

Supplementary Material

S1. Description of the most representative organisms of soil biota, considered as bioindicators, with a special focus on the context of the Ecuadorian Amazon described in Chapter 3.2.

Earthworms are perhaps the most important soil organisms, decisively influencing the decomposition of the organic material (OM), structure development, and nutrient cycles. With approximately 8,000 species in the soil, the most prominent and important genera are *Diplocardia* and *Lumbricus* [1]. The vast majority live a few centimeters from the surface without emerging, except for the *Lumbricus terrestris* species that emerges during the night to feed [2]. According to their type of food and habitat, they are classified as epigeas, which feed on plant residues, inhabit the surface or the litter, and facilitate the mineralization of surface residues; Anecics, which feed on plant and soil residues, live in tunnels facilitating the flow of water and nutrients to the deep layers, and incorporate the waste into the profile; and endogetic, which form extensive tunnel systems and feed on soil and fragmented OM that mix in the mineral soil surface [3][2].

Earthworms play a crucial role in the fragmentation, decomposition, and mixing of leaf litter with the earth, building galleries covered by the mucus they secrete that facilitates movement and respiration in the earth. Their intestines contain enzymes that facilitate the degradation of cellulose and polymers of chitin, so that the mineral matter that passes through the worms, and is deposited on the surface (rich in inorganic N), provides a favorable environment for microorganisms in relation to the surrounding soil [2]. This increases the mineralization of organic carbon (OC), improves ventilation, porosity, and water infiltration [3], and avoids the loss of surface soil from runoff [4][5]. In general, the higher the OC content in the soil, the greater the number of worms [2], but in the tropics, they are not as numerous [1] for the food and chemical conditions of the soil. The factors that reduce the abundance of worms are: the use of fungicidal and herbicidal chemicals that affect populations, liming (carbonate type pesticide), inorganic substances, acid rain (depresses populations), and some heavy metals (sublethal doses decrease growth and reproduction, affecting the trophic network) [6]. Species richness, density of individuals (ind/m²), and biomass (g / m²) of earthworms is proposed as an indicator to assess soil status [4][1][5].

Beetles, worldwide are the most widely used beetles (Scarabaeidae) in conservation and biodiversity studies, due to their wide distribution and habitability in almost all types of natural biomes [7]—they feed on decomposing OM, they can be found in the soil, leaf litter, bark and inside plants, under stones and trunks, in corpses, and mostly take advantage of a source of nutrients that increases in the presence of large herbivores (feces) [8]. Dung beetles dig and introduce decomposing organic compounds into the soil; in grasslands they bury up to 78% of cattle feces, which is equivalent to adding 175 kg ha⁻¹ of nitrogen [9][8]. They contribute to nutrient cycling, natural fertilization (inorganic N), soil aeration, water filtration, and make it easier for plants to absorb elements, such as nitrogen and phosphorus [10]. At the same time, they are very sensitive to environmental variations and the deterioration of ecosystems, and are considered as excellent bioindicators to evaluate anthropic intervention according to abundance, diversity, and rapid response to changes in the environment [3][4].

Ants are the most widely spread Hymenoptera (Formicidae) on the entire planet. Some species build their habitats in the form of mounds (nests), mainly in areas susceptible to periodic flooding, and others inhabit caves, old logs, or tree bark. Depending on the longevity, size, and type of colony, wastes on or around the mounds are rich in nutrients such as N, P, K, Ca, Mg, Mn, and Fe, favoring

the proliferation of microflora and micro-mesofauna, such as colonies of *Pogonomyrmex occidentalis* enriched with vesicular-arbuscular mycorrhizal fungi [11]. Ants affect the structure of the soil, mixing the horizons of the profile and recycling part of elements that are leached from the Surface [5]. They modify the physical (porosity, moisture retention) and chemical (pH, nutrient availability) properties of the soil. The importance of ants in the transport of materials varies with density and diversity per unit area [2][11].

Termites, in the tropics, are considered important organisms for soil biology. For some species, the main source of food is wood pulp and leaf litter, which is transported to their nests, and through protozoa in their entrails they are capable of producing enzymes to break down the cellulose. The rest of the species live in the humus and litter in decay, feeding on a fungus that grows in the macerated cellulose that they prepare themselves [4][2][12]. They have an active participation in the removal and decomposition of OM, and in the formation of soil aggregates by the excretion of solid and liquid waste [11]. Changes in temperature and humidity conditions are not a limiting factor for termites, which is why they are easily related to the implementation of crops, which allows the development of a Worm/Termite density index, in which: worm dominance means a conserved habitat and the prevalence of termites means a less conserved habitat. Some authors consider them opportunistic and resistant to induced disturbances [4].

Snails and slugs, Terrestrial gastropods, are not uniformly distributed, with populations ranging from 10 to 25 ind/m². Due to the great abundance and diversity of species registered in tropical ecosystems, it is estimated that the continuous incorporation of leaf litter and high soil moisture is the perfect habitat for these organisms, as they have a varied diet (omnivorous organisms) [2]. Moisture influences your vital functions (gas exchange, reproduction, feeding) [4][3]. They are part of processes of mineralization of OM, and the decomposition and fragmentation of leaf litter; when they mobilize they secrete mucus that provides a habitat for fungi and microorganisms to colonize [6]. This mucus helps aggregate formation, improving soil structure and properties [2]. Research reports that snails and slugs are very sensitive to sudden changes in humidity and temperature [4][3] and proposes the use of them as indicators to assess the state of the soil.

Centipede and millipede, humid forests are the habitat for the largest population of Miriápodos, living among the leaf litter or under the bark of trees and rocks. They play an important role as predators, some feed on decomposing plant matter, and others participate in litter fragmentation, accelerating the process of decomposition of the OM [2]. Changes in humidity and temperature influence their vital functions and abundance, hence, it is considered that they can indicate the state of soil disturbance [4][3].

Enquitraeidos worms are oligoquetos annelids, and in humid soils, there can be up to 100,000 / m². Their diet consists of microorganisms and certain dissolved OM, because they do not have enzymes to digest it. They participate in the maceration of leaf litter and plant remains, facilitating transport for those who are excavators [2]. They are organisms sensitive to drought.

The collembola are the most abundant and best distributed arthropods in all continents. Most species live in the soil, others among the leaf litter, in trees, and in the rainforest canopies [2]. Some species live on agricultural soils that are fertilized with OM. The food source of some species are the hyphae of fungi and decaying material, such as nematodes, feces, rotifers, and even other collembola [13]. Others feed directly on plant material, causing significant economic damage. Due to their consumption of pathogenic fungi, they are used in the control of fungal diseases.

They are considered a decisive element in the recycling of organic remains, capable of crushing and fractionating plant residues. They contribute to the soil structure. Their excretions participate in the formation of humus, facilitate the implantation of microflora, and facilitate the continuous release of nutrients [13][2]. The collembola population is closely related to moisture, are responsive to the presence of heavy metals, and decrease with the addition of inorganic compounds (cadmium and zinc) [14]. Species of collembola are used as indicators of ecological variations, due to environmental pollution, as their abundance allows the assessment of soil effects. Collembola and mites are bioindicators of soil stability and fertility [15]. They have densities that decrease due to the increase in compaction [13].

Mites are arthropods that have great diversity and abundance, from different taxonomic groups (Cryptostigmata, Uropodidae, Astigmata, Gamasidae and Prostigmata). Most live in the soils of temperate or tropical areas, where they are very abundant. Their function is to fragment dead leaves and dead wood, disperse the OM, microbial, and fungal spores (mycorrhizal spores) throughout the soil, and some species are predators of other microarthropods, nematodes, and mites [13][2]. Mites can be saprophagous when they feed on dead matter, microphages (fungi), and coprophages [13]. Those that stand out among them are:

- Oribatids (Cryptostigmata); facilitate the decomposition of the OM, fragment the remains of animal and plant origins, and are accessible to microbial action. They are very sensitive to anthropic disturbances and changes in the environment.

- Uropodinos (Uropodidae); considered saprophagous because of their morphological and bioecological characteristics. They are very demanding to the quality of habitat, are not abundant, and inhabit ecosystems with significant levels of OM, such as compost areas and decomposing logs. Together with the oribatids, they are considered as indicators of high productivity, and from their variations, the health of the environment can be estimated.

- Astigmados (Astigmata) belong to fungivores, live in unfavorable environmental conditions, and are considered to be indicators of disturbed soils.

- Gamasinos (Gamasidae) are predators, controlling the populations of nematodes and microarthropods. They have great sensitivity to disturbances and weather changes, which is why they are considered indicators of soil quality.

- Prostigmates (Prostigmata); predominantly occupy nutrient poor soils with low OM content and low humidity, and most are predators. They have sensitivity to water fluctuations in the substrate. Their reproductive potential is high and better in the absence of predators. Due to their adaptability to disturbances, they are considered good indicators of soil quality.

The abundance of mites per m² positions them as potential indicators of nature and ecosystem disturbances. They are affordable for collection and preservation, but some species are very sensitive to fungicides (decrease mycophagous mites) and dryness [13][14].

They are used as bioindicators of soil stability and fertility, together with the collembola, for which reason the specific composition, abundance, and density of the different groups have been evaluated [15]. Oribatids, gamasinos, and prostigmados have densities that decrease due to the increase in soil compaction [13]. Oribatids are biological indicators of moisture and the content of OM in forest ecosystems, forest plantations, and agroecosystems. They also indicate low values of heavy metals in non-intervened soils. Furthermore, they have low species density in soils under cultivation [14]. On the other hand, the implementation of agricultural practices generates changes in the composition of

the mite and collembola communities, considering them as bioindicators of herbicide treatments [15], as in the Brazilian Amazon [13].

Nematodes are small water-dependent invertebrates with wide diversity, abundant in terrestrial and aquatic habitats, mainly tropical [16]. Analysis of tropical soils in Ecuador position them as hotspots for the diversity of nematodes, revealing that they are important reservoirs of species not yet discovered [16]. Organic environments with a neutral pH are the ideal habitat for most of their populations. They concentrate on the roots, serving as food for the plants, or in search of food, and do not participate in the decomposition of the OM [2].

Nematodes reflect the availability of OM in different ecosystems, mainly in permanent grasslands, and are the food chain link between microorganisms and complex organisms [13]. According to some authors, nematodes can be: colonizers; resisting soil disturbances and chemical contaminants, and persistent; in which they are not very proliferative because of their sensitivity to contaminants and external disturbances [17]. They have a short life cycle, are parasites (threat to human health, colonize plant and animal tissue), and are free-living (prey on microinvertebrates and microorganisms) [2]. They are identified through their oral apparatus. Plant parasites have a needle-like stiletto, microphages have a fine and delicate stiletto that they enter into hyphae, bacteriophages lack stiletto but participate in the regulation of available nitrogen and phosphorus and influence the nodulation of Rhizobium bacteria, predators have a buccal cavity in the form of teeth or a stiletto, and omnivores (polyphagous) have a hollow stiletto [2][17].

They are important agents of the cycling of nutrients and regulators of fertility of the soil, and are responsible for the respiration of life in 10% of the soil. They provide agroecological services, acting as biological control agents for pests and insects, including entomophilic and entomopathogenic nematodes, qualifying them as powerful bioindicators of ecological conditions [17]. Through an appropriate analysis of the nematode community, the level of contaminant disturbance and changes in land use can be estimated [18], hence, they are considered as indicators for sensitivity.

The diploma, protura, and pauropoda are rare organisms, and little is known about their ecology; however, their morphology and trophic functions are considered as indicators of soil stability [13][2]. The Diplura are moisture-dependent soil microarthropods that live under stones, logs, and litter; some species inhabit caves, avoiding exposure to edaphic environment disturbances. The Protura are small insects that inhabit deep strata, have styliform mouthparts, feed on nutrients in aqueous solutions, have an ecological relationship with collembola, and whose presence may be related to mycorrhizae. The Pauropoda are tiny myriapods that feed on fungal microorganisms and hyphae, affirming their presence in the decomposition of OM, and are very sensitive to agricultural practices [13].

Microfauna; in this group are the most abundant invertebrates, with a high richness of species, mostly protozoa and archaea. They can be found in all types of crops as they are associated with the roots of plants as fodder. They are considered the most important predators of bacteria and fungi. They regulate microbial communities, and as pathogenic insects, represent an important biological control [6][2].

Fungi is one of the components of soil biomass, involved in processes of decomposition, mineralization, and cycling of nutrients. It is estimated that there may be about 1.5 million species worldwide and tropical forests are a source of new species [19][20]. They belong to the Fungi kingdom and are composed of small filaments called hyphae with a diameter of 3–8 μm that can reach large lengths to cover hectares of land, as well as being vegetative and fertile [2]. The branching

of hyphae contributes to the formation of stable soil aggregates and water–air relations [20]. Their source of food and energy is obtained by extracellular degradation and absorption of OM from the external environment.

The level of growth is fast since they present a set of enzymes that allow them to grow in various substrates and produce toxic inhibitor compounds to exclude competitors [6]. There are parasitic fungi or pathogens, mainly *Fusarium* and *Rhizoctonia*, that cause significant losses in agricultural crops every year [19][2]. However, the most common are saprophytes, which live on dead or decomposing OM. They produce extracellular enzymes capable of depolymerizing cellulose, hemicellulose, and lignin. There are pioneers, easily germinating, others that grow as a secondary organisms from the metabolic waste of colonizers, and tertiary or native growth [20]. The mycelium growth is well adapted to the heterogeneity of the soil, but they are very sensitive to external disturbances, such as plow that prevent the growth of hyphae, increases in the concentrations of N by fertilization, and exposure to UV-B radiation for deforestation [6].

Another important group is the mutualistic fungi that establish mutually beneficial relationships with another organism, either a cyanobacterium or algae (to form lichen), or with the root of a moss, fern, or upper plant (to form mycorrhizae) [20]. The fungi that are part of mycorrhizae are called mycorrhizal fungi, and these can be internal or endophytes, which constitute endomycorrhizae and external constituting ectomycorrhizae, also known as arbuscular mycorrhizal fungi (AMF) [21]. In the endomycorrhizas, the fungus grows inside the plant and serves as a shield against pathogens and insects. In the ectomycorrhizas, the fungus obtains some of its sugars from the plant, and this improves the intake of water and nutrients through the hyphae, mainly phosphorus; although also N, Zn, Cu, and S [21][22]. This occurs because the hyphae of AMF envelop the root hairs, increasing the root's absorption surface, favoring the health and growth of the plant, and strengthening its tolerance to heavy metals, root pathogens, drought, salinity, pH, and transplantation [21]. The presence of AMF, in addition to promoting the development of plants, increases soil aggregation and participates in the carbon cycle. For this reason, they are considered as an ecological soil restoration mechanism [20]. The distribution of AMFs is very wide, from very dry ecosystems to very humid tropical forests [21]. There is great diversity of species depending on climate, floristic composition, and soil type [17]. The most common genus is *Glomus* (*Zygomycetos*) [20]. In Amazonian soils, which are characterized by their acidity and low phosphorus availability, arbuscular mycorrhizae play a relevant role, allowing plants to survive and efficiently take up phosphorus from the soil. [22]. However, inadequate management techniques, such as the excessive application of phosphate fertilizers, prevent the formation of mycorrhizae [20]. Due to the multifunctionality that mycorrhizae perform, they are considered as an important indicator to assess soil quality [20]. The limiting factor is the conversion of land use that directly affects spore production and composition of AMF species. [21].

Algae are eukaryotic organisms, with chloroplasts associated with the endoplasmic reticulum and flagella. They are photosynthesizers. During the formation of life on Earth, they contributed to the elimination of carbon dioxide (CO₂) through the formation of calcium carbonate (CaCO₃) and the generation of OM. The role they play as colonizers contributes to primary production, OC compounds, and soil structure [2]. When associated with fungi, they form lichens and contribute to soil formation, degrading minerals such as silicates or rocks by the excretion of organic acids. They stop erosion by incorporating them into sandy or clayey soils. The carbohydrates they produce participate in the formation of aggregates. They are a source of food, placed almost at the beginning of the food chain [2]. The main types of soil algae are the following:

- Green eukaryotic terrestrial algae; which include the phyla Chlorophyta (especially from the Trebouxiophyceae class group) and Streptophyta (most of the Klebsormidiophyceae group) [23].
- Xanthophyceae; are yellow-green algae that belong to the group of heterocontos organisms. In their life cycles, dormancy or resistance phases are frequently present, with aspects very different from the rest of the vital stages. Their morphology varies from single-celled free-living or fixed to the substrate, to the microscopic, filamentous, branched, or siphonal colonial [24].
- Diatoms; belong to the Bacillariophyceae class [25][26], and like the rest of land algae, have been more studied in the soils of temperate areas of the Northern Hemisphere [27] than in tropical environments.

The identification of these algae has traditionally been carried out through enrichment cultures, followed by the development of pure unialgal cultures. Subsequently, the species were determined through microscopic observable characteristics (morphological, ultrastructural, microscopic). However, given the variable and morphologically similar nature of the majority, today, they are identified using molecular techniques [23][24][25].

Bacteria, they can be true or native residents, and migrants or introduced residents, that rarely contribute to biological activity. The bacterial prokaryotic domain primarily inhabits soils. They have many basic forms, their metabolism is inversely proportional to their size, and they can be considered as bags full of enzymes. They prefer warm soils and humidity [2]. They are so numerous in the soil that a soil sample can house more than 4000 genetically distinct bacteria. Some can play an important role in degrading chemical compounds, and others like *Rhizobium* form nodules in the roots of Legumes, fulfilling the function of fixing atmospheric nitrogen. In some cases, as in that of *Pseudomonas*, they can be pathogenic [2][28]. There are autotrophic bacteria (chemoautotrophs and photosynthesizers) and most are heterotrophic.

Soil conditions that affect the abundance and diversity of bacteria are humidity, temperature, aeration, pH, OM, and mineral substances. The main soil groups belong to the Eubacteria, and are:

- Cyanobacteria; microscopic blue-green algae, prokaryotes, photosynthesizers, and autotrophs. They contribute to the fixation of atmospheric N in soils through specialized structures called heterocistes. They are unicellular, colonial, or filamentous. Their union forms structures visible to the naked eye on the surface of the soil. The precise identification of this group is achieved through molecular techniques, and because their determination is based on morphology, it is currently considered imprecise or incomplete [29].
- Actinobacteria are bacteria that form filamentous colonies that look like mycelium fungus and have some characteristics similar to them, or intermediate between bacteria and fungus. This is why they are called Actinomycetes or actinomycetes. They have the ability to perform numerous biochemical reactions. In the soil, they degrade the OM to form humus. They participate in the mineralization process that benefits plants. Some species can fix atmospheric nitrogen in association with some tree species. In addition, they perform the function of regulating the composition of the microbial community in the soil, through antibiotic substances excreted abroad. They secrete enzymes that serve for the biological control of nematodes, insects, and other soil organisms. Their numbers in agricultural land are high (one million to one hundred million per gram of land). Their average weight is one

ton per hectare [19]. In tropical soils, they are one of the most important bacterial groups, as indicated by a recent review of soil bacteria globally [30].

Proteobacteria, Acidobacteria, Planctomycetes, Chloroflexi, and Verrucomycobiota have also been cited in the Global Atlas of dominant bacteria found in soils. These groups are much less significant in the tropics [30]. Their systematic classification is based on molecular techniques (16S rRNA sequencing) of soil microorganisms. The authors [30] suggests expanding the study of the Archaeobacteria group.

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