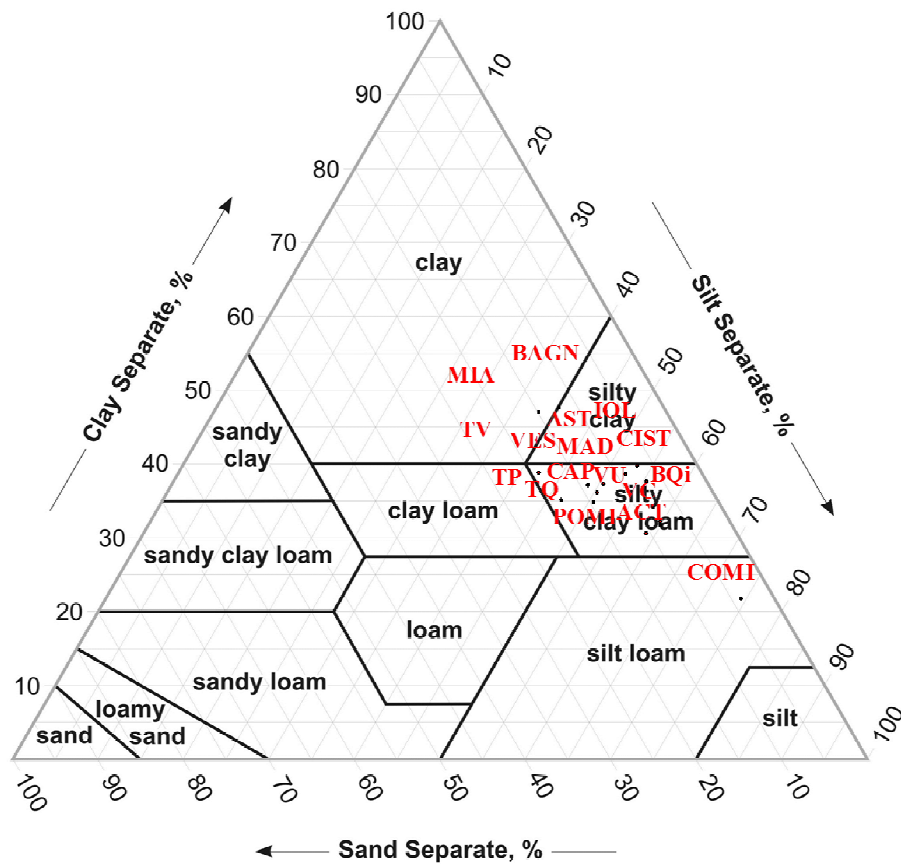
Figure 4.1. Textural composition of soils collected in Naples and surroundings, Italy.

For almost all the soils pH was around neutrality (Table 4.1), slightly acidic for VES (5.87) and slightly basic for TQ (8.30) and TP (8.30), on average pH was lowest at Natural sites. The values of bulk density (BD) were similar for all the investigated soils (Table 4.1), ranging from 0.44 (VES) and 1.03 (COMI). At agricultural soils, bulk density was higher than all the others site typologies (Table 4.1). Particularly

high content of organic matter were detected at VES, CAP, MIA, IOL and POMI (Table 4.1). On average, natural and urban soils showed the highest values of organic matter, whereas agricultural soils showed the lowest values (Table 4.1). Water holding capacity and cationic capacity exchange were higher at urban soils, with particularly high values measured at CAP and MIA (Table 4.1). Water holding capacity was positively (P < 0.001) correlated with soil silt content and negatively (P < 0.001) with sand content. C/N was higher at natural and agricultural soil (Table 4.1).

Table 4.1. Mean of soil physical and chemical characteristics measured in soils collected in Naples and surroundings in October 2011. The mean value (s.e.) reported under each soil typology indicate the mean for that typology. Different letters indicate statistically significant differences among soil typology mean (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

|		pH	BD (g cm^{-3} d.w.)	OM (% d.w.)	WHC (% d.w.)	C_{tot}/N_{tot}	CEC (cmol$_{(+)}$kg^{-1})
NAT	**VES**	5.87	0.44	25.3	44.1	21.9	21.3
	AST	6.99	0.56	13.3	55.4	14.2	31.0
	mean	**6.43 a** (0.56)	**0.50 a** (0.06)	**19.30 a** (6.00)	**49.77 a** (5.65)	**18.08 a** (3.87)	**26.15 a** (4.58)
URB	**CAP**	7.37	0.56	18.8	61.4	13.9	52.4
	ACT	6.95	0.71	8.34	47.0	17.0	19.3
	MIA	7.78	0.57	19.0	71.1	16.8	54.3
	MAD	6.94	0.70	8.89	50.4	13.8	23.8
	IOL	7.38	0.63	13.90	50.0	19.2	24.8
	mean	**7.28 a** (0.15)	**0.63 b** (0.03)	**13.79 b** (2.3)	**55.97 b** (4.50)	**16.14 b** (1.03)	**34.92 b** (7.59)
IND	**BAGN**	7.82	0.66	8.38	58.7	14.3	21.4
	BQi	7.10	0.85	4.84	35.5	15.1	12.2
	POMI	6.91	0.51	16.50	43.1	14.8	33.2
	TQ	8.30	0.75	7.32	43.2	23.0	22.2
	mean	**7.53 a** (0.32)	**0.69 b** (0.07)	**9.26 c** (2.5)	**45.14 a** (4.88)	**16.79 b** (2.06)	**22.25 a** (4.29)
AGR	**TP**	8.08	0.81	5.90	45.3	30.1	20.2
	COMI	7.41	1.03	3.53	35.0	14.4	11.0
	TV	7.84	0.89	4.12	43.0	32.9	16.0
	CIST	7.42	0.90	5.88	42.1	11.4	15.3
	VC	6.98	0.98	4.35	34.1	11.4	12.4
	VU	6.42	0.89	4.26	34.2	12.3	8.08
	mean	**7.36 a** (0.24)	**0.92 c** (0.03)	**4.67 d** (0.40)	**38.91 c** (2.06)	**18.74 ab** (4.07)	**13.66 c** (1.85)

4.1.2 Soil major and trace element concentrations

Concentrations of major elements (Al, Ca, Fe, Mg and Na) and trace elements (Cd, Cr, Cu, Ni, Pb and Zn) are reported in Figg 4.2, 4.3. Total Al and Fe

concentrations showed similar values for all the investigated soils, except at TP and
TV which were particularly low (Fig 4.2). Total Ca concentration also was similar
among the soils, but it was higher at TP and TV (Fig 4.2). Total Mg showed the same
trend of Al and Fe, showing higher values at VES and in all the agricultural soils,
except TP and TV (Fig. 4.2). Total Na was similar among the soils, although it was
lower in the agricultural soils (Fig. 4.2).

Available fractions of Al and Fe showed similar trends, being higher at VES and
VU (Fig. 4.2). Ca available fractions were higher at CAP and MIA, whereas MG
available fraction was particularly high at VES, AST, CAP, MIA, POMI, TQ and TV
(Fig. 4.2). On average the mobility of Mg decreased passing from natural to
agricultural soils (Fig. 4.2). Na available fraction was similar among all the soils,
with the exception of TV where it showed the highest values (Fig. 4.2).
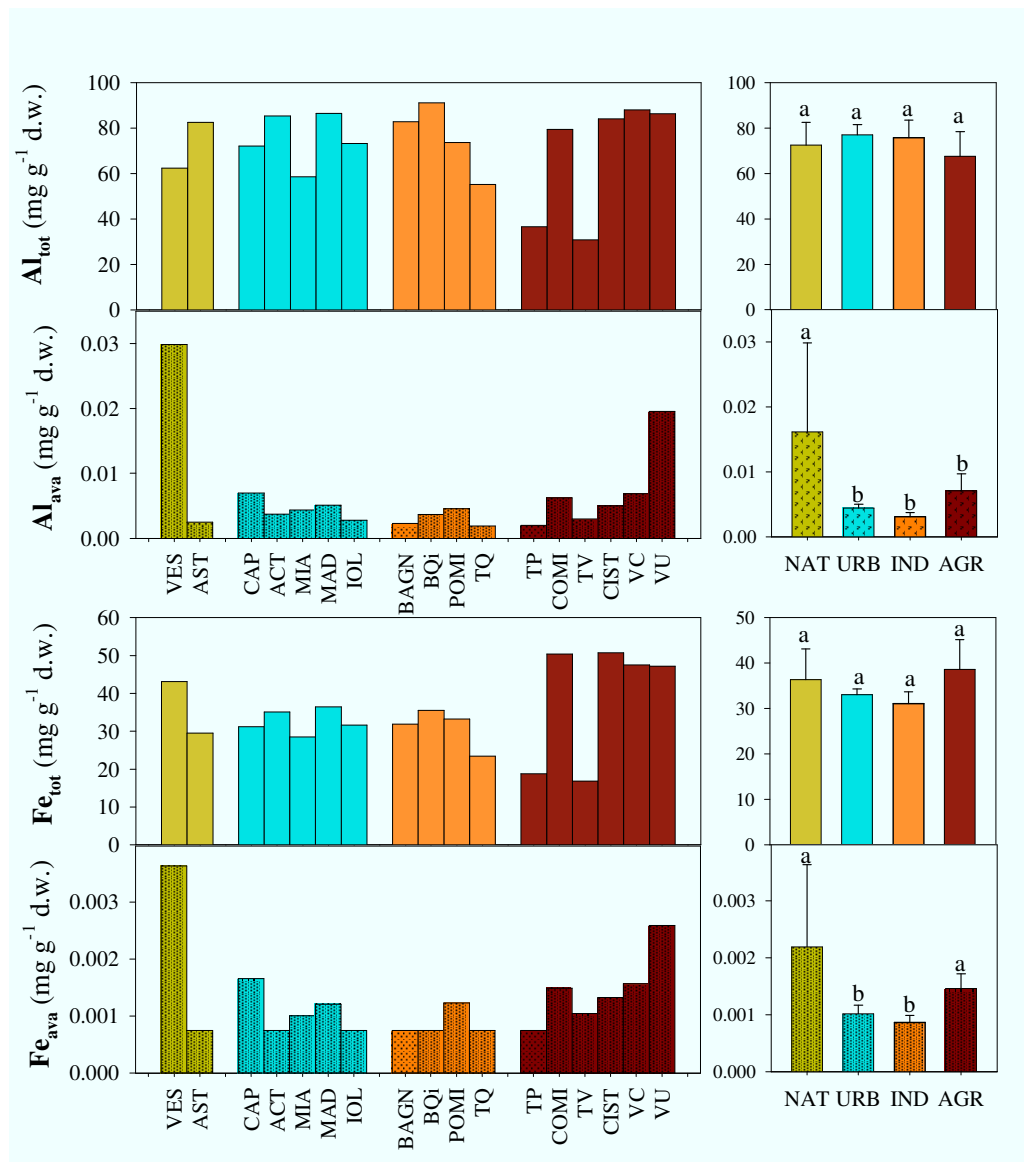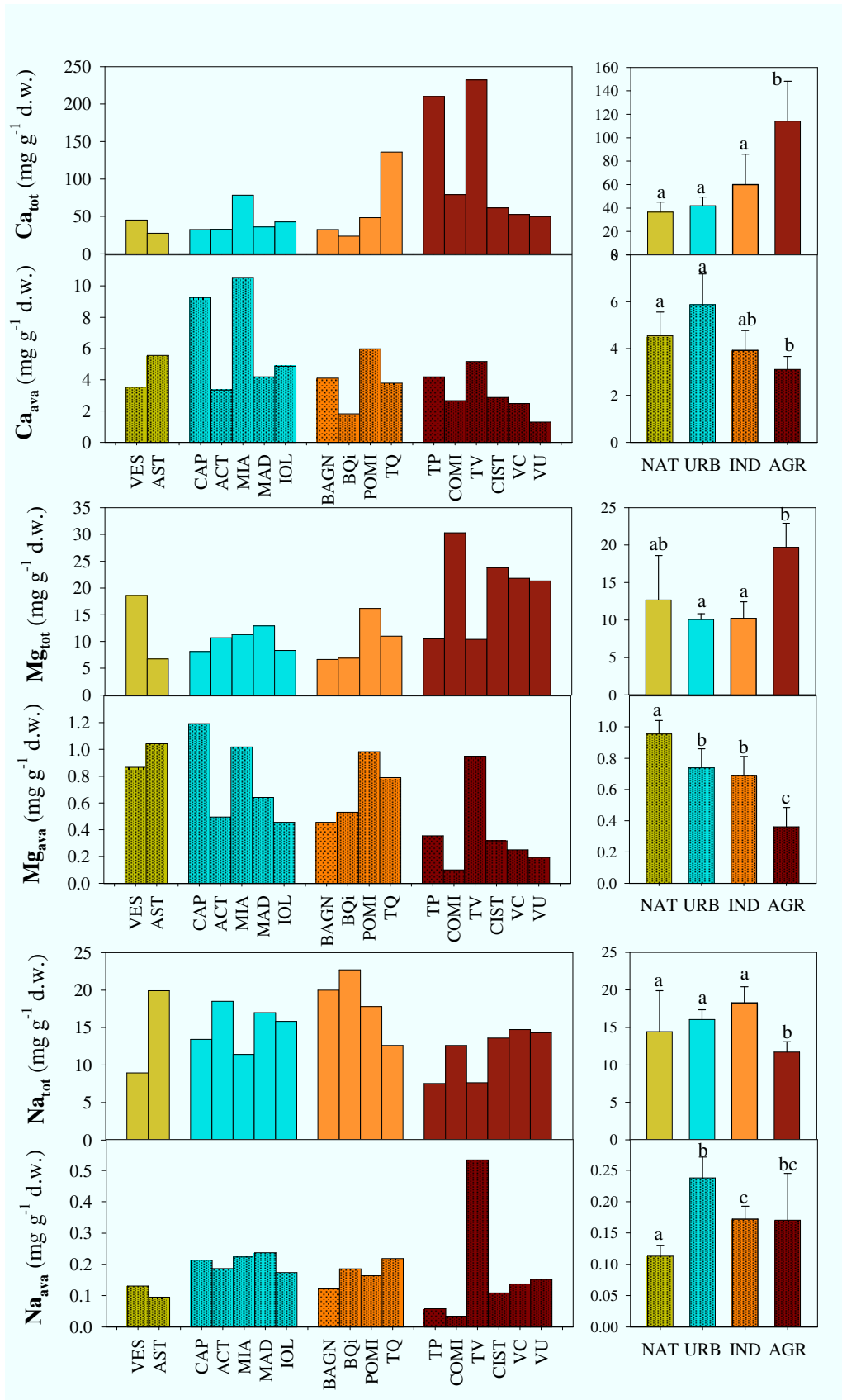
Figure 4.2. Mean of major element total (tot) and water-extractable (w.e.) concentrations measured in soils collected in Naples and surroundings in October 2011. The mean value (s.e.) reported near each elements indicate the mean for each soil typology. Different letters indicate statistically significant differences among soil typology mean (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

Total Cd concentrations showed similar values among the soils, with higher values detected at VES, COMI; CIST, VC and VU (Fig. 4.3). All the soils exceed the background level (straight line) detected for Cd. Cr concentrations were highly variable among the soils, showing the highest values at IOL, TP, CIST and VC (Fig. 4.3), although the soils never exceed the background level. The concentration of Ni was variable among the soils, and it was higher at POMI, COMI, CIST, VC and VU (Fig. 4.3), never exceeding the background level. Cu concentrations showed highest values at MIA and CIST. Agricultural soils as well as MIA, MAD and IOL exceeded the background level (straight line) detected of Cu in the same area (Fig. 4.3).
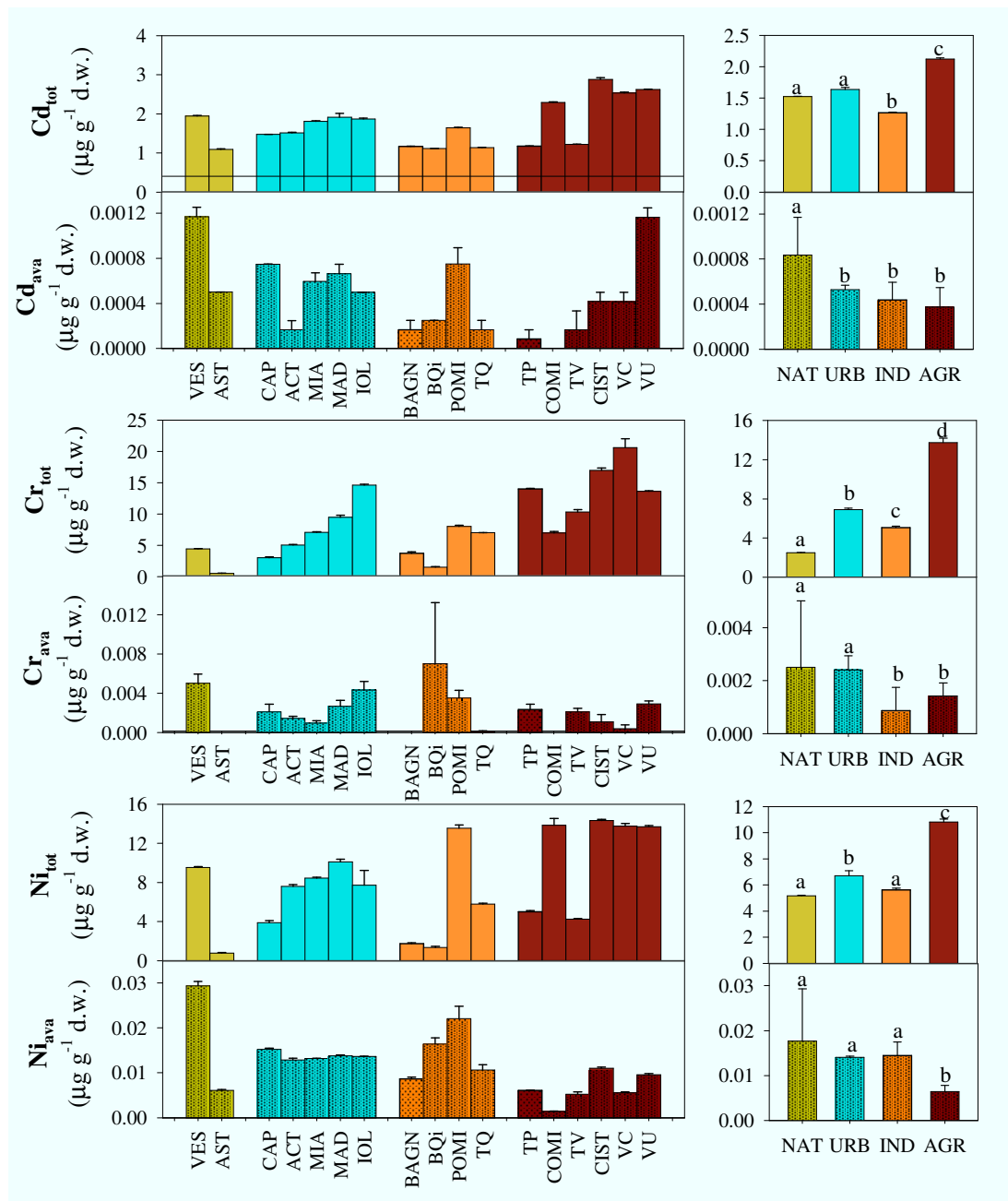
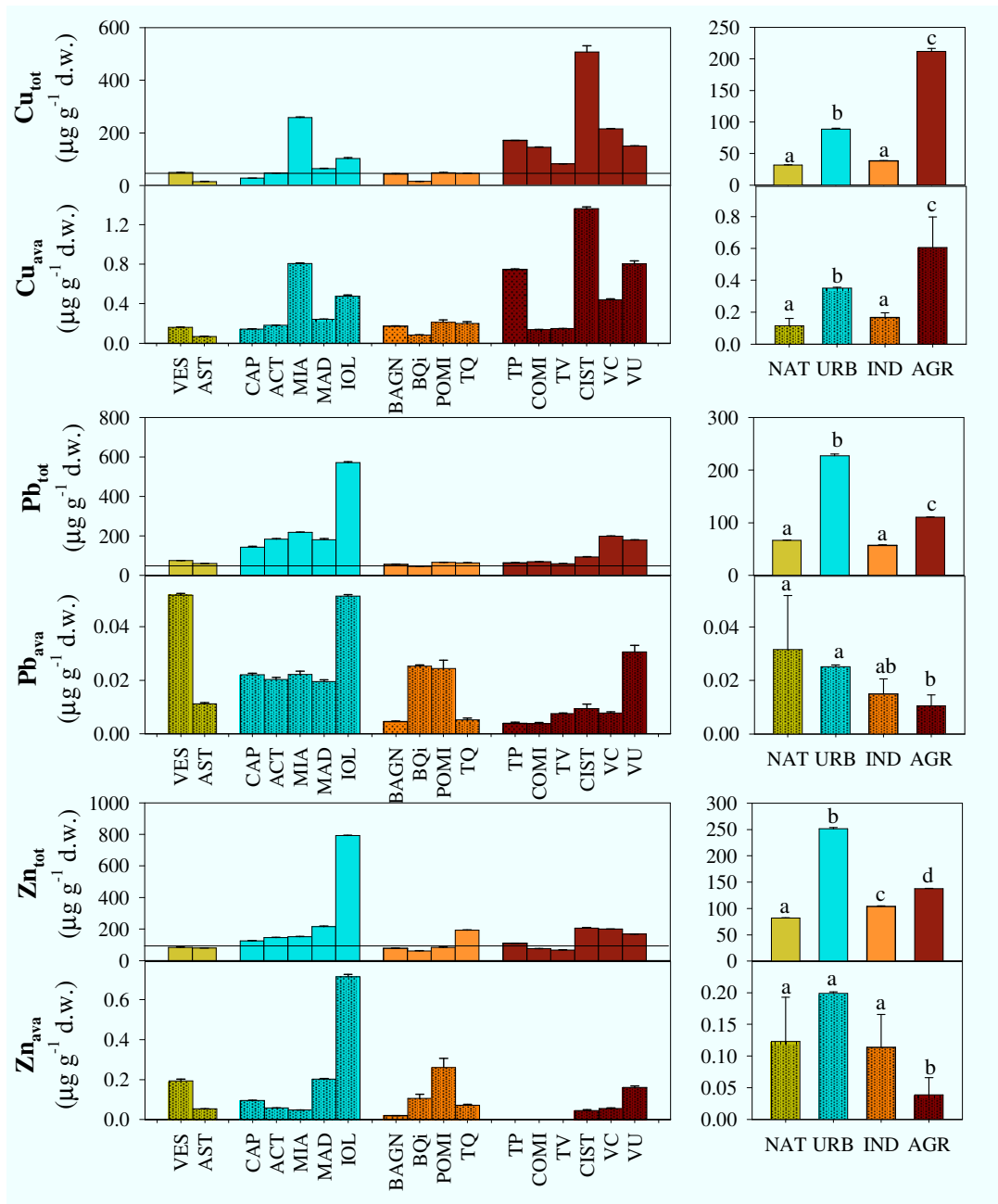**Figure 4.3.** Mean of trace element total (tot) and water-extractable (w.e.) concentrations measured in soils collected in Naples and surroundings in October 2011. The mean value (s.e.) reported near each elements indicate the mean for each soil typology. Straight line indicate the background concentration. Different letters indicate statistically significant differences among soil typology mean (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

Pb and Zn showed similar trends, showing the highest values at IOL (Fig. 4.3), but in all the urban soils and at CIST, VC and VU they exceed the background levels (straight line). Total Cd, Cr, Cu and Ni concentrations and total Pb and Zn concentrations were positively correlated (P < 0.01) among them.

The water-extractable fractions of Cd was higher at VES, POMI and VU, whereas Cr showed similar among all the soils (Fig. 4.3). Ni water-extractable concentration was higher at VES and POMI (Fig. 4.3). The water-extractable fraction of Pb was

highest at VES and IOL, where also the highest water-extractable Zn concentration was measured (Fig. 4.3).

Cd, Cr and Ni fractions negatively correlated ($P < 0.05$) with pH and positively ($P < 0.05$) with organic matter content. Pb water-extractable content was negatively correlated ($P < 0.05$) with pH and positively ($P < 0.05$) to organic matter and its total content. By contrast, Cu and Zn water-extractable concentrations were positively correlated ($P < 0.05$) with the total concentrations.

4.1.3 Soil PAH concentrations

The sum of PAHs showed the lowest value at Natural sites and the highest at Urban sites (Fig. 4.4).
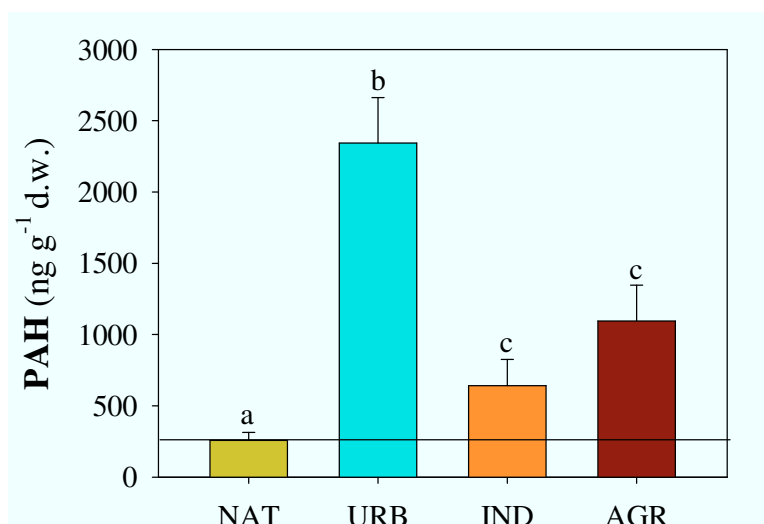
Figure 4.4. Mean (s.e.) of sum of PAH concentrations measured in each soil typology collected in Naples and surroundings in October 2011. Straight line indicates the background concentrations. Different letters indicate statistically significant differences among soil typology mean (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

Main differences in PAH profiles among soils were detected for particularly high content of dibenzo(a,h)anthracene in both natural soils, Fluoranthrene and Pyrene at ACT and benzo(g,h,i)perylene at IOL and acenaphtene at COMI (Fig. 4.5).

The percentage of low (LMW), medium (MMW) and high (HMW) molecular PAHs in different soils highlighted that PAHs with medium and high molecular weight were more abundant in all the soil typologies (Fig. 4.6). LMW PAHs were more abundant at natural and industrial soils, whereas MMW PAHs were more abundant in urban soils, and HMW were more abundant in agricultural soils (Fig. 4.6).
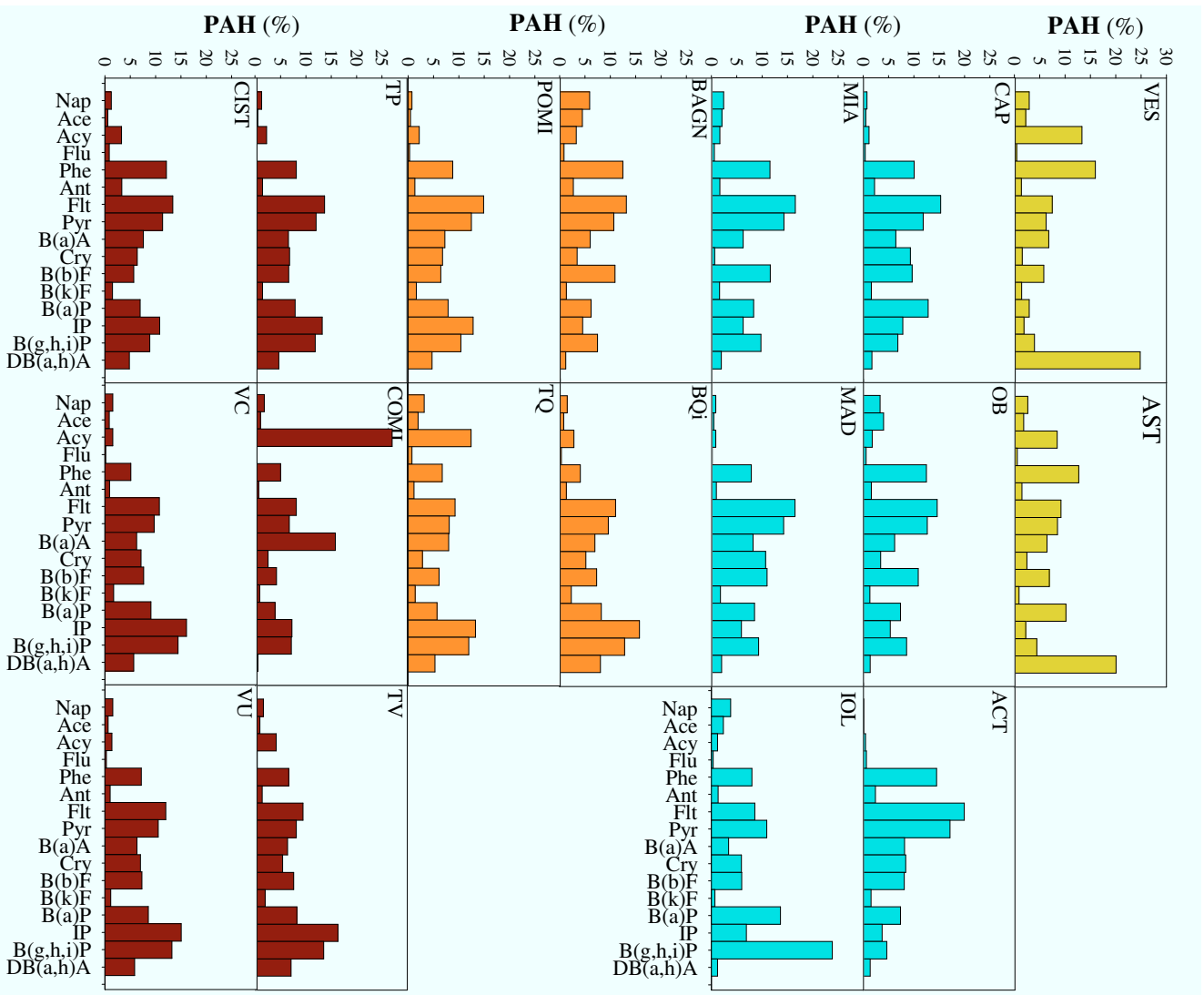
Figure 4.5. Percentage of each PAH detected in all the soils collected in in Naples and surroundings in October 2011.
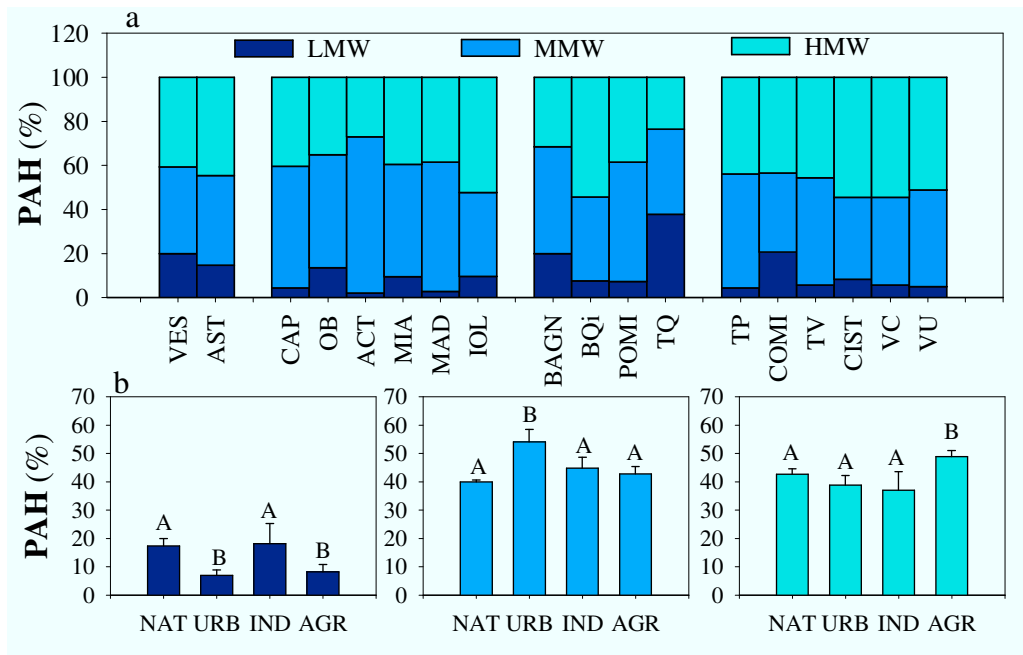
Figure 4.6. Percentage (a) of low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW) of PAHs detected in all the soils, and (b) mean (s.e.) of mean values of LMW, MMW, and HMW detected in each soil typology collected in in Naples and surroundings in October 2011.

PCA analysis was carried out to classify the all the soils on the basis of the physical and chemical properties. The PCA highlighted the presence of 6 clusters. Clusters 1 and 2 were composed mainly by urban (CAP, MIA, ACT, MAD) and industrial (BAGN, BQi, POMI) soils. Also a natural soil (AST) belongs to the cluster 2. These soils were characterized by high cationic exchange capacity, high water holding capacity, medium content of organic matter, scarce presence of trace elements (Fig. 4.7).

a   **Hierarchical clustering on the factor map**

Variables factor map (PCA)
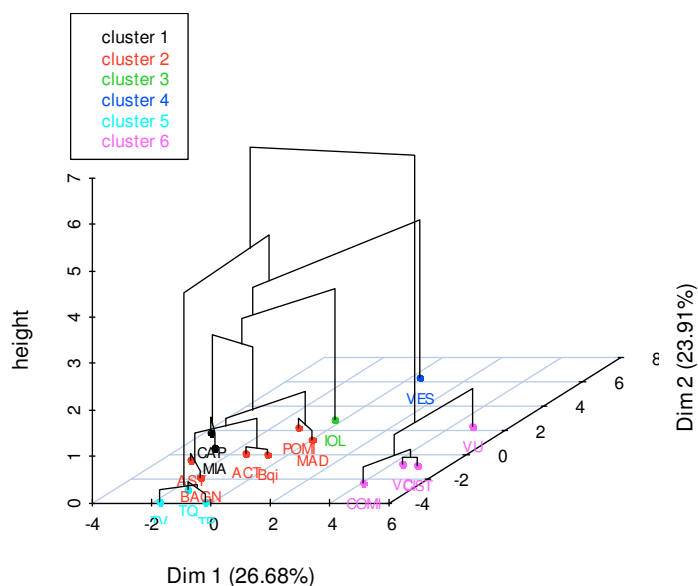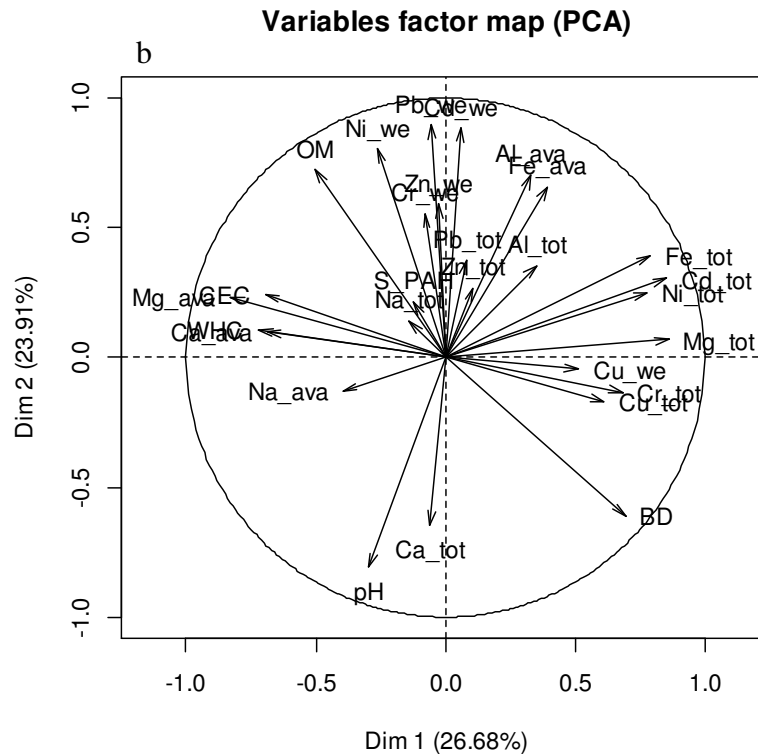
Figure 4.7. PCA results of (a) ordination of soils on the basis of physical and chemical properties of soils collected in Naples and surroundings in Ocotber 2011, and (b) circle of variable distribution used for performing the PCA.

Cluster 3 is composed by an urban soil (IOL) characterized by high concentrations of Pb and Zn and high PAH contents. Cluster 4 was formed by a natural soil (VES) with high organic matter content and low pH values, and high concentrations of soluble element fractions (Fig. 4.7). Cluster 5 is formed both by industrial (TQ) and agricultural (TP, TV) soils, which shared high pH values and high total Ca concentrations. The cluster 6 is formed only by agricultural (COMI, CIST, VC, VU) soils characterized by high bulk density and high total concentrations of Cd, Cr, Cu, Ni.

4.1.4 Discussion

The site separation obtained on the basis of physical and chemical properties, showed wide differences among the soils, which are partially due to land use management. In fact, the collected soil presented more or less expressed characters derived from volcanic materials, with clay and iron oxides accumulation (www.soilmaps.it). Textural composition and bulk density showed values typical of soils with andic properties (Di Gennaro and Terribile, 1999; World reference base for soil resources, 2006), indicating that the pedogenetic substrate of the investigated soils was similar. However, the higher bulk density measured in the agricultural soils

indicates that the agricultural management could lead to a soil compaction, as also reported by Hamza and Anderson (2005).

Organic matter content in the soils can be also linked to soil management, as its accumulation mainly at natural and urban soils appears to depend on litter deposition. In fact, at agricultural site, where vegetation is removed, the organic matter amount was significant lower than in the other site typologies. Variations in soil water contents were not related to organic matter content, but rather than to soil texture, as also confirmed by the found correlations. Little and colloids particles showed high capability to retain water (Bullini et al., 1998; Wu et al., 1990).

Al and Fe were the most abundant elements in the investigated soils, as they are also the most abundant elements in Earth's crust (Kabata-Pendias and Mukherjee, 2007). The scarce differences detected among the soils Al and Fe, as well as for Mg, are attributable to high content of allumino silicates and Fe-Mg silicates contained in general in the volcanic soils (Olafur et al., 2007). However, not all the investigated soils showed same Al and Fe values. TP and TV sites, presented low Al and Fe content and high Ca content indicating a calcareous character (Jakovljević et al., 2003), which explain also the high value of pH detected in the same sites. Although TP and TV are both agricultural soils, these differences cannot be attributable to the land use management, but they appear to be linked to a different substrate composition. With some exceptions, on average the major element content in the soils was similar, as all the investigated elements are present in silicate rock-forming minerals (Jakovljević et al., 2003), which are abundant in volcanic soils.

Trace element concentration in investigated soils showed wider variations than the major elements. The average background level reported for volcanic soils for Cd, Cr, Cu, Ni, Pb and Zn is respectively 0.42 μg g^{-1}, 47.84 μg g^{-1}, 64.45 μg g^{-1}, 16-33 μg g^{-1}, 52.16 μg g^{-1}, 103.50 μg g^{-1} (Maisto et al., 2006; Olafur et al, 2007). Therefore in the collected soils only Cd, Cu, Pb and Zn exceed the background level and can be considered as contaminants. Cd exceeded the background in all collected soils, which being andosoils, they are naturally contaminated by volcanic eruptions (Olafur et al, 2007). Cu concentrations exceed the background content in agricultural and urban soils. Cu enrichment in agricultural soils appears to be dependent on widespread agricultural amendments Cu-rich fertilizers (Olafur et al, 2007). In urban environment, by contrast, Cu, as well as, Pb and Zn accumulation appears to be dependent to vehicular traffic, as they are mainly emitted by traffic-related activities

(Wong et al., 2006). In particular, Cu is emitted by vehicle brakes, Pb is associated with coarse particles deriving from vehicular exhausts, and Zn comes from tyre wears (Davis et al., 2001). In contrast with the concentrations of major elements, which was no or scarcely influenced by soil land management, trace elements accumulation in soils seems mainly deriving from anthropic activities. In fact, even though Cr and Ni did not exceed the natural soil background their concentrations, as well as Cu and Cd concentration was higher in agricultural soils, suggesting a common emission source, as confirmed by the correlation found among them. Higher presence of Cd, Cr, Cu and Ni in agricultural soils can be linked to the fertilization practice, which may add to soils conspicuous amounts of Cd, Cr, Cu and Ni (Kabata-Pendias and Mukherjee, 2007) and therefore, these elements could be considered markers of agricultural management. By contrast, Pb and Zn, being higher in all urban soils could be considered markers of urban pollution.

Trace element water solubility and major element availability were not depending on soils land management, but they are highly governed by several soils properties. Differences in soluble fractions were mainly due to the pH and organic matter content of investigated soils, as also confirmed by several found correlations. Al, Fe, Cd, Cr and Ni are often associated with colloids and organic matter, which play a fundamental role in affecting their mobility (Kabata-Pendias and Mukherjee, 2007). Their presence at VES it is not surprisingly as in this soil the lowest pH and the highest organic matter content were detected. It is important to note, then, that even if VES is a natural it presents the highest content of element available fractions which distinguish it from AST, the other natural soil, and from all other kind of soils. The high Pb water-extractable fraction measured at VES is also due to the low pH values, as confirmed by the negative correlation found. In fact, the mobilization of Pb is usually slow, but the increase of acidity and the content of organic matter may increase its solubility (Kabata-Pendias and Mukherjee, 2007). However Pb soluble fraction is also related to its total content, as shown by its high content at IOL. This result suggests that available fraction of Pb is depending on acidity but also on its total concentrations. As confirmed by the found correlation, Cu and Zn water-extractable concentrations followed the same trend of total content, being higher at agricultural and urban soils. Although Cu and Zn mobility is controlled by several parameters, it is strictly depending on element form and soil structure. In fact, Cu

mobility in soils is depending on its form (Ponizovsky et al., 2006), whereas silicates may contribute to the Zn retention in soils (McBride et al., 1997).

The elevate abundance of PAHs in urban soils appear to be dependent to combustion processes occurring the urban area. In fact, combustion processes are the main responsible of the abundance and profiles of PAHs that enter the environment (Chen et al., 2005). Urban soils are more exposed to the PAHs produced by both stationary and diffused sources (Banger et al., 2010), produced by residential heating, traffic emissions, road byproducts such as wearing of tires and asphalt constituents. In particular, MMW PAHs and benzo(g,h,i)perylene at IOL (urban site), which are continuously emitted by diesel and gasoline combustion (Wang et al., 2007; Pies et al., 2008), can be considered as markers of urban pollution. Instead, the presence of LMW PAHs both at natural and industrial sites can be due to different emission sources. In natural environment, the presence of LMW PAHs mainly derives from natural sources such as plant and microbial metabolism (Maisto et al., 2006). By contrast, in industrial soils LMW PAHs derive from small industrial emissions. Also Pisupati et al. (2000), in fact, studying the emission of PAHs from small industries detected only acenaphthene, fluoranthene, and naphthalene. This is plausible in the collected industrial soils, as they are near to small industries. Moreover the higher percentage of HMW PAHs in agricultural soils appears to be dependent to the sewage irrigation. In fact, sewage sludge amendments have considerable amounts of 4-6-rings PAHs (Cerniglia, 1992; Juhasz and Naidu, 2000), which are difficult to be degraded, due both to the considerable share of their toxic form and their complex molecules (Oleszczuk, 2006).

Among the collected soils, three main differences in abiotic properties can be detected. Soils with higher bulk density and high total concentrations of Cd, Cr, Cu and Ni mainly belonged to agricultural soil category. Soils characterized by high concentrations of total Pb, Zn and PAHs, mainly belonging to urban soils. Soils with high organic matter content and high element soluble fractions are mainly composed by VES (natural soil). Industrial soils did not show distinctive features of abiotic properties and cannot be classified in any category.

4.2 Effects of urban soils on soil organisms: comparison between standard test protocols and in situ communities

4.2.1 Toxicity of urban soils through standard species exposition

After 28 d incubation in control soil, Cu, Pb and Zn concentrations in *E. andrei* were always lower than those in animals exposed to the investigated soils (Fig. 4.8). This was also the case for Pb and Zn concentrations in *E. crypticus* (Fig. 4.8). Animals differently accumulated metals; in particular, Cu accumulation in *E. andrei* and *E. crypticus* was higher when exposed to ACT, MIA and IOL soils, while in *F. candida* it was similar for all the soils (Fig. 4.8). Pb accumulation in *E. andrei* was higher when exposed to ACT, IOL and CAP soils, while in *E. crypticus* and *F. candida* it was higher only when exposed to IOL soil (Fig. 4.8). Zn bioaccumulation was higher in all the tested species exposed to IOL soil (Fig. 4.8).
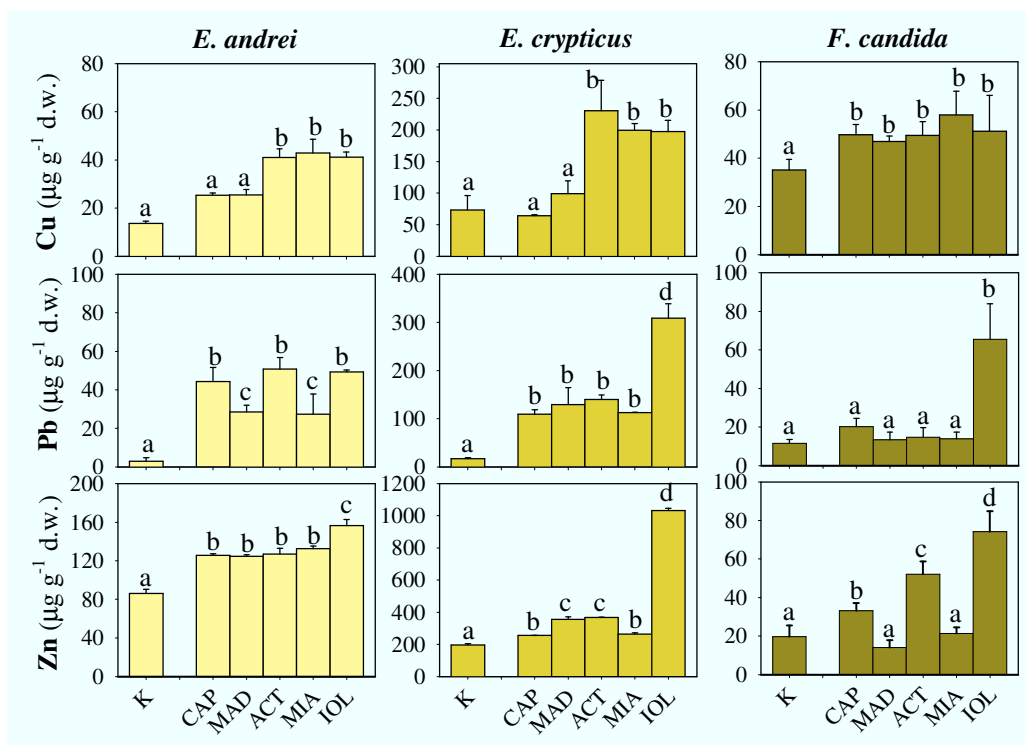
Figure 4.8. Mean (±s.e.) of body metal concentrations in *Eisenia andrei*, *Enchytraeus crypticus* and *Folsomia candida*, after exposure to urban soils from Naples, Italy. Different letters indicate statistically significant differences (P < 0.05) among the soils (one-way analysis of variance with Holm-Sidak posthoc test). K = Lufa 2.2 reference soil.

Zn body concentrations in *E. andrei* and *E. crypticus* were significantly correlated (P < 0.05) to soil total concentration. Similarly, Cu concentrations in *E. andrei* and *F. candida* were significantly correlated (P < 0.05) to soil total Cu concentration. Pb concentrations in *E. crypticus* and *F. candida* were significantly correlated (P < 0.05)

to soil total and water-extractable Pb concentrations (Fig. 4.8). No statistically significant correlations were found between body metal concentration and soil pH, WHC and OM.

Control survival in control soil was 100%, 95%, and 94% for *E. andrei*, *E. crypticus* and *F. candida*, respectively. Survival was high in all the field soils for all the three tested species (Fig. 4.9) and no significant differences with the control soil. Reproduction in control soil reached approximately 10, 920 and 400 juveniles per container for *E. andrei*, *E. crypticus* and *F. candida*, respectively (Fig. 4.9). *E. andrei* reproduction was highest in soil from MIA (29 individuals/container) and lowest in MAD (0.5 juveniles/container). For *E. crypticus* reproduction was low in all the soils compared to the control, showing the lowest value in MIA (377 juveniles/container). *F. candida* reproduction showed the highest value (435 ind./container) at CAP and the lowest (260 juveniles/container) in IOL (Fig. 4.9). Significant negative correlations ($P < 0.05$) were found between *F. candida* reproduction and soil total Zn concentration (Fig. 4.9). Control growth was 36%. Growth of *E. andrei* was highest (44%) in soil from MAD and lowest (8%) in IOL (Fig. 4.9). No significant correlations were found between the test results and the soil properties.
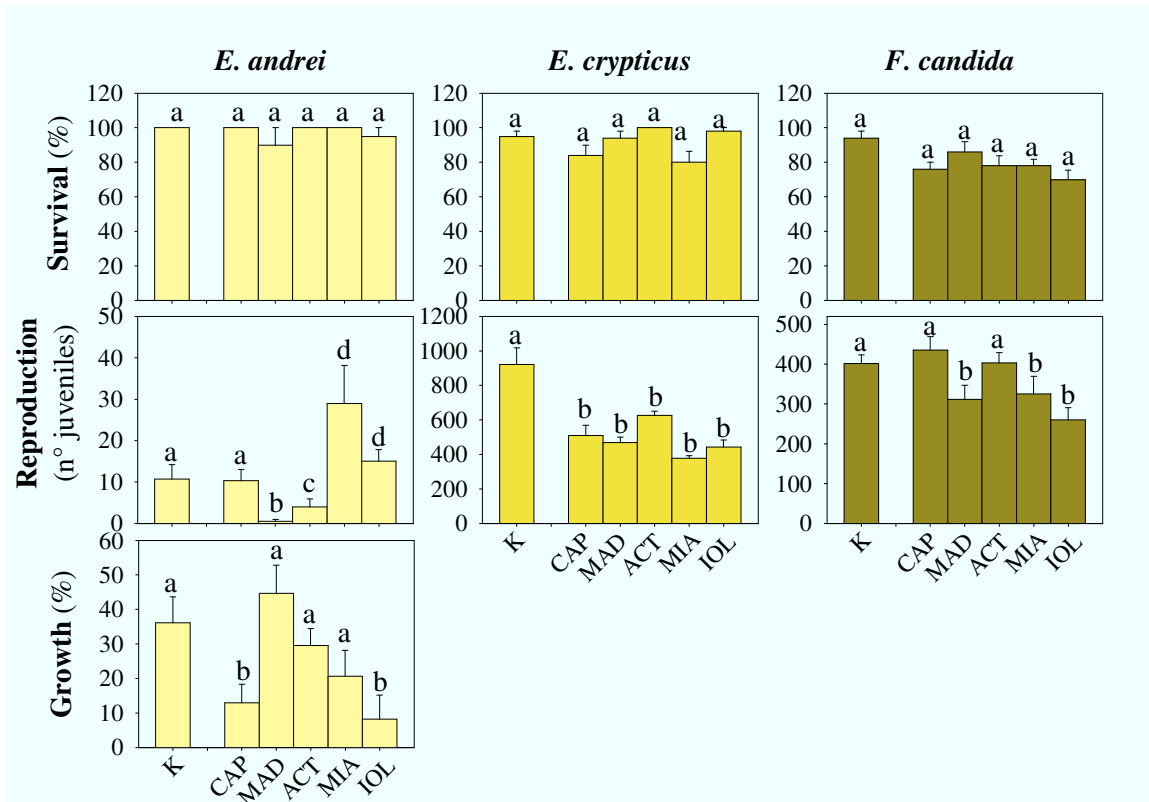
Figure 4.9. Mean values (±s.e.) of survival, reproduction and growth of *Eisenia andrei*, *Enchytraeus crypticus* and *Folsomia candida* after time test exposure to urban soils from Naples, Italy. Different letters indicate statistically significant differences ($P < 0.05$) among the soils (one-way analysis of variance with Holm-Sidak posthoc test).

The NMDS analysis (Fig. 4.10) performed on the bioassays results highlighted that all endpoint responses to metal contamination are similar, being grouped altogether in the graph (ellipse). Earthworm growth and reproduction showed different responses between them and in comparison with all the other tested endpoints (Fig. 4.10).
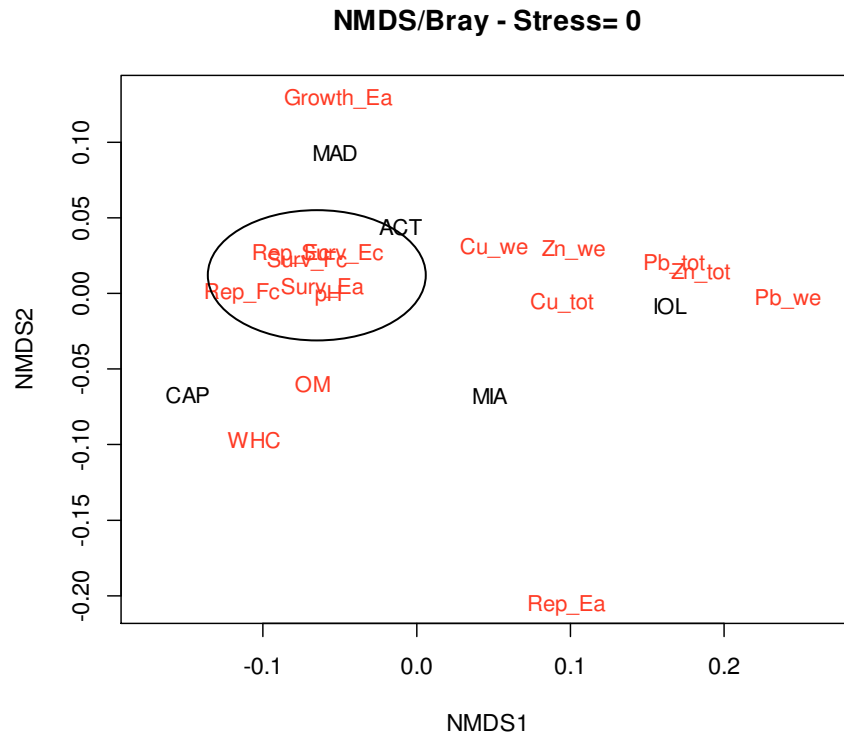
Figure 4.10. Results of ordination (NMDS) analysis of bioassay responses of *Eisenia andrei*, *Enchytraeus crypticus* and *Folsomia candida* exposed to soils sampled in downtown Naples, Italy.

4.2.2 Effects of urban soils on in situ arthropod communities

The highest density was observed for CAP, whereas the lowest values were observed for ACT.

Figure 4.11. Mean (s.e.) density and taxa richness of soil arthropod in urban soils from Naples, Italy. Different letters indicate statistically significant differences (p < 0.05) among the soils (One-way Analysis of Variance with HolmeSidak posthoc test).
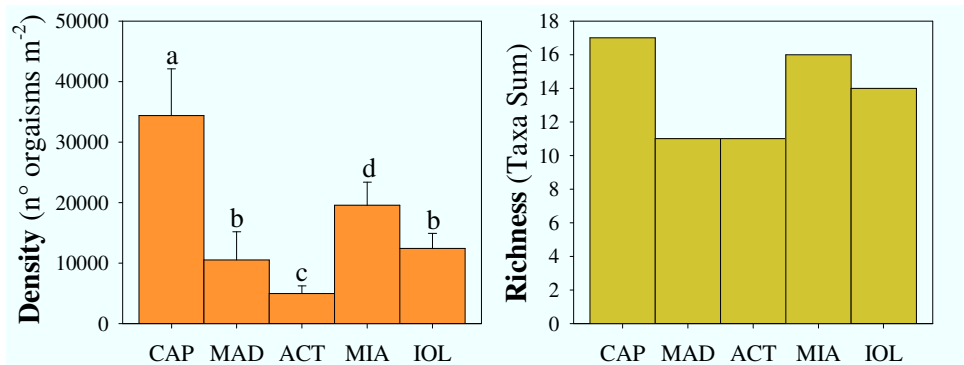
Altogether, 19 arthropod taxa were extracted with a minimum of 11 at ACT and MAD, and a maximum of 17 at CAP (Fig. 4.11).

The higher abundant taxa in all the collected soils were Acarina, Collembola (Table 4.2). Diplopoda, Coleoptera larvae, Chilopoda, Symphyla and Diptera larvae, and Isopoda, Lepidoptera larvae, Diplura, Araneae, Formicidae, Tysanoptera, Coleoptera, Pseudoscorpiones, Embioptera, Diptera, and Pauropoda, grouped on others, were the less abundant groups of organisms present in the collected soils (Table 4.2). Acarina abundance was higher at CAP and lower at ACT. Likely Collembola abundance was higher at CAP but lower at IOL, and it was negatively correlated ($P < 0.05$) with total and water-extractable Pb concentrations. The remaining taxa were very low abundant and except for Diplopoda and Diptera larvae, their abundances were higher at CAP (Table 4.2).

Table 4.2. Mean (± s.e.) taxa abundances found in urban soils from Naples, Italy. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

	CAP	MAD	ACT	MIA	IOL
Acarina	175 **A** (42.1)	56.4 **B** (27.5)	17.8 **C** (3.76)	92.8 **D** (27.2)	41.4 **E** (14.8)
Collembola	38 **A** (21.1)	12.8 **B** (4.27)	7.6 **C** (3.17)	10.4 **B** (4.75)	2.6 **D** (0.81)
Diplopoda	4.00 **B** (2.61)	3.4 **B** (1.12)	5.00 **B** (2.86)	3.20 **B** (1.11)	9.00 **A** (3.36)
Coleoptera larvae	1.00 **A** (0.44)	0.20 **B** (0.20)	0.20 **B** (0.20)	2.40 **A** (0.75)	0.00 **C** (0.00)
Chilopodi	1.80 **A** (0.73)	1.80 **A** (0.92)	0.0 **B** (0.0)	1.80 **A** (0.49)	0.80 **C** (0.37)
Symphyla	3.80 **A** (1.36)	0.20 **B** (0.20)	0.40 **B** (0.24)	0.40 **B** (0.40)	0.20 **B** (0.20)
Diptera larvae	3.00 **B** (0.71)	0.40 **C** (0.24)	1.20 **BC** (0.58)	5.20 **A** (1.56)	3.40 **AB** (2.23)
Others	3.58 **A** (0.96)	0.62 **B** (1.20)	0.57 **B** (0.36)	3.12 **A** (1.18)	3.35 **A** (1.20)

Even though with different individual abundances, arthropod community was dominated by Acarina in all the soils (Fig. 4.12). Collembola were present in all the soils with similar percentages, except at MIA and IOL where they were lower represented in the community (Fig. 4.12). Diplopoda were less represented at CAP (Fig. 4.12).
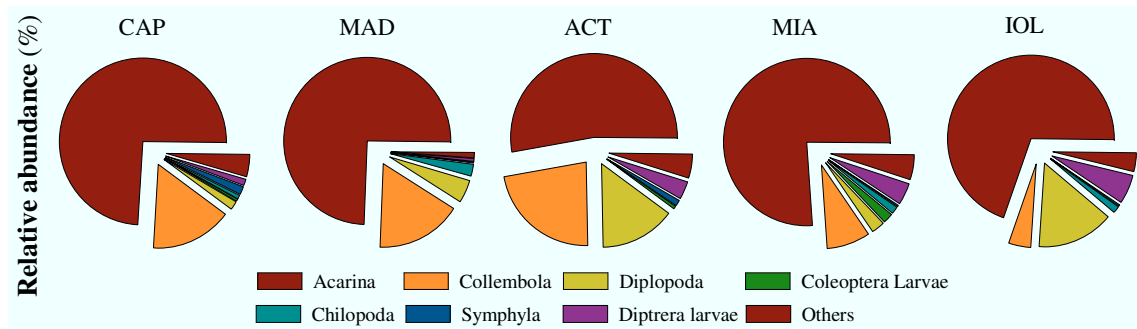
Figure 4.12. Relative abundance of the main soil arthropod taxa in urban soils from Naples, Italy Shannon (H) and Evenness (E) indices did not statically differ among the soils

The highest ratio between Acarina and Collembola (A/C) was observed for IOL and the lowest for ACT (Table 4.3). The QBS index indicated soil quality was highest at CAP and lowest at ACT (Table 4.3). QBS was positively ($P < 0.05$) correlated with density and taxa richness. No correlations were found between the index values and the soil physical and chemical properties and metal contents.

Table 4.3. Mean (± s.e.) Diversity index (H), Evenness (E), Acarina/Collembola ratios (A/C) and soil biological quality index (QBS) arthropod community in urban soils from Naples, Italy. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

	CAP	MAD	ACT	MIA	IOL
H	1.13 A (0.09)	0.97 A (0.09)	1.36 A (0.10)	1.24 A (0.11)	1.15 A (0.05)
E	0.43 A (0.04)	0.52 A (0.04)	0.60A (0.06)	0.52 A (0.05)	0.56 A (0.02)
A/C	5.21 AB (2.5)	4.15 AB (0.81)	1.98A (0.71)	14.5 AB (4.7)	15 B (2.9)
QBS	145.2 A (12.9)	83.2 B (8.04)	78 B (16)	133.2 AC (8.9)	92 BC (11.9)

4.2.3 Role of seasonality in affecting arthropod community in urban soils

In spring sampling arthropod density showed the highest value at CAP and the lowest at IOL (Fig. 4.13). 15 taxa of arthropods were extracted in spring, with higher values measured at CAP, MAD and MIA (Fig 4.13). On average, arthropod density and richness were statistically ($P < 0.05$) lower in spring, than in autumn (Fig. 4.13).
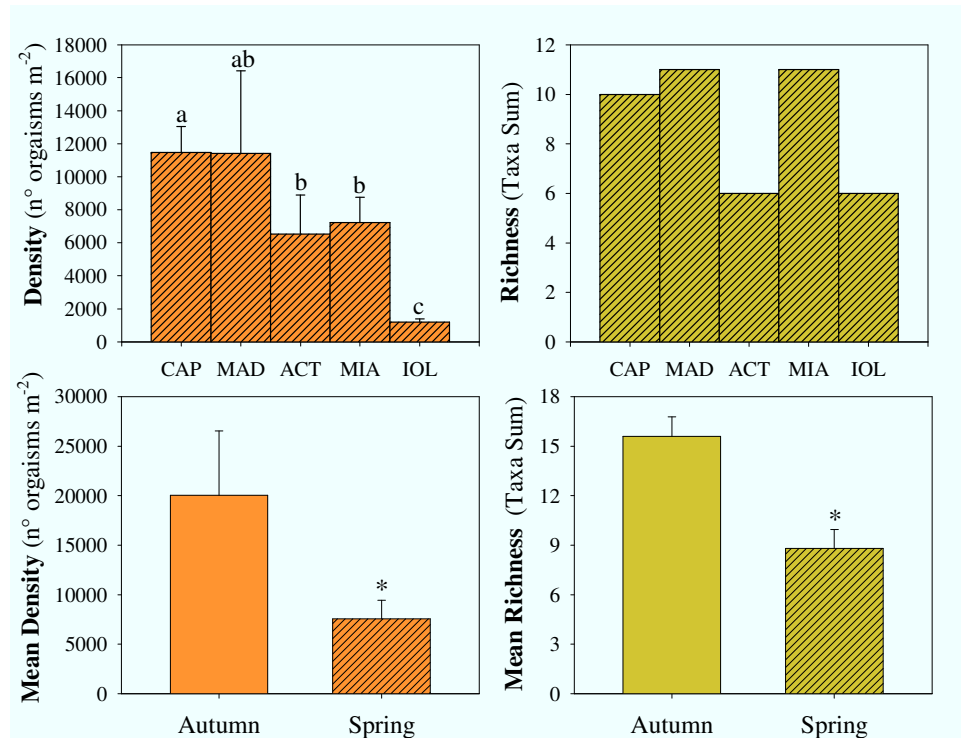
Figure 4.13. Mean (± s.e.) density and taxa richness found in spring, and average density and richness found in autumn and spring in urban soils from Naples, Italy. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05). Asterisks indicate the statistical differences between the seasons (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05)

Also in spring, Acarina and Collembola showed the higher abundances values within the community, being both higher at CAP and MAD and lower at IOL (Table 4.4). The abundances of remaining taxa are lower than those detected in autumn, and all of them were higher at CAP and MAD (Table 4.4).

Table 4.4. Mean (± s.e.) taxa abundances found in spring in urban soils from Naples, Italy. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

	CAP	MAD	ACT	MIA	IOL
Acarina	47.4 **A** (10.0)	37.2 **B** (12.6)	25.0 **C** (8.48)	30.4 **B** (7.70)	4.60 **D** (0.81)
Collembola	31.6 **A** (8.96)	44.5 **A** (30.0)	23.8 **B** (14.6)	19.4 **B** (5.09)	2.60 **C** (1.63)
Coleoptera larvae	0.60 **B** (0.24)	1.20 **A** (0.58)	1.40 **A** (0.40)	0.60 **B** (0.24)	0.20 **C** (0.20)
Diplopoda	5.40 **A** (1.12)	4.20 **B** (2.00)	0.00 **C** (0.00)	0.80 **D** (0.37)	1.00 **D** (0.32)
Chilopoda	1.40 **B** (0.40)	0.40 **B** (0.24)	0.00 **C** (0.00)	3.20 **A** (0.24)	0.00 **C** (0.00)
Symphyla	2.40 **A** (0.75)	0.80 **B** (0.37)	0.00 **C** (0.00)	0.80 **B** (0.58)	0.00 **C** (0.00)
Diptera larvae	0.00 **B** (0.00)	6.00 **A** (6.00)	0.20 **B** (0.20)	0.40 **C** (0.24)	0.00 **B** (0.00)
Others	0.15 **B** (0.11)	0.53 **A** (0.34)	0.10 **B** (0.01)	0.15 **B** (0.12)	0.12 **B** (0.07)

In spring, as well as in autumn, Acarina dominated the community, even though their percentage was significantly lower than in autumn; the percentage of Collembola, by contrast, was significantly higher in spring than in autumn (Fig. 4.14). The less abundant taxa showed similar percentages between the seasons (Fig. 4.14).
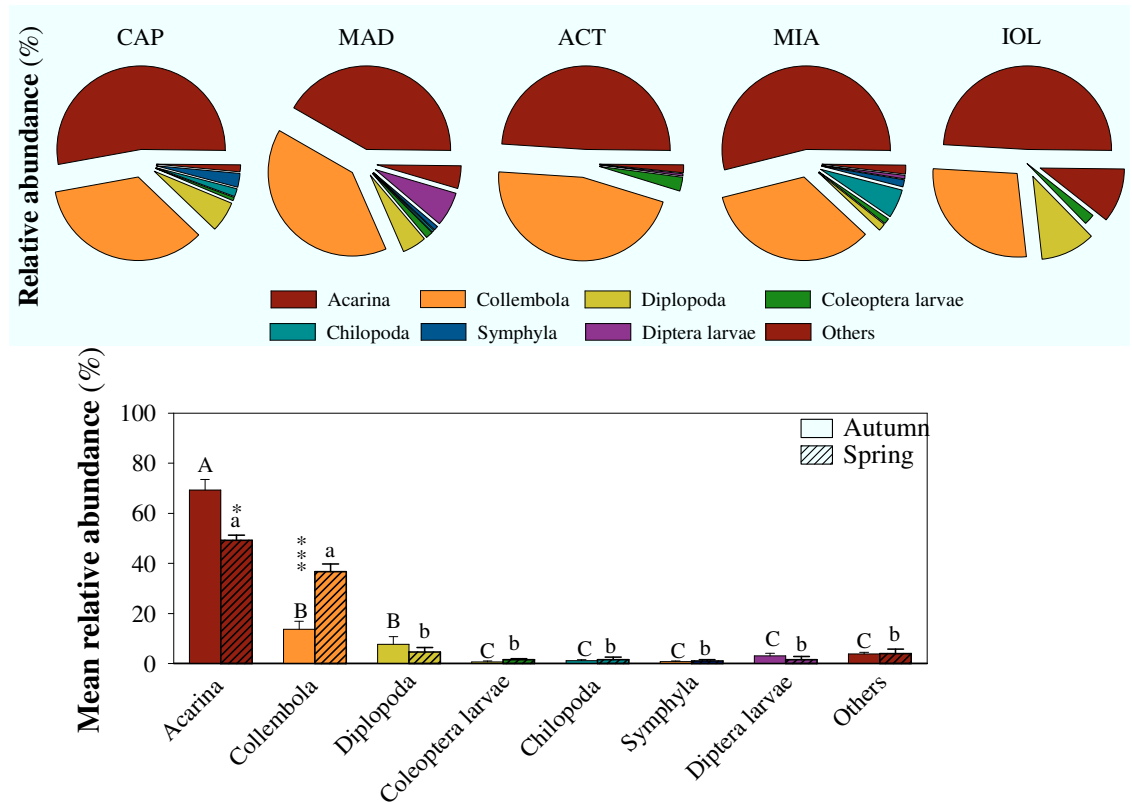
Figure 4.14. Relative abundance of the main soil arthropod taxa found in spring in urban soils from Naples, Italy.

In spring, as well as in autumn, Shannon (H) and Evenness (E) indices did not vary among the soils and they were not different between the seasons (Table 4.5). By contrast, in spring, A/C ratios did not vary among the soils but it was statistically lower than in autumn (Table 4.5). Compared to the autumn, QBS in spring showed a different trend among the soils, being higher at CAP and the lower at IOL (Table 4.5), and in contrast with autumn, it was positive correlated ($P < 0.05$) only with richness.

Table 4.5. Mean (± s.e.) Diversity index (H), Evenness (E), Acarina/Collembola ratios (A/C) and soil biological quality index (QBS) arthropod community in urban soils from Naples, Italy. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05). Asterisks indicate the statistical differences between the seasons (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05)

	CAP	**MAD**	**ACT**	**MIA**	**IOL**	**Autumn**	**Spring**
H	1.80 A (0.04)	1.10 A (0.18)	0.83 A (0.10)	1.12 A (0.12)	0.98 A (0.11)	1.17 (0.06)	1.17 (0.17)
E	0.62 A (0.03)	0.567 A (0.05)	0.62 A (0.03)	0.64 A (0.04)	0.78 A (0.02)	0.53 (0.02)	0.66 (0.03)
A/C	1.92 A (0.53)	2.44 A (0.19)	3.53 A (1.92)	1.9 A (0.60)	2.57 A (0.10)	8.17 (2.74)	2.48 * (0.30)
QBS	109 A (12)	86.8 B (13)	47 C (2)	98 B (4)	40 C (8)	106 (14)	76 * (14)

The NMDS analysis showed a clear site separation on the basis of sampling season, with all the soils collected in autumn on one side and all soils collected in spring on the other side (Fig. 4.15). In both seasons arthropod density, Acarina and collembolan abundances were higher at CAP, whereas richness was higher at MIA (Fig. 4.15). IOL presented the highest contamination and the lowest assessed biological parameters (Fig. 4.15).
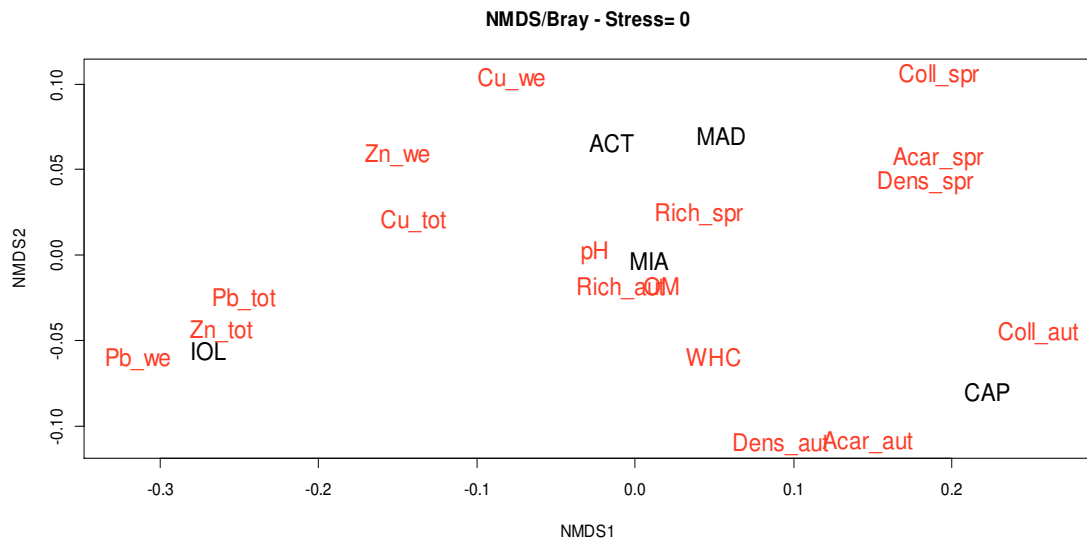
Figure 4.15. Results of ordination (NMDS) analysis of arthropod community sampled autumn and spring in urban soils of Naples, Italy.

4.2.4 Discussion

The main contamination found in the collected urban soils was characterized by Cu, Pb and Zn accumulation. As these contaminants are the markers of traffic, the highest concentrations of these metals were found in the soil near to the motorway

(IOL), which is busy by traffic, not only by cars but also by trucks and buses and near a very busy roadside (MIA). Metal accumulation of the organisms exposed to the soils varied among the different soils and the measured metals. In particular, bioaccumulation was higher in the most contaminated soils, indicating metal bioaccumulation strictly related to soil metal concentrations, as also confirmed by the correlations ($P < 0.05$). Found correlations showed also that metal body uptake was more depended on total metal concentration than on water-extractable metal concentration measured the soils. The strong relationship between bioaccumulation and total metal content occur because soil organisms can absorb metals not only from pore water, but also from soil contact and feeding (Van Vliet et al., 2006). In addition, measurements of metal pore-water concentrations are restricted to a single moment in the time and cannot reflect the dynamics of concentrations over the exposure period.

In addition, tested organisms accumulated differently the three measured metals, confirming the ability of different species to accumulate different amounts of the same metal, depending on the occupied habitat and species behavior (Vijver et al., 2004; Van Vliet et al., 2006). The higher amounts of all measured metals found in *E. crypticus* bodies, compared to *E. andrei* and *F. candida*, would indicate a better capability of earthworms and collembolans to regulate their metal body concentrations. This was also reported by Suthar and Singh (2008) for earthworms and by Bruus Pedersen et al. (2000) for collembolans. However, when *F. candida* was exposed to very high Pb and Zn concentrations metal uptake strongly increased, suggesting that its regulation ability is inhibited at very high soil metal concentrations (Vijver et al., 2001).

The ability of organism to exclude or accumulate metals can be reflecting on its physiological performances. In fact, as metal body accumulation, some organism responses also varied among the soils and on the basis of different sensitivities of the tested species. In particular, the low mortality found for the three exposed species agreed with the results reported by several authors (Lock and Janssen, 2003; Kuperman et al., 2004; Van Gestel et al., 2011) also at metal concentrations higher than those found in the collected soils. Therefore, mortality seemed to be not a very sensitive parameter to estimate effects of soil metal contamination on the tested species. In spite of no effects on survival, reproduction and growth of organisms differed among the soils. In particular, reproduction and growth of *E. andrei* showing

opposite responses to metal pollution exposition and agrees with Van Gestel et al. (1992). It is possible, in fact, that when *E. andrei* lives in stressed soils, is forced to choose on which process to spend its energy. In addition, reproduction of earthworms, being higher in the more polluted soils seemed to be not depended on metal contamination and body metal accumulation, but on other soil properties. In particular, soil organic matter content seems to play an important role in affecting earthworm's reproduction (Ávila et al., 2009; Van Gestel et al., 2011), as *E. andrei* is an organism living in organic matter. By contrast, *E. andrei* growth, *E.crypticus* and *F. candida* reproduction followed a decreasing trend with metal soil concentration increasing, as also found by several authors (Scott-Fordsmand et al., 2000; Reinecke et al., 2002; Spurgeon et al., 2004; Ávila et al., 2009). Although *E.crypticus* was the best accumulator, reproduction responses did not seem to be influenced by the higher bioaccumulation of this organism. In fact, *E.crypticus* reproduction was similar in all the collected soils. By contrast, the reproduction of *F. candida,* the best excluder organism, was more affected by metal contamination, as also confirmed by the negative correlation with soil total Zn concentration. This negative correlation suggests a negative effect on reproduction due to soil metal contamination (Sandifer and Hopkin, 1997; Smit and Van Gestel, 1997; Lock and Janssen, 2003; Xu et al., 2009; González et al., 2011). However, physiological parameters of the three organisms were never correlated to body metal accumulation, suggesting that the accumulation of metals in *E. crypticus* and the exclusion in *E. andrei* and *F. candida* are strategies of organisms to face the soil metal contamination.

Differences detected in arthropod community responses among the urban soils can be attributable not only to the contamination but also to several soils properties. Arthropod density, in fact, did not follow a linear trend with soil metal contamination, although it was higher in the less contaminated soil (CAP). This finding suggests that metal contamination is a driving force in affecting distribution of arthropod community (Battigelli and Marshall, 1993; Eitminaviciute, 2006; Gongalsky et al., 2010), even if it is not the only factor. Taxa richness, in fact, seemed to be more dependent on organic matter, suggesting the central role of organic matter in affecting arthropod richness (Wolters, 2001; Räty and Huhta, 2003; Mulder, 2006; Pizl et al., 2009). Likely to density and richness, arthropod assemblages also differed among the soils. The high abundance of Acarina and Collembola in all soils suggests that these taxa are tolerant to a wide range of soil

properties, even though they showed variations in individual abundance among the soils. Collembola, in particular, were lower in the soils with the higher metal concentrations (MIA and IOL), which can indicates that Collembola are more sensitive, than Acarina, to soil metal contamination as is well documented by several authors (Filser et al., 2000; Kuperman et al., 2007; Xu et al., 2009). The trend of Collembola among the soils is in according to what found in *F. candida* (Collembola) laboratory exposition. In fact, low *F. candida* reproduction found in more metal contaminated soils can explain the low abundance of Collembola found on collected soils, in the field investigations. However, metal sensitivity by Collembola can be very variable as other authors (Fountain and Hopkin 2004; Fiera, 2009) reported that Collembola abundance did not decrease in metal polluted sites because these organisms are capable to excrete metals. Moreover the abundance of collembolan in the soil can be linked to many parameters, and in addition, Collembola were able to disperse in soil and tended to settle in unpolluted areas (Sjögren, 1997). Hence, the absence of Collembola in the polluted soils of the investigated area can be due both to the sensitivity of these organisms and to their avoidance behaviour.

By contrast, the abundance of Acarina showed the same trend of the whole density of community, indicating that total community density was dependent from the abundance of Acarina. Like to density, Acarina distribution was affected by similar abiotic factors: organic matter content and soil metal pollution.

The abundances of remaining taxa are very scarce and did not seem to follow a linear trend with metal pollution, neither with pH or organic matter content. The presence of these taxa in the investigated area could be linked to the entire set of soil properties, since their distribution is not attributable just to one or few parameters.

Despite the differences of organism abundances among the soils, the diversity and the evenness were similar among the soils, suggesting that the assessed differences of the others parameters are not enough to bring variations on total diversity and evenness of the communities of urban soils. However, taxonomic index results should be interpreted carefully, as they are very sensitive to various factors, such as the sampling unit size and rare taxa, and are able to detect differences only in extremely different soils (Nahmani and Lavelle, 2002). By contrast, wide differences were observed for Acarina/Collembola (A/C) ratios and soil biological quality (QBS) index. These two indices differed from the others, since they are considered be

indicative of soil quality. High values of A/C should indicate high soil quality, because it has been established that in degraded soils the number of Acarina species decreases (Jacomini et al., 2000). This is not the case of the collected soils, in which A/C ratio showed positively correlation to soil metal contamination. These findings disagree with the idea that Acarina are more sensitive, than Collembola, to metal contamination (Menta et al., 2008), indicating that this index cannot always be used as index of high soil quality.

On the other hand, QBS index following the same trend of arthropod density and richness, as also confirmed by the positive correlations found between them. As QBS index evaluate the affinity of organisms to live in the soil, the strong relationships between QBS and density, and QBS and richness suggests that in urban area density and richness are heavily influenced by the abundance of euedaphic organisms.

As the study area of downtown Naples is inserted in a typical Mediterranean climate, it is characterized by strong fluctuations in temperature and precipitation between the seasons. The mean temperature and total amount of rainfall in the last three months before the samplings in autumn (September 2010) and spring (April 2011) were 25 °C and 100 mm and 10 °C and 250 mm, respectively. The different climatic conditions occurring in the sampling times can alter soil microclimate and indirectly modifying resource availability and food web composition (Block et al., 1990; Frampton et al., 2000). These modifications affect arthropod community structure, which differed between the seasons. In autumn, in fact, arthropod density seemed to be mainly linked to soil organic matter content and slightly to contamination, whereas in spring soil contamination seem to play a more important role in affecting arthropod density, as the lowest density value was found in the most polluted soil (IOL). Taxa richness, on the other hand, showed the similar trends between the seasons. Moreover, both density and taxa richness were lower in wet-cold conditions (spring) than in dry-warm conditions (autumn). This finding agreed with those reported for Mediterranean climatic conditions by various authors (Stamou et al., 2004; Antunes et al., 2008; Touloumis and Stamou, 2009) who found an increase of arthropod abundance and taxa richness in periods characterized by high temperature. In particular, Stamou et al. (2004) reported that in Mediterranean fields the optimum temperature for soil arthropods is around 20°C and lower values result in a significant decline of organism activity, which, in turn, affects arthropod growth and abundance.

Among all the taxa found in urban area, Acarina and Collembola are the most affected by seasonal climatic conditions. Although they dominated the community in both the seasons, Acarina were more abundant in dry-warm season (autumn) and Collembola in wet-cold season (spring). These results are in according to several authors (Cortet and Poinsot-Balaguer, 1998; Zhu et al., 2010; Kardol et al., 2011) which found Acarina density higher in warm conditions, while collembolan abundance higher in cold-wet conditions. The high Collembola abundance found after a rainy period could be due to higher fungal biomass (Hawkes et al., 2011) that represents Collembolan main food source (Hopkin, 1997). Moreover, the variations of Acarina and Collembola abundances between the sampling times can also be due to the organism life cycle that is partially dependent on the different climatic conditions.

The variations in abundances of the most abundant taxa did not influence the diversity and the evenness between the seasons. Probably, Acarina and Collembola fluctuations are not enough to provoke changes on the whole diversity and evenness of the urban soil community. By contrast, Acarina and Collembola variations exert a strong effect on A/C ratio and QBS, which showed different values between the seasons. A/C ratio, in fact, in spring was lower than in autumn because of the decrease of Acarina and the increase of Collembola.

The decrease of QBS detected in spring, on the other hand, may suggest that in spring, soil conditions are less favorable for the euedaphic organisms, compared to the autumn.

Overall, both in field and laboratory exposition investigation Collembola (represented as the Collembola in field and Folsomia candida in laboratory) showed the highest sensitivity to the metal pollution. However, field investigation were more powerful to discriminate the differences in metal pollution among the sites than the bioassay results.

4.3 Effects of urban soils on Collembola communities and comparison with different land uses

4.3.1 Collembola taxonomic structure in autumn sampling

Overall, density of Collembola was lower in industrial soils, although it showed a great heterogeneity among soils belonging to the same land uses (Fig. 4.16). Within urban soils lower densities were found at MIA and IOL. Density in agricultural soils showed great variability, reaching the higher values at VU (Fig. 4.16). By contrast species richness showed more homogeneous values, showing a decreasing trend passing from natural > urban = industrial > agricultural soils (Fig. 4.16). The lowest values of richness were detected in all the agricultural soils, at ACT (urban) and at POMI (industrial). Collembola species richness was negatively ($P < 0.05$) correlated with total concentrations of Cd, Cr and Ni.
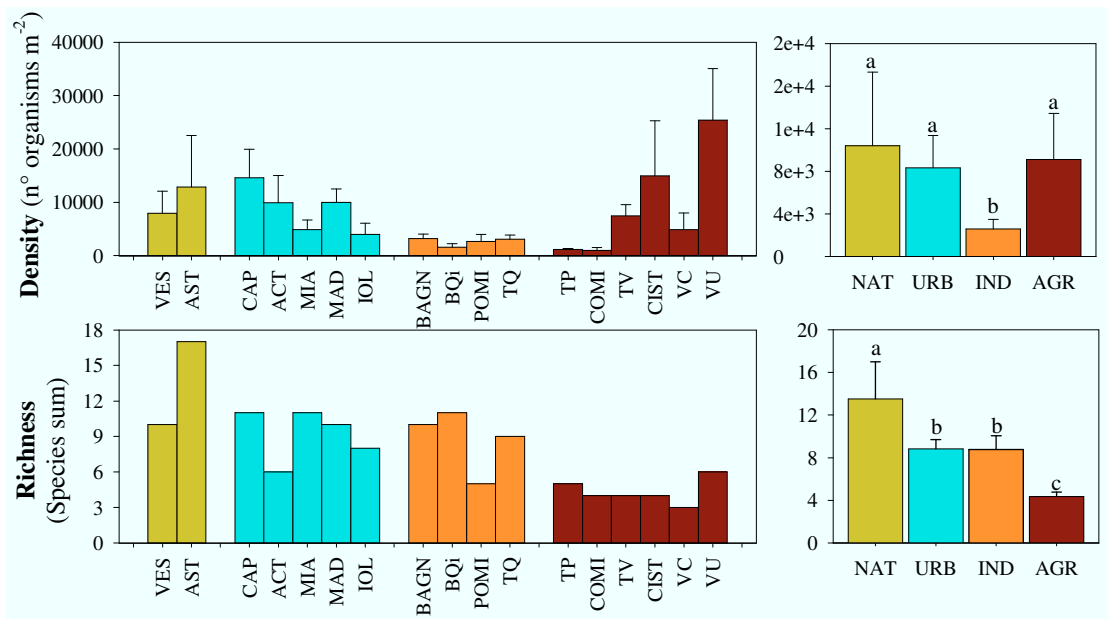
Figure 4.16. Mean (s.e.) density and richness of Collembola found in soils with different land uses in October 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

Forty-four species of Collembola species belonging to 6 different families (*Entomobyidae*, *Isotomidae*, *Onychiuridae*, *Poduridae*, *Sminthuridae*, *Sminthurididae*) were found in all the collected soils (Annexes, table 1). Collembola species composition showed wide variations among soil typologies. In the natural soils *Mesaphorura sp.* (MES_X) showed the highest density (Fig 4.17), although it was very abundant only at VES (Annexes, table 2). By contrast, at AST (Annexes, table 2) the higher abundant species were *Mucrosomia garretti* (MUC_GAR), *Parisotoma notabilis* (PAR_NOT) and *Folsomia inoculata* (FOL_INO). In urban

soils several species showed similar abundances (Fig 4.17). In particular, at CAP *Mesaphorura sp*, *Folsomia quadriloculata* (FOL_QUA) and *Mucrosomia garretti* were more abundant. At ACT showed higher density *Proisotoma minuta* (PRO_MIT) and *Folsomia litseri* (FOL_LIT), which were more abundant at MAD and IOL, respectively (Annexes, table 2). At industrial soils four species were dominant in the community (Fig. 4.17). *Mucrosomia garretti* was abundant at BAGN, *Mesaphorura sp.* at BQi, *Proisotoma minuta* at POMI and *Parisotoma notabilis* at TQ (Annexes, table 3). The agricultural soils were heavily dominated by *Prosotoma minuta* (Fig. 4.17) which was present in all the soils of the typology (Annexes, table 4). Only in natural (VES, AST) and urban soils (CAP, MIA, IOL) was present *Isotomiella minor*. *Mesaphorura sp.* was negatively (P < 0.05) correlated with bulk density and pH and positively (P < 0.05) with organic matter and nitrogen content. *Folsomia litseri* was positively (P < 0.05) correlated with total Pb concentration. *Proisotoma minuta* was positively (P < 0.05) correlated with total Cd and Cu concentrations. *Isotomiella minor* was positively (P < 0.05) correlated with organic and nitrogen content and negatively (P < 0.05) with bulk density.
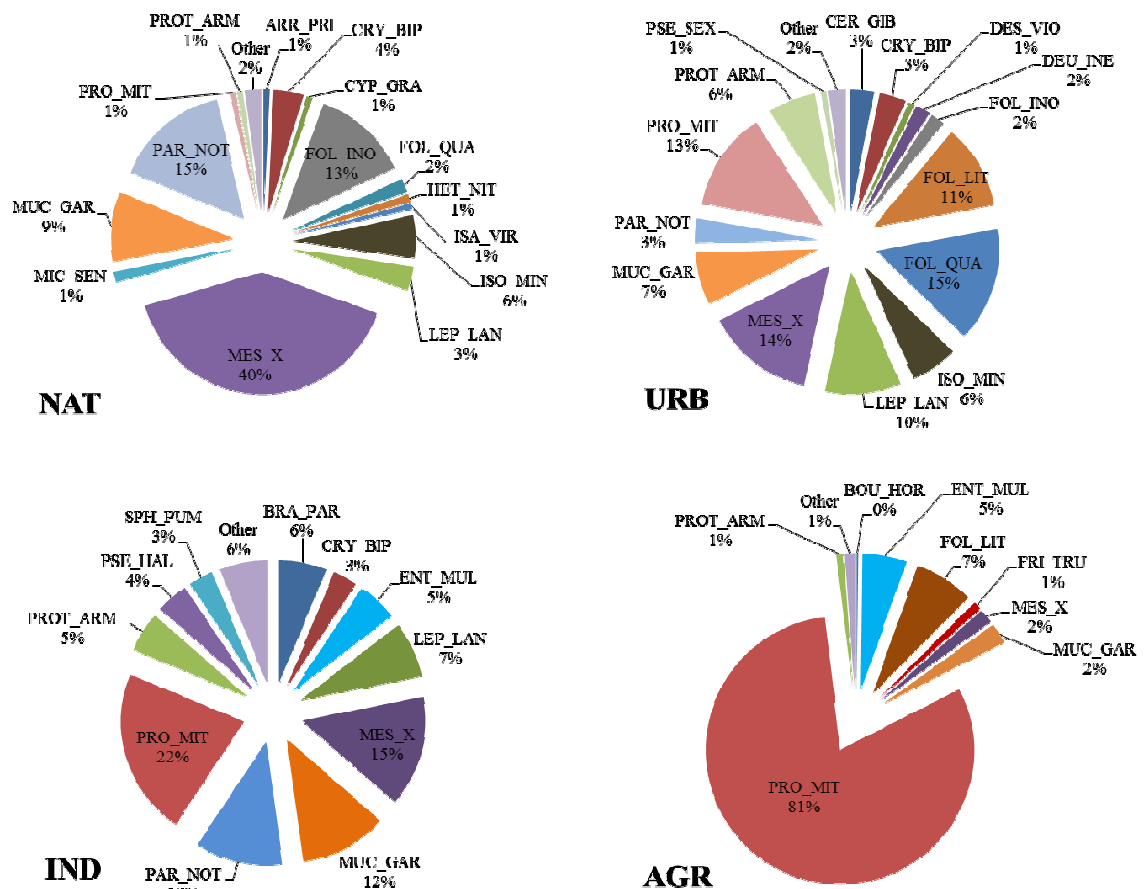
Figure 4.17. percentage composition of Collembola community found in soils with different land uses in October 2011.

The diversity (H) and evenness (E) were statistically lower in agricultural soils (Table 4.6).

Table 4.6. Mean (± s.e.) Diversity index (H), Evenness (E), of Collembola community in soils from different land uses. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

	NAT	URB	IND	AGR
H	1.05 **a** (0.16)	0.86 **a** (0.16)	0.80 **a** (0.24)	0.28 **b** (0.15)
E	0.68 **a** (0.10)	0.66 **a** (0.11)	0.71 **a** (0.18)	0.30 **b** (0.15)

4.3.2 Collembola functional structure in autumn sampling

Body sizes of Collembola showed wide variations among soils typologies and among soil of same typology.
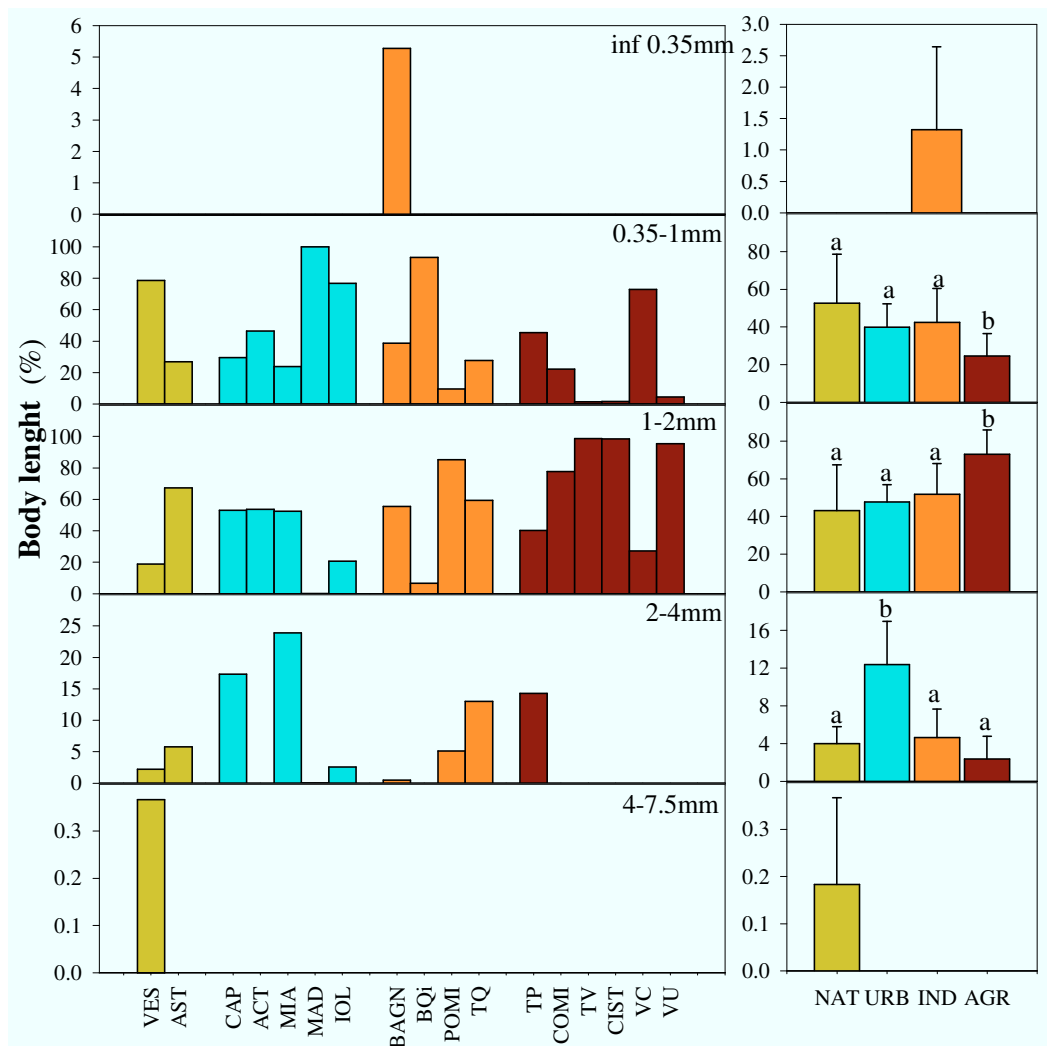
Figure 4.18. Percentage distribution of organisms with different body sizes measured in soils with different land uses sampled in October 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

Organisms less of 0.35 mm were only present at BAGN (industrial), whereas organisms between 4 and 7.5 mm were only present at VES (natural) (Fig. 4.18). On average, abundances of organisms between 0.35-1 mm was low at AST, CAP, MIA, POMI and in several agricultural (Fig. 4.18). The abundance of organisms between 1 and 2 mm increased passing from NAT to AGR. However, VES, IOL, BQi and VC showed similar values (Fig. 4.18).

The two classes of motion strategy were homogeneous among all the soils and did not statistically differ among land use typologies (Fig 4.19).
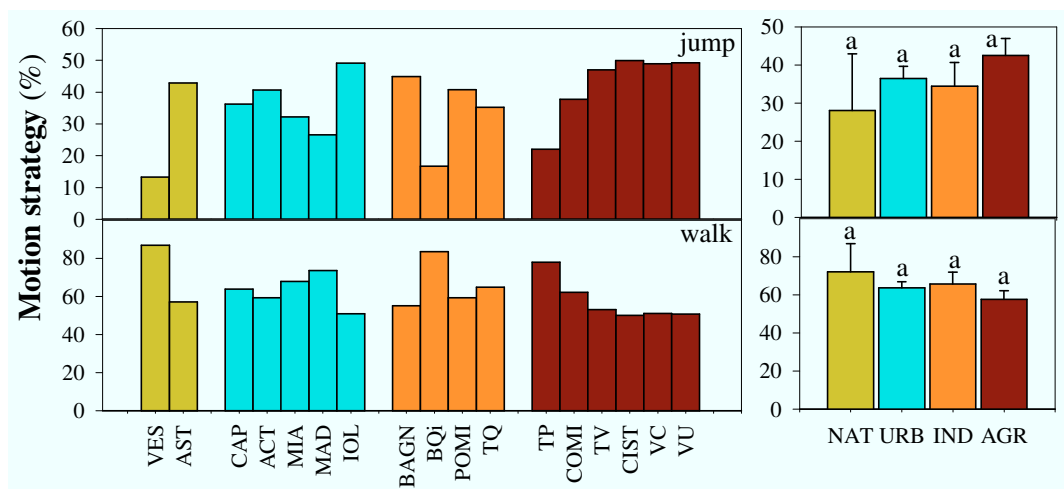
Figure 4.19. Percentage distribution of organisms with different motion strategies found in soils with different land uses sampled in October 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

The mouth part type highlighted the exclusive presence of Collembola without mandible at BAGN and BQi (industrial soils) (Fig. 4.20). Abundance of organisms with normal mandibles was higher in all the soils, whereas organisms with strong mandibles were more abundant at VES, AST (natural), CAP, MIA, IOL (urban), and VC (agricultural) (Fig. 4.20). Abundance of organisms with strong mandibles was negatively (P < 0.05) correlated with bulk density and positively (P < 0.05) with matter and nitrogen content.
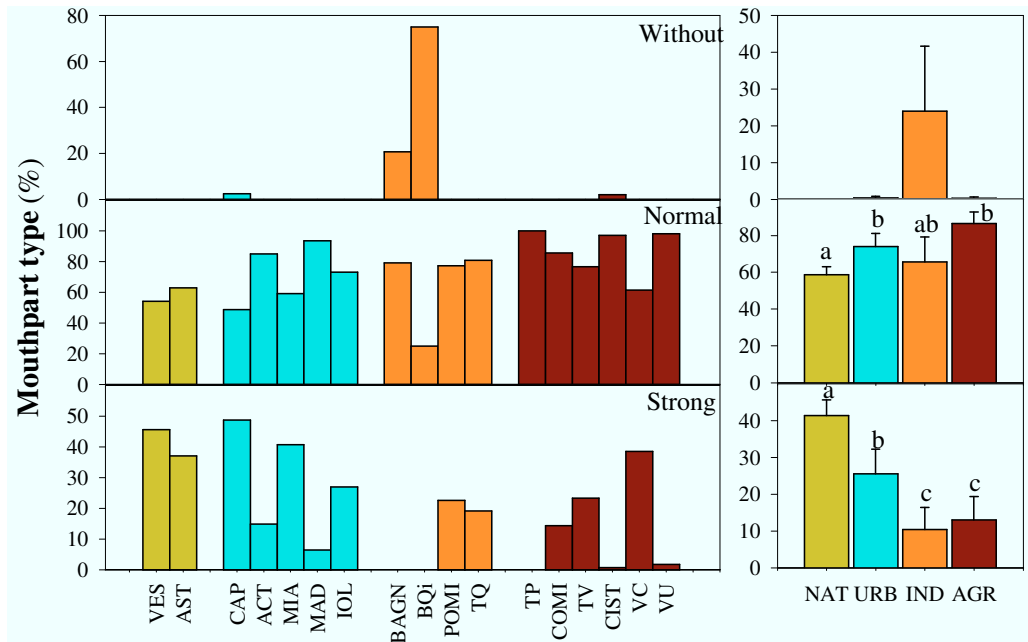
Figure 4.20. Percentage distribution of organisms with different mouthpart type found in soils with different land uses sampled in October 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

Pigmented and apigmented organism abundances showed opposite trend (Fig. 4.21). Pigmented and apigmented organisms showed the highest and the lowest values, respectively, at POMI (industrial), TV, CIST and VU (agricultural) (Fig. 4.21).
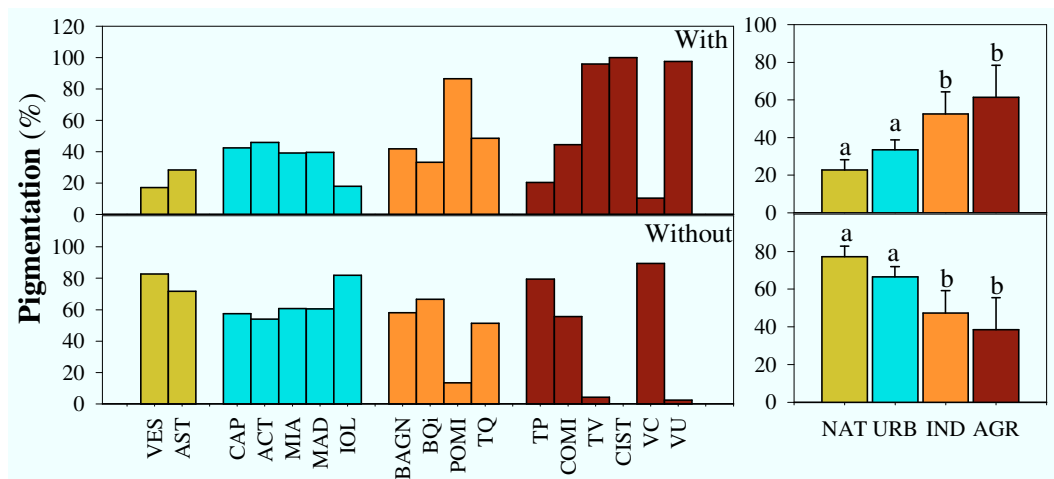
Figure 4.21. Percentage distribution of pigmented (With) and apigmented (Without) found in soils with different land uses sampled in October 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

Asexual reproductive organisms were more abundant at VES, AST (natural), BAGN, TQ (industrial) and TP (agricultural), and sexual reproductive organisms showed opposite trend (Fig. 4.22). Asexual and sexual reproductive organisms were,

respectively, negatively and positively ($P < 0.05$) correlated with total Cd, Cr, Cu, Ni concentrations.
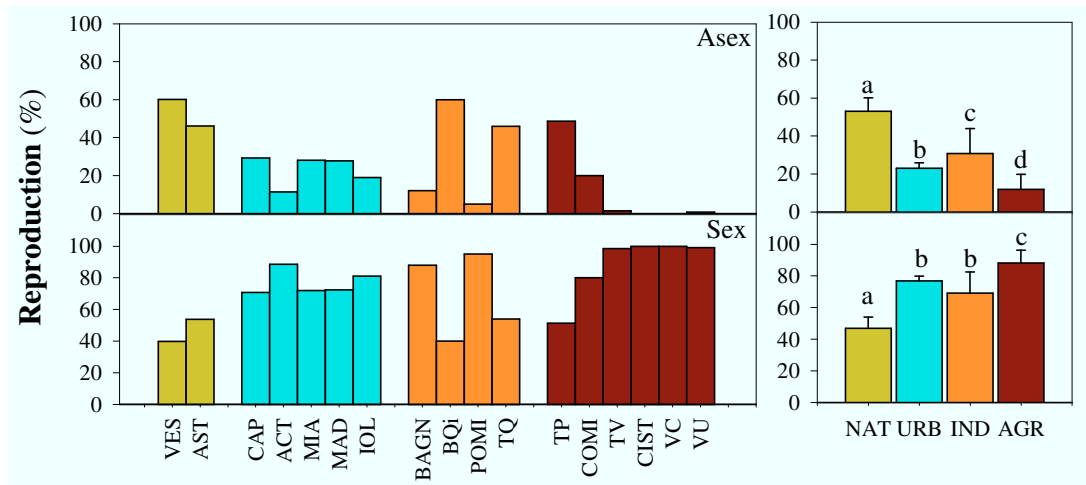
Figure 4.22. Percentage distribution of reproduction strategy (Asexual: Asex, Sexusual:Sex) of organisms found in soils with different land uses sampled in October 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

Redundancy analysis (RDA) was performed in order to assess the relationships between soil physical and chemical properties and Collembola functional traits (Fig. 4.23). This graph represent the sites (black), soil physical and chemical properties (red) and functional traits (blue). The abiotic properties and functional traits more distant to the centre are more correlated to the axe and are more correlated between them. The variance explained by the independent variables is 0.84.
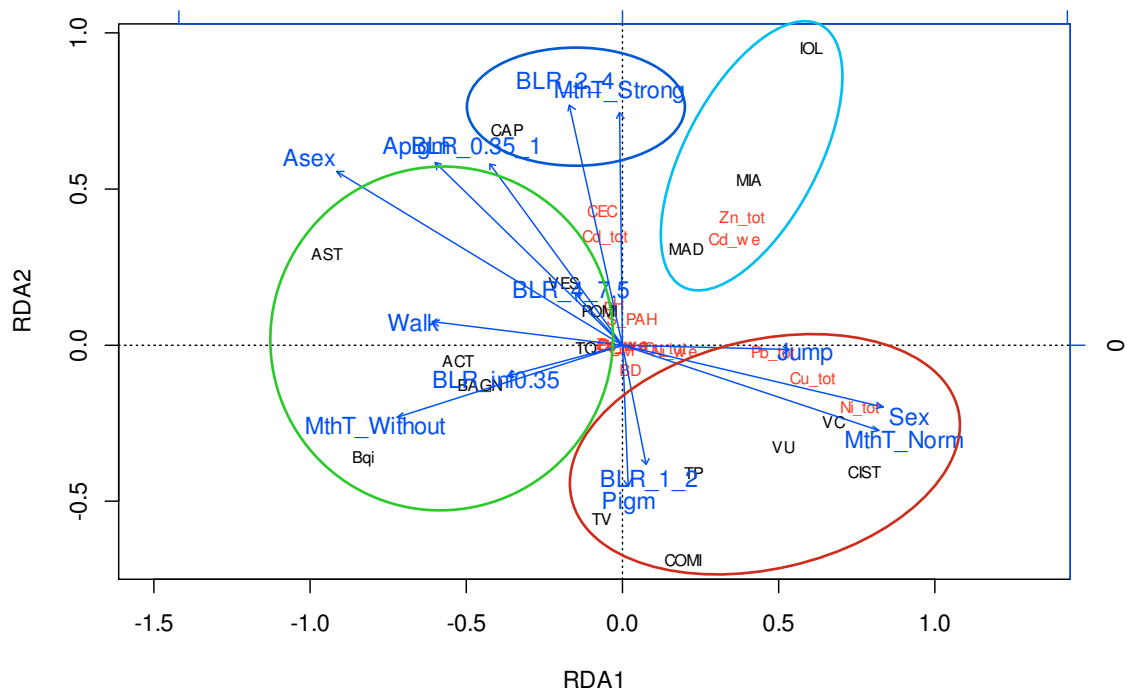
Figure 4.23. Results of ordination (RDA) analysis of data on the Functional traits of Collembola community in soils sampled in October 2011. RDA was analyzed taking into account sites, soil parameters and Collembola functional traits. Two tables were constructed with traits data and

environmental parameters. The figure represents the constraint ordination of the site samples, soil parameters (pH, bulk density, watr holding capacity, organic matter content, cationic capacity exchange, soil total and water-extractable metal content and sum of PAHs) and Functional traits of Collembola (Body size: BLRinf_0.35, BLR0.35_1, BLR1-2, BLR2-4, BLR4-7.5; Motion strategy: Jump, Walk; Mouthpart type: MthT_ Without, MthT_Norm, MthT_Strong; Pigmentation: Pig, Apig, Reproduction: Sex, Asex)

The analysis highlighted a separation between soils with scarce presence of metals and PAH concentrations (left) and soils with high presence of these compounds (right). On the left there are the soils characterized by higher pollutants content, on the right the soils scarcely polluted. The soils can be under-grouped in 4 groups: agricultural (red circle), urban (dark blue: urban park, light blue: urban gardens) and natural and industrial together (green circle). Functional traits also follow this site separation. Organisms with 1-2 mm body length, pigmented, with sexual reproduction, normal mandibles and jumping apparatus are more abundant in agricultural soils, with high concentrations of some metals and scarce vegetation cover was observed. Organisms with 2-4 mm body length and strong mandibles where higher at urban park (CAP), but not in all the urban soils, which showed high contamination but no distinctive functional triats. Organisms without pigmentation, asexual reproduction, without mandibles were abundant in less contaminated soils, represented by natural and industrial sites.

4.3.3 Effects of seasonality on Collembola community structure

4.3.3.1 Collembola taxonomic structure in spring sampling

In spring Collembola density was higher at AST, CAP, MIA, IOL, BAGN and VU. Overall, density was higher at NAT, URB and IND (Fig. 4.24). Richness showed the highest value at AST, and it linearly decreased passing from NAT to AGR (Fig. 4.24). Density and richness were not different between the seasons, except for the density at industrial soils which was higher in spring (Fig. 4.24).
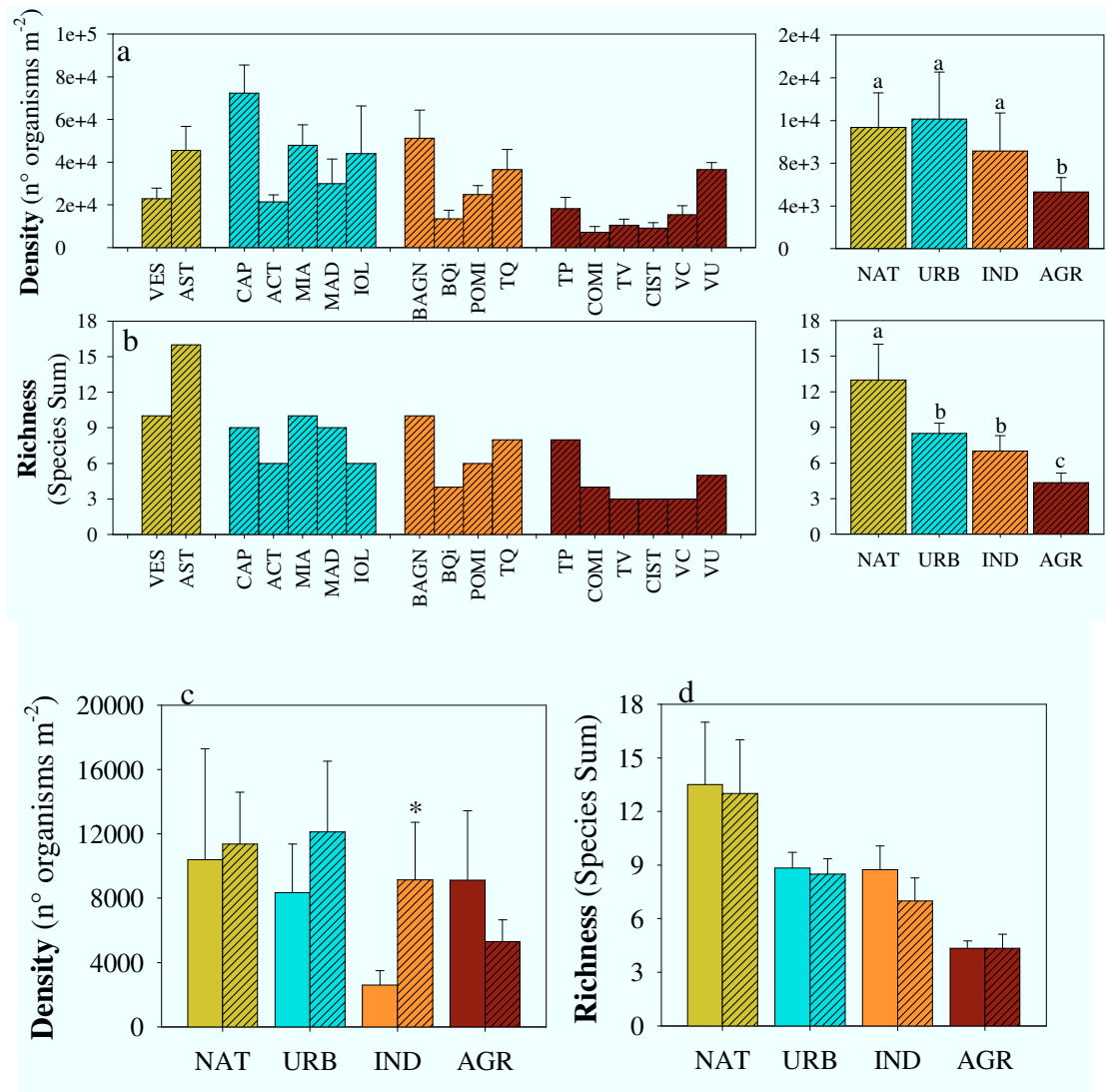
Figure 4.24. Mean (s.e.) density (a) and richness (b) of Collembola found in soils with different land uses in March 2012. Mean (s.e.) of density (c) and richness (d) detected in different land uses between autumn (no pattern) and spring (fine coarse pattern). Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

In spring species composition differed compared to the autumn. In natural soils, *Parisotoma notabilis* was dominant (Fig. 4.25), being abundant both at VES and AST. *Folsomia quadriloculata* and *Cryptopygus bipunctatus* were more abundant at AST and *Mesaphorura sp.* was higher at VES (Annexes, table 5). At urban soils, *Folsomia quadriloculata* was dominant (Fig. 4.25), but highly abundant at CAP (Annexes, table 5). Two species became abundant in spring: *Willemia denisi* at MIA and *Cryptopygus bipunctatus* at IOL (Annexes, table 5).
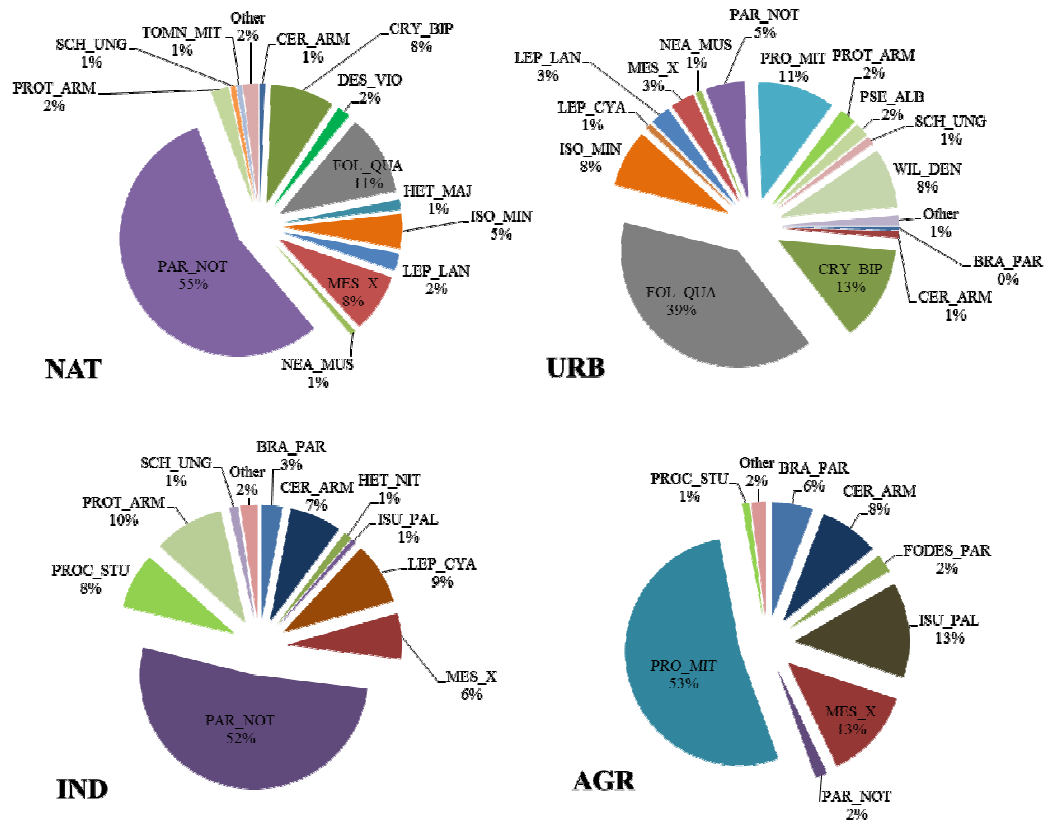
Figure 4.25. Percentage composition of Collembola community found in soils with different land uses in October 2011.

In spring, in all the industrial soils *Parisotoma notabilis* became abundant, whereas *Proctostephanus stukeni*, absent in all the soils in autumn, appeared at BAGN (Fig. 4.25, Annexes, table 6). In the agricultural soils, in spring, *Proisotoma minuta* dominated the community, although it decreased in abundance, whereas *Isotomurus palustris* abundance increased (Fig. 4.25, Annexes, table 7). *Cryptopygus bipunctatus* was positively (P < 0.05) correlated with Zn and Pb total concentrations, whereas *Proctostepanus stukeni* was positively (P < 0.05) correlated with low molecular weight PAHs.

In spring, diversity and evenness were higher in natural and urban soils, and showed similar values in industrial and agricultural soils (table 4.7).

Table 4.7. Mean (± s.e.) Diversity index (H), Evenness (E), of Collembola community in soils from different land uses. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

	NAT	URB	IND	AGR
H	1.07 **a** (0.19)	0.72 **a** (0.16)	0.65 **ab** (0.27)	0.41 **b** (0.13)
E	0.67 **a** (0.16)	0.59 **a** (0.15)	0.53 **ab** (0.21)	0.35 **b** (0.13)

4.3.3.2 Collembola functional structure in spring sampling

In spring, Collembola showed fewer differences among soils, regarding the body size, compared to the autumn. Abundances of Collembola between 0.35-1 mm and 1-2 mm showed inverse trends, although no differences were detected among soil typologies (Fig. 4.26). The lowest abundance of 0.35-1mm Collembola were measured at IOL, whereas the lowest abundances of Collembola between 1-2 mm were detected at ACT, BQi and VC (Fig. 4.26). At TP was measured the highest abundance of 2-4 mm organisms (Fig. 4.26).
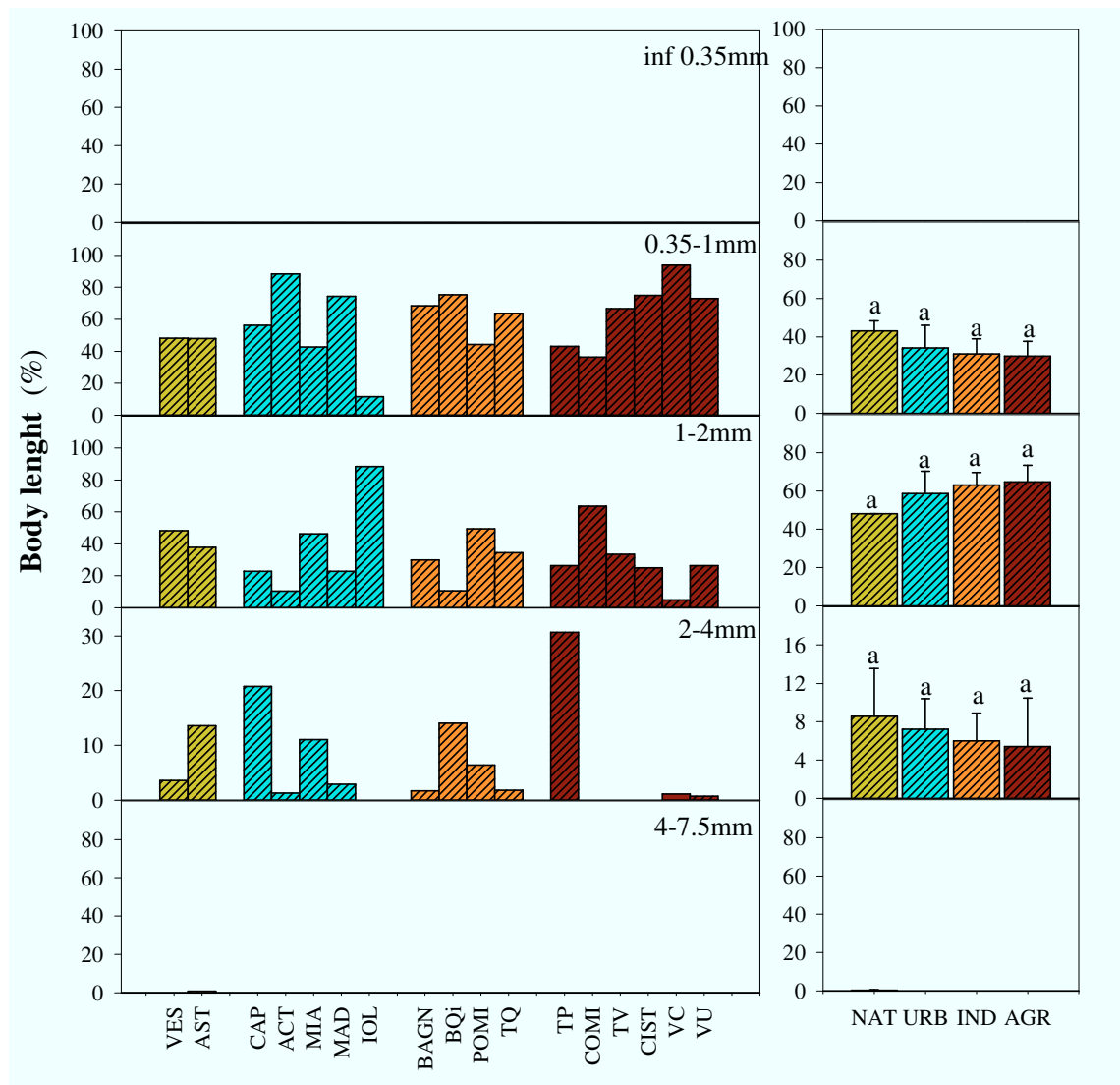
Figure 4.26. Percentage distribution of organisms with different body sizes measured in soils with different land uses sampled in March 2012. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

The two classes of motion strategy were homogeneous and did not showed differences among all the collected soils (Fig 4.27).
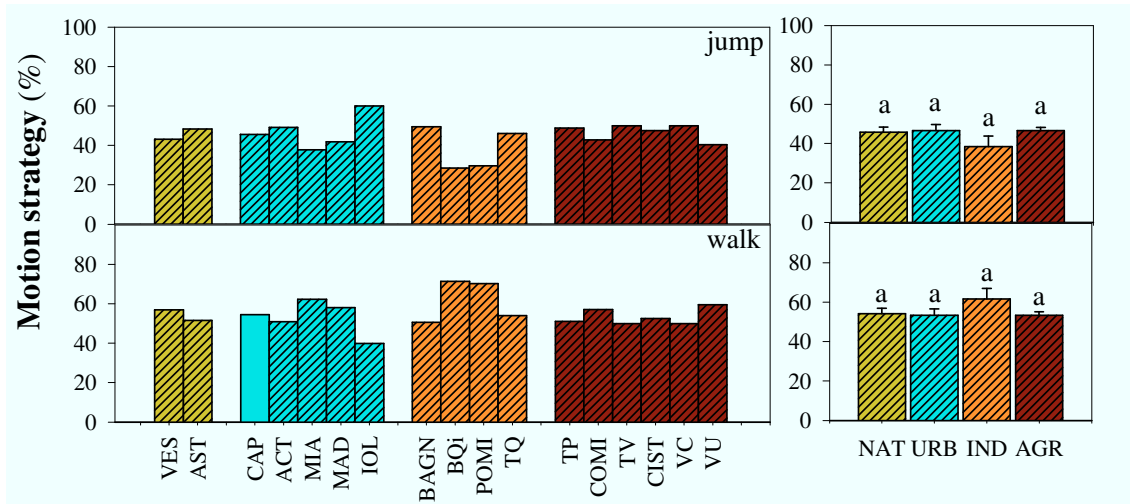
Figure 4.26. Percentage distribution of organisms with different Motion strategy found in soils with different land uses sampled in March 2012. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

In spring the abundance of organisms with strong mandibles decreased, whereas abundance of organisms without mandibles increased in some soils (Fig. 4.28).
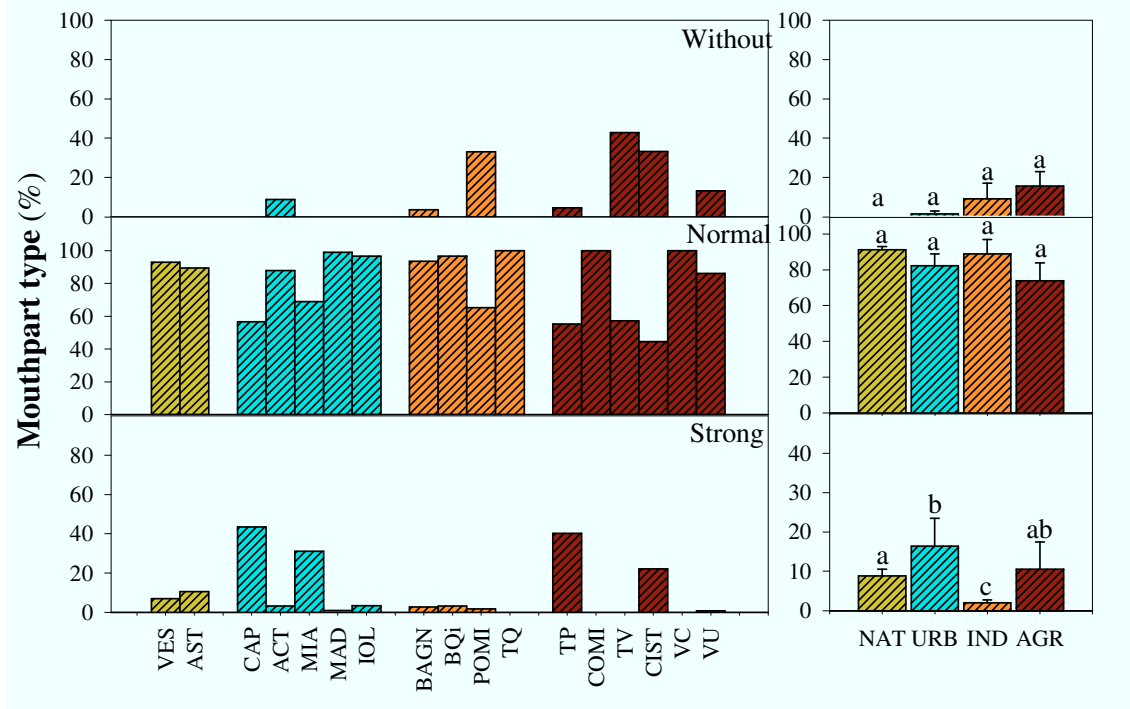
Figure 4.28. Percentage distribution of organisms with different mouthpart type found in soils with different land uses sampled in March 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

TV and CIST (agricultural) showed the highest abundance of organisms without mandibles, whereas CAP, MIA (urban) and TP and CIST (agricultural) the highest abundance of organisms with strong mandibles (Fig. 4.28). Organisms with normal mandibles were dominant in all the soils (Fig. 4.28), showing lower values at TP, TV, CIST (agricultural).

In spring all the agricultural soils showed the highest abundances of pigmented organisms (Fig 4.29), although their abundance increased, compared to the autumn, in all the soil typologies. Lowest values of pigmented organisms were detected at IOL and BQi, whereas agricultural soils showed the lowest values of apigmented organisms (Fig 4.29).
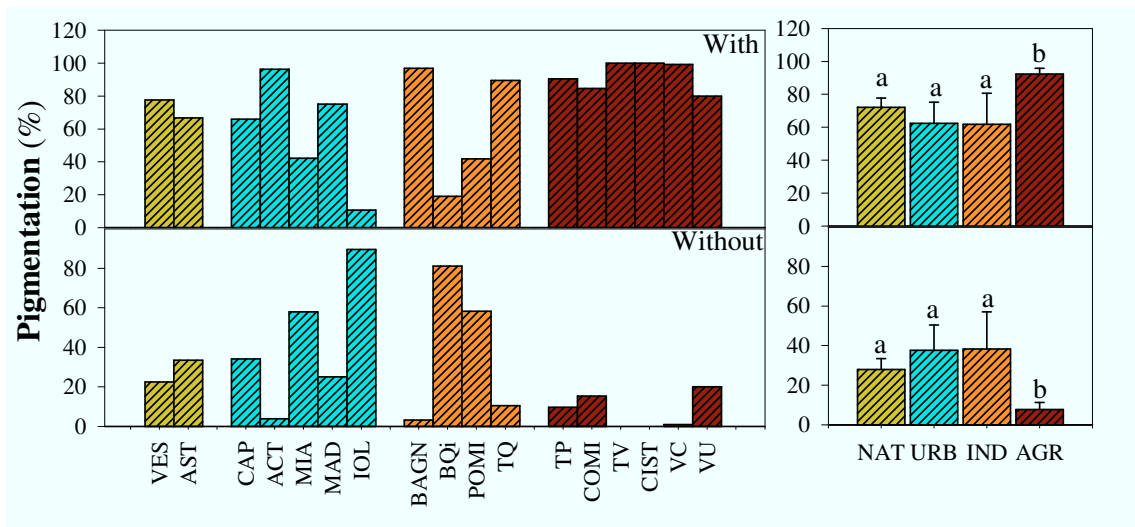
Figure 4.29. Percentage distribution of pigmented (With) and apigmented (Without) found in soils with different land uses sampled in March 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

Organisms with asexual reproduction showed the highest abundance at VES (natural), all the industrial soils and at COMI (agricultural). In spring the abundance of asexual reproductive organism increase in all the industrial soils and decreased in the urban soils (Fig. 4.30).
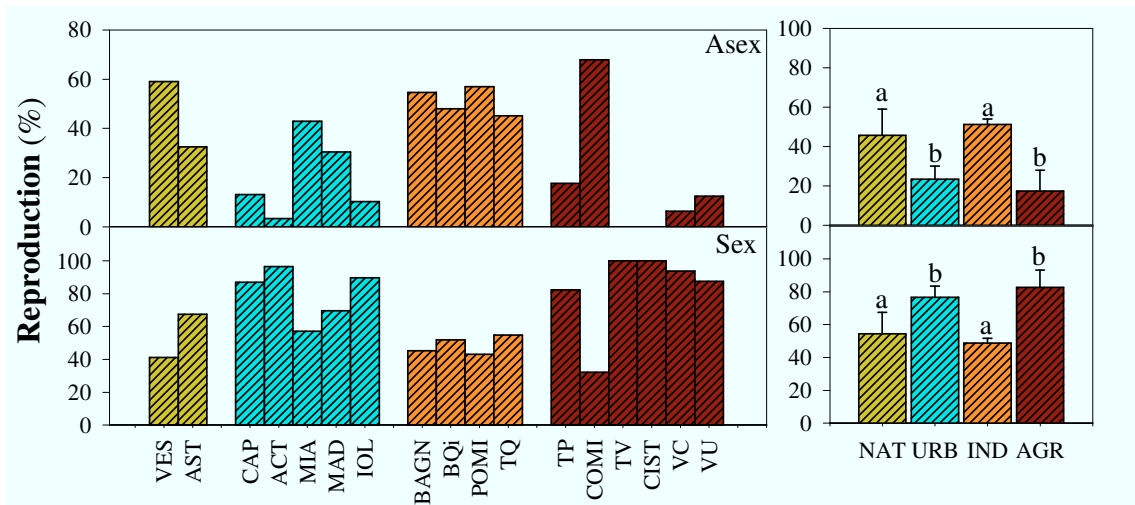
Figure 4.30. Percentage distribution of reproduction strategy (Asexual: Asex, Sexusual:Sex) of organisms found in soils with different land uses sampled in March 2011. Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at P < 0.05).

Between autumn and spring, statistical differences for the functional traits were detected for organisms with normal and strong mandibles, which increase and decreased, respectively, at natural sites, and for apigmented organisms which decreased at natural, urban and agricultural sites (Fig. 4.31).
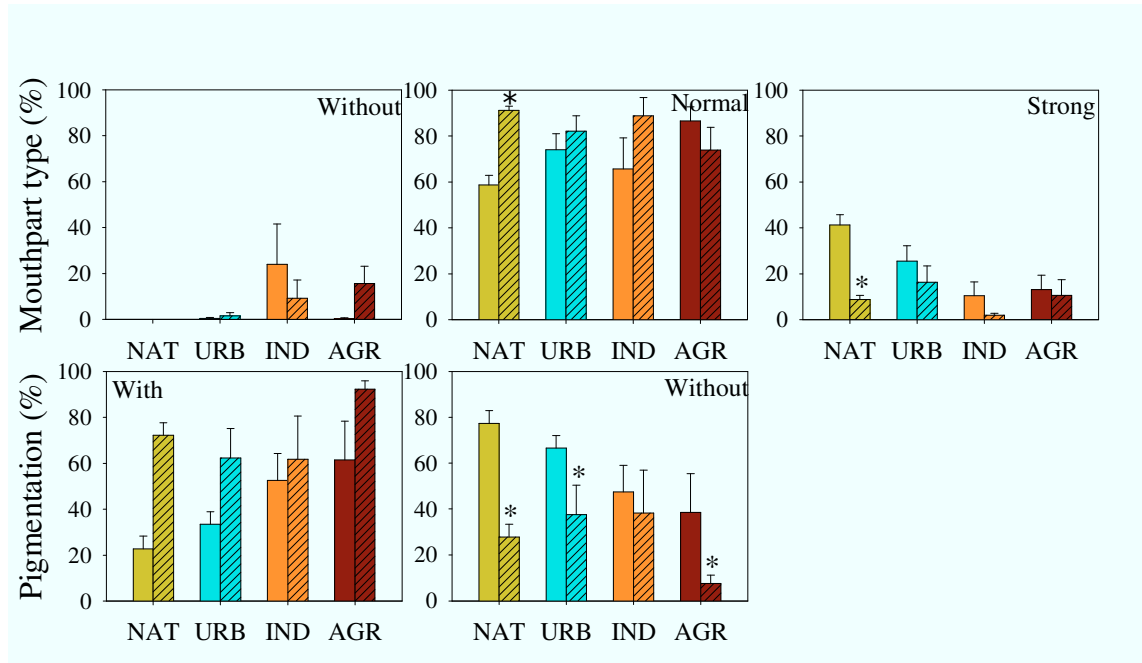
Figure 4.31. Percentage distribution of organisms with different mouthpart type and pigmentation found in soils with different land uses, in autumn (no pattern) and spring (fine coarse pattern) .Different letters indicate statistically significant differences among the sites (One-way Analysis of Variance with Holm-Sidak post-hoc test at $P < 0.05$).

The NMDS analysis (Fig. 4.32) was performed in order to highlight the relationships among Colembola functional traits between the two seasons of sampling. The main differences detected between the seasons regarding the abundance of organisms without mandibles (MthT_No) which in autumn were more abundant in industrial soils whereas in spring more abundant in agricultural soils. The abundance of other functional traits showed less or more similar trend among the soils between the seasons.

NMDS/Bray-Stress= 0.131
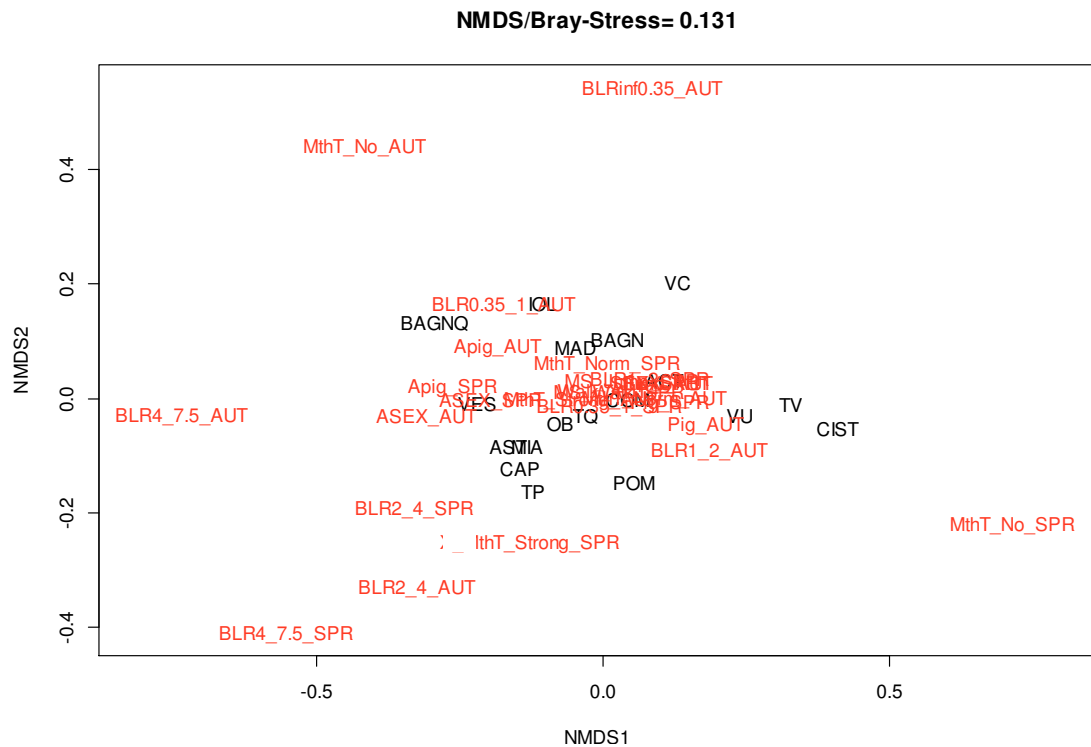
Figure 4.32. Results of NMDS analysis considering differences among Collembola functional traits between the seasons. Sites are represented in black; functional traits were represented in red (Body size in autumn : BLRinf_0.35_AUT, BLR0.35_1_AUT, BLR1-2_AUT, BLR2-4_AUT, BLR4-7.5_AUT and in spring BLRinf_0.35_SPR, BLR0.35_1_SPR, BLR1-2_SPR, BLR2-4_SPR, BLR4-7.5_SPR; motion strategy in autumn: MS_jump_AUT, MS_walk_AUT and in spring MS_jump_SPR, MS_walk_SPR; mouthpart type in autumn: MthT_No_AUT, MthT_Norm_AUT, MthT_Strong_AUT and in spring MthT_No_SPR, MthT_Norm_SPR, MthT_Strong_SPR; pigmentation in autumn: Pig_AUT, Apig_AUT and in spring Pig_SPR, Apig_SPR, reproduction in autumn Sex_AUT Asex_AUT and in spring Sex_SPR, Asex_SPR).

4.3.4 Discussion

Differences detected for the physical and chemical properties of investigated soils are reflecting on Collembola community structure and species assemblages. The scarce presence of Collembola in all the industrial soils, may suggest a sensitivity of collembolan to industrial management. This result is difficult to explain, since apparently industrial soils did not showed peculiar abiotic properties, being, in fact, for many aspects, similar to several categories of investigated soils. The low presence of Collembola in these kinds of soils can be linked several factors, such as several pollutants not assessed, or food resource availability or quality of organic matter. Also the landscape structure can be played a role in influencing Collembola distribution in industrial soils, as it has been showed Collembola community is also affected by landscape structure (Ponge et al., 2006). By contrast, density showed high variability among soils belonging to similar land uses. In particular, in urban environment density seemed to be dependent on soil contamination, as it was lower

at MIA and IOL. This result agreed with the above made hypothesis (4.2 section) that in urban environment Collembola are sensitive to metal contamination. On the other hand, in agricultural soils, Collembola density seems to be mainly linked to vegetation cover and to the litter accumulation. In fact, the lowest values of density were found in fields (TP, COMI) with orchard vegetation, where no litter layer was present. Despite low Collembola density in industrial soils, their richness, in the same soils, was comparable to urban soils, whereas it was very low in agricultural soils. The lower species richness found in agricultural soils, would suggest agricultural management and related inputs of metals heavily affected collembolan richness, as also confirmed by the found correlations between richness and total Cd, Cr and Ni concentrations. In addition, these results agreed with Postma-Blaauw (2010), which found that soil biota was generally negatively affected by conversion from natural to agricultural land. Moreover, the detected discrepancy between density and richness of Collembola happened because in soils with low richness, community can be composed by few species that better responding to habitat structures (McIntyre, 2001), becoming dominant in that environment. In fact, all the agricultural soils were dominated by *Proisotoma minuta*, which, being well adapted to live in open and disturbed contaminated fields (Potapow, 2001), seems to be a better competitor in agricultural soils. In addition its presence in soils with low organic matter content can indicate that it can tolerate the scarcity of organic matter. By contrast, species composition in soils of other land uses was more variable. *Parisotoma notabilis* and *Mucrosomia garretti,* being ubiquitous species (Potapow, 2001; Fjellber, 2007) were abundant both in natural, urban and industrial soils. The abundance of *Folsomia inoculata* in a mature forest (AST), can be linked to the high litter accumulation occurring in that site, as this species showed high density in humus soils (Potapow, 2001). At VES (natural) the high presence of *Mesaphorura sp.* could be due to acidic pH, as some *Mesaphorura* species are acidophilic (Ponge et al., 2003; Fjellberg, 2007). However *Mesaphorura* was present also in other kind of soil typologies, although its presence was very high in soils with high organic matter content, as confirmed by the found correlation. This finding may suggest that species of *Mesaphorura* can tolerate several soil managements, but not the scarcity of organic matter. *Folsomia quadriloculata* and *Folsomia litseri* were abundant species in urban soils, although the former was more present in less contaminated urban soil (CAP) and the latter in the most Pb-Zn contaminated urban soil (IOL). The

presence of *F. quadriloculata* at CAP is probably linked to the higher amount of litter deposition, as this species is positively related to organic matter content and high microbial biomass rates (Potapow, 2001). By contrast its low presence in other soils with high litter accumulation can be attributable to several factors, such as quality of organic matter and food resource or inter-specific competition. On the other hand, the positive correlation between *F. litseri* and total Pb concentration would suggest this species as tolerant to urban pollution, being also in accordance with Potapow (2001). In addition, although Potapow (2001) report that *Isotomiella minor* is sensitive to various pollutants, its presence in the collected soils was strongly related to the organic matter content and less to soil contamination.

Despite the differences in species composition diversity and evenness was lower only at agricultural soils, where an extreme simplification of the community occurred. It is important to note that, even if, collected agricultural soils presented different vegetation cover (orchards, vegetables, untilled) their Collembola community compositions were similar. It is arguable therefore, that agricultural management is more important, than vegetation, in affecting species assemblage. Farming management, in fact, can lead to a strong homogenization of the local communities composed often by same organisms, as only a small set of species is adapted to this kind of disturbance (Hendrickx et al., 2007).

However, differences in species assemblage among different soils were reflecting on functional structure. The presence of < 0.35mm organisms at industrial soils and of organisms of big dimension (4-7.5 mm) at natural sites, is supposed to be linked to the variability, rather than to a peculiarity of soil typology, given the very low detected abundances. By contrast, although organisms between 0.35-1mm were less abundant in agricultural soils, the classes of body size showed high variability among different soils, which indicates that properties of each soil, than land management, mainly influence the distribution of organism size.

Likely, motion strategy was not severely affected by land use. However the abundance of jumping organisms followed an increasing trend passing from natural to agricultural soils. The jumping strategy, in fact, is an adaptation to surface life and was typical of organisms showing high mobility and dispersal capacity (Hopkin, 1997). Mebes and Filser (1997) showed, in fact, a more large surface dispersal of Collembola living in agricultural fields compared to environment where litter began to accumulate.

The exclusive presence of Collembola without mandibles in industrial soils is due to abundance of *Brachystomella parvula*. The presence of this species in that environment could be linked to the presence of food resource. Collembola without mandibles eat by sucking their food (Adams, 1972), showing a piercing apparatus. Berg et al. (2004), studying the gut content of *B. parvula,* revealed the presence of nematodes and protozoa. As in disturbed sites the abundance of nematodes seems to increase (Turbè et al., 2010), the presence of a nematode feeder can be supposable, which can explain the presence of *B. parvula* in industrial soils. By contrast Collembola with normal mandibles feed on bacteria, fungi and organic decays (Hopkin, 1997). As this is the main feeding habit of Collembola, the constant presence of this category of organisms in all the soils was expected. On the other hand, the scarcity of Collembola with strong mandibles in agricultural soils, may suggest a sensitivity of these organisms to agricultural management. In fact, Collembola with strong mandibles seem to feed on living part of vegetables (Fjellberg, 2007). As herbivorous organisms are pests for agricultural plants (Sànchez-Moreno et al., 2011) the use of pesticides could cause a reduction of these organisms in agricultural fields. In addition, the abundance of herbivorous organisms is also related to plant diversity (Raupp et al., 2010), hence the homogenization of farming can bring to a decrease of these organisms.

Open sites such agricultural fields were characterized by the presence of pigmented and sexual reproductive organisms, which are well adapted to surface life (Hokin, 1997). Pigmentation is an adaptation to avoid predation and sexual reproduction needs easy-to-visit sites for the deposition of spermatophores by males and movement in search of mating partners using olfactory or tactile clues (Salmon and Ponge, 2012), which is easier in surface than in depth. In addition, sexual reproduction can be more useful in disturbed environment than in wood land. In fact, wood lands are usually habitats more stable than urban or agricultural ones, in which different kind of perturbation can occur. Therefore for the organisms should be better to have a larger genetic biodiversity which can allows it to better adapt to several disturbances.

Studied area usually showed wide variations in climatic conditions among the season. However, in the considered period (October 2011 and March 2012) climatic conditions showed wider differences for temperatures than for rainfalls. In fact, the mean temperature and total amount of rainfall in the last three months before the

samplings in autumn (October 2011) and spring (March 2012) were 22.6 °C and 120 mm and 9.7 °C and 150 mm, respectively. As it has been showed that Collembola density increased with rainfall rates (4.2.4 section), the absence of differences in rainfalls, detected between the seasons, did not strongly influence Collembola density and richness. However, at industrial sites density of Collembola increased, with respect to the autumn. It is arguable that the increase of density in industrial soils in spring, it is not linked to environmental conditions, rather than to the life cycle of some species present in the community. In fact, in spring *Parisotoma notabilis* become abundant both in natural than in industrial soils. Potapow (2001) reported that abundance peaks of this species occurred in spring and in summer. By contrast, the abundance of *Isotomurus palustris* and *Folsomia quadriloculata* in spring, in agricultural and urban soils, respectively, could be linked to the climatic conditions. In fact, although no difference were detected for rainfall between the seasons, in spring the humidity is generally higher than in autumn, which explain the increased abundance of two hygrophilic species (Potapow, 2001). In general, in collected soils species with epidaphic characteristics (*Parisotoma notabilis*, *Cryptopygus bipunctatus*, *Folsomia quadriloculata*) increased in abundance in spring. This shift in the Collembola community could be due to reduced vegetation coverage occurring in the previous period (winter), which could favor the presence of species well adapted to surface life. This shift in Collembola community in spring is reflecting on diversity and evenness of community which resulted more similar among soils than in autumn. In fact, in spring, in all the soils typologies one species was dominant than the others, resulting in a similar evenness values among the soil typologies.

In addition, also functional structure is affected by changing in community composition. In fact, for almost all the detected functional traits, differences among the soils were less pronounced in spring than in autumn. In general, main differences were detected in natural environment, in which there was an increase of functional traits typical of soil surface life, confirmed by the increase in abundance of pigmented and sexual reproductive organisms. The reduction of organisms with strong mandibles in natural soils also could be due to the reduction of groundcover vegetation. In fact, these organisms are supposed to eat on living parts of vegetables, therefore the reduction of vegetation is reflecting on decreasing of their abundance.

Land use changes affected Collembola community structure both taxonomically than functionally. Collembola richness, more than density, was negatively affected for the land use transformation. There were not detected species typical of natural habitats, whereas species indicators of disturbed habitats were found. *Proistoma minuta* and *Folsomia litseri* were respectively indicator species of agricultural management and urban pollution. Functional structure, than taxonomic structure, was more informative about the land management, discriminating the collected in three different groups: agricultural, urban, natural-industrial. Collembola community responded to seasonal variation with increasing species with epidaphic characteristics, probably due to the vegetation responses to the seasonality. However, taxonomic and functional structure of Collembola community was similarly distributed among the soils in both the seasons.

CHAPTER V

General Discussion and Conclusions

The increasing of human population size and the parallel extent and magnitude of human-mediated effects on the Earth has synergistically sparked a growth in ecologist interest about human-environment relationships. However, despite the research about urban ecology, scarce is the knowledge about the effects of urbanization on several processes, such as soil functionality. In urban environment, in fact, soil and soil biota actively participate to the global benefit of citizens. On the other hand soil organisms are sensitive to every changes occurring in the environment, and their distribution strongly vary in according soil characteristics, climatic conditions and habitat and landscape structures. Dynamics of soil organism communities in soils with particular characteristics, such as volcanic soils, and in peculiar climatic conditions remain still unknown, even more in anthropized soils.

The results obtained allowed to better understand how different land anthropization affect soil arthropod community and therefore quality and functionality of different soils.

The different kinds of anthropic impacts on soils affected first of all soil physical and chemical properties. Agricultural and urban lands represented the practices which mostly altered soil abiotic properties. Type and rate of vegetation coverage, habitat and landscape structure, as well as the presence of some compound and some physical properties can be considered as markers of agricultural or urban land use.

Arthropod community, strictly dependent on abiotic environment, was strongly differentiated among soils. In the urban soils, organic matter content and pollution were the main driving forces in affecting the distribution of arthropods. In fact, organisms were more abundant and diversified in soils rich of organic matter and poor in pollutant concentrations. Among the found taxa, Collembola seemed to be more sensitive to urban metal contamination in soils, as also confirmed by the laboratory exposition results. However, the results obtained during the bioassay

investigation highlighted less differences among the tested soils, compared to the arthropod community composition in field investigation. This indicates and confirms the power of field investigations in soil quality assessment. Moreover, in Mediterranean environment, arthropod community varied also between the seasons. Dry-warm (autumn) season seemed to be more favourable, than wet-cold (spring) season, for arthropods in Mediterranean soils. In spring, however, organisms living in surface soils were more favoured than in autumn.

Collembola community structure also widely responded to different land use practices. Species richness was heavily affected by agricultural practices. Taxonomic composition strongly varied between natural and agricultural managements, whereas industrial and urban land uses showed a taxonomical composition intermediate between natural and agricultural environments. On the other hand functional structure seemed to mainly respond to soil contamination. In fact soil mostly contaminated (agricultural and urban) showed a different functional structure of Collembola. This may suggest than the degree of pollution, more than land use, affected functionality of Collembola community and therefore soil functioning. Seasonal variations did not heavily change the structure of Collembola community, although in spring the organisms living on the surface were favoured.

Overall, the kind of land use mostly affected arthropod taxonomic structure, whereas soil contamination, independently on land use, influenced more the functional structure of soil organism community.

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ANNEXES

Table 1. List of Collembola species found in soils from Naples and surroundings in October 2011 and March 2012

COLLEMBOLA		
FAMILY	**SPECIES**	**CODE SPECIES**
Entomobyidae	*Cyphoderus albinus*	CYP_ALB
	Cyphoderus grassei	CYP_GRA
	Entomobrya multifasciata	ENT_MUL
	Heteromurus hexophtalmus	HET_HEX
	Heteromurus nitidus	HET_NIT
	Lepidocyrtus cyaneus	LEP_CYA
	Lepidocyrtus lanuginosus	LEP_LAN
	Pseudosinella halophyla	PSE_HAL
	Pseudosinella sexoculata	PSE_SEX
	Pseudanurophorus binoculatus	PSED_BIN
Isotomidae	*Cryptopygus bipunctatus*	CRY_BIP
	Cryptopygus thermophilus	CRY_THE
	Desoria violacea	DES_VIO
	Folsomides parvulus	FODES_PAR
	Folsomia fimetaria	FOL_FIM
	Folsomia inoculata	FOL_INO
	Folsomia litseri	FOL_LIT
	Folsomia quadriloculata	FOL_QUA
	Folsomia sexoculata	FOL_SEX
	Folsomia spinosa	FOL_SPI
	Isotoma virides	ISA_VIR
	Isotomiella minor	ISO_MIN
	Isotomurus palustris	ISU_PAL
	Mucrosomia garretti	MUC_GAR
	Parisotoma notabilis	PAR_NOT
	Proisotoma minuta	PRO_MIT
	Proisotomodes scalpelliferus	PRODES_SCA
Onychiuridae	*Protaphorura armata*	PROT_ARM
Poduridae	*Brachystomella parvula*	BRA_PAR
	Ceratophysella armata	CER_ARM
	Ceratophysella gibbosa	CER_GIB
	Deuteraphorura inermis	DEU_INE
	Friesea mirabilis	FRI_MIR
	Friesea truncata	FRI_TRU
	Mesaphorura	MES_X
	Micranurida sensillata	MIC_SEN
	Neanure muscorum	NEA_MUS
	Schoetella ununguiculata	SCH_UNG
	Willemia denisi	WIL_DEN
Sminthuridae	*Arrhopalites principalis*	ARR_PRI
	Bourletiella hortensis	BOU_HOR

	Sminthurus viridis	SMI_VIR
	Sminthurinus niger	SMINUS_NIG
Sminthurididae	*Sphaeridia pumilis*	SPH_PUM

Table 2. Mean (s.e.) of Collembola species abundances found in natural and urban soils from Naples and surroundings in October 2011

	NATURAL		URBAN				
	VES	AST	CAP	ACT	MIA	MAD	IOL
CYP_ALB			102 (102)				
CYP_GRA		204 (204)				102 (102)	
ENT_MUL		102 (102)					
HET_HEX		102 (102)					
HET_NIT		306 (204)					
LEP_CYA	50.9 (50.9)						
LEP_LAN	153 (77.8)	611 (249)	1428 (467)		1121 (374)	713 (444)	
PSE_HAL							
PSE_SEX			102 (102)			204 (204)	
PSED_BIN				102 (102)			
CRY_BIP	50.9 (50.9)	1019 (456)			204 (125)		102 (102)
CRY_THE						102 (102)	
DES_VIO			102 (102)				102 (102)
FODES_PAR							
FOL_FIM							
FOL_INO	459 (459)	2752 (2499)		815 (815)	102 (102)		
FOL_LIT				2853 (2727)	306 (204)		2446 (2446)
FOL_QUA		509 (227)	6624 (4242)		917 (691)		
FOL_SEX					204 (125)		
FOL_SPI		102 (102)					
ISA_VIR	102 (102)						
ISO_MIN	408 (238	815 (572)	1936 (1313)		713 (499)		408 (408)
ISU_PAL						102 (102)	
MUC_GAR		2650 (1760)	1529 (683)				204 (125)
PAR_NOT	968 (555)	2446 (1342)	102 (102)		408(408)	306 (204)	408 (250)
PRO_MIT	102 (67.9)			4484 (3505)	102 (102)	1936 (1332)	
PRODES_SCA		102		102			

	C1	C2	C3	C4	C5	C6	C7
		(102)		(102)			
PROT_ARM		204 (204)				2548 (1712)	
BRA_PAR			102 (102)				
CER_ARM		102 (102)					
CER_GIB						1325 (1088)	204 (204)
DEU_INE			306 (204)		611 (611)		
FRI_MIR							
FRI_TRU							
MES_X	5554 (2946)	408 (297)	2242 (1732)	1528 (1056)		2650 (1886)	102 (102)
MIC_SEN		408 (408)					
NEA_MUS					102 (102)		
SCH_UNG					102 (102)		
WIL_DEN							
ARR_PRI	102 (102)						
BOU_HOR							
SMI_VIR							
SMINUS_NIG							
SPH_PUM							

Table 3. Mean (s.e.) of Collembola species abundances found industrial soils from Naples surroundings in October 2011

	INDUSTRIAL			
	BAGN	BQi	POMI	TQ
CYP_ALB				
CYP_GRA				
ENT_MUL			408 (298)	102 (102)
HET_HEX				
HET_NIT				
LEP_CYA	102 (102)			
LEP_LAN			204 (125)	510 (280)
PSE_HAL				408 (298)
PSE_SEX				
PSED_BIN				
CRY_BIP	306 (125)			
CRY_THE				
DES_VIO				
FODES_PAR				
FOL_FIM				102 (102)
FOL_INO				

	col1	col2	col3	col4
FOL_LIT				102 (102)
FOL_QUA				
FOL_SEX				
FOL_SPI				
ISA_VIR				
ISO_MIN				
ISU_PAL				
MUC_GAR	1121 (632)			
PAR_NOT		170 (170)		1019 (279)
PRO_MIT	306 (306)		1732 (1254)	102 (102)
PRODES_SCA				
PROT_ARM	102 (102)			408 (408)
BRA_PAR	408 (298)	340 (170)		
CER_ARM				
CER_GIB	102 (102)			
DEU_INE				
FRI_MIR			102 (102)	
FRI_TRU				
MES_X	306 (204)	1019 (778)	204 (125)	306 (125)
MIC_SEN				
NEA_MUS				
SCH_UNG				
WIL_DEN				
ARR_PRI				
BOU_HOR				
SMI_VIR				
SMINUS_NIG	102 (102)			
SPH_PUM	306 (204)			

Table 4. Mean (s.e.) of Collembola species abundances found agricultural soils from Naples surroundings in October 2011

	AGRICULTURAL					
	TP	COMI	TV	CIST	VC	VU
CYP_ALB						
CYP_GRA						
ENT_MUL		102 (102)	1732 (125)	102 (102)	510 (280)	408 (408)
HET_HEX						
HET_NIT						
LEP_CYA						
LEP_LAN						
PSE_HAL						

	NAT		URBAN				
	VES	AST	CAP	ACT	MIA	MAD	IOL
PSE_SEX							
PSED_BIN							
CRY_BIP							
CRY_THE							
DES_VIO							
FODES_PAR	102 (102)						
FOL_FIM							
FOL_INO							
FOL_LIT					3567 (3318)	306 (306)	
FOL_QUA							
FOL_SEX							
FOL_SPI							
ISA_VIR							
ISO_MIN							
ISU_PAL							
MUC_GAR		306 (306)	204 (125)		815 (815)		
PAR_NOT							
PRO_MIT	102 (102)	306 (204)	5401 (2022)	14573 (9964)		23745 (9861)	
PRODES_SCA							
PROT_ARM	408 (250)						
BRA_PAR				204 (204)			
CER_ARM				102 (102)			
CER_GIB							
DEU_INE							
FRI_MIR							
FRI_TRU						510 (510)	
MES_X	408 (102)	204 (204)				306 (306)	
MIC_SEN							
NEA_MUS							
SCH_UNG							
WIL_DEN			102 (102)				
ARR_PRI							
BOU_HOR						102 (102)	
SMI_VIR	102 (102)						
SMINUS_NIG							
SPH_PUM							

Table 5. Mean (s.e.) of Collembola species abundances found in natural and urban soils from Naples and surroundings in March 2012

BRA_PAR				306 (306)			
CER_ARM		204 (125)		306 (204)			
CER_GIB						204 (204)	
CRY_BIP	204 (156)	2242 (676)	713 (499)		917 (297)		6828 (6701)
CYP_ALB							
CYP_BIN		102 (102)					
DES_VIO		510 (322)					
ENT_MUL							102 (102)
FODES_PAR							
FOL_CAN							
FOL_QUA	51 (51)	3261 (1026)	16917 (5571)		9478 (3176)		
HET_MAJ	204 (113)		102 (102)				
HET_NIT		102 (102)					
ISO_MIN	408 (250)	611 (374)	1732 (594)	102 (102)	3057 (837)		408 (250)
ISU_PAL				102 (102)			
ISU_PLU		102 (102)				102 (102)	
LEP_CYA					102 (102)	408 (408)	
LEP_LAN	102 (68)	510 (279)	1427 (590)		204 (204)		
MES_X	1070 (298)	306 (204)		102 (102)	204 (204)	713 (594)4	510 (161)
MUC_GAR			102 (102)			102 (102)	
NEA_MUS		204 (125)					
ORC_VIL		102 (102)					
PAR_NOT	5758 (1699)	5503 (4138)	204 (204)		204 (204)	2751 (2626)	306 (306)
PRO_MIT	51 (51)			4484 (1480)		2038 (1338)	510 (395)
PROC_STU							
PROT_ARM		713 (381)			204 (204)	1223 (472)	
PSE_ALB			510 (395)		815 (525)		
PSE_SEX							
SCH_UNG	51 (51)	102 (102)				611 (495)	
SMINUS_ELE	51 (51)						
TOMN_MIT		204 (204)					
WIL_DEN			815 (472)		4688 (1061)		

Table 6. Mean (s.e.) of Collembola species abundances found industrial soils from Naples surroundings in March 2012

	INDUSTRIAL			
	BAGN	**BQi**	**POMI**	**TQ**
BRA_PAR	204 (204)		815 (594)	
CER_ARM	2446 (1875)			102 (102)
CER_GIB				
CRY_BIP				
CYP_ALB				102 (102)
CYP_BIN				
DES_VIO	102 (102)			
ENT_MUL				
FODES_PAR				
FOL_CAN				102 (102)
FOL_QUA	102 (102)			
HET_MAJ				
HET_NIT				408 (297)1
ISO_MIN	102 (102)			
ISU_PAL	102 (102)	170 (170)		
ISU_PLU			102 (102)	
LEP_CYA				3057 (1258)
LEP_LAN				
MES_X		170 (170)	1019 (581)	1121 (762)
MUC_GAR				102 (102)
NEA_MUS				
ORC_VIL				
PAR_NOT	7439 (3363)	510 (205)	815 (594)	9682 (3642)
PRO_MIT				
PROC_STU	2650 (1036)		102 (102)	
PROT_ARM	306 (306)	2718 (2718)	1529 (1529)	
PSE_ALB				
PSE_SEX				
SCH_UNG	408 (408)			
SMINUS_ELE	102 (102)			
TOMN_MIT				
WIL_DEN				

Table 7. Mean (s.e.) of Collembola species abundances found agricultural soils from Naples surroundings in March 2012

	AGRICULTURAL					
	TP	COMI	TV	CIST	VC	VU
BRA_PAR	204 (125)		102 (102)	102 (102)		1427 (959)
CER_ARM	2344 (1146)	102 (102)	204 (204)			
CER_GIB						
CRY_BIP						
CYP_ALB						
CYP_BIN						
DES_VIO						
ENT_MUL				102 (102)		
FODES_PAR	815 (414)					
FOL_CAN						
FOL_QUA					102 (102)	
HET_MAJ						
HET_NIT						
ISO_MIN						
ISU_PAL	4076 (1879)					204 (204)
ISU_PLU						
LEP_CYA						
LEP_LAN						
MES_X	204 (204)	204 (125)				3669 (2320)
MUC_GAR						
NEA_MUS						
ORC_VIL						
PAR_NOT	306 (204)				204 (125)	
PRO_MIT	713 (260)	102 (102)	204 (125)	204 (204)	1630 (632)	13860 (3643)
PROC_STU		306 (306)				
PROT_ARM						
PSE_ALB						
PSE_SEX					204 (204)	
SCH_UNG						
SMINUS_ELE	204 (125)					
TOMN_MIT						
WIL_DEN						

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