

SOIL BIODIVERSITY IN SWITZERLAND

The BioDivSol Project

Swiss Biodiversity Strategy Action Plan

Project 4.2.2 Development of a soil strategy for Switzerland

Measure 3: Research on soil biodiversity

Synthesis report

Imprint

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The contractor bears sole responsibility for the content.

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The BioDivSol Project

Synthesis report for the project leaders of the Action Plan for Swiss Biodiversity Strategy

Summary

Soil organisms are key actors in maintaining soil health and ecosystem services essential to society and the economy, making their preservation crucial for sustainable development. The BioDivSol project represents the first extensive assessment of soil organism diversity at the national scale in Switzerland, revealing an extraordinary hidden biodiversity beneath our feet. The project was funded as part of the Swiss Biodiversity Strategy Action Plan (AP SBS) and Measure 4.2.2 of the Action Plan, which support the development of a Swiss National Soil Strategy. The objective of the project was to enhance our understanding of the biodiversity present in Swiss soils and to clarify the role this biodiversity plays in soil functionality.

The project examined six major organism groups (Archaea, Bacteria, Fungi, Protists, Nematodes, and Soil Arthropods including microarthropods) across 663 study sites covering all six Swiss biogeographical regions. A key aim of the project was to explore the potential of advanced molecular techniques, notably metabarcoding, combined in some cases with traditional morphological approaches to assess biodiversity of soil organisms across six land-use types, with particular focus on forests (31%), permanent grasslands (22%), and arable land (17%).

More than 35'000 taxa were detected in soils across Switzerland, with diversity ranging from 30'000 fungal taxa to 40 archaeal taxa. The project provided key information on species and taxa that were previously unrecorded or unknown in Switzerland. The proportion of undescribed taxa ranged from 24% for nematodes to 68% for bacteria. These data will be used to build and expand existing databases. Soil organism groups respond differently to various environmental factors. Distinct land-use types are associated with specific biodiversity patterns, for example increased ASV richness in soil arthropods but reduced bacterial diversity in forest soils, or ASVs found exclusively under particular land-uses. The current project on soil biodiversity highlights that, beyond pH, soil carbon metrics such as organic carbon (Corg), the C/N ratio, the Corg-to-Clay ratio, and total carbon (Ctot) are major determinants of biodiversity across the groups of organisms assessed. These parameters could provide a basis for establishing reference values and developing indicators, to support biodiversity monitoring and the assessment of soil biological health. Considering soil microorganisms, short-term agricultural practices (over six months) exert limited influence on microbial communities, except for organic amendments, which appear to slightly affect microbial activity and biomass. In contrast, long-term practices (over a decade) have a more pronounced impact, likely mediated through gradual modifications of soil properties such as organic carbon content, pH, and texture (clay content).

The project demonstrates that large-scale soil biodiversity monitoring is technically feasible with current molecular technologies, although further optimization is still required. Metabarcoding offers significant

advantages for routine assessment, providing comprehensive diversity data while being cost-effective for national-scale implementation.

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1. Context and goals

Soil is a crucial but finite resource laying at the foundation of life and vital for human basic needs. Fulfilling a large range of ecological functions, healthy soils sustain and promote food production and other ecosystem services essential to society and the economy. Many of these soil functions and ecosystem services are led by soil inhabiting organisms. Indeed, their diversity and interactions within the soil ecosystem place them as key actors responsible for important processes, such as nutrient transformation or plant resistance and defense, which in turn support ecosystem services (Figure 1) (Dell' Ambrogio et al., 2023; Creamer et al., 2022; Faber et al., 2021). Despite the importance of soil biota in maintaining soil health and soil quality (Creamer et al., 2022), the biodiversity of soil inhabiting species is largely underestimated. While soil is estimated to host 59% of Earth's species (Anthony et al., 2023), much of this diversity is still poorly understood. Accurate and quantifiable data on the specific contribution of each organism to soil functions is still lacking. It is therefore necessary to better understand and quantitatively estimate the diversity of soil organisms, and to include soil biodiversity in conservation programs in the same way as above-ground biodiversity. The BioDivSol project is a new milestone towards assessing soil biodiversity at a national level in Switzerland. The aim is to enable stakeholders and politicians to take the necessary decisions to preserve, protect and restore the overall biodiversity, including the diversity of soil organisms.

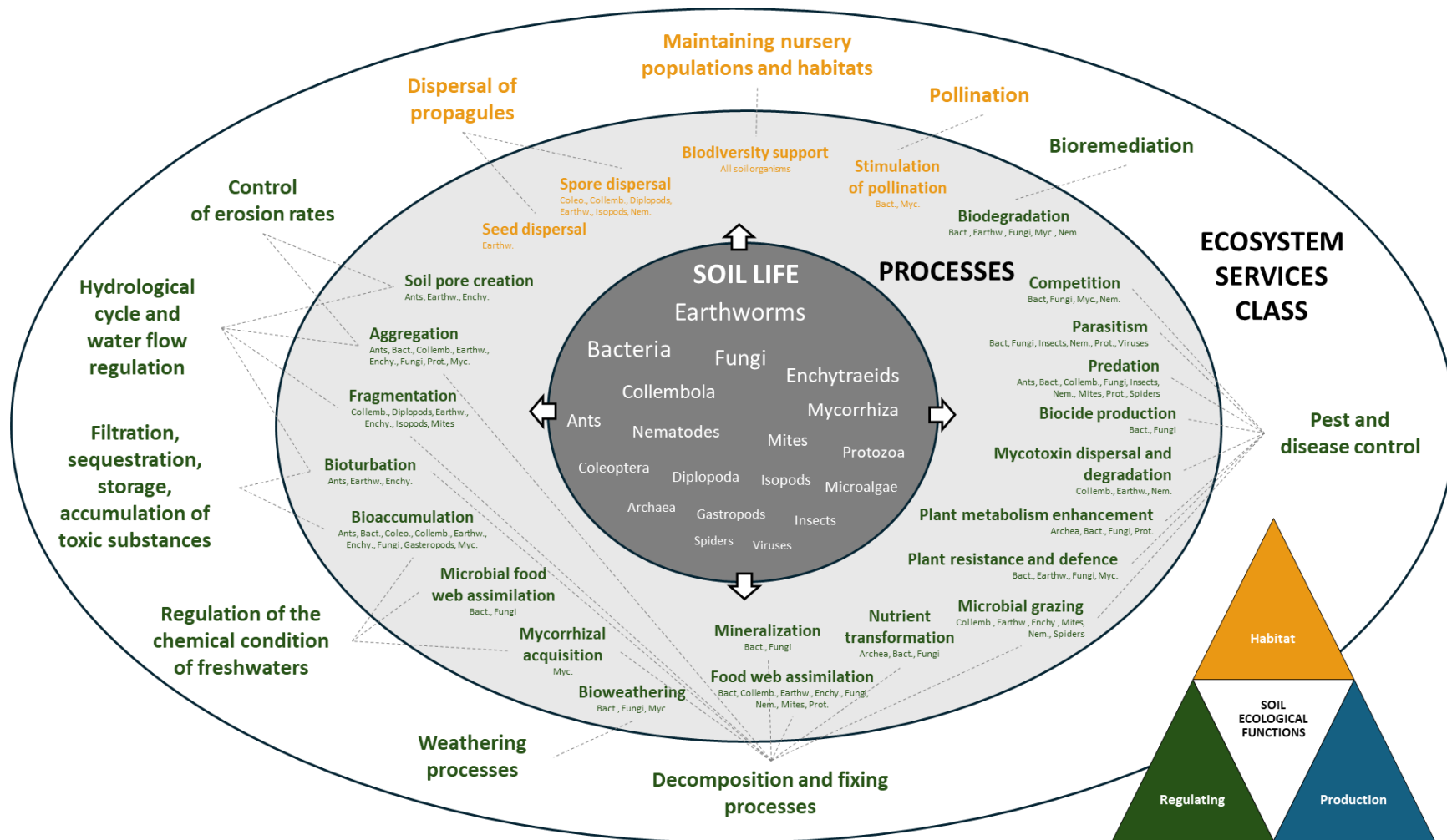


Figure 1: Importance of soil organism diversity for soil multifunctionality, based on Dell' Ambrogio et al. 2023. The key role played by soil organisms in soil processes, and their links to the corresponding ecosystem service classes (Common International Classification of Ecosystem Services (CICES)) is represented here. Processes and ecosystem service classes are categorized according to the soil ecological functions defined in the Swiss national soil strategy (Swiss Federal Council, 2020) (yellow: habitat function, green: regulating function). Since the production function is only related to plants, it is not included here.

1.1. Commitment of Switzerland for conservation and promotion of biodiversity

Reversing biodiversity decline has become one of the major economic and societal challenges of recent decades at both national and global levels. Biodiversity is recognized as "an indispensable basis for life on earth and hence also a central pillar of human existence" (FOEN, 2012), the loss of biodiversity leading to the disappearance of ecosystem services and a reduction in people's quality of life (FOEN, 2012; FOEN, 2017) (see Figure 1 for link between soil biodiversity, processes and ecosystem services). In 1992, Switzerland is committed to halting the decline in biodiversity by signing the Convention on Biological Diversity (CBD), which came into force in 1995 and aims for the conservation of biological diversity, the sustainable use of the components of biological diversity, the fair and equitable sharing of the benefits arising out of the utilization of genetic resources (CBD, 2011). To achieve these objectives of biodiversity conservation and promotion, and as part of the Federal Council's Sustainable Development Strategy, Switzerland adopted a Biodiversity Strategy (SBS) in 2012 (FOEN, 2012). The Biodiversity Strategy was reinforced in 2017 by an action plan to implement the 10 strategic goals set out in the Strategy and proposes a series of 27 measures to achieve these goals (FOEN, 2017) (Figure 2).

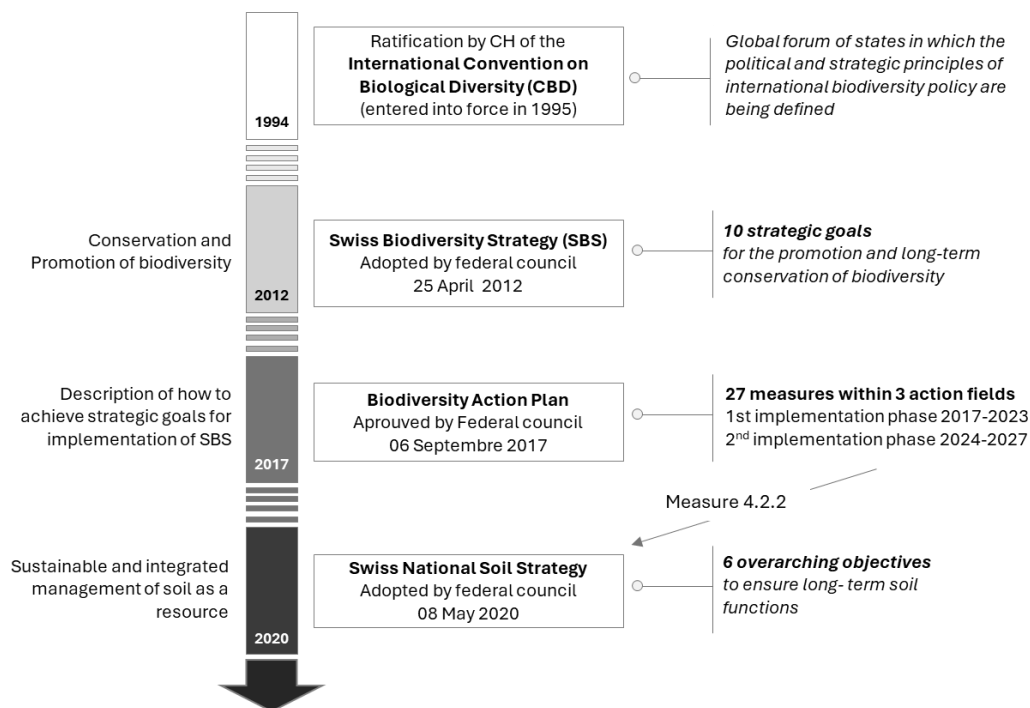


Figure 2: Switzerland commitment to biodiversity conservation at global and national level. Calendar of actions, objectives, and measures.

Biodiversity is defined by the SBS as the species diversity of animals, plants, fungi and microorganisms, the genetic diversity within the different species, the diversity of habitats, and the interactions within and between these levels. In brief, it encompasses the diversity of ecosystems, species, and genes (FOEN, 2012; FOEN, 2017) (Figure 3). The long-term conservation and promotion of biodiversity requires that sufficient knowledge about its status is available and that changes in biodiversity can be measured at each of these three levels. Indicators are therefore needed (FOEN, 2012; FOEN, 2017; Leung & Gonzalez, 2024).

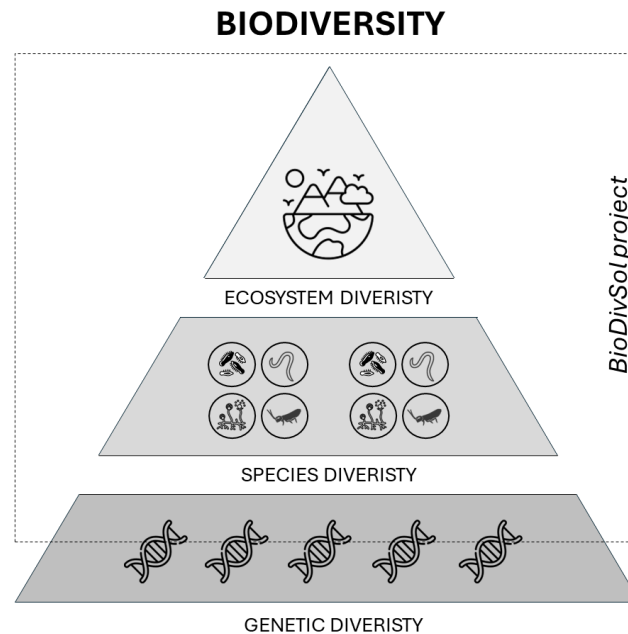


Figure 3: The three components of biodiversity working together to generate the complexity of life in relationship with the BioDivSol project.

Preserving biodiversity also means ensuring that ecosystems are healthy and protected from any adverse human impact. Consequently, and with the aim of preserving soil functions in the long-term for which soil life plays a key role, measure 4.2.2 of the Action Plan of the SBS asks for the development of a Soil Strategy for sustainable and integrated management of soil as a resource. The Soil Strategy was adopted by the Federal Council in May 2020 (Figure 2).

1.2. Swiss National Soil Strategy

The Swiss National Soil Strategy is a “guiding framework and decision-making aid” for cantonal and federal authorities to ensure sustainable soil management, that preserves soil long-term functions and the diverse services it provides (Figure 1) for the benefit of future generations (Swiss Federal Council, 2020). Among the 44 strategic orientations of the Swiss National Soil Strategy, six emphasize the need for improved consideration and enhancement of available information on soil biodiversity. The BioDivSol project aims to provide data to address these expectations.

1.3. The BioDivSol project

The BioDivSol project is a four-year project (2020-2024), funded as part of the Swiss Biodiversity Strategy Action Plan (AP SBS) and measure 4.2.2 of the Action Plan, which calls for the development of a Swiss National Soil Strategy. The project aims to gain a better understanding of the biodiversity of Swiss soils and the more precise role that biodiversity plays in soil functioning.

The main goals of the BioDivSol project are:

1. to test and validate methodologies for assessing soil biodiversity at different scales,
2. to acquire additional data and extend current knowledge on soil organism diversity in Switzerland,
3. to better understand the relationship between soil biodiversity and environmental factors (e.g. soil properties, agricultural practices).

The BioDivSol project comprises 3 research sub-projects:

Microscopic biodiversity in soils (Gschwend et al., 2022; Bouraoui et al., 2022)

The goal of this sub-project is to explore the feasibility of methods for studying the diversity of microscopic soil organisms to address knowledge gaps in soil biodiversity. This includes expanding the existing data repository on bacterial communities, acquiring data on fungal communities, and gathering data on the diversity of other soil organism groups, such as protists, nematodes, and microarthropods, while also validating molecular analytical methodologies for biodiversity monitoring.

This sub-project is hereinafter referred to as “Microscopic biodiversity”.

Reference values for soil organic matter (OM) content: organic matter pools, soil physical and biological quality (Deluz et al., 2024)

This sub-project aims to confirm reference values for organic matter content in agricultural soils and their impact on soil quality, particularly structural quality and links to organic matter pools, while also identifying key agronomic and soil factors that influence the biological quality of agricultural soils. To this end, the relationship between soil microbial diversity, microbial activity (biological quality), and various physico-chemical indicators of soil quality, while also considering the historical context of farming practices (both short- and long-term), has been analyzed.

This sub-project is hereinafter referred to as “Microbial properties & organic matter (OM)”.

National Inventory of Collembola and Acari (Mitchell et al., 2024)

This sub-project aims to explore and document the biodiversity of springtails, oribatid and gamasid¹ mites in Swiss soils through the elaboration of a taxonomic catalogue and a database on species distribution. It includes the creation of a reference collection of specimens and of the DNA sequences characterizing them (barcode). This work will contribute to the development of molecular methods (metabarcoding) for precise species identification and the assessment of their usefulness for soil biodiversity monitoring, together with the enhancement of taxonomic expertise in Switzerland for these microarthropods.

This sub-project is hereinafter referred to as “Microarthropod Inventory”.

2. Biodiversity of Soils – toward the assessment of biodiversity in Swiss soils

2.1. Scope of the 3 sub-projects

The BioDivSol sub-projects explore soil biodiversity from three distinct perspectives:

- Feasibility of tracking changes in soil organism biodiversity over time at the territorial scale using metabarcoding techniques.
- Identification and cataloging of present species.
- Better understanding of the factors (agronomic, pedological, land-uses) shaping biodiversity and, consequently, the biological quality of soils.

2.1.1. Organisms studied

2.1.1.1. Organism groups assessed and their importance for soil multifunctionality

The organisms studied in the BioDivSol project represent the major life forms found in the soil within all three domains of life, i.e. the prokaryotes (single-cell organisms lacking a nucleus) with the archaea and bacteria, and the eukaryotes (organisms whose cells have nucleus and organelles). The assessment of soil biodiversity focuses on six different groups of organisms ranging from microorganisms to soil mesofauna, i.e.:

- Archaea,
- Bacteria,
- Fungi,
- Protists,
- Nematodes,

¹ Oribatids and gamasids are two major groups of soil mites (Acari)

- Soil arthropods², with a particular focus on microarthropods, namely
- Collembola & mites (i.e. Oribatids and Gamasids)

Representing a significant fraction of the complex soil trophic network, these organism groups support soil multifunctionality and its related soil ecosystem services. They play a fundamental role in, for instance, decomposition and fixing processes (e.g. mineralization, nutrient transformation, fragmentation), hydrological cycle and water flow regulation or pest and disease control (Dell Ambrogio et al., 2023; Creamer et al., 2022; Jiao et al., 2021; Bloor et al., 2021). Figure 1 in Chapter 1 provides more insight into this point.

2.1.2. Approaches used for biodiversity assessment

Morphological and molecular methodologies are the two main approaches to characterize the diversity of soil organisms, each of which has its advantages and limitations with respect to the different groups of organisms present in the soil (see Geisen et al. 2019 for further information). Molecular methods based on high-throughput sequencing (HTS), such as metabarcoding (a specific application of amplicon sequencing), offer the potential to identify at the same time thousands of taxa within the same environmental sample (e.g. soil) or bulk sample (organism fraction extracted from soil sample) by isolating their genetic material (deoxyribonucleic acid, DNA) to study biodiversity. Metabarcoding involves the selective amplification and sequencing of specific genomic regions, namely barcodes, characteristic of different groups of organisms. Specific oligonucleotide primers are designed to amplify these regions of interest in the genome, enabling their detection (sequencing) and analysis to determine species identity and diversity. For that purpose, the sequences obtained must be compared with reference sequences in a database: each sequence present in the sample is matched against known sequences to determine which species it belongs to. This requires the species to be included in the database. However, certain taxa are much better represented than others and many species are still missing. If a taxonomic assignment is not possible, e.g., due to an incomplete database or an unknown taxon, ASVs (Amplicon Sequence Variants) or OTUs (Operational Taxonomic Units) are used. These unique DNA sequences serve as surrogates for species or taxa to classify DNA sequences obtained from sequencing. Metabarcoding is now the standard approach for assessing the diversity and community structure of soil microorganisms including bacteria, archaea and fungi. These approaches are however still less frequently used to study the diversity of larger soil organisms from the meso-, and macrofauna (Geisen et al. 2019). In the BioDivSol project, the reliability and development needs of using a metabarcoding approach to assess and monitor the diversity of the above-mentioned major groups of soil organism was investigated. An overview of the current state of metabarcoding research for each organism group studied is provided below.

² Soil arthropods mainly include insects, arachnids, Acari (mites), Collembola, Protura and Symphyla

***Current status of molecular approaches by organism groups for diversity monitoring
(Geisen et al., 2019; Gschwend et al., 2022; Mitchell et al., 2024)***

	– Metabarcoding technics well suited – Specific DNA barcodes and primers for Prokaryotes (Archaea and Bacteria) available
Archaea	– Large DNA sequence reference databases available – Direct DNA extraction from a soil sample – Very small amount of homogenized soil (e.g. 0.5 g for BioDivSol) are needed for the analysis³
	– Metabarcoding technics well suited and well established – Specific DNA barcodes and primers for Prokaryotes (Archaea and Bacteria) available
Bacteria	– Large DNA sequence reference databases available – Direct DNA extraction from a soil sample – Very small amount of homogenized soil (e.g. 0.5 g for BioDivSol) are needed for the analysis³
	– Metabarcoding technics well suited and well established – Specific DNA barcodes and primers for Fungi available
Fungi	– Large DNA sequence reference databases available – Direct DNA extraction from a soil sample – Very small amount of homogenized soil (e.g. 0.5 g for BioDivSol) are needed for the analysis³

³ DNA from Archaea, Bacteria, Fungi and Protists can be extracted within the same soil sample.

Protists

- Universal eukaryote primers for overview of protists diversity or specific primers available for selected monophyletic groups of protists
 - DNA sequence reference databases available and under development
 - Direct DNA extraction from a soil sample
 - Very small amount of homogenized soil (e.g. 0.5 g for BioDivSol) are needed for the analysis³
-

Nematodes

- Metabarcoding technics established. Traditionally, a morphological approach is employed to identify individuals.
 - Eukaryote primers are generally used. Some specific primers exist for certain nematode groups, but they do not cover the entire diversity of nematodes sequences reference database (work in progress)
 - Large DNA sequence reference databases
 - The use of active nematode taxa extracted from soil samples for DNA analysis is common. Direct DNA extraction from a soil sample, while still under development, is becoming more widespread (Donhauser et al., 2023)
 - Small amounts of homogenized soil are required for the analysis (e.g., 20 g for direct DNA extraction from a soil sample as in BioDivSol, or 100 g if nematodes are first extracted from the soil sample)
-

**Soil
Arthropods**

**Collembola
&**

Mites

- Soil arthropods are very often identified using morphological methods. However, molecular approaches have become widely adopted in recent years
 - Metabarcoding approaches are established but the uneven and spatially heterogeneous distribution, as well as low abundances of soil arthropods compared to, for example, bacteria and fungi, poses a challenge for robust assessment of diversity
 - Certain classes and groups of Arthropoda, like Protura, Malacostraca, and Ostracoda, but also to some extent Collembola and mites, are still underrepresented in DNA barcode reference databases, which limits taxon identification
 - The applied approach selects for living and active organisms prior to metabarcoding (organisms are extracted from the soil samples before proceeding with DNA analysis)
 - Relatively large volumes of soil are required to capture sufficient biodiversity to overcome the above-mentioned constraints. Seven to twenty-four intact soil cores (8 cm diameter, 9 cm depth) were collected per site in the BioDivSol project
-

2.1.3. Study sites

The BioDivSol project, through its three sub-projects, encompasses 663 study sites distributed across the entire Swiss territory, ensuring comprehensive coverage for analyzing soil organism diversity (taxa richness, taxonomy, community composition). Most of the studied sites are already established and integrated into observation programs or monitoring networks dedicated to the study of above-ground biodiversity and environmental conservation. Additionally, the project explored the influence of land-use types and environmental factors on the diversity of the studied organism groups. These aspects are described more in detail in the chapters below.

2.1.3.1. Biogeographical distribution of sites and connection with existing monitoring networks

The 663 study sites in the BioDivSol project are distributed across all six of Switzerland's main biogeographical regions, each distinguished by its own specific diversity of flora and fauna and varying levels of management intensities (FOEN, 2022) (Figure 4).

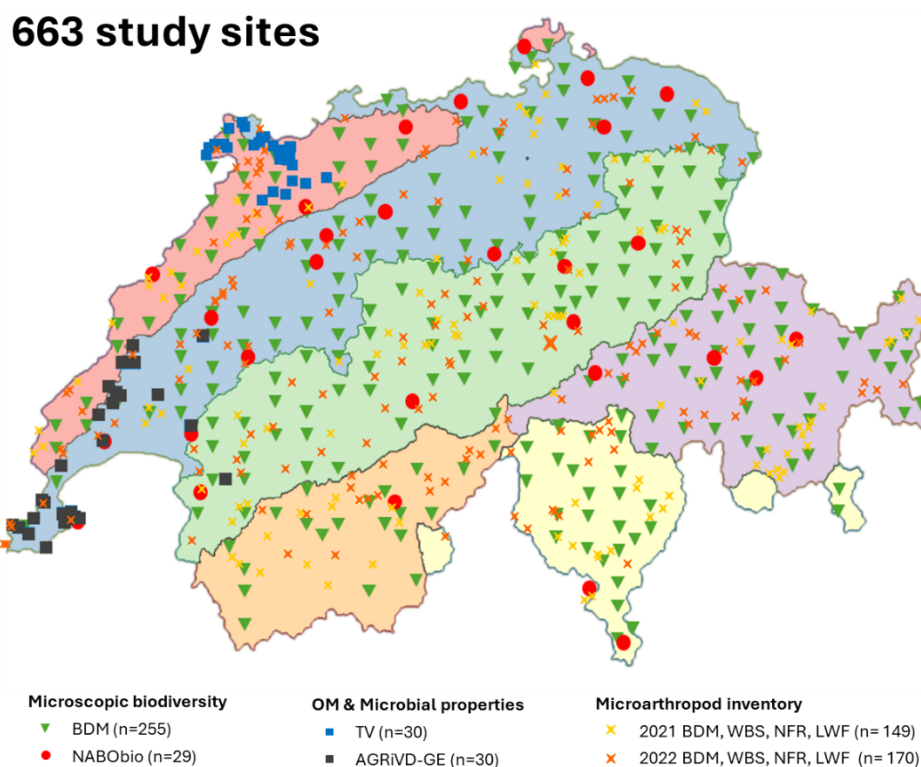


Figure 4: The locations of the 663 study sites of the BioDivSol project across Switzerland are shown, categorized by the six main Swiss biogeographical regions (FOEN, 2022): Jura (pink), Plateau (blue), Northern Alps (green), Western Alps (orange), Eastern Alps (ililac), and Southern Alps (yellow), as well as their affiliation with the three sub-projects. Monitoring networks and number of associated sites⁴ are indicated accordingly, with further details provided in Table 1.

⁴ For the microarthropod inventory sub-project, only the results from a subset of sites are presented, as the overall analysis is still ongoing (see chapter 2.2.1.7).

Most of these sites were selected from six existing national observation programs or monitoring networks. The data collected through BioDivSol will enrich the information already gathered by these programs, with additional sites in the cantons of Geneva and Vaud further complementing the datasets. Table 1 and Figure 4 provide an overview of the monitoring networks and observation programs involved in BioDivSol, detailing the organisms studied and the number of sites for each program, their affiliation with the three sub-projects, and their distribution across Switzerland's biogeographical regions.

Table 1: Existing observation programs or monitoring networks in Switzerland and their relationships to the BioDivSol project.

Observatory network	Abbreviation	Organism assessed (BioDivSol)	Sub-project	Number of sites	Reference
Biodiversity Monitoring	BDM	Fungi	Microscopic biodiversity	255	www.biodiversitymonitoring.ch
		Collembola and mites	Microarthropod inventory	100	www.biodiversitymonitoring.ch
Monitoring the Effectiveness of Habitat Conservation	WBS	Collembola and mites	Microarthropod inventory	179	https://biotopschutz.wsl.ch/en/
Program for sustainable use of natural resources "Terres Vivantes"	TV	Archea Bacteria Fungi	OM & Microbial properties	30	https://www.frij.ch/terres-vivantes-2/
Swiss Soil Monitoring Network – Biological Soil Monitoring	NABObio	Archea Bacteria Fungi Protozoa Nematodes Soil arthropods	Microscopic biodiversity	29	https://www.agroscope.admin.ch/agroscope/en/home/topics/environment-resources/soil-bodies-water-nutrients/nabo/monitoring/nabobio.html
Natural forest reserves	NFR	Collembola and mites	Microarthropod inventory	21	https://www.wsl.ch/en/forest/biodiversity-conservation-and-primeval-forests/natural-forest-reserves/
Long-Term Forest Ecosystem Research Program	LWF	Collembola and mites	Microarthropod inventory	19	https://www.wsl.ch/en/forest/forest-development-and-monitoring/long-term-forest-ecosystem-research/
Additional sites					
Agricultural sites VD & GE	AGRIVD-GE	Archea Bacteria Fungi	OM & Microbial properties	30	
Total number of sites				663	

2.1.3.2. Represented land-uses

The BioDivSol project covers seven main land use types, with forests (31%), permanent grasslands⁵ (22%) and arable land (17%) together accounting for 70% of the sites studied. The remaining sites are distributed among alpine grasslands (10%), alluvial sites (9%), raised bogs & fens (9%), and settlements⁶ (1%) (Figure 5). The diversity of all organism groups included in the project was assessed for the three most predominant land-use types (forests, permanent grasslands, and arable lands), though not all together at all sites within a land-use. For the other land uses, only fungal and/or collembola & mite diversity was assessed (see Chapter 2.1.3.3 for information). Table 2 shows the land-use types assessed in each of the three BioDivSol sub-projects.

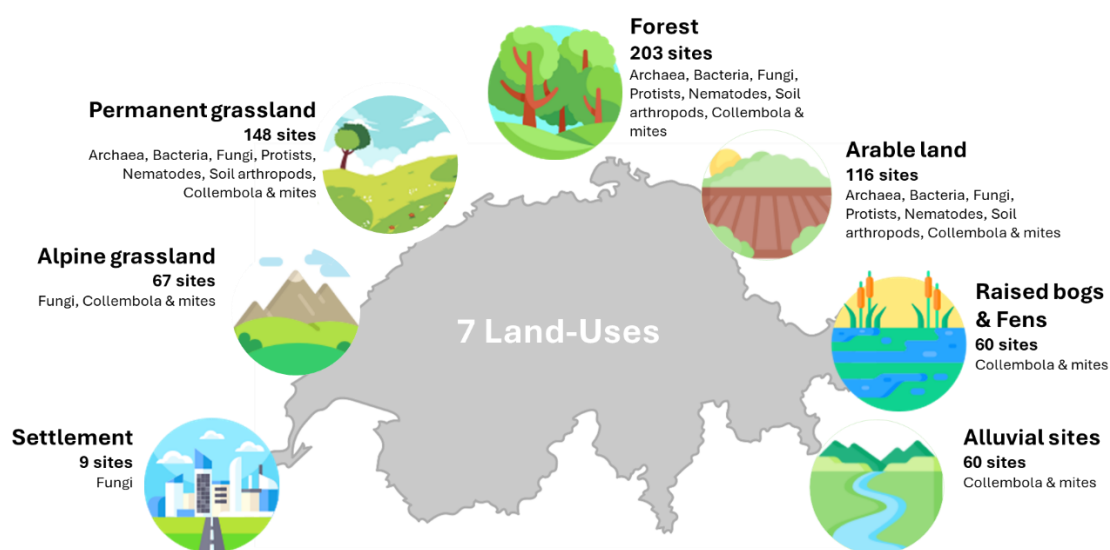


Figure 5: Land-use types investigated in the BioDivSol project, and number of sites and organism groups studied for each land use. Not all groups of organisms have been analyzed at a single site. Settlements were in the end excluded from the results analysis due to the limited number of sites.

⁵ Permanent grasslands also include “Dry Meadows & Pastures” areas described in the WBS monitoring (Microarthropod inventory sub-project).

⁶ Settlements were in the end excluded from the results analysis due to the limited number of sites (n=9).

Table 2 : Land-uses evaluated in each of the 3 BioDivSol sub-projects.

Sub-project	Observatory network	Land-uses investigated
Microscopic biodiversity	NABObio	Arable land, Forest, Permanent grassland
	BDM	Alpine grassland, Arable land, Forest, Permanent grassland, Settlement
Microbial properties & OM	TV, AGRiVD-GE	Arable land
Microarthropod inventory	WBS, BDM, NFR, LWF	Alpine grassland, Alluvial sites, Arable land, Dry meadows & pastures, Forest, Raised bogs and fens

2.1.3.3. Organism-to-site ratio

The BioDivSol project investigated soil organism diversity across all 663 study sites. Fungal diversity was assessed at more than half of the sites (52%), while Collembola and mite diversity was analyzed at 48%. Bacteria and archaea were studied at 13% of the sites, with nematodes, protists, and soil arthropods assessed at 4%. The diversity of up to six groups of organisms was evaluated at a same site (e.g. NABObio sites; see Chapter 2.1.3.2 for details) (Figure 6).

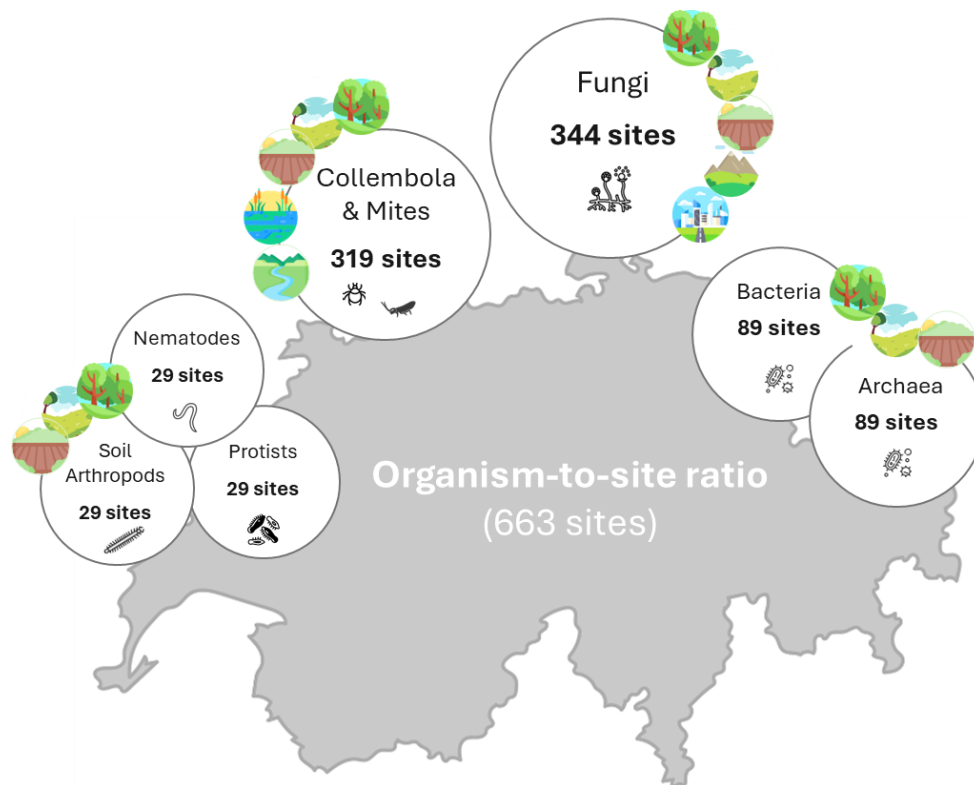


Figure 6: Organism groups studied and number of sites and land-use types for which they were assessed. Up to 6 groups of organisms were analyzed at the same site.

2.1.3.4. Environmental factors considered for biodiversity assessment

In addition to the different land-use types, various environmental factors (soil properties, climatic conditions, and agricultural practices) were considered to investigate and/or explain the diversity of soil organisms. Table 3 summarizes the different environmental factors taken into account in the 3 sub-projects.

Table 3: Environmental factors included in the analysis of soil organism diversity

Sub-project	Observatory network	Indicators considered	Used for
Microscopic biodiversity	NABObio	pH, Ctot, Corg, Ntot, C/N ratio, Bulk density, Silt, Clay, Sand, Elevation, Mean annual temperature, Mean annual precipitation, Precipitation in 2019, Temperature in 2019	Relationship to organism diversity
	BDM	Corg, pH, Ntot, C/N ratio, Bulk density, Silt, Clay, Sand, Soil water content, Slope, Elevation, Exposition, DNA content, Landolt plant ecological indicators (moisture, variability of moisture, nutrients, pH), mean annual temperature, SD of inter-annual temperature, mean annual precipitation, SD of inter-annual precipitation, mean annual solar radiation, SD of inter-annual solar radiation	
Microbial properties & OM	TV, AGRiVD-GE	<u>Soil properties</u> : Clay, pH, Corg, Soil organic matter thermal pools, Soil structural quality (coreVESS) <u>Agricultural practices</u> : Temporary meadow, cover crop species, crop species in the rotation, fallow period, OM input, Nmin input, Soil Tillage Intensity Rating (STIR) indicator, Herbicide application	Site selection & Relationship to microbial properties
Microarthropod inventory	WBS, BDM, NFR, LWF	Elevation, slope, soil physico-chemical properties (pH, Corg, Ntot, silt and clay), Landolt plant ecological indicators (nutrients, moisture, soil reaction), plant communities (beta diversity), geographical distance between sites, bio-climatic factors	Site selection

For the "Microarthropod Inventory" sub-project, environmental factors were used prior to sampling to select appropriate sites in line with the project's objectives (inventory of microarthropods at the Swiss national scale). A clustering approach was applied to each monitoring network to group similar sites and select only those with significant differences, aiming to encompass the largest possible proportion of microarthropod diversity across a wide range of environments.

In the "Microscopic Biodiversity" sub-project, environmental factors were integrated into statistical models to analyze the relationships with the assessed organism diversity.

The "Microbial Properties & OM" sub-project followed a combined approach, where some environmental factors were used to select the study sites, and all chosen factors were subsequently utilized to examine the relationship with microbial properties.

2.2. Project outcomes

2.2.1. Diversity of soil organisms

The main results obtained regarding the diversity of the different groups of organisms studied in Swiss soils are presented in the sections below. The first sections include the observations made in the “Microscopic Biodiversity” sub-project on:

- The ASV⁷ richness (number of identified taxa) of the different groups of organisms present in the respective monitoring programs and the influence of land-use types and associated environmental factors.
- The community composition, in relation to land-use types and associated environmental factors, and classification success of these organism groups.

The results of the other two sub-projects are presented in the last sections of the chapter and explore with greater focus:

- The influence of soil properties and agricultural practices on microbial parameters in agricultural environments (sub-project “Microbial Properties & OM”).
- The diversity, in the form of an inventory, of the particularly important arthropod subgroups collembola & mites in Swiss soils (sub-project “Microarthropod Inventory”).

⁷ ASVs, or Amplicon Sequence Variants, are unique DNA sequences obtained through metabarcoding (High-throughput sequencing analysis). While they do not directly correspond to species, they serve as surrogates for species or taxa in biodiversity studies.

2.2.1.1. Archaea

Figure 7 illustrates the diversity of Archaea analyzed across 29 sites within the NABObio monitoring program, encompassing three types of land-uses: arable land, permanent grassland, and forest. The applied metabarcoding methods identified 40 taxa (ASVs) in the studied soils. These findings are particularly valuable for improving our knowledge of Archaeal diversity in Swiss soils, a group of organisms that remains largely understudied. To date, no comprehensive study on Archaeal diversity in Swiss soils has been conducted (Gschwend et al., 2022).

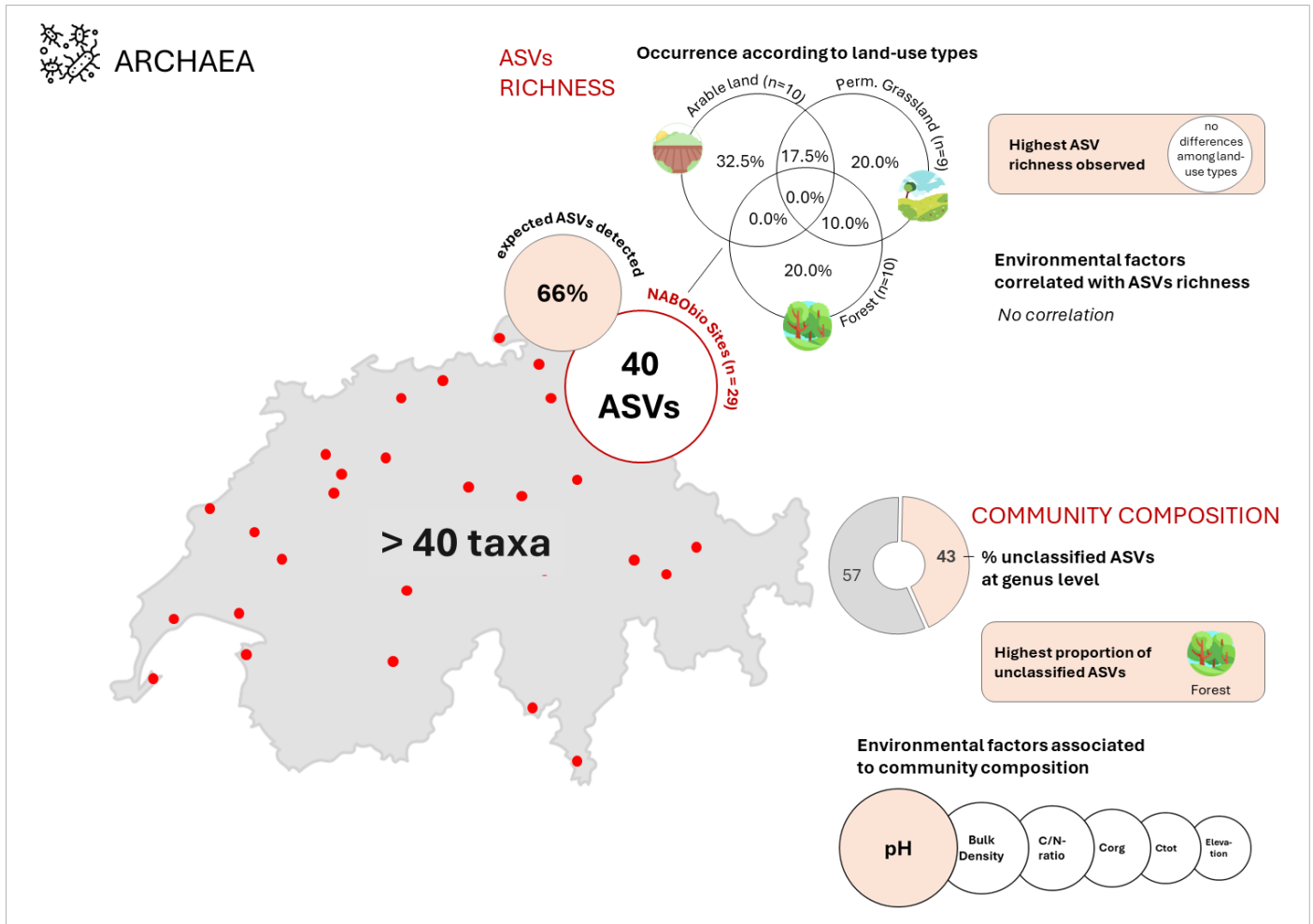


Figure 7: ASV richness, classification success and environmental factors (in order of importance) associated to community composition of Archaea across the 29 sites of the NABObio monitoring network. Venn Diagrams illustrate the percentage of archaeal ASVs found in each land-use type (arable land, permanent grassland and forest).

The data reveal that the majority of Archaeal taxa are specific to a single type of land-use, with 73% of taxa exclusively found in either land-use. Furthermore, the study shows that no Archaeal taxa are shared among the three studied land-use types (arable land, permanent grassland and

forest) and that Archaea ASV richness did not differ among these land-uses. Rarefaction analyses⁸ show that with 29 sampled sites, only 66% of Archaea diversity was detected. It would require more than doubling the number of sampling sites to encompass the full diversity of Archaea for the studied land-uses (88% of estimated ASVs is detected by doubling the number of sites; Gschwend et al., 2022). Molecular identification shows that 43% of Archaea ASVs are not yet classifiable at the genus⁹ level, the forest land-use revealing the highest proportion of unknown genera. Soil pH plays a predominant role among the environmental factors influencing the composition of Archaea communities.

Data available at this stage are limited, as they rely on universal prokaryote PCR primers. More specific molecular approaches are needed to better capture archaeal diversity and could be used to create a more comprehensive dataset across Switzerland, for example by utilizing the DNA extracts from the BDM. Until these additional analyses are performed, reliably estimating the total soil archaeal diversity in Switzerland is not feasible.

2.2.1.2. Bacteria

Despite studies such as those of the BDM (255 sites) which identified 48'568 Bacteria "taxa" (ASVs) in Swiss soils (Mayerhofer et al., 2021), a comprehensive estimate of general bacterial soil diversity across Switzerland is still lacking. The BioDivSol analysis of bacterial diversity across the 29 NABObio sites led to the identification of 15'660 taxa (ASVs) in the soils of arable land, permanent grassland, and forest land-uses providing valuable information for enhancing our understanding of bacterial diversity. The data reveal that the majority of bacterial taxa were detected in only a single land-use type, with just a small fraction (3.2%) shared across all three land-use types. Bacterial richness is highest in permanent grassland and arable land and seems sensitive to soil textural fractions (i.e. silt and sand). However, the 29 sites of the NABObio monitoring network are insufficient to cover the total estimated bacterial diversity for these land-uses (only 51% of the expected ASVs detected) and sampling additional sites would be required. For comparison, with the 255 sites of the BDM, total coverage (99.99%) is reached for bacteria, and 29 sites were sufficient to detect 87% of all detected bacterial ASVs for the five land-uses assessed (Gschwend et al., 2022; Mayerhofer et al., 2021). Sixty-eight percent of Bacteria ASVs remain unclassified at the genus level, with no land-use-related differences. Bacterial community composition was best explained by pH and C/N ratio (Figure 8).

A large volume of data has been generated from multiple studies conducted across Switzerland, resulting in relatively large datasets for the main land-use types. These datasets could be used to define and characterize soil bacterial diversity baselines in greater detail and relatively comprehensively. However, some habitats remain undersampled, for example settlement areas.

⁸ Rarefaction analyses allow for the estimation of species diversity (number of detected ASVs) based on sampling effort (number of sampling sites). The generated rarefaction curve initially shows a rapid increase in the number of species detected when sampling effort is low, but as the sampling effort increases, the curve tends to stabilize indicating that fewer new species are being discovered. This helps determine the minimum number of sites to sample in order to detect the majority of the taxa present.

⁹ The genus is the taxonomic rank directly above species.

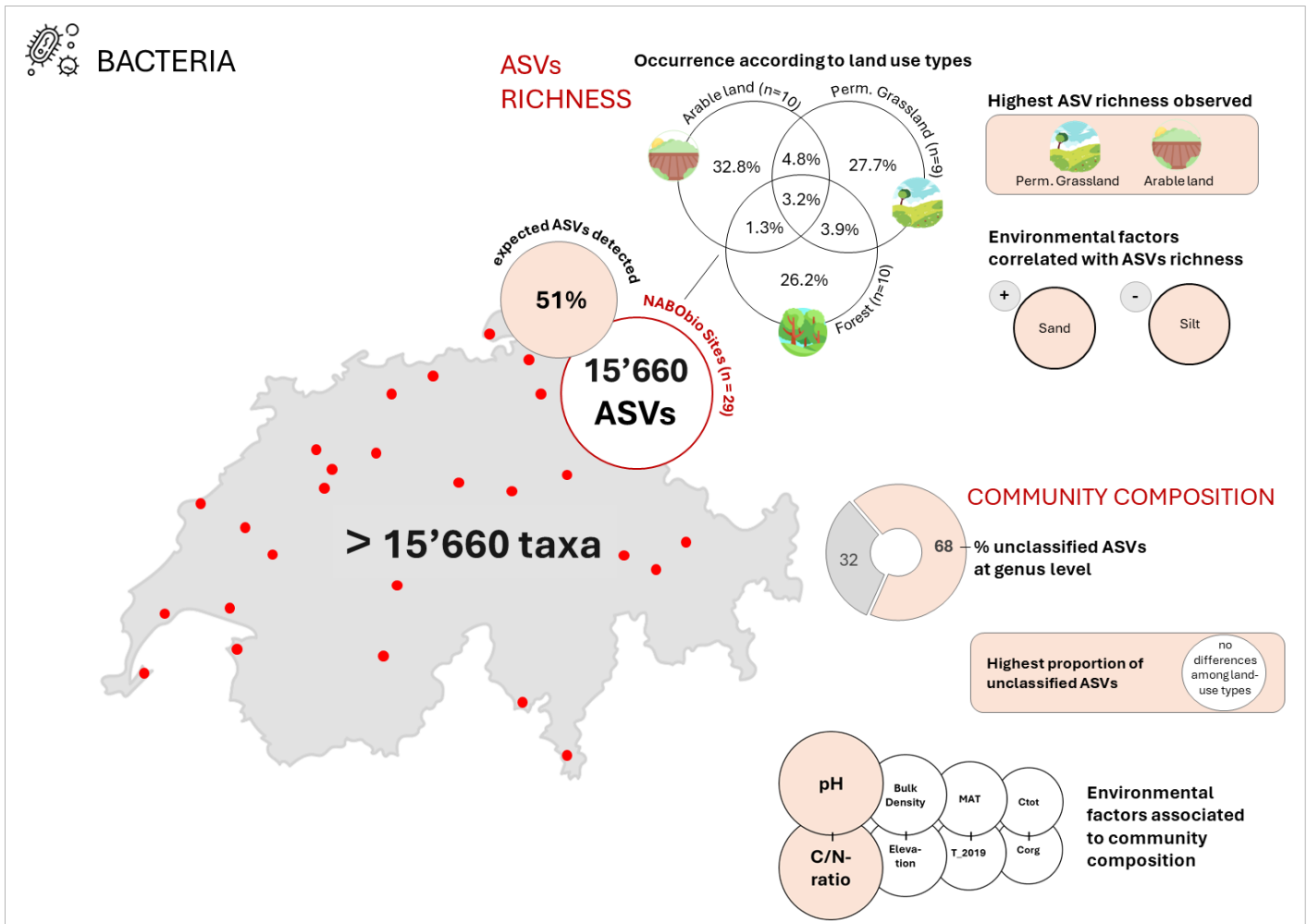


Figure 8: ASV richness, classification success and environmental factors (in order of importance) associated to community composition of Bacteria across the 29 sites of the NABObio monitoring network. Venn Diagrams illustrate the percentage of bacterial ASVs found in each land-use type (arable land, permanent grassland and forest). MAT= Mean annual temperature, T_2019 = Mean temperature in 2019.

2.2.1.3. Fungi

Figure 9 illustrates the results of fungal diversity obtained from the two monitoring networks investigated, with 8'159 taxa detected across the 29 NABObio sites and 28'085 taxa across the 255 BDM sites. Taken together, it can then be inferred that fungal diversity in Switzerland is estimated to exceed 30'000 taxa, whereas existing data indicate that approximately 15'000 fungal species (macrofungi producing fruiting bodies) have been documented in Switzerland (FOEN 2019, Gschwend et al., 2022, Bouraoui et al., 2022). As with other organism groups, both studies highlight the necessity of sampling a minimum number of sites to fully address the diversity of the soil organism present. Rarefaction analyses conducted for the two monitoring networks reveal that, with the 29 NABObio sites, only 50% of the expected fungal taxa were detected, while the 255 BDM sites captured nearly all (99%) of the estimated fungal diversity across all land-uses considered. However, when analyzed individually, some land-use types would require a larger sampling effort to ensure comprehensive coverage of their fungal diversity (see Bouraoui et al., 2022 for further details). Increasing the number of sampling sites also allows for more accurate

conclusions about land-use types and the environmental factors associated with fungal diversity. The BDM monitoring program, for instance, shows that the highest fungal taxa richness is found in arable land and permanent grassland, while alpine grassland and forest exhibit lower richness (settlements could not be included in the final analysis due to the low number of sites sampled). The 29 NABObio sites did not reveal a predominant land-use type in terms of fungal richness. Similarly, while NABObio identified only one environmental factor (i.e. mean temperature in 2019) associated with fungal richness, the BDM monitoring identified seven environmental factors¹⁰. Both studies show that a significant portion of fungal taxa remains unclassified at the genus level, with 48% and 66% of ASVs unidentifiable to this level in NABObio and BDM monitoring network, respectively. Finally, pH arises as an important environmental factor strongly influencing fungal community composition in both monitoring networks. The BDM system also reveals that plant communities and soil nutrient status are important drivers of soil fungal diversity.

As for soil bacterial diversity, it would be possible to create a more comprehensive overview on soil fungal diversity across Switzerland if datasets from existing studies were combined. Furthermore, for soil fungal diversity estimates, comparisons with morphological surveys (i.e. swissfungi) should be considered.

¹⁰The BDM monitoring considers a greater number of environmental factors than NABObio (see Table 3 for details), as well as a larger number of sites, which provides increased statistical power when comparing environmental factors with biodiversity patterns.

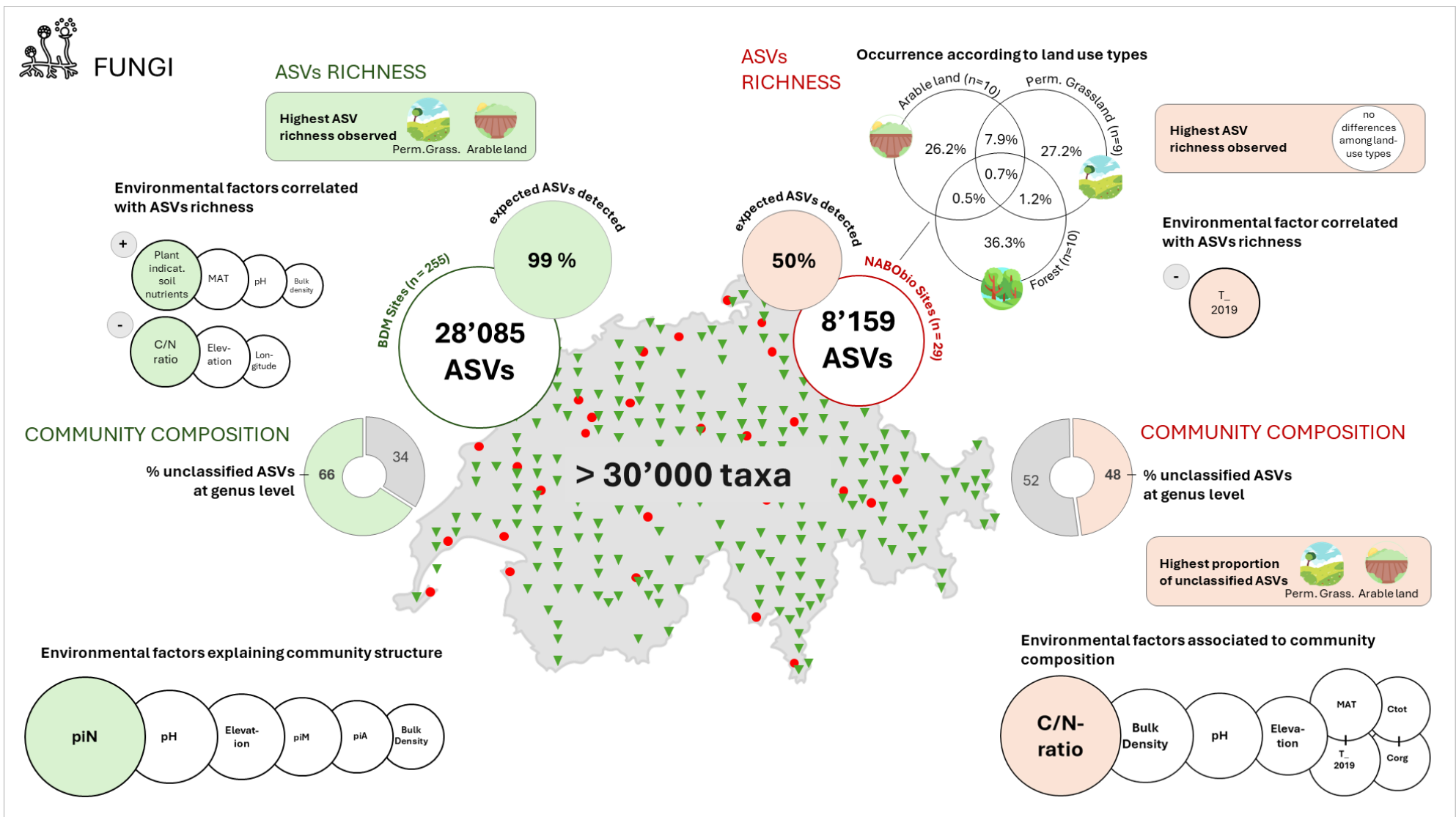


Figure 9: ASV richness, classification success and environmental factors (in order of importance) associated to community composition of Fungi across the 29 NABObio and 255 BDM sites. Venn Diagrams show the percentage of fungal ASVs occurring in each land-use type (arable land, permanent grassland and forest) for the NABObio sites. MAT= Mean annual temperature, piA=Plant indicator for soil pH, piM=Plant indicator for soil Moisture, piN= Plant indicator for soil nutrients, T₂₀₁₉ = Mean temperature in 2019.

2.2.1.4. Protists

Figure 10 summarizes the results obtained for protist diversity across the 29 NABObio sites, with 1'580 taxa (ASVs) detected in the studied soils. Recent studies highlight the remarkable diversity and functional roles of protists in soils. However, their diversity in Switzerland remains largely unexplored, with no existing estimates for Swiss soils (Gschwend et al., 2022). The collected data will provide essential information to expand our understanding of protist diversity.

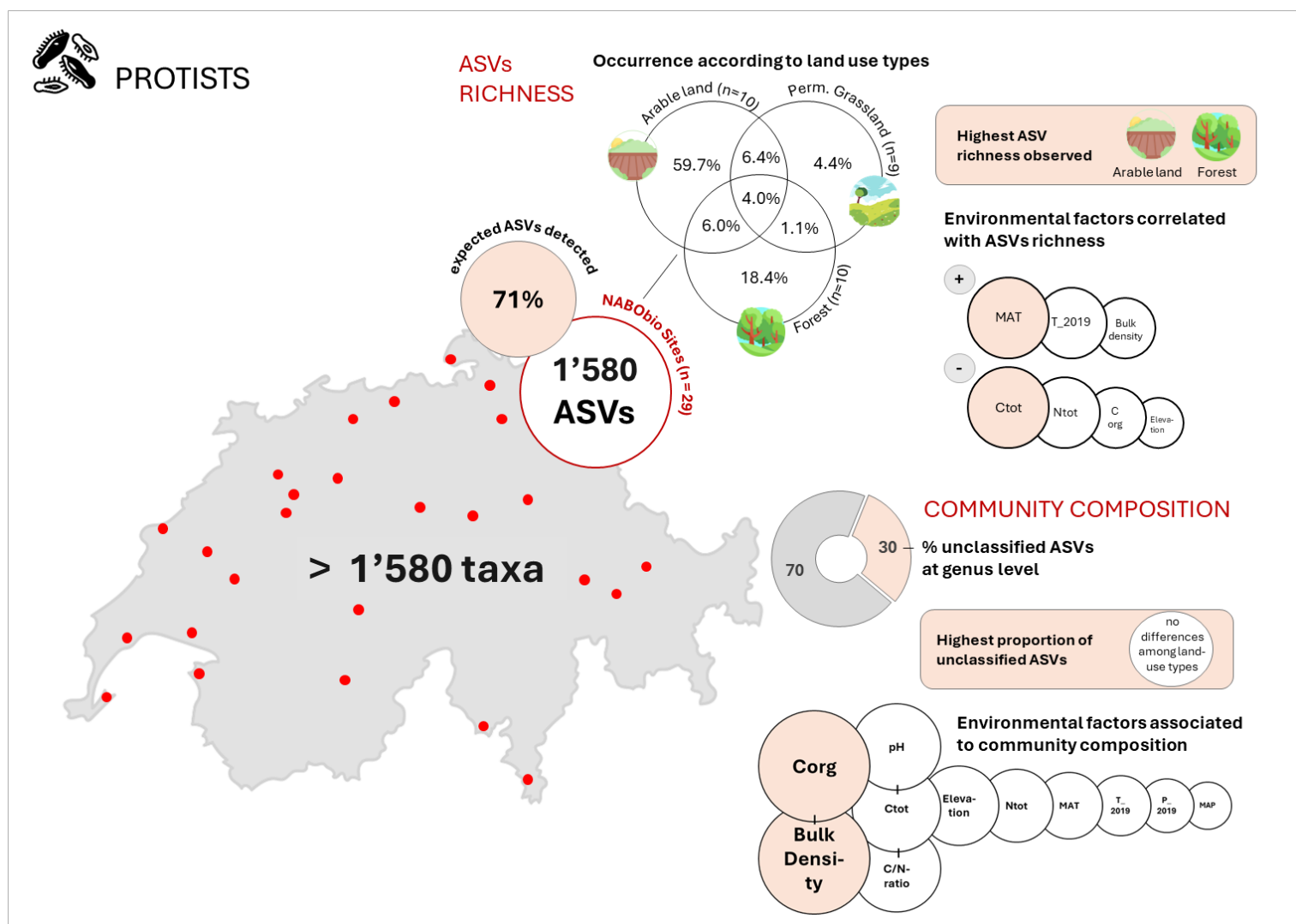


Figure 10: ASV richness, classification success and environmental factors (in order of importance) associated to community composition of Protists across the 29 sites of the NABObio monitoring network. Venn Diagrams illustrate the percentage of protist ASVs found in each land-use type (arable land, permanent grassland and forest). MAP = Mean annual precipitation, MAT= Mean annual temperature, P_2019 = Precipitation in 2019; T_2019 = Mean temperature in 2019

Arable soils had the highest proportion of protist ASVs specific to a single land-use (60%), and along with forest soils, exhibited the highest observed ASV richness. Within the NABObio monitoring program, protists are the organism group most strongly linked to environmental factors, with ASV richness correlated with seven out of 14 factors (see Table 3 for details), and most strongly (negatively) correlated with soil organic carbon. Among the studied organism groups, protists, together with nematodes, exhibit the highest diversity coverage (71%) relative to the number of sites sampled, with only one-third of taxa remaining unclassified at the genus level. Among the numerous environmental factors identified, soil organic carbon (Corg) and bulk density play a key role in shaping community composition.

Detecting the full diversity of soil protists remains challenging, and current data only provide a limited general overview. Larger surveys, such as those using BDM samples, would be required to confirm the results and to better estimate protist diversity in Swiss soils. Group-specific surveys are also needed to validate findings from universal metabarcoding.

2.2.1.5. Nematodes

For nematodes, metabarcoding techniques allowed the detection of 392 taxa in the soils of permanent grassland, arable land, and forest within the NABObio program. In comparison, it is estimated that 2'500 nematode species exist in Switzerland (Gschwend et al., 2022; FOEN, 2019). It is the group of organisms that shares the highest proportion of taxa (6%) across the three studied land-uses and has the lowest percentage of unclassified taxa at the genus level (24%). Nematode community composition was significantly correlated with nine environmental factors and was best explained by the carbon-to-nitrogen ratio (Figure 11).

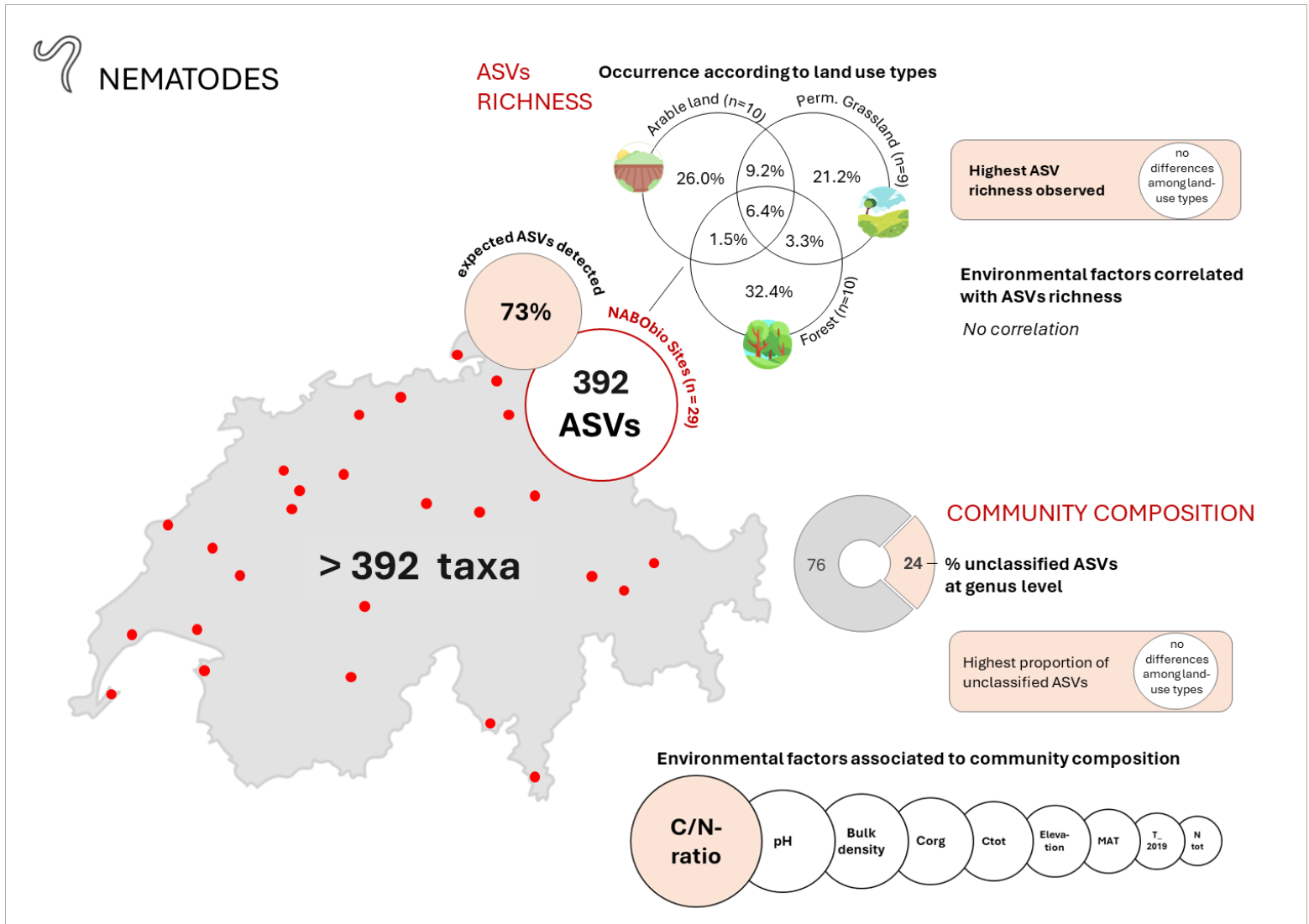


Figure 11: ASV richness, classification success and environmental factors (in order of importance) associated to community composition of Nematodes across the 29 sites of the NABObio monitoring network. Venn Diagrams illustrate the percentage of nematodes ASVs found in each land-use type (arable land, permanent grassland and forest). MAT= Mean annual temperature, T₂₀₁₉ = Mean temperature in 2019.

Compared to soil microbial organisms, assessing soil nematode diversity requires adjustments. For example, larger soil volumes are needed. Metabarcoding of soil nematodes offers strong potential for evaluating soil quality and health, particularly through the use of existing ecological indices.

2.2.1.6. Soil arthropods

Slightly more than 9'000 arthropod taxa were detected in the soils of the 29 NABObio sites, which, according to rarefaction analyses, represent only 19% of the expected taxa. Doubling the number of sampling sites would result in only a 10% increase (Gschwend et al., 2022) in the additional detected ASVs, remaining insufficient to fully capture the expected richness at these sampling locations. Like Archaea, soil arthropods share virtually no common ASVs (only 0.1%) across all three studied land-use types. Forest land-use appears to be particularly noteworthy for soil arthropods, as forest sites exhibit both the highest number of ASVs unique to a single land-use and the greatest ASV richness among soil arthropods. Half of the detected taxa remain not classified at the genus level, with forest land-use showing the greatest proportion of unclassified ASVs. The carbon to nitrogen ratio is positively correlated with taxa richness and influences as well the community composition of soil arthropods (Figure 12).

Metabarcoding methods are appropriate and can be applied to larger-scale surveys. However, a more extensive survey would be needed to comprehensively assess soil arthropod diversity across Switzerland. Such an effort should be combined with DNA barcoding initiatives (e.g., mite and collembola inventories), as many undescribed species are expected.

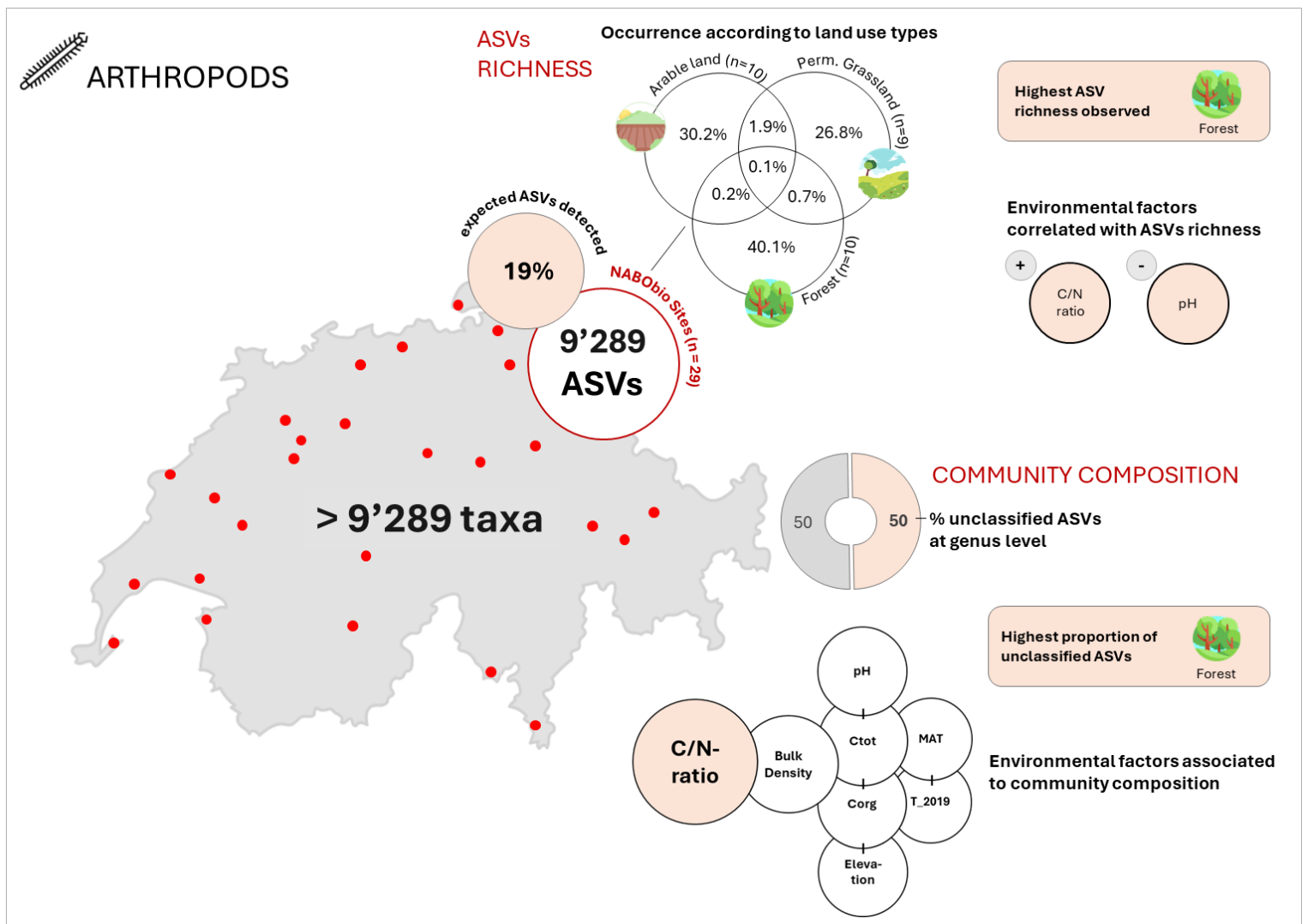


Figure 12: ASV richness, classification success and environmental factors (in order of importance) associated to community composition of soil arthropods (insects, arachnids, Acari (mites), Collembola, Protura and Symphyla) across the 29 sites of the NABio monitoring network. Venn Diagrams illustrate the percentage of soil arthropod ASVs found in each land-use type (arable land, permanent grassland and forest). MAT = Mean annual temperature, T_2019 = Mean temperature in 2019.

2.2.1.7. Microarthropods (collembola and mites)

The morphological analyses and the development of molecular methods (barcoding and metabarcoding) implemented in the sub-project "National Inventory of Collembola and Acari" have already enabled the inventory of a large number of Collembola and Acari species. Figure 13 shows the initial results obtained regarding collembola diversity across the different analyzed sites, while Figure 14 focuses on the observed diversity of Oribatids and Gamasids, which represent two major orders of soil mites. A morphological identification of the individuals extracted from the collected soil samples was carried out under the microscope (morphological diversity) and complemented by DNA barcoding (molecular diversity) for each new species not yet cataloged.

Collembola

Figure 13 illustrates the observed diversity of collembola extracted from soil samples collected from the first 45 sites analyzed out of the 319 sites included in the national microarthropod inventory. The 575 identified specimens of collembola belong to 170 different species. Ongoing DNA sequencing has already generated DNA barcodes for 70 of them. Among the identified Collembola species, 105 had not yet been listed in the Swiss Global Biodiversity Information Facility (Swiss GBIF) database for Swiss microarthropods, which currently lists 68 collembola species. The obtained data will therefore contribute to supplement this database as well as the "regional GBIF" (Switzerland and its neighboring countries). Since 50% of the identified species were recorded at only a single site, these preliminary results further support the generally reported high spatial heterogeneity in Collembola distribution. Accumulation curves indicate that with slightly less than 15% of the sites analyzed, approximately 63% of the expected soil collembola diversity has already been covered. The 319 sites sampled for the national microarthropod inventory appear to be sufficient to represent the diversity of collembola in Swiss soils, taking into account the different habitats and environmental factors considered. Among the land-use types studied (forest, arable land, raised bogs and fens, alluvial sites, alpine grasslands, dry meadows & pastures), forest is the land-use that holds the greatest number of collembola species.

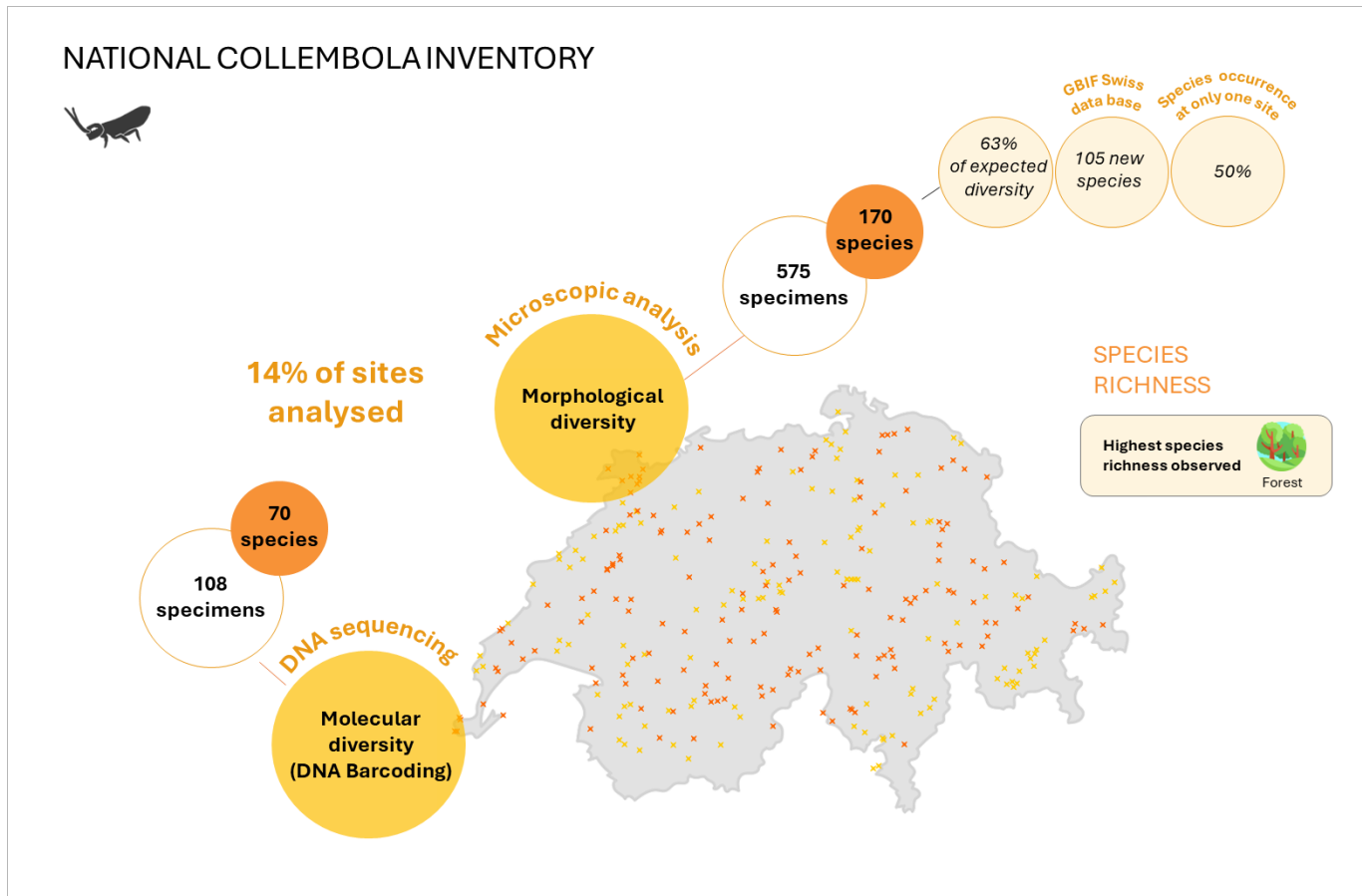


Figure 13 : Diversity of collembola across 45 out of the 319 sites from the “National Inventory of Collembola and Acari”, characterized using morphological and molecular approaches. Of the 916 individuals collected from these soil samples, 575 specimens were morphologically identified under the microscope and classified to the species level, resulting in 170 species. A DNA barcode is planned for each new species not yet cataloged. Out of the 108 collembola samples sent for sequencing, a DNA barcode was successfully obtained for 70 species. (GBIF = Global Biodiversity Information Facility).

Mites

The results of the mite inventory (oribatids and gamasids) from the analysis of around thirty sites from the project are presented in Figure 14. The observed species richness for the 617 identified oribatid specimens and 295 identified gamasid specimens reaches 221 and 113 species, respectively. A DNA barcode has already been generated for 31 oribatid species and 41 gamasid species. Among the mite species found, 75% of oribatid species and 88% of gamasid species were not yet listed in the Swiss GBIF database. The proportion of mite species recorded at only a single site (51% for oribatids and 60% for gamasids) is relatively high and similar to that observed for collembola, further supporting the potential strong spatial heterogeneity in species distribution and/or the high ecological specialization of microarthropods. The analysis of only 10% of the sites already covers approximately

61% of the expected diversity for oribatids and 54% for gamasids, according to the calculated accumulation curves. As with collembola, forest land-use supports the highest number of mite species, with oribatids showing a significantly higher species richness than gamasids. Similarly, oribatids are significantly richer in species than gamasids in dry meadows and pastures.

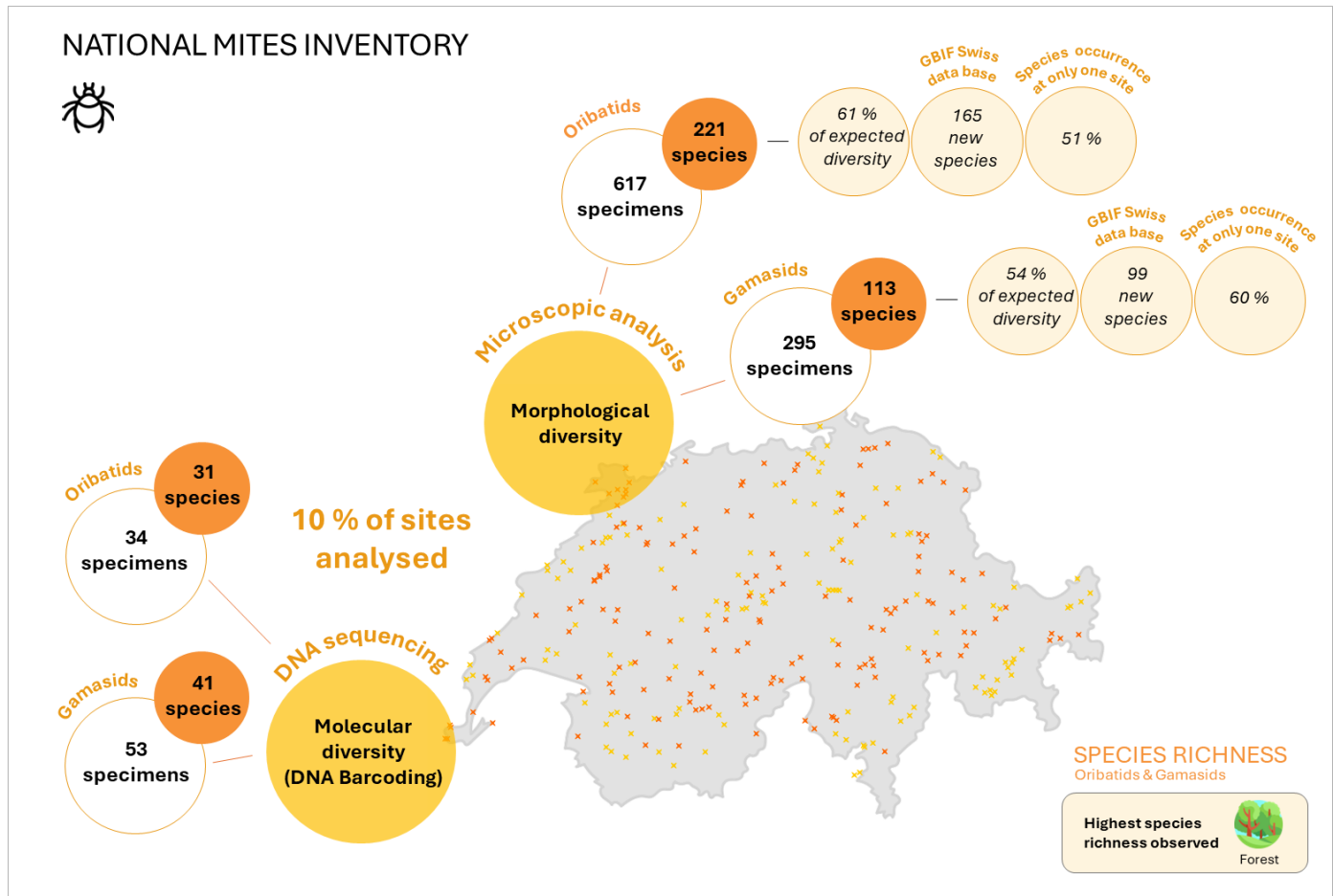


Figure 14 : Diversity of oribatid and gamasid mites across around 30 sites out of the 319 sites from the “National Inventory of Collembola and Acari”, characterized using morphological and molecular approaches. Among the 671 extracted oribatid specimens, 617 were morphologically identified and classified into 221 species, with DNA barcodes obtained for 31 of them. For gamasids, 295 out of the 336 extracted specimens were identified, resulting in 113 species. A DNA barcode was obtained for 41 of them. (GBIF = Global Biodiversity Information Facility).

The first results obtained also show that the species richness of gamasids and oribatids is strongly correlated with each other and, to a lesser extent, slightly correlated with that of collembola. In contrast, no significant correlation was observed with the species richness of vascular plants, suggesting that these groups of organisms have a different bioindication potential than what is generally estimated for vascular plants. Mites appear to form more or less distinct species communities depending on habitat

types (land-uses), whereas this trend is less pronounced for collembola. Beyond GBIF, the data obtained will complement other existing collections and databases on the diversity of Swiss Collembola and Acari, such as museum collections or Info Fauna for information on identified specimens, as well as BOLD (Barcode of Life Data System) and GenBank for barcoding data.

2.2.1.8. Influence of soil properties and agricultural practices on microbial parameters

This part of the BioDivSol project (sub-project “Microbial Properties & OM”) aimed to characterize microbial habitat quality and microorganism diversity (Archaea, Bacteria, and Fungi) in agricultural systems using a multidisciplinary approach. The results presented below focus more closely on the relationships between microbial diversity and activity, and soil properties as well as agricultural practices, across different spatial and temporal scales.

Microbial responses to soil properties

Figure 15 presents the key findings on the relationships between microbial biomass and activity, enzymatic activity¹¹, microbial diversity (richness and community composition), and soil structure and organic matter properties (soil microstructure, soil structure quality and vulnerability, and soil organic matter pools). Microbial life (biomass and activity) is sensitive to soil microstructure and positively associated with fine structural pores (diameters of 10 to 60 µm). Soils in poor structural condition (CoreVESS¹² > 3) exhibit a significant reduction in microbial biomass and activity. On the other hand, soils with a good Corg-to-Clay ratio (soil structural vulnerability) support higher microbial life. These indicators of soil structure quality and vulnerability are directly linked to soil organic carbon content (Corg). Enzymatic activity shows no relationship with soil microstructure, soil structure quality or vulnerability. The organism groups studied in the context of agricultural system show different sensitivities to soil properties. More specifically, fungal species richness is primarily and positively influenced by soil carbon content (Corg) and pH, whereas the species richness of prokaryotes (Archaea and Bacteria) is primarily and negatively affected by clay content and fine porosity, soils with a well-developed microstructure resulting in a lower prokaryote species richness. The composition of prokaryotic communities is influenced by the Corg-to-Clay ratio (soil structural vulnerability), whereas fungal community composition is not affected. Microbial life is not especially affected by the organic matter pools¹³.

¹¹ Four extracellular hydrolytic enzymes were studied: beta-glucosaminidase (cellulose hydrolysis), beta-xylosidase (hemicellulose hydrolysis), N-acetylglucosaminidase (chitin hydrolysis), and phosphatases (phosphorus solubilization)

¹² CoreVESS (Visual Evaluation of Soil Structure on Soil Cores) is a method to evaluate soil structure quality based on visual evaluation. The quality of soil structure is assessed on a scale from 1 to 5 (1 being the best score and 5 the worst) using criteria mainly linked to structural porosity observable to the naked eye (Johannes & Boivin, 2019).

¹³ The biogeochemical stability of soil organic matter (SOM) can be characterized using thermal analysis techniques such as Rock-Eval®, which allow for quantifying SOM content and differentiating between labile, resistant (intermediate), and refractory thermal pools.

SOIL AS A HABITAT - MICROBIAL RESPONSES TO SOIL PROPERTIES

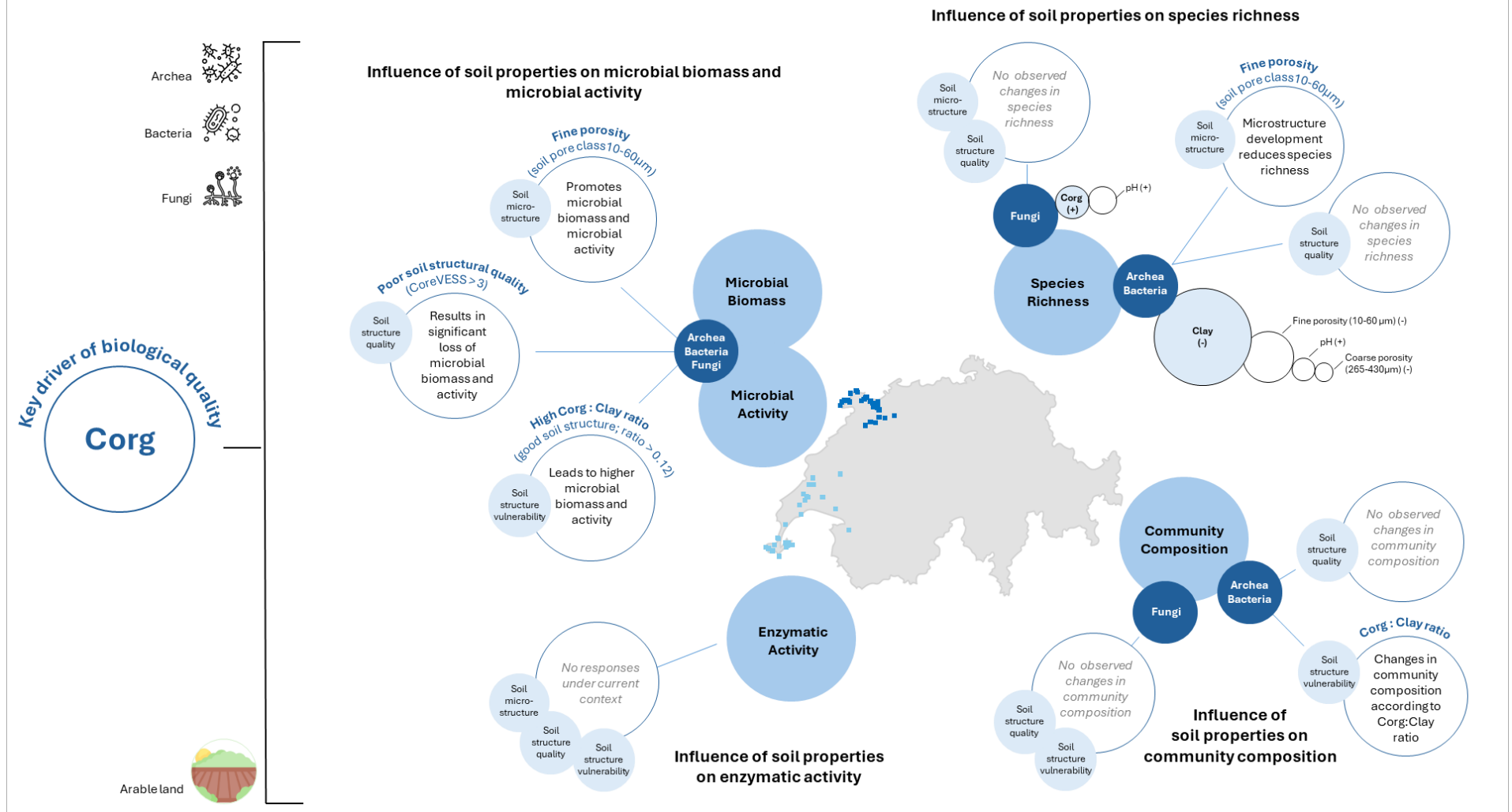


Figure 15: Responses of Archaea, Bacteria, and Fungi to soil properties in an agricultural context. Microbial parameters – including microbial biomass and activity, enzymatic activity, species richness, and community composition – were analyzed across the 60 agricultural sites from the sub-project “Microbial Properties & Organic Matter”. This figure illustrates the characterization of these parameters in relation to soil microstructure (porosity), soil structure quality and vulnerability. Soil properties influencing species richness are shown in order of importance (the size of the circles is scaled proportionally to their relative contribution to the observed variance). The relationship between community composition and soil microstructure, as well as between species richness and soil structure vulnerability, are not shown here.

Microbial responses to agricultural practices

The combined effect of soil properties and short-term (6 months) or long-term (10 years)¹⁴ agricultural practices on microbial life is presented in Figure 16. The results show that in the short term, agricultural practices (crop rotation diversity, soil tillage intensity, pesticide use; see Table 3 for more details) have little impact on microbial life. Only the application of organic amendments appears to influence microbial activity and biomass, with a shift in dynamics observed between seasons. Microbial life also does not show seasonal variations, except for enzymatic activity, which decreases in autumn. The impact of agricultural practices on microbial responses (activity and biomass, species richness, and community composition) is more pronounced over the long term, on timescales of about a decade, in connection with changes in the properties of the microbial habitat, namely the modification in soil parameters like Corg, pH, or texture (clay content). These results further highlight the importance of organic carbon for microbial parameters, a factor that evolves very slowly over multi-annual to decadal timescales rather than at a seasonal scale.

¹⁴ The agricultural operations (technical itineraries) were recorded over the 10 years preceding the sample collection for long-term effect observations, and over a period of 6 months for the three sampling seasons considered, namely spring 2021, autumn 2021, and spring 2022, for short-term effect observations.

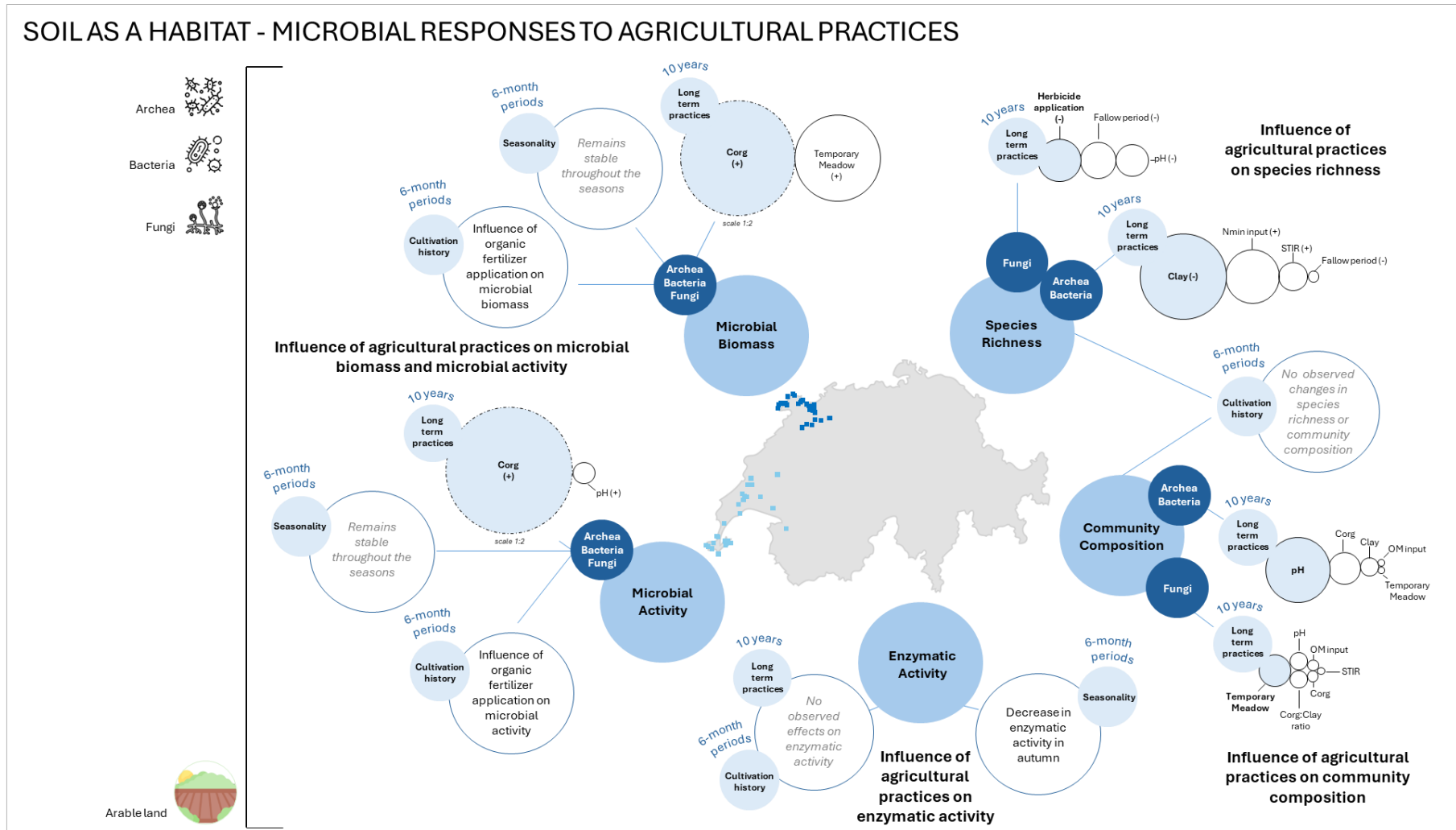


Figure 16 : Responses of Archaea, Bacteria, and Fungi to agricultural practices in interaction with soil properties. Microbial parameters – including microbial biomass and activity, enzymatic activity, species richness, and community composition – were analyzed across the 60 agricultural sites from the sub-project “Microbial Properties & Organic Matter”. This figure illustrates the characterization of these parameters in relation to long-term (10 years) and short-term (6-month cultivation history) agricultural practices, and seasonality. Soil properties associated to long-term practices influencing the specified microbial parameters are shown in order of importance (the size of the circles is scaled proportionally to their relative contribution to the observed variance). The relationship between community composition and seasonality is not shown here.

3. Contributions of the BioDivSol project to current needs for the Swiss biodiversity strategy

The BioDivSol project shows that an impressive diversity of organisms is present in Swiss soils, much of which remains previously unexplored (Edaphobase; FOEN, 2022). With over 35'000 taxa detected, it is the first study of this magnitude, focusing extensively on soil organisms.

BioDivSol main strengths are:

1. Nationwide coverage: assessment of soil organism diversity at larger scale, exhaustive of all biogeographical regions of Switzerland
2. More than 660 sampled sites: increased representativeness and enhanced robustness of the results
3. 6 land-uses investigated: allows an understanding of the diversity of organisms associated with the specificity of soil use and management
4. 6 organism groups assessed: key actors of soil multifunctionality mainly represented

Beyond evaluating the applicability of molecular approaches to assess the diversity of soil organisms (see below), the BioDivSol project provides a remarkable amount of data on species richness (α -diversity) and on the composition of organism communities in Swiss soils in relation to different land-use types and environmental factors.

BioDivSol contributes to provide:

5. Key information on species and/or taxa not yet recorded or still unknown in Switzerland:
The BioDivSol findings indicate that 24% to 68% of the detected taxa by organism groups remain undescribed, with the lowest proportion in nematodes and the highest in bacteria.
6. Habitat indicators: Different factors act differently across organism groups. Land-use types with group-specific biodiversity values have been identified, such as a higher ASV richness in soil arthropods but lower bacterial diversity in forests, or the presence of specific ASVs at only one land-use. These findings may create further opportunities for the development of bioindicators for soil habitats.
7. Deeper understanding of the link between soil organism biodiversity and environmental factors: Among the environmental factors considered in the three sub-projects on soil biodiversity, aside from pH, soil carbon (Corg, C/N ratio, Corg-to-Clay ratio, Ctot) emerges as one of the main drivers of diversity for the studied organism groups. Reference values based on soil carbon, for example through the indicators of soil structural quality (CoreVESS) and soil structural vulnerability (Corg:Clay ratio), could be developed for monitoring the state of organism diversity and soil biological quality. Considering soil microorganisms, short-term agricultural practices have minimal effects, whereas long-term practices influence microbial communities as a result of changes in soil properties such as organic carbon, pH, and texture.
8. Information for the development and/or enrichment of databases: referencing of species, reference database for DNA sequences, occurrence and distribution maps, specimen collection, etc.

The metabarcoding methods applied in the BioDivSol project demonstrate that this approach is suitable for large-scale monitoring to assess the diversity of various soil organism groups, although some adaptations are still required, notably for the assessment of archaea, protists, and nematodes. These methods are particularly advantageous for microscopic organisms or others whose morphological identification remains especially difficult, enabling an inventory of taxa that have been little or not studied until now. In this sense, they offer a significant gain in both information and time, as morphological identifications are often time-consuming. Furthermore, when the analysis can be performed directly on an environmental sample (eDNA), metabarcoding provides a more comprehensive view of diversity. Non-mobile or dormant species, as well as resting species (organisms physically absent but still detectable through their biological traces), can also be detected.

4. Conclusions

Data on taxonomic richness and community composition for six of the main soil organism groups were collected and correlated with land-use types and environmental factors, covering an extensive number of sites at the national scale. These analyses provide insights into the influence of these variables on soil biodiversity and the biological quality of soils. For microorganisms (Archaea, Bacteria, and Fungi), additional focus was placed on identifying key pedological and agronomic factors shaping microbial diversity in agricultural context, highlighting their impact on soil biological quality. Similarly, special emphasis was given to carry out an inventory and build DNA reference datasets for microarthropods. Part of the molecular and morphological analyses is still ongoing, so some uncertainties remain regarding the exact numbers presented in this report. Nevertheless, the main conclusions are unchanged.

The use of metabarcoding as a universal DNA sequencing approach across the three BioDivSol subprojects demonstrated its potential to cover the diversity of multiple organism groups at the same site or even within a single soil sample (i.e. microorganisms). However, for metabarcoding to enable effective large-scale biodiversity monitoring, standardization of methodologies is required to ensure comparability between studies. For these approaches to be applied in routine biodiversity assessments, the specificity of certain PCR primers for some organism groups would also need to be improved. An exhaustive assessment of soil biodiversity across Switzerland appears feasible with a reasonable number of sites (approximately 300) to represent the diversity of all organism groups. Nonetheless, gaps remain in reference databases, particularly for taxa not yet identified at the genus level. These gaps highlight the need for further efforts to enrich existing databases and describe new species. To achieve a fully comprehensive overview of Swiss soil biodiversity, it would also be highly informative to combine genetic and morphological surveys for the groups where this is technically feasible, such as fungi, arthropods (e.g., mites and collembola), and potentially nematodes.

In conclusion, the BioDivSol project successfully demonstrates the feasibility of using metabarcoding for large-scale soil biodiversity evaluations. While adjustments are still needed, the universal nature of this approach offers a powerful tool for advancing our understanding of soil ecosystems and supporting routine assessments of soil health and biodiversity.

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