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Earthworms

our partners for resilient, living soil in the mountains

Jean-Jacques Brun, Sébastien De Danieli

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March 2020

In memory of our colleague Bernard Juvy
Assistant engineer, earthworm specialist
and discoverer of the new epigeic species *Octodrilus juvyi*

© J.-J. Brun

Bernard spraying a mustard solution to capture earthworms in the soil of the Bois de Tavanet woods (Chaudun near Gap) in October 2001

Foreword

According to the latest IPCC reports, our planet's soil resources are in an alarming state. This is caused by massive losses due to erosion and soil handling, removal for many different reasons, rapid development, pollution from various toxic chemical inputs, the drop in levels of organic matter and the disappearance of soil life.

It is worth reminding ourselves here that soil life relies on a complex, fragile network of living organisms which work in close interdependence with one another (bacteria, fungi, arthropods, earthworms). The real conductors of this underground symphony are earthworms.

Industrial and chemical agriculture (dependent on oil) has completely sidelined the presence and role of these tiny organisms. With agro-ecology, the trump card of the ecological transition, they are once more becoming a central focus for an agricultural sector which wants to encourage the soil's natural fertility. By contributing to freeing farmers from their dependency on chemical inputs of fossil fuel origin, earthworms are becoming real partners in a process to reconquer the quality of our soils.

This booklet aims to offer a closer look at the unique position occupied by these animals in the ecosystems on which we depend for our survival. We will be particularly focusing on mountain soils, long neglected due to the climatic and geographical constraints limiting their fertility. Special attention will also be given to the forest ecosystems. We will discover the unique, little-known role of earthworms in our forests despite the considerable constraints imposed by gravity and the weather confronting them in the mountains.

Acknowledgements

Like soil life, scientific life is based on teamwork and sharing information. Without this spontaneous and voluntary cooperation, there would be no transformation of raw materials (living soil) and no knowledge would be revealed (scientific research).

Our thanks are therefore extended to our research colleagues, PhD students and Irstea students with whom we worked in the field:

F. Auray, A. Bédécarrats, G. Boudin, V. Breton, L. Caner, N. Cassagne, L. Cécillon, Ph. Delcros, N.A. De Mello, L. Didier Feltgen, G. Favier, J.M. Gonzalez, J. L. Grossi, B. Juvy +, G. Loucougaray, E. Mermin, S. Meynier, J. Puissant, A. Saenger, C. Sagot, M. Stauffer, P. Tardif, S. Vanpeene, M. Vennetier.

Thank you also to our soil and life sciences partners who supported our work:

U. Babel +, J. Berthelin, A. Bispo, M. Blouin, M. B. Bouché, A. Brêthes, F. Bureau, Th. Gauquelin, J.-M. Gobat, B. Jabiol, J.-F. Ponge, G. Rovera, B. Souchier, P. Taberlet, F. Toutain, A. Zanella.

Contents

What is the purpose of this booklet?

Following numerous articles which have recently been published in the mainstream media about soil life, many people, from farmers, gardeners, foresters and naturalists to hikers who are simply curious to know how nature works, have been wondering about the knowledge available to assess the quality of living soil. It could even be said that there is a real quest for earthworm quality (abundance and richness in earthworms) for those who wish to exploit, manage and preserve resilient, living soil.

This information booklet therefore aims to provide these people with some answers and more broadly to answer the following questions:

- Is this soil living soil?
- Are there earthworms in my soil? Which ones? How many?
- How do I recognise earthworms? And how do I protect and keep them?

Among the rapid collapses of biodiversity and related services[1] reported by the mainstream media on an almost daily basis is also the issue of soil biodiversity. As we will see, soil biodiversity guarantees the creation of humus, the capture and storage of carbon from the atmosphere and slow maturing which enriches and stabilises the Earth system.

In the wake of this rising awareness, a new form of knowledge is emerging from composters (those boxes where our green waste is digested to transform it into "brown gold"). It reminds us that soil fertility has always been a mark of prosperous, sustainable societies and that its loss has always been the first sign of collapses which have marked the end of civilisations[1].

We hope that this booklet will be a modest contribution towards building this new knowledge and putting the living soil revolution into action.

[1] *Blouin M., Hodson M. E., Delgado E. A., Baker G., Brussaard L., Butt K. R., Dai L., Dendooven L., Peres G., Tondoh E., Cluzeau D., Brun J.-J. (2013) – A review of earthworm impact on soil function and ecosystem services, European Journal of Soil Science, 64, 161-182.*

[2] *Diamond J. (2009) - Effondrements, Folio Essais, 2009, 880 p.*

The importance of earthworms in the mountains

In our temperate landscapes of northern Europe, earthworms are often referred to as valuable assistants for the farmers and gardeners who cultivate the soils of the plains. It is frequently said that earthworms are the largest animal biomass in these environments which have often been farmed by humans for centuries.

But what do we know about them if we venture into the mountains, particularly the Alps? Aren't the steep slopes, winter cold and sometimes thin, often stony soil too much for their fragile conditions for existence?

Information about them is often rarer and yet it has to be recognised that they are present (almost) everywhere, even at high altitude. Our Soil Ecology team even discovered some earthworms in a pasture 2,800 metres above sea level in Maurienne.

Little work has been done concerning these species in the Alps. Without being exhaustive, this includes the work of Pietro Omodeo and Emilia Rota in Italy on the taxonomy of some earthworms in alpine soils[1] and that of Rudolf Hofer in Austria to educate the public on the hidden life of the soil (Federal State of Tyrol)[2].

In Switzerland, the earthworm specialist Gérard Cuendet[3] confirms that they also represent the largest animal biomass in the mountains, particularly in the alpine meadows which he studied in the Swiss National Park between 2,400 and 2,500 metres above sea level. In his opinion, the importance of the presence of earthworms in the mountains depends on:

- the nature of the bedrock, as very acidic soils have the fewest earthworms,
- the volume and frequency of precipitation (soil moisture and snow cover when freezing temperatures return),
- the zoo-geographical conditions and farming and grazing practices.

[1] *Omodeo P. et Rota E. (2004) - Observaciones taxonomicas sobre las lombrices de tierra de los Alpes Occidentales in A. G. Moreno et S. Borges (dir.), Avanas en taxonomia de lombrices de tierra, Editorial Complutense, Madrid, p. 189-220*

[2] *Hofer R. (2017) - Die verborgene Welt des Bodentiere, Alpine Druck GmbH, Innsbruck, 56 p.*

[3] *Cuendet G. (1994) - Importance des vers de terre (Lumbricidae) dans les écosystèmes alpins, Revue valdôtaine d'histoire naturelle, n°48, p. 381-386*

Little work has been done in France on earthworms in the alpine mountain environment. However, mention should be made of the research conducted by Jean-François Ponge and his collaborators into the involvement of earthworms in the dynamics of a spruce stand at high altitude[1].

We felt it would be of interest to provide some additional knowledge based on work led by Jean-Jacques Brun (Research Director Emeritus) and the scientists from the Irstea Grenoble Soil Ecology team, particularly Sébastien De Danieli (Active Design Engineer) and Bernard Juvy (Retired Assistant Engineer).

[1] *Ponge J.-F., André J., Zackrisson O., Nilsson M.-C., Gallet C. (1998) - The Forest regeneration puzzle, BioScience, vol 48 n 7, 523-530*

Extraction of earthworms using mustard © S. De Danieli

Capture of earthworms in the mountains © J.-J. Brun

Knowledge of earthworms

- Soil, worms, humus and humans

© J.-J. Brun

- So what is an earthworm?

© S. De Danieli

- Earthworm quality in the mountains: input of the work of the Soil Ecology team

© N. Sardat

Soil, worms, humus and humans

Soil, the fragile epidermis of the biosphere

Degraded soil © J.-J. Brun

Soil is the living epidermis of the Earth's biosphere. It forms very slowly over thousands of years but can degrade very quickly in the space of just a few years if no precautions are taken.

Currently in France, the equivalent of a county in soil surface area is lost every ten years. This soil haemorrhage will severely reduce our resilience and our ability to adapt to climate change.

It is important to remind ourselves here that the quality and fertility of soil are linked to the abundance and diversity of the aggregates it contains. The manufacture of these aggregates is largely controlled by earthworm activity.

These aggregates intimately bind together organic matter and mineral matter. In this way, the nutrients essential to plant growth are better conserved and carbon, which intensifies the greenhouse effect in excess, is better sequestrated and stored longer in the soil[1].

[1] *Gobat J.-M., Aragno M., Mathey W. (2017) - Le sol vivant : bases de pédologie-biologie des sols, Presses Universitaires Romandes, 848 p.*

Soil life, an underground symphony

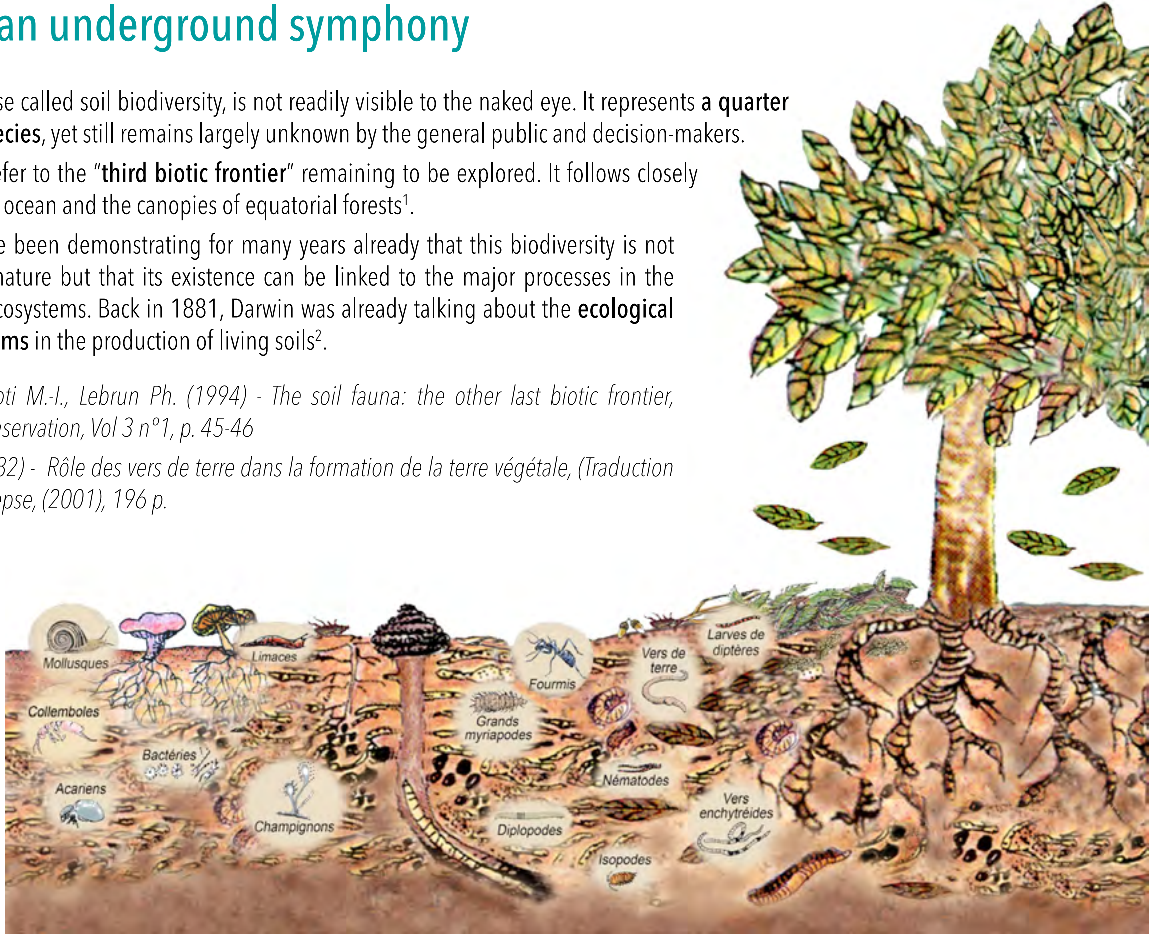
Soil life, otherwise called soil biodiversity, is not readily visible to the naked eye. It represents **a quarter of all known species**, yet still remains largely unknown by the general public and decision-makers.

Scientists now refer to the "**third biotic frontier**" remaining to be explored. It follows closely behind the deep ocean and the canopies of equatorial forests[1].

Researchers have been demonstrating for many years already that this biodiversity is not just a whim of nature but that its existence can be linked to the major processes in the functioning of ecosystems. Back in 1881, Darwin was already talking about the **ecological role of earthworms** in the production of living soils[2].

[1] *André H.M., Noti M.-I., Lebrun Ph. (1994) - The soil fauna: the other last biotic frontier, Biodiversity & conservation, Vol 3 n°1, p. 45-46*

[2] *Darwin C.R. (1882) - Rôle des vers de terre dans la formation de la terre végétale, (Traduction A. Berra), Ed. Syllepse, (2001), 196 p.*



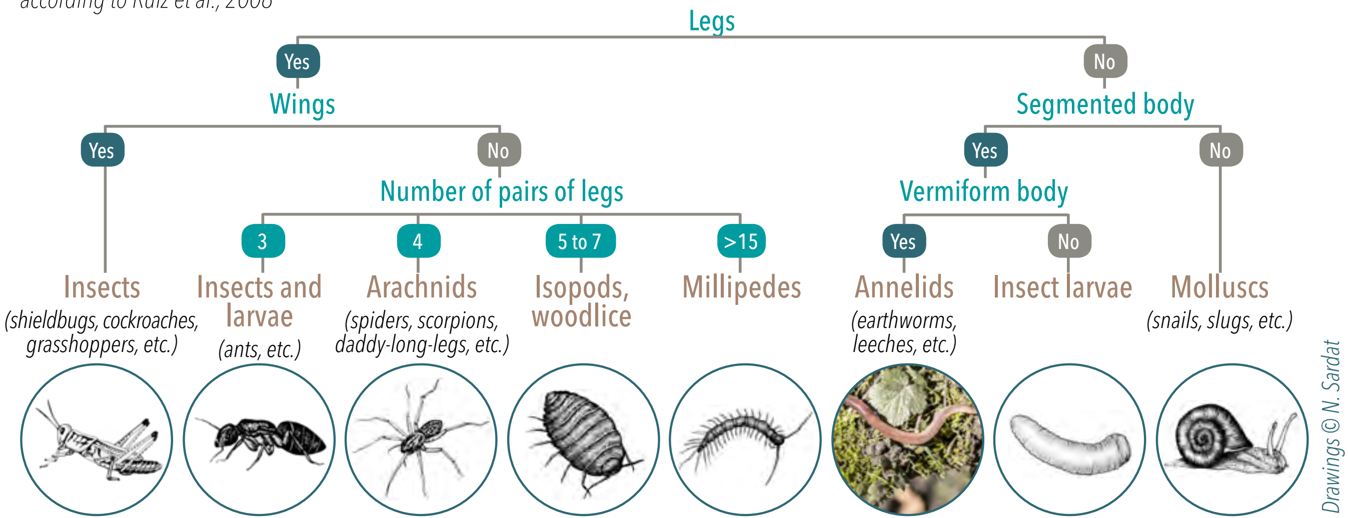
Forest litter
© N. Sardat

Earthworms, animals that are easily recognisable

It is easy to recognise an earthworm. With no wings or legs, its segmented, elongated body can include from 60 to 150 rings depending on the species, hence the name “annelid” from the Latin anellus, meaning "little ring". Each ring has four pairs of bristles enabling it to move horizontally by gripping the substrate. Powerful longitudinal muscles are also used for creeping, enabling it to slowly extend and to shorten quickly if necessary to escape from a threat. Each ring also has circular muscles so that the worm can make itself very thin and slide into cracks, or swell like a pneumatic compressor, enlarging crevices in the soil into which it enters. These impressive muscles working with a powerful digestive tube lined with a multitude of bacteria results in the famous mixture of dead organic matter and soil mineral matter on which soil fertility is based. Of all the fauna in the soil, earthworms have the biggest impact on soil quality. Endogeic and anecic worms are real navvies, tunnellers and biochemical activators of the edaphic compartment, thus facilitating soil resilience (ability to withstand disruption without malfunctioning).

Simplified key to soil macrofauna

according to Ruiz et al., 2008



Earthworms, ecosystem engineers and "tunnellers"

The different burrows according to the ecological category of earthworm © N. Sardat

All categories of earthworm modify the soil in which they live by constructing burrows which vary in width and length. However, small epigeic worms only contribute very marginally to these holes in the soil.

It is the endogeic and anecic worms which really deserve to be described as "tunnellers".

Endogeic worms create fairly horizontal burrows in the top 30 centimetres of soil.

Anecic worms create vertical burrows which can be over one metre in depth and even up to three or four metres depending on the soil.

To give an idea, in a temperate meadow, anecic worms are considered to be capable of constructing up to 4,000 kilometres of burrows per hectare[1].

All these stable, vertical or sometimes sloping burrows improve infiltration and drainage of surface water. If the soil is deep, this could even be described as having a flood control effect. By limiting runoff and erosion, the subterranean burrow network enhances water storage. The water retention capacity of an earthwormless soil can be improved by 25% in ten years by introducing worms.

[1] *Bouché M. B. (2014) - Des vers de terre et des hommes : découvrir nos écosystèmes fonctionnant à l'énergie solaire, Actes Sud, p. 187*

Earthworms, ecosystem engineers and "navvies"

Anecic worm casts © J.-J. Brun

By ingesting soil and the organic matter which falls onto it, earthworms create a thoroughly mixed blend with remarkable nutritional properties.

This edaphic elixir, also referred to as "lombrimix"[1], gives the soil its fertility, thus enabling ecosystems to deploy the full range of their diversity. Moreover, agro-ecosystems are able to establish their high productivity based on the preservation and enhancement of this valuable mixture.

All the droppings which make up this vegetable mould and which are deposited in the soil (in the burrows) and on top of the soil in the form of piles called "casts" are the result of this process known as bioturbation.

In permanent pastures, this can represent a layer 5 to 6 cm thick which is the equivalent of 500 to 1,000 tonnes of earth per hectare[2]. For this reason, earthworms can be described as real "navvies" or "biological labourers", as there is little to choose between their free work (renewable solar energy) and powerful, energy-thirsty agricultural machinery.

Earthworm activity fosters a virtuous process conserving the soil and maintaining soil fertility. This is why earthworms are referred to as ecosystem engineers[3].

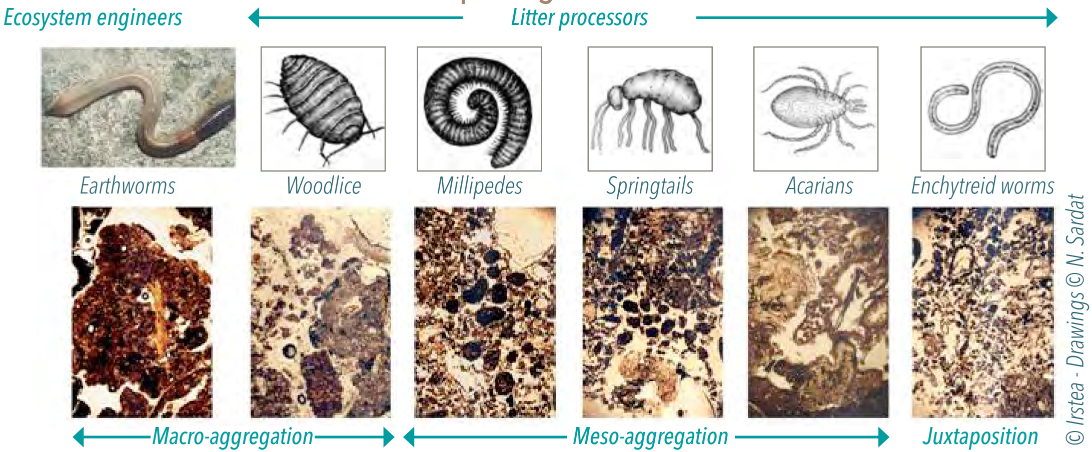
[1] *Bouché M. B. (2014) - Des vers de terre et des hommes, Actes Sud, p. 182*

[2] *Bouché M. B. (2014) - Des vers de terre et des hommes, Actes Sud, p. 183*

[3] *Lavelle P. et al. (1997) - Soil function in a changing world : the role of invertebrate ecosystem engineers. European Journal of Soil Biology 33, 159-193*

The entire soil fauna contributes to creating living soil

Micro-morphological mini-atlas

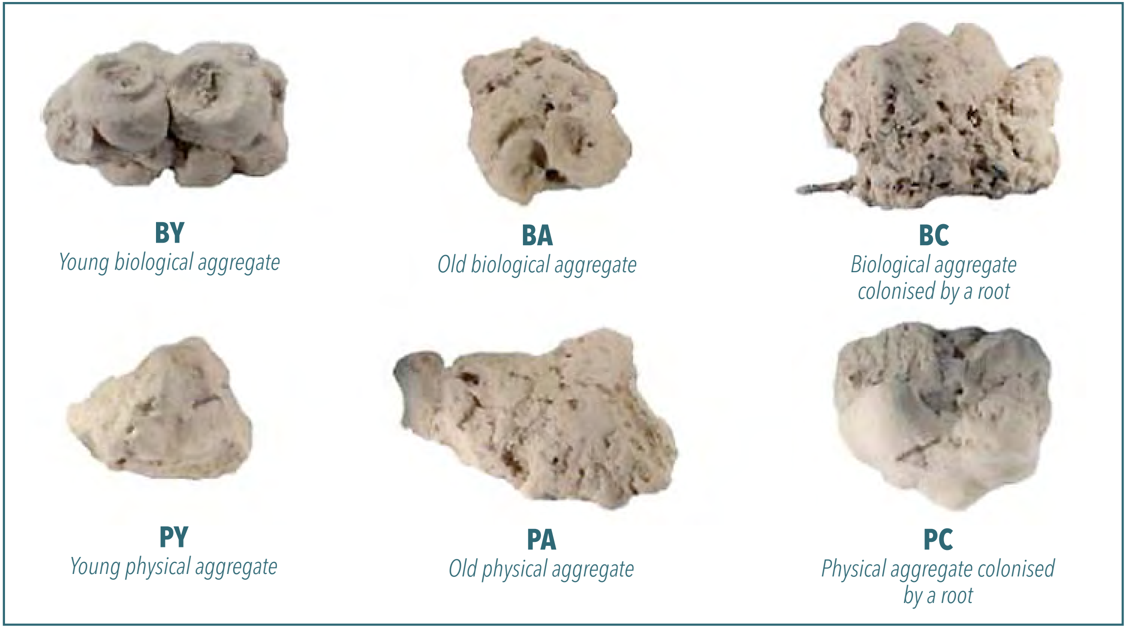


In fact, the entire soil fauna contributes to creating living soil through its aggregation activity deployed in time and space. Our observations via thin sections of humus (micromorphology) have shown that at a given time, mosaics with several different types of juxtaposed aggregates form within humus. Several types of biological aggregates co-exist:

- **Macro-aggregates** (2 cm - 250 microns) are produced by earthworms and woodlice. Earthworms are the only living organisms able to thoroughly mix organic and mineral matter. In this way they produce those organic and mineral complexes which are the key to natural soil fertility. This is called a macro-aggregated structure.
- **Meso-aggregates** (250 microns - 25 microns) are produced by various organisms (millipedes, springtails, acarians), which break down plant debris, process it and excrete it in the form of oval-shaped light-brown to dark-brown droppings. They participate in meso-aggregation.
- **Micro-aggregates** (< 25 microns) are mainly produced by enchytreid worms. Their droppings are frequent in very acid soils and are often the only inclusions of organic matter in the mineral matrix. This is why this is referred to as a juxtaposition structure.

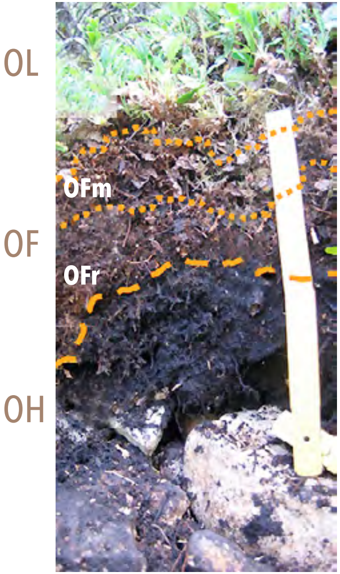
In cold soil in the mountains (sporadic permafrost), our observations show a combination of two types of aggregates: biological and also physical resulting from the microclimatic conditions (alternating freeze/thaw) in the humus analysed[4].

[4] *Cécillon L., de Mello N. A., De Danieli S., Brun J.-J. (2010) - Soil macroaggregate dynamics in a mountain spatial climate gradient, Biogeochemistry, 97: 31-43*

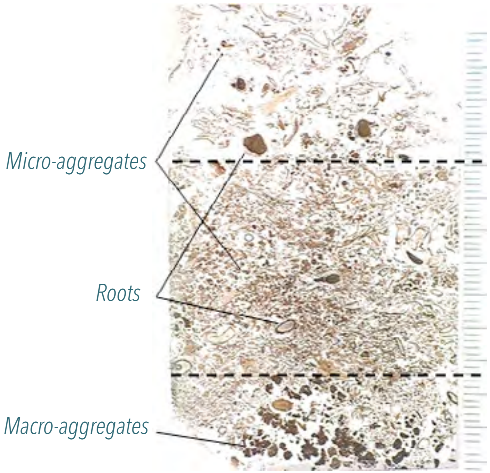


Example of a diversity of aggregates (size around 10 mm) observed in cold mountain soil[4]

Humus is a visual compendium of all the processing activity of soil fauna



Macro-morphology



Micro-morphology

Humus visual compendium © N. Cassagne and J.J. Brun

OFr horizon

- loose structure, dominated by plant material (debris, roots)
- fine textured, dispersed OM in low amounts

OFm horizon

- more compact structure, still dominated by plant material (debris, roots)
- greater micro-aggregation

OH horizon

- considerable plant material (some debris, a lot of roots)
- micro- and macro-aggregation

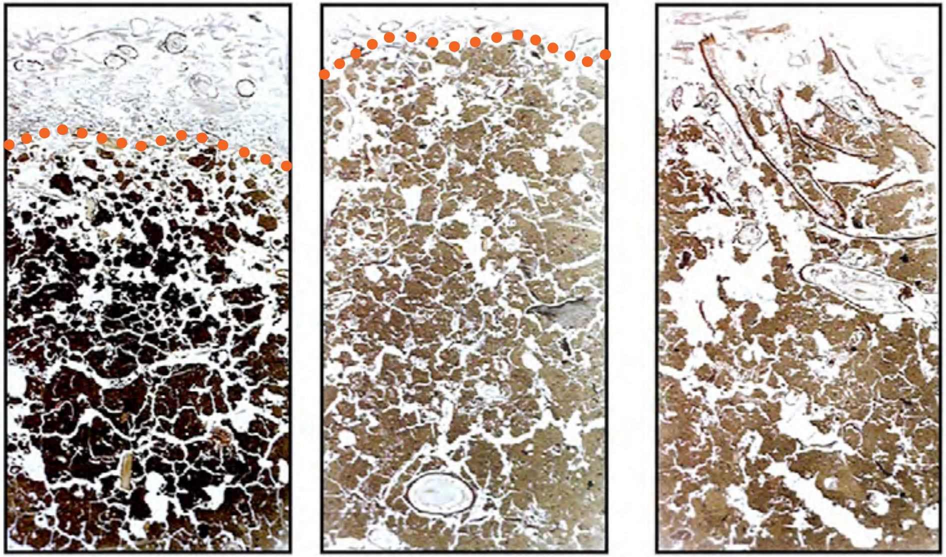
OL horizons: organic litter horizons
OF horizons: organic fragmentation horizons
OH horizons: humified organic horizons
OM: organic matter

The forms of humus[1] most frequently found in the mountains (Amphimull and Moder) often bring together all the aggregation systems. The OF and OH horizons include the greatest quantity of these aggregates.

[1] *Jabiol B., Brêthes A., Ponge J.-F., Toutain F., Brun J.-J. (2007) - L'humus sous toutes ses formes (2e édition), AgroParisTech, 67 p.*

In the most favourable forest sites (deep soil and weak acid pH), the entire soil is composed of macro-aggregates produced by anecic worms

1 **2** **3**



Humus dominated by anecic worm activity (L. Caner et J.-J. Brun)

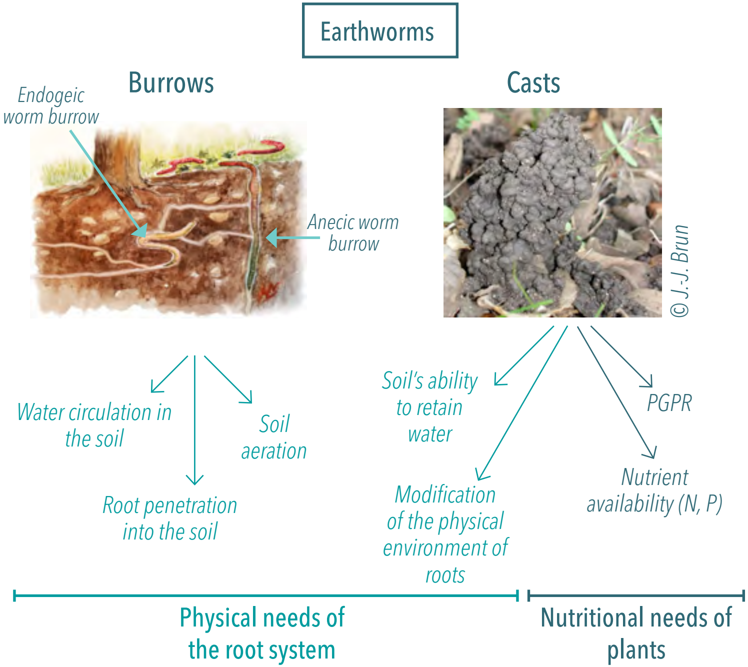
Increasing anecic activity incorporating litter into the soil:

1 - Litter still present in the form of OL horizon

2 - Partly incorporated relectual litter

3 - Fully incorporated litter

The health of ecosystems mainly depends on beneficial interaction between earthworms and plants



It would be reasonable to believe that soil life and plant life have been working hand-in-hand for a long time. Could plants have developed without earthworms?

Earthworms satisfy two main categories of plants' major needs[1]:

- physical needs to establish root systems,
- nutritional needs of the entire phytomass.

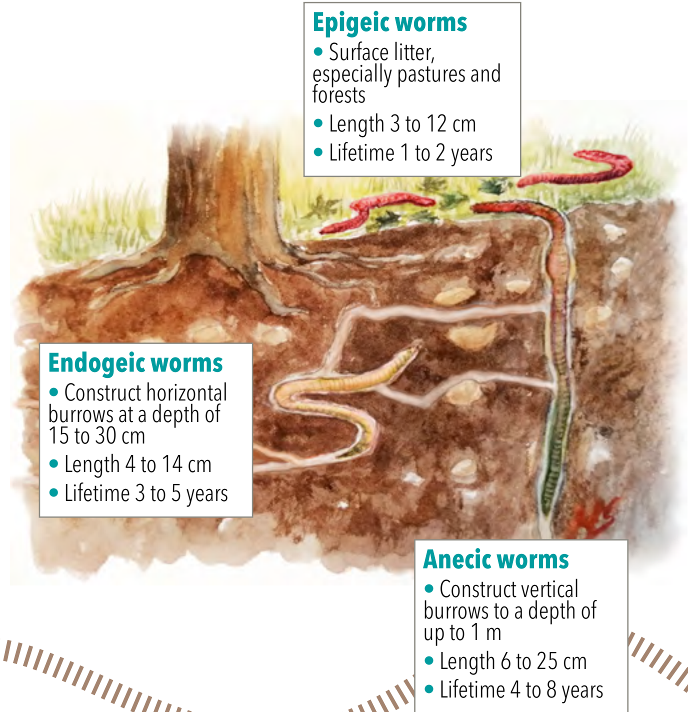
This makes it easier to understand the importance of earthworm quality mentioned above, particularly with regard to agro-ecosystems which have to specifically enhance the ecological functions produced by this earthworm quality to guarantee efficient plant productivity.

Earthworm quality also concerns natural ecosystems. Their major carbon-sequestration functions, both in above-ground biomass and in the soil aggregates by trapping above-ground carbon, will be one of the keys sought by climate change mitigation policies (4 per 1000 programme)[2].

[1] *Hedde M. and Zwicke M. (2020) - Soil fauna and plant production*
https://planet-vie.ens.fr/thematiques/ecologie/production-agricole-agrosystemes/faune-du-sol-et-production-vegetale
[2] *https://www.4p1000.org/fr*

So what is this earthworm?

The three ecological categories



Those interested by a more in-depth identification of earthworms can refer to the identification sheets.

In Europe there are 400 different species of earthworm, many of which are to be found in the soil of the alpine regions. In 1971, Marcel B. Bouché proposed an initial classification of earthworms in 3 ecological categories, which are adaptive as many species are in intermediate positions between these three categories.

The morphological characteristics (pigmentation, muscles, etc.) and physiological characteristics (such as the ability to go into diapause) are used as a basis to determine the categories and sub-categories informing us of their current way of life[1].

In the mountains, earthworm samples taken by our team in the different mountain regions around Grenoble show:

- for forests, the presence of 6 to 8 species on average,
- for pastures, 10 to 12 species,
- for hay fields, 14 to 16 species.

[1] *Bouché M. B. (1972) - Lombriciens de France : écologie et systématique, Ann. Zool., n°Hors série, INRA, n°72-2, 672 p.*

Epigeic worms

Epigeic worms represent 5% of the total earthworm biomass.

They are some of the smallest (1 to 5 cm) and most colourful (dark colour: red, brown).

They live on the surface in litter (forests) or the remains of crops (fields) and root mats (pastures).

Their role in the ecosystem is to break fresh organic matter down into fine fragments, however:

- they do not mix it with mineral matter,
- they do not construct burrows,
- they have a short lifetime (maximum of 6 months),
- they have a high mortality (multiple predators),
- they do not enter diapause.

© S. De Danieli

Endogeic worms

Endogeic worms represent between 20% and 40% of the soil's earthworm biomass.
Their size ranges from 1 to 20 cm
Their colouring is pale but characteristic:

- some are grey,
- others are pink,
- some can be a greenish shade.

They live in the top thirty centimetres of soil and come to the surface if their burrows are flooded.

- they construct horizontal and subhorizontal burrows,
- they excrete their organic faeces (droppings) in these burrows.

© S. De Danieli

Anecic worms

Anecic worms represent between 30% and 40% of the earthworm biomass in the soil in temperate regions.

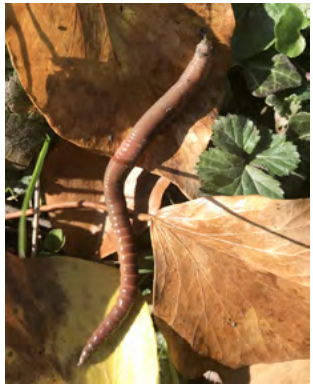
They are some of the largest (10 cm to 110 cm) and some species can exceed 150 cm.

They vary from red to dark brown, with the colour fading from the animal's head towards its tail.

They have a long lifetime (between 5 and 8 years).

They have a vital ecological role:

- they construct stable vertical burrows and draw fresh or decomposing plant residues into them. Runoff water also infiltrates these burrows, reducing dangerous levels of excess rainwater.
- they line the walls of their tunnels with humus, mucus and micro-organisms, thus facilitating the decomposition process and the extension of plant roots.
- they excrete casts on the surface which loosen packed soil and limit gravity erosion.



© J.-J. Brun

The two main categories of anecic worm

© J-L. Grossi

Strict anecic earthworms

Also called blackhead worms, they have important specific characteristics:

- they can enter diapause, which means their physiological functions shut down from June to September,
- they construct highly branched networks of burrows.

Epi-anecic earthworms

Also called redhead worms, they are the most common:

- they are reactive and react quickly to sudden changes in their physical environment (pressure, temperature, humidity),
- they do not enter diapause, but can enter quiescence (slowing of functions),
- the network of burrows which they construct has few branches.

Earthworms can withstand harsh environmental conditions

Earthworm in quiescence © J.-J. Brun

When the external conditions are demanding, earthworms implement two important adaptive mechanisms:

- **quiescence** is triggered directly by an environmental factor, either drought (aestivation) or cold (hibernation). This dormancy process is ended when the cause disappears. All earthworms can enter quiescence.
- **diapause** is controlled by a neuro-hormonal system linked to abundant photoreceptive cells in earthworm's head. It is triggered to make the animal lethargic during the longest days. In earthworms, only strict anecic worms enter diapause.

These faculties enable earthworms to protect themselves from climatic extremes and are therefore very useful in mountain environments. The soil in these regions is often rather thin and subject to alternating freeze/thaw and wet/dry periods. The earthworm potential of mountain soil is preserved by a large part of the earthworm population entering quiescence.

Earthworm quality in the mountains: contribution of the work of the Soil Ecology team

Earthworms and the health of ecosystems

© J.-J. Brun

As already stated, the presence of earthworms is recognised as a reliable indicator of the health of soil and more broadly that of ecosystems[1]. Databases of species are being compiled for agro-ecosystems in particular (e.g. Brittany).

However, given the slow pace at which this data is acquired by traditional scientific inventories, simplified participatory inventories have been launched for efficient capture and recognition of earthworms.

More specifically, this is the current mission of participatory observatories such as the OPVT (participatory earthworm observatory) from the OSU (Sciences of the Universe Observatory) at the University of Rennes in France[2], led by Daniel Cluzeau.

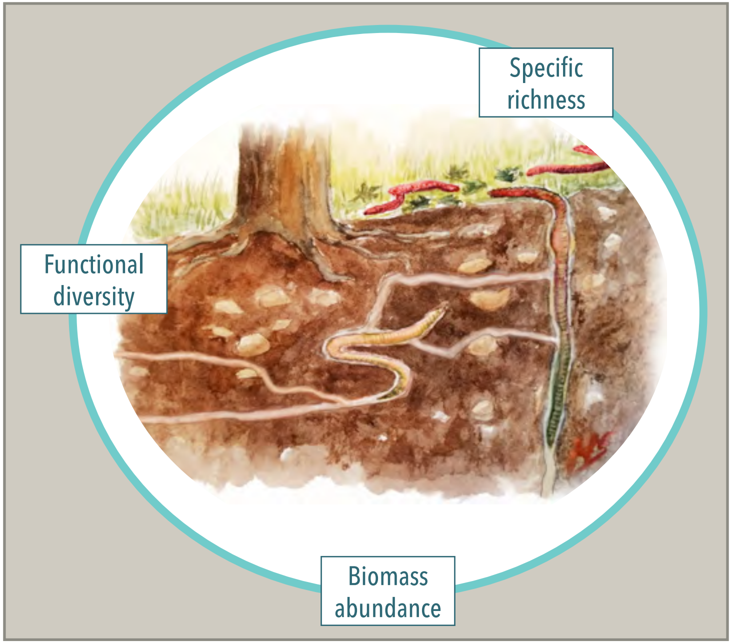
In mountain regions, given the difficulties to implement taxonomic inventories, genetic approaches (DNA barcodes) offer interesting possibilities for diagnoses in small areas with a strong heritage identity[3]. The earthworm quality of soils can represent an ecological dimension in these areas to be monitored in the long term.

[1] *Römbke J., Jänsch S., Didden W. (2005) - The use of earthworms in ecological soil classification and assessment concepts, Ecotoxicology and environmental safety, 62, 249-265*

[2] *https://ecobiosoil.univ-rennes1.fr/*

[3] *Pansu J., De Danieli S., Puissant J., Gonzalez J.-M., Gielly L., Cordonnier T., Zinger L., Brun J.-J., Choler P., Taberlet P., Cécillon L. (2015) - Landscape-scale distribution patterns of earthworms inferred from soil DNA, Soil Biology and Biochemistry, 83, 100-105*

Diagnosis of earthworm quality



Sound references for mountain regions are still lacking. However, studies we have conducted in which earthworms were taken into account can be used. These works are considered as being contributions to the diagnosis of soil earthworm quality. It should be specified that for us, this diagnosis includes three supplementary areas of information:

- that linked to specific richness which provides information on soil biodiversity,
- that linked to functional diversity which provides information about ecological integrity,
- that linked to abundance and biomass, which provides information on the bioturbation potential linked to fertility.

In the three examples chosen below, this information is presented in a simplified manner, sometimes in diagram form, to line with our chosen booklet form. Readers who wish to know more can consult the articles mentioned in reference.

Studies conducted in a mountain setting

© J.-J. Brun

- Earthworms in farming environments

- Earthworms and forest environments

© J.-J. Brun

© J.-J. Brun

- Earthworms and degraded environments

Earthworms in farming environments

The decline of agriculture in the mountains: towards new forests?

Saint-Michel-de-Maurienne 1935

Saint-Michel-de-Maurienne 2004 © J.-J. Brun

Mountain landscapes are often characterised by areas of fallow land of varying size due to a decline in farming. Afforestation quickly colonises these fallow areas.

In some valleys such as the Maurienne valley, agricultural landscapes have switched to forest landscapes (since the 1950s) with mainly deciduous trees (ash, maple and cherry).

The ecological diagnosis requested from the Soil Ecology team consisted in determining if these new deciduous afforestations, some of which were over 80 years old, constitute forests which are already mature or if they were still in the recolonisation phases.

To support our appraisal, we looked at the state of the earthworm population to know if these deciduous forests resulting from the decline in farming had become real forests from the point of view of the soil, or if they had remained as pastures, or wooded pastures.

Earthworms and farming decline in Maurienne

The richness and abundance of earthworms in Maurienne reveals that the new deciduous forests have a higher earthworm quality than that of the pastures on which they are growing. These environments therefore constitute carbon sinks of utmost importance. By comparing the earthworm quality of recent deciduous forests with that of old coniferous forests, it is possible to confirm that from the point of view of soil quality, these deciduous forests are more like pastures than old forests growing on the hillsides.

Article of reference: Grossi J.-L. et Brun J.-J. (1997) - Impact of climate and vegetation on lumbricid populations in the french Alps, Soil Biology and Biochemestry, 29 (3/4) : 329-333.

Earthworms and forest environments

1 ■ Earthworm quality in the forest soils of Chartreuse

In 2018, the Chartreuse forest was awarded the first AOC (registered designation of origin) in France for the wood it produces. This label awarded on 23.10.2018 covers 28,000 hectares of forest and concerns 134 municipalities in Isère and Savoie.

The quality of the Chartreuse wood is linked to the exceptional ecological conditions which prevail in the mountain range: high rainfall and the quality of the soil on which the forests grow.

By means of the "earthworm quality", the Soil Ecology team wanted to shed new light on this soil quality revealed indirectly by the Chartreuse wood AOC.

The inventory targeted two major components of the Chartreuse forest landscape:

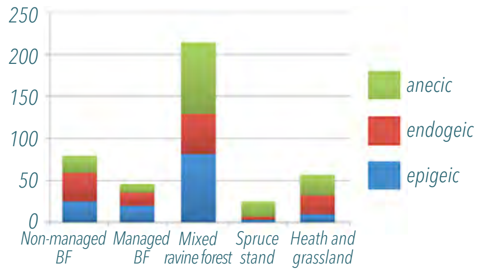
- productive managed forests: beech-fir woods and spruce stands,
- natural forests with high heritage value: mixed ravine forests.

The level of management intensity was taken into account for beech-fir woods, distinguishing between:

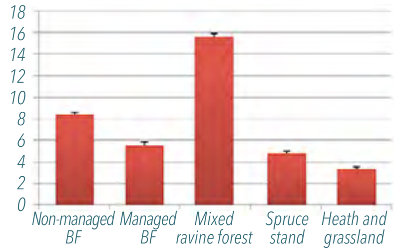
- managed beech-fir woods
- non-managed beech-fir woods

The inventory results reveal the astonishing richness of mixed ravine forests followed by the non-managed beech-fir woods, managed beech-fir woods close to heaths and grassland and finally the spruce stands.

La Grande Chartreuse national forest © J.-J. Brun

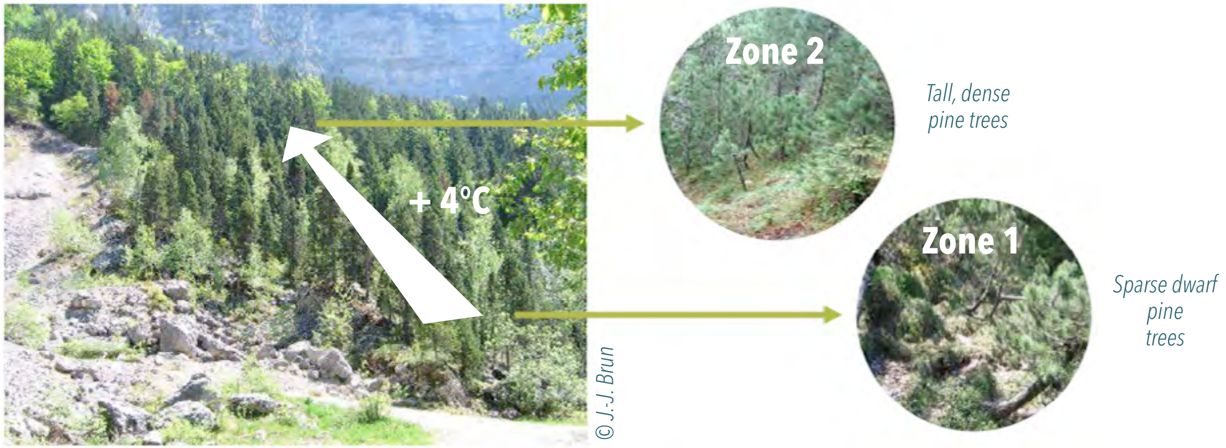


Density of earthworm ecological categories by habitat



Biomass (g/m²)

2 ■ Earthworms and climate change in dwarf pine forests



Using a short-distance altitudinal transect on a scree in Chartreuse, we were able to simulate the impact of an increase in soil temperature of 4°C in a dwarf pine forest. This type of simulation with space-for-time substitution is often used in ecology as it partially offsets the lack of means for monitoring over time.

Zone 1, which has a cold micro-climate linked to the presence of a permanent ice lens, has an average annual soil temperature 4°C lower than zone 2 which lies outside the ice lens and 50 metres higher, also with dwarf pine coverage. The main issue explored in this research was to know if this temperature argument could modify the earthworm community and therefore the earthworm quality on this scree.

Article of reference: Cassagne N., Spiegelberger T., Cécillon L., Juvy B., Brun J.-J. (2008) - The impact of soil temperature increase on organic matter and faunal properties in a frozen calcareous scree in the French Alps. Geoderma, 146 (1-2), 239-247

Results on the potential modification of earthworm communities following a rise in soil temperature of 4°C

	Stage	Ecological group	Biomass
Zone 1			
Dendrodrilus rubidus (Savigny, 1826)	adult	epigeic	0.158
Dendrodrilus sp.	poor condition		
Lumbricus friendi (Cognetti, 1904)	adult	epianecic	1.713
Lumbricus sp.	juvenile	epianecic	0.168
Lumbricus sp.	juvenile	epianecic	0.603
Octodrilus sp.	juvenile	epigeic	0.08
		Total biomass	2.722
Zone 2			
Dendrobaena octaedra (Savigny, 1826)	adult	epigeic	0.18
Dendrobaena sp.	juvenile	epigeic	0.11
Lumbricus friendi (Cognetti, 1904)	adult	epianecic	1.171
Lumbricus meliboeus (Rosa, 1884)	adult	epianecic	1.418
Lumbricus sp.	very poor condition		
Lumbricus sp.	juvenile	epianecic	0.543
Lumbricus sp.	juvenile	epianecic	0.77
Lumbricus sp.	juvenile	epianecic	0.268
Lumbricus sp.	juvenile	epianecic	0.315
Lumbricus sp.	juvenile	epianecic	0.33
Lumbricus sp.	juvenile	epianecic	0.14
Lumbricus sp.	juvenile	epianecic	0.41
Nicodrilus caliginosus caliginosus (Savigny, 1826)	juvenile	endogeic	0.331
Nicodrilus caliginosus caliginosus (Savigny, 1826)	adult	endogeic	0.565
Nicodrilus caliginosus tuberculatus (Eisen, 1875)	adult	endogeic	0.603
Nicodrilus sp.	juvenile	endogeic	0.27
Octodrilus juvyi (Zicsi, 2005)	adult	epigeic	0.438
Octodrilus juvyi (Zicsi, 2005)	adult	epigeic	0.236
Octodrilus juvyi (Zicsi, 2005)	adult	epigeic	0.491
Octodrilus juvyi (Zicsi, 2005)	adult	epigeic	0.603
		Total biomass	9.192

Octodrilus juvyi earthworm © J.-J. Brun

Discovery of a new endemic species in Chartreuse: *Octodrilus juvyi*

In Chartreuse with +4°C, the earthworm biomass increases from 2.7g to 9.2g. More epigeic and anecic worms are found and endogeic worms appear.

Media focus on the discovery of a new earthworm in Chartreuse

Mixed ravine forests growing on mountainsides are part of the forests considered at European level as rare habitats of priority interest. Cemagref researchers discovered a new soil "engineer" there.

Their conservation therefore plays an important role in maintaining biodiversity (habitats directive, code 9180). These forests develop on steeply sloping terrain made unstable and mobile by rock slides, rock falls or avalanches. On these sites, the forest is a means of fixing the soil. This type of ecosystem is present in the alpine foothills of the Grande Chartreuse region.

How can these maples, a species which is normally post-pioneer or nomadic, colonise these mobile substrates and manage to persist?

This is the question asked by the Cemagref researchers for research funded by the Chartreuse regional nature park. They sought to understand the biological functioning and the dynamic of these sites, a preliminary step to any conservation management procedure.

The study conducted by the Soil Ecology team from the "Mountain ecosystems" research unit, had two stages. It was conducted on three high-altitude sites (1,000 and 1,200 m) differentiated by their level of instability. The first stage led to a precise assessment of the plant heritage of these sites. The second aimed to understand the population dynamics of the mixed ravine forests.

© Irstea

Discovery of a new soil engineer: Octodrilus juvyi

3 ■ Earthworms and forest fires

Burnt forest in Italy © S. Dupire

With climate change, forest fires which were limited to the Mediterranean strip have become a worrying reality for the alpine area which had been spared up to now.

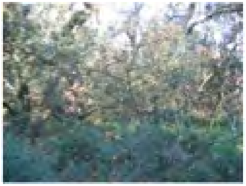
In August 2003 with the Mont Néron fire close to Grenoble, we witnessed a radical change in the plant landscape which considerably increased the risk of rock falls close to many houses.

The Grenoble Soil Ecology team was then able to study the earthworm quality of the burnt soil through its involvement in a large European project, the IRISE project (led by Michel Vennetier), dedicated to the study of the impact of fire recurrence in the Maures mountain range in the period 2005-2008.

Article of reference: Vennetier M. et coauteurs (2008) - Étude de l'impact d'incendies de forêt répétés sur la biodiversité et sur les sols. Recherche d'indicateurs. Rapport final, DOI: 10.13140/RG.2.1.1450.3923

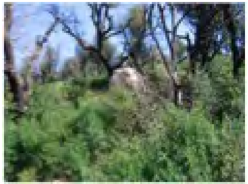
Results concerning earthworm quality according to a fire intensity gradient (Maures mountain range)

Control (no fire)



1 to 2 fires (1990)

2 fires (2003)



3 fires (1990)

3 to 4 fires (2003)

© M. Vennetier

Control (no fire)	1 to 2 fires (1990)	2 fires (2003)	3 fires (1990)	3 to 4 fires (2003)
4 - 9	**5 - 9**	**4**	**3 - 4**	**1 - 2**

Species of earthworm present in the soil

Nicodrilus nocturnus, the only anecic worm surviving the maximum number of fires burrows and brings to the surface sufficient fertile soil on its own to enable the Maures forest to recover after these traumatic upheavals.

© J.-J. Brun

© J.-J. Brun

Article of reference: Cécillon L., Cassagne N., Czarnes S., Gros R., Vennetier M., Brun J.-J. (2008) - Predicting soil quality indices with near infrared analysis in a wildfire chronosequence. Science of the Total Environment, 407 (3), 1200-1205

A challenge: conserve anecic worms to fight climate extremes

© J.-L. Grossi

This work shed interesting light on the ability of the soil to withstand the ecological trauma linked to fires. However, the observed drop in earthworm populations, particularly anecic worms which ensure the resilience of fertile soil, could be threatened in a context of worsening climate change. It is therefore necessary to keep as many anecic worms as possible in the soils which have them naturally, as they ensure soil resilience and stability, a major issue for the Alps.

Some recommendations to conserve or stimulate anecic worm populations

(Applicable to gardens or the restoration of degraded environments)

To preserve the habitat of anecic worms, particularly in gardens

- use a broadfork rather than a spade,
- shallow-plough,
- sow directly into the soil without ploughing.

To stimulate anecic worms (biostimulation) and the other partners of soil life

- apply RCW (ramial chipped wood),
- apply green waste compost,
- apply organic fertilisers (manure).

To restore earthworm potential in degraded environments (fire, eroded areas, etc.)

- set up a mesh of biostimulation islands (at 30 m intervals),
- create these islands on a reduced scale (3 m x 3 m depending on the amount of material available),
- spread materials in a non-compact layer (maximum thickness of 10 cm).

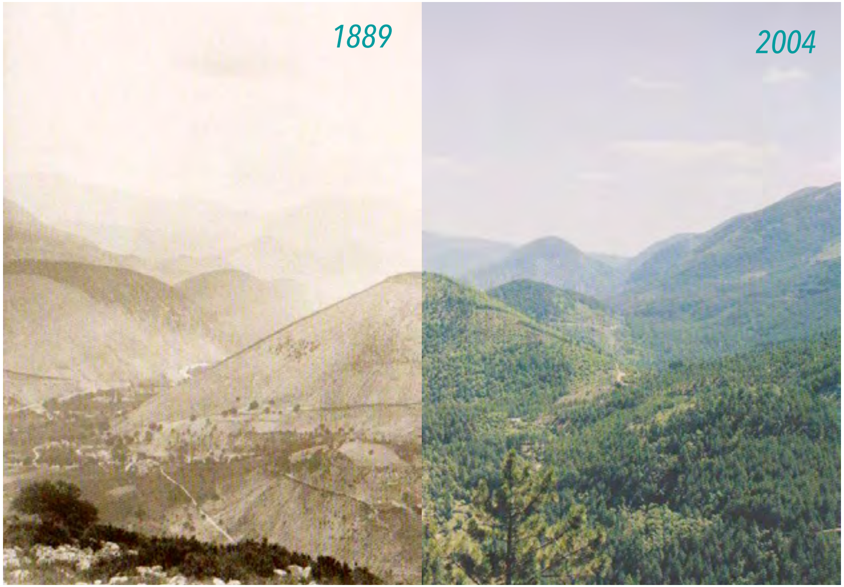
Experimental biostimulation plots in Switzerland
© J.-J. Brun

Reference work: Leclerc B. (2002) - Les jardiniers de l'ombre : vers de terre et autres artisans de la fertilité, Éditions Terre vivante, 126 p.

Earthworms and degraded environments

1 ■ Earthworm quality in eroded, reforested areas of the Southern Alps

Photomontage of the same landscape in the Southern Alps in 1889 and then in 2004



Source: "Restoring the mountain" SOMOGY/ Éditions d'Art

© J.-J. Brun

Eroded soils with few earthworms — *Restored soils with earthworms*

Article of reference: Vallauri D., Grossi J.-L., Brun J.-J. (1998) - Diversité, distribution et dynamique des communautés lombriciennes un siècle après restauration forestière dans le bassin du Saignon (Alpes-de-Haute-Provence). Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie, 321 : 1023 - 1033

Result: earthworm quality after 120 years of reforestation

© J.-J. Brun

1885 → **1950** → **1985** → **2004**

Black pine plantation

The restoration of mountain land by reforestation carried out 120 years ago on eroded soil with no fauna (no earthworms) facilitated the slow natural return of earthworm quality with a few epigeic and anecic worms first of all, then finally endogeic worms.

Reconquering this earthworm quality resulted in an increase in the biodiversity of woody species in relation to the initial coniferous plantations (Austrian black pine only) thanks to the arrival of numerous deciduous understorey species (coronilla, mountain ash, hawthorn, etc.). A new ecosystem was recreated.

Time dynamic of earthworms

Year	*Soil*	*Earthworms*			
		Biomass ($g.m^2$)	*Species*		
			Epigeic worms	*Anecic worms*	*Endogeic worms*
1885	*eroded soil*	*0*	-	-	-
1950	*dysmull**	*1*	+	+	-
1985	*oligomull**	*20*	+	+++	-
2005	*mesomull**	*27*	+	++	+

** Forms of humus in temperate climates. Cf document Jabiol B., Brêthes A., Ponge J.-F., Toutain F., Brun J.-J. (2007) - L'humus sous toutes ses formes (2e édition), AgroParisTech, 67 p.*

2 ■ Earthworms and the quarries in mountain valleys

The soil of quarries is affected by subsurface exploitation. The topsoil (surface humus) is often partially recovered and poorly stored, thus reducing the fertility of the reconstituted land. To compensate for these losses which can sometimes be total for some thin soil, mineral waste (washing sludge) and organic waste (compost made from shredded household waste) can be used, spreading them one on top of the other[1].

The introduction of earthworms (natural labourers) is a free, natural means of mixing these two layers of waste which are very sensitive to settling, and thus recreate soil worthy of the name.

This was the situation at the Passy quarry for which the Soil Ecology team was brought in during the period 1980-1985. The earthworms were captured by M. B. Bouché in a nearby farmed pasture and reintroduced into the artificial soil, respecting the initial equilibrium between the three ecological categories of the source pasture (epigeic, anecic and endogeic worms). Protective nets were placed over the areas where the worms had been introduced to prevent predation by birds.

The results were spectacular, since one year after introduction, analysis of the casts produced by the anecic worms on the surface of the recreated land displayed an improvement in the main soil fertility indicators.

Surface reconstitution at Passy (Haute-Savoie) © J.-J. Brun

Passy quarry © J.-J. Brun

[1] *Vanpeene S., Piedallu C., Delory I. (2002) - Réaménagement agricole des carrières de granulats*

Result: the reconstitution of an earthworm community restores earthworm quality to reconstituted land by the bioturbation performed by the earthworms

Earthworm biostimulation

A - Reconstituted land

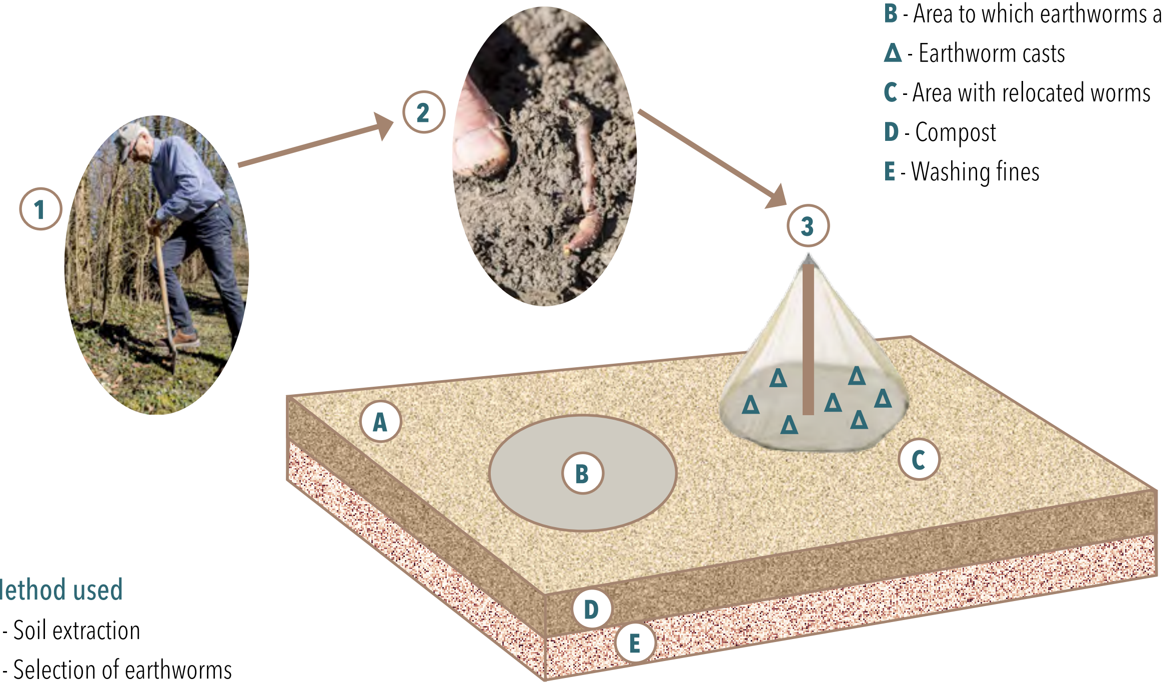
B - Area to which earthworms are relocated

Δ - Earthworm casts

C - Area with relocated worms

D - Compost

E - Washing fines



Method used

1 - Soil extraction

2 - Selection of earthworms

3 - Introduction of the earthworms (protection net)

Anecic earthworm transferred to quarry soil
© J.-J. Brun

Results	
Soil descriptors	*Cast quality/soil quality*
Soil carbon	↗
C/N	↗
Soil stability	↗
pH	↗
CEC (cation exchange capacity)	↗

Article of reference: Brun J.-J., Cluzeau D., Trehen P., Bouché M.B. (1987) - Biostimulation : perspectives et limites de l'amélioration biologique des sols par stimulation ou introduction d'espèces lombriciennes. Revue d'écologie et de biologie du sol, 24 (4) : 685-701

The techniques

- Extraction techniques

  Extraction using formalin

  Extraction using mustard

  Simplified extraction (spade test and manual sorting)

© J.J. Brun

- Methods of recognition

  The standard taxonomic method
  The genetic method

© S. De Danieli

Extraction techniques

There are many ways to capture earthworms all based on the use of a physical vector (manual or instrumental), electric (electrodes) or chemical (solutions) means, the aim of which is to disturb the animals and make them come up to the surface.

Among these techniques are:

1 • vibrations by repeatedly banging the ground,

2 • electric current applied using electrodes planted in the ground,

3 • irritant solutions:

- formalin: exhaustive references but few in number
- mustard: more references but less exhaustive

4 • a volume of soil is extracted using a spade. This is then manually sorted to extract the animals:

- These are simplified evaluation techniques which are not exhaustive but are significantly on the increase for more generalised monitoring of the state of the earthworm population in France

In this booklet we will only present techniques of types **3** and **4**.

Extraction using formalin

Capture in areas of grassland

Earthworms are collected using the Bouché method (1972) and ISO 23611-1 standard, adapted to the agro-pedological context (Cluzeau et al. 1999 and 2003).

This involves spraying an area of one square metre three times with a formalin solution (3 x 10 litres) of increasing concentration (0.25%, 0.25%, 0.40%) at 15-minute intervals.

The earthworms are captured as they emerge from the ground. The surface of the ground is then scratched to a depth of 1 cm to recover any individuals which have not fully emerged on the surface.

Finally, a block of soil (0.25 x 0.25 x depth of 0.20 cm) is dug out for manual sorting to dislodge individuals which have hidden in the most protective clumps of earth.

NB: information given in "Tool worksheet F2" of the ADEME CGDD GESSOL guide entitled "The hidden life of the soil", to which the reader may refer.

Extraction using formalin in a mountain region © J.-J. Brun

Capture in mountain regions

The soil in mountain regions is more heterogeneous in terms of layers colonised by earthworms. Capture may therefore require more spraying and does not include the final extraction of a block of earth.

On grassland plains, soil organisation in layers prevails whereas in the mountains organisation is in slices with fairly deep pockets of soil but which are sometimes spatially disconnected from each other.

The method used is that adapted to the alpine mountain environment by Grossi et al.[1]

The ground surface is cleared of undergrowth over an area measuring one square metre and the perimeter is cleaned somewhat.

The soil is sprayed four times with a solution of water and formalin (0.25%, 0.25%, 0.40%) at 15-minute intervals. Between the third and fourth application, the soil surface is scratched to facilitate penetration of the solution.

[1]Grossi J.L., Chenavier. L., Delcros Ph., Brun J.J. (1995) - Effects of landscape structure on vegetation and some animal groups after agriculture abandonment, Landscape and Urban Planing 31 (1-3): 291-301

Extraction using mustard

NB: Given the environmental toxicity of formalin, capture techniques are increasingly moving towards the use of mustard. The solutions are easy to make by purchasing jars of mustard from the shops.

UMR Ecobio OPVT[1] Univ. Rennes method

At least one month after any work on the soil, the vegetation is cut to ground level over an area of one square metre and the debris is cleared away.

The square is sprayed with a solution of 300 grammes of strong fine mustard diluted in 10 litres of water. Wait 15 minutes.

Spray again with 10 litres of mustard solution on the same square. Wait 15 minutes and capture the earthworms.

Finally a soil block measuring 30 x 30 x a depth of 20 cm is extracted and is crumbled by hand, collecting all the worms still present.

[1] *Participatory Earthworm Observatory*

Irstea Univ. Grenoble Alpes method

The uniqueness of mountain environments is linked to the significant horizontal and vertical heterogeneity of the soil covering. In these conditions, it may be useful to increase the number of sampling patches and the number of spraying applications to improve capture rates.

An area measuring one square metre is selected. It is cleared of undergrowth and the surface is cleaned. It is then sprayed four times with 10 litres of mustard solution at 15-minute intervals to capture the escaping earthworms.

Finally, a block of soil measuring 33 x 33 x a depth of 20 cm extracted from the centre of the square is sorted manually.

Extraction using mustard © J.-J. Brun

Simplified extraction (spade test and manual sorting)

"Earthworm spade test" protocol OSUR[1] Univ. Rennes 1 and OPVT

1 – Position the soil blocks

2 – Extract the 6 soil blocks (20 x 20 x a depth of 25 cm)

3 – Sort the 6 soil blocks

4 – Identify the earthworms in each block in 4 categories: epigeic, epi-anecic, strict anecic, endogeic

5 - Photograph the earthworms

6 – Store the earthworms

7 – Transfer the data to the University of Rennes 1

Extraction of a soil block using a spade © J.-J. Brun

[1] *Sciences of the Universe Observatory at the University of Rennes*

SOLAB[2] Earthworm ITAB[3] and ISARA Lyon[4] protocol

Step 1: sampling and manual sorting

Step 2: identify, count and establish the ecological classes

NB: simplified identification key focusing on skin pigmentation

Manual sorting © J.-J. Brun

[2] *Study of the effects of the different innovative modes of soil management in organic farming on fertility and its assessment methods*

[3] *Institut technique de l'agriculture biologique (Organic Farming Technical Institute)*

[4] *École d'ingénieurs en agronomie, agroalimentaire et environnement (Agronomy, Agrifood and Environment Engineering School)*

Example of the use of simplified techniques for national assessment of the soil earthworm quality in France (1972-2022)

- The # Bouché 2022 participatory earthworm sampling project. The aim of this new national capture campaign is to have an image of the evolution of the earthworm potential in French soil over the 50 years following the initial work produced by M. B. Bouché.
- Project management: TEBIS network on the ecological and biological characteristics of soil organisms.

Methods of recognition

The standard taxonomic method

This method is based on the anatomical observation of animals collected using the techniques described above. The body of the earthworm is cylindrical. It is formed of a succession of similar segments (or rings) between a cephalic lobe (the prostomium, which can be likened to the head) and a terminal lobe.

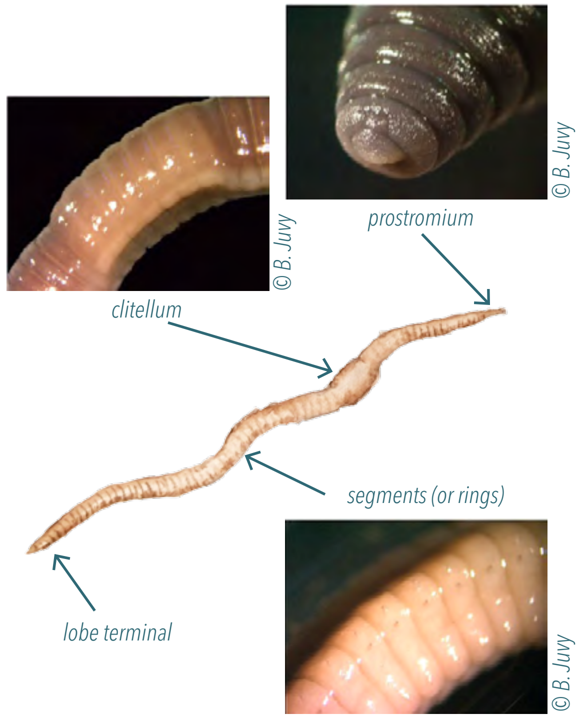
The identification of species is performed on adult individuals. The adult has a more swollen part called the clitellum which is in the form of a "ring". It can be whitish, orange-red or reddish-brown, depending on the species. It becomes orange when individuals are ready to mate.

Ultimately, it is the number of segments separating the clitellum from the first segment (the prostomium), a characteristic specific to each species, which is usually used to identify the species collected[1].

Simplified methods of earthworm identification also exist. They are intended for non-specialists wishing to assess how many of the ecological categories (epigeic, anecic and endogeic) are present in their soil.

For France, the most comprehensive key was produced by the OPVT (Participatory Earthworm Observatory), the fact sheets for which can be downloaded at the following address:

https://ecobiosoil.univ-rennes1.fr/OPVT_documents.php



[1] *Bouché M. B. (1972) - Lombriciens de France : écologie et systématique, Ann. Zool., n° hors série, INRA, 72-2, 672p.*

The genetic method

Traditionally, earthworms are captured manually, counted and identified by specialists. These techniques involving considerable logistics are difficult to implement in the field and take a lot of time (spraying and manual sorting). These constraints are even greater in the mountains (slopes, difficult to access). Recent technical developments in genetics are in the process of revolutionising the world of fauna inventories, particularly for earthworms.

For around a decade, this barcoding technique (use of a fragment of DNA) has proved its worth in the field of taxonomy (the science of naming, describing and classifying organisms). Ecologists have adopted the technique and tested it for earthworms in particular.

Samples of residual DNA in plain soil © J.-J. Brun

Sterilisation of sampling equipment © J.-J. Brun

Earthworms living in the soil leave traces of DNA behind them. These small pieces of DNA are found freely in the environment and are therefore called residual DNA. The first tests on this earthworm residual DNA were performed by the Irstea Grenoble team in collaboration with the team of P. Taberlet at the LECA (alpine ecology laboratory at CNRS, France's national scientific research centre). Their initial results[1] show the complementarity of the methods. Molecular analysis detects two species of endogeic worm which the standard method was not able to recover. Conversely, the standard method detects two species of epigeic worm which escaped the genetic method. In future, the improvement of the genetic method and reduced analysis costs will make this method a valuable asset for the inventory of earthworms in mountain soils.

[1] *Bienert F., De Danieli S., Miquel C., Coissac E., Poillot C., Brun J.-J., Taberlet P. (2012) - Tracking earthworm communities from soil DNA, Molecular ecology, 21, 2017-2030*