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Study of the impact of different green roof types on soil biodiversity in Île-de-France

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Chaire Agricultures Urbaines

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List of abbreviations

APUR: Atelier Parisien d'Urbanisme

BIOTOV: BIOdiversité des Toitures Végétalisées

CEC: Cation-Exchange Capacity

FAO: Food and Agriculture Organization of the United Nations

INRAE: Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement

IPCC: Intergovernmental Panel on Climate Change

MB: Microbial Biomass

MBC: Microbial Biomass Carbon

MEA: Millennium Ecosystem Assessment

NMDS: Non-metric Multidimensional Scaling

OM: Organic Matter

PCA: Principal Components Analysis

PLFA: PhosphoLipid Fatty Acid

PLU: Plan Local d'Urbanisme

RDA: Redundancy Analysis

TME: Trace Metallic Element

WRB: World Reference Base for Soil Resources

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Foreword

For several decades, the Île-de-France region has been developing a policy to promote the development of green roofs and green spaces in urban areas in general, with the aim of promoting biodiversity. The concept of "Zero Net Artificialization" appeared in 2018 in the French government's biodiversity plan. Supported since 2011 by the European Commission to improve biodiversity in cities and decrease climate change, it aims to find a balance between artificialization and renaturation (Institut Paris Région 2020). There is therefore a synergy between the development of new green infrastructure and the development of urban biodiversity (Clergeau et al. 2020). Projects for greening roofs and walls are becoming more and more widespread this millennium, and interest in them is growing, both from an environmental and societal point of view.

Numerous research projects have been launched over the past two decades to quantify the environmental impact of greening urban environments. The “Chaire Agriculture Urbaine”, created by the AgroParisTech Foundation in 2018, has financed several projects aimed at studying urban areas, including green roofs. Thus, BIOTOV *(BIOdiversité sur les TOitures Végétalisées)* project was born in 2021, on which I carried out my 6-month internship from the 13rd of February to the 28th of July 2023, at the INRAE (Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement) Agro-Paris-Saclay campus from Palaiseau (UMR ECOSYS). It focused on the study of the impact of green roofs on soil organisms (microorganisms, micro-, meso-, and macrofauna) in Ile-de-France. The stakes are high in Ile-de-France, where urbanization is very strong, and the expansion of green spaces is anchored in current policies, to alleviate the many climatic and environmental problems encountered in the region. Sékou Coulibaly, post-doctor in biodiversity and ecosystem services of vegetated and cultivated green roofs, and Sophie Joimel, researcher in soil ecology, supervised me during my internship.

Figure 1: **Millennium Ecosystems Assessment (MEA) categorization of urban ecosystem services** (Srdjevic, Srdjevic, and Lakicevic 2019)

Introduction

Representing 54% of the world's population (Guilland et al. 2018) for only 3% of the Earth's surface (MEA 2005), urban areas have been the pinnacle of anthropization for several decades, and have a strong impact on ecosystems (Elmqvist et al. 2013). According to the IPCC (Intergovernmental Panel on Climate Change), urbanization is *"the conversion of land from a natural or managed natural state to cities; a process driven by net rural-to-urban migration whereby an increasing percentage of a nation's or region's population moves to settlements that are defined as urban centers"* (IPCC 2007).

The urban area is very heterogeneous and anthropized, consisting of the juxtaposition of several ecosystems interacting more or less with each other (Cerema 2018). City is shaped for the most part of inert materials (e.g., buildings, streets), provoking urban heat islands with the increase of the atmospheric temperature (Oberndorfer et al. 2007). Water cycle in urban areas is also disrupted by soil sealing linked to the imbalance between water infiltration and runoff (Elmqvist et al. 2013). This environment has led to a deep change of virgin landscapes and has negatively impacted and reduced all form of biodiversity (Haase et al. 2014). The main causes of biodiversity decline are therefore pollution (Guilland et al. 2018), climate change, urban land artificialization, urban sprawl (Awada 2021), or the resulting fragmentation of natural systems (IPCC 2007).

However, urban areas provide ecosystem services. It allows both the aspects of nature and social aspects to be encompassed (Blanc 1998), and is then defined as *"the system of relations between ecological systems and social systems in urbanized areas"* (Cerema 2018), and answer to four services (Clergeau et al. 2020; Elmqvist et al. 2013) (*Figure 1*). Urban ecosystem services are still few studied, but more than decades ago (Haase et al. 2014). Before the 1960s, city was viewed by ecologists as an environment hostile to wildlife (Cerema 2018), it changed when the notion of urban ecosystem appeared, and with the first scientific researches (Duvigneaud 1974). Policies based on the environment in urban areas emerged in the 1980s, raised awareness of global environmental issues, and promote nature in the city (Blanc 1998).

The major turning point in ecosystem valuation in urban areas came in the early 2000s with the Millennium Ecosystem Assessment (MEA), which redefined the vision of ecology, and is nowadays widely used (Clergeau et al. 2020). Governmental bodies such as the FAO (Food and Agriculture Organization of the United Nations) in 2020 set out sustainability development goals to be achieved, including *"[the] creation a more sustainable future for cities and other urban communities"* (e.g., Joimel, Jules, and Vieublé Gonod 2022). Before 1995, only 100 publications addressed ecology and the urban environment, today it is 14% of scientific paper (Joimel, Jules, and Vieublé Gonod 2022).

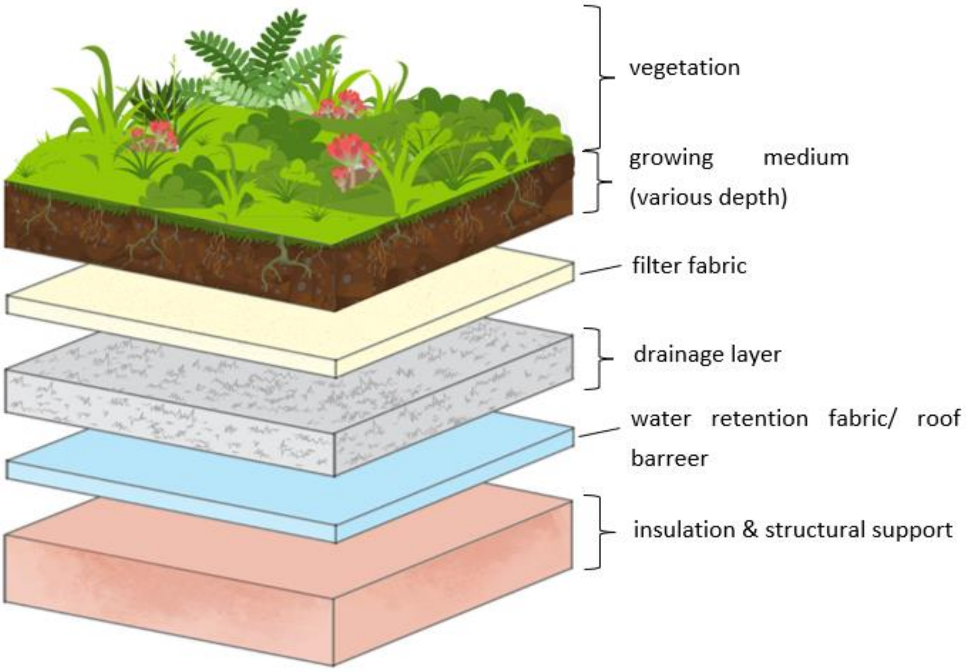

Figure 2: **type of conventional green-roofing technology** (Levaillant 2023). *Underneath the soil thickness is a filter material to keep fine particles, so that there is no risk of clogging with the layer below, which is the drainage layer. This layer protects the roof from damage caused by root penetration and is supported by an impermeable layer/root barrier that also retains excess water* (Getter and Rowe 2006; Mann 1994). *Finally, these different layers rest on top of an insulating layer and the structural support of the roof (Oberndorfer et al. 2007).*

Vegetation in the city allows for the sequestration and storage of carbon in the soil. It also makes it possible to manage runoff, regulate flooding, mitigate heat islands, and improve air quality (Awada 2021). The first desire of the French population on the expectations of the cities of tomorrow is to *"put nature back in the heart of cities"* (53% of positive responses), followed by *"a city that does not pollute"* (42%) and *"a city that offers a good mix between economic and social life, between work and housing"* (30%) (Clergeau et al. 2020). The presence of green spaces in the city is crucial: urban parks and gardens, sports fields, urban wastelands, or green walls and roofs. The challenge today is to provide a viable habitat in the city with vegetation, both for humans and for other living beings in this ecosystem (Braaker et al. 2014). Cities must be resilient, that is, they must *"return to normal ecosystem functioning after being disturbed"* (Elmqvist et al. 2013). The transformation must be profound, nature in the city does not only have an aesthetic role, but must also be functional (Barra 2020).

1. Is greening rooftops in urban areas just a fad, or a real interest for the ecosystem and biodiversity? – focus on soil biodiversity

1.1. Green roofs, a growing type of green space: their characteristics, and constraints

In the 1920s, Le Corbusier introduced the concept of the flat roof into architecture, and theorized the use of vegetation in places that previously lacked it. From a simple green cover to a real urban vegetable garden, green roofs have continued to develop in recent decades (Clergeau et al. 2020). The growing ecological awareness in the 1970s allowed the rise of green roofs (Madre et al. 2014). In 2002, 100,000m² of green roofs were counted, compared to over 1,000,000m² in 2011 (Joimel et al. 2022). In highly urbanized areas where space is in short supply, finding places to vegetate is not easy, hence the growing interest in green roofs, as buildings represent a large potential surface area (Joimel et al. 2022). It appears to be a solution to *"greening the gray"* (Francis and Lorimer 2011), but also to allow for the replacement of the vegetation footprint that was lost (Braaker et al. 2014), and that has been destroyed by the construction of cities and buildings (Getter and Rowe 2006).

The installation of green roofs could also be a tool to increase urban biodiversity (Oberndorfer et al. 2007), as they are sometimes qualified as *"green lungs"* of cities (Schrader and Böning 2006). According to the APUR (Atelier Parisien d'Urbanisme), 80 ha of roofs in Paris have a strong potential to host vegetation (APUR 2013). Emerging phenomena such as urban agriculture have allowed rooftops to become plots for growing food goods and have thus increased the interest in green roofs (Madre et al. 2014). However, one should not forget the importance of being part of a coherent urban ecology with green roofs, and therefore be careful with the growing "fad" that green roofs are sometimes built without a real objective of answering to ecosystem services (Awada 2021).

Table 1: **comparison of extensive, semi-intensive and intensive green roofs, the three most common green roofs types** (Levaillant 2023), based on many sources: [(1)] (Oberndorfer et al. 2007); [(2)] (SECC 2019); [(3)] **(Adianens 2022)**; [(4)] (Joimel et al. 2018) ; [(5)] (Madre et al. 2014) ; [(6)] (Awada 2021) ; [(7)] (SECC 2019) ; [(8)] (Joimel et al. 2022) ; [(9)] (Getter et Rowe 2006) ; [(10)] (Mann 1994). *Photos:* ©Nolwen Levaillant 2023

Characteristic	**Extensive Roof**	**Semi-intensive roof**	**Intensive roof**
			
Purpose	Functional; storm-water management, thermal insulation, fireproofing [(1)(8)]	Functional and aesthetic (ornamental); increased living space [(1)(8)]; pedagogical and productive (urban agriculture) [(3)(8)]	Functional and aesthetic (ornamental); increased living space [(1)(8)]; pedagogical and productive (urban agriculture) [(3)(8)]
Support element	Wood, concrete [(2)]	Wood, concrete [(2)]	Wood, concrete [(2)]
Structural requirements	80-180 kg/m² [(2)]	150-350 kg/m² [(2)]	>600 kg/m² Planting trees >1,5t/m² [(2)]
Soil type	Lightweight; high porosity, low organic matter [(1)]	Lightweight to heavy; high porosity, higher organic matter rate [(1)]	Lightweight to heavy; high porosity, higher organic matter rate [(1)]
Soil composition	For the most part inert mineral substrate (e.g., pozzolan, gravel, expanded clay balls, bricks) [(4)(10)]	For the most part inert mineral substrate (e.g., pozzolan, gravel, expanded clay balls, bricks) [(4)]. Presence of organic substrates (e.g., compost, potting soil, wood chip) [(6)]	For the most part inert mineral substrate (e.g., pozzolan, gravel, expanded clay balls, bricks) [(4)]. Presence of organic substrates (e.g., compost, potting soil, wood chip) [(6)]
Average soil depth	5-10cm [(2)]	10-30cm [(2)]	>30cm [(2)]
Plant communities	Low-growing communities of plants and mosses selected for stress-tolerance qualities (e.g., *Sedum* spp.) [(1)(5)(10)]	Few restrictions other than those imposed by substrate depth, climate, building height and exposure, and irrigation facilities [(1)(5)]	No restrictions other than those imposed by substrate depth, climate, building height and exposure, and irrigation facilities [(1)(5)(10)]
Irrigation	Most require little or no irrigation [(1)(10)]	Quite often require irrigation [(1)]	Often require irrigation [(1)(4)(10)]
Maintenance	Little or no maintenance required [(1)(10)]	Few maintenance [(1)(7)]	Nearly same maintenance requirements as similar garden at ground level [(1)(10)]
Accessibility	Not accessible, not circulable [(2)(9)]	Sometimes accessible and circulable [(2)(9)]	Typically accessible ; bylaw considerations [(1)(9)]

A green roof is composed of several layers serving multiple functions (*Figure 2*). Three main types of green roofs exist (*Table 1*). The differentiation is made at the level of the thickness of the soil, the type of management and irrigation, and finally the vegetation layer present (Awada 2021). The majority of green roofs are extensive because they are lightweight, easy to install, costs are relatively low, and they require little maintenance (Awada 2021). The installation system for intensive roofs is more complex than for the first type of roof, and they are more expensive to implement (SECC 2019).

Soil on green roof is very diverse and particular (Joimel and Grard 2021). According to the World Reference Base for Soil Resources (WRB), these man-made soils are categorized as constructed Technosols. Soils in this category contain a lot of anthropogenic materials from industrial and artisanal manufacturing. These soils are young compared to agricultural soils, and appeared with human activity (Cerema 2018). However, compaction, contamination by metallic elements (e.g. lead, zinc, copper) (Awada 2021), pollution, low presence of vegetation or artificialization of soils are elements depicting the low agronomic interest for these soils (Clergeau et al. 2020). Most urban soils contain few major nutrients (N, P, K) and are often very compacted and anoxic. Since the effervescence of urban agriculture, the quality of urban soils on green roofs is nowadays studied (Joimel and Grard 2021). Organic substrates or compost are added to these cultivated Technosols to improve their agronomic properties, being favorable to both cultivations and the development of biodiversity (Guilland et al. 2018). Thus, agronomic qualities (e.g., Organic Matter (OM); carbon, nutrient), differ on the green roof type, and seem to be better and higher on intensive roofs than on extensive roofs (Joimel et al. 2022).

The initial development of green roofs was mainly to reduce runoff and increase the longevity of roofs (Guilland et al. 2018). Thanks to vegetation, they seem to improve the urban living environment on many points (Adianens 2022; Mann 1994): contribution of biodiversity, sound insulation caused by human activities (Oberndorfer et al. 2007; Getter and Rowe 2006), depollution of urban environments (Madre et al. 2014; Getter and Rowe 2006), protection against climatic conditions (Oberndorfer et al. 2007), and cooling down temperatures (Clergeau et al. 2020). Vegetation also absorbs runoff water thanks to its high-water retention capacity (Madre et al. 2014; Getter and Rowe 2006). From both an ecological and economic point of view, installing vegetation rather than leaving a roof bare is globally more interesting (Piccinini Scolaro and Ghisi 2022). Green roofs then may represent islands of habitation for this urban biodiversity.

Few studies have also proven that soil organisms are more abundant and diverse than in conventional asphalt roof (Partridge and Clark 2018; Wooster et al. 2022; Coulibaly et al. 2023), or rely on the response of soil organisms in such Technosols on green roofs (Joimel et al. 2018), either at the soil microbial (Molineux et al. 2015) or fauna scale (Hedde et al. 2019; Joimel et al. 2021, 2022),

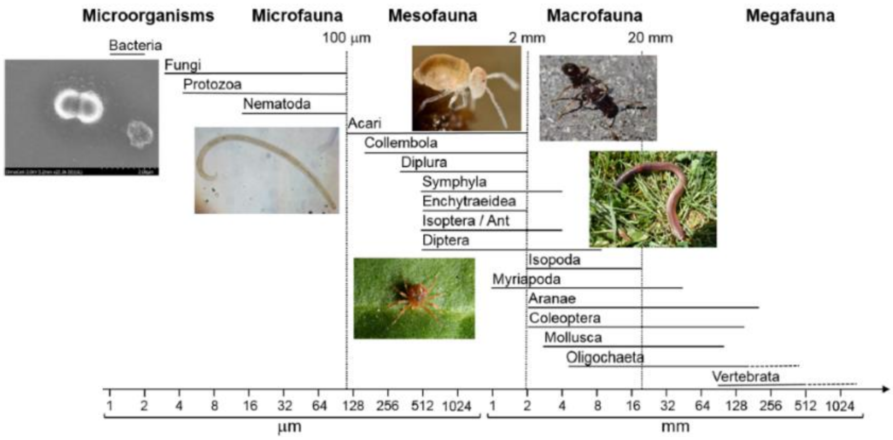

***Figure 3*: classification of soil organisms according to their size (Decaëns et al. 2006)**

***Table 2:* characteristics of the four different soil groups of organisms (Levaillant 2023)**, based on these sources: [1] (Awada 2021); [2] (Lavelle 1996); [3] (Bertrand et al. 2019); [4] (Molineux et al. 2015); [5] (Hedde et al. 2019); [6] (Socarrás 2013)

Soil organism group	Size	Example of organisms	Trophic regime	Roles in soil and for biodiversity
microorganisms	1-100µm	fungi, archaea, bacteria		Are involved in most of ecosystem services: OM mineralization [1][3], litter transformation [3], carbon and nutritive element (P, N) cycle, primary production, atmospheric gas regulation, water supply, maintenance of other organisms' habitats [1]. Can digest any substrate in the soil [2]. Symbiosis with plant roots [3], positive impact on plant growth and diversity [4].
microfauna	<0,2mm	protozoa, nematodes	phytophagous, bacterivous, predators, omnivorous, fungivorous	OM decomposition [1], stimulation and mineralization [2]. Predation [2]
mesofauna	0,2-2mm	enchytraeids, acarids, springtails *(collembola)*	Detritivorous, herbivorous, fungivorous, predators [6]	*Springtails*: colonization of other areas with their furcula, major role in the first steps of OM degradation [1], litter transformers [2], involved in provisioning and supporting services, increase of fertility and micropores [1]. Incubator for microbial activities [2]. *Acarids:* OM decomposition, soil good quality indicators, microfauna regulation [6].
macrofauna	>2mm	earthworms, arthropods, isopods, diplopods, gastropods, spiders	predators, phytophagous, detritivorous, zoophagus, phytophagous, microbivorous	"Engineers" of soil ecosystems [5], only soil organisms able to modify soil physical properties [3]. *Earthworms*: Better soil porosity [1][3] and soil impermeability [3], decrease of soil erosion, OM decomposition [1] and mineralization [2], limitation of erosion and runoffs [3]. Can dig in the soil and produce organo-mineral structures (casts) and also a large variety of pores, creating burrows [2]. *Carabids and spiders*: pest regulation, soil good quality indicators [2].

nor on the potential of green roof to provide habitats for biodiversity (Braaker et al. 2014; Coulibaly et al. 2023). The presence and functioning of soil organisms is thus essential to the productivity of ecosystems, on which we rely a lot (Awada 2021). The particular conditions and heterogeneities of anthroposols do not *a priori* favor the proliferation of organisms living in the soil on green roof (Clergeau et al. 2020).

1.2. The importance of soil biodiversity

Soil is one of the richest habitats of terrestrial ecosystems in terms of species (Decaëns et al. 2006), supporting a multitude of organisms, from macrofauna to the smallest of microorganisms (André, Noti, and Lebrun 1994). Soil biodiversity represents nearly 25% of the global biodiversity (Bertrand et al. 2019), with instrumental values of biodiversity in economic terms, with both direct (e.g., species cultivated for food), and indirect (e.g., ecosystem services) uses (Decaëns et al. 2006).

Their role is important, because the structures they create are one of the main keys for the ecosystem functioning, and are the initiation of all basic soil processes. However, they are deeply affected in urban areas by human activity and this is the major cause of biodiversity loss (Lavelle 1996). Spatial organization (Guilland et al. 2018), road transport and urban sprawl are obstacles to the development of soil biodiversity in cities (Clergeau et al. 2020). Understanding how soil biodiversity is living on green roofs is essential, so as to develop further strategies to improve ecological and functional values of this urban habitat for soil organisms (Braaker et al. 2014), and best to promote them (Wooster et al. 2022).

From microorganisms invisible to the naked eye to soil organisms several tens of centimeters long, each one plays a specific role in the soil (Guilland et al. 2018). They are mainly involved in the decomposition, transport and transformation of organic matter, in biogeochemical cycles, and in the formation and maintenance of soil structure (Guilland et al. 2018). There are four groups of organisms present in the soil (*Figure 3*): microorganisms, microfauna (e.g., nematodes), mesofauna (e.g., collembola) and macrofauna (e.g., arthropods, earthworms), which cover several orders of magnitude in terms of body size (*Table 2).*

Organisms on green roofs have adapted to survive in this disturbed environment and Technosols (e.g., high temperatures, drought, pollutants), including developing specific adaptive characteristics (Joimel and Grard 2021). Some springtails for instance have developed new morphological and physiological characteristics in urban rooftop soils that are adapted to hot, dry environments (Joimel et al. 2018), or microorganisms that can be able to dissolve contaminants from the Technosols' high metal concentration, and some develop metal-resistance (McGuire et al. 2015).

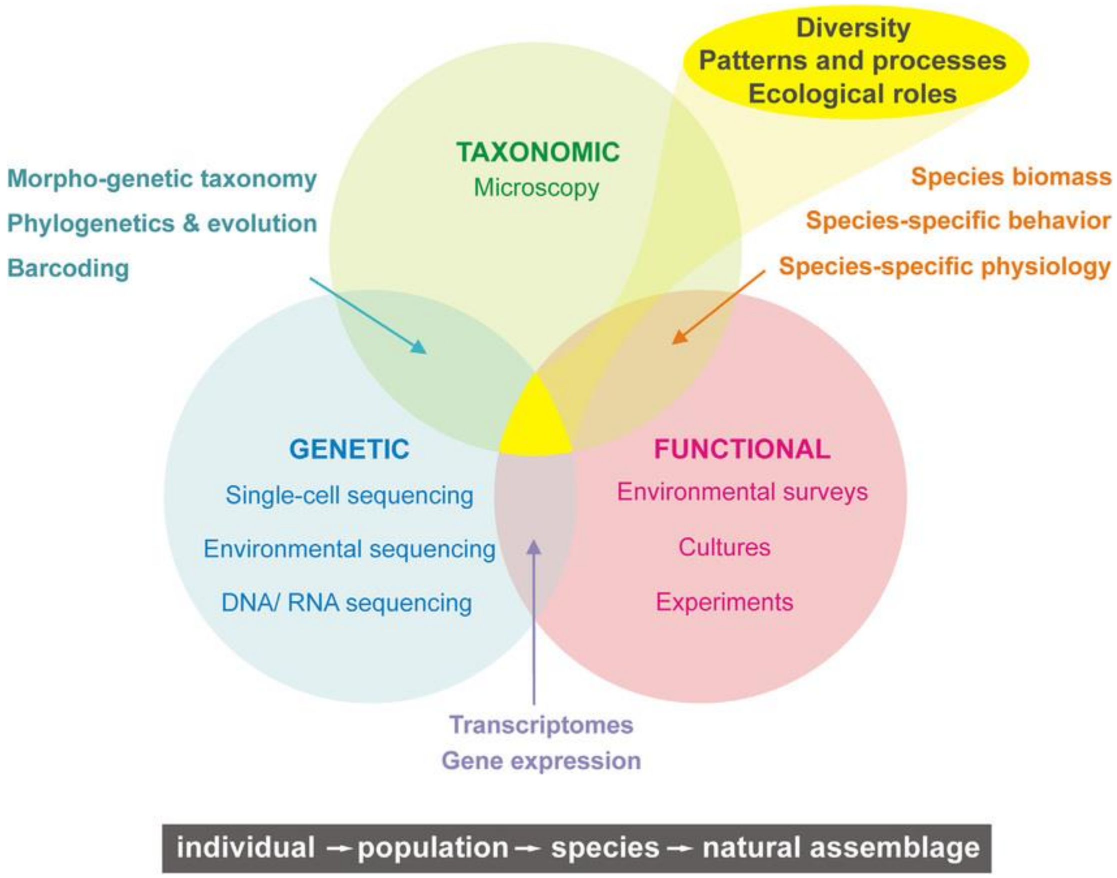

***Figure 4*: Integration of the taxonomic, genetic, and functional dimensions of biodiversity:** a three-dimensions case study. Different methods are used to quantify their diversity and ecological processes (Santoferrara and McManus 2017).

1.3. Different ecological approaches to characterize (soil) biodiversity

Three main approaches exist to quantify (soil) biodiversity, using the taxonomic, the phylogenetic, or the functional one (Garnier and Navas 2013). The differentiation of these approaches relies on the level of relationship studied (Moore 2013). For the taxonomic characterization (which is the most commonly used approach), the abundance and diversity of groups or species is studied (Bevilacqua et al. 2021), while phylogenetics is used to understand species evolutive history and to incorporate species differences through genetical studies (Vellend et al. 2011). Functional ecology allows to understand how organisms are distributed and living in an ecosystem, and how communities are assembled. It uses traits, which are defined as a *"morphological, physiological, or phenological measurable approach at the individual scale, without any reference to other organizational level"* (Garnier and Navas 2013).

Taxonomies or functional attributes-based approaches of species are all human constructions, and are constantly being under debate (Moore 2013). However, studying biological with the combination of different approaches allows to better understand it, and to improve the assessment and monitoring of ecological changes in time and space (Bevilacqua et al. 2021) (*Figure 4*), especially in urban area where biodiversity is not well understood (Clergeau et al. 2020). For instance, Joimel et al. 2018 used both taxonomic and functional approach to understand collembola colonization on green roof in urban area, by quantifying both species diversity and community assemblage.

1.4. Study of soil biodiversity on green roofs: premise and results of previous studies

Many heterogeneities on results obtained by studies made on soil biodiversity on green roofs are observed (Awada 2021), and show sometimes contradictory results (Schindler et al. 2019). A difference would remain between the different green roof types. Extensive roofs are a very limiting environment (Awada 2021). The shallow substrates and low organic matter content or water retention of extensive roofs would decrease the diversity of soil organisms, especially arthropods (Madre et al. 2014). Biodiversity would likely to be favored on semi- intensive and intensive roofs (Rumble et al. 2013; Clergeau et al. 2020), as earthworms, that have a low movement capacity and colonization, and a high sensitivity to urban fractionation (Guilland et al. 2018; Rumble and Gange 2013; Clergeau et al. 2020). Better establishment of soil organisms would be identified on intensive roofs, as well as a lower plant amount and diversity present in green roofs does not favor soil fauna (Guilland et al. 2018). Organic matter content is likely to be a key to soil biodiversity abundance (Joimel et al. 2022). Greater substrate depth and structural complexity of green roof vegetation also influence all soil biodiversity (Partridge and Clark 2018; Schindler et al. 2019; Madre et al. 2014), as does moisture (Mann 1994), the roof size (Partridge and Clark 2018; Schindler et al. 2019), or the age: macrofauna would be most impacted by the age of the roofs according to Mann 1994. Dispersal and colonization are currently

limited on green roofs, because roof soils are young (Joimel et al. 2022): older is the roof, more diversified and abundant seems to be biodiversity (soil organisms and vegetation) (Mann 1994).

Old roofs are likely to promote a more stable environment because of the advanced soil formation (Schrader and Böning 2006). However, roofs with similar architecture and substrate composition would have the same community of organisms overall according to Rumble and Gange 2013). Soil texture disturbance and compaction could also be a limiting condition for soil biodiversity (Rumble, Finch, and Gange 2018; Clergeau et al. 2020), such as trace metallic elements (TME) that would likely also decrease the abundance of springtails and nematodes (Joimel et al. 2022; Clergeau et al. 2020; Guilland et al. 2018).

Some similarities between the different green roof types have been observed. According to Joimel et al. 2022, the level of abundance and diversity would be higher on extensive roofs for microorganisms, which can be explained by a higher organic carbon content (e.g., (Awada 2021; Joimel et al. 2022). Predation and competition between different organisms are almost absent on green roofs, especially on extensive roof, due to the low presence of macrofauna, regulation is then very low according to Clergeau et al. 2020. This would favor the proliferation of microorganisms, bacteria, and other micro- and mesofauna (Joimel et al. 2022). Collembola abundance can still be very high on green roofs (Guilland et al. 2018; Joimel et al. 2018), but their abundance and diversity seem to decrease with the increase of temperatures and with low soil moisture (Rumble, Finch, and Gange 2018; Rumble and Gange 2013). Some studies show a difference between different green roof types for the presence of springtails (e.g., Awada 2021), others prove the opposite (e.g., Joimel et al. 2018).

1.5. Presentation of the BIOTOV project: goals and hypotheses

BIOTOV project (*see Foreword)* tends to show results that have never been proven on green roofs, and is innovative, insofar as many different types of green roofs types and soil organisms are studied, which has had never been done before. It is also to understand the contradictory results that have been studied during previous studies on green roof soil biodiversity. The project was trying to answer to the following question: **What is the impact of roofs revegetation on soil biodiversity in urban areas, and thus similarities observed between different taxonomic groups according to different green roof types?**

BIOTOV project has many purposes, the evaluation of soil biodiversity according to different green roof types is the major one, and then try to evaluate which group is most found in urban area. Thus, it would be possible to determine their potential of habitat for soil biodiversity. It also allows to characterize the differences encountered depending on the roof itself and puts the emphasis on the most

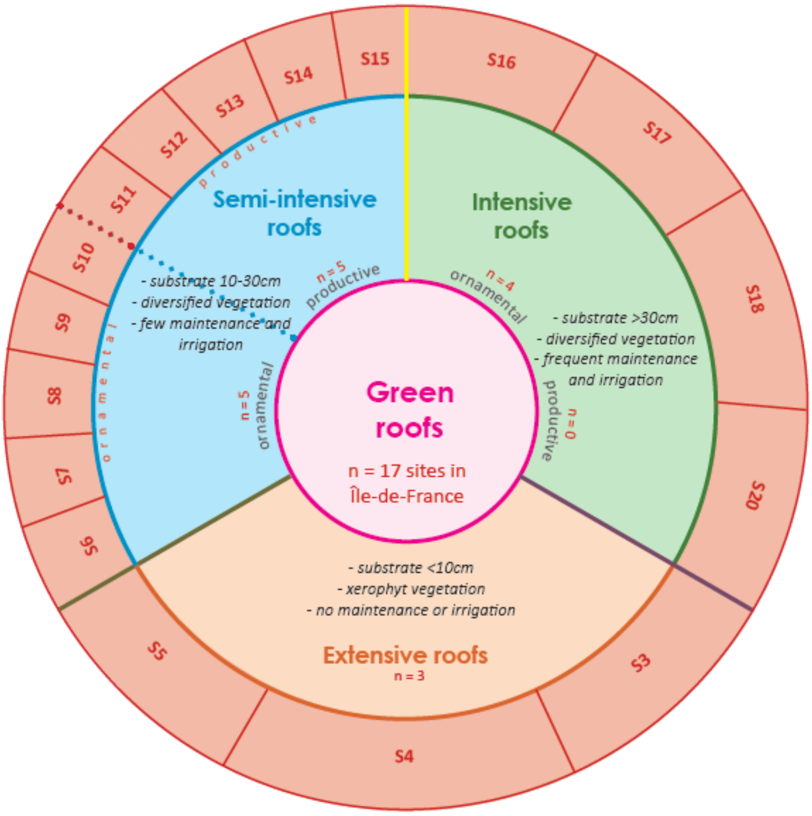

***Figure 5*: diagram of the 17 sites studied, from S3 to S20 according to four modalities** (EXT, SEMI, PROD, INT) for the BIOTOV project (Levaillant 2023)

determining factors for the presence of soil biodiversity (e.g., green roof type, soil physicochemical properties). Taxonomic and functional approaches are used to quantify soil organism diversity.

Based on the results obtained from the different studies, some hypotheses can be put forward:

- **H1:** Soil biodiversity is in general more abundant and diversified on intensive or semi-intensive roofs than on extensive roofs. Soil depth is then a major factor.
- **H2:** Macrofauna is very little present on (extensive) green roofs, compared to other smaller organisms that are favored and more present in green roof Technosols.
- **H3:** The roof structure itself affects soil biodiversity, particularly the Technosol physicochemical composition: soil OM, pH, nutrient content, fertilization and irrigation increase the abundance and diversity of soil organisms.

The set up of the experimentation is explained in the part 2: materials and methods.

2. BIOTOV Project: materials and methods

The study started in February 2023, and will be finish and the end of the year 2023.The data on green roofs were collected during the field phase in Ile-de-France, mostly in Paris. The field phase took place for eleven weeks, from the 6th of March to the 12th of May 2023. The roofs selected for sampling were at least two years old (in the exception of one roof), as this is the time needed for biodiversity to reach its peak (Ksiazek-Mikenas et al. 2018). After the field phase, samples were analyzed in laboratory of the UMR ECOSYS at the INRAE of Palaiseau.

2.1. Experimental and sampling design

The types of roofs selected for the project are those most encountered in the Paris region, namely extensive, semi-intensive and intensive roofs (*Figure 5*). The sites were provided free of charge by various organizations, companies and associations that were contacted before February 2023. At least three, and up five replicates by green roof type were studied.

Their usage can differ, the experimentation thus includes four modalities which are:

- **Extensive roofs (EXT):** green roofs with little diversified and xerophytic vegetation cover, soil depth <10cm (n=3, S3 to S5)
- **Semi-intensive roofs:** soil depth between 10 and 30cm, few maintenances and irrigation (n=10)

***Figure 6*: map of the 17 sites in Île-de-France for the BIOTOV project** (Levaillant 2023)

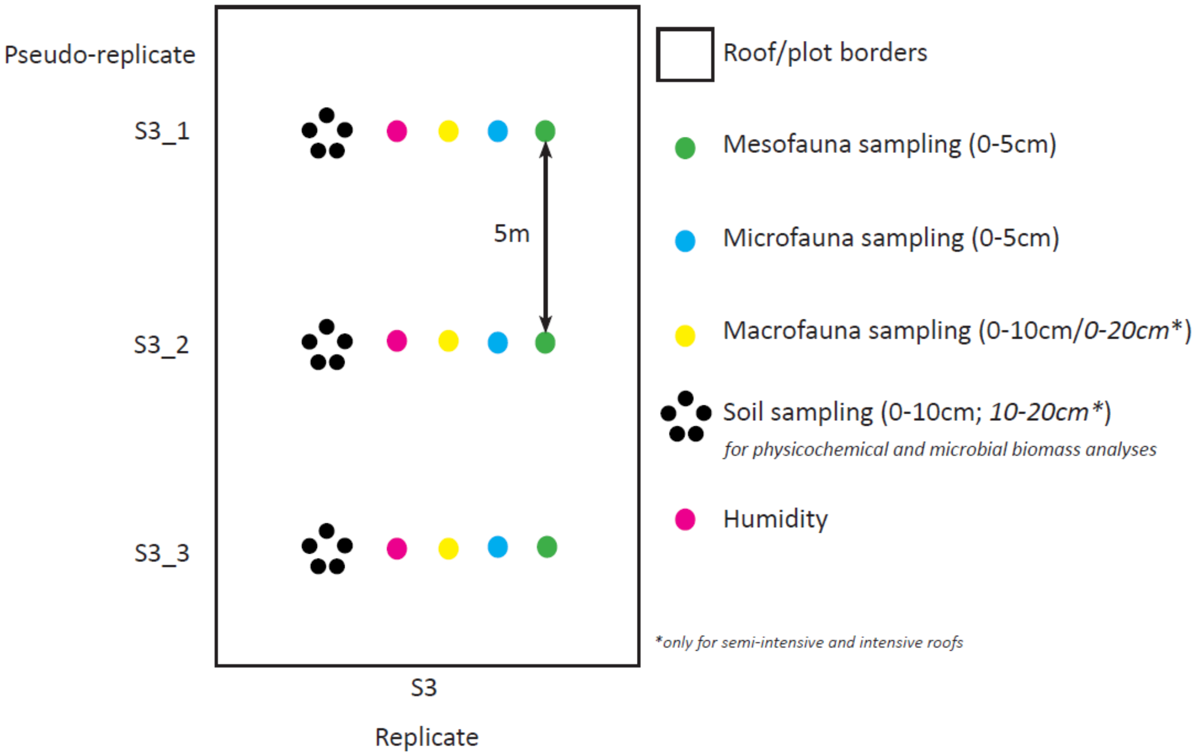

***Figure 7*: Diagram of the field surveys on the 17 roofs** (Levaillant 2023)

Ornamental (SEMI): diversified vegetation for aesthetic purpose (n=5, S6 to S10)

Productive (PROD): diversified vegetation for food production (n=5, S11 to S15)

- Intensive roofs:

  Ornamental (INT): diversified vegetation for aesthetic purposes, soil depth >30cm, regular maintenance and irrigation (n=4, S16 to S18 and S20)

For each roof, much information was collected, such as the construction date of the green roof, the type, the surface, the average substrate depth, the type of vegetation, management practices, the irrigation, the fertilization and the use of chemical inputs (*Annex I*). The 17 green roofs are mostly located in Paris (14), two in Val-de-Marne and one in Hauts-de-Seine (*Figure 6).*

2.2. Physical, chemical and biological characteristics studied of green roof soils

On each green roof site, many parameters are studied with five sampling methods to address the initial problem, through several processes:

Physicochemical parameters:

- **Soil sampling**: study the chemical, physical and microbiological parameters of the soil (physicochemical properties, and soil moisture).

Biological parameters:

- **Microorganisms sampling**: soil sampling with further laboratory analysis to determine the microbial biomass.
- **Sampling of microfauna:** collection of soil samples to extract the microfauna (nematodes) in the laboratory.
- **Sampling of mesofauna** (different groups of acarids, other microarthropods, and collembola): realization of soil samples in order to extract the mesofauna in laboratory.
- **Sampling of macrofauna**: sorting of substrate monoliths to collect the macrofauna present in the soil (e.g., arthropods, earthworms).

Samples were collected according to *Figure 7*. Three pseudo-replicates were made on each roof for the five sampling methods, with minimum 5m of distance between the different sampling points (e.g., S3_1, S3_2 and S3_3 are three pseudo-replicates of the replicate S3). The sampling of micro-and mesofauna was done first, then macrofauna sampling, for finish with soil samplings. Biodiversity samplings and analysis have been made in the frame of my missions, except for microbial biomass which was purchased by a ECOSYS technician. Soil analyses were sent to an external laboratory (Teyssier Laboratory in Bourdeaux). The sampling plans are in the *Annex II.*

Figure 8**: nematodes extraction before fixation with formaldehyde (left), and nematodes counting under binocular magnifier (right)** (©Nolwen Levaillant 2023)

Figure 9: **a nematode observed under the binocular magnifier** (©Nolwen Levaillant 2023)

2.3. Analysis of soil sampling for physicochemical properties

For intensive and semi-intensive roofs, soil was sampled at 0-10cm depth, and until the maximum depth for extensive roofs (*Annex I*). Soil was sieved to 2mm, then air dried for few weeks, depending on the initial moisture of the soil. The three pseudo-replicates were pooled together by site, and analyzed to determine the CEC (Cation-Exchange Capacity)-Metson (X 31-130 standard), pH-water, OM (10693 standard), C, N, and Olsen-Phosphorous (X31-160 standard). The amount sampled has varied from roofs, but 100gr of dried soil of each site was sent the for analysis. Concerning the soil moisture, a sample of soil of about 50-80g was taken in a tare box, then weighed to obtain the fresh weight. The box was placed for 24 hours at 105°C in an oven to determine the dry weight of the soil.

2.4. Soil biodiversity analysis

All groups (microorganisms, microfauna, mesofauna and macrofauna) are studied in this experiment, but only a few are analyzed with more details. Nematodes, acarids, collembola, and earthworms have been therefore identified as good bioindicators in urban areas, for their sensibility to soil physicochemical properties (Lavelle 1996; Santorufo et al. 2012). A deeper focus is made on these four groups.

2.4.1. Sampling and analysis of microorganisms

Soil samples used for microbial biomass analysis with the fresh soil samples are collected and are sieved to 4mm. Microbial Biomass Carbon (MBC) was estimated by means of the fumigation-extraction method. 20g are shaken in a solution of K2S04 at 0,05M during 1h, and then filtered at 0,45µg, and finally analyzed for dissolved Corg on Shimatzu-TOC-5050A.

2.4.2. Sampling and identification of microfauna (nematodes)

The nematodes were sampled with a 5cm diameter and 4cm deep corer which is inserted in the first centimeters of the soil. In the laboratory, the nematodes were extracted in water using the Baermann-modified method, for 48h. The volume of extracted nematodes was weighted. Then, the nematodes were counted alive after maximum 7 days. The method consists of counting three times on three 5mL-samplings of the extracted solution (*Figure 9*). An estimation of the total number of nematodes in the volume extracted can be calculated then. After counting, the nematodes were extracted again for 24h (*Figure 8*) to be conserved for a further identification to the trophic group.

***Figure 10*: McFayden high gradient extractor** (©Nolwen Levaillant 2023)

***Figure 11*: identification of collembola** (left: counting under binocular magnifier; middle: identification under microscope) and earthworms (right: under binocular magnifier) (©Nolwen Levaillant 2023)

***Figure 12*: monolith for macrofauna sampling** (left: extensive roof, 4cm-depth; middle: semi-intensive roof, 15cm depth; right: intensive roof, 20cm depth) (©Nolwen Levaillant 2023)

2.4.3. Sampling and identification of mesofauna

The mesofauna was sampled in the same way as the microfauna, with a deep corer. They were then extracted according to NF/ISO 23611-2, using a McFayden high gradient extractor for 8 days (*Figure 10*). Collembola were counted for each pseudo-replicate and identified at the species level with a key (Hopkin 2007) (*Figure 11*). The method for the identification is detailed in the *Annex III.* For the other groups, such as acarids, larva or microarthropods were counted as well, but only identified until the class. For acarids they are classified as *Oribatidae*, *Acarididae*, *Actinididae* and *Gamasidae* orders.

2.4.4. Sampling and analysis of macrofauna

Macrofauna sampling was done by digging in the soil and taking a 20x20cm monolith (*Figure 12*) for S6, S7, S9, S10, S11, S12, S14, and for the intensive roofs; and at the maximum depth for S8, S13, S15, and for the extensive roofs. Macrofauna was sampled on the site, by manual sorting. The identification of individuals was done in the laboratory with a binocular magnifying glass, down to the species for earthworms (Sherlock 2018). The identification method is explained in the *Annex IV*. After identification, earthworms were weighted to calculate their biomass. Other macrofauna, such as larvae and arthropods, were identified to the major group/family/order.

2.5. Ecological approaches of the data analysis

Taxonomic and functional approaches were used in this experimentation to analyze soil biodiversity. The taxonomic characterization of biodiversity (for micro-, meso-, and macrofauna) consists of determining the density (D) of the group, and, when the group is identified down to the species, to compute the species richness (S), the species diversity with the Shannon index (H), and the equitability of Piélou (E). The functional approach was used to determine the functional composition of the collembolan community. Traits were chosen because they are typical characteristics of collembola: reproduction, number of ocelli, furcula length, pigmentation, body length, PAO, body shape, and scale (*Annex III*). The functional traits information was collected on the BETSI database (Biological and Ecological functional Traits of Soil Invertebrates), which collects and compute values for functional traits derived from sources such as identification keys. Statistical analyses were executed to interpret the results of both taxonomic and functional approaches.

2.6. Statistical analysis

Data did not match the basic assumptions of normality and homoscedasticity, the Kruskal-Wallis non-parametric test was used for the statistical analysis. The p-value used for the tests is 0,05. For the taxonomic approach of all organisms, “kruskal.test”, “print(kruskal)”, and “kruskalmc” were used

***Table 3*: Soil physicochemical characteristics of the BIOTOV experimentation according to four green roof types (±standard deviation)**, for a same characteristic, the lowercase letters show the significative differences between green roof types (*print(kruskal)* and *kruskalmc*). EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

	EXT	SEMI	PROD	INT
CEC (meq/100g) p-value: 0,232	21,6 (±4,3)	13,2 (±4,4)	18,2 (±5,5)	17,0 (±6,4)
pH-water p-value: 0,02831	7,1 (±0,4)^{c}	7,5 (±0,2)^{bc}	7,8 (±0,3)^{a}	7,6 (±0,3)^{ab}
Organic Matter (%) p-value: 0,06749	13,2 (±6,4)	7,6 (±4,8)	19,1 (±6,0)	9,8 (±7,0)
P_2O_5Olsen (mg/kg) p-value: 0,006724	100,7 (±52,2)^{bc}	85,8 (±55,0)^{c}	324,6 (±42,4)^{a}	160,3 (±26,4)^{b}
N (mg/kg) p-value: 0,04168	6158,3 (±2700,1)^{ab}	3104,0 (±1884,5)^{b}	8612,2 (±2854,3)^{a}	4350,5 (±3158,9)^{ab}
C (g/kg) p-value: 0,06749	76,5 (±37,3)	44,0 (±27,9)	110,5 (±34,8)	56,8 (±40,7)
C/N p-value: 0,119	12,3 (±1,1)	13,9 (±1,0)	12,9 (±0,8)	13,2 (±0,5)
Soil moisture (%) p-value: 0,1689	53,0 (±18,5)	70,6 (±17,3)	72,8 (±13,8)	81,3 (±7,2)

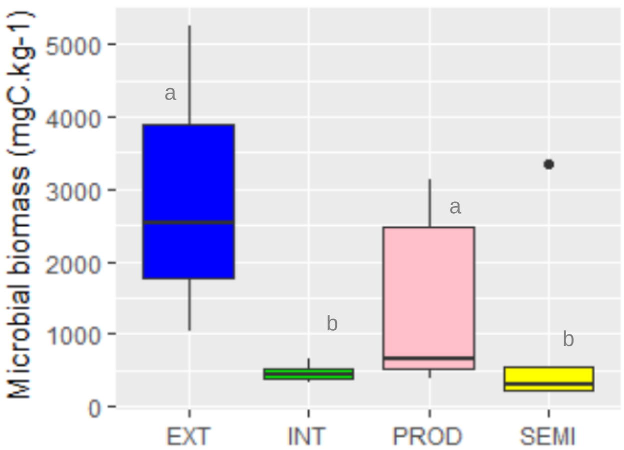

***Figure 13*: Graph showing the microbial biomass (mgC/kg) of the BIOTOV experimentation soils according to four green roof types**, for a same characteristic, the lowercase letters show the significative differences between green roof types (*print(kruskal)*). Blue boxplot: EXT, green boxplot: INT, pink boxplot: PROD, yellow boxplot: SEMI. EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

to show the differences of D, S, H, and E between roof types. Collembola taxonomic statistical analysis was performed with a Non-metric MultiDimensional Scaling (NMDS), to analyze the effects of green roof types on collembola species composition, and to explore dissimilarities between communities (Joimel et al. 2022). ANOSIM, a permutational analysis of similarities, was used to determinate the differences in species composition. For the functional approach, collembola functional composition was performed with a Principal Components Analysis (PCA), and with ANOSIM to determine significant differences. Finally, a Redundancy Analysis (RDA) was performed to visualize relationships between soil organism community variables as response matrix (density of nematodes, acarids, collembola and macrofauna excluding earthworms, functional richness of springtails, earthworm biomass) (Y) and soil physicochemical parameters (CEC, OM, C/N, P_2O_5Olsen, soil moisture, pH, C, N) and microbial biomass as explanatory matrix (X). RDA is an extension of multiple regressions that allows one to explain the variation of a multivariate response data table using explanatory variables. RDA were computed on log-transformed data, y'=log (y+1). The software RStudio 4.2.0 was used to compute the different data analysis. The packages “emmeans”, “vegan”, “pgirmess”, “indicspecies”, “ggplot2”, “ade4”, “agricolae”, “nlme”, “lme4”, “MuMin”, “multcomp”, and “car” have been used. Data analysis was performed on the raw data obtained from the pseudo-replicates, with the exception of the collembola and physicochemical soil parameters analysis, which were performed per replicate.

3. Results

The different species and groups of identified organisms are gathered in the *Annex V.*

3.1. Soil physicochemical parameters

The physicochemical characteristics whose values were measured are presented in *Table 3*. For the nine physicochemical parameters studied, only three have shown significant results (POlsen, N, pH). The highest pH-value measured was for PROD (7,8), the lowest for EXT (7,1). Extensive and semi-intensive productive roof type have a significative pH difference, semi-intensive ornamental and semi-intensive productive as well. N is also significantly different for SEMI (3104,0 mg/kg) and PROD (8612,2 mg/kg). EXT and INT have in between N values. P_2O_5Olsen has a significative difference between PROD-SEMI, PROD-INT, SEMI-INT, and PROD-EXT. Values vary from 85,8 mg/kg (SEMI) to 324,6 mg/kg (PROD). For the non-significative results, CEC values go from 13,2 meq/100g (SEMI) to 21,6 meq/100g (EXT), OM from 7,6% (SEMI) to 19,1% (PROD), C from 44,0 g/kg (SEMI) to 110,5 g/kg (PROD), C/N from 12,3 (EXT) to 13,9 (SEMI), and finally soil moisture from 53,0% (EXT) to 81,3% (INT). PROD has nearly all highest values compared to other green roof types, except for CEC, C/N, and the soil moisture.

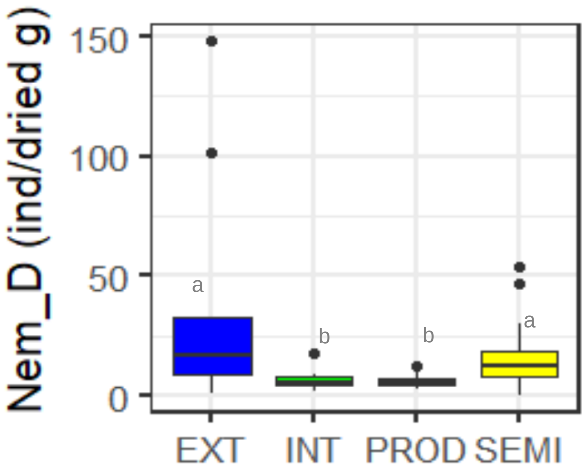

***Figure 14*: boxplot of nematodes density (Nem_D, ind/dried g) of the BIOTOV experimentation soils according to four green roof types (±standard deviation),** for a same characteristic, the lowercase letters show the significative differences between green roof types (*print(kruskal)*). Blue boxplot: EXT, green boxplot: INT, pink boxplot: PROD, yellow boxplot: SEMI. EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

***Table 4*: Taxonomic indices of collembolan communities of the BIOTOV experimentation soils according to four green roof types (±standard deviation),** no significant difference has been observed (*kruskalmc*). EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

Roof type	Density (10^3 ind/m²)	Species richness S	Diversity H	Equitability E
	p-value: 0,2789	p-value: 0,4907	p-value: 0,7839	p-value: 0,4062
EXT	18,3 (±11,5)	6,3 (±1,0)	1,1 (±0,3)	0,52 (±0,5)
SEMI	7,6 (±4,7)	6 (±2,1)	1,4 (±0,2)	0,72 (±0,7)
PROD	11,6 (±7,1)	7,6 (±1,8)	1,3 (±0,3)	0,61 (±0,6)
INT	7,8 (±5,1)	6 (±3,0)	1,3 (±0,3)	0,66 (±0,7)

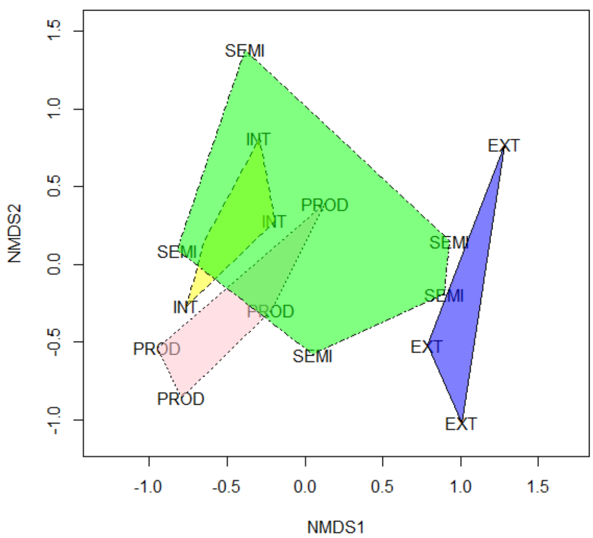

***Figure 15*: Graph showing the two first composition NMDS axes of the collembolan communities according to four green roof types,** differences between green roof types, differences between green roof types are significative (*ANOSIM, distance=bray, permutation=9999, p-value=0,0079)*. Blue: EXT, green: SEMI, pink: PROD, yellow: INT. EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

3.2. Soil biological parameters

3.2.1. Soil microorganisms

There is a significative difference between extensive and intensive green roofs, between extensive and semi-intensive ornamental roofs, and between semi-intensive productive and semi-intensive ornamentals roofs (chi-squared=21,8, df=3, p-value = 7,219e^{-5}) (*Figure 13*). Microbial biomass average values vary from 474,0 mgC/kg (INT) to 2939,0 mgC/kg (EXT). EXT is the highest value, which is 3,2x higher than SEMI (924,9 mgC/kg), 2,0x higher than PROD (1492,3 mgC/kg), and 6,2x higher than INT.

3.2.2. Soil microfauna

The nematodes density showed a significant difference between green roof types (chi-squared=13,01, df=3, p-value=0,004615). Difference is significant between EXT-INT, EXT-PROD, SEMI-INT, and SEMI-PROD (*Figure 14*). EXT has in average the highest nematodes density (39,2 ind/dried g), following by SEMI (16,9 ind/dried g). PROD and INT have the lowest density values, with both 6,4 ind/dried g. This is 6,1x less than EXT.

3.2.3. Soil mesofauna

3.2.3.1. Collembolan communities

Taxonomic approach:

In total, 46 collembola species have been identified. The three more abundant species were *Proisotoma minuta, Cryptopygus thermophilus,* and *Folsomides angularis* for EXT; *C. thermophilus, P. minuta* and *Parisotoma notabilis* for SEMI; *P. notabilis, Folsomia angrelli* and *Cryptopygus bipunctatus* for PROD; and finally *P. notabilis, Lepicocyrtus lanuginosus* and *Isotomiella minor* for INT. Taxonomic indices of collembolan communities, such as density, species richness, density, and equitability does not show any significant differences *(Table 4).* Average collembola densities vary from 7,6e10^3 ind/m² (SEMI) to 18,3e10^3 ind/m² (EXT); species richness from 6 (INT and SEMI) to 7,6 (PROD); diversity from 1,1 (EXT) to 1,4 (SEMI), and finally the equitability varies from 0,52 (EXT) to 0,72 (SEMI). Collembolan communities sampled were analyzed with NMDS (*Figure 15*). The differences between the green roof type collembolan composition are significative (p-value=0,0079). Since that the distance between EXT and INT/PROD polygons is high, the collembolan community composition is different for these three green roof types. SEMI has the largest collembolan composition then the green polygon area is the biggest. The species explaining the most this significant difference are *Sminthurinus elegans* (p-value=0,022), *Sphaeridia pumilis* (p-value=0,022), and *Folsomides angularis* (p-value=0,014) for the extensive roofs; and *Isotomodes parvulus* (p-value=0,022) for semi-intensive productive roofs.

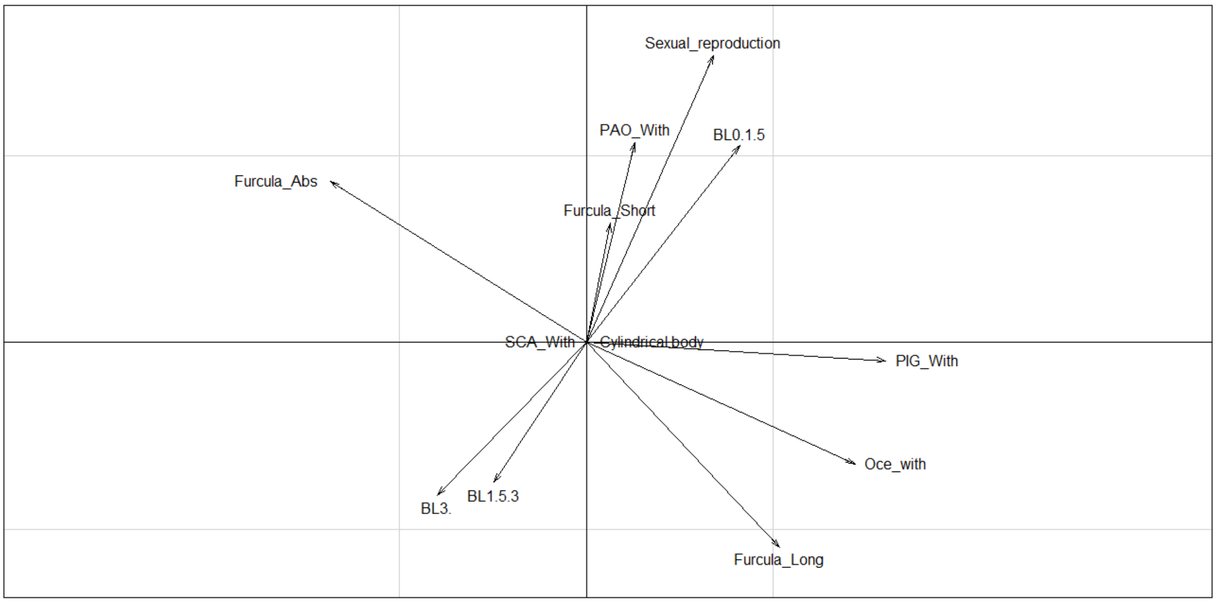

***Figure 16*: PCA showing the functional composition according to 12 traits of the collembolan communities on the BIOTOV experimentation soils.** Functional traits shown in the PCA are the furcula length (Furcula_Abs, Furcula_Short, Furcula_Long), the body length (0,5-1,5mm; 1,5-3mm; >3mm), the sexual reproduction, the presence of PAO (PAO-With), the presence of ocelli (Oce_with), the cylindrical body, the presence of scale (SCA_With), and the presence of pigmentation (PIG_With). *As a reminder, when two arrows point in opposite directions, this means that the two traits are inversely correlated. For example, here, the more sexual reproduction tends to be, the smaller the body, as the BL3. and BL1.5.3 arrows point in the opposite direction to the Sexual_Reproduction arrow.*

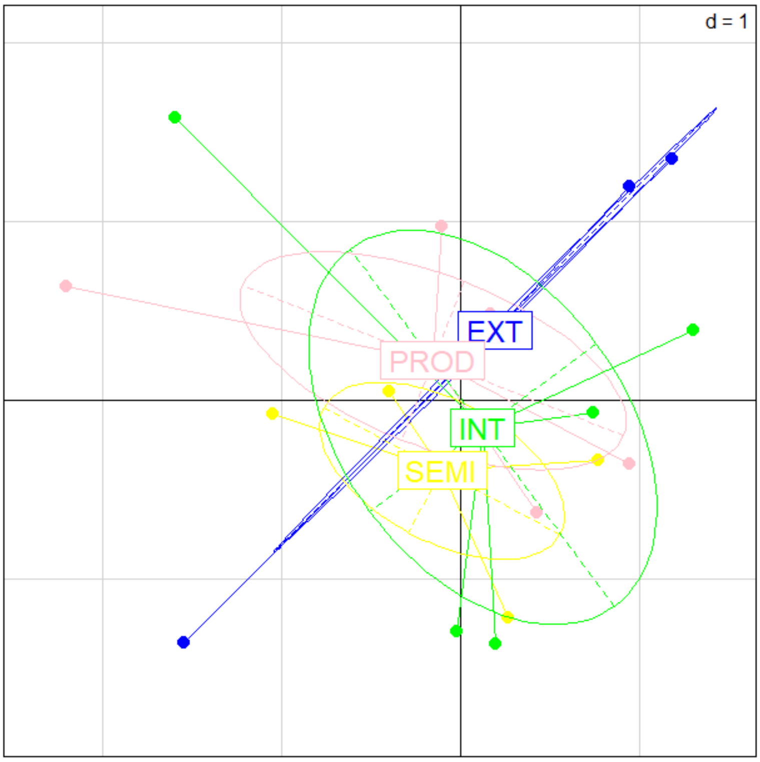

***Figure 17*: Graph showing the functional composition of the collembolan communities according to four green roof types,** differences between green roof types are note significative (*ANOSIM, distance=bray, permutation=9999, p-value=0.0583)*. Blue: EXT, green: INT, pink: PROD, yellow: SEMI. EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

Functional approach:

Functional composition between green roof types does not show any significant difference (p-value=0,0583). According to *Figure 16*, certain traits are correlated (e.g., sexual reproduction, presence of PAO, short furcula, and body length 0-1,5), and other are opposed (e.g., body length 0-1,5 with body length 1,5-3 and >3; or absence of furcula with presence of furcula). The SCA_With and Cylindrical body traits are poorly represented. To understand the *Figure 17* with the functional composition of the collembolan communities, it is compared with the *Figure 16*. Despite the absence of significant differences between the green roof types for the functional composition, the p-value is very close to 0,05. Some tendences could be therefore discussed. Extensive roofs show a functional collembola composition with the least different traits, as it can be seen by its linear shape representation (*Figure 17*, in blue). PROD, SEMI, and INT have a more diversified functional composition, especially INT which has the biggest and roundest area on the graph (*Figure 17*, in green). If the two figures information are combined, extensive roof collembola communities have then most opposite traits according to the sampling site, comparing to the three other green roof types. The other collembola composition for SEMI, EXT, and INT is more homogenic and covers more different traits. But it is difficult to draw tendences without significant differences.

3.2.3.2. Other mesofauna

Acarids is the most present group of mesofauna, following by collembola. The acarids density percentage varies from 46,6% (INT) to 71,0% (PROD) of the total mesofauna density, whereas microarthropods density represents only 6,3% (PROD) to 33,6% (INT) of the total mesofauna (*Annex VI*). No significant difference has been observed between the four acarid orders for the density (p-value>0,05 for all acarids groups) (*Table 5*). *Gamasidae* is the most present order for all green roof type (7,4 to 14,4 e10^3 ind/m²), which is between 39% (SEMI) and 56% (EXT) of the total acarids density. The exception is for intensive ornamental roof, where *Oribatidae* has the highest density (7,7e10^3 ind/m², 42% of the total density). *Acarididae* is the least frequent order identified, its density varies from 0,5e10^3 ind/m² (SEMI) to 4,1e10^3 ind/m (PROD).

3.2.4. Soil macrofauna

3.2.4.1. Earthworms

The taxonomic indices of earthworms show a significative difference for the density, the species richness, and the biomass (p-values<0,05). EXT shows a significant difference with SEMI, PROD, and INT for the density and the species richness (*Table 6*). The lowest value of earthworm density is on extensive roof is 1,8e10^3 ind/m², which is 126x less than PROD, 79,3x less than SEMI and 80x less than INT. Species richness is 0 for EXT, and the same value for the other modalities (0,7). Diversity and

***Table 5*: Acarids density (10^3 ind/m²) of the BIOTOV experimentation soils according to four green roof types (±standard deviation),** differences between green roof types are note significative (*kruskalmc*). EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

Roof type	Density (10^3 ind/m²)				
	Actinididae	*Acaridiae*	*Gamasidae*	*Oribatidae*	*Total*
EXT	7,2 (±7,8)	0,5 (±0,7)	14,4 (±5,5)	3,7 (±2,3)	25,7 (±11,9)
SEMI	2,7 (±2,1)	0,1 (±0,3)	7,4 (±6,8)	8,5 (±5,8)	18,9 (±13,2)
PROD	4,8 (±2,0)	1,5 (±1,6)	15,8 (±10,3)	14,2 (±19,8)	36,2 (±20,7)
INT	4,4 (±4,8)	0,3 (±0,3)	6,0 (±1,9)	7,7 (±5,1)	18,3 (±6,5)
p-value = 0,4126	0,1057	0,1144	0,4991	0,432	

***Table 6*: Taxonomic indices of macrofauna communities of the BIOTOV experimentation soils according to four green roof types (±standard deviation),** For a same characteristic, the lowercase letters show the significative differences between green roof types (*print(kruskal)* and *kruskalmc*). EXT: extensive green roof; SEMI: semi-intensive ornamental green roof; PROD: semi-intensive productive green roof; INT: intensive ornamental green roof.

Roof type	Earthworms					Other macrofauna
	Density (10^3 ind/m²) p-value=0,0057	*Species richness* p-value =0,03699	*Diversity* p-value =0,2324	*Equitability* p-value =0,2324	*Biomass (g/m²)* p-value =0,003913	*Density (10^3 ind/m²)* p-value=0,008682
EXT	1,8 (±5,3)[b]	0,0 (±0,0)[b]	0,0 (±0,0)	0,0 (±0,0)	0,0 (±0,1)[b]	16,0 (±17,9)[b]
SEMI	142,9 (±175,1)[a]	0,7 (±0,7)[a]	0,1 (±0,1)	0,1 (±0,2)	7,1 (±9,7)[a]	203,7 (±247,5)[a]
PROD	226,1 (±397,1)[a]	0,7 (±0,8)[a]	0,1 (±0,2)	0,2 (±0,3)	15,9 (±26,8)[a]	467,2 (±890,3)[a]
INT	144,0 (±133,7)[a]	0,7 (±0,5)[a]	0,0 (±0,0)	0,0 (±0,0)	9,6 (±8,3)[a]	205,3 (±193,1)[a]

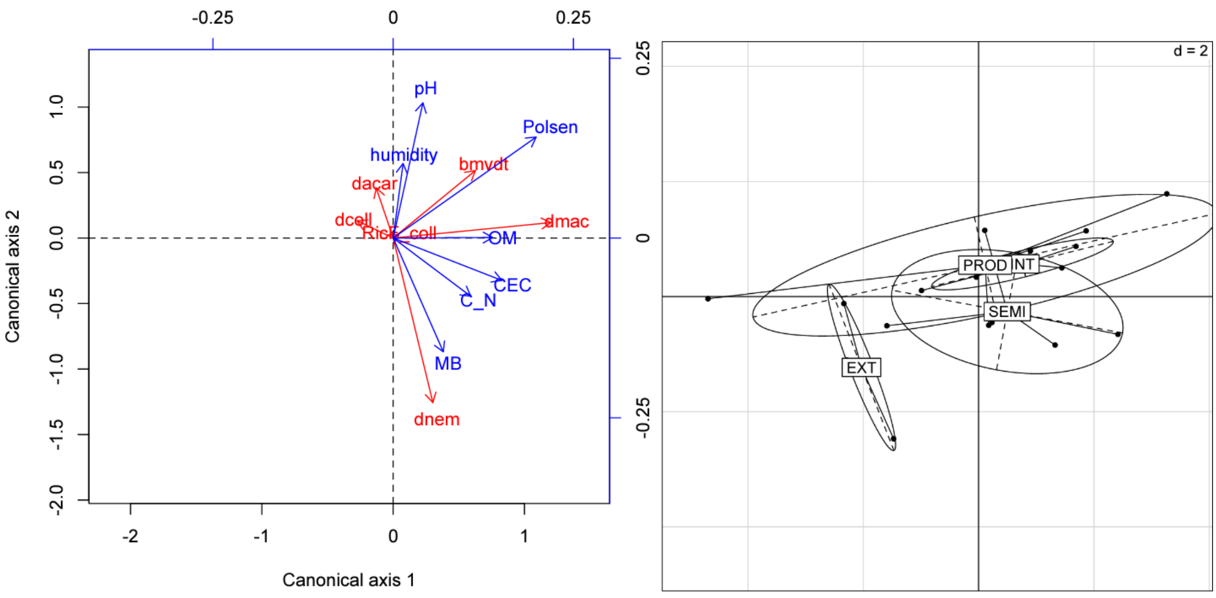

***Figure 18*: RDA showing the interaction between physicochemical parameters and microbial biomass with the soil organisms of the BIOTOV experimentation soils, according to different green roof types.** Blue parameters are soil physicochemical parameters (pH, soil moisture, Polsen, OM, CEC, C/N) and microbial biomass (MB); red parameters are soil organism densities and biomass (density of nematodes: dnem, acarids: dacar, collembola: dcoll, macrofauna without earthworms: dmac), collembola functional richness (RicF_coll), and earthworm biomass (bmvdt).

equitability are reaching 0 for all green roof types. For the biomass, there is also a significant difference between EXT (0,0 g/m²), with SEMI (7,1 g/m²), PROD (15,9 g/m², which is the highest value) and INT (9,6 g/m²). Only five species have been identified on all roofs of the experimentation.

3.2.4.2. Other macrofauna

There is a significant difference between green roof types for the macrofauna without earthworms (p-value= 0,008682). The significant difference is between EXT-INT, EXT-SEMI, and EXT-PROD. The values go from 16,0e10^3 ind/m² (EXT) to 467,2e10^3 ind/m² (PROD). SEMI and INT other macrofauna density is around the same (respectively 203,7e10^3 and 205,3e10^3 ind/m²). Excluding earthworms, the most found macrofauna were diplopods and isopods.

3.3. Soil physicochemical properties x soil biological properties

The proportion of the total variance in the RDA, which is explained by all environmental parameters, is 32.8%. The first axis accounted for 13.8% of variance and the second only for a further 9.3% (*Figure 18*). Nearly all arrows are pointing to the right side of the vertical axis, except for mesofauna density (acarids and collembola). Mesofauna is associated with soil moisture and pH, macrofauna and earthworm biomass is related to POlsen and OM. CEC and C_N are quite related to micro- and macrofauna, but less than the other parameters previously named. No organisms are opposite to physicochemical parameters. Nematodes density is the least related to physicochemical characteristics but is very related to microbial biomass.

The area for productive roofs is the largest, which means that the parameters explaining PROD are numerous and diversified (*Figure 18*, right). On the contrary, EXT has the smallest area, and does not cross that of SEMI and INT. The latter two are quite similar, so the parameters describing SEMI and INT are relatively identical. EXT is opposed to macrofauna and soil physicochemical properties, while the other semi-intensive and intensive roofs are more closely associated, especially PROD, which is the roof type most closely linked to physicochemical properties and macrofauna. EXT is the roof type most associated with nematode density and microbial biomass, followed by SEMI.

4. Discussion

In general, there is little significance of results despite a significant difference between values. This can be explained by the raw data, which have a very high standard deviation, which can bias the significance of the results. This is due to the considerable heterogeneity of the roofs selected. It is therefore sometimes difficult to discuss on the results obtained.

***Table 7*: Average values soil chemical properties on the 17 green roof of the BIOTOV project comparing to agricultural soils**, based from [1] (Jolivet et al. 2006) and [2] (Fenton 2008)

Soil chemical properties	Green roof soils *(average of the 17 sites of the BIOTOV project)*	Agricultural soils
CEC (meq/100g)	17,5	14,0 [1]
pH-water	7,5	6,7 [1]
Organic Matter (%)	12,4	4,5 [2]
P_2O_5 Olsen (mg/kg)	167,9	72,0 [1]
N (mg/kg)	5556,3	-
C (g/kg)	72,0	19,2 [1]
C/N	13,1	10,0 [1]

4.1. Technosols on green roof have particular physicochemical properties

The chemical properties of green roof soils have all higher values comparing to what could be found in agricultural soils (*Table 7*). The pH measured is alkaline, which is common for green roofs. Values between 7,3 and 7,6, and similar to that of this study, have been recorded on other roofs (Joimel et al. 2022). Technosols from all 17 sites show a very high level of elements (C and POlsen) and OM, respectively 3,8x, 2,3x and 2,8x higher than agricultural soils (*Table 7*). This is due to the addition of compost or fertilizer which increase the pH and other chemical soil values, otherwise Technosols on green roof would be very poor (Guilland et al. 2018).

Between the different green roof types, only three chemical parameters are significant. However, the difference between OM, C or soil moisture (p-value close to 0.05) remains high. Some studies have shown that C values are significantly higher for productive roofs than for extensive roofs (e.g., Joimel et al. 2022), and this trend is reflected in the results obtained for the BIOTOV project. There is a considerable variability in physicochemical properties across the four roof types. In fact, pH, OM, POlsen, N and C levels are the highest on semi-intensive productive roofs, as the Technosol is more composted and fertilized for food cultivation. These elements are in lower content on the other three types of ornamental roofs, as the need to add nutrients is less important. The significant difference in pH between productive and extensive has also been proven by (Joimel et al. 2022). So, there is still a difference in terms of roof use: productive roofs are those with the highest nutrient and OM content, while ornamental roofs are poorer in nutrients and OM (Guilland et al. 2018).

The difference in content is less marked between EXT, SEMI and INT, this is really the productive VS ornamental use that will come to influence the physicochemical parameters. We might have expected more significant differences between EXT and INT, as the structure and vegetation are very different, but this is not the case. The difference between EXT and the other modalities could be the soil moisture, which is lower, but the difference is still not significant. This could be justified by the fact that no artificial irrigation is applied, unlike the other roof types, which are irrigated more or less regularly (*Annex I*). There are significant differences between physicochemical parameters depending on the type of green roof, and these must be taken into account further in the analysis, by comparing them with the organisms present in the soil, as these could have an impact on their presence.

4.2. Green roofs are providing habitats for soil biodiversity – comparison with other urban and non-urban areas

The presence of soil organisms on green roofs is very variable, it shows sometimes similarities with other urban areas or natural soils, but it can also be very different. The role of green roofs as providing habitat for soil biodiversity can therefore be discussed.

The microbial biomass found on the green roofs in the experiment is in average higher than that found in agricultural or post-industrial soils (McGuire et al. 2013; Molineux et al. 2015), or than in other urban areas. The average microbial biomass in urban area is about 574,7 mgC/kg (Guilland et al. 2018). Only SEMI and INT have average MB values reaching this level, other green roof types have much higher average values. In rural places, the average microbial biomass is less, about 443,7 mgC/kg, which is proving that urban areas, particularly green roofs, are welcoming microorganisms (Awada 2021).

About the nematodes, in urban areas, the average density is 45 ind/dried g, and 60 ind/dried g in rural places (Pavao-Zuckerman and Coleman 2007). Only extensive roofs in our study have a nematode density (39,2 ind/dried g) approaching the mean of urban areas, other roof types have a lower density, rather in urban or in rural places.

In terms of collembola density, the average found on green roofs is 19,000 ind/m², in urban parks this average rises to 20,000 ind/m² (Guilland et al. 2018); other studies, however, record a much higher density of springtails, up to 56,000 ind/m² on average (Schrader and Böning 2006). For all four types of roofs, collembola density is below than the average found in urban area. Only EXT (18,300 ind/m²) is almost the same as the Guilland et al. 2018 average. According to Rumble and Gange 2013, the density counted on extensive roofs was 20,637 ind/m², which is similar to our average measured. The density of PROD is 0.6x the reference value of Guilland et al. 2018, and finally SEMI and INT are 0.4x this value. About the collembolan species richness, the average is few for all roof types compared to other urban areas where the average is about 22 different species (Guilland et al. 2018), and compared to some other studies on green roofs: Schrader et al. 2006 found 25 different species. Rumble and Gange 2013 has found 5 species on average on extensive roofs, which is nearer what is found on the 17 green roofs of BIOTOV project (between 6 and 8). Due to the low species richness, the diversity and equitability on the green roofs is low as well. To go deeper on species description, the collembola species found on the 17 green roofs are common in urban environments. According to Rumble and Gange 2013, Symphipleone species, in particular *Sminthurinus aureus*, D*euterosminthurinus pallipes*, and *Bourletiella hortensis*, are resistant to heat and drought, and are therefore often adapted to these environments. *Parisotoma notabilis*, one of the most common species found on BIOTOV project roofs, is the species most frequently encountered on green roofs, and in urban areas in general, for its resistance to harsh conditions (Rumble, Finch, and Gange 2018; Joimel, Jules, and Vieublé Gonod 2022). Some species encountered are uncommon in semi-natural or natural environments, but are found on green roofs, for instance *Folsomides angularis*. This is a species that has been recorded in dry locations, and is capable of living in very aridic places for some time (Joimel et al. 2018).

Concerning the rest of the mesofauna, acarids is the most dominant group. Rumble and Gange 2013 showed that acarid density averaged is 12,360 ind/m². On all 17 roofs, the total acarids density average is around two times higher than what was shown by Rumble and Gange 2013. Even within the same urban green space, huge differences can be drawn, especially with acarids density.

According to Guilland et al. 2018, the average value for earthworm biomass in an urban environment is very variable, the earthworm biomass varies therefore from 4,69 to 107 g/m². Only PROD comes close to the highest average but is still lower, then it is INT which is 1.8x less than the average value, 2.4x less for SEMI, and EXT is the furthest from it as its biomass is 0. Earthworms are likely to be less present on green roofs than on other urban green spaces apart from PROD which would come closest to what can be found on the ground. In rural areas, the average earthworm biomass goes from 17 to 94,12 g/m² (Guilland et al. 2018). More earthworms could be found on urban areas, but the minimum found in rural places is in average higher. This is therefore difficult to determine if earthworms are more present in rural or in urban green spaces (other than green roofs), because earthworms are still few studied in urban areas (Guilland et al. 2018). Species earthworms diversity is very low, which is common in Technosols, as earthworms are sensitive to disturbed environments (Lavelle 1996), only five species were encountered in this study, and two of which were only observed on S11 and S12 (*Eiseniella tetraedra* and *Dendrobaena veneta*). The species found are adapted to OM-rich habitats such as composts and litter (Sherlock 2018), and are often found in urban environments. *Eiseinia fetida* is a very hardy species, tolerant of variations in temperature and humidity, so it thrives in disturbed environments such as Technosols (Grard et al. 2018), and is the species most commonly found on green roofs and on those of the BIOTOV study. Talking about other macrofauna, carabid beetles and other flying arthropods were few observed, mainly endogeic species were collected. Epigeic macrofauna on green roofs cannot therefore be compared with other green spaces. The main endogeic groups found in this study were isopods and diplopods, and were found in an important number in other urban places (Korsos et al. 2002). Macrofauna in general is less present on urban areas than in rural places (Guilland et al. 2018).

Finally, green roofs represent a viable habitat for all organisms, but in a different level of importance. The organisms that are more likely to live in urban areas and on green roof, and that are more found than in other green spaces, are microorganisms. Microfauna and mesofauna is found more or less the same than other urban areas, but macrofauna is less present on green roofs than on other green spaces in general. But these conclusions can always be discussed, because even within different studies on green roof soil biodiversity, some results can be totally different. The identification down to the species for collembola and earthworm have demonstrated that some specific species are found only on green roofs, and in urban areas. These green spaces create an habitat for species that sometimes

cannot be found in rural places, it proves that the difficult conditions on green roofs are not always an obstacle for soil life. Differences and similarities between different green spaces have been observed, it is the same within green roof types themselves. According to the type of green roof, some differences can be observed in the soil biodiversity.

4.3. Impact of green roof types on soil biodiversity

The green roof types have a more or less important impact on soil biodiversity, and is therefore necessary to be discussed. With the four green roof types of this study (EXT, SEMI, PROD, INT), the goal is to show if either the depth or the usage of the roof can have an impact on the soil living organisms.

First, there is a significative difference about the microbial biomass (MB) on the different green roof types: both on the roof type depth (EXT VS INT/SEMI), and the usage (productive VS ornamental). Microbial biomass is influenced by factors other than soil depth, in particular by roof usage, since the second highest average MB value is found on productive roofs. According to Joimel et al. 2022, microorganisms might prefer extensive roofs because competition with other soil organisms is lower. The high presence of microorganisms on productive green roofs could be linked with the physicochemical parameters and therefore the high content of OM and nutrients, it will be discussed after. However, it is not possible to know which type of microorganism is present on the roofs (fungal or bacterial), which could have been a good indicator of the differences encountered on green roof types. A PLFA (PhosphoLipid Fatty Acid) will be conducted later to determine the microbial composition of Technosols.

More nematodes are found on EXT (Joimel et al. 2022), as for microorganisms, they seem to prefer this type of roof. SEMI also has a significantly higher density than PROD and INT, but lower than EXT. The depth is not only the main factor of their presence. On the other hand, PROD had a higher density of microbial biomass than SEMI and INT, and in terms of microfauna, PROD was the modality with the lowest density of nematodes. Nematodes therefore appear to be less sensitive to roof usage than microorganisms (productive VS ornamental), this is then not a factor that can be taken into account in nematodes density. This absence of any significant difference in nematode density between urban land uses was shown by Pavao-Zuckerman and Coleman 2007. For the nematodes analysis, there are only density results, the trophic regime identification will be performed later. As for microorganisms, it could have been interesting to know more about the trophic nematodes regime and better understand the green roof types taxonomic composition, but the identification has not been done because of lack of time.

There is no difference on taxonomic indices of collembola according to different green roof types. This is not a factor influencing their presence, the green roof itself is a factor impacting springtails abundance, in comparison to other urban or non-urban soil. According to (Rumble and Gange 2013), extensive roofs are less able to host a high density of collembola than semi-intensive and intensive roofs, due to their more difficult conditions. In this study, it's the opposite: EXT is the roof with the most springtails, but the results are not significantly different – this is speculative. To summary about, the green roof types do not influence neither density, species richness, diversity or equitability.

Despite the absence of significance for these parameters, the composition of collembola communities show significant results and can be discussed further. In terms of taxonomic composition, for PROD and EXT, the most abundant species are similar to those found on the same green roof types in Joimel et al. 2018 study: *Proisotoma minuta* and *Folsomides angulari*s in common for EXT, *Parisotoma notabilis* in common for PROD. The most significant species in our study are *Sminthurinus elegans*, *Sphaeridia pumilis* (two Symphipleone species) and *Folsomides angularis*. Taxonomic composition differs between EXT and the other roof types, as can be seen on the NMDS, where EXT has the taxonomic composition furthest removed from those of the other roof types. This taxonomic composition of extensive roofs is in line with other studies, notably that of Joimel et al. 2022, which showed that there were more Symphipleone collembola on extensive roofs than on other roof types. For PROD, the taxonomic composition is significantly different for *Isotomodes parvulus*, but the presence of this species on productive roofs has not been noted in any study. SEMI has the most diverse composition, and also encompasses the taxonomic composition of PROD and INT. This means that similar species will be found on these roof types, which is not the case for EXT which is the green roof with the most taxonomic composition differences. The type of roof therefore influences which taxonomic community of collembola is found, and it is above all the assemblage of Collembolan communities that will differ between roof types. The parameters explaining this differentiation are not easy to pinpoint, and may include both depth and roof usage, as previous studies have shown (Joimel et al. 2018; 2022).

The functional approach of collembola does not show any significant differences between green roof types. Joimel et al. 2018 proved that the functional composition of springtail communities was very similar on green roofs. In this paper, it was shown that the most dominant traits on extensive and productive roofs were small size, parthenogenetic reproduction, the presence of ocelli and furca, yet these are not the traits found for BIOTOV roofs. EXT is said to have collembola communities with ocelli and long furcula, but sexual reproduction and body size are variable. PROD, on the other hand, tends not to have furcula and ocelli, and sexual reproduction is favored, plus the body is small. The absence of ocelli and furcula is consistent with what was reported in Joimel et al. 2018 on productive roofs.

However, in the absence of significance, it is impossible to say whether these traits are different between roof types, only trends can be drawn. No study has demonstrated the functional approach of springtails traits on semi-intensive ornamental and intensive ornamental roofs, but overall, their traits look rather similar to PROD. It is therefore very hard to draw conclusions on the functional approach about the green roof type differences. This approach is still little used, and even less so in urban environments. This is nevertheless an excellent tool to better understand a community and its interaction with the surrounding environment.

No significant difference between green roof types on acarids was shown, so this is not the factor that will impact their presence, they are present similarly in each roof type. This is the same conclusion than collembola. All mesofauna reacts the same to green roof types. Acarids were more present in PROD, then in EXT, but no conclusions can be drawn because of the absence of significance. In terms of composition, two groups were predominantly present (*Oribatidae* and *Gamasidae*). *Gamasidae* is the acarid group most commonly found in soils (Socarrás 2013).

Nearly no earthworms were found on extensive roofs, which proves that the green roof type, particularly the soil depth, has a strong impact on earthworm presence. The earthworm species found on semi-intensive ornamental and productive roofs were mostly epigeous, i.e., living on the surface. *Aporrectodea caliginosa* is an endogenous species found on SEMI and INT, i.e., roofs with a substrate depth of at least 15cm. The depth influences which species can be found, as well as the general presence or absence of earthworms. Nearly no earthworms were observed on the extensive roofs, proving that soil depths on this type of roof are not suitable for earthworm development (Awada 2021). According to Schrader and Böning 2006, earthworms are only present from 12cm soil depth, then in the SEMI, PROD and INT modalities. The usage of the roof could also have an impact on the presence of earthworms, even if no significant difference was shown. The biomasses of INT and SEMI are identical, while PROD biomass is higher. Probably, when there is more tillage (e.g., watering, fertilizing, composting), earthworms could be present in greater numbers. However, the most decisive factor in the presence of earthworms remains soil depth. For macrofauna excluding earthworms, the same pattern of organism presence occurs: very little macrofauna is found on the extensive roofs, the most on the productive ones, and similar occurrences on SEMI and INT. All macrofauna behave in the same way and are affected identically by the type of roof. When this is below a certain depth, there is little or no macrofauna, which means on extensive roofs.

The hypothesis **H1**, which stated that soil organisms are more present and diversified on roofs with greater substrate depths (in semi-intensive and intensive roofs) can be discussed now. The finding of a greater abundance of microorganisms and nematodes on roofs with shallow soil depths, such as

extensive roofs, goes in the opposite direction to **H1**. They are more present on extensive roofs than on other roof types, depth is not a clustering factor for all soil organisms. Since that no difference were shown between mesofauna taxonomic indices on green roof types, **H1** is also rejected. But taxonomic compositions of collembola differ between green roof type, this is on semi-intensive and intensive roofs that are found the most diversified taxonomic composition, **H1** can be here validated. **H1** is also true for macrofauna, because extensive roofs are less likely to promote an habitat for this group. Soil depth could be therefore a factor explaining soil biodiversity differences but is only pertinent for macrofauna. Initial usage of the roof is another factor that could affect the presence of organisms. For instance, it has an importance for microbial biomass, productive roofs are welcoming many microorganisms, and also for earthworms that are likely to prefer productive roofs (but no significance about it). On the other hand, on INT/SEMI/PROD, each group of organisms is present, even if only to a small extent, whereas on EXT, macrofauna is virtually absent. Specific diversity is therefore greater on SEMI/INT/PROD. **H1** is true on the point of the greater diversity of different group organisms found on semi-intensive and intensive roofs. On PROD, all groups of organisms are present, and in relatively large numbers each time, so this is the green roof type where diversity is the greatest. Once again, productive VS ornamental usage must be taken into account when characterizing soil biodiversity. Joimel et al. 2022 has shown significant differences in the various communities of organisms between extensive and intensive roofs, but also between ornamental VS productive usage. It has been proven here that soil biodiversity differences on green roof types could be explained by the soil depth and the green roof usage, but other biotic and abiotic factors are explaining these differences.

4.4. Abiotic and biotic factors influencing soil biodiversity on green roofs

4.4.1. Strong interactions between soil organisms

A biotic factor explaining differences observed on green roofs soil biodiversity are the interactions between organisms. The presence of macrofauna is opposed to the presence of microorganisms and microfauna. Green roofs rich in macrofauna have then in general fewer small organisms. A hypothesis can then be put forward concerning the competition and predation of macrofauna on the rest of soil biodiversity. The presence of macrofauna would then reduce their presence, but when it is not present, and therefore in the absence of competition, small organisms proliferate much more (Joimel et al. 2022; Guilland et al. 2018).

However, this hypothesis needs to be qualified, as for mesofauna, the presence of macrofauna does not seem to have an impact on the density of collembola and acarids. Even if the trends show that the most springtails are found on EXT, where macrofauna is the least established, the results are not significant. According to Schrader and Böning 2006, the presence of earthworms would reduce the

presence of collembola. But according to Joimel, Jules, and Vieublé Gonod 2022, the opposite is shown: earthworms are likely to favor the presence of collembola, as they would feed on earthworm excrement and use their galleries to move around and hide. It is therefore difficult to conclude on the collembola presence comparing to other soil organisms because of the absence of significance.

A general conclusion can be drawn: the same communities may be found on the different types of roofs. One type of roof contains small organisms (microorganisms, microfauna, in a way collembola) (EXT), while another contains small, and bigger organisms (SEMI/PROD/INT). The second hypothesis **H2** pointed out that macrofauna was little found on green roofs, especially on extensive ones comparing to other smaller organisms that are favored, is verified. This is true for extensive roofs, were nearly no macrofauna was found, but on other green roof types, their abundance depends more on the interactions between organisms for instance. **H2** also said that smaller organisms are favored on green roof, which is also true. Generally speaking, the organisms best suited to urban environments are microorganisms, micro- and mesofauna, as they are found on all types of roofs (Santorufo et al. 2012). They are therefore more resistant to the limiting conditions of urban environments than macrofauna, which is more sensitive to urban areas (Lavelle 1996).

4.4.2. An important abiotic factor impacting soil organisms: soil physicochemical parameters

It has been proven here that soil physicochemical properties could have an impact on soil organisms. Least impacted soil organisms by physicochemical properties are nematodes, and then microorganisms. Microorganisms and microfauna are really close and related to each other, probably because nematodes are feeding microorganisms. However, it has been seen that the second highest average MB is on semi-intensive productive roofs. The green roof usage could however play in a way on the average MB on semi-intensive productive roofs, as this is the second highest value. Between EXT and PROD, the C quantity is higher than PROD, and it has been proven that carbon could favor the presence of microorganisms (Molineux et al. 2015). Microbial biomass is relatively correlated to C/N ratio in the RDA, which can confirm this theory that the usage could have a positive impact on microorganism presence. Nematode density cannot be correlated with the usage of the roof, since that this in on PROD that the density is the lowest.

Other organisms that do not seem to be that much impacted by soil physicochemical parameters are mesofauna. Their representation on the RDA is bad, it is difficult to conclude about the impact of these abiotic factors. The nearest physicochemical parameters in the RDA are the pH and the soil moisture, and then POlsen. According to Rumble and Gange 2013, collembola are sensitive to low soil moisture and therefore their density would decrease. pH and Phosphorous would also be an important factor influencing the community composition (Joimel, Jules, and Vieublé Gonod 2022). It could be in this

study the explaining parameters on collembola presence. However, the lowest soil moisture is in EXT, and this is in this green roof type that collembola are the most present (even if again no significance has been shown). Collembola communities would also be more abundant in soil with high water and OM contents (Santorufo et al. 2012; Joimel, Jules, and Vieublé Gonod 2022). It cannot be verified in this study. It is then difficult to conclude, other parameter not studied here are probably explaining the presence of collembola. It is the same conclusion for acarids. *Gamasidae* and *Oribatidae* would likely to be negatively impacted by soil moisture (Rumble and Gange 2013; Socarrás 2013), pH, and OM content (Socarrás 2013). Without significant correlation between acarid density, nothing can be concluded. However, the high OM content and soil moisture on the BIOTOV green roof Technosols could explain the high presence of acarids within the mesofauna.

The most impacted organisms to soil physicochemical parameters are macrofauna. They are strongly correlated to OM, POlsen, and C/N. The usage of the green roof could be an important factor as well, because once again there is more OM in PROD, and this is in this green roof type that the most earthworm biomass is encountered. OM is an important factor determining earthworm presence, more is the OM, more abundant are earthworms (Hedde et al. 2019). Other than earthworms, the same conclusion about their more important presence could be drawn: more OM content and nutrients on PROD would favor macrofauna presence. This high OM content is favorable for isopods and diplopods as well (Korsos et al. 2002).

Soil sampling were sent for TME analysis, but results were not available at the moment of the redaction of this report. It has nevertheless been proven by many studies that all soil organisms are more or less impacted by soil contaminants (Guilland et al. 2018; Joimel et al. 2022). Physicochemical characteristics of the BIOTOV Technosols is then an important abiotic factor in the presence of soil organisms, especially for macrofauna, and then more or less for the smaller organisms.

The hypothesis **H3** can be verified. However, OM content was identified as one of the most important physicochemical parameters impacting soil biodiversity (Joimel et al. 2022), but here only macrofauna is sensitive to it. Soil physicochemical parameters are not the only important factor impacting soil biodiversity, some have not been studied during this study, but it could have been interesting to do a deeper focus. It could also be a tool to explain some differences that could not have been solved.

4.4.3. Other possible biotic and abiotic factors impacting soil biodiversity on green roof that have not been studied

Due to a lack of time and resources for the project, certain biotic and abiotic parameters could not be studied, despite their proven impact on soil biodiversity. Age was considered a neutral factor here, as

it was considered old enough to have stable soil biodiversity. However, as demonstrated by (Mann 1994), the young age of roofs can be a limiting factor, especially for macrofauna, as young Technosols are less stable and formed (Schrader and Böning 2006). As the roofs in the BIOTOV project ranged in age from 1 to 16 years old (*Annex I*), it might have been interesting to classify the roofs according to their age, thus shifting the comparative studies from types of green roofs to their more or less advanced age.

Another aspect of the project that was difficult to get to grips with was the spacing between different roofs, and hence the study of species colonization between green roofs. The "urban mosaic" effect created by the heterogeneous spatial organization of cities would likely disadvantage the presence of soil organisms (Guilland et al. 2018), and the provision of green spaces at relatively close distances could promote dispersal and colonization (Joimel, Jules, and Vieublé Gonod 2022). Some of the BIOTOV project roofs were located less than 50m apart, such as S6, S7, S16, and S17, or S11 and S12, and similar species of collembola and earthworms were identified. However, this hypothesis of community similarity between nearby roofs has not been studied here.

In terms of the structural composition of roofs, various surface area (from 5 to 2500m²) and height (2nd to 17th floor) have been measured, but again not studied, even though these abiotic factors have been identified as having an impact on soil biodiversity (Partridge and Clark 2018; Schindler et al. 2019; Awada 2021). Vegetation was also surveyed (*Annex I*), and was found to be very diverse on the roofs, ranging from simple *Sedum* ground cover, to spontaneous flora, to planted trees several meters high. Only the productive VS ornamental use of vegetation was studied here. It might be interesting to see whether the number of different plant species, or even whether vegetation cover has an impact on the presence of soil biodiversity.

4.5. Critical aspects of the project: problems and difficulties encountered

The initiation of the experimentation was not easy on some points. First, the initial number of roofs surveyed was 20, but some green roofs providers no longer responded, or were no longer available on the dates we had provided to take the samples. There must have been 5 roofs of each type. EXT and INT modalities were therefore only composed of 3 and 4 roofs respectively. Extensive roofs being those most frequently found, it would have been more relevant to have a greater number of this green roof type. It has not been possible to find other roofs to replace those that could not be done.

Then, the sampling period for soil organisms was spread over several months, which can induce differences in development due to the different temperature and humidity conditions of the seasons. Initially, the sampling period was to take place over 6 weeks, it had to be extended to 13 weeks due to

the vagaries of the sites. The samples could not be taken according to the schedule prepared in January 2023. It could have been interesting to measure the variation of temperatures, and to compare it with the data collected. As the project lasted only six months, a choice had to be made about the organisms to be studied in more detail. The identification of nematodes in the trophic regime, as well as TMEs and PLFA will be carried out later, making sometimes more difficult to analyze results. This is the same for other abiotic factor that were supposed to be studied, such as density and soil profile, but by lack of time, these data have not been analyzed. These difficulties encountered have nevertheless made it possible to obtain rather conclusive results on the impact of green roofs on soil biodiversity.

5. Conclusion and perspectives

The role of green roofs as habitat suppliers in urban areas for soil biodiversity is undeniable, despite particular pedological and climatic conditions, and they even welcome species not found in agricultural or natural areas. However, the quality of this habitat is not the same for all soil organisms. Macrofauna, because of its high sensibility to disturbed area such as urban places, is less present than the smallest organisms (microorganisms, microfauna, mesofauna) which are found in any type of green roof. Hypothesis **H2** is then verified. The type of roof is indeed one of the most impacting factors, extensive roofs are the least able to accommodate macrofauna because of the few depth, nevertheless they are largely preferred by microorganisms or nematodes. Mesofauna is the only group not affected by green roof types. The hypothesis **H1** is rejected. Soils depth of the semi-intensive and intensive roofs provides a living space for all organisms in general. Productive roofs compared to ornamental roofs are more likely to host a significant abundance and diversity of organisms, due to their greater presence of organic matter and other nutrients necessary for the survival of organisms. In addition to soil depth and roof usage, other biotic and abiotic parameters affect soil biodiversity and should not be overlooked, such as physicochemical properties that affect particularly macrofauna, or the interactions and competitions between organisms. The hypothesis **H3** is validated.

This study made it possible to demonstrate certain explanatory aspects of soil biodiversity encountered in urban areas on green roofs, but it would be interesting, in case that the study is purchased, to study other parameters influencing soil organisms that are not addressed here (e.g., surface, height, vegetation, spatial connectivity). Long-term monitoring taking into account several seasons or years would make it possible to assess the viability and colonization of biodiversity in a given time. More functional approach on soil organisms cumulated with the taxonomic approach could also be very interesting to better understand rather composition and interaction of soil communities in urban ecosystems.

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Annex

Annex I: Characteristics of the different green roofs of the BIOTOV project; blue: extensive; yellow: semi-intensive ornamental; pink: semi-inensive productive; green: intensive ornamental.

S3 | 14/04/2023

Installation year: 2007
Vegetation surface: 600m^2
Height: 4th floor
Substrate: max 6cm, vegetation with pre-grown carpet, clay balls
Use: -
Management practices: -

Sampling point	Depth (cm)	Vegetation
S3-1	6	*Sedum sp.*
S3-2	4	
S3-3	4	

S4 | 06/04/2023

Installation year: 2016
Vegetation surface: 110m^2 semi-intensive ornamental (SEMI), 14m^2 extensive (EXT)
Height: 6th floor
Substrate: max 10cm (EXT), max 20cm (SEMI), vegetation with pre-grown carpet, clay balls
Use: ornamental
Management practices: -

Sampling point	Depth (cm)	Vegetation
S4-1	10	*Sedum sp.*
S4-2		
S4-3		

S5 | 20/04/2023

Installation date: 2014
Surface: 500m^2
Height: 5th floor
Substrate: max 10cm, vegetation in box, pre-grown carpet or in pots, organic
Use: biosolar panels
Management practices: -

Sampling point	Depth (cm)	Vegetation
S5-1	9	*Sedum sp.*
S5-2	8	
S5-3	10	

S6 | 06/03/2023

Installation year: 2018
Surface: 150m^2
Height: 3rd floor
Substrate: max 25cm, substrate "jardilight" (with brick debris), clay balls, vegetation in box
Use: ornamental
Management practices: drip watering

Sampling point	Depth (cm)	Vegetation
S6-1	24,5	Ornamental species planted
S6-2	25	
S6-3	25	

S7 | 06/03/2023

Installation date: 2019
Surface: 20m²
Height: 6th-7th floor
Substrate: max 20cm
Use: ornamental, biofiltration
Management practices: 2 x/year, drip watering

Sampling point	Depth (cm)	Vegetation
S7-1	20	*Asarum europaeum, Nepeta faasenii, Melica ciliata, Prunella grandiflora, Carex acutiformis, Carex pendula*
S7-2		
S7-3		

S8 | 07/04/2023

Installation date: 2015
Surface: 800m² (EXT), 400m² intensive ornamental (INT), 300m² SEMI
Height: 5th-6th floor
Substrate: max 20cm (SEMI), substrate "Id Flor sp", pozzolan and 30% compost, organic
Use: biodiversity, ornamental, recreation space
Management practices: -

Sampling point	Depth (cm)	Vegetation
S8-1	14,5	More than 10 species of spontaneous weeds
S8-2	15,5	
S8-3	14	

S9 | 19/04/2023

Installation date: 2018

Surface: 200m² SEMI, 430m² INT & EXT
Height: 7th floor
Substrate: max 30cm (SEMI), vegetation in pre-grown carpet or in box, organic, mulch
Use: ornamental, recreation space
Management practices: regularly, drip watering

Sampling point	Depth (cm)	Vegetation
S9-1	25	Ornamental plantations
S9-2		
S9-3		

S10 | 20/04/2023

Installation date: 2015
Surface: total of 2500m², some boxes INT
Height: 3rd floor
Substrate: max 25cm (SEMI), vegetation in box
Use: experimental site, ornamental
Management practices: -

Sampling point	Depth (cm)	Vegetation
S10-1	20	Spontaneous weeds, *Sedum sp.*
S10-2	19	
S10-3	21	

S11 | 21/03/2023

Installation date: 2020

Surface: -
Height: 3rd floor
Substrate: max 25cm, shell debris
Use: productive (fruits and vegetables), sell
Management practices: regularly, drip watering

Sampling point	Depth (cm)	Vegetation
S11-1	20	*Orchard, vegetables (leek, cabbage, etc.)*
S11-2	20	
S11-3	20	

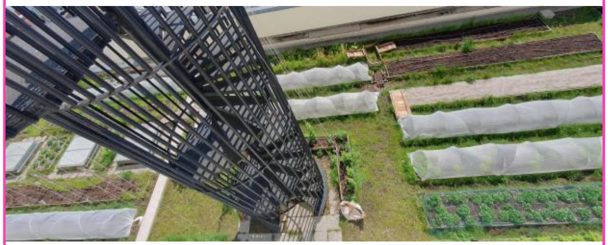
S12 | 21/03/2023

Installation date: 2020

Surface: -
Height: 3rd floor
Substrate: max 15cm, shell debris
Use: productive (fruits and vegetables), sell
Management practices: regularly, drip watering

Sampling point	Depth (cm)	Vegetation
S12-1	20	*Aromatic plants, vegetables (leek, cabbage, etc.)*
S12-2	20	
S12-3	20	

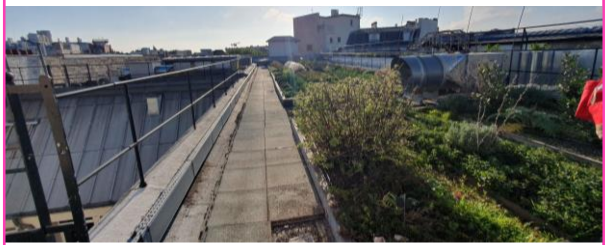
S13 | 02/05/2023



Installation date: 2018
Surface: 2500m² (many green roofs on the same building)
Height: 5th floor
Substrate: max 20cm, vegetation on substrate layer, organic
Use: productive (vegetables and fruits)
Management practices: regularly, drip watering

Sampling point	Depth (cm)	Vegetation
S13-1	18	Cabbage
S13-2	19	
S13-3	18	Garlic 'tulbachia', *Sedum sp.*

S14 | 07/04/2023



Installation date: 2017

Surface: 800m²
Height: 6th floor
Substrate: max 30cm, vegetation in a vegetal substrate with pozzolan and clay balls, organic
Use: productive (more than 100 species of vegetables and aromatic plants)
Management practices: regularly, drip watering

Sampling point	Depth (cm)	Vegetation
S14-1	20	Aromatic plants
S14-2		
S14-3		

S16 | 06/03/2023

Installation date: 2018
Surface: 5m²
Height: 7th floor
Substrate: max 80cm, vegetation in box
Use: ornamental (small fruit trees)
Management practices: 2 x/year, drip watering

Sampling point	Depth (cm)	Vegetation
S16-1	78	Lingonberries, strawberries, cranberries, gooseberries
S16-2		
S16-3		

S17 | 06/03/2023

Installation date: 2018
Surface: 8,35m²
Height: 8th floor
Substrate: max 50cm
Use: ornamental
Management practices: 2 x/year, drip watering

Sampling point	Depth (cm)	Vegetation
S17-1	50	*Carex acutiformis, Anthemis tinctoria, Nepeta faasenii, Campanula poscharskyana, Prunella grandiflora*
S17-2		
S17-3		

S18 | 29/03/2023

Installation date: 2017
Surface: 250m²
Height: 2nd floor
Substrate: max 50cm, vegetation on a substrate layer or in box
Use: ornamental (including trees), pedagogical
Management practices: regularly, drip or sprinkler watering

Sampling point	Depth (cm)	Vegetation
S18-1	50	Ornamental plantations, trees
S18-2		
S18-3		

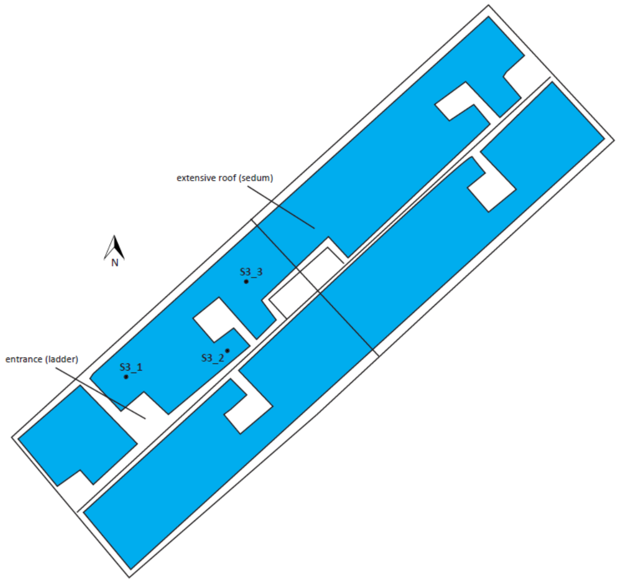
S20 | 13/04/2023

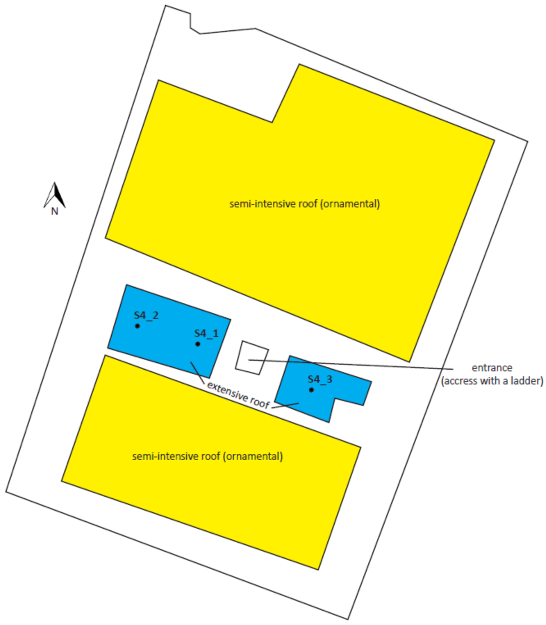
Installation date: 2016
Surface: 116m²
Height: 17th floor
Substrate: max 100cm, vegetation in box
Use: biodiversity, ornamental (including trees)
Management practices: 4-5 x/year, drip watering

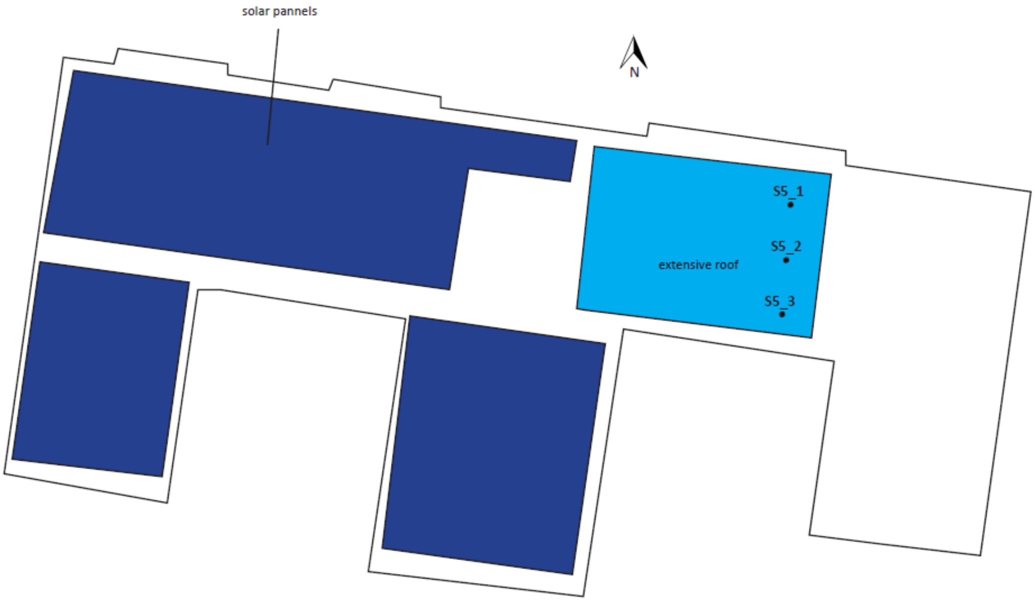
Sampling point	Depth (cm)	Vegetation
S20-1	100	Ornamental plantations, trees
S20-2		
S20-3		

Annex II: plan of 16 green roofs of the BIOTOV project: from S3 to S20 (Levaillant 2023)

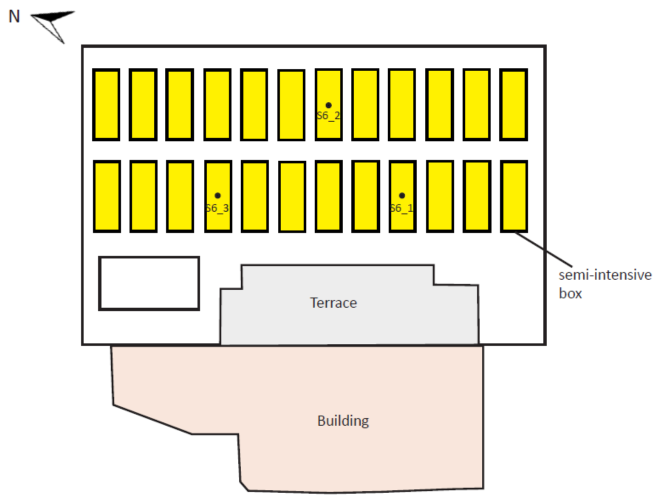
<u>II.1. S3, S4, and S5: extensive green roofs (blue)</u>

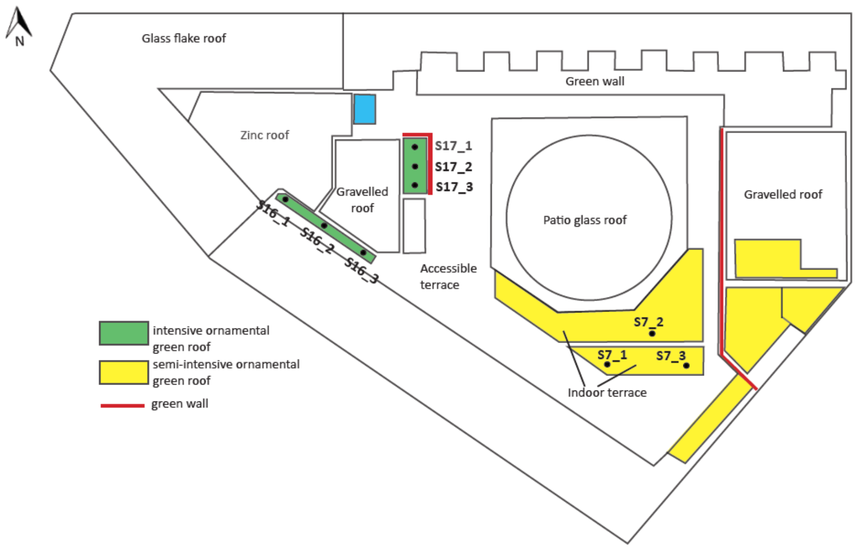


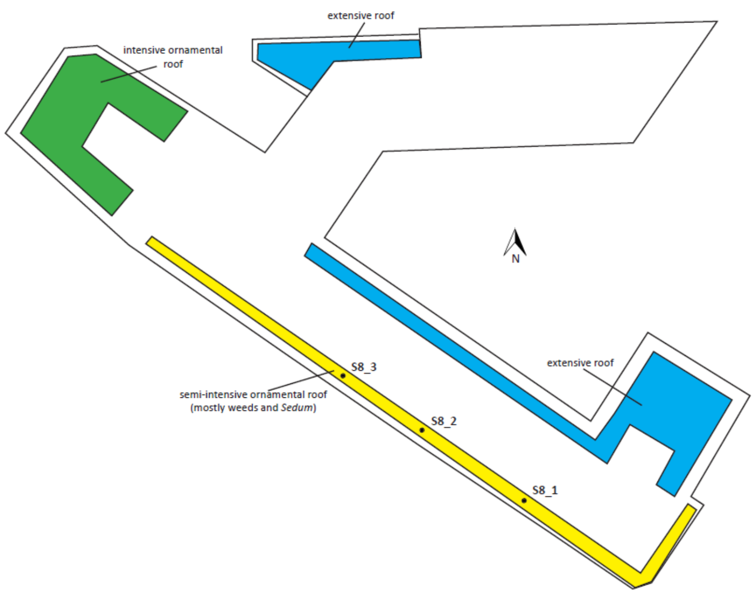


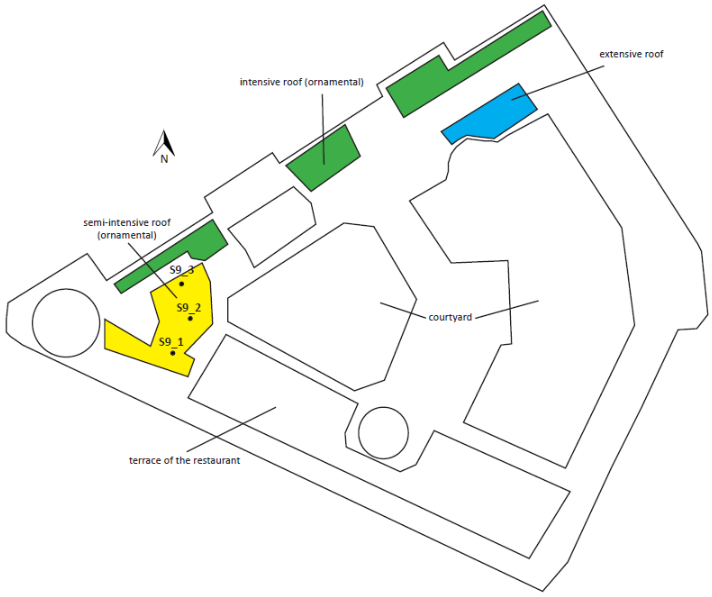


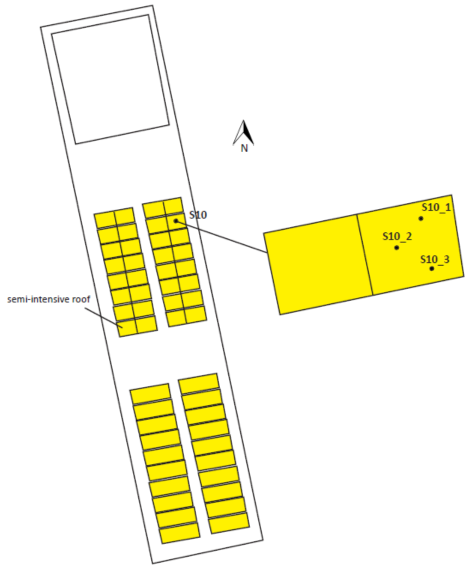
II.2. S6, S7, S8, S9, and S10: semi-intensive ornamental roofs (yellow); S16 and S17: intensive ornamental roofs (green)



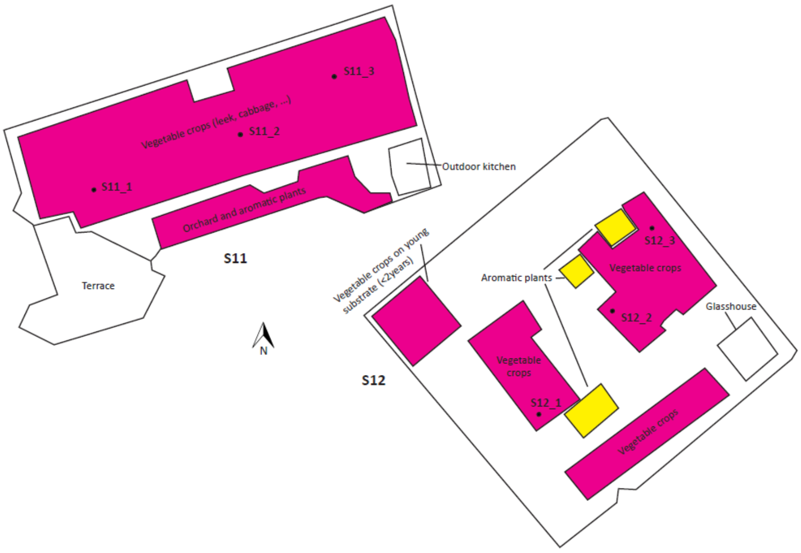


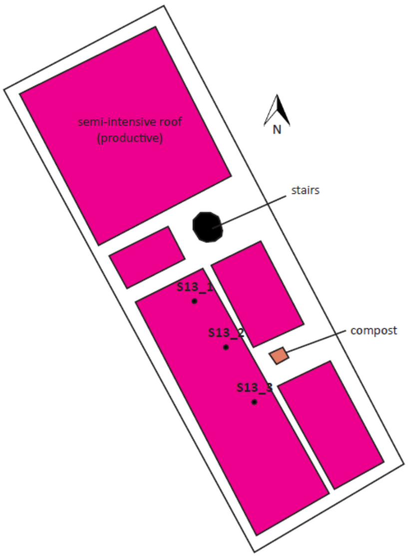


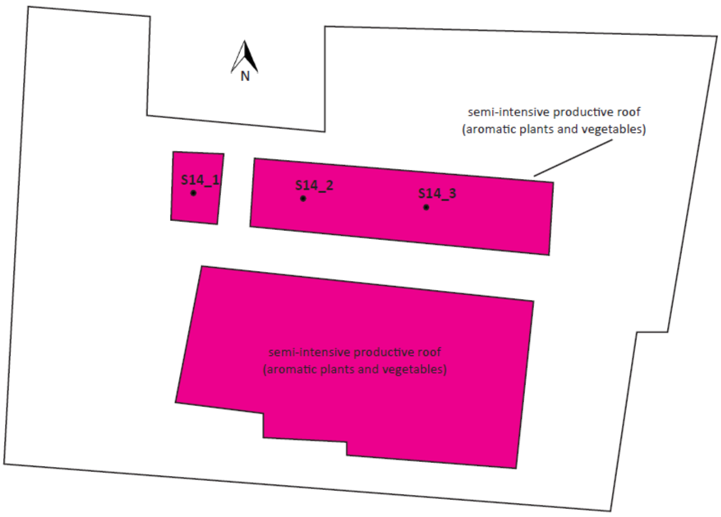




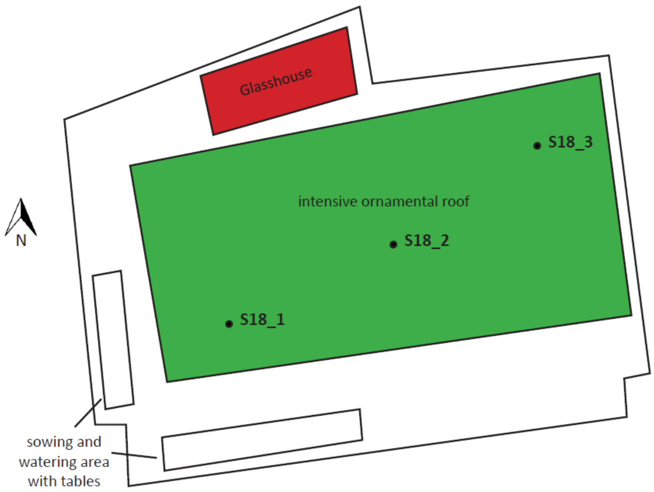
II.3. S11, S12, S13, S14: semi-intensive productive roofs (pink)







II.4. S18 and S20: intensive ornamental roofs (green)



Annex III: Collembola identification down to the species level (Boyer 2022; Hopkin 2007)

Collembola are divided into 4 distinct orders: Poduromorphs, Entomobryomorphs, Symphypleones and Neelipleones. These four orders can be distinguished after a quick examination on the basis of several criteria, the most important of which is the general shape of the body. Once the springtails have been identified to an order, other criteria are used to refine identification down to species level (e.g., respective size and fusion of abdominal or antennal segments, presence of setae or scales, number of ocelli).

III.1. Poduromorphs

Poduromorphs have a pudgy body, short antennae and a dorsally developed first thoracic segment (*Figure 19*).

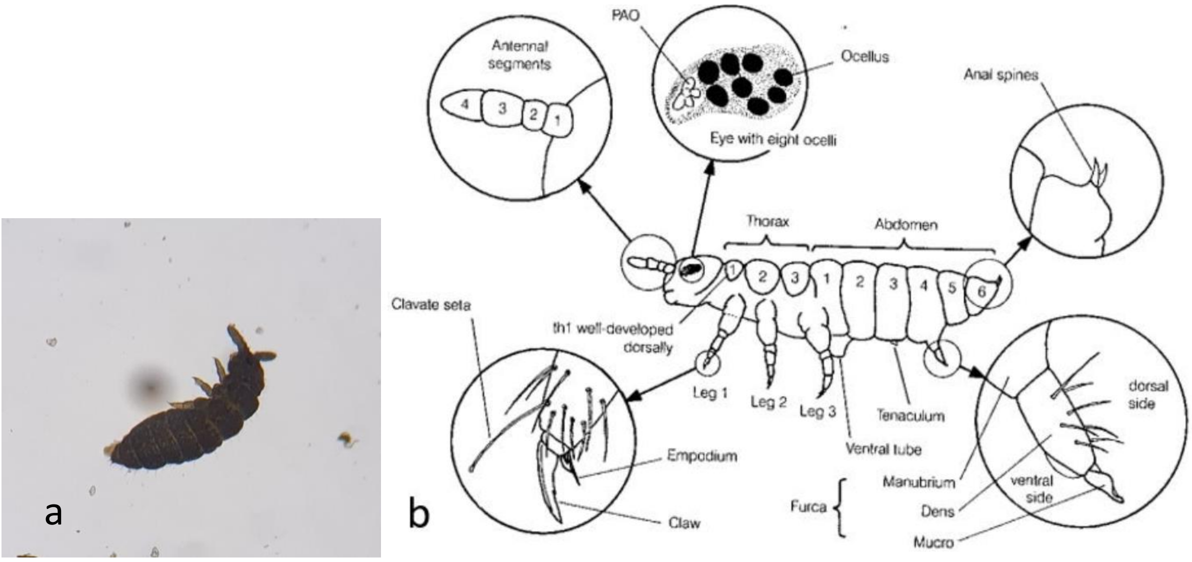


***Figure 19*: Poduromorph Collembola** (a: ©Nolwen Levaillant, b: (Hopkin, 2007 p.2), a: Poduromorph Collembola under binocular magnifying glass (actual size 1 mm), b: morphological characteristics of a Poduromorph

III.2. Entomobryomorphs

Entomobryomorphs have a longer body than Poduromorphs, long antennae and a dorsally reduced first thoracic segment (*Figure 20*).

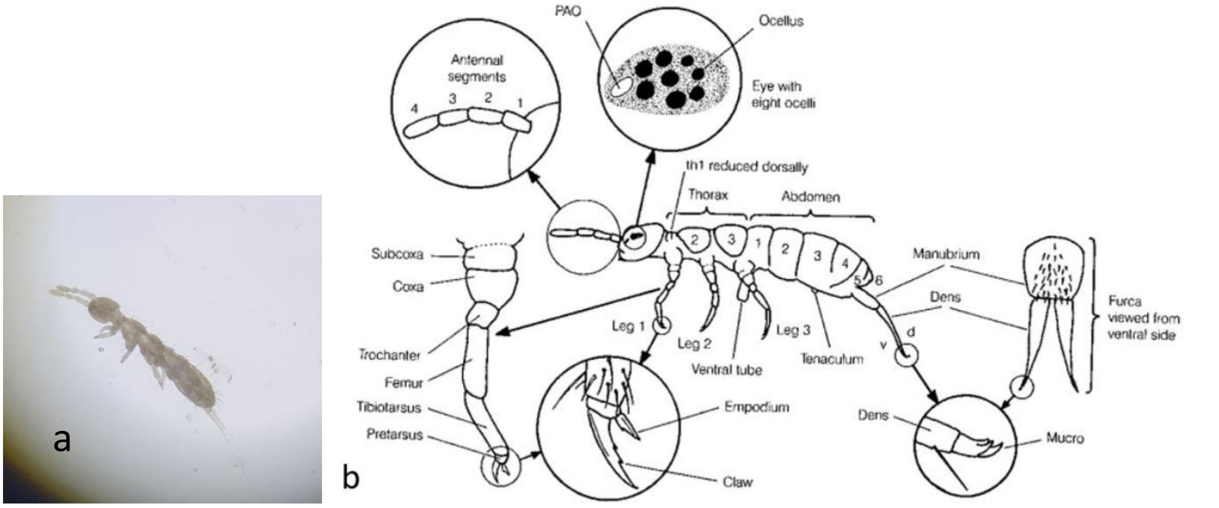


***Figure 20*: Entomobryomorph Collembola** (a: ©Nolwen Levaillant, b: (Hopkin, 2007 p.2), a: Entomobryomorph Collembola under binocular magnifying glass (actual size 4,5mm), b: morphological characteristics of an Entomobryomorph

III.3. Symphipleones

Symphypleones have a round head and abdomen (the segmentation of the latter is difficult to see). The distal segment of the antennae is longer than the others (*Figure 21*). They are usually epigeal.

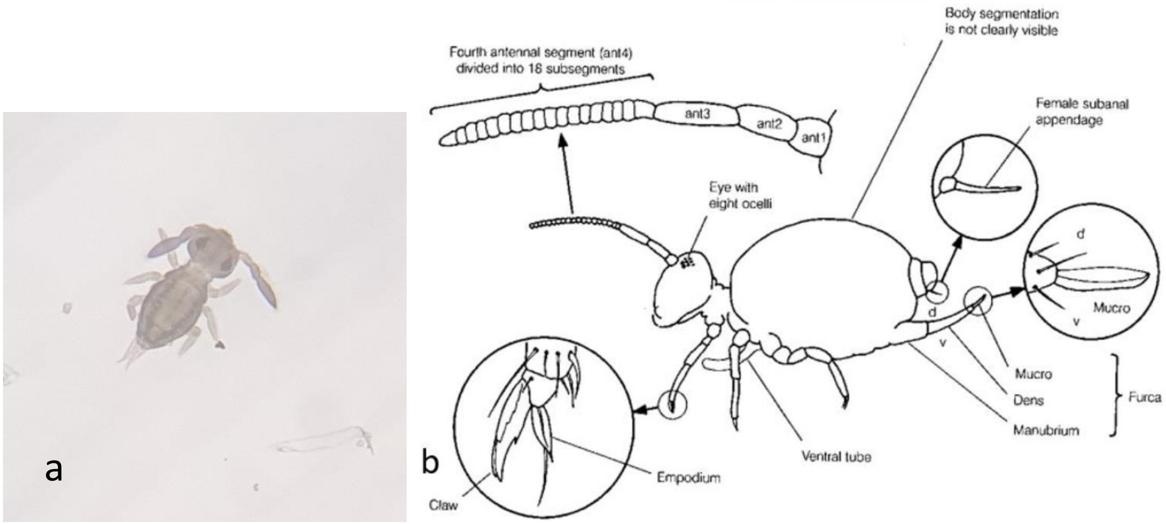


Figure 21**: Symphipleone Collembola** (a: ©Nolwen Levaillant, b: (Hopkin, 2007 p.3), a: Symphipleone Collembola under binocular magnifying glass (actual size 2,5mm), b: morphological characteristics of a Symphipleone

III.4. Neelipleones

Neelipleones are small (0.4 mm), with no ocelli or clear body segmentation. The antennae are smaller than the head (*Figure 22*). This order contains only one family, with only sixty species.

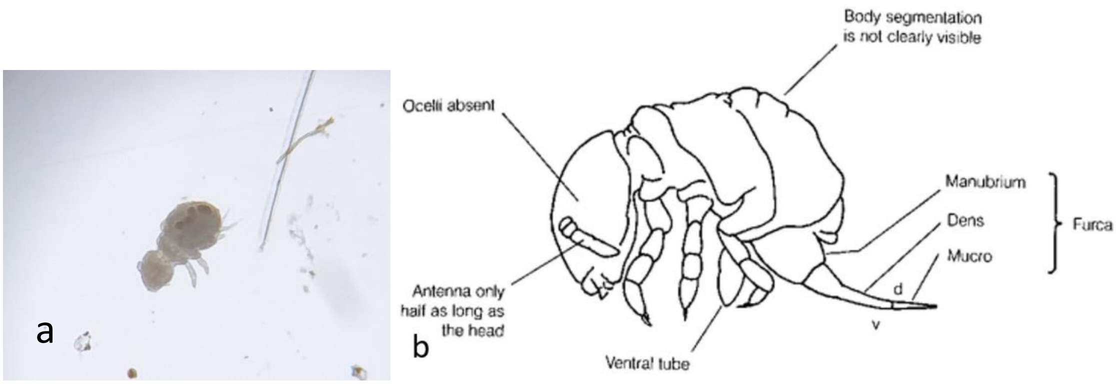


Figure 22**: Neelipleone Collembola** (a: ©Nolwen Levaillant, b: (Hopkin, 2007 p.3), a: Neelipleone Collembola under binocular magnifying glass (actual size 0,4mm), b: morphological characteristics of a Neelipleone

Annex IV: earthworm identification down to the species level (Sherlock 2018)

Earthworms are distributed into three group:

- The epigenic: surface-dwelling earthworms, red in color, and found under leaf litter, in rotting logs and for some species in areas of very high organic matter such as compost.

- The endogenic: soil dwellers earthworms, and are rarely present on the surface. They have a small adult size, and are grey, green or pink in color.
- The anechoic: are deep burrowing worms, red or black colored. These are the biggest earthworms.

The earthworm identification is mostly done at the adult stage, where the clitellum is present. To determine the species, many organs are looked, such as the number of segments, the head shape (tanylobic or epilobic), the clitellum location, the presence of male pore, and the setae.

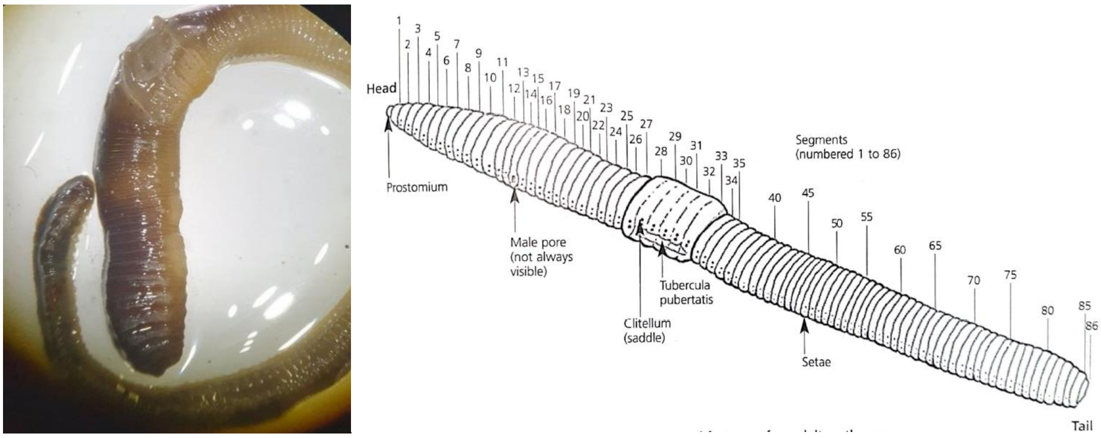


***Figure XXX*: earthworm under binocular magnifier (left)** (©Nolwen Levaillant) **and the external features of an adult earthworm (right)** (Sherlock 2018)

Annex V: list of the identified soil organisms of the BIOTOV project

V.1. Collembola species found on the 17 green roofs studied for the BIOTOV project (Hopkin 2007)

Species name	**Group**	**Frequency**	**Pigmentation**	**Body shape**	**Body size (mm)**
Bourletiella arvalis	symphypleona	common	Yes	Cylindric	1,5
Bourletiella hortensis	symphypleona	common	Yes	Cylindric	1,3
Brachystomella parvula	poduromorpha	common	Yes	Spheric	1
Cryptopygus bipunctatus	entomobryomorpha	moderately common	No	Spheric	0,7
Cryptopygus thermophilus	entomobryomorpha	moderately common	Yes	Spheric	1
Cyphoderus albinus	entomobryomorpha	common	No	Spheric	1,6
Deuteraphorura inernis	poduromorpha	common	No	Spheric	2,3
Deuterominthurus pallipes	symphypleona	several	Yes	Cylindric	1
Dicyrtoma fusca	symphypleona	common	Yes	Cylindric	2
Entomobrioides myrmecophilus	entomobryomorpha	rare	Yes	Spheric	2
Entomobrya albocinta	entomobryomorpha	moderately common	Yes	Spheric	2
Entomobrya lanuginosa	entomobryomorpha	moderately common	Yes	Spheric	2
Entomobrya multifasciata	entomobryomorpha	very common	Yes	Spheric	2
Entomobrya nivalis	entomobryomorpha	extremely common	Yes	Spheric	2,5
Folsomia agrelli	entomobryomorpha	rare	No	Spheric	1,3
Folsomia binoculata	entomobryomorpha	rare	No	Spheric	1,5
Folsomia inoculata	entomobryomorpha	rare	No	Spheric	1,9
Folsomia similis	entomobryomorpha	very rare	No	Spheric	1,4
Folsomides angularis	entomobryomorpha	rare	No	Spheric	0,9

Folsomides parvulus	entomobryomorpha	rare	No	Spheric	0,9
Hypogastrura purpurescens	poduromorpha	very common	Yes	Spheric	2,3
Isotoma anglicana	entomobryomorpha	common	Yes	Spheric	4
Isotoma antennalis	entomobryomorpha	rare	Yes	Spheric	3
Isotomiella minor	entomobryomorpha	extremely common	No	Spheric	1,2
Isotomodes productus	entomobryomorpha	common	Yes	Spheric	0,9
Isotomurus palustris	entomobryomorpha	very common	Yes	Spheric	2,5
Lepidocyrtus cyaneus	entomobryomorpha	common	Yes	Spheric	1,5
Lepidocyrtus lanuginosus	entomobryomorpha	very common	Yes/No	Spheric	2
Lepidocyrtus lignorum	entomobryomorpha	moderately common	No	Spheric	2
Lepidocyrtus paradoxus	entomobryomorpha	very rare	Yes	Spheric	3
Lepidocyrtus violaceus	entomobryomorpha	moderately common	Yes	Spheric	1,6
Megalothorax minumus	neelipleona	very common	No	Cylindric	0,4
Metaphorura affinis	poduromorpha	moderately common	No	Spheric	1,3
Parisotoma notabilis	entomobryomorpha	extremely common	Yes	Spheric	1
Proisotoma minuta	entomobryomorpha	common	Yes	Spheric	1,1
Protaphorura armata	poduromorpha	very common	No	Spheric	2,5
Pseudosinella alba	entomobryomorpha	very common	No	Spheric	1
Pseudosinella decipiens	entomobryomorpha	moderately common	No	Spheric	2
Pseudosinella immaculata	entomobryomorpha	very common	No	Spheric	2,8
Sminthurinus aureus	symphypleona	very common	Yes/No	Cylindric	1
Sminthurinus elegans	symphypleona	common	Yes/No	Cylindric	0,7
Sphaeridia pumilis	symphypleona	very common	Yes	Cylindric	0,5
Thalassaphorura encarpata	poduromorpha	very rare	No	Spheric	1,4
Tomocerus minutus	entomobryomorpha	rare	Yes	Spheric	2,0
Tomocerus minor	entomobryomorpha	very common	Yes	Spheric	4,5
Willemia denisi	poduromorpha	rare	No	Spheric	0,7

V.2. Earthworm species found on the 17 green roofs studied for the BIOTOV project (Sherlock 2018)

Species name	**Group**	**Frequency**	**Habitat**	**Length (mm)**	**Color**
Aporrectodea caliginosa	Endogenic	Very common	Many anthropogenic habitats	40-180mm	Pink/grey
Dendrobaena octaedra	Epigenic	Uncommon	Liiter, bark, stones	20-60mm	Dark red to pale
Dendrobaena veneta	Epigenic	Common	Well drained soils	15-45mm	Deep red
Eiseinia fetida	Epigenic	Common	Compost	26-130mm	Reddish dark
Eiseniella tetraedra	Endogenic	Common	Waterlogged soils	20-80mm	Green-brow, pale

Annex VI: Percentage of density of all identified mesofauna

	% of density for all identified mesofauna		
Roof type	*Collembola*	*Acarids*	*Microarthropods*
EXT	29,9	41,9	28,2
SEMI	25,9	64,5	9,6
PROD	22,7	71,0	6,3
INT	19,8	46,6	33,6

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Nb pages : 25 Annexe(s) : VI	
Année de soutenance : 2023	

Titre français : Etude de l'impact de différents types de toits verts sur la biodiversité des sols en Île-de-France

Titre anglais : Study of the impact of different green roof types on soil biodiversity in Île-de-France

Résumé (1600 caractères maximum) :

Les toitures végétalisées sont un type d'espace vert qui, depuis plusieurs décennies, se développe de plus en plus pour pallier les enjeux environnementaux et sociétaux rencontrés en milieu urbain. L'étude de la biodiversité en milieu urbain, en particulier de la biodiversité des sols sur les toits verts est très restreinte. Or les organismes du sol sont essentiels et répondent à de nombreux services écosystémiques. Le projet BIOTOV étudie l'impact de différents types de toitures végétalisées sur la biodiversité des sols (microorganismes, micro-, méso-, et macrofaune) en Île-de-France. Les résultats de cette étude ont montré que ce sont les plus petits organismes (nématodes et collemboles), mais surtout les microorganismes qui sont le plus adaptés en toiture. Ils sont par ailleurs retrouvés en densité similaire voire beaucoup plus élevée que d'autres espaces urbains ou naturels. La profondeur des sols de toitures impacte particulièrement la macrofaune (e.g., vers de terre) qui n'est quasiment pas présente à faible profondeur, mais elle impacte beaucoup moins les plus petits organismes. D'autres facteurs viennent influencer la présence de la biodiversité des sols, comme la compétition entre organisme, l'usage de la toiture (productif VS ornemental), ou encore les paramètres physicochimiques des sols.

Abstract (1600 caractères maximum):
Green roofs are a type of green space which, for several decades, have been developing more and more to overcome the environmental and social issues encountered in urban areas. The study of biodiversity in urban areas, in particular soil biodiversity on green roofs, is very limited. However, soil organisms are essential and respond to many ecosystem services. The BIOTOV project studies the impact of different types of green roofs on soil biodiversity (microorganisms, micro-, meso-, and macrofauna) in Île-de-France. The results of this study showed that it is the smallest organisms (nematodes and springtails), but above all microorganisms that are the most suitable for roofing. They are also found in similar or even much higher density than other urban or natural spaces. The depth of the roof soils particularly impacts the macrofauna which is almost not present at shallow depths, but it impacts the smallest organisms much less. Other factors influence the presence of soil biodiversity, such as competition between organisms, the use of the roof (productive VS ornamental), or the physicochemical parameters of the soil.

Mots-clés : toits verts, biodiversité des sols, milieux urbain, microorganismes, mésofaune, microfaune, macrofaune

Key Words: green roofs, soil biodiversity, urban areas, mesofauna, microorganisms, microfauna, mesofauna, macrofauna