# PHUSICOS

## According to nature

Deliverable D.4.5

Evaluation of ecosystems and ecosystem services for alternative landscape scenarios with plan designs

Work Package 4 – Technical innovation to design a comprehensive framework
Task 4.2. 4.2 – Monitoring of ecosystem services

Deliverable Work Package Leader:
UNINA

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## Note about contributors

| | |
|---|---|
| Lead partner responsible for the deliverable: | CREAF |
| Deliverable prepared by: | Pilar Andrés |
| | |
| Partner responsible for quality control: | UNINA |
| Deliverable reviewed by: | Francesco Pugliese |
| | |
| Other contributors: | CREAF, Enrique Doblas, Stefania Mattana, Roberto Molowny, Jordi Vayreda, Joan Pino<br>Universitat de Barcelona, Moisès Guardiola |

## Project information

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| Project coordinator: | Norwegian Geotechnical Institute, (NGI project no.: 20180404) |

## Project partners:

# Summary

This deliverable summarizes the work made by the CREAF team in the framework of WP4. The objectives of the work were:

- To propose soil and vegetation properties sensitive to NBSs addressed to reduce hydrogeological risks in European landscapes
- To determine which of these properties may be used as indicators of effects of the NBSs on fundamental environmental services provided by soil and vegetation. Specifically, we concentrated our efforts on indicators informing about carbon sequestration in soil and in aboveground plant biomass and about biodiversity provision.
- To apply our indicators to predict (ex-ante) the effect of the NBSs proposed in three study cases of the PHUSICOS project: the risk of snow avalanches in the Capet Forest and the instability of the Santa Elena roadcut (both in the Pyrenees), and the pollution of the Massaciuccoli lake by sediments from agricultural soils.

The main conclusions can be summarized as follows:

- In the two study cases in mountain forests, the studied NBSs are expected to improve carbon sequestration in soil and aboveground vegetation. In the agricultural area of the Massaciuccoli lake, the effect may be positive of negative depending on carbon content in soil and on soil texture.
- In the two mountain cases, effects of the NBSs implemented are expected to be positive on plant cover and plant biodiversity.
- Soil microbes and invertebrate functional groups are very sensitive to the NBSs evaluated in this work. Different groups should be used for mountain forests and agricultural areas.

Soil biodiversity is expected to increase as a consequence of the NBSs implemented in the two mountain forest cases. As for carbon sequestration, in the agricultural area of the Massaciuccoli lake, effects of the evaluated NBS will depend on soil initial characteristics (mainly organic carbon content and texture.

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# Appendices

# 1 Introduction

## 1.1 Objective of this report

The aim of this report is to provide a framework to evaluate Nature Based solutions designed to minimize hydrogeological risks for their effect on environmental services whose influence extends beyond the direct impact of the NBS.

The selected environmental services were some of those provided by soil and vegetation. As a starting point, we proposed the indicators shown in Table 1.

*Table 1 Preliminary indicators suggested in the preliminary stages of the project*

| Ecosystem compartment | Ecosystem service | Indicator | Desired evolution |
|---|---|---|---|
| Soil | Below ground C sequestration | Total organic carbon in topsoil | Maximize |
| | | Soil organic carbon chemically protected | Maximize |
| | | Soil organic carbon physically protected | Maximize |
| | Physical resilience | Soil loss by water erosion | Minimize |
| | | Soil resilience to erosion: aggregate stability | Maximize |
| | | Soil water holding capacity | Maximize |
| | Fertility | Soil nutrients | Maximize |
| | | Soil texture | Adequate |
| | Biodiversity provision | Microbial diversity | Maximize |
| | | Microbial functional diversity | Maximize |
| | | Microbial community level physiological profiling | Maximize |
| | | Invertebrate functional diversity | Maximize |
| | | Carbon mineralization by the soil food web | Maximize |
| | | Soil ecosystem stability | Maximize |
| Vegetation | Above ground C sequestration | Above ground carbon stock | Maximize |
| | Biodiversity provision & treats | Plant species diversity | Maximize |
| | | Invasive species | Minimize |
| | Soil protection | Total vegetation cover | Maximize |
| | | Non-Woody plant cover | Maximize |
| | Wildfire risk mitigation | Plant Moisture Index | Maximize |
| | | Plant Flammability Index | Minimize |
| Green Infrastructure | Landscape connectivity / fragmentation | Hanski's Index | Maximize |

At the time, the main questions guiding our work where:

a) How do specific NBSs affect soil and plant carbon stocks and biodiversity?
b) Which are the most sensitive and user-friendly indicators we can recommend for the ex-ante evaluation of effects of a given NBS?

c) Are soil and plant indicators of universal application to any type of NBS and environment?
d) For a given indicator, can we stablish universal desirable trends and reference values?

To address these questions, we selected three demonstrator cases of the PHUSICOS project in which the previewed solutions were expected to alter significantly the aboveground and belowground carbon stocks and biodiversity.

Among the available cases meeting these criteria, we selected those for which a detailed description of the NBS to be applied was available before the end of 2020.

In this sense, a thorough understanding of the operations and the final solution is essential to correctly design the field samplings necessary to describe the baseline (the pre-operative value) of the indicators, as well as to simulate their post-operation evolution and expected values in the medium and long term.

Moreover, given the manifest seasonality that characterizes temperate climates and its significant effect on plant and soil activity, the value of most indicators significantly varies with seasons, and sampling campaigns must be carefully scheduled if the value of the indicators has to be representative of the functioning of the ecosystem.

Eventually, the cases that best fitted our requirements were:

- Reforestation of the upper parts of the Capet Forest (Barèges, French Pyrenees)
- Stabilization of a roadcut in Santa Elena (Biescas, Spanish Pyrenees)
- Erosion in the agricultural area around the Massaciuccoli lake (Lucca province, Italy)

## 1.2 Work method

At each site, our work can be drawn as shown in Box 1.


*BOX 1. Work scheme*

At the time of writing, the solution finally chosen to stabilize the roadcut of Sta Elena has not yet been implemented. In the cases of the Capet Forest and the Massaciuccoli lake, the solutions were completed in the second half of 2021, but the measures have not yet had time to take effect, due to the slow response of soil and vegetation to manipulation.

In this sense, it is worth to note that, when soil is very disturbed, it is not advisable to initiate the monitoring plan immediately after the implementation of the measure. Works cause great disturbance in the soil-plant system and the value of most indicators at the end of the operations will most probably indicate deterioration instead of progress. Therefore, the reference state to evaluate the effect of the applied measures must be the pre-operative baseline of the indicators and not their port-operation value.

## 1.3 Soil and plant indicators: meaning, previewed evolution, and methodology

Based on the preliminary field visits to the three study sites, we reconsidered the pertinence of the indicators proposed at the beginning of the PHUSICOS project. The resulting list of soil and plant properties that we finally studied in the project for their potential use as indicators is shown in Table 2.

Some of these properties resulted of low interest for their study in a particular site or were evaluated in specific ways depending on the site. The reason for these decisions will be explained in specific sections of this report.

The effects of the studied NBSs on landscape connectivity were not assessed because of the low spatial extent of the measures which minimizes their impact on the green infrastructure of the landscape

Finally, we did not calculate the indicators informing about effects on wildfire risk because, as explained in Deliverable 4.2, they vary at each dry season depending on plant cover but also on the year's special weather conditions. Therefore, the wildfire risk must be estimated every fire season at the Capet Forest and the Sta Elena roadcut. These indexes are not applicable to the case of the Massaciuccoli lake.

The following sections are intended to clarify the meaning of our indicators.

Soil indicators are particularly hard to explain because soil is a very cryptic environment. Therefore, talking about soil is talking about concepts that are totally new for most people and, what is worse, it means talking about things for which there is not a sensorial register in people’s brains.

Whereas everybody has visual and olfactive images and concepts associated to words as “open air” or “tree”, “soil porous space” or “root environment” (in fact their below-ground equivalent) do not evoke anything, because people has never accessed the underground.

*Table 2 Soil and plant properties considered for their potential use as indicators*

| SECTOR | ECOSYSTEM SERVICE | INDICATOR | UNITS | Applicability to the study cases | | |
|---|---|---|---|---|---|---|
| | | | | Capet forest | Sta Elena | Massac. |
| Soil | Carbon sequestration | Total organic carbon stock (Total $C_{org}$) | $g\ C\ .\ m^{-2}$ | YES | YES | YES |
| | | Labile organic carbon ($C_{org}$ in the fast pool) | $g\ C\ .\ m^{-2}$ | YES | YES | YES |
| | | Recalcitrant organic carbon ($C_{org}$ in the slow poo | $g\ C\ .\ m^{-2}$ | YES | YES | YES |
| | | Physically protected organic C | % versus Cmin | YES | YES | YES |
| | Soil physical resilience | Soil erodibility (aggregate stability) | mm | YES | YES | YES |
| | | Water erosion | t soil $ha^{-1}\ yr^{-1}$ | NO | NO | NO |
| | Soil fertility | Soil bulk density | $g\ cm^{-3}$ | YES | YES | YES |
| | | Nitrogen content | % | NO | NO | NO |
| | | Content of calcium, magnesium, phosphorous, potassium, sodium | $mg\ kg^{-1}$ | NO | NO | NO |
| | Biodiversity provision | Microbial diversity | | | | |
| | | Microbial species richness (bacteria) | number sps | YES | YES | YES |
| | | Microbial species diversity (bacteria) | unitless | YES | YES | YES |
| | | Microbial species eveness (bacteria) | unitless | YES | YES | YES |
| | | Microbial catabolic diversity | unitless | YES | YES | YES |
| | | Invertebrate functional diversity | | | | |
| | | Flagellates | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Amoebae | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Ciliates | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Total protists | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Bacterial feeder nematodes | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Fungal feeder nematodes | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Plant-feeder nematodes | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Omnivore nematodes | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Predatory nematodes | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Total nematodes | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Predatory Mites | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Nematophagous Mites | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Nematophagous prostigmatic mites | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Collembola | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Fungivorous cryptostigmatic mites | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Fungivorous Prostigmata | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Diplura | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Symphyla | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Protura | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | | Total microarthropods | $mg\ C\ g^{-1}$ soil | YES | YES | YES |
| | Biodiversity functions | Carbon mineralization by the soil food web | $g\ C\ m^{-2}\ y^{-1}$ | YES | YES | YES |
| | | Theoretical soil food web stability | $y^{-1}$ | YES | YES | YES |
| Plants | Aboveground C sequestration | Total aboveground carbon stock | $t\ C\ ha^{-1}$ | YES | YES | NO |
| | Biodiversity provision & treats | Species richness | number sps | YES | YES | NO |
| | | Species diversity | unitless | YES | YES | NO |
| | | Eveness | unitless | YES | YES | NO |
| | | Invasive species | Number ofspecies | YES | YES | NO |
| | Wildfire risk mitigation | Plant moisture index | | NO | NO | NO |
| | | Plant flammability index | | NO | NO | NO |
| | Soil protection | Soil vegetation cover | % | YES | YES | NO |
| Green Infrastructure | Landscape connectivity | Hanski's Index | unitless | NO | NO | NO |

## 1.4 Soil Indicators

### 1.4.1 Indicators informing about carbon sequestration in soil

Atmospheric carbon dioxide ($CO_2$) is removed from the atmosphere through photosynthesis, a process by which gaseous $CO_2$ is fixed by plants and retained in their body (the living plant biomass).

The removed carbon will be later delivered by plants to soil in the form of root exudates by the living plants, and eventually in the form of dead plant debris, including death roots. Soil microbes feed on these organic debris and return to the atmosphere a portion of the carbon they contain as $CO_2$ through respiration. Other fractions of the organic carbon that came into soil, together with secondary metabolites resulting from microbial metabolism of the primary organic residuals, remain in soil protected from microbes' attack by different physical and chemical mechanisms.

The quantity of organic carbon that a given soil can hold and the length of time this carbon stays in the soil before returning to the atmosphere (as gas) or water (in a dissolved form) determines soil capacity for "carbon sequestration".

The total quantity of organic carbon that a given soil can hold eventually depends on "soil texture", the proportion of mineral particles of different size (clay, silt and sand - from the smallest to the greatest)- in a soil, and on the thickness of the soil profile. Soil texture and soil thickness are assumed to be stable for a given soil at the human time scales, although the two of them can be affected by erosion in the long term due to climate change, management and catastrophic hydrogeological events.

At the human time scale, the length of time carbon remains in the soil varies significantly depending on the type and composition of the plant cover and on land management. Plants that contain high proportion of carbon in an unattractive chemical form for microbes (such as lignin and aromatic or phenolic compounds) will provide to soil "recalcitrant" debris of long residence time; plants with high proportion of carbon in forms attractive as food for microbes (such as sugars) will provide to soil "labile" carbon that will be quickly eaten (Jastrow et al., 2007)

Since the industrial revolution, global emissions of carbon to the atmosphere have been estimated at about 270 Pg from fossil fuel combustion and about 136 Pg due to land use change and soil cultivation. The depletion of the soil organic carbon (SOC) pool has contributed about 78 Pg C to the atmosphere, and this depletion is being exacerbated by soil degradation caused by land mismanagement (Lal, 2004; Lal et al., 2015). Globally, soil organic carbon stocks are estimated at an average of 1500 Pg C in the upper 1 m of soil (Scharlemann et al., 2014), which is more carbon than is contained in the atmosphere and terrestrial vegetation combined (FAO & ITPS, 2015).

Thus, restoring degraded soils or implementing nature-based solutions leading to recover proper soil-plant systems will contribute to remove CO2 from the atmosphere while enhancing other environmental services, such as biodiversity provision (Jackson et al., 2017).

Soil carbon content (a carbon pool) and carbon sequestration in soil (a process) are different things. Effects of a given NBS on soil carbon content may be directly measured from soil samples. Effects on carbon sequestration are more difficult to assess and monitor, because changes in soil C stocks are slow and imply deep soil layers.

The following is a short description of the indicators of carbon content and carbon sequestration in soil that we have used in the PHUSICOS project

#### 1.4.1.1 Total organic carbon in topsoil

Definition and relevance of the indicator. Total organic soil carbon is the sum of three carbon forms: elemental carbon (graphite and soot derived from natural or anthropogenic incomplete combustion), inorganic carbon (mostly carbonates and bicarbonates) and organic carbon.

Organic carbon is the main component (58% in average in topsoil) of soil organic matter (SOM) and is often used as a proxy for it. SOM is made up of plant and animal materials in various stages of decomposition, of microbial cells and microbial products, and is vital for soil to maintain its functions and to correctly deliver its ecosystem services. SOM turnover plays a crucial role in soil fertility, terrestrial ecosystem functioning and global warming mitigation. Organic matter is critical for the stabilization of soil structure, retention and release of plant nutrients, and maintenance of water-holding capacity and, hence, is a key indicator for agricultural productivity and environmental resilience (Lefèvre et al., 2017). Therefore, soil organic carbon content is a fundamental parameter in the calculation of soil quality indexes and is always included in post-restauration monitoring programs (Mukhopadhyay et al., 2014).

Expected evolution of the indicator. Land use intensification and land degradation result in losses of soil organic carbon (Matson et al., 1997), and highly degraded terrestrial ecosystems typically have soils with low organic carbon content. Inversely, soil quality assessment of post-restauration chronosequences shows that soil organic carbon increases with reclamation time as vegetation recovers (Mukhopadhyay et al., 2014). Therefore, in our study cases, soil organic C is expected to increase over time in the areas restored with NBSs. As ecosystem recovery progresses, the SOC values in these zones are expected to converge with those measured in the mature land units chosen as a reference for the middle and long-term scenarios. In Europe, organic carbon content in the topsoil ranges from <1% in degraded soils and natural soils of the arid and semi-arid zone, to >70% in organic soils, mainly located in the northern cool and wet regions (de Brogniez et al., 2015) and in peat soils under warmer climate (see the case of the Massaciuccoli study case in this report).

Recommendations for the monitoring program. Follow the evolution of soil organic carbon every 5 years.

Analysis method. Soil organic carbon has been measured in this project from soil samples taken at 0-15 cm depth. Before analysis, the samples were air-dried, homogenized and sieved at <2mm. The analytical process starts with the elimination of all inorganic carbon by acidification of the sample. The resulting product is then totally oxidized by combustion with pure oxygen at about 1000 ºC. The resulting $CO_2$ is transported by helium, separated in a selective column and measured in an elemental micro-analyser.

#### 1.4.1.2 Carbon sequestration in soil: fast, slow, and recalcitrant carbon fractions

Definition and relevance of the indicator. Soil organic matter mineralization (or "decomposition") is the consequence of microbial nutrition and metabolism. Heterotrophic microbes exploit soil organic matter as a source of energy and, as a result of their metabolism and respiration, a part of the carbon contained in the soil organic matter is released as $CO_2$. The quantity of carbon respired as $CO_2$ per unit of soil weight and time is the SOM decomposition rate ($k$) that highly depends on microbial biomass and activity and on SOM quantity and quality.

SOM is composed by a great variety of chemical forms that show different decomposition rates. Three different fractions can be identified: (a) a small but very active *labile* fraction that is very actively utilized by the micro-organisms (1% to 5% of total SOM); this fraction originates from new residues and living organisms (including dead micro-organisms) and has a turnover within some weeks and about 3 years, (b) a *slow* fraction, with a turnover of 20-40 years, consisting of organic compounds that are either chemically resistant to decomposition or physically protected, and (c) a large *passive* fraction chemically stable with a turnover greater than 2500 years.

The labile fraction (also called "fast C pool") is extremely sensitive to changes in plant composition and activity, climate and management, and the slow fraction is very responsive to soil manipulations that disrupt soil physical structure (such as tillage). The passive pool is the least likely to be influenced by changes in management practice.

Expected evolution of the indicator. Carbon decomposition rates can evolve within a few hours depending on local microclimate (soil temperature and water content) and resources availability, and are extremely sensitive to changes in climate, land use and soil management. However, this sensitiveness does not affect with the same intensity the entire soil C pool, being the labile fraction much more responsive to management than the more recalcitrant parts (Conant et al., 2012). Therefore, the proportion of soil organic C belonging to different recalcitrance/lability classes is highly informative of carbon stability in soil and of soil potential for carbon sequestration. Early impacts of management on soil carbon dynamics and on soil capacity to supply nutrients to plants can be assessed based on changes in the proportion of total soil carbon that is labile. However, recalcitrant C is the bests indicator of carbon sequestration in soil in the middle and long term.

The proportion of labile soil carbon (related to total C) fraction is hight under herbaceous vegetation, and the amount of the recalcitrant fraction is expected to increase as plant cover matures and includes increasing proportions of woody species (Haynes, 2000, Pregitzer & Euskirchen, 2004).

Recommendations for the monitoring program. It can take several decades before effects of afforestation or cessation of cultivation on SOC pools can be observed in deep soil horizons (Shi et al., 2013), but effects on the labile fraction of the upper organic soil layers are measurable after 3 to 5 years. The size of the recalcitrant and labile soil C pool should be included in the monitoring plans every 5 years. The proportion of labile C exhibits great seasonal variability associated with changes in soil moisture, rainfall, temperature, rhizodeposition and leaf fall (Haynes, 2005) and, therefore, sampling must always be made in the same period of the year in order to make measures comparable. Samples should always be taken at the same depth, preferably in the upper 0-30 cm of the soil. When working in agricultural ecosystems, and in particular when changes in crop type and tillage regime are implemented, soil sampling for C sequestration should include total root profile or, at least, the upper soil 30 cm. In both cases, sampling must be spatially stratified, with samples taken above and below tillage level.

Analysis method. There are several methods to calculate the fractions of soil organic C belonging to the active, slow and stable pools. In this project, and depending on the soil type, we have applied two of them: (a) long-term soil incubations, and (b) organic matter digestion with increasingly aggressive acids.

### Soil incubation

Long-term soil incubation under optimal conditions (a constant temperature of 25 ºC and about 50% of soil water holding capacity) is the cheapest method to measure the relative abundance of soil C pools, although the analyses can take many months in organic soils. The method demands putting a known quantity of soil to respire into a closed bottle and measuring the $CO_2$ evolved at increasing time intervals. In general terms, the rate of $CO_2$ production over time (e.g. $CO_2$-C $g^{-1}$ $day^{-1}$) follows an exponential decay curve from which the size of the three C pools and their independent decomposition rates may be calculated as follows (Robertson & Paul, 2000):

$$\text{Mineralized C} = k_1\,(C_1 e^{-k1t}) + k_2\,(C_2 e^{-k2t}) \qquad [1]$$

where $C_1$ is the C content of the labile C pool, $k_1$ is the decomposition rate constant for the $C_1$ pool, $C_2$ is C content of the slow turnover pool, $k_2$ is the decomposition rate constant for the intermediate pool, and $t$ is incubation time in days.

To calculate the size of the recalcitrant pool ($C_3$), an analysis of total organic carbon (TOC) is required. The recalcitrant pool can be then calculated as:

$$C_3 = TOC - (C_1 + C_2) \qquad [2]$$

### Soil acid hydrolysis

We have also used a two-steps $H_2SO_4$ acid hydrolysis procedure (Rovira & Vallejo, 2002, slightly modified) to determine the size of the soil labile C pool and of the recalcitrant C pool. Briefly, soil samples are first hydrolysed with 2.5 M $H_2SO_4$ at 105 °C for 30 min. Subsequently, the remaining residue is hydrolysed with 13 M $H_2SO_4$ and shaken overnight at room temperature. Then, distilled water is added to dilute the acid concentration to 1 M, and the sample is hydrolysed at 105 °C for 3 h. The hydrolysate is regarded as labile pool. The remaining soil residue is rinsed twice with distilled water and dried at 60 °C. This fraction is considered the recalcitrant SOM pool. The C concentration in the labile and recalcitrant C pools are determined using a TOC analyser.

#### 1.4.1.3 Physically protected organic carbon

Definition and relevance of the indicator. Stabilization and of organic carbon in soil aggregates is a key mechanism of physical protection of soil carbon against microbial attack. An increase in SOM is generally associated with an increase in C found in macro-aggregates, and long-term carbon sequestration depends on its stabilization in soil micro-aggregates (Six et al., 2000).

Expected evolution of the indicator. Revegetation, soil restoration and soil conservation practices are expected to enhance the passive C pool and to improve soil structure. Hence, we expect increasing carbon incorporation into soil micro-aggregates.

Recommendations for the monitoring program. Carbon physical protection in soil aggregates should be evaluated every 5 years.

Analysis method. Before analysis, the samples were air-dried and sieved at <5mm. We have used the wet sieving method to separate the aggregates into three size classes: macro-aggregates (>212 µm), meso-aggregates (53–212 µm) and micro-aggregates (20–53 µm) (Klute et al., 1986). For each size class, we determined TOC and calculated the proportion of physically protected C as the ratio between C in crushed samples and C in undisturbed samples. We measured carbon physically protected in soil stable meso-aggregates (those resistant to wet sieving) as the difference of SOC mineralization in crushed aggregates (aggregates disrupted, SOC not protected and mineralized) and intact aggregates (intact aggregates, SOC protected). Briefly, 2 g of soil samples were placed into a 0,4 jars and soil moisture was maintained with deionised water at 40% of WHC. $C0_2$ accumulated in the jar was measured regularly for 21 days using an infra-red gas analyser (IRGA).

### 1.4.2 Indicators informing about soil physical resilience

#### 1.4.2.1 Soil aggregate stability as a proxy measure for soil erodibility

Definition and relevance of the indicator. Soil aggregate stability is a key indicator of the stability of soil structure that also informs about soil resistance to physical degradation. In general, soil structure depends on the presence of stable soil aggregates. Soil aggregates, that are the basic units of the soil structure, are made of primary mineral particles held together by diverse organic and inorganic binding agents (including diverse microbial polysaccharides and proteins, root airs, fungal hyphae, etc.). The stability of an aggregate is its ability to remain stable under physical stresses such as soil tillage, soil swelling and shrinking processes following wetting/drying cycles and, in general, any mechanical or physical or chemical event promoting aggregate disintegration. Structure is an important soil propriety with direct influence on soil ecosystem services such as supporting plant growth and animal life, sequestering atmospheric $CO_2$ and guaranteeing water quality (Bronick & Lal, 2005). Favourable soil structure and soil aggregate stability are important factors that influence soil fertility, preserve soil productivity, and reduces soil susceptibility to erosive events.

Expected evolution of the indicator. Land use intensification and land degradation usually result in declining soil structure and increasing soil erodibility (Bronick & Lal, 2005). Therefore, in the PHUSICOS study cases, the proposed NBSs are expected to improve soil structure over time and, consequently, to increase aggregate stability.

Recommendations for the monitoring program. Analyse soil aggregate stability every 5 years.

Analysis method. We have measured aggregate stability from soil samples taken at 0-15 cm depth. Before analysis, the samples were air-dried, homogenized, and sieved at <5 mm. We have used the wet sieving method to evaluate aggregate stability of macro-aggregates (mesh at > 212 µm), meso-aggregates (53–212 µm) and micro-aggregates (20–53 µm) (Klute et al., 1986). We have calculated one index expressing soil aggregate stability: mean weighted diameter (MWD) (Le Bissonais, 1996) The MWD (mm) of water-stable aggregates was calculated using the following equation:

$$MWD = \sum_{i=1}^{n} \mathrm{w}i\bar{X}i \qquad [3]$$

where $\bar{X}i$ is the mean diameter of each size fraction (mm) and $w_i$ the proportion of total water-stable aggregates in the corresponding size fraction.

The mean weight diameter (MWD) is a measure of the size distribution of the stable aggregates that remained on each sieve after the wet sieving.

#### 1.4.2.2 Soil loss by water erosion: specific approaches by study case

Definition and relevance of the indicator. Soil erosion can be defined as the accelerated removal of topsoil from the land surface by water, wind, or tillage (FAO, 2015).

Water erosion is estimated to be the most extensive form of erosion occurring in Europe. At each site, the extend of water erosion depends on several factors, including rainfall erosivity (or aggressiveness), land surface slope, soil plant cover, land management and soil erodibility (or susceptibility to erosion) that, in turn, emerges from soil intrinsic properties such as texture and organic matter content. Silty soils are the most erodible, and organic matter protects soil against erosion. Soil plant cover and land use are among the most important factors explaining the intensity of soil erosion, by far ahead of rainfall intensity and slope (García-Ruiz, 2010).

Deforestation, overgrazing, and construction of buildings and roads are among the most powerful erosive factors in mountains while, in agricultural zones, inappropriate management causes critical soil losses and contributes to water pollution by sediments and associated chemicals (Grimm et al., 2001). The mean rate of soil loss in European erosion-prone lands (agricultural, forests and semi-natural lands) is about 2,46 t $ha^{-1}$ $yr^{-1}$, resulting in a total soil loss of 970 Mt $yr^{-1}$ (Panagos & Borrelli, 2017). Reported rates of soil formation are within a range of approximately 0,3 to 1,45 t $ha^{-1}$ $yr^{-1}$ for European soils (Verheijen et al., 2009), which can be considered the maximum tolerable erosion rate to maintain a stable soil pool. Since the informed soil loss rates are 1,7 to 8,2 times higher than soil formation rates, erosion control is a priority in environmental protection.

<u>Analysis methods</u>. We used different approaches depending on the characteristics of each study case.

We found severe difficulties when trying to measure the baseline of the erosion levels in the Sta Elena roadcut. Due to the extreme angle of inclination of the roadcut, we were not able to find appropriate erosion models applicable to this case, and other approaches, based on stability models, were followed by other members of the PHUSICOS consortium. An alternative way to estimate the pre-operative exportation of soil sediments from the roadcut would have consisted at intercepting the drainage ditch at the base of the roadcut during rain episodes of diverse intensity and at weighting the exported sediments in relation with the rain characteristics of every event. But applying this method was complicated and risky in a road in operation. Therefore, erosion was not calculated in this study case.

In the study case of the Massaciuccoli Lake, field measurement (by means of erosion micro-plots) of sediments exported from cultivated plots to the drainage system would have been desirable to settle the baseline of the erosion before implementing the NBS. Temporal and financial restrictions prevented our research team from applying this method. Pre-operation and post-operation erosion were therefore simulated.

<u>Expected evolution of the indicator</u>. Since soil plant cover plays a key role in erosion control, increasing soil protection by revegetation of mountain slopes is expected to result in decreasing erosion rates. In the Massaciuccoli case, sediment exportation during rain events is expected to diminish due to the impact of the VFS on runoff.

<u>Recommendations for monitoring programs</u>. Erosion taxes may be higher immediately after NBS application than just before, due to soil disturbance by works and slow development of the protective plant cover. To correctly evaluate the effect of the NBSs on this parameter, erosion values should be registered before NBS implementation, immediately after application and then yearly during five years in the Massaciuccoli lake and the Santa Elena roadcut. In the case of the Capet Forest, where soil and plant cover

disturbance have been minimal during operations, erosion can be assessed every two years after NBS application.

### 1.4.3 Indicators informing about soil fertility

In preliminary reports, we had placed great importance on providing indicators of soil fertility, defined as "the ability of a soil to sustain plant growth by providing essential plant nutrients and favourable chemical, physical, and biological characteristics as a habitat for plant growth".

From the specific characteristics of our three study cases, it is clear that soil fertility, with the abovementioned agronomical focus, is not a soil service that can be significantly affected by the applied solutions.

In the specific case of our study cases, soil bulk density and nutrient concentration in soil, including nitrogen) have been used as soil properties useful to explain the behaviour of the calculated indicators. Therefore, we have measured these properties in soil in the three cases. In particular, measuring soil bulk density is necessary to extrapolate lab results to the landscape scale (i.e. to transform soil C content from g C per 100 g soil, to g C per $m^2$ at 15 cm depth).

In preliminary reports we had also proposed measuring soil water holding capacity, as an indicator of soil ability to provide water to plants. This is an important indicator in arid and semiarid ecosystems, where drought limits plant production and therefore the amount of C that enters the soil system. However, climogrames show that hydrological deficit is not a concern in the two study cases of the Pyrenees and that, in the Massaciuccoli area, soil is flooded a part of the year and is irrigated during the dry season. Nonetheless, we performed preliminary soil analyses that showed no differences in soil water holding capacity between soils degraded to varying degrees at each of our study sites. Therefore, the indicator was discarded.

### 1.4.4 Indicators informing about biodiversity provision by soil

<u>Definition and relevance of the indicators</u>. Soil harbours a large part of the world's biodiversity. By far the most abundant group of organisms are soil microbes (e.g., viruses, bacteria, archaea and fungi) that, together with soil invertebrates (mainly protozoa, nematodes, mites, springtails, enchytraeids and earthworms), underlie crucial soil ecosystem processes, such as carbon sequestration, water cycle regulation, nutrient cycling, plant diversity regulation, decontamination and bioremediation, pest control or plant and human health (Turbé et al., 2010). Soil biodiversity evaluation is particularly important to estimate the ability of the ecosystem to respond to changing environmental conditions and to assess its resilience and sustainability.

#### 1.4.4.1 Soil microbial diversity

<u>Definition and relevance of the indicators</u>. Soil microbial communities play a pivotal role in terrestrial ecosystems by reintegrating essential nutrients into biogeochemical

cycles, and by regulating plant growth and the quality of the atmosphere and the hydrosphere. Microbial functional diversity can be defined as 'the sum of the ecological process, and/or the capacity to use different substrates developed by microorganisms of a community' (Nannipieri et al., 2003; Campbell et al., 2003). The diversity of functions performed by the organisms within ecosystems has been recognized as the missing link between biodiversity patterns and ecosystem functions. There is growing recognition that patterns of functional diversity can provide more powerful indication of ecosystem health than taxonomic richness.

Metagenomic analysis is a remarkable tool for studying the taxonomic composition and functional capacities of the soil microbial community. Among all available metagenomic techniques, "Shotgun Metagenome Sequencing" can reveal taxonomic profiling (diversity and abundance), as well as the functional attributes of soil microbes. Functional gene analysis is included in the list of powerful indicators aimed to monitor soil biodiversity and ecosystem function across Europe (Griffiths et al., 2016).

In PHUSICOS, effects of the NBSs on soil microbial diversity have been studied by shotgun sequencing of soil microbial DNA. We performed these analyses on soil samples taken at 0-15 cm depth and stored at -20ºC. DNA was extracted from soil with the Soil Microbe Microprep Kit (Zymo, USA, https://www.zymoresearch.com/), purified and quantified by PCR before sequencing with Illumina HiSeq 2500 technology getting 2 x150 paired-end reads.

We studied microbial biodiversity from two complementary approaches: the taxonomic and the functional approach. The taxonomic approach seeks to identify microbial species present in soil samples. Since knowledge on soil microbial species still is very poor, identification must be done at different taxonomic levels. The functional approach aims to identify the presence of microbial genes that encode proteins that in turn perform specific functions in soil metabolism. This functional approach was performed on 16s rRNA sequences and informs specifically about environmental functions performed by soil prokaryotes (bacteria and archaea).

From the taxonomic approach, we calculated the following indexes:

**Microbial species richness** informs about the number of microbial taxa

**Microbial species diversity** (Shannon index, Shannon, 1949) informs about the number of microbial taxa and about the relative abundance of each taxon. We calculated the Shannon index as:

$$H' = \Sigma\, p_i \cdot \ln(p_i) \qquad [4]$$

where, for each taxon found in a soil sample, $p_i$ is the proportion of total microbial DNA contributed by this taxon to total microbial DNA extracted. The Shannon index is 0 when all DNA belongs to a unique taxon, increases with the number of taxa, and is maximized for a given number of taxa when proportions are equal.

**Microbial species evenness** informs about how evenly or unevenly the relative abundance of microbial taxa is distributed in soil. We calculated evenness as:

$$J' = \frac{H'}{H'_{max}}, \qquad [5]$$

with $$H'_{max} = -\sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S \qquad [6]$$

were S in the total number of taxa.

J' ranges from 0 and 1. The more dominant a single species is (or a few species are), the lower is J', and J' increases with the equitable sharing of species abundance.

Recommendations for the monitoring program. Soil microbial biodiversity should be monitored every five years, following the same calendar proposed for soil invertebrate biodiversity (see section 2.1.6).

Expected evolution of the indicators. It is not easy to propose an optimal pattern of evolution for the post operational values of S, H' and J'. In fact, microbial species composition, and the characteristics of the microbial consortium are more significant than the absolute number of species or their relative abundance. Together with these synthetic indexes a statistic study of the microbial community is required (see examples of this approach in the sections corresponding to the study cases).

#### 1.4.4.2 Microbial catabolic diversity

Definition and relevance of the indicator. Soil microbial catabolic diversity is often known as *Soil microbial community level physiological profiling (CLPP).* Community-level physiological profiles of the soil microbial community can be assessed by measuring microbial utilization of a wide range of carbon sources. As said before, organic carbon appears in soil under a variety of chemical forms of different lability/recalcitrance depending, among other factors (such as how long carbon has been in soil, the metabolic transformations it has undergone, etc.), on the maturity and composition of the plant cover that determines the chemical composition of plant debris. Since soil and plants evolve together, the ability of soil microbes to decompose carbon species of different recalcitrance is an indicator of the maturity of the plant-soil system.

We have evaluated the affinity of soil microbes for diverse carbon chemical species by the MicroResp™ test (Campbell et al., 2003), that is included in the list of the most powerful indicators recommended to monitor soil biodiversity and ecosystem functions across Europe (Griffiths et al., 2016). Brief, this method measures microbial respiration rates induced by a range of diverse carbon sources (Chapman et al., 2007). The amount of carbon utilised indicates the quantity of microbial biomass able to utilise a specific carbon source. The greater the diversity of the microbial community the wider the range of carbon source utilisation.

Recommendations for the monitoring program. Repeat the analysis every 5 years.

Expected evolution of the indicator. We posit that the ability of the soil microbial community to metabolize increasingly recalcitrant chemicals will increase with time and ecosystem maturation after the application of the proposed NBSs.

Analysis method. The Microresp™ assay was applied to soil samples taken at 0-15 cm depth, sieved at <2 mm and stored at 4 ºC. Soils were adjusted to 40% of their WHC and loaded into 1,2 ml deep-well plates (ca. 0,35 g soil per well). Subsequently, the samples were stored for 5 days at 25 ºC within a $CO_2$ trap, as recommended by the fabricants. Physiological profiles were determined using 15 different sources of carbon: two simple sugars (D-glucose, D-fructose); one disaccharide (sucrose); one polysaccharide (cellulose); three amino acids (γ-aminobutyric acid, L-proline, L-arginine); three carboxylic acids (α-ketoglutarate, citric acid, L-malic acid); one aromatic carboxylic acid (protocatechuic acid); one polymer (a-cyclodextrin); one chiral (mannitol), one polyol (glycerol) and one sugar alcohol (meso-erythriol).

#### 1.4.4.3 Soil invertebrate functional biodiversity

The abundance and variety of belowground organisms is overwhelming (Figure 1), and often very difficult to handle even by experts. The dimensions of soil microbial diversity are probably the best known. Bacteria and archaea amount to 4 to 10 x $10^9$ genome equivalents per $cm^3$ of soil, and fungi to 200-235 OTUs (operational taxonomic units) per gram of soil.

However, the diversity of soil invertebrates is less often considered, though 1 $m^2$ of soil can shelter up to 12.000 to 311.000 enchytraeids, 1 to 5 x $10^4$ collembolans, and 1 to 10 x$10^4$ oribatid mites (Bardgett & Van Der Putten, 2014) among other less abundant groups.

Since soil microbes provide most soil living biomass (together with plant roots), they are the main direct contributors to soil respiration and carbon transformation. However, microbial communities are top-down controlled to a great extent by soil invertebrates that feed on them. These invertebrates are also key actors in creating and maintaining soil structure at different levels (from soil aggregates to soil aeration and drainage channels) and in making a suitable environment for microbial development.

A practical way of reducing this complexity to handy levels is to substitute taxonomic diversity by functional diversity, i.e. by the diversity of guilds of organisms that share similar characteristics, realize the same functions and show similar metabolic or behavioural responses to important environmental factors (such as temperature or water availability). In fact, it has been argued that it is functional diversity rather than taxonomic diversity that is important for the long-term stability of an ecosystem (Walker, 1992).

In PHUSICOS, we have approached soil invertebrate diversity from a functional perspective and following a food web perspective.

*Figure 1 Living forms frequently found in soil. Sources: (a) [https://esdac.jrc.ec.europa.eu/themes/soil-biodiversity](https://esdac.jrc.ec.europa.eu/themes/soil-biodiversity); (b) Geisen et al., 2019.*

<u>Definition and relevance of the indicators</u>. Soil trophic webs depict food relationships between different groups of the soil biota (basically, who eats whom and how much each one eats of the other) and, therefore, the forces predators exert on their prey and vice versa (Moore et al., 1988). In their simplest form, food webs picture links between feeding guilds (trophic species) by drawing arrows between prey and predator (Scheu, 2002). An example of this representation is shown in Figure 2.

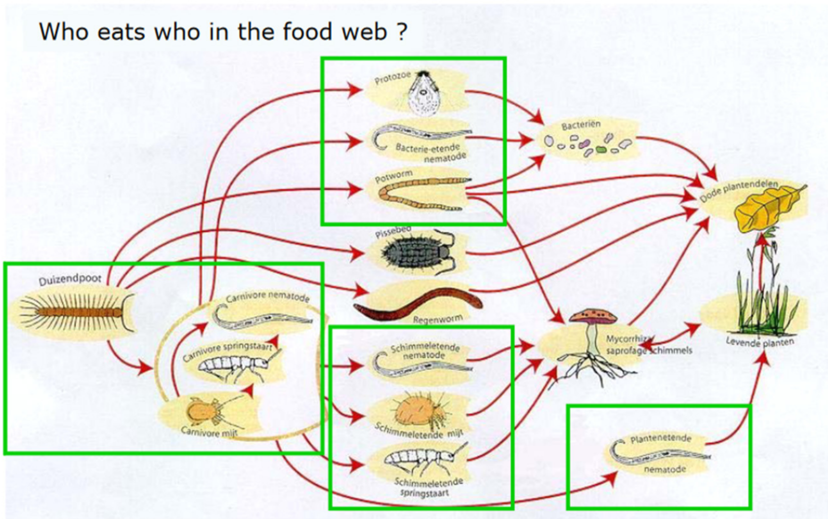


**Figure 1. Diagram of the soil food web**
*The groups not shown in a box are those involved in decomposition processes and primary production. The groups in green boxes represent microherbivores, fungal-feeders and bacteria-feeders. The last box on the left represents the predators and top-predators (figure from R. de Goede).*

*Figure 2 Simplified scheme of the soil food web. Source: Rutgers et al., 2008.*

A key advantage of this ordination of soil biodiversity is that the flux of carbon through the soil biological system can be simulated from the biomass of each trophic group (calculated from field samples) and their chemical and metabolic characteristics. Soil food webs normally include the following trophic groups, that are almost always present in soils: bacteria, fungi, protists (flagellates, amoeba and ciliates), nematodes (plant feeders, bacterial feeders, fungal feeders, predatory and omnivores), springtails, and detritivore, fungivore and predatory mites. Any other less ubiquitous invertebrate group found in a relevant percentage of the soil samples must also be included.

Some of the groups used to build up trophic web models, as well as the relative importance of their biomass can also be independently used as indicators of soil quality, maturity, and post-disturbance recovery. Good ecosystem response indicators can be extracted from nematodes (Neher, 2001), protists (Foissner, 1999) or fungal to bacterial biomass ratios (Bailey et al., 2002).

In PHUSICOS, we have tested the response of 17 trophic groups of soil invertebrates (see the list in Table 2) fort their possible usefulness as bioindicators of NBS effects.

<u>Analysis method</u>. To prepare data for their future use in food web modelling, we expressed the abundance of each trophic group in the form of mg C per gram of dry soil. However, other more intuitive forms of expression are acceptable, as for example the number of individuals of each group per unit area. Before sorting and counting, each group must be extracted from soil samples, which demands specific extraction methods.

Protists (sorted into ciliates, amoebas, and flagellates) were extracted from soil, and their abundance was estimated by the "most probable number" method (Darbyshire et al., 1974). We extracted nematodes from soil samples with Baermann funnels for three days, and micro-arthropods from the whole soil cores in Tullgren funnels for 7 days. We sorted them into trophic groups (following Moore et al., 1988) under classic (for microarthropods) or inverted (for nematodes) optical microscopes. All individuals included in each functional group were attributed the same individual biomass, metabolic rate and feeding preferences based on literature. Biomass-C density was calculated for each group by multiplying its abundance by half the individual body weight attributed to the group, since we assume that 50% of the dry weight of the soil living biomass is made of carbon.

Recommendations for monitoring. Soil invertebrate indicators should be ideally monitored every 5 years. Belowground populations fluctuate seasonally, with the highest size and activity occurring during the plant growing period. Therefore, the sampling campaigns should be conducted in April-May or September-October in the two study cases of the Pyrenees (preferably in the fall to get results comparable with those of the baseline), and in September-October in the Massaciuccoli Lake. In this last case, the sampling dates must be adapted to the agricultural calendar, to take advantage of the short resting period before tillage.

Expected evolution of the indicators. The abundance of soil invertebrates is expected to increase with the maturity of the soil-plant system. The same is expected for the abundance of groups belonging to high levels of the food web (predators).

### 1.4.5 Indicators informing about soil food web functions

Classical outputs of the soil food web models are simulated $CO_2$ emissions from soil biota to the atmosphere (De Ruiter et al., 1993) and the theoretical stability of the web. Food web stability can be described as a measure of the likelihood of the persistence of the interacting soil species or functional groups following disturbances or environmental impacts. Stability guarantees maintaining biodiversity and preserving the provision of soil environmental services in front of environmental fluctuations, which is primeval under current climate uncertainty (Schwarz et al., 2017).

The debate about which food web metrics determine the stability of the ecosystem is long-lasting (see Dunne, 2006). At the beginning of the twentieth century, stability was supposed to correlate with species diversity, based on the observation that low diversity ecosystems (as recently restored or agricultural environments) are more prone to destructive oscillations than riche ecosystems, and more vulnerable to invasions. This supposition is no longer relevant, and stability is now most often justified based on the relative importance of the bacterial food channels that exhibit, on average, more abundant and weaker interactions among groups (Rooney et al., 2006).

Expected evolution of the indicators. $CO_2$ emissions from the soil food web are expected to decrease as the soil carbon metabolism becomes increasingly conservative. Stability is expected to decrease with increasing accumulation of C in soil.

Recommendations for the monitoring program. Unfortunately, studying the soil trophic web requires expertise for identification and computation of all groups and for further modelling. Therefore, monitoring the post-operation evolution of the soil food-web is very desirable but not feasible unless the monitoring plan includes funds for contracting expert assistance. We are seeking for reliable relationships between soil food web indicators and other biological indexes of soil quality easier to calculate.

Analysis method. To run soil food web models, soil bacterial and fungal biomasses are necessary, we obtained these data for our soils from direct count of slides under epifluorescence microscope (Bloem, 1995). Once the carbon abundance of each soil trophic group has been calculated from our soil samples, we ran the food web model described in depth in Moore & de Ruiter (2012). The suitability of this model to predict real N and C mineralization rates has been tested by comparing values for k obtained by simulation with those obtained from lab incubation under controlled conditions (Schröter et al., 2003).

## 1.5 Plant indicators

### 1.5.1 Indicators informing about aboveground carbon sequestration: change in plant carbon stocks

Definition and relevance of the indicator. The stock of carbon in forest trees and woody vegetation is a consequence of the balance between its increase, as a result of tree growth, and its decrease by tree exploitation and mortality (Vayreda et al., 2012). If tree growth surpasses losses, the result is C accumulation; on the contrary, if losses exceed growth, the stock of C decreases. If forest management is designed to obtain energy, the whole C stock is immediately released into the atmosphere as $CO_2$. On the contrary, if forest management is oriented to produce wood for long-lasting products, such as furniture, the stored C remains sequestered throughout the product life. On the other hand, the C contained in dead trees is gradually released into the atmosphere because of their decomposition at a rate that depends on multiple factors of which temperature, humidity, the position of the tree (standing or lying down) and its size are the most determining (Harmon, 2009).

Nearly 86% of the terrestrial above-ground carbon is stored in forests (Rodger, 1993). Forests play a key role in the global carbon cycle by sequestering a substantial amount of $CO_2$ from the atmosphere. C sequestration is more active in the initial phases of the natural (or human induced) succession from herbaceous vegetation to forest systems dominated by woody plants, and in young forest, where the trees grow the fastest.

When measuring aboveground carbon stocks, the estimated biomass components usually include the aboveground live biomass (trees and shrubs excluding the roots) and dead above-ground biomass (woody litter and fallen branches or stems). Carbon contained in herbaceous vegetation is not included, due to its fast turnover.

Plant carbon-stock estimation provides an idea of the quantity of carbon present in vegetation at a given time but does not inform about future trends, which is necessary to assess carbon sequestration. However, measuring carbon stocks is essential for tracking changes in the carbon stock through monitoring, which is a basis to estimate carbon sequestration by the stock-change method, as proposed by the IPCC (2003) or by modelling.

In this project, plant carbon stocks include the amount of carbon stored in the aboveground of living trees and shrubs expressed in tonnes per hectare.

<u>Expected evolution of the indicator</u>. Carbon storage increases during the process of plant succession, when woody plants take over from herbs and shrubs, and when large trees take over from smaller ones. Therefore, in the absence of high intensity disturbances (unsustainable exploitation, snow slides, windstorms, wildfire...) it is expected that the C stock of the aboveground biomass will increase over time until final stabilization.

<u>Recommendations for the monitoring program</u>. Follow the evolution of aboveground tree C stock every 5 years.

<u>Method</u>. We calculated the baseline of plant C stocks in the Capet Forest and in the Sta Elena roadcut. The method was the same in both sites, although the number of plots varied depending on local plant cover characteristics. We simulated post-operation carbon sequestration by vegetation in specific ways for each case, depending on diverse plant evolution scenarios (see it for each study case).

*Field inventories*. The baseline C stocks were obtained from sampling plots randomly located in forests and shrublands found in the study areas. With a GPS, the centre of each plot was located with an error of 5-10 m. Trees larger than 2,5 cm DBH (diameter of trunk at breast height) were checked within a radius of 5 metres (distance corrected for slope). For each shrub species or tree regeneration (trees taller than 1 m and DBH < 2,5 cm) present in the plot, the species, the vegetation cover in percentage (1% accuracy) and the mean height (10 cm accuracy) were noted.

For small trees (DBH between 2,5 cm and 7,5 cm) the "distance to $n^{th}$ nearest neighbour" method was used. For each tree up to the $n^{th}$ distance the following was noted: the species, the horizontal distance to the centre of the $6^{th}$ tree of the species closest to the plot centre if it was less than 5 m away. Otherwise, the distance to the $3^{rd}$ nearest tree was averaged whatever this distance is. For all trees up to the $6^{th}$ or $3^{rd}$ tree, as appropriate, the DBH (in cm) and the height (in m) were recorded. For each large tree (DBH $\geq$ 7,5 cm) present in a circular plot with a radius of 5 metres (distance corrected for slope), the species, the DBH (in cm) and the height (in m) were recorded.

*Data processing.* For each shrub species or tree regeneration (DBH < 2,5 cm), the biovolume (volume occupied by vegetation, in $m^3$) was calculated by multiplying the vegetation cover by its mean height. The resulting volume was transformed to biomass (in kg) by multiplying by the ratio between dry weight and biovolume ($kg/m^3$) which is species-specific (Armand et al., 1993, Pasalodos-Tato et al. 2015). Total biomass (in t $ha^{-1}$) was obtained as the sum of the biomass of all woody species. The value was multiplied by 0,5 (1 kg OM = 0,5 kg C) to obtain the C stock (in tC $ha^{-1}$).

For small trees (DBH between 2,5 cm and 7,5 cm) the tree density (D, in trees·$ha^{-1}$) was calculated according to:

$$D = v \cdot 10{,}000/(\pi \cdot d^2) \qquad [7]$$

where: v is the $n^{th}$ nearest neighbour and d is the distance to this tree from the plot centre.

The total aboveground biomass of each tree (kg/tree) was obtained from the equation that relates this variable to DBH and height and is species-specific (Gracia et al., 2004, Ibañez et al., 2005). Total aboveground biomass (t $ha^{-1}$) is the average biomass of all measured trees multiplied by tree density. The value was multiplied by 0,5 (1 kg OM = 0,5 kg C) to obtain the C stock (tC $ha^{-1}$).

For each large tree (DBH $\geq$ 7,5 cm) the corresponding total above-ground biomass was calculated by applying the equation described above. All values were summed to obtain the biomass per plot and multiplied by 127.32 to obtain the value equivalent to 1 hectare (ratio between 1 ha and the area of the sampling plot), r = 5 m, A = 78,53 $m^2$. The value was multiplied by 0,5 (1 kg OM = 0,5 kg C) to obtain the C stock (tC $ha^{-1}$).

### 1.5.2 Indicators informing about plant biodiversity provision and threats

Definition and relevance of the indicator. Plant biodiversity greatly influences ecosystem functions and services and sustain ecosystem multifunctionality, and its preservation and restoration are a priority in most conservation programs and restoration plans. In improved vegetated areas or in newly created plant spots resulting from land restoration, plant cover characteristics immediately after operation will depend on the density and species composition of the plantation. Trying to stablish mature vegetation from the beginning is unsuccessful and, usually, pioneer communities are promoted by seeding herbaceous species and planting young seedlings of woody species. Vegetation is then let to evolve spontaneously towards mature formations through interaction with the restored soil. When soil restoration has been correctly addressed, and where the structure of the landscape favours the progressive colonization of the restored area by native elements, the succession is expected to progress towards communities fully integrated into the surrounding vegetation. Although some trajectories are predictable (i.e. increasing importance of woody species), species composition must be tracked over time to alert to the possible presence of unwanted species (as in the case of alien and invasive species) or of species indicating undesirable ecological processes (i.e. ruderal species …)

#### 1.5.2.1 Plant species biodiversity. Shannon Index

Definition and relevance of the indicator. The Shannon Index (H') is a measure of diversity and is a function of the number of plant species and their proportion (Shannon, 1949). This indicator is dimensionless. The index was calculated as:

$$H' = \Sigma\, p_i \cdot \ln(p_i) \qquad [8]$$

where $p_i$ is the proportion of total plot area covered by plants belonging to the $i^{th}$ species.

Expected evolution of the indicator. Between the initial and older stages of the post-operation plant succession, species richness usually fluctuates, as well as species biodiversity (Isbell et al., 2011). For example, forest succession shows higher species numbers in intermediate stages of succession, and different successional stages may harbour very different sets of species (Teurlincx et al., 2018). In forest successions, increasing presence of woody and tree species is a foreseeable trend (Figure 3).

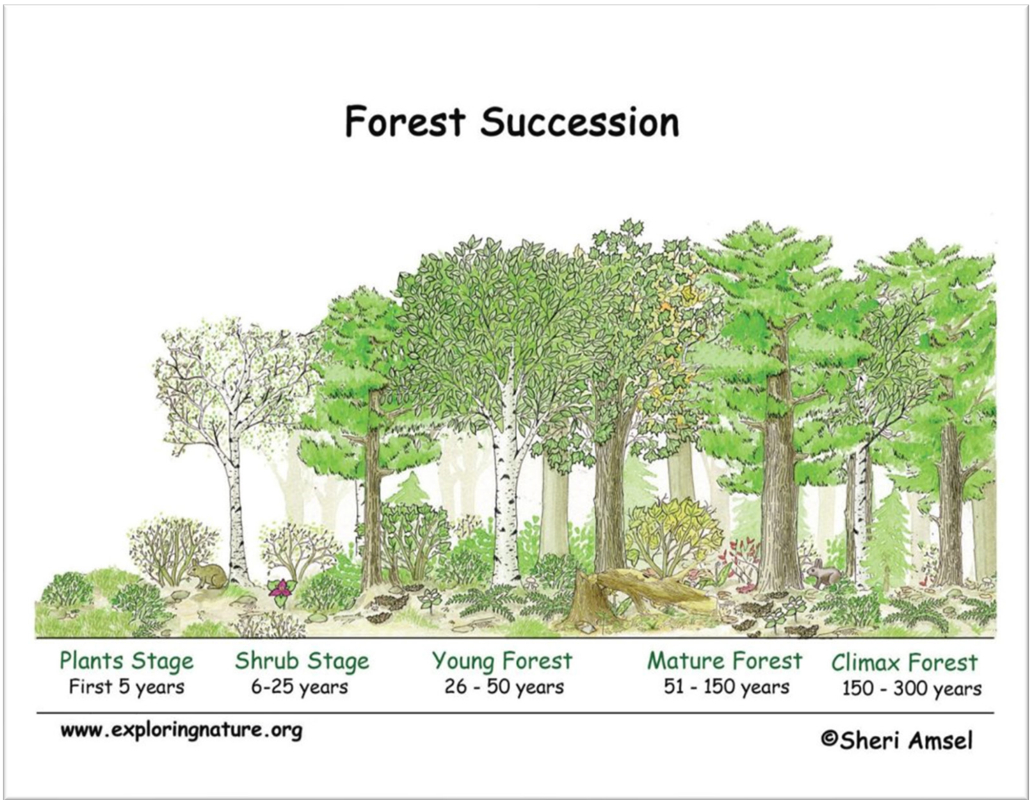


*Figure 3 Scheme of the ideal evolution of the structure of plant communities over time*

Recommendations for the monitoring program. Follow the evolution of species diversity every 2 years.

Sampling method. At each study site, vegetation inventories were made in circular field plots taking soil sampling points as plot centres. We sampled soil and plant in the same days. For the purpose of this project and given the variety (from threes to herbs) of plant forms, plant abundance was not estimated from the number of individuals per species, but from the estimated proportion of the plot area covered by each species.

#### 1.5.2.2 Invasive species

Definition and relevance of the indicator. The proportion of non-native invasive tree species is an indicator of the degree of ecosystem disturbance. A species is considered invasive when it rapidly colonizes and occupies a space by altering its ecological integrity and ecosystem services (Charles & Dukes, 2008; Pejchar & Mooney, 2009) and by hindering the regeneration, establishment, and growth of native species. Once established, even in small proportions, their eradication is almost always very difficult. Moreover, invasive species disrupt the fundamental structure and function of the ecosystem food webs, and consequently reduce native biodiversity (Ehrenfeld, 2010). Considerably negative impacts for socioeconomic and human welfare have been reported for invasions (Pimentel et al., 2005; Vilà et al., 2010, Andreu & Vilà, 2011).

The presence of invasive tree species is usually related to altered habitats that provide open spaces available to rapid colonization. Riparian habitats tend to be very auspicious spaces especially after the alteration caused by large avenues of water. Another factor that determines their establishment is the proximity to urban areas or roads (González-Moreno et al., 2012).

Expected evolution of the indicator. In the short term, the likelihood of establishment of invasive species will depend primarily on the proximity of propagules and on high intensity disturbance of the colonizable habitat.

Recommendations for the monitoring program. To detect straight away the presence of invasive species, it is highly recommended to carry out a follow-up every 2 years making exhaustive paths of all the monitored area.

Sampling method. The sampling method consists of detecting the presence of invasive tree species at any development stage, from seedlings to adult trees.

## 1.6 Simulating the post-operational evolution of the indicators over time: scenario-building

The established method to estimate effects of any action addressing ecological restoration consists of: (a) selecting environmental indicators sensitive to the action, (b) measuring their pre-operational value, (c) measuring their post-operational value, (d) defining the ideal trajectories and value (when pertinent) expected for the indicator, (e) calculating the difference between the pre- and post-operational value of the indicator, and (f) evaluating how our action has contributed to reduce the distance between the pre-operational value of the indicator and its desired value.

However, when dealing with environmental services, the process is not as straightforward as this. As we have indicated when presenting many of our indicators, the response of most environmental services is gradual and slow. This is particularly true for services provided by soil and plants, that coevolve following successional patterns difficult to foresee due to multiple ecosystem interactions and subsequent possible trajectories.

Therefore, the effect of restorative measures cannot be assessed by simply comparing the value of a set of indicators immediately after operation (their post-operation state) with their value before operation (their baseline state).

In many cases, and particularly when soil is replaced or recreated or when plantation works produce soil disturbance, operations cause temporary negative impacts on the system that demand some time to begin a clear process towards recovery.

An additional limitation to take note of when working with biodiversity is the scarcity of models useful to preview its evolution over time, which is particularly problematic for soil biodiversity.

To overcome these limitations, in this project we decided to proceed, for each study case, as follows:

- we mapped the plant cover in the affectation area and classified it into uniform units of increasing maturity (increasing age).

- we proposed the most likely scenarios for plant cover distribution in the affectation area at different times after restoration.

- based on time series of satellite images, we identified in the region spots of vegetation of different degrees of maturity, with soil type, topography, microclimate, height above sea level and exposition similar to those of the affectation area.

- we sampled these spots for all selected indicators and considered that the obtained values are the reference values expected at different times after restoration.

Examples of the application of this approach to specific situations are shown in the sections corresponding to the three cases studied in PHUSICOS.

# 2 Evaluation of the NBSS in three study cases

## 2.1 The Capet Forest (Barèges, French Pyrenees)

### 2.1.1 Case description

Barèges (Hautes-Pyrénées, 42º 53’47.4”N; 0º 3’ 48.05”E, 1250 m a.s.l.) is located in the Bastan River Valley, at the foot of the Capet mountain (2328 m a.s.l.) (Figure 4). In 2016, the stable population included 170 people distributed in 86 households. Besides, 922 holiday homes are registered (INSEE, 2019) which indicates the touristic interest and high frequentation of this town, associated to sky resorts.


*Figure 4 The Barèges area*

The town has been historically threatened by hydrogeological risks, including catastrophic flooding, and repeated destructive snow avalanches reported since 1644 (Lanusse, 1988). The most threatening avalanches originate in the Midaou and Theil avalanche corridors (Figure 5) that are being managed for risk mitigation since the second half of the XIX$^{th}$ century.

The Capet Forest, the public national forest that occupies 147 ha in the 1930-2120 m a.s.l. fringe above Barèges, was designed as a protective forest by Napoleon III in 1860, which prompted and active reforestation of this area with coniferous trees during the 1880-1920 period (Lanusse, 1988). The top sector of the slopes was planted with native *Pinus uncinata* (mountain pine), while mixed forests of *Picea abies* (silver fir) and *Larix decidua* (European larch) were introduced in the lowest parts. *L. decidua* seeds were directly spread on the snow since these trees require bare soil for recruitment and early establishment. Unfortunately, this property makes this species of very limited use for avalanche prevention.

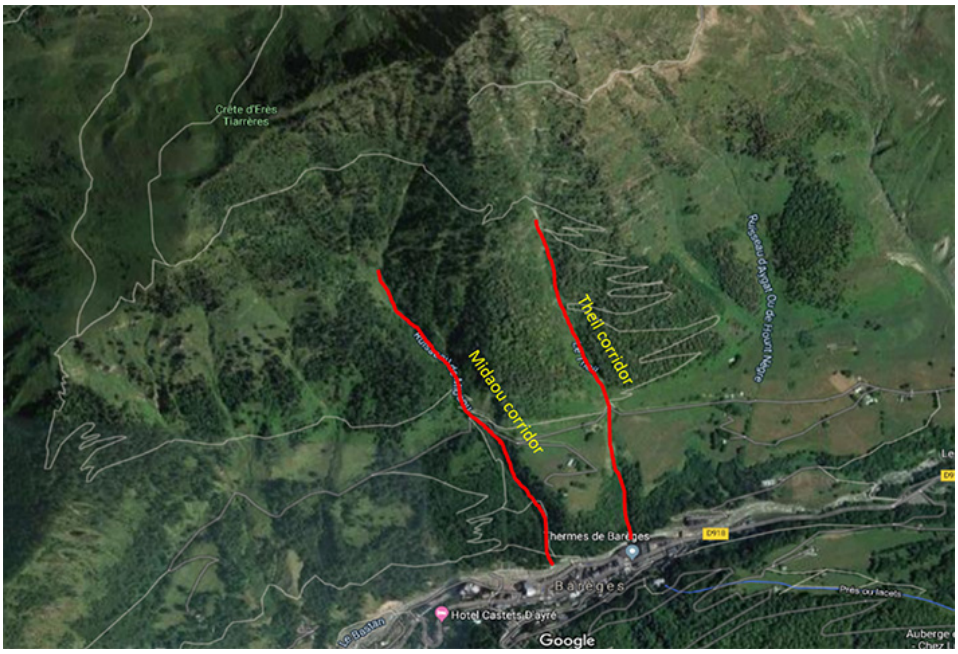


*Figure 5 The Miadou and Theil avalanche corridors above Barèges*

To reinforce the anti-avalanche protection, dry stone walls were introduced in 1892 and, from then on, the defence system has been significantly improved and densified. Currently, together with the former stone walls, a dense network of cast-iron snow rakes is apparent on the mountainside, particularly concentrated in the Thiel corridor (Figure 6).

*Figure 6 Anti-avalanche defences in the Theil corridor*

About 900 protective structures (5232 lineal meters) are currently maintained by the ONF/RTM staff (Anonymous, 2011). Notwithstanding the deployment of protective measures, avalanches continue to cause damages, as was the case in the winter of 2013 (Figure 7).

*Figure 7 Barèges covered by a snow avalanche in 2013*

### 2.1.2 The implemented solution

A new approach to avalanche risk reduction is now being tested, consisting at preventing snow avalanches from the very beginning of their formation at the steeply sloped (more than 45 degrees) top of the avalanche corridors. These unfriendly environments are usually deforested because of tree establishment during the juvenile phase is challenged by snow gliding.

The implemented solution consists of slope reforestation supported with snow glide tripods. This NBS aims to increase the soil surface roughness, to prevent snow gliding and to favour tree establishment and growth. This strategy is very common in the Alpine region, and in its original version, the tripods are placed in groups where the distance between tripods should not exceed 1,5 m to mimic the clumpy structure of the Alpine forests (Rudolf-Miklau et al., 2014). Under each tripod, seedlings are planted following the "nucleation" strategy. Once planted, the resulting small patches of trees will act as focal areas for forest recovery. From the ecological point of view, nucleation is an attractive option that mimics natural successional processes to aid woody plant recolonization and to restore deforested habitats into heterogeneous landscapes, including patches of the current herbaceous and shrubby vegetation on skeletal unstable soils, and forest groves in the most favourable microsites. The final spatial pattern is thought to significantly increase soil surface roughness then slowing down snow gliding at its origin and preventing the snowpack to gain momentum (Corbin & Holl, 2012).

The original method has been tailored to fit the characteristics of the Capet Forest. 300 tripods will be stablished 10 m away of each other and, under their shadow, the plantation area will be drop-shaped, as shown in Figure 8. The 2,5 long wooden tripods will be made of non-chemically treated European larch or Douglas fir (*Pseudotsuga menziesii*) stems, both very resistant to microbial and insect attacks (the generalized use of copper will be avoided). The poles will measure about 2,5 m. The structure of the tripods is shown in Figure 8.

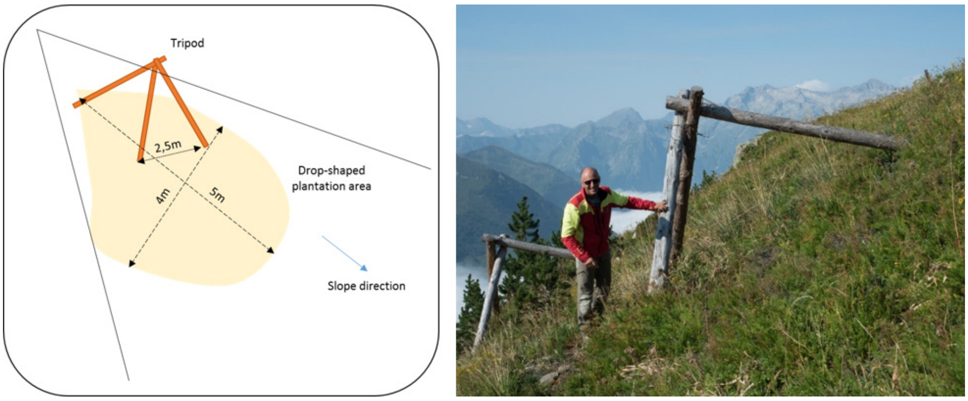


*Figure 8 Plantation units under tripods (left) and a built-up tripod (right)*

30 to 50 tree seedlings will be planted downhill of each tripod in a drop-shape framework. The composition of the whole plantation is shown in Table 3.

*Table 3 List of trees planted. Number of units and number of tripods*

| Species | Origin | N of plants | N of tripods |
|---|---|---|---|
| Pinus uncinata | Native | 2100 | 70 |
| Pinus sylvetris | Native | 200 | 7 |
| Pinus bougetii | Native | 300 | 10 |
| Pinus cembra | Non-native | 1200 | 40 |
| Larix decidua | Non-native | 300 | 10 |
| Picea engelmanii | Non-native | 600 | 20 |
| Cedrus deodara | Non-native | 300 | 10 |
| Pinus ponderosa | Non-native | 800 | 27 |
| Abies concolor | Non-native | 600 | 20 |
| Betula pendula | Native | 250 | 8 |
| Populus tremula | Native | 250 | 8 |
| Sorbus aucuparia | Native | 400 | 13 |
| Sorbus aria | Native | 100 | 3 |
| Sorbus chamaemespilus | Native | 100 | 3 |
| Acer opalus | Native | 200 | 7 |
| Quercus petraea | Native | 200 | 7 |
| Tilia platyphyllos | Native | 100 | 3 |
| Total | | 8000 | 266 |

Six of the 17 planted species (35,3% of the seedlings) are non-native. The introduction of these exotic species aims to guarantee the anti-avalanche efficiency of the plantation in case of high mortality of native trees by forest pests, in particular by *Cronartium* *flaccidum* ("blister rust"). The seedlings were manually planted in 40-50 cm wide micro-terraces to avoid soil disturbance forming two types of plant nuclei big nuclei of 30 plants, and small nuclei of 16 plants, organized as shown in Figure 9.

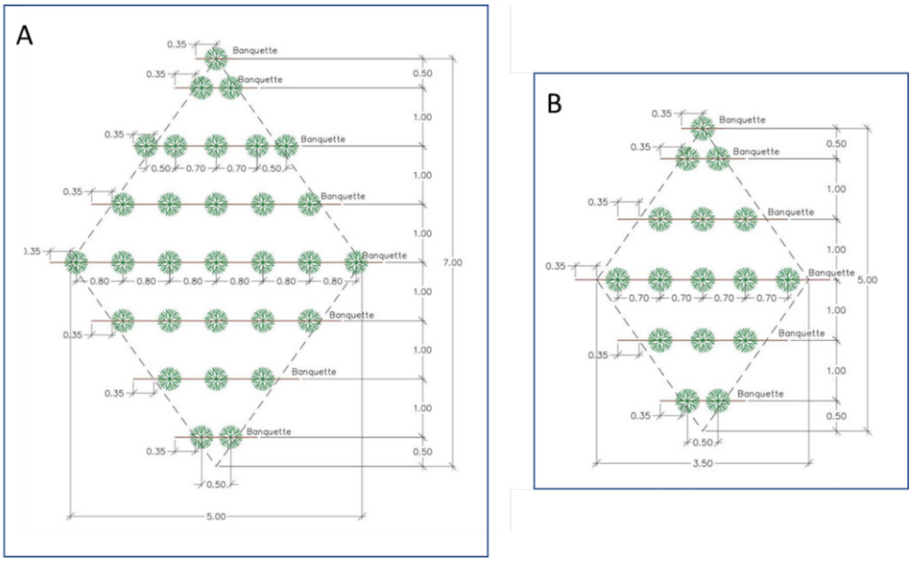


*Figure 9 Distribution of plants in two types of nuclei (provided by the Office National des Fôrets-Service RTM Savoie.*

The plantations were done at the headwaters of the Midaou corridor, with an intervention area of about 32 ha with an altitudinal range between 1900 and 2100 m a.s.l. (Figure 10).

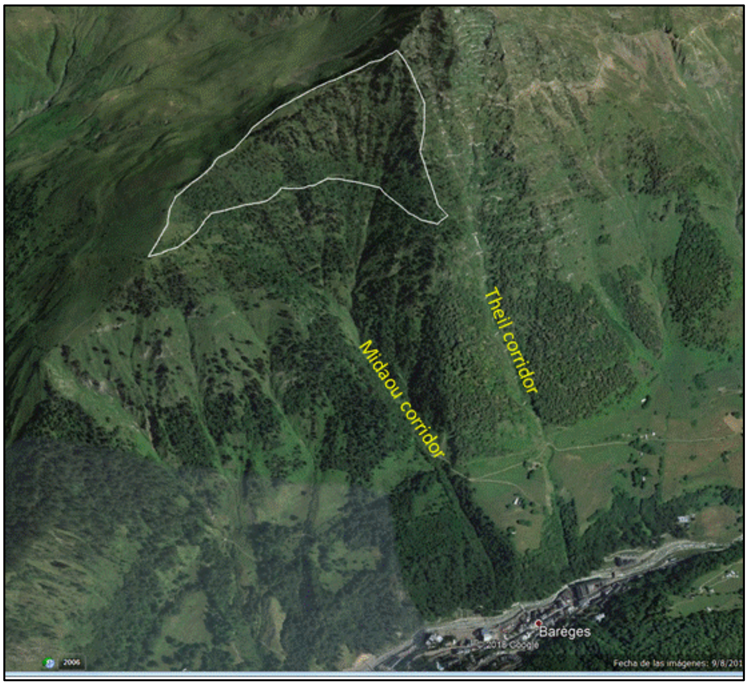


*Figure 10 Perimeter of the intervention area (in white)*

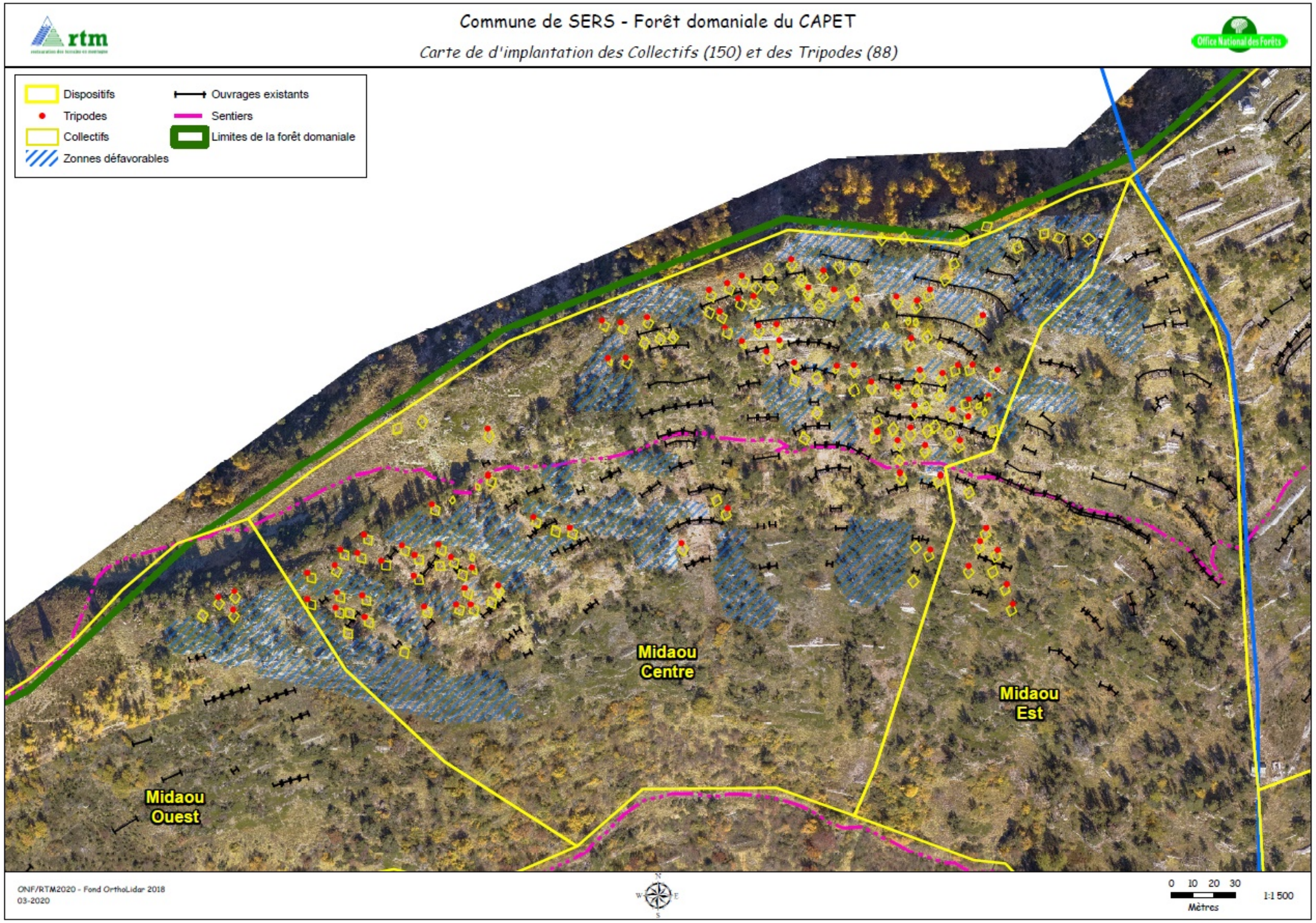


*Figure 11 Distribution of the planted nuclei in the Midaou corridor.*

### 2.1.3 Field sampling

To measure the pre-operative value of our indicators we mapped the vegetation from satellite images to classify it into homogeneous types.

The plant cover consists of a matrix of flowery alpine prairies dominated by grasses (dominated by *Festuca panniculata* -alpine violet fescue-, *Festuca eskia* -endemic to the Pyrenees and Cantabrian range- and *Brachypodium pinnatum* –tor-grass), with scattered thickets of bearberry (*Arctostaphylos uva-ursi*), rusty-leaved alpenrose (*Rhododendron ferrugineum*), bilberry (Vaccinium myrtillus), heather (Calluna vulgaris) and decumbent juniper (Juniperus nana) (Figure 12).

*Figure 12 Prairies and bushes cover the intervention area*

The rare remains of the old plantations of mountain pine are only visible in the ridges and rocky outcrops (Figure 9a) while spots of mixed forest of pines and European larches can be found in the lower part of the hillside (Figure 13).


*Figure 13 Small mountain pine spots in the ridges of the top of the Midaou avalanche channel (a); mixed forests of Pinus uncinata and Larix decidua in the lower part of the channel (b)*

Only where the snow rakes have performed well (most often in the Thiel avalanche channel), soil starts to stabilize, which is indicated by clumps of young aspens (*Populus tremula*), silver birches (*Betula pendula*) and mountain ashes (*Sorbus aucuparia*) (Figure 14).

*Figure 14 Soil stabilization in the Thiel corridor, as proved by the presence of dense 25-year-old groups of aspens, silver birches and mountain ashes below the snow rakes*

Table 4 shows the extension and relative importance of these types of soil cover in the area of intervention.

*Table 4 Extension of diverse plant cover types found in the area of intervention*

| Land cover | m² | % |
|---|---|---|
| Prairie | 188637 | 58,3 |
| Shrubs | 41475 | 12,8 |
| Pine groves | 54074 | 16,7 |
| Aspen groves | 32599 | 10,1 |
| Rocks & bare soil | 6538 | 2,0 |
| **Total** | **323322** | **100** |

To assess the baseline of the indicators, a field campaign was carried out in September 2019. We selected 32 sampling points (8 per vegetation type) corresponding to each of the four plant cover types identified in the Midaou micro-watershed. To distribute the points, we avoided the western sector of the intervention area, where the baseline was altered by preliminary plantations done one year ago. In the central and eastern zones, the sampling points were spaced equidistantly along three parallel paths that cross the ridge and the middle and lowest part or the intervention zone. The spatial distribution of the sampling points is shown in Figure 15.

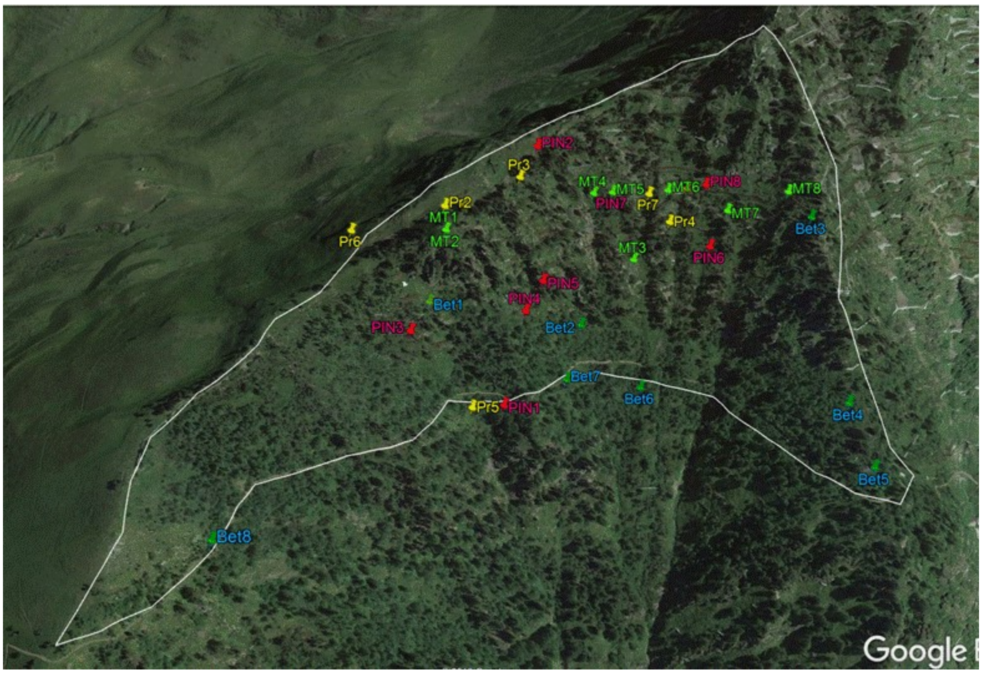


*Figure 15 Situation of the sampling points in the Midaou channel*

Due to the sharp topography of the work area, the sampling campaign required the cooperation of the Land Restoration Service for Mountainous Regions of the French National Forestry Office (ONF-TRM) that provided helicopters for transportation of samples and researchers, personnel for sampling, and shelter in their mountain camp (Figure 16).

*Figure 16 Sampling campaign in the Capet Forest*

For soil indicators, three contiguous samples (5 cm in Ø and 15 cm deep) were extracted at each sampling point. The first sample was allocated to physical and chemical analyses, the second one to micro-arthropod extraction, and the last one to microbial biodiversity and micro-invertebrates (nematodes and protists). A small cylindrical soil core was also extracted from the sampling points for bulk density determination.

For plant biodiversity, at least one botanical inventory was made per sampling point, taking these points as the center of the inventory plot. For herbs and shrubs, the relative abundance of each species was estimated based on its relative soil coverage. Average species height was also measured for woody species to estimate carbon stocks. In the pine groves, cores were extracted from the trunk trees with an increment corer for dating.

Soil samples were stored in field coolers for immediate transport to Barcelona for analysis, following the methods explained in section 1.4 for each indicator.

### 2.1.4 The baseline of the indicators and their expected post-operation evolution

Table 5 shows the value of all soil and plant properties measured for the four types of plant cover present in the work area. The table also shows which properties are significantly affected by plant cover, and only these properties sensitive to plant cover will be considered as potential indicators. In this sense, please remember that the expected progression towards plant-soil ecosystem plantation is expected to follow these two chronosequences:

*Prairie > Shrubs > Pine forests (in well-developed soils soils)*

*Aspen & Birch groves > Pine forests (in stony and rocky soils under stabilized prairies).*

*Table 5 Value of all soil and plant properties measured in the four types of plant cover at the Capet Forest. Values are expressed as mean ± standard deviation. For properties showing very low values below 0, we have used the scientific notation to avoid long decimal numbers. For better understanding, 2,14 E-5 means 0.0000214 and 1,31 E-3 means 0,00131. p represents the significance of differences between plant units after analyses of the variance. When differences are significant (red values of p) red letters indicate differences between pairs of units: units sharing one letter are equal and units that have no common letters are different.*

| ECOSYSTEM SERVICE | INDICATOR | Unit | VEGETATION TYPES | | | | |
|---|---|---|---|---|---|---|---|
| | | | **Praire** | **Shrub** | **Aspen & Birch groves** | **Pine groves** | ***p*** |
| Total organic carbon in topsoil | TOTAL $C_{org}$ | g C . m$^{-2}$ | 7292,7 ± 1759,1 b | 6613,1 ± 1654,5 b | 7866,3 ± 4144,2 b | 15619,9 ± 7066,4 a | <0,01 |
| | $C_{org}$ Fast Pool | g C . m$^{-2}$ | 2537,8 ± 879,2 b | 1720 ± 1027,4 bc | 1318,5 ± 864,9 c | 3049,7 ± 697,4 a | <0,01 |
| Chemicaly protected SOC | $C_{org}$ Slow Pool | g C . m$^{-2}$ | 4754,8 ± 1547,8 b | 4893,1 ± 1880,6 b | 6547,7 ± 3821,2 b | 12570,3 ± 6887,7 a | <0,01 |
| Physically protected SOC | C in crushed vs intact soil aggregates | % | 28,2 ± 9,4 | 51,2 ± 5,8 | 48,8 ± 6 | 47,7 ± 11,9 | ns |
| Soil erodibility | Aggregate stability: MWD | mm | 2,2 ± 0,2 | 2,7 ± 0,3 | 2,6 ± 0,3 | 2,8 ± 0,2 | ns |
| | Soil bulk density | g cm$^{-3}$ | 0,5 ± 0,1 | 0,4 ± 0,1 | 0,7 ± 0,2 | 0,5 ± 01 | ns |
| | N | % | 4,9 ± 0,6 | 6,2 ± 1,1 | 4,1 ± 0,6 | 6,2 ± 1,1 | ns |
| | Ca | mg kg$^{-1}$ | 2,0 ± 0,8 | 3,6 ± 0,8 | 2,1 ± 0,6 | 2,1 ± 0,7 | ns |
| Soil fertility | Mg | mg kg$^{-1}$ | 177,1 ± 66 | 241,8 ± 36 | 177,1 ± 66 | 153,1 ± 43 | ns |
| | P | mg kg$^{-1}$ | 12,3 ± 1,1 b | 12,6 ± 1,6 b | 11,4 ± 1,1 b | 19,5 ± 3,3 a | <0,05 |
| | K | mg kg$^{-1}$ | 222,5 ± 33 | 226 ± 40 | 157 ± 35,6 | 199,3 ± 32,5 | ns |
| | Na | mg kg$^{-1}$ | 34,5 ± 2,7 | 47,3 ± 6,1 | 28,6 ± 2,4 | 41,3 ± 8,1 | ns |
| | Microbial species richness (Bacteria) | number sp | 140,8 ± 6,7ab | 147,4 ± 5,9 b | 154,0 ± 5,5 a | 125,6 ± 6,6 b | <0,05 |
| | Microbial species diversity (Bacteria) | Unittless | 4,03 ± 0,6 | 4,12 ± 0,1 | 4,16 ± 0,5 | 3,97 ± 0,9 | ns |
| | Microbial species eveness | Unittless | 0.41 ± 0.1 | 0,43 ± 0,2 | 0,42 ± 0,1 | 0,43 ± 0,2 | ns |
| | Microbial catabolic diversity | Unitless | 2,73 ± 0,1 | 2,74 ± 0,1 | 2,72 ± 0,1 | 2,74 ± 0,1 | ns |
| | Invertebrate functional diversity | | | | | | |
| | Flagellates | mg C g$^{-1}$ dry soil | 1,98 $E^{-6}$ ± 8,35 $E^{-7}$ | 1,82 $E^{-6}$ ± 7,0 $E^{-7}$ | 2,05 $E^{-6}$ ± 6,72 $E^{-7}$ | 9,79 $E^{-7}$ ± 2,71 $E^{-7}$ | ns |
| | Amoebae | mg C g$^{-1}$ soil | 1,82 $E^{-4}$ ± 1,06 $E^{-4}$ | 2,04 $E^{-4}$ ± 6,99 $E^{-5}$ | 1,44 $E^{-4}$ ± 6,51 $E^{-5}$ | 4,17 $E^{-5}$ ± 1,82 $E^{-5}$ | ns |
| | Ciliates | mg C g$^{-1}$ soil | 0 | 1,15$E^{-5}$ ± 1,11 $E^{-5}$ | 3,53 $E^{-7}$ ± 3,53 $E^{-7}$ | 0 | ns |
| | Total Protists | mg C g$^{-1}$ soil | 1,84 $E^{-4}$ ± 1,06 $E^{-4}$ | 2,17 $E^{-4}$ ± 7,52 $E^{-5}$ | 1,47 $E^{-4}$ ± 6,53 $E^{-5}$ | 4,27 $E^{-5}$ ± 1,82 $E^{-5}$ | ns |
| | Bacterial feeder nematodes | mg C g$^{-1}$ soil | 6,12 $E^{-5}$ ± 2,77 $E^{-5}$ | 3,39 $E^{-5}$ ± 9,16 $E^{-6}$ | 2,83 $E^{-5}$ ± 1,26 $E^{-5}$ | 2,14 $E^{-5}$ ± 5,22 $E^{-6}$ | ns |
| | Fungal feeder nematodes | mg C g$^{-1}$ soil | 9,77 $E^{-6}$ ± 4,01 $E^{-6}$ | 1,33 $E^{-5}$ ± 3,69 $E^{-6}$ | 1,15 $E^{-5}$ ± 4,48 $E^{-6}$ | 1,09 $E^{-5}$ ± 1,79 $E^{-6}$ | ns |
| | Plant-feeder nematodes | mg C g$^{-1}$ soil | 2,47 $E^{-5}$ ± 1,08 $E^{-5}$ | 8,79 $E^{-6}$ ± 3,48 $E^{-6}$ | 1,37 $E^{-5}$ ± 8,46 $E^{-6}$ | 1,66 $E^{-6}$ ± 6,50 $E^{-7}$ | ns |
| | Omnivore nematode | mg C g$^{-1}$ soil | 2,71 $E^{-5}$ ± 1,32 $E^{-5}$ | 1,51 $E^{-5}$ ± 5,18 $E^{-6}$ | 1,34 $E^{-6}$ ± 1,34 $E^{-6}$ | 8,33 $E^{-6}$ ± 6,33 $E^{-6}$ | ns |
| Biodiversity provision | Predatory nematodes | mg C g$^{-1}$ soil | 2,24 $E^{-6}$ ± 2,24 $E^{-6}$ | 2,00 $E^{-6}$ ± 2,00 $E^{-6}$ | 5,46 $E^{-6}$ ± 4,01 $E^{-6}$ | 5,22 $E^{-6}$ ± 5,22 $E^{-6}$ | ns |
| | Total nematodes | mg C g$^{-1}$ soil | 1,25 $E^{-4}$ ± 3,95 $E^{-5}$ | 7,31 $E^{-5}$ ± 1,69 $E^{-5}$ | 6,03 $E^{-5}$ ± 2,44 $E^{-5}$ | 4,75 $E^{-5}$ ± 8,36 $E^{-6}$ | ns |
| | Predatory Mites | mg C g$^{-1}$ soil | 3,26 $E^{-6}$ ± 3,26 $E^{-6}$ | 0 | 0 | 3,27 $E^{-6}$ ± 3,27 $E^{-6}$ | ns |
| | Nematophagous Mites | mg C g$^{-1}$ soil | 5,16 $E^{-4}$ ± 1,12 $E^{-4}$ a | 8,67 $E^{-4}$ ± 2,87 $E^{-4}$ a | 5,23 $E^{-4}$ ± 9,99 $E^{-5}$ a | 2,86 $E^{-3}$ ± 7,11 $E^{-4}$ b | <0.0001 |
| | Nematophagous Prosti | mg C g$^{-1}$ soil | 4,40 $E^{-5}$ ± 1,07 $E^{-5}$ | 7,78 $E^{-5}$ ± 3,03 $E^{-5}$ | 3,14 $E^{-5}$ ± 9,87 $E^{-6}$ | 1,46 $E^{-4}$ ± 5,92 $E^{-5}$ | ns |
| | Collembola | mg C g$^{-1}$ soil | 1,55 $E^{-4}$ ± 7,73 $E^{-5}$ | 1,01 $E^{-4}$ ± 2,85 $E^{-5}$ | 1,15 $E^{-4}$ ± 3,93 $E^{-5}$ | 2,63 $E^{-4}$ ± 6,68 $E^{-5}$ | ns |
| | Fungivorous Cryptostigmata | mg C g$^{-1}$ soil | 5,62 $E^{-4}$ ± 1,20 $E^{-4}$ | 1,54 $E^{-3}$ ± 5,93 $E^{-4}$ | 5,71 $E^{-4}$ ± 1,10 $E^{-4}$ | 6,12 $E^{-3}$ ± 4,56 $E^{-3}$ | ns |
| | Fungivorous Prostigmata | mg C g$^{-1}$ soil | 2,39 $E^{-6}$ ± 1,27 $E^{-6}$ | 4,75 $E^{-5}$ ± 3,01 $E^{-5}$ | 1,07 $E^{-5}$ ± 5,49 $E^{-6}$ | 3,07 $E^{-5}$ ± 9,39 $E^{-6}$ | ns |
| | Diplura | mg C g$^{-1}$ soil | 1,44 $E^{-5}$ ± 1,44 $E^{-5}$ | 0 | 0 | 0 | ns |
| | Symphyla | mg C g$^{-1}$ soil | 1,27 $E^{-5}$ ± 8,40 $E^{-6}$ | 0 | 3,0 $E^{-5}$ ± 1,26 $E^{-5}$ | 4,41 $E^{-6}$ ± 3,03 $E^{-6}$ | ns |
| | Protura | mg C g$^{-1}$ soil | 0 | 0 | 2,08 $E^{-6}$ ± 1,36 $E^{-6}$ | 2,72 $E^{-6}$ ± 2,72 $E^{-6}$ | ns |
| | Total arthropods | mg C g$^{-1}$ soil | 1,31 $E^{-3}$ ± 2,27 $E^{-4}$ | 2,64 $E^{-3}$ ± 8,67 $E^{-4}$ | 1,28 $E^{-3}$ ± 2,45 $E^{-4}$ | 9,4 $E^{-3}$ ± 5,15 $E^{-3}$ | ns |
| | C mineralization by soil food webs | g C m$^{-2}$ y$^{-1}$ | 3503,2 ± 454,7 b | 4383,4 ± 834 b | 3796,4 ± 178,5 b | 14180,6 ± 2250,1 a | <0.0001 |
| | Soil ecosystem stability | y$^{-1}$ | 1,65 ± 1,17 | 0,27 ± 0,03 | 0,30 ± 0,01 | 1,53 ± 0,63 | ns |
| Aboveground C sequestration | Aboveground carbon stock | t C ha$^{-1}$ | 0,24 ± 0,10 d | 2,27 ± 0,55 c | 7,44 ± 3,15 b | 48,69 ± 16.50 a | <0.0001 |
| | Species richness | number of sp | 18,4 ± 3,7 | 16,7 ± 2,8 | 17,4 ± 4,8 | 15 ± 3,7 | ns |
| Biodiversity provision & treats | Species diversity | bits | 1,98 ± 0,41 | 1,64 ± 0,25 | 1,89 ± 0,33 | 1,84 ± 0,29 | ns |
| | Eveness | unitless | 0,68 ± 0,1 | 0,59 ± 0,09 | 0,67 ± 0,08 | 0,69 ± 0,06 | ns |
| | Invasive species | number of sp | 0 | 0 | 0 | 0 | ns |
| Soil protection | Plant cover | % | 100 | 100 | 100 | 100 | ns |

Among the properties sensitive to plant cover and that, therefore, will be affected by the post-operation evolution of the vegetation towards maturity, we have selected for their interest as indicators those that show a pattern coherent with vegetation maturity, i.e. those that have been observed to increase or to decrease with vegetation maturity. The resulting selection is shown in Table 6 for the chronosequences *Prairie > Shrubs > Pine forests* and *Aspen & Birch groves* separately.

*Table 6 Soil and plant indicators for the Capet Forest and their expected evolution in the plated prairies (above) and in current bare stony soils (below) during their maturation towards pine forests. For each indicator, the intensity of the cell color increases as the indicator increases.*

| | | | | >>>>> post-operation maturity pattern >>> | | |
|---|---|---|---|---|---|---|
| **Compartment** | **Environmental servive** | **Indicator** | **Unit** | **Praire** | **Shrubs** | **Pine groves** |
| Soil | Belowground carbon sequestration | Total $C_{org}$ | $g\,C\,.\,m^{-2}$ | 7292,7 | 6613,1 | 15619,9 |
| | | $C_{org}$ Fast Pool | $g\,C\,.\,m^{-2}$ | 2537,8 | 1720 | 3049,7 |
| | | $C_{org}$ Slow Pool | $g\,C\,.\,m^{-2}$ | 4754,8 | 4893,1 | 12570,3 |
| | Soil fertility | Phosphorous | $mg\,kg^{-1}$ | 12,3 | 12,6 | 19,5 |
| | Biodiversity provision | Microbial species richness (Bacteria) | number of sp | 140,8 | 147,4 | 125,6 |
| | | Nematophagous Mites | $mg\,C\,g^{-1}$ soil | 0,00052 | 0,00087 | 0,0028 |
| | Soil food web functions | C mineralization by soil food webs | $g\,C\,m^{-2}\,y^{-1}$ | 3503,2 | 4383,4 | 14180,6 |
| Plants | Aboveground C sequestration | Aboveground carbon stock | $t\,C\,ha^{-1}$ | 0,24 | 2,27 | 48,69 |

| | | | | >> post-operation maturity pattern >>> | |
|---|---|---|---|---|---|
| **Compartment** | **Environmental servive** | **Indicator** | **Unit** | **Aspen & Birch groves** | **Pine groves** |
| Soil | Belowground carbon sequestration | Total $C_{org}$ | $g\,C\,.\,m^{-2}$ | 7866,3 | 15619,9 |
| | | $C_{org}$ Slow Pool | $g\,C\,.\,m^{-2}$ | 6547,7 | 12570,3 |
| | Soil fertility | Phosphorous | $g\,C\,.\,m^{-2}$ | 11,4 | 19,5 |
| | Biodiversity provision | Nematophagous Mites | $mg\,C\,g^{-1}$ soil | 0,00053 | 0,00286 |
| | Soil food web functions | C mineralization by soil food webs | $g\,C\,m^{-2}\,y^{-1}$ | 3796,4 | 14180,6 |
| Plants | Aboveground C sequestration | Aboveground carbon stock | $t\,C\,ha^{-1}$ | 7,44 | 48,69 |

The indicators shown in these tables are of direct use for evaluating the effect of the applied solution on the environmental services they represent by simply comparing their post-operative value in a given moment of the monitoring program with their pre-operative value. For reforested prairies, the baseline for comparison will be, for any indicator, its value in prairies and the expected maximum value will be the value of the indicator in the mature pine forest.

For currently bare unstable soils, all indicators are expected to increase progressively. Their expected value 30 years after operation should approach the baseline value of the aspen and birch groves and, as before, the expected maximum value will be the value of the indicator in the mature pine forests.

### 2.1.5 Additional indicators of plant and soil biodiversity

Together with these synthetic indicators that can be expressed by a single number, two statistic approaches to soil biodiversity and plant diversity are recommended.

For plant diversity, a principal component analysis of the results of the plant inventory is very interesting to track significant changes in the species composition over time (Figure 17).

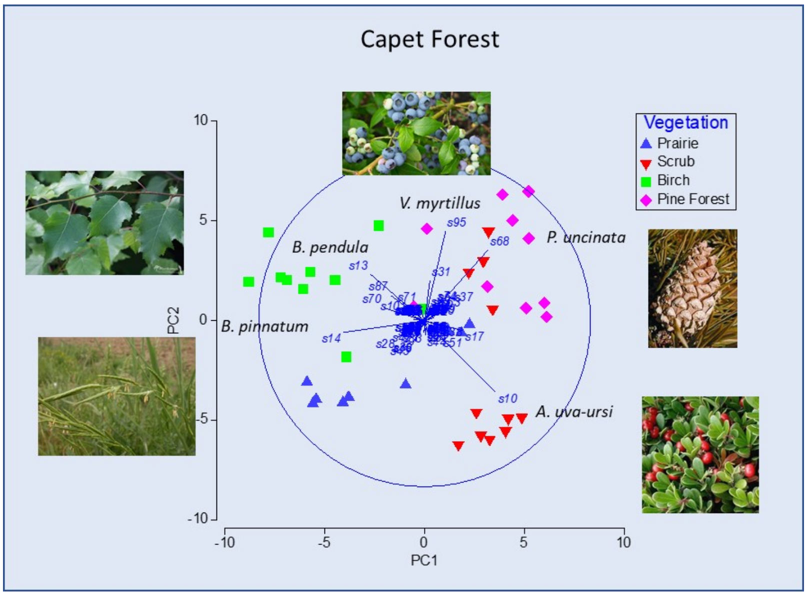


*Figure 17 PCA of the plant community at different phases of the plant succession in the Capet Forest. The most significant species of each stage are shown.*

The list of plant species inventoried at each vegetation type is shown in Table 7.

*Table 7 List of species found at each type of plant cover in the study area of the Capet forest. Blue shade indicates presence.*

| | Prairie | Shrubs | Pine groves | Birch groves | | | Prairie | Shrubs | Pine groves | Birch groves |
|---|---|---|---|---|---|---|---|---|---|---|
| Acer pseudoplatanus | | | | | 51 | Juniperus nana | | | | |
| Achillea millefolium | | | | | 52 | Knautia cf. arvernensis | | | | |
| Agrostis capillaris | | | | | 53 | Larix decidua | | | | |
| Amelanchier ovalis | | | | | 54 | Laserpitium sp. | | | | |
| Anemone hepatica | | | | | 55 | Lathyrus cf. linifolius | | | | |
| Anthoxanthum odoratum | | | | | 56 | Leucanthemum gr. vulgare | | | | |
| Anthyllis vulneraria | | | | | 57 | Leuzea centauroides | | | | |
| Aquilegia vulgaris | | | | | 58 | Lilium martagon | | | | |
| Arabis cf. glabra | | | | | 59 | Lotus corniculatus | | | | |
| Arctostaphylos uva-ursi | | | | | 60 | Luzula nutans | | | | |
| Asphodelus albus | | | | | 61 | Melampyrum pratense | | | | |
| Avenula sp. | | | | | 62 | Meum athamanticum | | | | |
| Betula pendula | | | | | 63 | Origanum vulgare | | | | |
| Brachypodium pinnatum | | | | | 64 | Phyteuma spicatum | | | | |
| Bupleurum cf. falcatum | | | | | 65 | Pinus sylvestris | | | | |
| Calamagrostis arundinacea | | | | | 66 | Pinus uncinata | | | | |
| Calluna vulgaris | | | | | 67 | Plantago lanceolata | | | | |
| Campanula glomerata | | | | | 68 | Populus tremula | | | | |
| Campanula scheuchzeri | | | | | 69 | Potentilla erecta | | | | |
| Carduus defloratus | | | | | 70 | Potentilla rupestris | | | | |
| Carex cf. caryophyllea | | | | | 71 | Pulsatilla alpina | | | | |
| Centaurea nigra | | | | | 72 | Rhododendron ferrugineum | | | | |
| Cerastium arvense | | | | | 73 | Rosa cf. pimpinellifolia | | | | |
| Cicerbita plumieri | | | | | 74 | Rosa pendulina | | | | |
| Cotoneaster integerrimus | | | | | 75 | Rubus idaeus | | | | |
| Crepis pyrenaica | | | | | 76 | Sedum cf. anglicum | | | | |
| Cruciata glabra | | | | | 77 | Sedum rupestre | | | | |
| Dactylis glomerata | | | | | 78 | Sempervivum tectorum | | | | |
| Daphne cneorum | | | | | 79 | Senecio adonidifolius | | | | |
| Deschampsia flexuosa | | | | | 80 | Silene rupestris | | | | |
| Dianthus hyssopifolius | | | | | 81 | Silene vulgaris | | | | |
| Echium vulgare | | | | | 82 | Solidago virgaurea | | | | |
| Empetrum nigrum | | | | | 83 | Sorbus aria | | | | |
| Erysimum sp. | | | | | 84 | Sorbus aucuparia | | | | |
| Euphorbia cf. dulcis | | | | | 85 | Stachys alopecuros | | | | |
| Festuca eskia | | | | | 86 | Stachys officinalis | | | | |
| Festuca paniculata | | | | | 87 | Stellaria holostea | | | | |
| Galium verum | | | | | 88 | Succisa pratensis | | | | |
| Gentiana gr. acaulis | | | | | 89 | Teucrium pyrenaicum | | | | |
| Gentiana lutea | | | | | 90 | Thesium pyrenaicum | | | | |
| Geranium pyrenaicum | | | | | 91 | Thymus gr. serpyllum | | | | |
| Geranium sylvaticum | | | | | 92 | Vaccinium myrtillus | | | | |
| Globularia nudicaulis | | | | | 93 | Vaccinium uliginosum | | | | |
| Helianthemum nummularium | | | | | 94 | Veronica chamaedrys | | | | |
| Hieracium pilosella | | | | | 95 | Veronica fruticulosa | | | | |
| Hieracium sp. | | | | | 96 | Veronica officinalis | | | | |
| Hippocrepis comosa | | | | | 97 | Vicia cf. orobus | | | | |
| Hypericum richeri subsp. burseri | | | | | 98 | Vicia pyrenaica | | | | |
| Iris latifolia | | | | | 99 | Viola sp. | | | | |
| Iris latifolia | | | | | | **number of sp** | **65** | **58** | **48** | **66** |

A similar approach can be applied to soil microbial biodiversity. Although the Shannon Index calculated for the taxonomic of functional microbial biodiversity was not sensitive to ecosystem maturation, a more detailed analysis alerts of microbial groups (Figure 18A) that are only present in the latest stages of the succession, and of groups of functional genes characteristic of mature pine forests (Figure 18B).

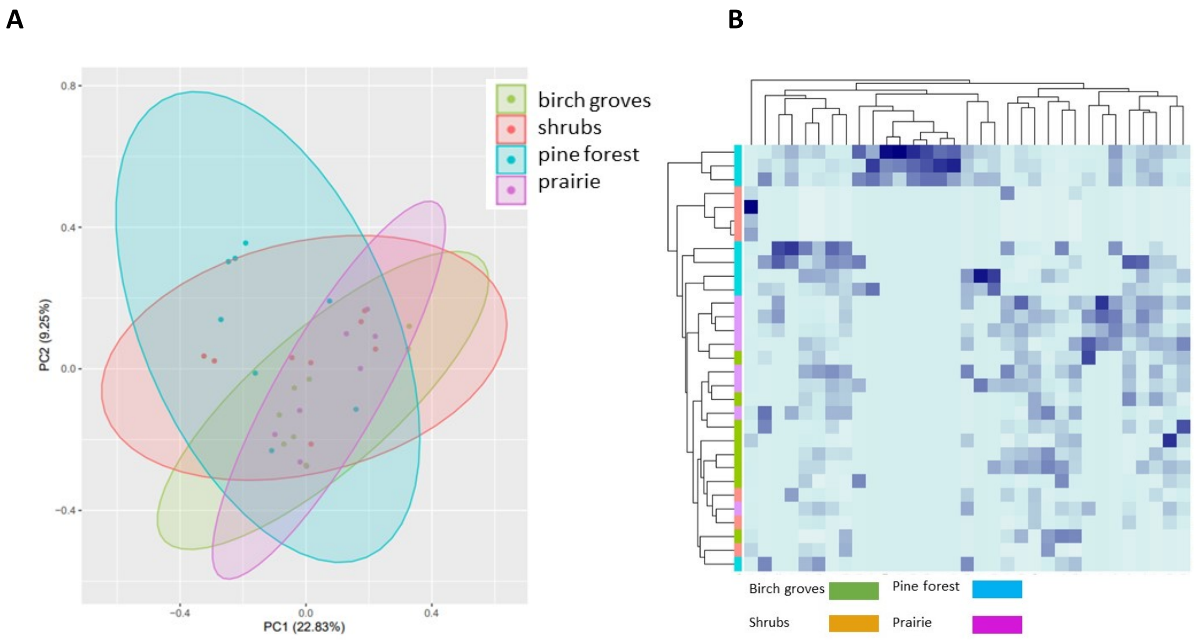


*Figure 18 PCA plot for soil microbial genera (A) and heatmap plot showing the clustering (scaled Kegg pathways relative abundances) for functional genes (B) in the soil of the four stages of vegetation sampled in the Capet Forest.*

### 2.1.6 Expected effect of the reforestation on aboveground and belowground C stocks

The post-operational forecasted scenarios in the medium and long term emerged from the interaction of forest experts of the CREAF and the French Forest Service.

- *In the medium term (30 years).* Based on the observed initial plant survival rate and on the observed plant dynamics in the area, we can expect that the plantation will be successful. The increased soil surface roughness induced by the new plants will cooperate to stabilize the stony soil currently unfavourable to plant development. We postulate that the bare soil spaces will behave as the areas that have been successfully protected by the anti-avalanche rakes of the Thiel corridor and that, 30 years after planting, the dominant matrix below this new protective vegetation will look much like the aspen, silver birch and mountain ash thickets found in the Thiel Channel. After a period of soil stabilization and accretion by addition or organic matter, vegetation will converge with the native pine forests. The plantations that have been done on grassland patches that are expected to evolve to bushes and to pine forests.

- *In the long term (100 years).* Within 100 years, a mountain pine forest is expected to cover the whole intervention area, with environmental characteristics comparable to the current 100-year-old pine forest coppices found in the neighbouring hillslopes.

To construct the future plant cover scenarios, we have assumed that (a) the planted seedlings will stablish and progress at the rate observed in comparable zones of the Pyrenees, (b) there will not be catastrophic hydrogeological events or wildfires affecting the zone in the next 100 years and that (c) grazing is suppressed during the following 10 years in the restored area.

Based on these assumptions, we have simulated the probable evolution of total (aboveground plant C + organic soil C at 15 cm depth) carbon stocks in the 32,33 ha of the intervention area (Table 8).

*Table 8 Aboveground plant C stocks in the area of intervention of the Capet Forest. Measured values for the baseline, and simulated values 30 years and 100 years after restoration.*

| BASE LINE C STOCKS | | | | | |
|---|---|---|---|---|---|
| | | **Aboveground C** | **Soil $C_{org}$** | **Tot. aboveground C** | **Tot. soil $C_{org}$** |
| Land cover | ha | tC $ha^{-1}$ | gC $m^{-2}$ | tC | tC (15 cm depth) |
| Prairie | 18,9 | 0,25 | 7292,7 | 4,72 | 1375,67 |
| Shrubs | 4,1 | 2,27 | 6613,1 | 9,41 | 274,28 |
| Pine groves | 5,4 | 48,69 | 15619,9 | 263,29 | 844,63 |
| Aspen groves | 3,3 | 7,44 | 7866,3 | 24,25 | 256,43 |
| Rocks & bare soil | 0,7 | 0 | 0 | 0,00 | 0,00 |
| **Total** | **32** | | | **302** | **2751** |

| SIMULATED C STOCKS AFTER 30 YEARS | | | | | |
|---|---|---|---|---|---|
| | | **Aboveground C** | **Soil $C_{org}$** | **Tot. aboveground C** | **Tot. soil $C_{org}$** |
| Land cover | ha | t C $ha^{-1}$ | g C . $m^{-2}$ | tC | tC (15 cm depth) |
| Prairie | 18,9 | 0,25 | 7292,7 | 0,00 | 0 |
| Shrubs | 4,1 | 2,27 | 6613,1 | 52,24 | 1522 |
| Pine forest | 5,4 | 48,69 | 15619,9 | 422,01 | 1354 |
| Aspen groves | 3,3 | 7,44 | 7866,3 | 4,86 | 51 |
| Bare soil | 0,7 | 0 | 0 | 0,00 | 0 |
| **Total** | **32** | | | **479** | **2927** |

| SIMULATED C STOCKS AFTER 100 YEARS | | | | | |
|---|---|---|---|---|---|
| | | **Aboveground C** | **Soil $C_{org}$** | **Tot. aboveground C** | **Tot. soil $C_{org}$** |
| Land cover | ha | t C $ha^{-1}$ | g C . $m^{-2}$ | tC | tC (15 cm depth) |
| Pine forest | 32 | 48,69 | 15619,9 | 1574,25 | 5050,26 |
| **Total** | **32** | | | **1574** | **5050** |

### 2.1.7 Case description

The study case is an unstable roadcut in the transnational A-136 road from Biescas (Spain) to Laruns (France). The roadcut is located 4,7 Km north of Biescas (42.659479, -0.323979, 1060 m.a.s.l.), and is excavated perpendicular to a quaternary moraine produced by the Gállego glacier. The moraine lies on top of Eocene flysch deposits (Barrère 1966). These glacial sediments consist of disorganized-looking accumulations of clays and sands with clasts and blocks of varying dimensions.

The 35 m high cutting is triangularly shaped with a base about 150 m long (Figure 19). The surface is highly uneven and scarcely vegetated, and rock falls are frequent creating a great threat for the very busy road.

*Figure 19 Front view of the Santa Elena roadcut.*

### 2.1.8 The solution

The chosen solution was inspired by a successful restoration program undertaken in 1903 in the 160-ha watershed of the Arratiecho torrent (Biescas, 42.629104, -0.308481) over colluvial flysch sediments, with an average gradient of 53% (Fábregas et al., 2014).

At the beginning of the 20th century (1903-1904), the watershed was deforested and totally degraded (Figure 20). The restoration works included the stabilization of the hillsides by terracing, implementing drainage systems and reforesting the terraces with *Pinus sylvestris*.

The project was implemented between 1903 and 1907 and, currently, the restored area is successfully integrated in the surrounding landscape. The erosion rate is much reduced, and the zone is a popular destination for recreational uses (Figure 21).

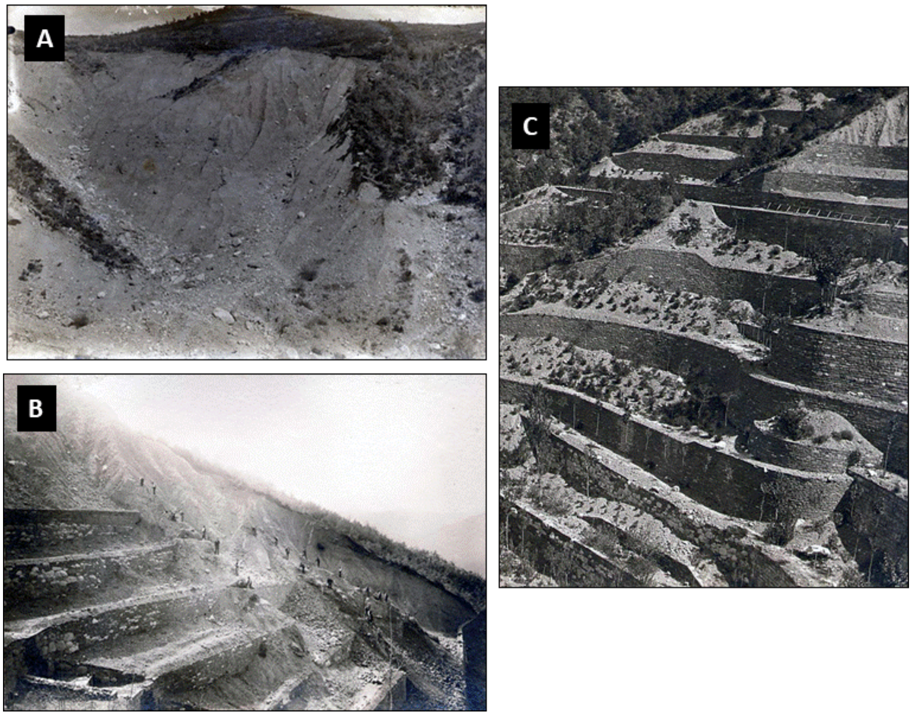


*Figure 20 The Arratiecho watershed in (A) 1902-1904, and during its restoration in 1903-1907 (B-C) (Pictures from the Tomás Ayerbe collection).*

*Figure 21 Current appearance of the Arratiecho watershed*

At the time of the writing of this report, the project has been accepted but not yet executed and there is some uncertainty about some important facts that will determine the post-operation trajectory of the restored front.

The front view of the projected restoration of the roadcut is shown in Figure 22, and the transversal profile of the project in Figure 23.

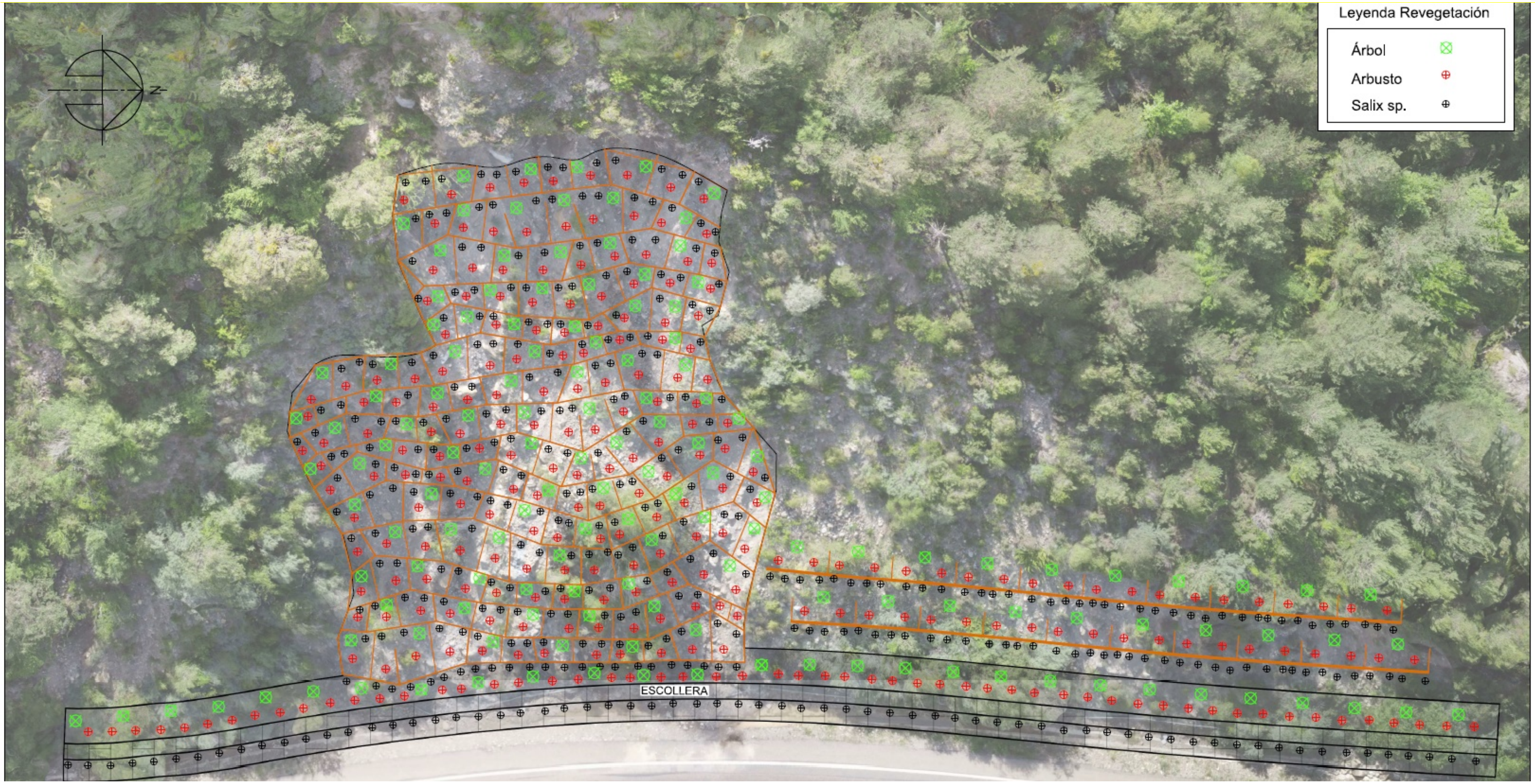


*Figure 22 Front view of the projected solution for the Santa Elena roadcut.*

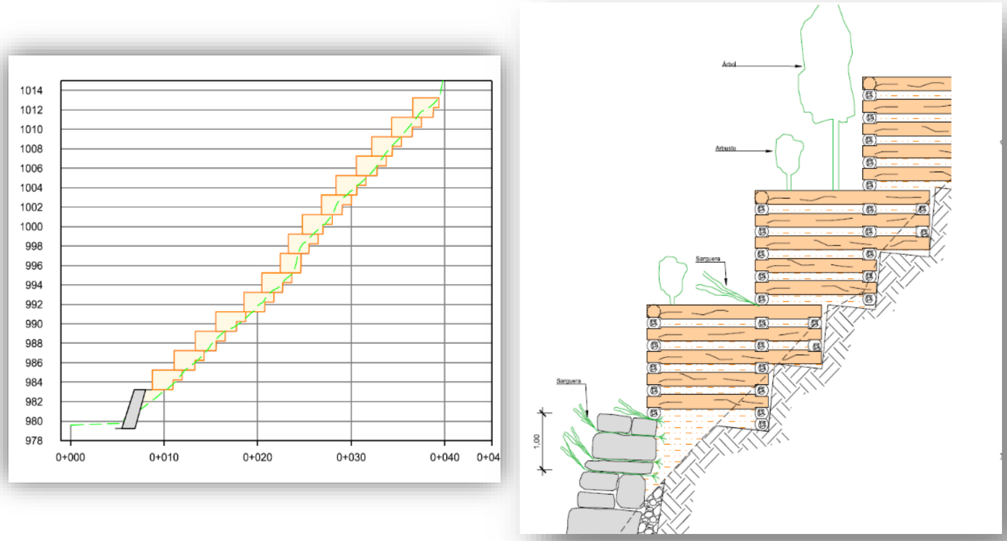


*Figure 23 Transversal profile (general and detailed) of the projected solution in the Sta Elena roadcut.*

Brief, the solution consists of eliminating all unstable rocks that break the surface of the current front of the moraine and of creating 16 terraces 3 m wide that provide the base for 16 jardinieres that will be planted with trees and shrubs.

Plantations of trees and shrubs are previewed with undetermined density and with the species composition shown in Table 9. Additionally, undetermined mixtures of local grasses and legumes will be sown in vertical surfaces. Total revegetation area is estimated at 5700 $m^2$.

*Table 9 Plant species previewed for plantation*

| Plant species | % |
|---|---|
| **Trees** | |
| *Pinus sylvestris* | 60 |
| *Betula pendula* | 10 |
| *Sorbus aria* | 10 |
| *Populus nigra* | 10 |
| *Salix capraea* | 10 |
| **Bushes** | |
| *Hippophae ramnoides* | 50 |
| *Salix eleagnos* | 50 |

The jardinieres will be filled with about 184,7 $m^3$ of a mixture of local excavation materials plus additional excavated materials of undefined provenance and quality.

This practice is being currently reviewed in depth in the EU due to undesirable consequences for soil and environment of spreading topsoil and excavation materials of unknown properties anywhere.

In the project, what is referred as "soil" is a totally undetermined material of which the only information provided is the percentage of particles of different sizes from a civil engineering approach. In the absence of any data about texture, acidity, organic matter content and nutrients, we cannot anticipate advantages of risks of this intervention on carbon sequestration and soil and plant diversity. The same can be said about the previewed utilization of an undetermined material to fill up the plantation holes. This material is defined as "sandy topsoil, clean, sieved and fertilized, provided in bulk". Provenance and chemical and physical characteristics are again missing.

To compensate for the lack of local topsoil, we have previewed that soil from the surrounding mature pine forest will be collected and sprayed on top of the fill material of the terraces to favour colonization by native soil biota and plant propagules (Wubs et al., 2016).

There are other deficiencies in the project that hinder our evaluation of real effects of the proposed solution on the environment. Specifically, there are no data about the volume and destination of excess material from excavation. There are no restoration plans for the areas affected by accesses of the machinery to the roadcut and of the temporary machinery depots and temporary stockpile sites.

All these considerations, that are mandatory in environmental impact assessment, must be respected as well in the implementation of nature based solutions comparable in execution demands to classic public works.

### 2.1.9 Field sampling

To assess the baseline of the environmental indicators at the roadcut, as well as to quantify their values in the reference vegetation units for further modelling, we conducted a field campaign in September 2019.

We also sampled neighboring vegetation units that were considered representative of the regenerative plant-soil succession in the roadcut. The distribution of the sampling points is shown in Figure 24.

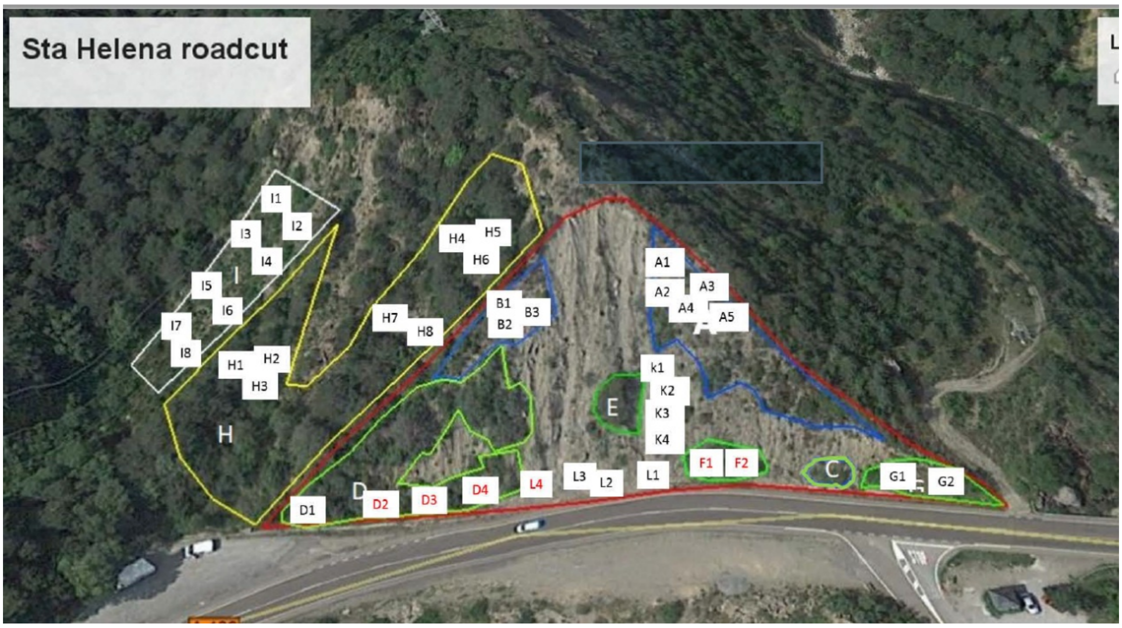


*Figure 24 Distribution of the sampling points in the roadcut of Santa Elena by types of plant cover. In the surface of the roadcut, units K and L correspond to bare soil, units A and B correspond to very immature low bushes, and units C, D, E, F and G correspond to dense tall bushes. On the left side of the picture, outside the affectation area, units I and H represent the reference scenarios expected for plant cover in the roadcut five and 60 years after restoration (see the text for further explanation).*

Sampling was risky due to the extreme slope of the surface (50% to 150% in some points) and the instability of the outcropping rocks. Our work therefore required the cooperation of experts in rope access work provided by AECT (Figure 25).

*Figure 25 Soil sampling campaign in the Santa Elena roadcut*

The extension of the three types of plant cover identified in the roadcut are shown in Table 10.

*Table 10 Extension of the three types of plant cover in the Santa Elena roadcut*

| Soil cover type | area ($m^2$) | % |
|---|---|---|
| Total tall shrub patches | 798,2 | 27,3 |
| D | 520,5 | |
| E | 62,9 | |
| F | 86,4 | |
| G | 128,4 | |
| Total low shrub patches | 569,1 | 19,5 |
| A | 410,9 | |
| B | 132,8 | |
| C | 25,4 | |
| Bare soil | 1552,7 | 53,2 |
| **Total area** | **2920** | **100,0** |

In the roadcut, we selected 24 sampling points and, at each of them, we extracted 4 cylindrical soil cores (5 cm in diameter and 15 cm long) for chemical and biological analyses and an additional soil core (7 cm Ø and 5 cm long) for soil bulk density measurement. The plant inventories were done with binoculars from the base of the cut.

We also found two areas representative of the vegetation that is expected to cover the restored front within 5 years (vegetation had been cut 5 years ago underneath the power line) and within 60 years (the age of the oldest pines we measured in the field). Here, soil sampling included 8 sampling points per plant type and 4 soil cores per sampling point. Plant inventories were done around every soil sampling point, including species cover and height and tree age.

### 2.1.10 The baseline of the indicators

The main properties of soil and plants for each vegetation type are shown in Table 11, and Table 12 shows those properties sensitive to the successional stage of the soil-plant system.

The first comment to make on Table 12is that no plant biodiversity properties are included. Indeed, when measuring plant biodiversity by synthetic indexes (i.e. number of plant species, Shannon biodiversity and Evenness indexes) no significant effects of plant community maturation are apparent.

However, successional changes are obvious when looking at the species composition of the community (see Table 13). From the table it follows that the 60-year-old reference forest is characterised by the exclusive presence of some species of deciduous trees: common maple (*Acer campestre*), Italian maple (*Acer opalus*), olive willow (*Salix eleagnus*) and whitebeam (*Sorbus aria*).

*Table 11 Soil and plant properties measured in the three types of plant cover at the Santa Elena roadcut and in 5-year-old brushes and 20-year-old pine forests considered as references for post operation succession in the restored surface. Values are expressed as mean ± standard deviation. For properties showing very low values below 0, we have used the scientific notation to avoid long decimal numbers. For better understanding, 3,72E-5 means 0,0000372. "p" represents the significance of differences between vegetation units after analyses of the variance. When differences are significant (red values of p) red letters indicate differences between pairs of units: units sharing one letter are equal and units that have no common letters are different.*

| ECOSYSTEM SERVICE | INDICATOR | Unit | Vegetation types | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Bare soil** | **Low shurbs** | **Tall shurbs** | **5 y old shrubs** | **60 y old forest** | ***p*** |
| Belowground C sequestration | TOTAL $C_{org}$ | g C . m$^{-2}$ | 8752,9 ± 3173,6 | 4391,6 ± 1578,9 | 5610,1 ± 2507,7 | 6687,5 ± 3083,4 | 7372,4 ± 5064,4 | ns |
| | $C_{org}$ Fast Pool | g C . m$^{-2}$ | 8027,4 ± 3012,8 a | 2281,2 ± 800,0 b | 1578,5 ± 692,2 b | 1976,8 ± 864,5 b | 1967,7 ± 1916,3 b | <0,005 |
| | $C_{org}$ Slow Pool | g C . m$^{-2}$ | 545,5 ± 199,8 b | 2110,4 ± 1344,2 a | 4031,6 ± 3047,2 a | 4710,7 ± 2587,8 a | 5404,7 ± 4181,4 a | <0,005 |
| | C in crushed vs intact soil aggregates | % | 10,8 ± 7,3 | 14,8 ± 6,3 | 37,3 ± 10,6 | 30,6 ± 6,5 | 37,3 ± 10,6 | ns |
| Soil physical stability | Aggregate stability | mm | 1,14 ± 0,1 b | 1,39 ± 0,2 b | 2,1 ± 0,3 a | 2,1 ± 0,1 a | 2,1 ± 0,2 a | <0,01 |
| Soil fertility | Soil bulk density | g cm$^{-3}$ | 1,7 ± 0,09 a | 1,4 ± 0,06 b | 1,35 ± 0,23 b | 0,8 ± 0,1 c | 0,52 ± 0,16 c | <0,0001 |
| | N | % | 0,47 ± 0,08 b | 0,86 ± 0,1 b | 1,22 ± 0,19 a | 2,95 ± 0,37 a | 2,96 ± 0,46 a | <0,0001 |
| | Ca | mg kg$^{-1}$ | 6,2 ± 0,2 b | 6,1 ± 0,2 b | 6,1 ± 0,2 b | 6,4 ± 0,3 b | 8,7 ± 0,7 a | <0,001 |
| | Mg | mg kg$^{-1}$ | 209 ± 19 b | 172 ± 27 b | 209 ± 48 b | 148 ± 14 b | 443 ± 129 a | <0,05 |
| | P | mg kg$^{-1}$ | 5,0 ± 0 b | 5,0 ± 0 b | 5,3 ± 0,24 b | 6,3 ± 0,7 b | 12,4 ± 3,5 a | <0,05 |
| | K | mg kg$^{-1}$ | 40 ± 1,8 b | 78,5 ± 8,6 b | 86,2 ± 10 b | 133,8 ± 12 b | 235 ± 74 a | <0,01 |
| | Na | mg kg$^{-1}$ | 20,33 ± 1,9 a | 19,66 ± 0,84 a | 21,5 ± 1,14 b | 23,66 ± 1,05 b | 29,5 ± 3,23 a | <0,01 |
| Biodiversity provision | Microbial species richness | number sp | 183,3 ± 5,1 a | 149,6 ± 6,2 b | 168,3 ± 6,2 a | 133,5 ± 4,1 b | 148,2 ± 4,1 b | <0,0001 |
| | Microbial species diversity | Unittless | 4,17 ± 0,1 | 4,09 ± 0,5 | 4,2 ± 0,4 | 3,9 ± 0,3 | 4,2 ± 0,5 | ns |
| | Microbial species eveness | Unittless | 0,37 ± 0,4 | 0,40 ± 0,1 | 0,40 ± 0,1 | 0,40 ± 0,1 | 0,45 ± 0,1 | ns |
| | Microbial catabolic diversity | Unitless | 2,75 ± 0,01 a | 2,75 ± 0,03 a | 2,73 ± 0,01 ab | 2,69 ± 0,3 b | 2,72 ± 0,0 ab | <0,05 |
| | Invertebrate functional diversity | | | | | | | |
| | Flagellates | mg C g$^{-1}$ soil | $3,13\,E^{-8} \pm 1,19\,E^{-8}$ | $4,13\,E^{-6} \pm 2,52\,E^{-6}$ | $3,13\,E^{-6} \pm 1,19\,E^{-6}$ | $7,44\,E^{-7} \pm 4,86\,E^{-7}$ | $1,73\,E^{-6} \pm 6,8\,E^{-7}$ | ns |
| | Amoebae | mg C g$^{-1}$ soil | $4,63\,E^{-7} \pm 3,19\,E^{-7}$ | $1,31\,E^{-4} \pm 9,04\,E^{-5}$ | $2,98\,E^{-5} \pm 1,59\,E^{-5}$ | $3,27\,E^{-4} \pm 3,04\,E^{-5}$ | $1,51\,E^{-4} \pm 9,29\,E^{-5}$ | ns |
| | Ciliates | mg C g$^{-1}$ soil | 0 b | $2,20\,E^{-6} \pm 1,68E^{-6}$ b | $7,53\,E^{-7} \pm 3,95\,E^{-7}$ b | $6,83\,E^{-6} \pm 6,78\,E^{-6}$ b | $7,75\,E^{-5} \pm 2,96\,E^{-5}$ a | <0,001 |
| | Total Protists | mg C g$^{-1}$ soil | $3,37\,E^{-7} \pm 1,57\,E^{-7}$ | $1,38\,E^{-4} \pm 8,94\,E^{-5}$ | $3,37\,E^{-5} \pm 1,57\,E^{-5}$ | $4,03\,E^{-5} \pm 2,95\,E^{-5}$ | $2,30\,E^{-4} \pm 1,17\,E^{-4}$ | ns |
| | Bacterial feeder nematodes | mg C g$^{-1}$ soil | $1,70\,E^{-7} \pm 1,13\,E^{-7}$ b | $4,28\,E^{-6} \pm 8,71E^{-7}$ b | $2,71\,E^{-5} \pm 1,27\,E^{-5}$ b | $3,06\,E^{-5} \pm 1,24\,E^{-5}$ b | $1,32\,E^{-4} \pm 4,68\,E^{-5}$ a | <0,001 |
| | Fungal feeder nematodes | mg C g$^{-1}$ soil | $2,03\,E^{-7} \pm 1,50\,E^{-7}$ | $3,14\,E^{-6} \pm 1,85\,E^{-6}$ | $1,60\,E^{-5} \pm 6,46\,E^{-6}$ | $1,35\,E^{-5} \pm 6,09\,E^{-6}$ | $1,87\,E^{-5} \pm 8,05\,E^{-4}$ | ns |
| | Plant-feeder nematodes | mg C g$^{-1}$ soil | 0 a | $6,18\,E^{-6} \pm 2,96\,E^{-6}$ b | $8,80\,E^{-6} \pm 1,89\,E^{-6}$ b | $1,84E^{-5} \pm 6,29\,E^{-6}$ b | $1,32\,E^{-4} \pm 4,65\,E^{-5}$ a | <0,0001 |
| | Omnivore nematode | mg C g$^{-1}$ soil | 0 b | $1,25\,E^{-6} \pm 0,25\,E^{-6}$ b | $9,05\,E^{-6} \pm 4,28\,E^{-6}$ ab | $1,15\,E^{-5} \pm 4,64\,E^{-6}$ ab | $3,52\,E^{-5} \pm 1,50\,E^{-5}$ a | <0,05 |
| | Predatory nematodes | mg C g$^{-1}$ soil | 0 b | 0 b | $1,44\,E^{-6} \pm 1,44\,E^{-6}$ b | $1,37\,E^{-5} \pm 5,69E^{-6}$ ab | $3,97\,E^{-5} \pm 1,97\,E^{-5}$ a | <0,05 |
| | Total nematodes | mg C g$^{-1}$ soil | $3,73\,E^{-7} \pm 1,86\,E^{-7}$ b | $1,48\,E^{-5} \pm 3,55\,E^{-6}$ b | $6,24\,E^{-5} \pm 2,09\,E^{-5}$ b | $8,77\,E^{-5} \pm 3,23E^{-5}$ b | $3,57\,E^{-4} \pm 1,15\,E^{-4}$ a | <0,0001 |
| | Predatory Mites | mg C g$^{-1}$ soil | 0 | 0 | 0 | $9,29E^{-6} \pm 6,52\,E^{-6}$ | 0 | ns |
| | Nematophagous Mites | mg C g$^{-1}$ soil | $1,94\,E^{-6} \pm 1,27\,E^{-6}$ b | $7,25\,E^{-5} \pm 2,94\,E^{-5}$ b | $1,29\,E^{-4} \pm 6,37\,E^{-5}$ b | $2,51\,E^{-4} \pm 5,29\,E^{-5}$ b | $1,05\,E^{-3} \pm 3,62\,E^{-4}$ a | <0,0001 |
| | Nematophagous Prosti | mg C g$^{-1}$ soil | $1,96\,E^{-6} \pm 5,34\,E^{-7}$ b | $1,96\,E^{-6} \pm 9,63\,E^{-7}$ b | $7,24\,E^{-6} \pm 6,26\,E^{-6}$ b | $1,57\,E^{-5} \pm 9,37\,E^{-6}$ b | $5,50\,E^{-5} \pm 1,81\,E^{-5}$ a | <0,001 |
| | Collembola | mg C g$^{-1}$ soil | 0 b | $1,23\,E^{-5} \pm 6,22\,E^{-6}$ b | $8,19E^{-6} \pm 4,66\,E^{-6}$ b | $4,71\,E^{-5} \pm 2,88\,E^{-6}$ ab | $1,37\,E^{-4} \pm 4,21\,E^{-5}$ a | <0,0001 |
| | Fungivorous Cryptostigmata | mg C g$^{-1}$ soil | $2,15\,E^{-6} \pm 9,39\,E^{-7}$ b | $3,72\,E^{-5} \pm 1,20\,E^{-5}$ b | $1,27\,E^{-5} \pm 4,22\,E^{-5}$ b | $5,23\,E^{-4} \pm 1,25\,E^{-4}$ b | $1,94\,E^{-3} \pm 5,27\,E^{-4}$ a | <0,0001 |
| | Fungivorous Prostigmata | mg C g$^{-1}$ soil | 0 b | $1,06\,E^{-6} \pm 1,06\,E^{-6}$ b | $3,98\,E^{-7} \pm 2,95\,E^{-7}$ b | $1,42\,E^{-6} \pm 8,39\,E^{-7}$ b | $2,35\,E^{-5} \pm 1,18\,E^{-5}$ a | <0,05 |
| | Diplura | mg C g$^{-1}$ soil | $4,01\,E^{-6} \pm 4,01\,E^{-6}$ | 0 | 0 | $2,73\,E^{-5} \pm 2,73\,E^{-5}$ | $2,87\,E^{-5} \pm 1,62\,E^{-5}$ | ns |
| | Symphyla | mg C g$^{-1}$ soil | 0 | $1,14\,E^{-6} \pm 1,14\,E^{-6}$ | $3,61\,E^{-6} \pm 2,71\,E^{-6}$ | $8,53\,E^{-6} \pm 4,38\,E^{-6}$ | $3,13\,E^{-6} \pm 2,32\,E^{-6}$ | ns |
| | Protura | mg C g$^{-1}$ soil | 0 | 0 | $9,92\,E^{-7} \pm 6,90\,E^{-7}$ | $3,86\,E^{-6} \pm 3,86\,E^{-6}$ | $1,65\,E^{-6} \pm 1,89\,E^{-6}$ | ns |
| | Total arthropods | mg C g$^{-1}$ soil | $1,01\,E^{-5} \pm 3,96\,E^{-6}$ b | $1,26\,E^{-4} \pm 4,11\,E^{-5}$ b | $2,77\,E^{-4} \pm 1,04\,E^{-4}$ b | $8,72\,E^{-4} \pm 1,90\,E^{-4}$ b | $3,25\,E^{-3} \pm 9,08\,E^{-4}$ a | <0,0001 |
| | C mineralization by soil food webs | g C m$^{-2}$ y$^{-1}$ | 44,9 ± 9,0 b | 440,1 ± 48,9 ab | 117,5 ± 14,3 b | 539 ± 101,8 ab | 1011,2 ± 459,2 a | <0,05 |
| | Soil ecosystem stability | y$^{-1}$ | 0,131 ± 0,027 a | 0,004 ± 0,001 b | 0,002 ± 0,00 b | 0,008 ± 0,001 b | 0,036 ± 0,02 b | <0,0001 |
| Aboveground C sequestration | Aboveground carbon stock | t C ha$^{-1}$ | 0 | 0,9 ± 0,59 b | 2,53 ± 1,16 b | 1,42 ± 0,43 b | 44,12 ± 19,87 a | <0,0001 |
| Biodiversity provision & treats | Species richness | number of sp | 0 | 8,71 ± 2,81 c | 7,38 ± 1,6 c | 14,25 ± 0,96 b | 23,3 ± 1,73 a | <0,0001 |
| | Species diversity | unitless | 0 | 1,04 ± 0,28 b | 1,22 ± 0,32 a | 1,54 ± 0,12 a | 0,87 ± 0,26 c | <0,05 |
| | Eveness | unitless | 0 | 0,49 ± 0,12 a | 0,62 ± 0,12 a | 0,58 ± 0,30 a | 0,28 ± 0,08 b | <0,001 |
| | Invasive species | number of sp | 0 | 0 | 0 | 0 | 0 | ns |
| Soil protection | Plant cover | % | 0 | 100 | 100 | 100 | 100 | ns |

*Table 12 Potential soil and plant indicators for the Santa Elena roadcut and their value in vegetation patches representing different stages on the succession. For each indicator, the intensity of the cell color increases as the indicator increases*

| ECOSYSTEM SERVICE | INDICATOR | Unit | >>>>>>>> post-operation maturity pattern >>>>>>> | | | | |
|---|---|---|---|---|---|---|---|
| | | | Bare soil | Low shurbs | Tall shurbs | 5 y old shurbs | 60 y old forest |
| Aboveground C sequestration | Total $C_{org}$ | g C . $m^{-2}$ | 8752,9 | 4392 | 5610 | 6687 | 7372 |
| | $C_{org}$ Fast Pool | g C . $m^{-2}$ | 8027 | 2281 | 1578 | 1977 | 1968 |
| | $C_{org}$ Slow Pool | g C . $m^{-2}$ | 545 | 2110 | 4032 | 4711 | 5401 |
| Soil physical stability | Aggregate stability | mm | 1,14 | 1,39 | 2,1 | 2,1 | 2,1 |
| Soil fertility | Soil bulk density | g $cm^{-3}$ | 1,7 | 1,4 | 1,35 | 0,8 | 0,52 |
| | N | % | 0,47 | 0,86 | 1,22 | 2,95 | 2,96 |
| | Ca | mg $kg^{-1}$ | 6,2 | 6,1 | 6,1 | 6,4 | 8,7 |
| | Mg | mg $kg^{-1}$ | 209 | 172 | 209 | 148 | 443 |
| | P | mg $kg^{-1}$ | 5 | 5 | 5,3 | 6,3 | 12,4 |
| | K | mg $kg^{-1}$ | 40 | 78,5 | 86,2 | 133,8 | 235 |
| | Na | mg $kg^{-1}$ | 20,33 | 19,66 | 21,5 | 23,66 | 29,5 |
| Biodiversity provision | Microbial species richness | number sp | 183,3 | 149,6 | 168,3 | 133,5 | 148,2 |
| | Microbial catabolic diversity | Unitless | 2,75 | 2,75 | 2,73 | 2,69 | 2,72 |
| | Ciliates | mg C $g^{-1}$ soil | 0 | 0,00000220 | 0,00000075 | 0,00000683 | 0,00007738 |
| | Bacterial feeder nematodes | mg C $g^{-1}$ soil | 0 | 0,00000428 | 0,00002715 | 0,00003062 | 0,00013163 |
| | Plant-feeder nematodes | mg C $g^{-1}$ soil | 0 | 0,00000618 | 0,00000880 | 0,00001841 | 0,00013171 |
| | Omnivore nematode | mg C $g^{-1}$ soil | 0 | 0,00000125 | 0,00000905 | 0,00001145 | 0,00003523 |
| | Predatory nematodes | mg C $g^{-1}$ soil | 0 | 0 | 0,00000144 | 0,00001372 | 0,00003969 |
| | Total nematodes | mg C $g^{-1}$ soil | 0,00000037 | 0,00001485 | 0,00006239 | 0,00008773 | 0,00035693 |
| | Nematophagous Mites | mg C $g^{-1}$ soil | 0,00000194 | 0,00007253 | 0,00012902 | 0,00025126 | 0,00105144 |
| | Nematophagous Prostigmata | mg C $g^{-1}$ soil | 0,00000196 | 0,00000196 | 0,00000742 | 0,00001570 | 0,00005497 |
| | Collembola | mg C $g^{-1}$ soil | 0,00000000 | 0,00001229 | 0,00000819 | 0,00004705 | 0,00013708 |
| | Fungivorous Cryptostigmata | mg C $g^{-1}$ soil | 0,00000215 | 0,00003718 | 0,00012712 | 0,00052304 | 0,00193659 |
| | Fungivorous Prostigmata | mg C $g^{-1}$ soil | 0,00000000 | 0,00000106 | 0,00000040 | 0,00000142 | 0,00002348 |
| | Total arthropods | mg C $g^{-1}$ soil | 0,00001006 | 0,00012616 | 0,00027676 | 0,00087195 | 0,00325258 |
| | C mineralization by soil food webs | g C $m^{-2}$ $y^{-1}$ | 44,90 | 440,100 | 117,500 | 539,000 | 1011,20 |
| | Soil ecosystem stability | $y^{-1}$ | 0,131 | 0,004 | 0,002 | 0,008 | 0,036 |
| Aboveground C stock | Aboveground carbon stock | t C $ha^{-1}$ | 0 | 0,900 | 2,530 | 1,420 | 44,12 |

A principal component analysis of the plant communities (Figure 26) revealed the important presence of *H. rhamnoides* and *S. atrocinera* at the base of the roadcut where carbon rich soil sediments are relatively stabilized thanks to the moderate incline. In this sense, planting *H. ramnoides* and willows species seems a good election to stabilize the substrate of the jardinieres.

*Table 13 Plant species present at each type of vegetation cover in the Santa Elena roadcut and in the reference sites (5- and 60-year-old natural communities). Blue shade indicates presence.*

| | **Species** | **Low shrubs** | **Tall shrubs** | **5 y old shrubs** | **60 y old forest** |
|---|---|---|---|---|---|
| 1 | Acer campestre | | | | |
| 2 | Acer opalus | | | | |
| 3 | Amelanchier ovalis | | | | |
| 4 | Arctostaphylos uva-ursi | | | | |
| 5 | Berberis vulgaris | | | | |
| 6 | Betula pendula | | | | |
| 7 | Buxus sempervirens | | | | |
| 8 | Clematis vitalba | | | | |
| 9 | Cornus sanguinea | | | | |
| 10 | Coronilla emerus | | | | |
| 11 | Corylus avellana | | | | |
| 12 | Crataegus monogyna | | | | |
| 13 | Cytisophyllum sessilifolium | | | | |
| 14 | Daphne laureola | | | | |
| 15 | Fraxinus excelsior | | | | |
| 16 | Genista scorpius | | | | |
| 17 | Hedera helix | | | | |
| 18 | Hippophae rhamnoides | | | | |
| 19 | Ilex aquifolium | | | | |
| 20 | Juniperus communis | | | | |
| 21 | Lonicera etrusca | | | | |
| 22 | Lonicera xylosteum | | | | |
| 23 | Pinus sylvestris | | | | |
| 24 | Populus nigra | | | | |
| 25 | Populus tremula | | | | |
| 26 | Prunus mahaleb | | | | |
| 27 | Quercus ballota | | | | |
| 28 | Quercus faginea | | | | |
| 29 | Rhamnus alaternus | | | | |
| 30 | Rhamnus alpina | | | | |
| 31 | Rhamnus saxatilis | | | | |
| 32 | Rosa sp. | | | | |
| 33 | Rubus idaeus | | | | |
| 34 | Rubus ulmifolius | | | | |
| 35 | Salix atrocinerea | | | | |
| 36 | Salix eleagnos | | | | |
| 37 | Santolina chamaecyparissus | | | | |
| 38 | Satureja montana | | | | |
| 39 | Sorbus aria | | | | |
| 40 | Thymus vulgaris | | | | |
| | **number of sp** | **23** | **16** | **23** | **36** |

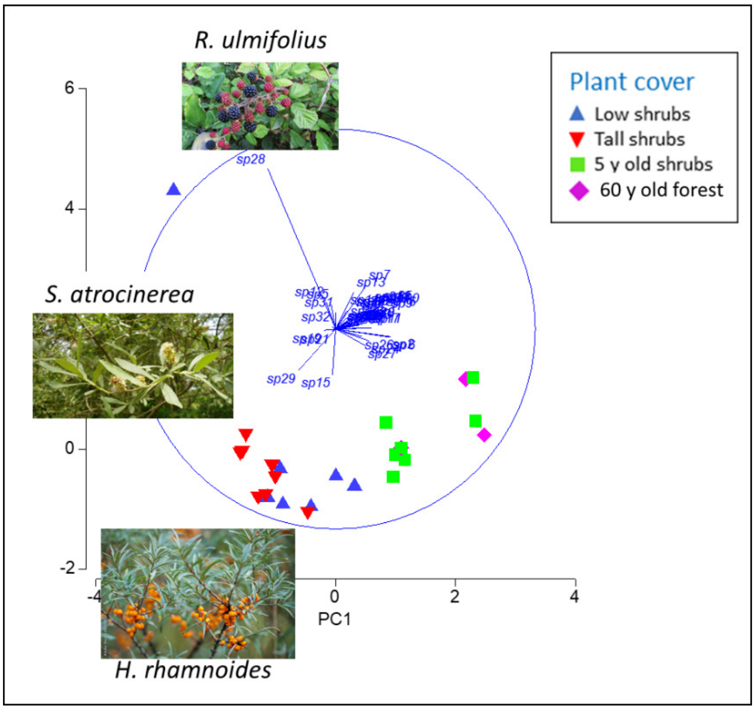


*Figure 26 PCA of the species composition of plant communities of different maturity in the Santa Elena roadcut. The most significant species of each stage are shown.*

A second important conclusion from Table 12is that soil carbon stocks in the surface of the roadcut follow patterns inconsistent with plant succession, according to which soil organic carbon content is expected to increase in accordance with the sequence: *bare soil < low shrubs < tall shrubs < 5-year-old shrubs < 60-year-old forest*. This inconsistence can be easily explained by the continuous translocation of soil materials across the surface of the cutting and by large inputs of dissolved and particulate soil carbon from forest soil above the cutting. A clear example of this importation is provided by very high organic carbon content in the bare soil, almost totally attributable to labile carbon. We hypothesize that this carbon has reached the bare surfaces in a dissolved form and that its presence is transient. Leaving aside the great spatial and temporal instability of carbon in the bare zones, soil carbon stocks increase with maturation of the soil-plant system as expected.

Concerning soil fertility, soil nutrients are not good indicators of progress towards mature systems. Despite being sensitive to ecosystem maturity when taken one by one, they are not good informers of the functioning of natural ecosystems and they must be considered in block or in the form of stochiometric relationships, since soil stoichiometry has been found to regulate the multifunctionality of the system together with plant biodiversity (Lucas-Borja & Delgado-Baquerizo, 2019). As a rule, soil nutrients should be monitored taking their value and stoichiometry in forest soil as benchmark to be met in the long-term.

Soil invertebrates are very promising for monitoring since they show a consistent increasing trend as the restorative succession progresses. Since carbon mineralization by the food web depends on the size and activity of soil biota, this property also increases with maturity. As expected, stability is the lowest in the very early stage of the ecosystem development, since it has been posited that increasing availability of nutrients would destabilize the system.

Soil microbial biodiversity progression towards maturity is not correctly assessed by species richness or microbial catabolic activity.

As for vegetation biodiversity, more accurate information is provided by the detailed study of the results of the shotgun analyses. Figure 27A shows that two microbial taxa are specific of the bare soil and that the community composition is the same across all later successional stages. Not surprisingly, when studying functional genes (Figure 27B) the reference forest shows a very characteristic range of functional capacities, that may be used as reference for microbial functional diversity post-operational progress.

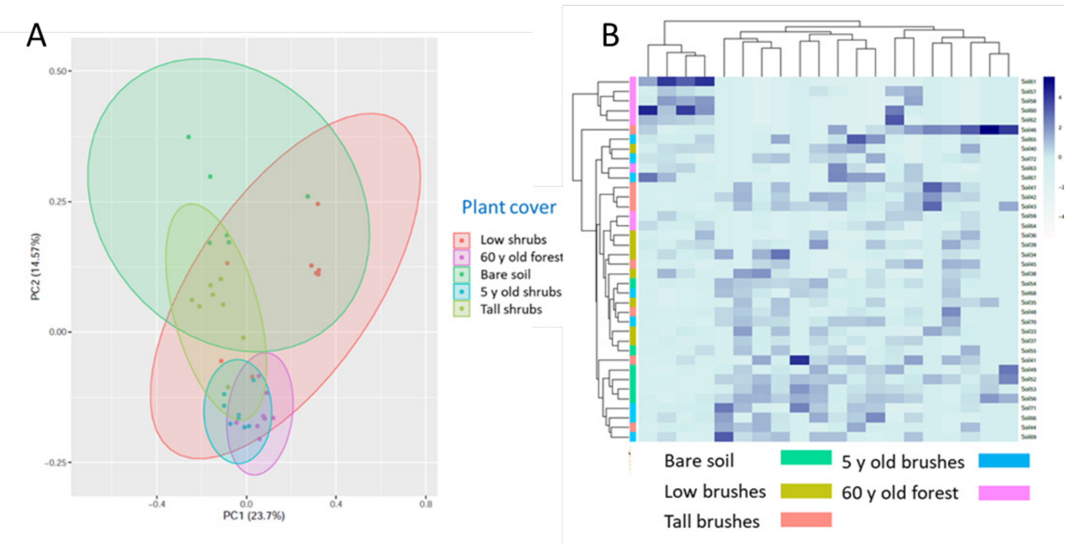


*Figure 27 PCA plot for soil microbial genera (A) and heatmap plot showing the clustering (scaled Kegg pathways relative abundances) for functional genes (B) in the soil of all maturity stages of the vegetation sampled in the Sta Elena roadcut.*

### 2.1.11 Expected effect of works on aboveground and belowground C stocks

To simulate effects of the accepted solution on the organic carbon stocks in the restored area, we have assumed that:

- as stated in the restoration project, 5700 m$^2$ of flat jardinieres will be created and planted
- the substrate used to fill the jardinieres is favourable to plant growth

- the composition of the initial plantation is comparable to that of the tall shrubs described in the baseline
- this initial plantation, in interaction with the surrounding source communities will converge in the middle and long term with the 5-year-old and 60-year-old soil-plant systems prospected next to the roadcut.

Our simulation considers aboveground plant carbon (root carbon is not included) and organic carbon in the top 15 cm of the soil.

To simulate the effect of the restoration works on carbon sequestration, we have calculated the difference between total carbon in the cutting 5 and 60 years after the operations and total carbon in the cutting in the baseline. Carbon sequestration has been simulated including the slow (recalcitrant and stable) soil carbon pool only, instead of including total (slow + fast) soil carbon stocks. Table 14show the resulting values.

*Table 14 Baseline and projected C stocks in the Sta Elena roadcut, including only recalcitrant organic carbon stocks in the upper 15 cm of the soil. Carbon in plant roots not included.*

| | Soil slow C stocks (15 top cm) | Aboveground plant C stocks | Total C stocks |
|---|---|---|---|
| **Base line** | **t C** | **t C** | **t C** |
| Bare soil | 0,8 | 0,0 | 0,8 |
| low shrubs | 1,2 | 0,1 | 1,3 |
| tall shrubs | 3,2 | 0,2 | 3,4 |
| **Base line total** | **5,3** | **0,3** | **5,5** |
| | | | |
| | Soil slow C stocks (15 top cm) | Aboveground plant C stocks | Total C stocks |
| **Projections** | **t C** | **t C** | **t C** |
| 5-year-old shrubs | 26,9 | 0,8 | **27,7** |
| 60-year-old forest | 30,8 | 25,1 | **55,9** |

From Table 14 it follows that the projected solution will have positive effects on local carbon sequestration. Currently, measured the organic carbon stock in the cutting is 5,5 tones and, if plant-soil succession progresses as expected, this stock is expected to amount to 27,7 tones 5 years after operations and to 55,9 tones 60 years later.

Vegetation will contribute to carbon accumulation much more that soil, as 60 years after restoration, plant carbon will be 83 times higher than in the baseline estate, while soil carbon will be only 5,8 times greater. However, it must not be forgotten that recalcitrant soil carbon is much more stable in time than plant C and contributes much more to climate change mitigation.

## 2.2 The case of the Massaciuccoli lake agricultural area (Lucca, Italy)

### 2.2.1 Case description

The Massaciuccoli lake (Lucca, Italy, 43.9833379, 10.333081) and its surrounding marshlands constitute one of the most important Tuscan Ramsar wetlands. The area is included in the Tuscan regional park "Migliarino-San Rossore-Massaciuccoli" (Figure 28) and makes part of the Natura 2000 network as the Special Area of Conservation named "Lago e Padule di Massaciuccoli".


*Figure 28 Situation of the study site in the protected area of the Massaciuccoli Lake.*

The Massaciuccoli lake is of coastal origin and is separated from the shoreline by a sand dune. The lake is shallow (no more than 5 m deep) and measures about 7 km$^2$. The surrounding palustrine zone (about 13 km$^2$) has been managed since Roman times and, since the fourteenth century has undergone repeated attempts of reclamation (Linoli, 2005). Since 1930, the lake basin is drained by a complex network of artificial channels, ditches, and pumping stations. Because of reclamation subsidence began leaving the lake perched above the drained area, that is now 0 to -3 m below the sea level. Subsidence persists at a rate of about 3 to 4 cm $yr^{-1}$ (Pistocchi et al., 2012) and is caused by the decrease of peat porosity following desiccation and, especially by biochemical oxidation and humification of soil organic matter following soil aeration by drainage and tillage (Serva & Brunamonte, 2007).

In the lake, most environmental problems (eutrophication, salinization, over-exploitation of the groundwater, hydraulic risk and presence of exotic species) are attributable to the surrounding intensive agriculture. Soil particles and agrochemicals reach the lake and accumulate there due to the functioning of the hydraulic network. In winter, the lake collects water from the surrounding areas thanks to the system of artificial channels (Figure 29) while, in spring and summer, the water is returned from the lake to the agricultural area and used for irrigation. The recirculated water is increasingly enriched in suspended solids (mainly eroded soil particles), nutrients and agrochemicals.

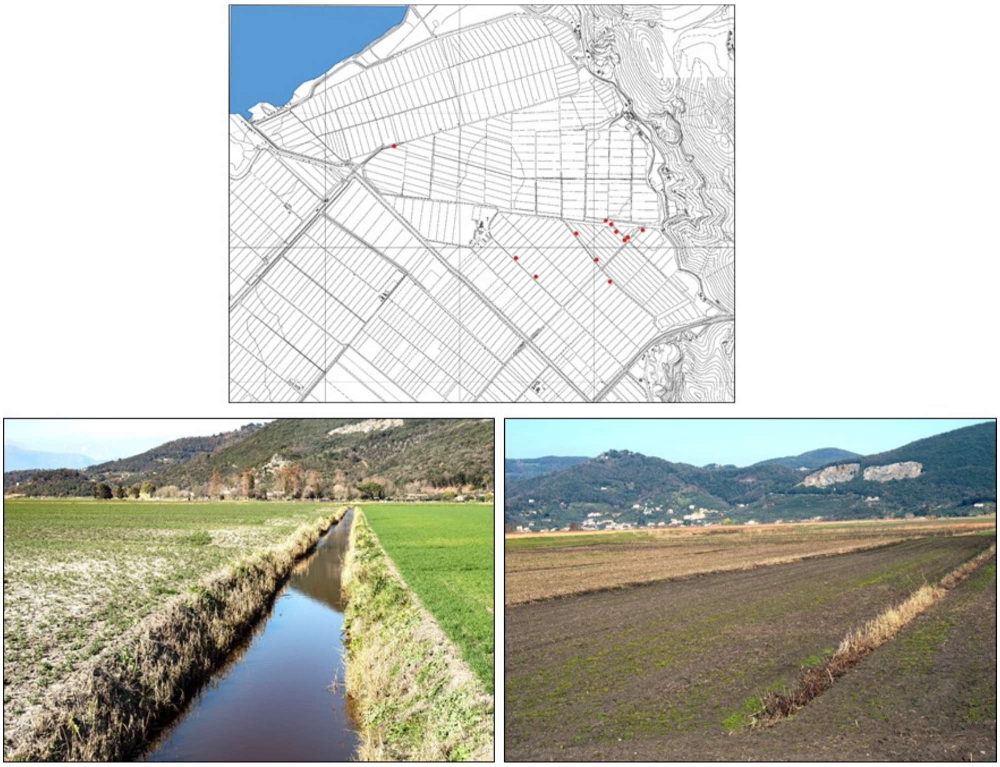

*Figure 29 Drainage network of the study site (above); primary (below, left) and secondary (below, right) drainage channels.*

## 2.2.2 Proposed solutions

Several complementary solutions have been proposed to cope with the abovementioned problem: (a) planting vegetation strips in the area of “La Costanza”; (b) planting vegetation strips in the “Studiati” area; (c) improving the management of the Fossaccio and Fossetto channels, including reshaping and stabilizing the channel banks; (d) creating a retention basin, and (e) implementing a “NBS lab” about potential strategies to mitigate the erosion of the eastern slopes around the Lake Massaciuccoli.

Because of budgetary limitations, we proposed to evaluate only the two first measures, that actually are a unique measure applied to two different zones of the agricultural area (Figure 30).

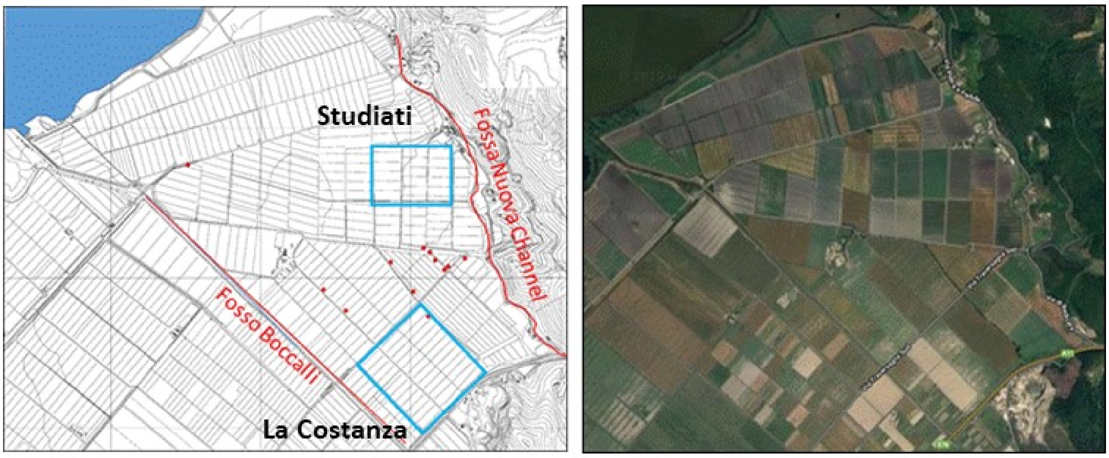


*Figure 30 Location of the two demonstrator zones (Studiati and La Costanza) in the agricultural area of the Massaciucoli lake.*

The proposed vegetation strips are land areas of either indigenous or planted vegetation (usually grasses) created down slope of the cropland to filter nutrients, sediments and pesticides from the runoff before it reaches the water drainage system. These strips are proven solutions for the removal of sediments and other suspended solids from runoff, provided that the water flow is shallow and uniform and that the strips have not been previously inundated with sediment (Dillaha et al., 1989). Other important factors affecting the effectiveness of the filters are the dimensions (length and width) of the filter (Abu-Zreig et al., 2004), the kind of the incoming pollutant, slope, volume and type of water flow and vegetation characteristics.

### 2.2.3 Field sampling

From previous field works (data provided by Dr. N. Silvestri, Università di Pisa) we knew that, from the point of view of soil carbon, the study area can be divided in three zones of different soil type and texture and contrasting soil organic matter (SOM) content (Figure 31).

In the high and medium carbon richness zones, soil is peaty, and the SOM content is as high as 30%-40% and 15-30%, respectively. At the other end of the agricultural area, the SOM content is significantly lower and ranges from 3 to 4%. Soil pH describes a N-S transect from acidity (pH about 4 by the lake) to basicity (pH about 8 in the southernmost end). The water table is maintained by pumping stations at a level ranging from 0,40 to 0,60 m below the soil surface (Pellegrino et al., 2015). Therefore, the upper soil layer is seldom subject to water saturation except during flood events. The distance between the water table and the soil surface also increases from the lake towards the south and shows seasonal variations. During our sampling campaign, in the fall of 2019, the water table was visible 15 to 20 cm below the soil surface in many points of the area.

Also, from data provided by Dr. Silvestri, we know that area has undergone substantial changes in crop management in recent years under the stimuli of the EU Common Agricultural Policy. Indeed, in 2012 an important proportion of the study area (mainly in the north part) was occupied by stable corn crops while, in the south part, the traditional wheat-sunflower rotation system was the dominant land use. From 2016 to 2020, a part of the previously stable corn fields has been managed in rotation with soybean, with important consequences for soil quality (Figure 31).

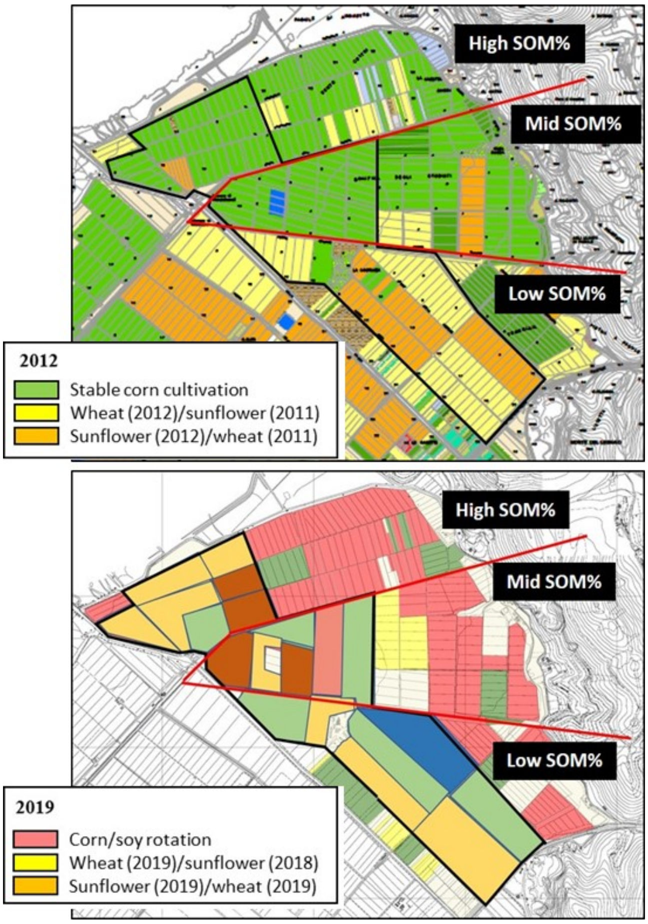


*Figure 31 Crop map in 2012 and 2019. The red line delimitates three zones with low, medium and high SOM content. The thick black line delimitates the "Constanza" agricultural farm (see text for explanation)*

The area is cultivated to the smallest corner, except for some grassy fringes that protect the embankments (made of unknown compacted soil materials) alongside the main channels and for some margins of natural soil in the periphery of the crop units let unseeded to provide access for machinery (Figure 32).

*Figure 32 Non-cultivated fringes in the study area: embankments alongside the main channel (left) and vegetated accesses for machinery (right).*

During the execution period of PHUSICOS, filters will be implemented in two very small demonstrator areas of the study site.

In October 2019, we launched a pre-operational sampling campaign. The sampling plan was designed to cover the great spatial heterogeneity of the soil. Given the absence of natural plant communities, plant characteristics were not included. To let the baseline described for future expansion of the filters, we sampled the whole agricultural area for all proposed soil properties.

The sampling date was carefully chosen to find all fields in their resting period, when soil was bare. The fields had not yet been sown but some of them had already been tilled.

As mentioned before, there have been recent changes in the agricultural management of the area and, at sampling time, two main agricultural strategies were represented: wheat/sunflower rotation and corn/soy rotation. To cover the main combinations of current crops, cropping history and soil organic matter and texture classes, we distributed 53 sampling points in 14 fields along three N-S transects, one per SOM content zone. At each of the three SOM zones, we also settled sampling points in non-cultivated strips on natural soil. Unfortunately, adequate non-cultivated fringes were very scarce, and for some statistical purposes the sampling is unbalanced. The sampling plan is shown in Figure 33.

All samples were taken within the boundaries of “la Constanza” farm, because of this was the only sector of the study area for which information about land use and current management was available from the owners.

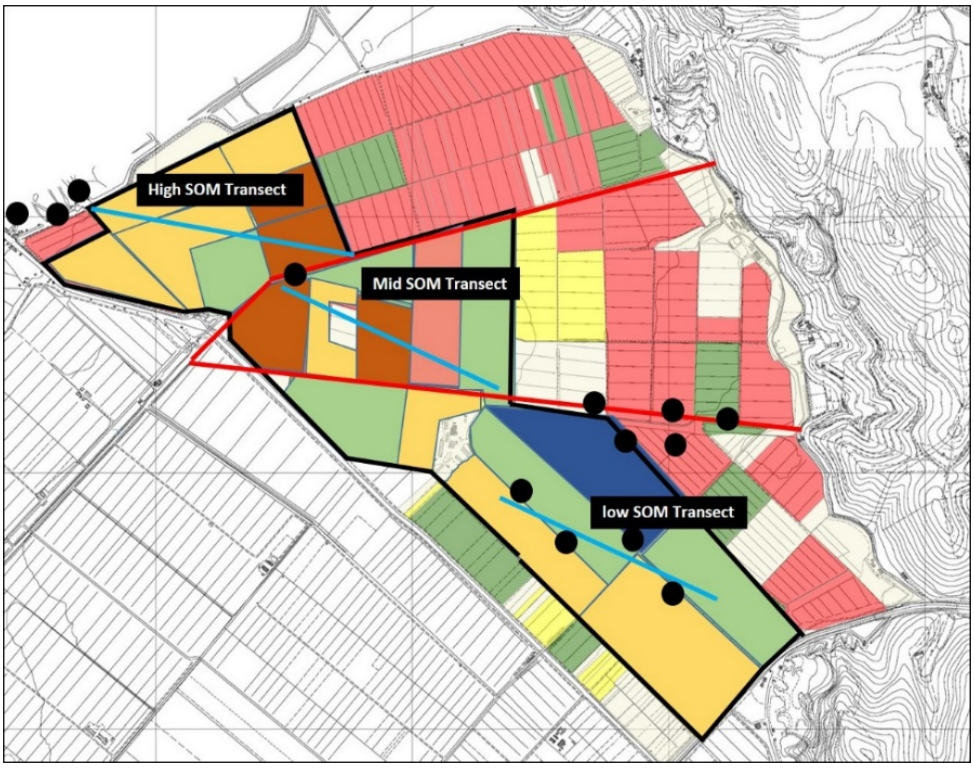


*Figure 33 Sampling plan in the Massaciuccoli area. The three blue lines indicate our soil sampling transects. Black dots show sampling points in non-cultivated soils. The red line delimitates three zones of low, medium, and high SOM content, respectively. The thi*

At each sampling point, four soil cores / 5 cm in Ø and 15 cm deep were taken and allocated to physical and chemical analyses, microbial biodiversity and functioning, food web groups and bulk density, respectively.

For the purposes of this report, and to make reading and interpretation easier, we have not included the data of the medium C concentration zone and we have ordered our results with a view to facilitate the comparison between cropped soil and soil under future vegetated strips in the richest soil C zone and in the poorest soil C zone.

### 2.2.4 The baseline on the indicators and their expected post-operation evolution

All studied properties are shown for these treatments in Table 15, and soil properties showing significant differences between cropped soils and soils under vegetated strips are displayed in Table 16.

*Table 15 Soil and plant properties measured in cropped soils and in soils under vegetated strips in the zones of high and low soil C content, around the Massaciuccoli lake. Values expressed as mean ± standard deviation. For properties showing very low values below 0, we have used the scientific to avoid long decimal numbers. For better understanding, 3,72E-5 means 0,0000372. "p" represents the significance of differences between vegetation units after analyses of the variance. When differences are significant (red values of p) red letters indicate differences between pairs of units: units sharing one letter are equal and units that have no common letters are different.*

| ECOSYSTEM SERVICE | INDICATOR | Unit | Sampled soil units | | | | |
|---|---|---|---|---|---|---|---|
| | | | Low C crop | Low C strips | High C crop | High C strips | *p* |
| C sequestration belowground | Total $C_{org}$ | mg C . $g^{-1}$ soil | 36,90 ± 4,3 b | 70,83 ± 7,3 b | 236,88 ± 7,5 a | 263,94 ± 7,6 a | <0.0001 |
| | $C_{org}$ Fast Pool | mg C . $g^{-1}$ soil | 5,27 ± 1,1 b | 18,64 ± 4,9 b | 39,89 ± 4,03 a | 32,93 ± 13 b | <0.0001 |
| | $C_{org}$ Slow Pool | mg C . $g^{-1}$ soil | 31,63 ± 3,9 b | 52,19 ± 8,8 b | 196 ± 5,4 a | 231 ± 47 a | <0.0001 |
| | C in crushed vs intact soil aggregates | % | 55,31 ± 4,1 a | 27,35 ± 17,1 c | 36,8 ± 3,1 b | 24,58 ± 6 d | 0,0004 |
| Soil physical stability | Aggregate stability | mm | 0,7 ± 0,4 c | 1,83 ± 0,2 b | 1,94 ± 0,1 b | 2,44 ± 0,2 a | <0.0001 |
| Soil fertility | Bulk density | g $cm^{-3}$ | 1,12 ± 0,02 a | 1,00 ± 0,1 b | 0,65 ± 0,2 c | 0,50 ± 0,7 d | <0,0001 |
| | N | % | 2,64 ± 0,17 c | 3,49 ± 0,37 c | 9,90 ± 0,05 a | 8,77 ± 1,23 b | <0,0001 |
| | Ca | mg $kg^{-1}$ | 9,4 ± 0,1 b | 8,9 ± 0,2 b | 13,3 ± 1,1 a | 12,1 ± 1,7 b | <0,01 |
| | Mg | mg $kg^{-1}$ | 211,06 ± 9,8 c | 312 ± 27,8 c | 735 ± 71 b | 1264 ± 454 a | <0,0001 |
| | P | mg $kg^{-1}$ | 33,08 ± 1,8 | 39,22 ± 15,5 | 35,96 ± 4,7 | 62,48 ± 24,2 | ns |
| | K | mg $kg^{-1}$ | 270,1 ± 27,7 c | 391,5 ± 75 ab | 430,6 ± 34 b | 712,6 ± 185 a | <0,001 |
| Biodiversity provision | Na | mg $kg^{-1}$ | 45,83 ± 0,8 b | 88,33 ± 11 ab | 247,6 ± 77,8 a | 134,4 ± 46 ab | <0,05 |
| | Microbial species richness (Bacteria) | number of ssp | 157,4 ± 4,0 | 152 ± 8,0 | 144 ± 3,2 | 154 ± 7,3 | ns |
| | Microbial species richness (Bacteria) | Unitless | 4,03 ± 0,03 | 3,98 ± 0,1 | 3,97 ± 0,03 | 4,13 ± 0,7 | ns |
| | Microbial species eveness | Unitless | 0,36 ± 0,01 b | 0,35 ± 0,01 b | 0,37 ± 0,1 b | 0,41 ± 0,2 a | <0,01 |
| | Microbial catabolic diversity | Unitless | 2,72 ± 0,01 | 2,70 ± 0,1 | 2,73 ± 0,01 | 2,73 ± 0,01 | ns |
| | Invertebrate functional diversity | | | | | | |
| | Flagellates | mg C $g^{-1}$ soil | 2,39 $E^{-5}$ ± 6,30 $E^{-6}$ | 4,64 $E^{-6}$ ± 4,38 $E^{-6}$ | 4,39 $E^{-5}$ ± 1,03 $E^{-5}$ | 5,48 $E^{-5}$ ± 3,21 $E^{-5}$ | ns |
| | Amoebae | mg C $g^{-1}$ soil | 4,39 $E^{-4}$ ± 1,92 $E^{-4}$ | 1,91 $E^{-6}$ ± 1,91 $E^{-6}$ | 1,24 $E^{-5}$ ± 3,91 $E^{-5}$ | 2,24 $E^{-3}$ ± 12,12 $E^{-3}$ | ns |
| | Ciliates | mg C $g^{-1}$ soil | 3,22 $E^{-5}$ ± 1,43 $E^{-5}$ b | 5,59 $E^{-6}$ ± 2,51 $E^{-6}$ b | 5,72 $E^{-5}$ ± 2,08 $E^{-5}$ b | 3,05 $E^{-4}$ ± 2,08 $E^{-4}$ a | <0,01 |
| | Total Protists | mg C $g^{-1}$ soil | 4,95 $E^{-4}$ ± 2,02 $E^{-4}$ | 1,21 $E^{-5}$ ± 5,25 $E^{-6}$ | 1,34 $E^{-3}$ ± 3,96 $E^{-4}$ | 2,60 $E^{-3}$ ± 2,10 $E^{-3}$ | ns |
| | Bacterial feeder nematodes | mg C $g^{-1}$ soil | 2,39 $E^{-5}$ ± 8,32 $E^{-6}$ | 2,75 $E^{-5}$ ± 5,22 $E^{-6}$ | 2,60 $E^{-5}$ ± 8,57 $E^{-6}$ | 8,36 $E^{-5}$ ± 4,01 $E^{-5}$ | ns |
| | Fungal feeder nematodes | mg C $g^{-1}$ soil | 1,51 $E^{-5}$ ± 6,58 $E^{-6}$ | 1,45 $E^{-5}$ ± 4,87 $E^{-6}$ | 1,37 $E^{-5}$ ± 4,96 $E^{-6}$ | 2,66 $E^{-5}$ ± 1,30 $E^{-5}$ | ns |
| | Plant-feeder nematodes | mg C $g^{-1}$ soil | 5,22 $E^{-6}$ ± 1,28 $E^{-6}$ b | 9,1 $E^{-6}$ ± 2,94 $E^{-6}$ b | 1,27 $E^{-5}$ ± 4,30 $E^{-6}$ b | 3,72 $E^{-5}$ ± 1,73 $E^{-5}$ a | <0,001 |
| | Omnivore nematode | mg C $g^{-1}$ soil | 2,54 $E^{-6}$ ± 2,54 $E^{-6}$ | 2,01 $E^{-5}$ ± 7,60 $E^{-6}$ | 2,14 $E^{-5}$ ± 6,78 $E^{-6}$ | 2,11 $E^{-5}$ ± 1,66 $E^{-5}$ | ns |
| | Predatory nematodes | mg C $g^{-1}$ soil | 0 b | 0 b | 3,37 $E^{-6}$ ± 1,83 $E^{-6}$ ab | 1,09 $E^{-5}$ ± 6,62 $E^{-6}$ a | <0,05 |
| | Total nematodes | mg C $g^{-1}$ soil | 4,67 $E^{-5}$ ± 1,74 $E^{-5}$ | 7,12 $E^{-5}$ ± 1,20 $E^{-5}$ | 7,72 $E^{-5}$ ± 1,96 $E^{-5}$ | 1,79 $E^{-4}$ ± 8,95 $E^{-5}$ | ns |
| | Predatory Mites | mg C $g^{-1}$ soil | 0 | 0 | 0 | 0 | ns |
| | Nematophagous Mites | mg C $g^{-1}$ soil | 3,77 $E^{-5}$ ± 1,67 $E^{-5}$ b | 1,43 $E^{-4}$ ± 3,98 $E^{-5}$ ab | 4,78 $E^{-4}$ ± 1,51 $E^{-4}$ a | 4,13 $E^{-4}$ ± 1,05 $E^{-4}$ ab | <0,05 |
| | Nematophagous Prosti | mg C $g^{-1}$ soil | 2,51 $E^{-6}$ ± 9,85 $E^{-7}$ | 8,34 $E^{-6}$ ± 5,63 $E^{-6}$ | 6,13 $E^{-6}$ ± 1,87 $E^{-6}$ | 2,56 $E^{-6}$ ± 1,66 $E^{-6}$ | ns |
| | Collembola | mg C $g^{-1}$ soil | 3,03 $E^{-6}$ ± 1,14 $E^{-6}$ | 8,95 $E^{-5}$ ± 1,88 $E^{-5}$ | 2,0 $E^{-4}$ ± 7,94 $E^{-5}$ | 1,47 $E^{-4}$ ± 7,48 $E^{-5}$ | ns |
| | Fungivorous Cryptostigmata | mg C $g^{-1}$ soil | 1,69 $E^{-5}$ ± 4,43 $E^{-6}$ | 1,72 $E^{-4}$ ± 6,84 $E^{-5}$ | 2,28 $E^{-4}$ ± 5,82 $E^{-5}$ | 4,46 $E^{-4}$ ± 1,65 $E^{-4}$ | ns |
| | Fungivorous Prostigmata | mg C $g^{-1}$ soil | 2,71 $E^{-6}$ ± 2,26 $E^{-6}$ | 1,04 $E^{-5}$ ± 9,83 $E^{-6}$ | 7,46 $E^{-6}$ ± 3,25 $E^{-6}$ | 1,37 $E^{-5}$ ± 1,17 $E^{-5}$ | ns |
| | Diplura | mg C $g^{-1}$ soil | 0 | 0 | 8,76 $E^{-6}$ ± 8,76 $E^{-6}$ | 1,53 $E^{-5}$ ± 1,53 $E^{-5}$ | ns |
| | Symphyla | mg C $g^{-1}$ soil | 4,07 $E^{-6}$ ± 2,46 $E^{-6}$ b | 1,02 $E^{-5}$ ± 1,47 $E^{-6}$ b | 5,62 $E^{-6}$ ± 2,36 $E^{-6}$ b | 2,47 $E^{-6}$ ± 2,24 $E^{-5}$ a | <0,05 |
| | Protura | mg C $g^{-1}$ soil | 0 | 2,68 $E^{-6}$ ± 2,68 $E^{-6}$ | 4,76 $E^{-7}$ ± 4,76 $E^{-7}$ | 0 | ns |
| | Total arthropods | mg C $g^{-1}$ soil | 6,69 $E^{-5}$ ± 2,53 $E^{-5}$ | 4,28 $E^{-4}$ ± 1,26 $E^{-4}$ | 9,35 $E^{-4}$ ± 2,54 $E^{-4}$ | 1,06 $E^{-3}$ ± 2,81 $E^{-4}$ | ns |
| | Carbon mineralization by soil food webs | g C $m^{-2}$ $y^{-2}$ | 65,69 ± 18,21 b | 148,51 ± 25,27 a | 52,21 ± 5,88 b | 65,46 ± 25,29 b | <0,01 |
| | Soil ecosystem stability | $y^{-1}$ | 0,096 ± 0,014 b | 0,137 ± 0,013 b | 0,22 ± 0,02 a | 0,124 ± 0,011 b | <0,0001 |

*Table 16 Potential soil and plant indicators for effects of vegetated strips in the zones with high and low soil C content around the Massaciuccoli lake. For each indicator, the intensity of the cell color increases as the indicator increases.*

| ECOSYSTEM SERVICE | INDICATOR | Unit | Low C content soils | | High C content soils | |
|---|---|---|---|---|---|---|
| | | | cropland | strips | cropland | strips |
| Carbon sequestraton | Total $C_{org}$ | mg C . g$^{-1}$ soil | 6133,7 | 11688,1 | 23178,5 | 171112 |
| | $C_{org}$ Fast Pool | mg C . g$^{-1}$ soil | 869,6 | 2810,6 | 3899,9 | 1591,4 |
| | $C_{org}$ Slow Pool | mg C . g$^{-1}$ soil | 5264 | 8877,5 | 19278,6 | 15521,1 |
| | C in crushed vs intact soil aggregates | % | 55,31 | 27,35 | 36,8 | 24,58 |
| | Aggregate stability | mm | 0,7 | 1,83 | 1,94 | 2,44 |
| Soil fertility | Bulk density | g cm$^{-3}$ | 1,12 | 1 | 0,65 | 0,5 |
| | N | % s.m.s. | 2,64 | 3,49 | 9,9 | 8,77 |
| | Ca | mg kg$^{-1}$ | 9,4 | 8,9 | 13,3 | 12,1 |
| | Mg | mg kg$^{-1}$ | 211,06 | 312 | 735 | 1264 |
| | K | mg kg$^{-1}$ | 270,1 | 391,5 | 430,6 | 712,6 |
| | Na | mg kg$^{-1}$ | 45,83 | 88,33 | 247,6 | 134,4 |
| | Microbial species eveness | Unitless | 0,36 | 0,35 | 0,37 | 0,41 |
| | Ciliates | mg C g$^{-1}$ soil | 0,00003222 | 0,00000560 | 0,00005718 | 0,00030531 |
| | Plant-feeder nematodes | mg C g$^{-1}$ soil | 0,00000522 | 0,00000906 | 0,00001277 | 0,00003725 |
| | Predatory nematodes | mg C g$^{-1}$ soil | 0 | 0 | 0,00000338 | 0,00001091 |
| | Nematophagous Mites | mg C g$^{-1}$ soil | 0,00003772 | 0,00014300 | 0,00047835 | 0,00041340 |
| | Symphyla | mg C g$^{-1}$ soil | 0,00000407 | 0,00000147 | 0,00000563 | 0,00002237 |
| | C mineralization by soil food webs | g C m$^{-2}$ y$^{-2}$ | 65,69 | 148,51 | 52,21 | 65,46 |
| | Soil ecosystem stability | Unitless | 0,096 | 0,137 | 0,22 | 0,124 |

One on the most interesting remarks to be made is that almost all indicators behave very differently in carbon rich and carbon poor soils. Therefore, in this agricultural area, soil carbon content is essential for correct design, implementation, monitoring and evaluation of any intervention.

Focusing on organic carbon content, soil in richer in C under vegetated strips than under crops in the “low carbon” zone but the reverse is true in the “high carbon” peaty soil zone. Therefore, the vegetated strips will be positive for C sequestration in the low carbon zone and negative in the high carbon zone. Transforming crop soils into vegetated soils will favor soil physical structure and soil resistance to erosion everywhere. Strips will also be beneficial for soil nutrients in the low C zone but detrimental in the rich C zone, with some exceptions.

Interestingly, this pattern is inverted when looking at soil biodiversity indicators. Only plant-feeder nematodes are favored by vegetated strips across the whole area, which is easily explained by the fact that these nematodes are associated to plant roots, that are much more abundant under herbaceous plant covers that in cropped soils. When assessing the effect of creating vegetated strips, nematophagous mites can be used as indicators in the low C zone and predatory nematodes and ciliates in the high C zone.

As observed in other study cases mentioned in this report, the Shannon index (or other equivalent biodiversity indexes) applied to the results of the shot-gun analysis is not useful to assess the effect on soil microbial diversity of changes in plant cover. And, again, and more detailed multivariate analyses are much more enlightening (Figure 34).

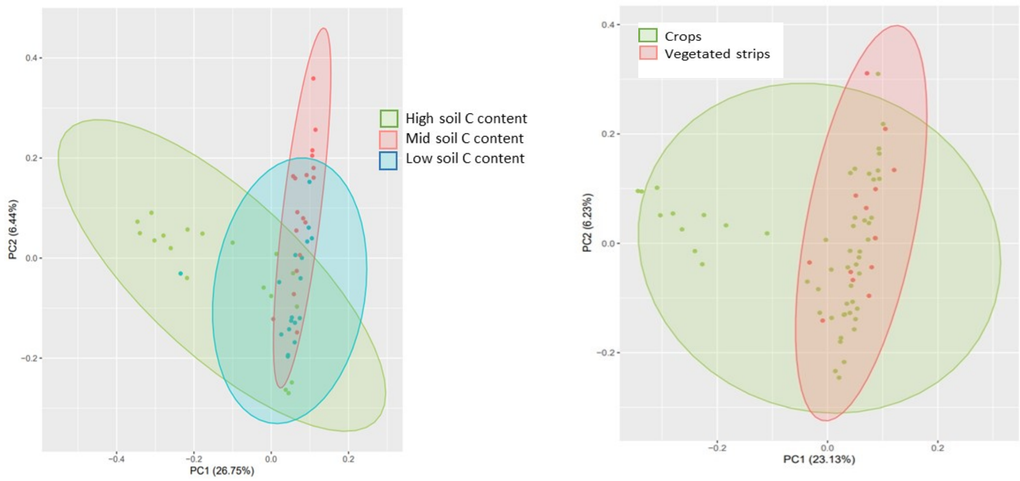


*Figure 34 PCA plot for soil microbial genera in the soil of the agricultural zone of the Massaciuccoli lake per zones of different soil C content -strip and crop soil pooled- (left), and per type of use -or plant cover- (right).*

From Figure 34, it appears that carbon rich soils have different microbial taxonomic composition than soils with medium and low carbon content.

Another important conclusion is that the microbial community of the cropped soils is richer in genera than the community developed in the soil of the vegetated strips. This fact seems contradictory with the general trend of soil microbial diversity, that usually increases with soil carbon content. The explanation arises from a careful study of Figure 35.

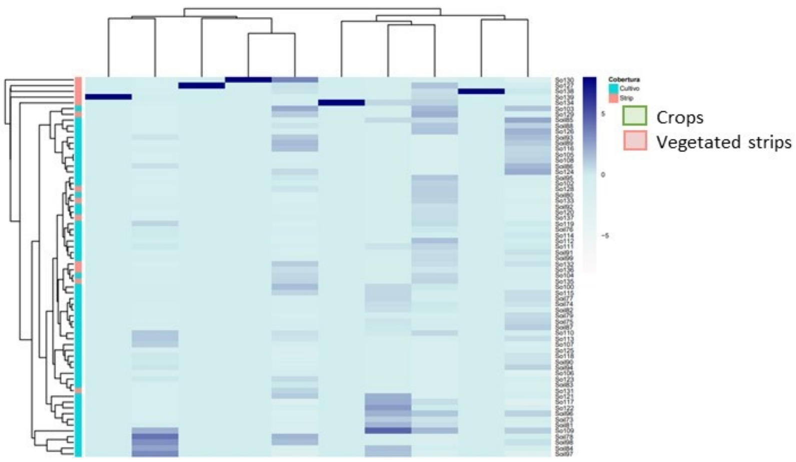


*Figure 35 Heatmap plot showing the clustering (scaled Kegg pathways relative abundances) of microbial functional genes found in cropped soils and in soils under vegetated strips.*

As it can be seen in Figure 35, the vegetated strips hold a particular group of functional genes, different from those found in cropped soils. When searching for the functions they fulfil, we found that one of them codifies the degradation of DDT

(1,1,1−Trichloro−2,2− bis (4−chlorophenyl) ethane) which means that this product, currently banned in the EU, has been applied to the non-cropped spots of the zone. DDT is very persistent and remains highly toxic for decades after cessation of application.

In light of our results, we must highlight that if pesticides were applied to the recently seeded vegetated strips, all their benefits for soil biodiversity will be lost.

### 2.2.5 Simulation of the effect of the vegetated strips on sediment exportation from cropped soils

This section explains our attempt to simulate the effect of vegetated strips on the exportation of soil from cropped soils to the drainage system of the Massaciuccoli agricultural area.

This simulation has been made using an approach different from that used be the UNINA team, also for PHUSICOS and that is included in other deliverables.

For reasons explained below, the simulation has only been made for the pilot area of the peaty areas south of lake Massaciuccoli.

In some points, our results diverge despite having used the same topographic base. Therefore, an in-deep review and comparison of both methodologies may be advisable.

Since this simulation follows a methodology totally different that the used for estimation of soil carbon and soil biodiversity, we include below the complete report.

**Massaciuccoli area and proposed NBS**

Figure 36 gives a schematic view of the whole area with data obtained from the UNINA team. Lake Massaciuccoli can be seen as a large light-blue patch at the top, whereas other smaller water pools and ponds, also in light-blue color, are distinguishable across the field of view. The Serchio river bends its way from East (right) down to South and then up to its mouth at the Tyrrhenian Sea (not colored), West (left) in the picture. The Studiati and la Costanza study areas are shown outlined in red on the upper half of the field, the latter below the former. The Viareggio city is located right on the West side of the Massaciuccoli lake, whereas smaller urban centers and isolated houses can be found across the region. Finally, highways, roads and smaller pathways crisscross the area.

The NBS proposal consists of surrounding each small rectangular crop plot at Studiati and la Costanza farms with a vegetated filter strip (VFS) or buffer zone along three of the four sides of each rectangle. The objective of this proposal is to increase sediment deposition from runoff waters moving through those strips.

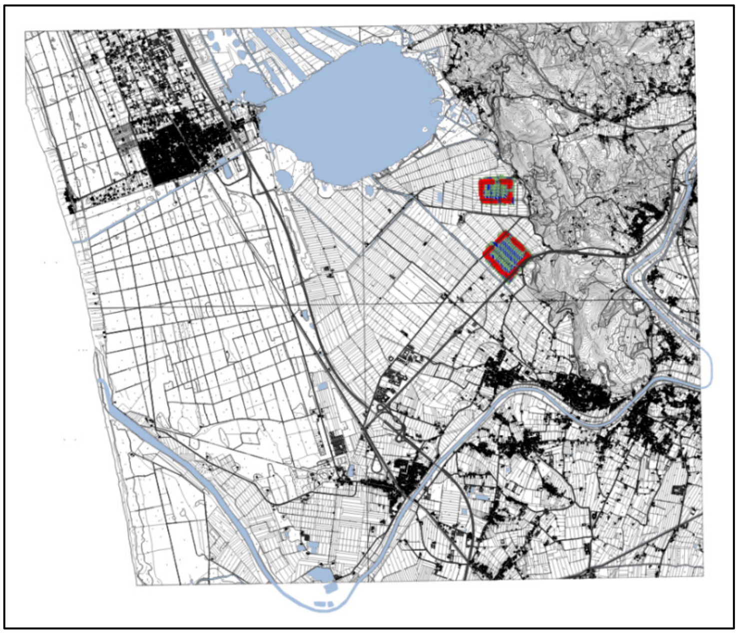

*Figure 36 View of the Massaciuccoli area. Orientation is such that North is up and East is right.*

Crop plots are thus divided into two distinct areas (Figure 37). In the larger one, so-called source area, crops are planted depending on selected agricultural management practices (i.e. conservational or conventional). Surrounding this rectangular source area, and on three of its four sides, there is a 3m-wide VFS/buffer zone where only grass crops are grown to retain suspended solids in surface waters.

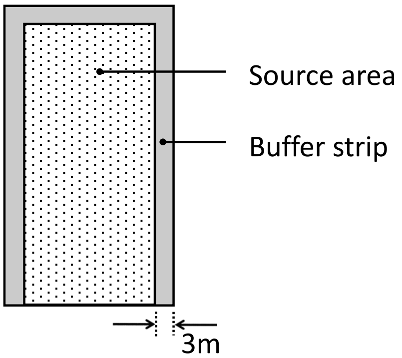


*Figure 37 Layout of the vegetated filter strips. Rectangular plots are surrounded on three of their four sides by 3-m wide buffer filters or VFS.*

Surface slope in the source and VFS areas must be such that precipitation falling onto the former runs naturally towards any of the three sides where VFS have been placed. If the slope is such that water rather flows to the side without VFS, no sediment retention can obviously take place. If the slope is correct, however, water that has already run through those VFS areas then goes into bigger channels, which must also have the right slope to direct water away and towards Lake Massaciuccoli.

We have checked the feasibility of implementing VFS in the crop land at the Studiati and la Costanza study areas. Field observations and experiments to check the performance of those VFS are arguably difficult to set up and run and, consequently, were discarded from the beginning. Instead, we opted for carrying out numerical simulations of surface water runoff and sediment retention, for several combinations of precipitation events and crop management strategies, with appropriate simulation software.

**Material and methods**

For the present study, we used a high-quality Digital Terrain Model (DTM) with a pixel size of 0.2 x 0.2 m obtained from the UNINA team. The UNINA data set also included, among others, a land cover map of the area, plus a detailed vector map outlining crop plots and the buffer filters around them and the drainage channels, as shown in Figure 36.

A preliminary analysis of the DTM data for selected crop plots indicated that both Studiati and la Costanza areas are located below sea level. As shown in Figure 38, the average height *under* sea level of the Studiati fields is about 2 m, which is in fact more than 1 m below those of la Costanza. Therefore, soils become saturated or flooded with water much more often at Studiati during the rainy season (September to December) or spring thaw. Flooding negates the advantages of the VFS strategy, since sediments are then not transported downslope towards the VFSs. Consequently, we limited our simulation analysis to the la Costanza field only.

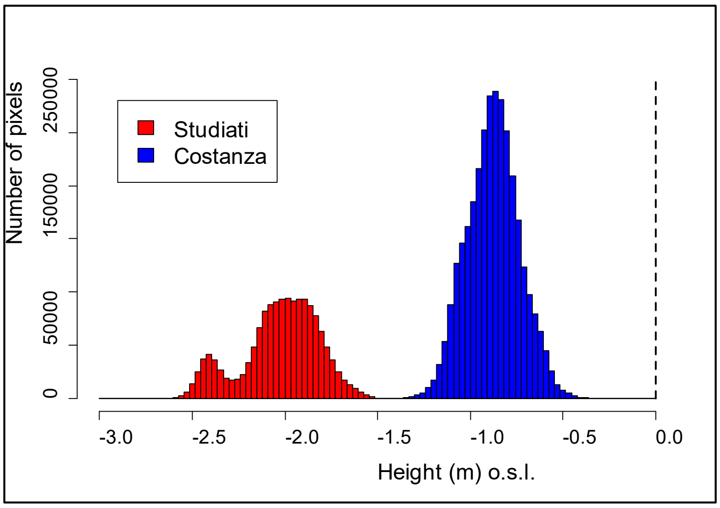


*Figure 38 Distribution of heights o.s.l. in meters of the Studiati and la Costanza study areas. The mean and 95% quantiles of the two distributions are (-2.03, -2.47, -1.67) for Studiati and (-0.88, -1.15, -0.59) for la Costanza. The dashed line on the rightmost side of the figure indicates sea level.*

**Soil data**. Sand, Silt and Clay content (in percentage) were obtained from field work at 20 sampled locations in la Costanza study area by the CREAF team. Additional sampling points were provided by Dr. N. Silvestri. Soil organic matter content (SOM, also in percentage) was also obtained in situ at the same locations during those field campaigns. Values from soil samples were then interpolated with thin plate splines (see the "Tps" and "interpolate" functions from the "fields" and "raster" R packages, respectively) across the study area to derive 2D maps of those four variables.

**La Costanza study area.** The la Costanza study area consisted of a series of rectangular crop plots distributed as shown in Figure 39. In the figure, the E76 road can be seen as a ribbon crossing from bottom center to left center. A wide channel that flows into the Lake Massaciuccoli can be seen as a thick light-blue straight stripe on the bottom left corner. Small, rectangular crop plots are shown in the la Costanza area in light green or purple colors. Those rectangular plots are outlined by small channels (in blue).

*Figure 39 Illustration of the la Costanza study area, which is enclosed by a double dashed red line. Geographic North and East are up and right, respectively.*

We are interested in those plots in Figure 40 for which vegetated filter strips were planned. In Figure 40 we have labelled those plots that were used in the analysis below.

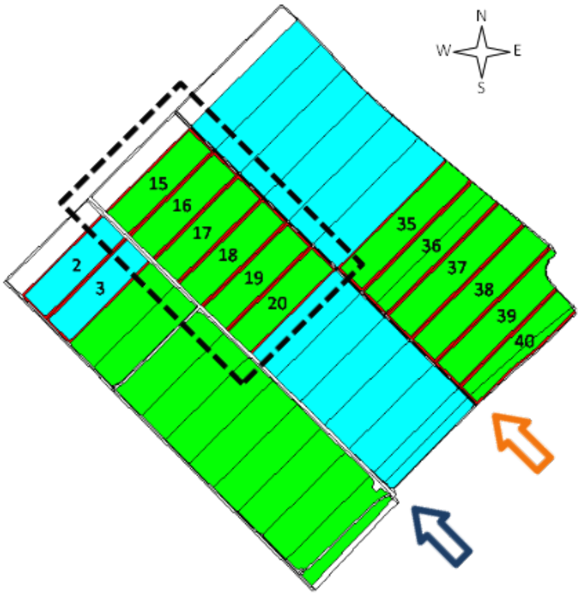


*Figure 40 Same as Figure 39, but with numeric labels on plots to identify those plots that have been used in the analysis. The two large arrows on the lower right-hand side indicate the location of a road (lower arrow, dark blue) and a discharge channel (upper arrow, orange) which run all across the la Costanza area. A thick dotted rectangular encloses the crop plots that were selected for study (see text for description).*

**Numerical simulations.** We used the Vegetative Filter Strip Modeling System (VFSMOD-w) simulation software package (Muñoz-Carpena & Parsons, 2004) to calculate the amount of sediment that would enter the proposed VFS and the efficiency of those vegetated filters (i.e. the percentage of input sediment that would be retained).

The VFSMOD-w is an open-source software package that can be retrieved freely from https://abe.ufl.edu/faculty/carpena/vfsmod/. It consists of a graphic user interface (GUI), a set of functions that perform the main calculations, as well as a set of auxiliary functions to handle input and output files.

The runoff that enters the buffer strip is first calculated in VFSMOD-w via a Modified Universal Soil Loss Equation (MUSLE) model. This utility, called UH in the VFSMOD-w documentation, accepts an ASCII input file describing topographic and hydraulic characteristics of the study area. It also allows users to choose between a set of rainfall events, either predefined for the USA or as a user-defined rainfall episode. The output files from the MUSLE modelling component of the VFSMOD-w software are then employed as inputs to the filter strip simulation utility, called VFSM. This utility also requires additional inputs describing the buffer strip and the input overland flow, as well as parameters related to computational aspects. The final output of the simulation is split into several different files providing sediment filtration characteristics and filter performance parameters.

**The Rvfsmod R package.** To help with the analysis we devised and implemented a companion R package called “Rvfsmod”, which is currently under development. This package is actually not an implementation of the mathematical equations that are used by the VFSMOD-w software to calculate runoff and sediment removal by buffer strips. Rather, it uses specially designed R functions to prepare input files and to read output files such that part of the analysis can be done with the aid of R scripts. This includes creating the content and format of all VFSMOD-w input files and writing them to disk, running the MUSLE model and the vegetated filter strip simulations and reading the results of those simulations back into the R session for further statistical analysis. MUSLE and filter calculations are done by external calls to the aforementioned UH and VFSM utilities, called “uh.exe” and “vfsm.exe” respectively, which are available in VFSMOD-w as Fortran-compiled executable files. Those calls are handled via a direct Windows-shell call from within an R session.

**Determination of average slope**. In order to study sediment transport into the buffer strips we must determine the slopes along and across the main axis of the crop plot, which runs approximately from the lower left to the upper right side of the figure. At pixel scale, any small depressions or pit in the DTM would give rise to a local sink that, in turn, may affect drainage routes in our simulations by hindering a correct slope calculation. Those pits, nevertheless, may actually be real or due to inaccurate height data. In this work, instead of applying numerical pit-removal techniques to the DTM to fill them in, we decided to calculate average slopes across plots to minimize their effect. Mean slopes were thus computed by averaging the slopes along and across the main axis of the land plots (Figure 41, dotted lines). Notice that, although only a few dotted lines are visible for the sake of clarity, that process was actually carried out by averaging 100 segments in each direction.

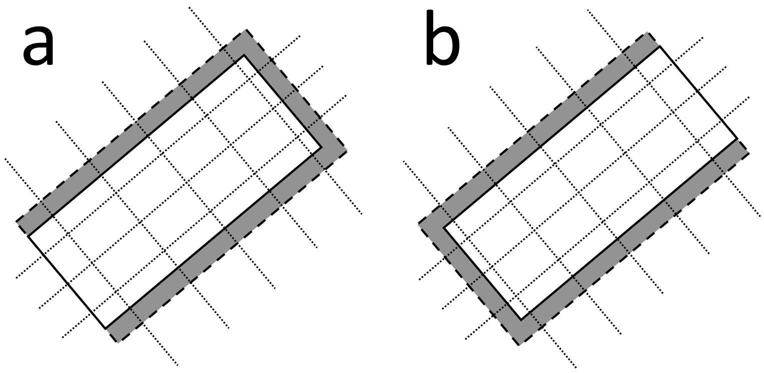


*Figure 41 Illustration of the procedure to calculate average slopes along and across the longest land plot axis. In both figures, the contour of the rectangular land plot is marked with a thick solid line. Vegetated strips (in gray) then surround three of its four sides, as in Figure 3 above. The orientation is such that North is up and East is right, as in Figs. 36, 39 and 40 above. Mean slope was calculated by averaging 100 segments in each direction.*

To determine those slopes, we first fitted a simple regression line $y = a + b \cdot x$ to each sliver (dotted lines in Figure 41) across the crop land, where $y$ is height o.s.l. and $x$ is position along the sliver. The coefficient $b$ then yielded the slope for that example. Mean slope was then obtained by averaging the $b$ coefficients from 100 fits. Given that the topography of crop plots along their shorter side showed that the central part of the plot was higher than the two sides, we divided those plots into two halves, fitted two regression lines independently (see figures in Appendix for details) and averaged the result.

**Crop data and rotation strategies.** We ran numerical simulations for the rotation scenarios shown in Figure 42. The rain event in Year 1 takes place right after fallow and at the beginning of the wheat-sowing season. Consequently, we assumed fallow conditions for that simulation.

Sunflower crops are labelled "Row crops" in VFSMOD-w manual. Hydrologic soil group is always D, i.e. Silty clay loam.

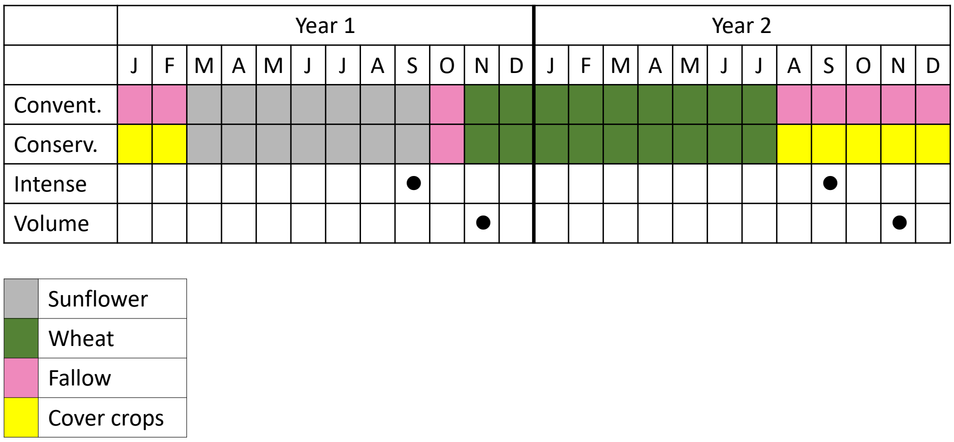


*Figure 42 Agricultural strategies that have been used for the NBS buffer strip modeling. "Convent." and "Conserv." labels on the left-most column indicate conventional and conservational agriculture, respectively. Large black dots on the two bottom rows of the upper figure indicate what rain episode is used in the simulations. Color codes in the bottom figure specify the type of management applied.*

**Storm events.** We applied two different storm events:

1. Intense short-term storm event (Figure 43a): a relatively short rain episode with a very high rainfall intensity peak but a moderate total volume.
2. Volume long-term storm event (Figure 43b): a long rain episode with lower intensity and a larger total volume.

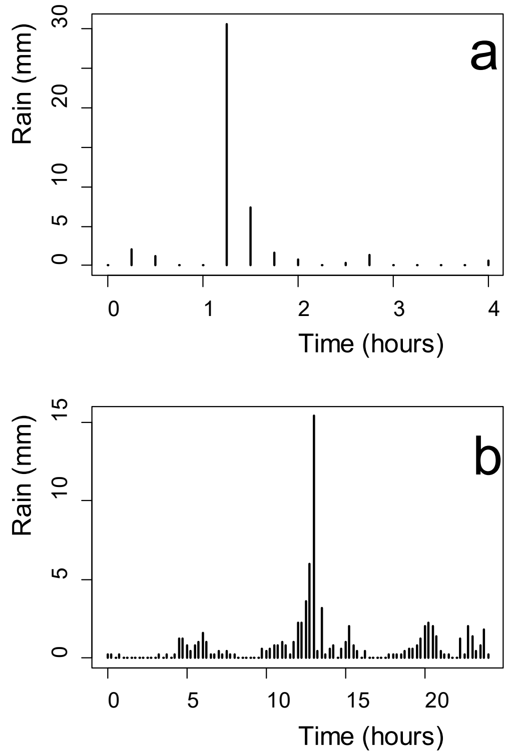


*Figure 43 Hyetograph of the a) intense short-term and b) volume log-term rain events. Parameters for those precipitation events are given in Table 17.*

Storm data were provided by the UNINA team and corresponded to typical rain conditions in locations close to or around the Massaciuccoli area. Table 17 gives the main parameters of the two storm events.

*Table 17 Description of the two rain events*

| | Duration (h) | Volume ($mm/m^2$) | Max. intensity (mm/h) |
|---|---|---|---|
| Intense short-term | 4 | 46 | 40,4 |
| Volume long-term | 24 | 77,8 | 27,2 |

The original long-term storm event lasted for 35 hours and had a total volume of 110 $mm/m^2$, but due to limitations of the VFSMOD-w software we had to select the first 24 hours only.

**Results**

**Maps of main soil characteristics.** Figure 44 shows the 2-D interpolated maps of percentage of silt, sand, clay and soil organic matter (SOM) for la Costanza. There are visible gradients across the area in all four variables, although for Silt and SOM those differences are relatively smaller. For Silt, Clay and SOM the gradient axis is East-West, whereas for Sand it has a North-South orientation.

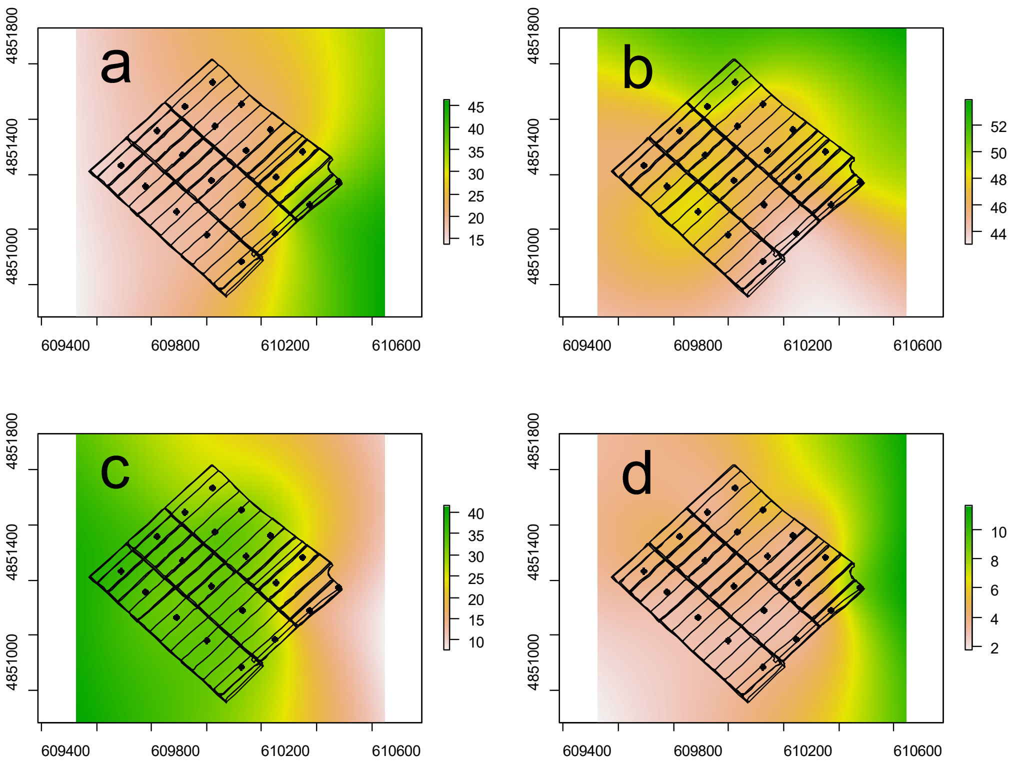


*Figure 44 La Costanza 2D maps of soil data interpolated from soil samples taken at 20 locations: a) Sand, b) Silt, c) Clay and d) Organic matter. All values are given in percentages, indicated by the color bar on the right of each map. Solid black points inside the perimeter of the study area identify locations where soil data were obtained.*

**Average slopes along and across plots.** A visual inspection of the DEM at la Costanza reveals that there is a mild slope downward from SW to NE. Figures in Annex A to this document show examples of average slopes along and across plots, together with least squares fits to the data. Our analysis revealed that many of the proposed VFS at la Costanza were oriented upslope, i.e. simulated water runoff should necessarily flow in the direction opposite to where the proposed VFS had been placed. This happens in plots 2, 3, 35, 36, 37, 38, 39 and 40. This applied only to the VFS located at the shorter side of the plot, since sideway slope correctly made water flow towards the sides. However, even in these latter cases, filtered water would have to run uphill to reach a discharge channel, which is not available for those plots. Only crop plots labelled 15, 16, 17, 18, 19 and 20 in Figure 40 above were well placed to collect surface waters from the source area, filter them with the VFSs and discharge them into a channel (blue arrow in Figure 40). Consequently, we focused our simulations on those plots.

We distinguish between the strips found at the NE side (i.e. the shorter side) of the crop plots and those distributed on both sides (i.e. the longer sides) of the plots. Table 18 lists the main characteristics of the inner area (i.e. the “source” area), to which a MUSLE model is applied in order to calculate runoff into the vegetated strip areas. Slope along the shorter axis, which runs along the NW-SE direction, is much higher than the slope along the longer SW-NE axis, although the length of the corresponding sides of the rectangular plot has the opposite effect. Total area, in addition, is approximately 7500 $m^2$ on average, or 0,75 ha.

*Table 18 Average slopes along and across crop plot axis. Land plot identifiers listed in the first column correspond to labels in Figure 40.*

| | SW-NE (longer) axis | | NW-SE (shorter) axis | | Area ($m^2$) |
|---|---|---|---|---|---|
| **Land plot id.** | **Slope (°)** | **Length (m)** | **Slope (°)** | **Length (m)** | |
| 15 | 0,06 | 171,21 | 0,46 | 43,9 | 7516,04 |
| 16 | 0,08 | 171,42 | 0,36 | 43,71 | 7493,3 |
| 17 | 0,1 | 171,33 | 0,79 | 43,83 | 7509,54 |
| 18 | 0,09 | 172,05 | 1 | 44,22 | 7607,58 |
| 19 | 0,05 | 171,99 | 0,94 | 44,23 | 7606,31 |
| 20 | 0,1 | 172,06 | 0,87 | 43,76 | 7530,15 |

**Sediment retention.** Table 19 shows total sediment retention for simulation along and across long axis for the selected land plots. Results include outputs for short-term intense rain as well as long-term mild rain. Values for the NW-SE shorter axis have already been multiplied by 2 to take into account that there are VFSs at the two sides of each plot.

*Table 19 Results of the VFSMOD-w numerical simulations. Numbers correspond to sediment input, output and retained along the two axes of the selected crop plots in Figure 40. Main parameters of the simulations: Crop = Sunflower; Manning' roughness coefficient = 0,18; Curve number = 89; Storm type = Intensity.*

| | SW-NE (longer) axis | | | NW-SE (shorter) axis | | |
|---|---|---|---|---|---|---|
| **Land plot id.** | **Input** | **Output** | **Retained** | **Input** | **Output** | **Retained** |
| 15 | 134,78 | 4,21 | 15 | 207,94 | 0,22 | 207,71 |
| 16 | 167,95 | 5,91 | 16 | 174,34 | 0,18 | 174,16 |
| 17 | 185,19 | 6,82 | 17 | 324,38 | 0,36 | 324,02 |
| 18 | 187,6 | 6,96 | 18 | 400,59 | 0,47 | 400,12 |
| 19 | 136,46 | 4,22 | 19 | 378,31 | 0,44 | 377,86 |
| 20 | 185,69 | 6,87 | 20 | 349,45 | 0,4 | 349,05 |

*Table 20 Same as Table 19. Main parameters of the simulations: Crop = Fallow; Manning' roughness coefficient = 0,25; Curve number = 94; Storm type = Volume.*

| | SW-NE (longer) axis | | | NW-SE (shorter) axis | | |
|---|---|---|---|---|---|---|
| **Land plot id.** | **Input** | **Output** | **Retained** | **Input** | **Output** | **Retained** |
| 15 | 372,92 | 19,41 | 353,51 | 1395,14 | 4,5 | 1390,64 |
| 16 | 463,47 | 27,4 | 436,07 | 1158,73 | 3,06 | 1155,67 |
| 17 | 511,56 | 31,57 | 480 | 2229,57 | 8,01 | 2221,56 |
| 18 | 521,94 | 32,18 | 489,76 | 2728,48 | 12,71 | 2715,76 |
| 19 | 376,57 | 18,57 | 358,01 | 2587,96 | 11,57 | 2576,39 |
| 20 | 509,13 | 31,08 | 478,05 | 2375,74 | 8,96 | 2366,78 |

*Table 21 Same as Table 19. Main parameters of the simulations: Crop = Fallow; Manning' roughness coefficient = 0,25; Curve number = 94; Storm type = Intensity.*

| | SW-NE (longer) axis | | | NW-SE (shorter) axis | | |
|---|---|---|---|---|---|---|
| **Land plot id.** | **Input** | **Output** | **Retained** | **Input** | **Output** | **Retained** |
| 15 | 211,86 | 11,94 | 199,93 | 557,63 | 1,16 | 556,47 |
| 16 | 234,72 | 14,36 | 220,36 | 465,07 | 0,95 | 464,12 |
| 17 | 258,22 | 15,73 | 242,48 | 877,93 | 1,89 | 876,04 |
| 18 | 261,9 | 15,89 | 246,01 | 1067,64 | 2,46 | 1065,18 |
| 19 | 214,41 | 11,76 | 202,65 | 1030,12 | 2,38 | 1027,73 |
| 20 | 258,91 | 15,83 | 243,08 | 927,25 | 2,06 | 925,19 |

## Discussion and conclusions

**The slope problem.** Some of the VFS proposed as NBS to retain sediment at la Costanza areas near Lake Massaciuccoli may not work as expected. For VFSs to work correctly a monotonic and smooth downhill slope is required. In that respect, we have detected two main issues that may compromise the way those buffer strips reduce runoff and retain sediment:

1. terrain irregularities (real or numerical) at small scale that create sinks where water may stop and accumulate.
2. wrong location of proposed VFS due to water running in the opposite direction.

Although the first issue cannot be settled without more accurate height measurements, its effects can be minimized in simulations by taking average slopes, as we did. However, in actual use, those irregularities must be smoothed out to avoid the formation of ponds and the subsequent infiltration they may give rise to.

The second issue, however, is more important and may make some of the proposed VFS useless. We have noticed that the design of the VFSs, in many cases, is such that they are located uphill, against the normal flow of water. Even when those VFSs are located downhill (as e.g. the buffer strips on the long sides of the crop plots), the small channels that drain the filtered water away discharge into areas without other channels. Therefore, further in-situ works should be carried out to make sure that those discharges are directed towards the lake.

**Sediment retention, rain events and rotation strategies.** Simulation results depicted in Tables 19, 20 and 21 reveal that volume rain events always generate larger amount of sediments than intensity rain events. This is true for runoff along both the SW-NE and the NW-SE axes. Noticeably, the MUSLE calculations predict a much higher runoff towards the sides of the plots. Therefore, those small channels that run parallel to the long axis of the plots and that separates them are key to discharging the output water into the larger channels.

Tables 19, 20 and 21 also show a very high buffer filter efficiency. In all cases, the vegetated filter strips will retain most of the sediments that flow along or across the long axis of the crop plots (ratio between output and input sediment >90% always).

**Limitations of the simulation results.** The accuracy of the VFSMOD-w numerical simulations could be increased by using more precise height data with better spatial resolution. In this way, some of the irregularities present in the DTM, and which give rise to small local sinks, may disappear. Moreover, better soil data should allow a better characterization of soil properties which, in turn, would improve the VFSMOD-w calculations. Finally, the so-called volume rain event could not be used in its entirety due to limitations of the VFSMOD-w software. Arguably, had we used the whole rain event input, those values in Table 20 would have been higher.

**Conclusion**

Proposed NBS solutions for the la Costanza study site consist of 3-m wide vegetated strips to retain sediment runoff from cropped areas. Those strips are located around three sides of individual rectangular crop plots at la Costanza, i.e. one strip at one of the shorter sides and the other two on each longer side. The idea behind the NBS approach is that runoff will be directed either to each side or all the way along the longer axis of the crop plot. Sediment retention will then take place in the buffer strips. Similar solutions for the Studiati study site have been discarded due to its very low height o.s.l. (-2 m), which makes the Studiati site prone to flooding.

Our analysis has revealed that many of the proposed strips are, in fact, located uphill relative to the predominant local slope, which would cause part of the sediment to run directly to the lake. In addition, in some cases filtered water discharge into channels with uphill slopes.

Simulations of sediment retention calculated with the VFSMOD-w software for plots with correctly oriented VFSs show that the efficiency of the filters may be large, reaching efficiencies of 90% or higher.

# 3 Conclusions and recommendations

1. In mountain forest areas, when the measures to be taken will impact the soil-plant system, their effect on soil and plant biodiversity and on aboveground and belowground stocks of organic carbon can be simulated by taking as reference comparable vegetation units in different maturity stages.
2. Interventions that impact soil can have positive and negative effects on carbon sequestration depending on the initial characteristics of soil. In particular, soil organic carbon and texture are determinant of soil response and must be carefully defined in the baseline of the ecosystem.
3. In the Capet Forest and in the Santa Elena study cases, the nature-based solutions designed to reduce the effect of hydrometeorological risks will contribute to carbon sequestration both in soil and vegetation.
4. In the Massaciuccoli Lake, the vegetation filters designed to reduce the input of soil sediments to the drainage system will be efficient in some parts of the agricultural area, but may be inefficient in very flat zones and also in these parts of the area far below the sea level subject to flooding.
5. In the Capet Forest the plantations are expected to expand the vegetated area, provided that grazing is banned during at least 10 years after works. Several exotic species have been introduced as tools to minimize snow avalanches in case the native species are destroyed by plant pests. Although this is not an optimal solution from the point of view of landscape biodiversity, there is low risk that the exotic species chosen to become invasives.
6. When aiming to monitor effects of the interventions on carbon sequestration in soil, it is advisable to measure not only total carbon, but also the size of the recalcitrant and labile carbon pools. This is particularly important when herbaceous species, that provide soil with strong pulses of labile carbon are involved
7. When working in agricultural areas, indicators that inform of soil structure (bulk density, aggregation, etc...) are fundamental to monitor effects on soil health and productivity.
8. When dealing with plant biodiversity, synthetic indicators such as the Shannon Index or the Evenness Index are not good indicators of the correct post-operation evolution of vegetation. Multivariate statistics applied to species (taxonomic or functional) is much more indicative of the evolution of the plant cover.
9. “Shot Gun” is a very strong metagenomic method to study effects of NBSs on soil microbial biodiversity. Combined with multivariate analyses (as for vegetation, much more advisable that synthetic indexes) it provides an in-deep view of progress of the affected soil towards maturity. As all metagenomic analyses Shot Gun is expensive and therefore sampling must be carefully designed to maximize cost-efficiency. Microrresp is a significantly cheaper approach to soil microbial functional biodiversity, but it has not been sensitive to the measures evaluated in this work.
10. Soil invertebrates considered at the level of trophic group are very appropriate and universal indicators of effects of the NBSs on soil bio-

diversity. Different groups are advisable for indication and show different sensitiveness depending on the type of ecosystem. Nematodes and protozoa are suitable for agricultural ecosystems. Soil microarthropods are very advisable in forest ecosystems. In our two study cases in mountain areas the number of sensitive indicators based on soil microarthropods in higher in Sta Elena than in the Capet Forest. This may be explained by larger difference between the initial and the expected soil quality in Sta Elena than in the Capet Forest.

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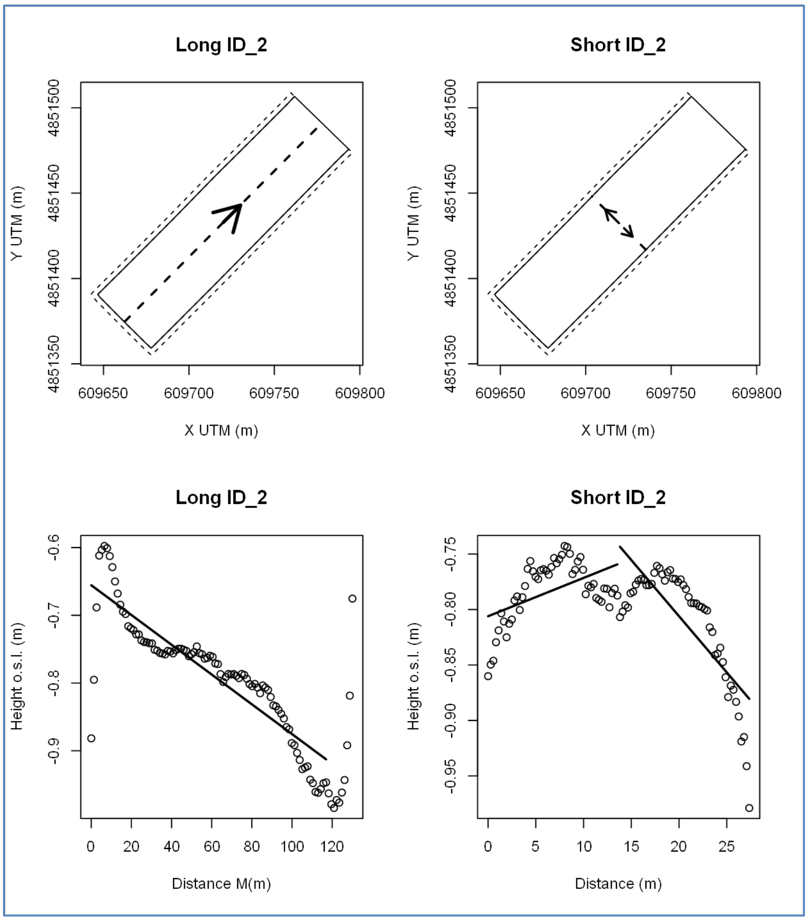
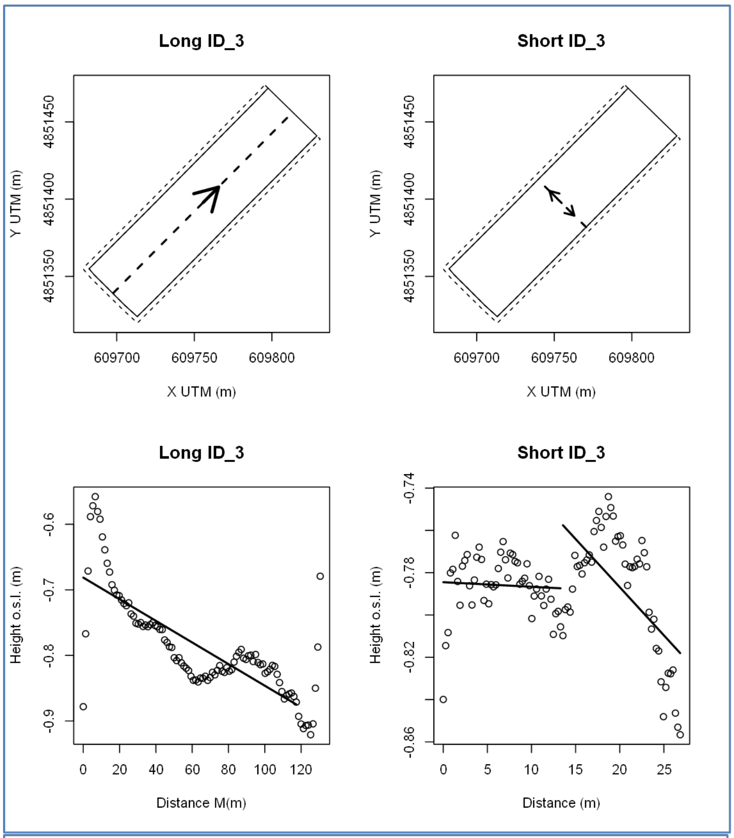
# Appendix A

Topography of the plots included in la Costanza to simulate the effects of the VFS on the exportation of soil sediments

All figures below show the height in meters along (labelled Long) and across (labelled Short) the main axes of selected plots at la Costanza. We have grouped figures belonging to the same plot within dashed-blue frames for ease of clarity. Numbers in the main title point to the labelled land plots in Figure 4 in the main text. Plot 39 is not shown below due to its irregular sides, which made average slope computation difficult. The two top figures within each frame display the long and short axes together with plot outer limits. The two bottom figures within each frame, then, correspond to the average height o.s.l. along each axis. For the long segments, a single linear fit has been added. For the two short segments, one for each half, two linear fits have been added. A negative sign in the Y-axis of the bottom figures reveal that the corresponding heights are, in fact, slightly below sea level. The proposed locations of the 3m vegetated filter strips are shown by a dotted line surrounding three of the four sides of the polygon.

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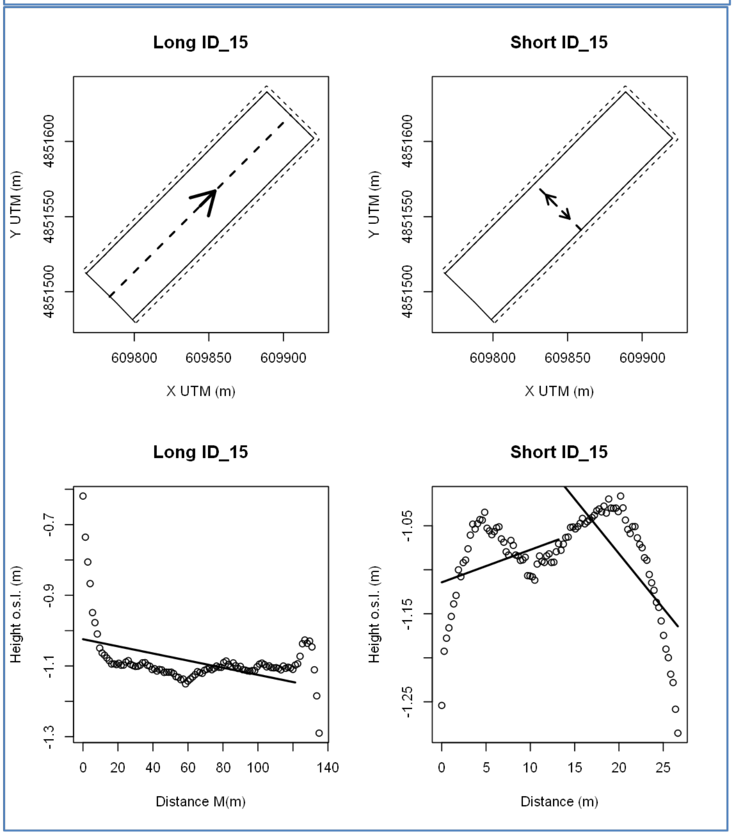
Short ID_3
Y UTM (m)
X UTM (m)
Long ID_3
Short ID_3
Height o.s.l. (m)
Distance M(m)
Distance (m)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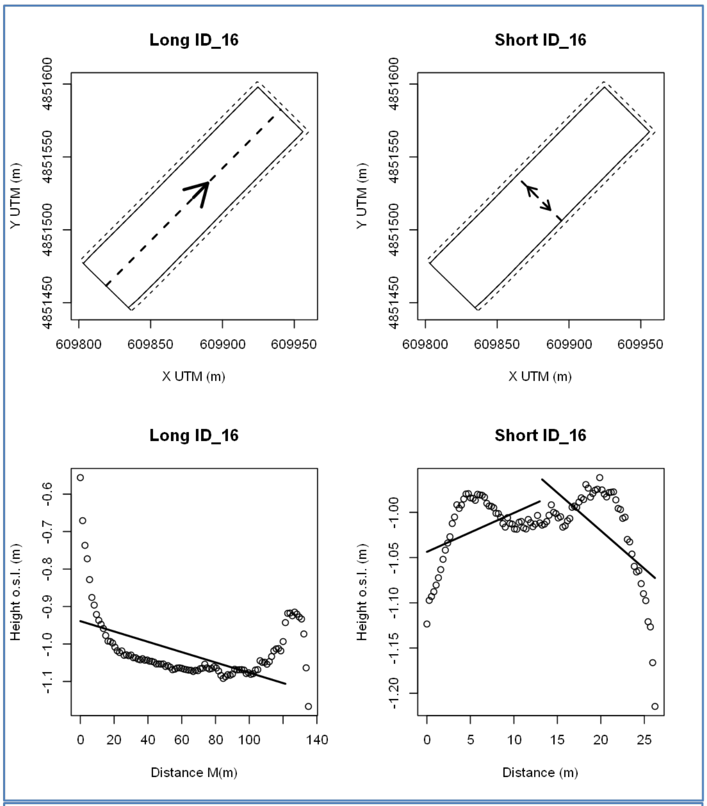
X UTM (m)
Long ID_15
Short ID_15
Height o.s.l. (m)
Distance M(m)
Distance (m)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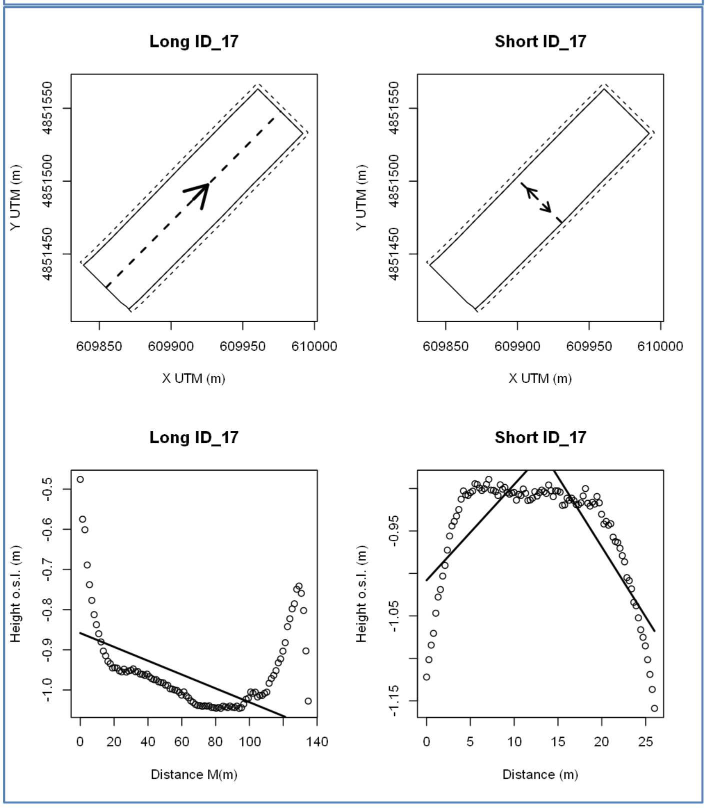
Distance (m)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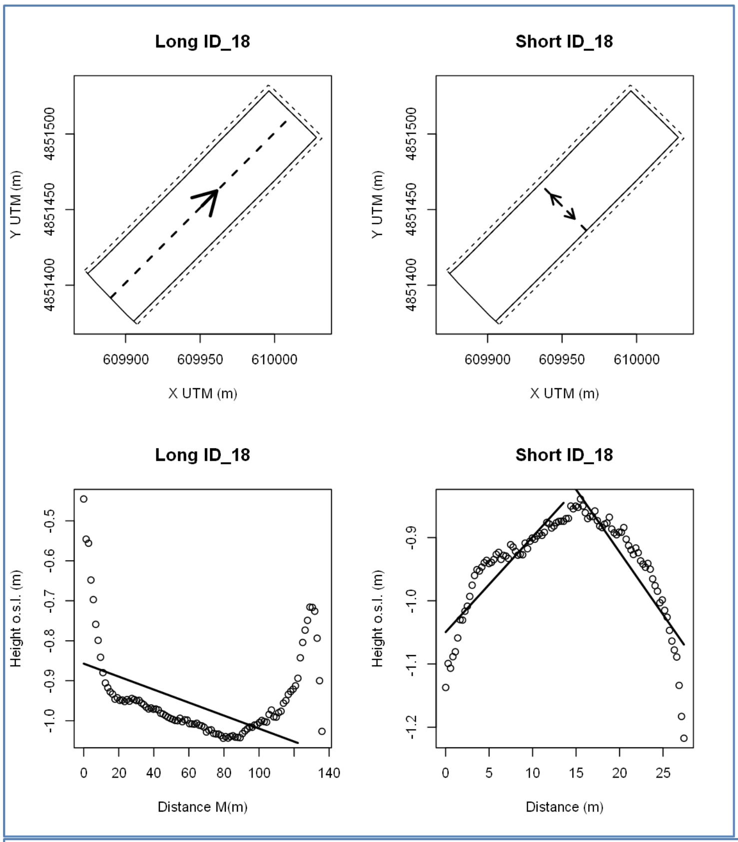
Distance (m)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Distance (m)
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20
40
60
80
100
140
0
5
10
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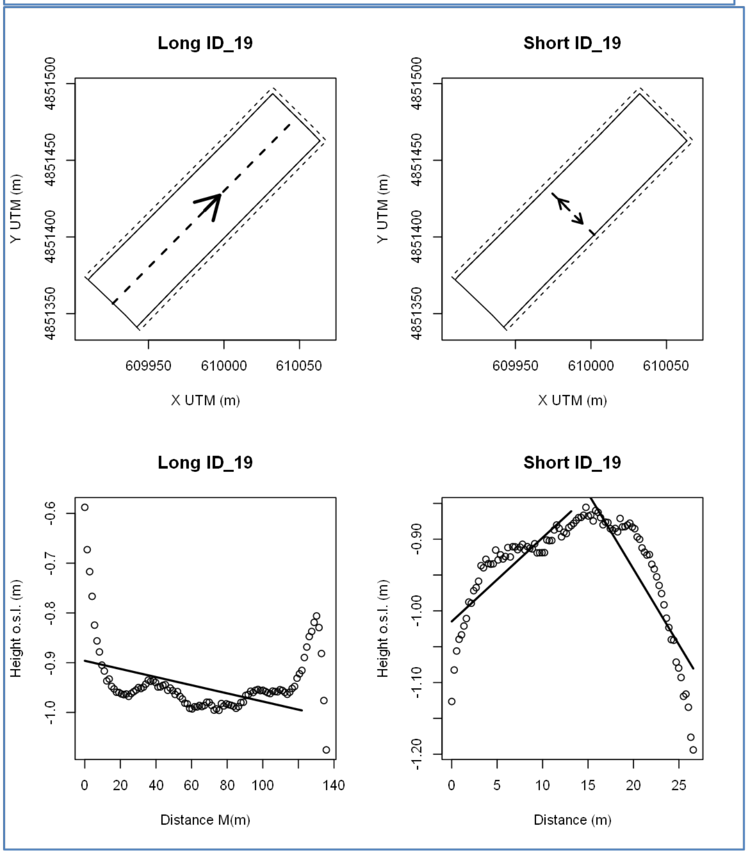
-1.1
-1.2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610050
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Short ID_19
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Distance M(m)
Distance (m)
0
20
40
60
80
100
140
0
5
10
15
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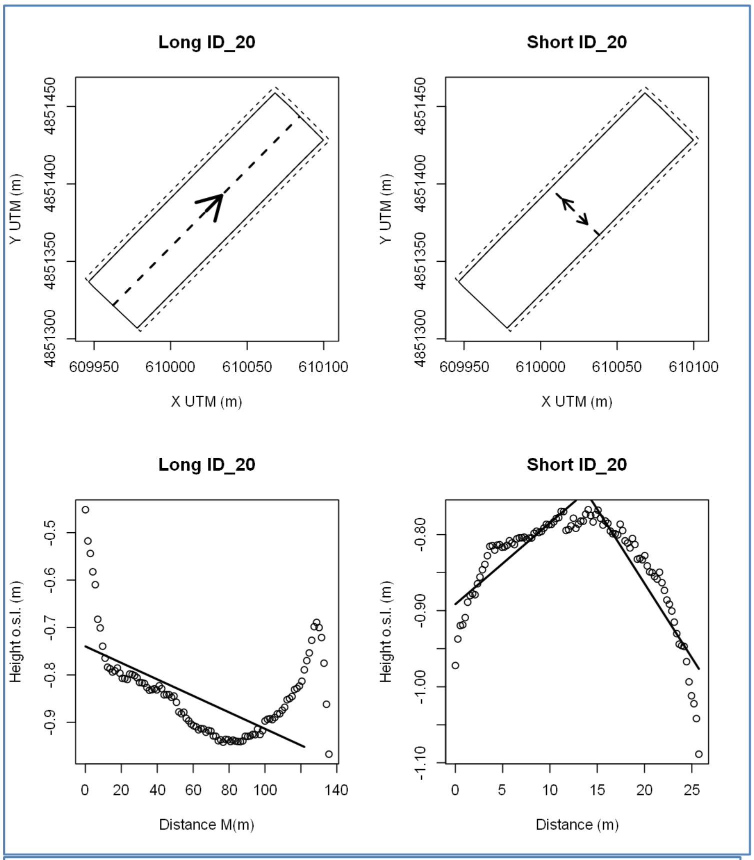
-1.00
-1.10
-1.20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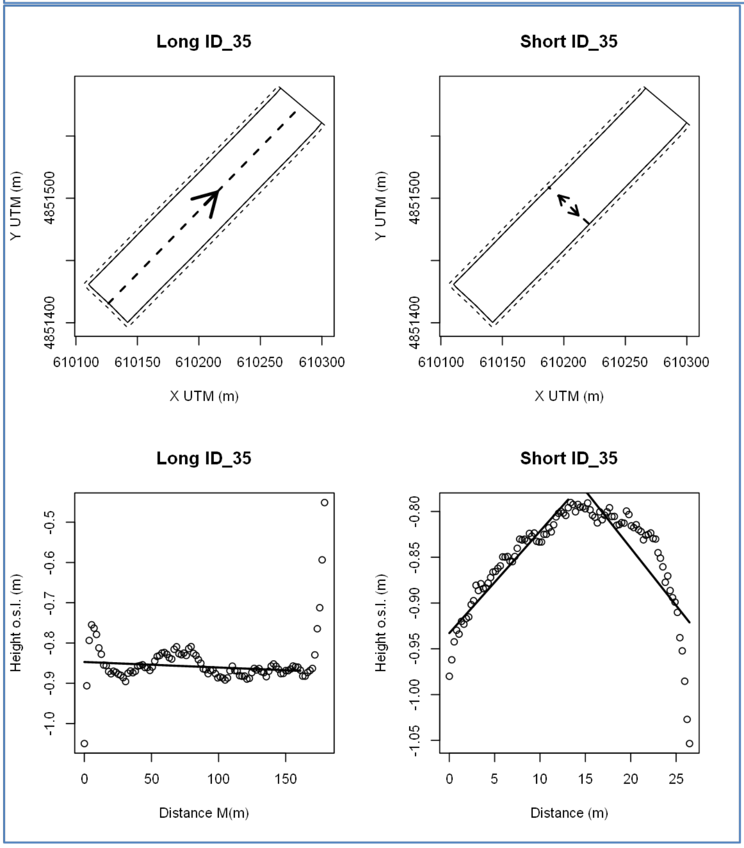
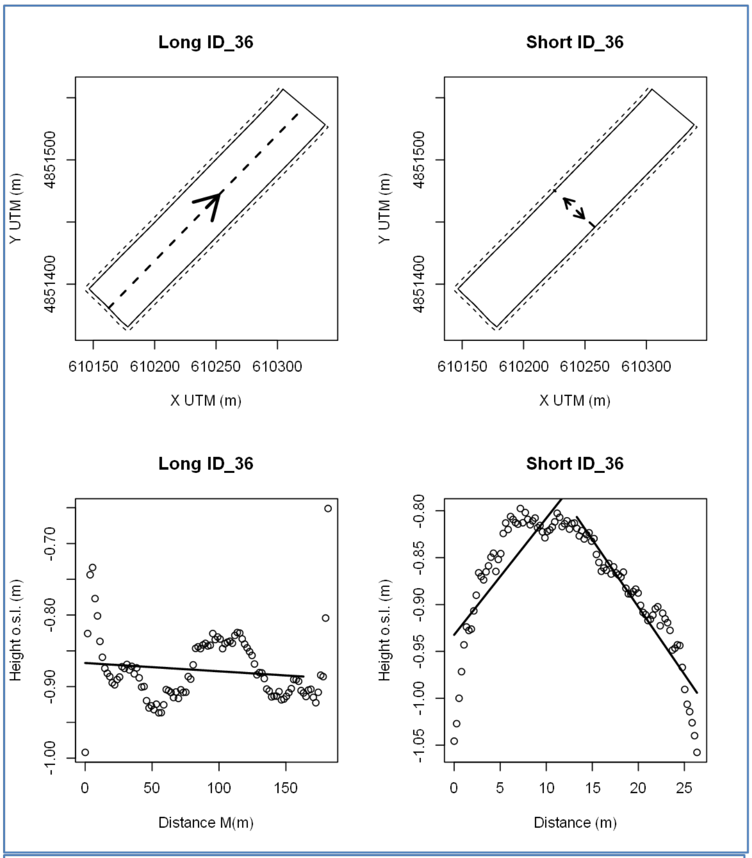
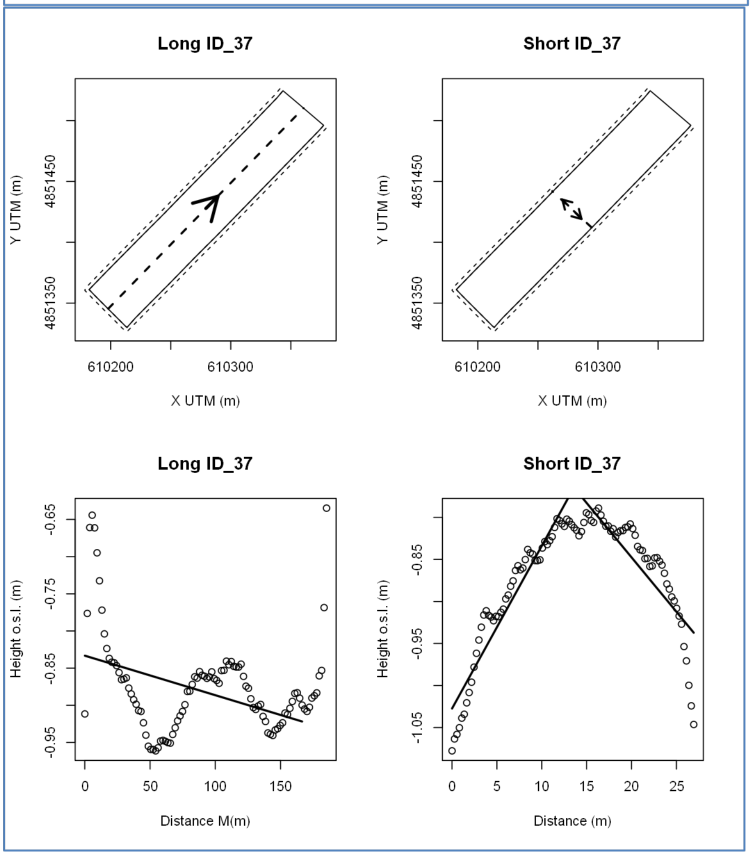
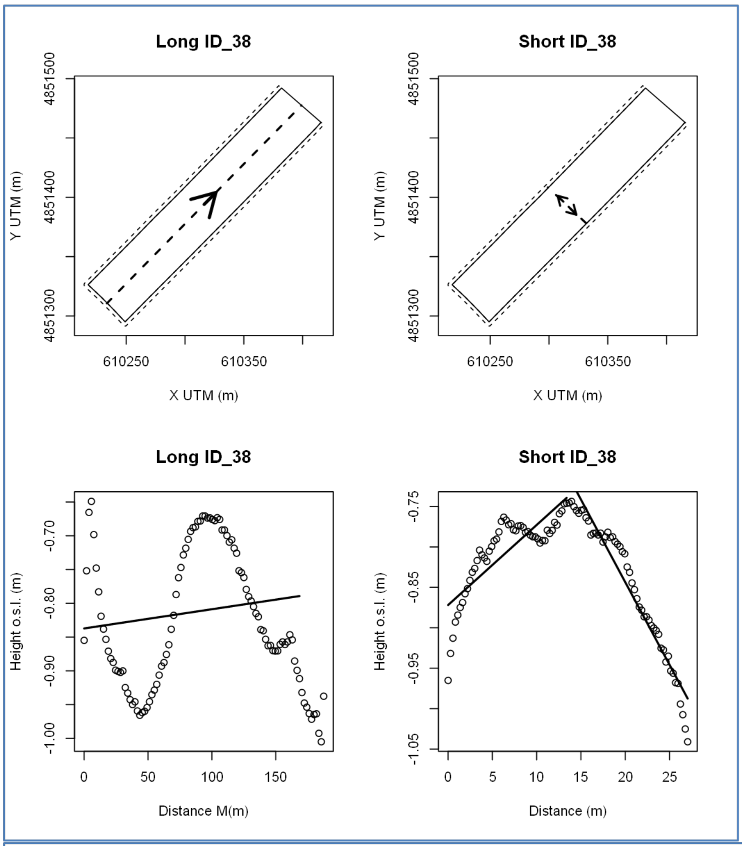
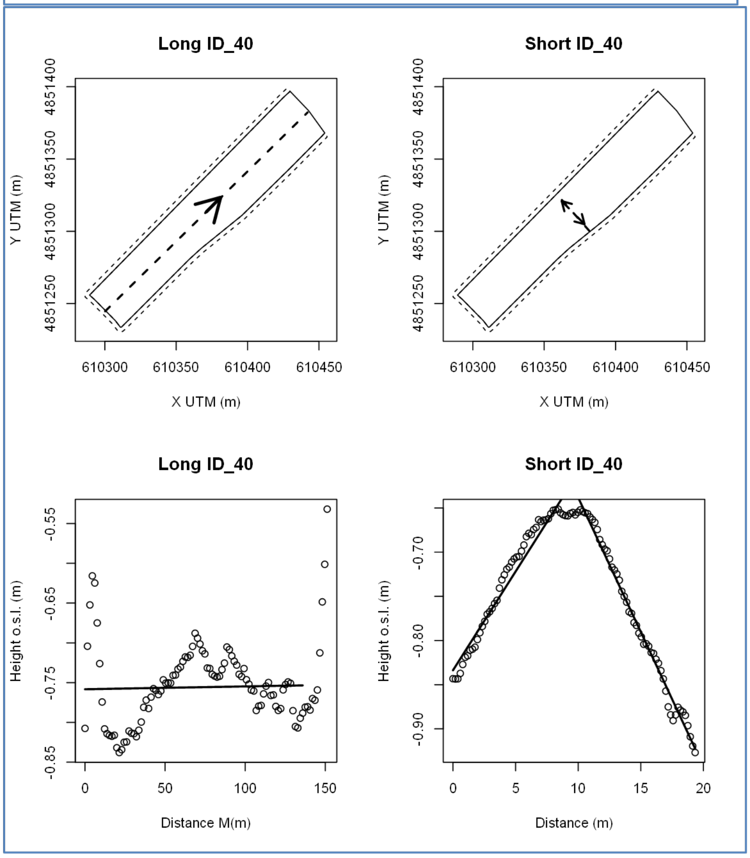
610100
X UTM (m)
Long ID_20
Short ID_20
Height o.s.l. (m)
Distance M(m)
Distance (m)


Long ID_35
Short ID_35
Y UTM (m)
4851400
4851500
610100
610150
610200
610250
610300
X UTM (m)
Long ID_35
Short ID_35
Height o.s.l. (m)
Distance M(m)
Distance (m)


Long ID_36
Short ID_36
Y UTM (m)
4851500
4851400
610150
610200
610250
610300
X UTM (m)
Long ID_36
Short ID_36
Height o.s.l. (m)
-0.70
-0.80
-0.90
-1.00
0
50
100
150
Distance M(m)
-0.80
-0.85
-0.90
-0.95
-1.00
-1.05
0
5
10
15
20
25
Distance (m)


Long ID_37
Short ID_37
Y UTM (m)
4851450
4851350
610200
610300
X UTM (m)
Long ID_37
Short ID_37
Height o.s.l. (m)
-0.65
-0.75
-0.85
-0.95
0
50
100
150
Distance M(m)
-0.85
-0.95
-1.05
0
5
10
15
20
25
Distance (m)


Long ID_38
Short ID_38
Y UTM (m)
4851300
4851400
4851500
610250
610350
X UTM (m)
Long ID_38
Short ID_38
Height o.s.l. (m)
-1.00
-0.90
-0.80
-0.70
0
50
100
150
Distance M(m)
-1.05
-0.95
-0.85
-0.75
0
5
10
15
20
25
Distance (m)


Long ID_40
Short ID_40
Y UTM (m)
4851250
4851300
4851350
4851400
610300
610350
610400
610450
X UTM (m)
Long ID_40
Short ID_40
Height o.s.l. (m)
-0.85
-0.75
-0.65
-0.55
0
50
100
150
Distance M(m)
-0.90
-0.80
-0.70
0
5
10
15
20
Distance (m)

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