CALCULATION OF BIODIVERSITY GAINS IN AGRARIAN LANDSCAPES

Fundación Global Nature Methodology

Fundación Global Nature

MEASURE AND MAXIMIZE YOUR IMPACT ON BIODIVERSITY

A practical, robust, and verifiable tool to quantify biodiversity gains or losses in agrarian ecosystems, aligned with corporate goals and the CSRD.

EXECUTIVE SUMMARY

The primary goal of this methodology is to establish a robust, verifiable, and comparable system for projecting and quantifying biodiversity gains (or losses) in agrarian ecosystems resulting from specific management practices aligned with corporate needs. This approach has no geographical restrictions, provided that the Intervention site is located within an agrarian ecosystem.

THE METHODOLOGY

This methodology uses the **Biodiversity Matrix**, created by FGN, to quantify changes in biodiversity within a given area using a pre-selected set of biodiversity metrics (biodiversity groups). Biodiversity gains (or losses) are measured as a percentage change (%) and expressed in Biodiversity Units per hectare per year (BU ha^{-1} $year^{-1}$). Each Biodiversity Unit (BU) represents a 1% increase or decrease in the aggregated biodiversity metrics included in the selected set.

The document outlines what to measure, when to measure it, how to measure it, the appropriate measurement scale, and the conditions under which measurements should be taken. It also details how to aggregate data to ensure that the calculated biodiversity gains (or losses) accurately reflect changes in land use, land management, and/or the landscape of the Intervention site, rather than being influenced by other variables present in highly dynamic agrarian ecosystems (e.g., crop variations, soil parameters, water usage, climate factors, and broader landscape features).

1 BU HA^{-1} YEAR^{-1} = 1% TOTAL BIODIVERSITY CHANGE

STARTING FROM YEAR 1

ASSESS AND MONETIZE BIODIVERSITY GAINS

This methodology accommodates two types of approaches, depending on the timeframe and objectives of the user:

Short-term projects (1–5 years), focused on assessing biodiversity gains associated with specific interventions.

Long-term projects (20+ years), aimed at consolidating and potentially monetizing biodiversity gains linked to land management practices.

SHORT-TERM PROJECTS

(1-5 YEARS)

Uncertainty regarding the impact of specific interventions on biodiversity prevents companies from taking proactive action and hinders efficient capital allocation—for example, investments in nature restoration or mitigating biodiversity-related risks in supply chains. Traditional approaches rely on long-term data collection (typically 10+ years) to demonstrate the positive or negative impact of a given intervention at a specific site. However, this extended timeframe often delays the implementation of effective conservation and restoration strategies.

In short-term projects, the efficacy of interventions can be quantified by comparing the average value of the biodiversity indicators included in the Biodiversity Matrix between different land uses and/or management approaches (i.e., Reference Sites vs. Intervention sites). These gains can be estimated within one year, but they must be reassessed every five years. This approach provides guidance on the expected outcomes of each intervention and serves as a foundation for reporting compliance under the EU Corporate Sustainability Reporting Directive (CSRD). Additionally, it supports informed decision-making for financial resource allocation, maximizing the positive impact of interventions. Biodiversity gains are measured as a percentage change (%) and expressed in Biodiversity Units per hectare per year (BU ha^{-1} $year^{-1}$).

LONG-TERM PROJECTS

(20+ YEARS):

When there is a clear strategy for the types of interventions to be implemented at a Intervention site, and the ambition extends beyond merely assessing biodiversity gains and ensuring compliance with the EU CSRD Directive, projects can adopt the second type of approach (long-term project).

This long-term approach aims at consolidating and potentially monetizing biodiversity gains linked to sustainable land management practices.

AGILE RESULTS AND LONG-TERM PLANNING TO PROTECT BIODIVERSITY

BIODIVERSITY PROJECT'S ROADMAP

How should a biodiversity project develop? What are the necessary steps? The figure below summarizes the key steps involved in the development of a biodiversity project (BP stands for Biodiversity Partner, and PRO stands for Promoter).

FGN'S BIODIVERSITY MATRIX

How does FGN's Biodiversity Matrix look like? What type of data needs to be provided? The figure below shows the key metrics and indicators involved in the calculation process (_Ref stands for reference site, _Int stands for intervention site, SoilInv stands for Soil invertebrates, AboveInv stands for Aboveground invertebrates, and BU stands for Biodiversity Units).

Indicator/ Gain	SoilInv_Ref	SoilInv_Int
QBS-ar	QBS-ar_Ref	QBS-ar_Int
Gain_QBS (%)	Min(5;QBS_Int/QBS_Ref-1) (%)	
Gain_Metric (%)	μ Gain (QBS) (%)	

Indicator/ Gain	Flora_Ref	Flora_Int	AboveInv_Ref	AboveInv_Int	Birds_Ref	Birds_Int
Abundance (A)	μ % soil coverage per monitoring point	μ % soil coverage per monitoring point	μ Abundance per monitoring point	μ Abundance per monitoring point	μ Abundance per monitoring point	μ Abundance per monitoring point
Gain_A (%)	Min(5;μ%coverage_Int/μ%coverage_Ref-1) (%)		Min(5;μ Abunudance_Int/μ Abundance_Ref-1) (%)		Min(5;μ Abunudance_Int/μ Abundance_Ref-1) (%)	
Richness (R)	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point
Gain_R (%)	Min(5;μ nb. species_Int/μ nb. Species _Ref-1) (%)		Min(5;μ nb. species_Int/μ nb. Species _Ref-1) (%)		Min(5;μ nb. species_Int/μ nb. Species _Ref-1) (%)	
Interest (I)	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point
Gain_I (%)	Min(5;μ Interest_Int/μ Interest_Ref-1) (%)		Min(5;μ Interest_Int/μ Interest_Ref-1) (%)		Min(5;μ Interest_Int/μ Interest_Ref-1) (%)	
Gain_Metric (%)	μ Gain (A, R, I) (%)		μ Gain (A, R, I) (%)		μ Gain (A, R, I) (%)	

Biodiversity Gain (BU ha^{-1} year^{-1})	**μ Gain (Soil invertebrates, Flora, Aboveground invertebrates, Birds) * 100 (BU ha^{-1} year^{-1})**

Fundación Global Nature

VICEPRESIDENCIA TERCERA DEL GOBIERNO
MINISTERIO PARA LA TRANSICIÓN ECOLÓGICA Y EL RETO DEMOGRÁFICO

This research has been funded by the Ministry for Ecological Transition and Demographic Challenge, but it does not reflect its official opinion.

Fundación Global Nature methodology

CALCULATION OF BIODIVERSITY GAINS IN AGRARIAN LANDSCAPES

Version 2.0 (June 2025)

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Index

This document constitutes version 2.0 of the methodology Calculation of biodiversity gains in agrarian landscapes. This methodology is subject to ongoing review and updates; therefore, future versions may incorporate technical improvements, conceptual adjustments, or feedback from field implementation. It is recommended to ensure that the most recent version is being used.

1. Summary

The primary goal of this methodology is to establish a robust, verifiable, and comparable system for projecting and quantifying biodiversity gains (or losses) in agrarian ecosystems resulting from specific management practices aligned with corporate needs. This approach has no geographical restrictions, provided that the Intervention site is located within an agrarian ecosystem.

This methodology quantifies changes in biodiversity within a given area using a pre-selected set of biodiversity metrics (biodiversity groups). Biodiversity gains or losses are measured as a percentage change (%) and expressed in Biodiversity Units (BU). One Biodiversity Unit per hectare per year ($BU \cdot ha^{-1} \cdot year^{-1}$) represents a 1% increase or decrease in the aggregated biodiversity metrics included in the selected set.

The document outlines what to measure, when to measure it, how to measure it, the appropriate measurement scale, and the conditions under which measurements should be taken. It also details how to aggregate data to ensure that the calculated biodiversity gains (or losses) accurately reflect changes in land use, land management, and/or the landscape of the Intervention site, rather than being influenced by other variables present in highly dynamic agrarian ecosystems (e.g., crop variations, soil parameters, water usage, climate factors, and broader landscape features).

This methodology accommodates two types of approaches, depending on the timeframe and objectives of the user:

(1) **Short-term projects** (1–5 years), focused on assessing biodiversity gains associated with specific interventions.

(2) **Long-term projects** (20+ years), aimed at consolidating and potentially monetizing biodiversity gains linked to land management practices.

2. Calculation of biodiversity gains in agrarian landscapes

Biodiversity encompasses the vast diversity of biological forms on Earth, ranging from the smallest genetic variations to species and ecosystems. Recently, the FAO introduced the term *Biodiversity for Food and Agriculture (BFA)*, defining it as the subset of biodiversity that contributes, directly or indirectly, to agriculture and food production. This concept includes both domesticated biological forms incorporated into production systems (e.g., crops, livestock, aquaculture species) and resources harvested from various ecosystems (e.g., forestry, fisheries). A novel aspect of this definition is the inclusion of "associated biodiversity", which refers to the wide array of biological forms that inhabit and support food and agricultural production systems, sustaining them and enhancing their output (FAO, 2019).

There is a strong scientific consensus that all businesses depend on nature and its services, whether through direct operations or their value chains (World Economic Forum, 2020). However, among all productive sectors, food systems exhibit one of the most direct and profound dependencies on BFA. Global pressures such as climate change, population growth, and market globalization exert continuous and diffuse effects on biodiversity. However, one of the primary drivers of biodiversity loss worldwide is food production, alongside key sub-drivers such as land use change, pollution, and the excessive use of external inputs (e.g., pesticides, fertilizers, water) (Rasmussen et al., 2018). It is estimated that agriculture alone threatens 86% of species at risk of extinction (IPBES, 2019).

Despite these challenges, experts highlight that agrifood systems have significant potential to contribute to what has been termed *nature-positive system transformation* (WBCSD, 2023), meaning the ability to reverse or at least mitigate negative impacts on biodiversity. The review of *The State of the World's Biodiversity for Food and Agriculture* (FAO, 2019) indicates that 80% of reporting countries implement biodiversity-focused practices. However, assessing the extent of these efforts remains difficult due to the diversity of scales and contexts involved, as well as the lack of standardized data and appropriate assessment methodologies (FAO, 2019).

In response to this situation, biodiversity has become a central focus for both public and private organizations. However, it is only in the past decade that efforts have concentrated on developing protocols for biodiversity accountability (e.g., Kunming-Montreal Global Biodiversity Framework, Natural Capital Coalition, Biological Diversity Protocol, Taskforce for Nature-related Financial Directive). These frameworks emphasize key aspects such as identifying impacts and dependencies, setting transparent, time-bound, specific, and science-based targets, and committing to action through the mitigation hierarchy principles (avoidance > mitigation > restoration > compensation).

These initiatives contribute to and expand upon one of the fundamental aspects of biodiversity accountability: the development of objective, science-based, and transparent indicators to assess agroecosystem conditions, with a particular focus on evaluating nature-positive system transformations. This approach assumes that biodiversity, and certain operational taxonomic units (OTUs), can serve as indicators of the health status of agricultural ecosystems and the ecosystem services they provide—an assertion extensively supported by the scientific community, particularly in intensive production systems (e.g., Billeter et al., 2008; Lomba et al., 2022).

However, biodiversity monitoring programs for food and agriculture remain limited, and most available datasets lack the resolution needed to assess the impact of specific practices at the appropriate scale. Numerous indices estimate ecosystem functionality or species status at a global level, but they are not designed to evaluate the management practices adopted by farmers at the plot scale, whether incentivized by public policies or private-sector initiatives. This limitation often leads to high investment risks and inefficient use of public resources.

Bioindicators (OTUs), selected for their ability to reflect environmental changes, have been used to assess and predict management strategies in agricultural landscapes. However, their use has not been standardized, and it is often difficult to ensure that they meet essential criteria, such as relative abundance in the ecosystem, appropriate response time, trophic level representation, strong scientific validation, and suitability for the scale of analysis. As Billeter et al. (2008) points out, no single group of organisms can fully reflect landscape structure and management changes. Therefore, umbrella species—if they exist—are unlikely to provide comprehensive insights.

At the agricultural plot and farm level, the use of indicators presents additional challenges. First, these are highly managed environments, making it extremely complex to isolate experimental variables from those not under evaluation. Additionally, these systems undergo frequent resets—annually or even more frequently—making it difficult to select indicators that accurately reflect their dynamic nature. Establishing reference conditions for evaluating changes is also challenging. In fact, reference areas can only be considered as the negative condition of the intervention or experimental management performed, as non-anthropized/natural environments are not valid for comparison.

Given these challenges, this study relies on the concept of a Basket of Metrics—a set of OTUs designed to capture the broadest possible range of bioindicator attributes described in scientific literature. This approach ensures a balance between scientific rigor, the reporting needs and timelines of the food sector, and biodiversity accounting initiatives, with a strong emphasis on assessing agronomic management practices at the plot scale.

3. Fundación Global Nature (FGN)

The vision and mission of Fundación Global Nature center on halting biodiversity loss and preserving ecosystem services, particularly in wetlands and agrarian environments. To achieve this, FGN prioritizes finding solutions to key threats such as climate change and the intensification of agrarian practices. This mission requires supporting those who work on the ground while also promoting public and private sustainability policies and strategies. The overarching goal is to create social value based on a fundamental premise: nature is the foundation of all economic activity.

In the Iberian Peninsula, approximately 50% of biodiversity is found in agrarian ecosystems (Díaz et al., 2021). For over 30 years, FGN has been restoring and preserving agrarian landscapes, including highly valuable natural habitats within them, such as wetlands. FGN works to ensure that these landscapes are recognized not only as biodiversity hotspots within iconic regions (Tierra de Campos, La Mancha Húmeda, Laguna de El Hito, Prat de Cabanes-Torreblanca, Marjal dels Moros, Marjal de Pego-Oliva, Monfragüe Natural Park, and Parameras de Molina) but also for their critical resources—such as water and soil—and their role in climate mitigation.

Across these landscapes, various projects converge to generate social and economic benefits, while strengthening natural and social capital.

Building on this foundation, FGN aims to scale its efforts and make them more accessible to a broader audience, particularly farming communities and corporations, which are ultimately responsible for environmental impacts or highly dependent on biodiversity.

4. Acronyms and glossary of terms

Acronyms

BG: Biodiversity Gain.

BP: Biodiversity Partner.

BU: Biodiversity Unit.

EMI: Eco-Morphological Index.

GIS: Geographic Information System.

LSSI: Landscape Structural Suitability Index.

OTU: Operational Taxonomic Unit.

PRO: Promoter.

Glossary of Terms

Abundance (A): the number of individuals (or percentage of soil coverage, in the case of Flora) present in a sample collected in a specific monitoring location.

Agrarian ecosystems (agroecosystems): agricultural landscapes, including both cultivated and non-cultivated areas, where interactions occur among species, edaphoclimatic conditions, and human-driven management practices.

Basket of metrics: a set of bioindicators (e.g., soil invertebrates, aboveground invertebrates, birds) used at the plot and/or landscape level to evaluate overall biodiversity gain associated with a specific land management practice.

Biodiversity Gain (BG): the relative increase in biodiversity resulting from specific land management practices, expressed as a percentage (%) and calculated using a basket of biodiversity metrics.

Biodiversity Unit (BU): a unit of measurement for biodiversity increase associated with specific land management practices, assessed through a basket of metrics. One Biodiversity Unit per hectare per year (BU $ha^{-1} \cdot year^{-1}$) corresponds to a 1% increase in Biodiversity Gain (BG).

Comparable situation: a scenario in which non-experimental variables are controlled across multiple plots where biodiversity gains are measured. In such cases, observed differences are expected to result solely from management practices, ensuring optimal comparability conditions.

Experimental variables: the specific variable(s) isolated from non-experimental factors to measure biodiversity gain.

Inter-annual comparisons: comparisons of biodiversity indicators within the same year to detect annual differences between control and experimental plots.

Interest (I): the ecological or conservation significance of a species, taxon, or habitat, often based on rarity, endemism, or ecosystem function. In this study, interest is weighted on a scale from 1 to 5.

Intra-annual comparisons: comparisons of biodiversity indicators in plots over multiple years to analyze long-term trends.

Landscape-level metrics: a set of bioindicators used at the landscape scale.

Landscape metrics: a set of quantitative measures used in landscape ecology to assess the spatial composition and configuration of a landscape.

Metric: a measurement standard for a specific bioindicator or OTU (e.g., soil invertebrates, aboveground invertebrates, birds). Each metric specifies:

- The group of organisms assessed.
- The sampling protocol.
- The data processing method.

Monitoring event: the time window in which plots are sampled using all project metrics. In this study, at least two sampling events are required, typically coinciding with peak biological activity (e.g., in temperate zones of the Northern Hemisphere: spring and autumn).

Non-experimental variables: external factors (e.g., edaphoclimatic, biological, agronomic) that must be controlled at the plot level to prevent interference with the variable(s) being assessed for biodiversity gain.

OTU (Operational Taxonomic Unit): the classification unit chosen by researchers, which may correspond to a species or higher taxonomic level (e.g., Order, Family) depending on the study. Different OTUs are used depending on the metric:

- Soil biodiversity metric: focus on various invertebrates based on their soil-related habits (without a unique taxonomic designation).
- Aboveground invertebrate's metric: consider specific insect families and genus.
- Bird metric: include all organisms within the class Birds.
- Flora metric: include all organisms within the kingdom Plantae.

Plot-level metrics: the set of bioindicators used at the plot scale.

Intervention site: plots in which agro-environmental management activities for biodiversity enhancement (e.g., tillage, new product application, crop management, flower strip installation, habitat restoration) are implemented. Their biodiversity indicators are compared to Reference sites in the Biodiversity Matrix.

Project site: the geographical area where biodiversity interventions are conducted. It includes all Intervention sites.

Reference site: the geographical area used as a control to compare against the Intervention sites where interventions occur.

Richness (R): the total number of different species (or taxa)—also known as species diversity—present in a sample collected in a specific monitoring location.

Structural metric: a control metric used to assess whether conditions for measuring biodiversity gains are valid.

Thesis: the experimental approach designed to evaluate a specific management practice and its associated methodological aspects, such as:

- Selection of Reference and Intervention plots.
- Applied metrics.
- Sampling times.

5. Applicability

The proposed methodology utilizes the Biodiversity Matrix created by FGN to assess changes associated with specific land management practices within a defined area. These changes can be directly or indirectly related to ecosystem services, ecosystem structure, and other environmental disruptions.

Biodiversity gains (or losses) are measured as a percentage change (%) and expressed in Biodiversity Units per hectare (BU), where one Biodiversity Unit per hectare per year (BU $ha^{-1} \cdot year^{-1}$) corresponds to a 1% increase or decrease in the biodiversity indicators of the Biodiversity Matrix.

The methodology supports two main applications, depending on the timeframe and objectives of the user:

(1) **Short-term projects** *(1–5 years)*: focused on assessing biodiversity gains resulting from specific interventions.

(2) **Long-term projects** *(20+ years)*: aimed at consolidating and potentially monetizing biodiversity gains linked to sustainable land management practices.

(1) Short-term projects (1-5 years):
Assessing biodiversity gains resulting from specific interventions

Uncertainty regarding the impact of specific interventions on biodiversity prevents companies from taking proactive action and hinders efficient capital allocation—for example, investments in nature restoration or mitigating biodiversity-related risks in supply chains. Traditional approaches rely on long-term data collection (typically 10+ years) to demonstrate the positive or negative impact of a given intervention at a specific site. However, this extended timeframe often delays the implementation of effective conservation and restoration strategies.

In shorter-term projects, the efficacy of interventions can be quantified by comparing the average value of the biodiversity indicators included in the Biodiversity Matrix between different land uses and/or management approaches (i.e., Reference Sites vs. Intervention sites). To ensure a valid comparison between different land management practices and land uses (i.e., Reference Sites vs. Intervention sites), comparability conditions are established (Section 7.1.2. and Section 7.3).

Biodiveristygains (or losses) can be estimated within one year, but they must be reassessed every five years. This approach provides guidance on the expected outcomes of each intervention and serves as a foundation for reporting compliance under the EU Corporate Sustainability Reporting Directive (CSRD). Additionally, it supports informed decision-making for financial resource allocation, maximizing the positive impact of interventions. Biodiversity gains or losses) are measured as a percentage change (%) and expressed in Biodiversity Units (BU). While biodiversity gains can be used to demonstrate the potential impact of interventions, they cannot be monetized unless there is a long-term commitment to maintaining interventions for at least 20 years (see Long-term projects). However, a project may initially start as a short-term initiative and later be upgraded to a long-term project.

(2) Long-term projects (20+ years):
Consolidating and potentially monetizing biodiversity gains linked to sustainable land management practices

When there is a clear strategy for the types of interventions to be implemented at a Intervention site, and the ambition extends beyond merely assessing biodiversity gains and ensuring compliance with the EU CSRD Directive, projects can adopt the second type of approach (long-term project).

To ensure a valid comparison between different land management practices and land uses (i.e., Reference Sites vs. Intervention sites), comparability conditions are established (Section 7.1.2. and Section 7.3).

BOX 1: Gains and Losses - Averting Biodiversity Decline Trends

Site interventions are designed to achieve a net increase in biodiversity. However, it is also expected that, in some cases, regional biodiversity may decline over the course of the project (Pereira et al., 2024). If intervened sites maintain higher biodiversity levels than non-intervened sites, this difference can be recognized as biodiversity gains, representing the prevention of biodiversity loss.

Plot-level metrics will be measured every five years, with biodiversity unitsclaimed annually, based on the project's stage of development. In contrast, landscape-level metrics (e.i., birds) will be measured annually, but biodiversity units will only be claimed in years 5, 10, 15, and 20, following the trendline analysis for those respective periods. This time lag in biodiversity claims is necessary to establish a robust trendline that accounts for data gaps in the years between assessments.

Under this methodology, two different types of sites are considered: Intervention site and Reference site, meant to be compared against each other. The requirements for the selection of the sites are detailed in **Tables 01–02**, and those for the selection of project interventions in **Table 03**.

***Table 01**: Requirements on the selection of Intervention and Reference sites.*

Intervention and References sites requirements applicable to (1) short-term projects (1-5 years), (2) long-term projects (20+ years).
• Sites must be agroecosystems and/or be located within a broader agrarian landscape matrix. • Sites must be comparable, as outlined in Section 7.1.3 of this document. Non-experimental variables must be fixed and controlled, while experimental variables must differ in terms of specific land use and/or land management under study, in accordance with Section 7.1.3. • Reference sites must not be deliberately altered to artificially reduce existing biodiversity for the purpose of claiming a later increase. To ensure consistency, Farm Register Books from the past three agricultural campaigns must confirm that land management practices have remained unchanged. • Intervention sites must not include newly converted agrarian areas where land-use changes have negatively impacted existing biodiversity in the past five years. • Intervention sites must consist of a set of continuous or non-continuous agrarian plots distributed within the agrarian landscape matrix area. • There are no specific surface area requirements, but at least three monitoring points per site are required to calculate relevant coefficients. • There can be as many Intervention and Reference sites as the number of assessments a projects intends to make (i.e. number of thesis under study). Reference sites include the land use/land management that is being substituted through the project interventions, Intervention sites include the land use/land management considered as project intervention.

Table 02: *Requirements on the Project duration.*

Project duration requirements applicable to (1) Short- term projects (1-5 years)	**Project duration requirements applicable to (2) Long term-projects (20+ years)**
• One year of comparable measurements for the thesis under study, aimed at assessing potential biodiversity gains or losses under highly comparable conditions. These potential biodiversity gains will remain valid for a five-year period following the conclusion of the study.	• 20+ years. Focused on the consolidation and monetization of biodiversity gains associated with long-term land management..

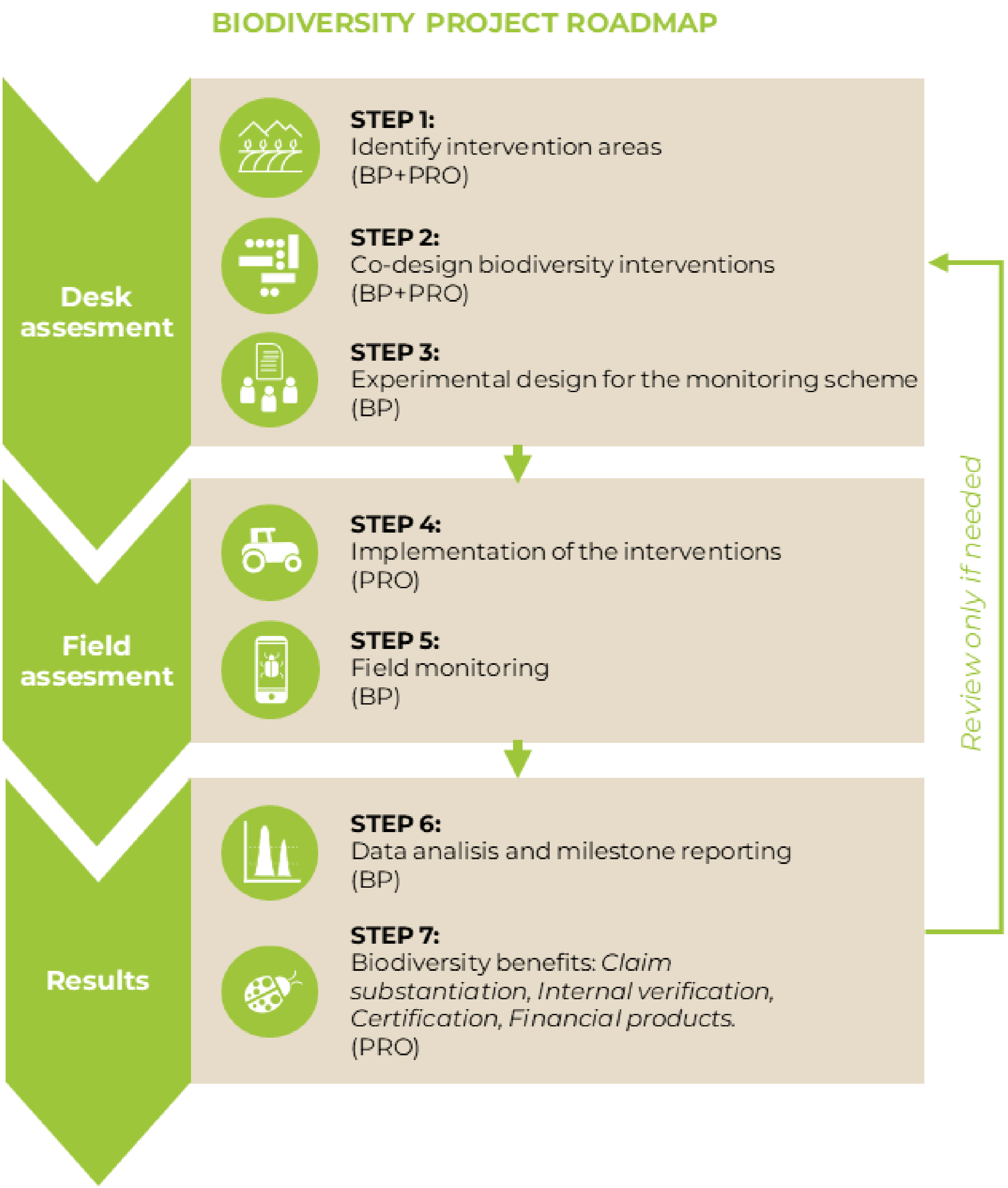
Table 03: *Requirements on the Project interventions.*

Project inerventions requirements applicable to (1) Short- term projects (1-5 years)	Project interventions requirements applicable to (2) Long term-projects (20+ years)
• The interventions studied should serve as representative measures with high replicability potential within the ecosystem and region under study, ensuring that the experimental variables of interest are properly isolated, as outlined in Section 7.1.2 of this document. • Local-scale interventions must focus on implementing best agrarian practices, or restoration actions suited to the agrarian context under study. • Interventions must not negatively impact surrounding areas and should be aligned with biodiversity conservation while maintaining the representativeness of agrarian habitats in the region. • Project interventions will be designed at the plot scale. Potential biodviersity gains can be used to demonstrate the potential impact of specific interventions, with a validity period of five years. However, they cannot be monetized unless there is a commitment to maintain interventions for at least 20 years and new monitoring events are conducted as part of a long-term project. A project may initially start as a short-term project and later be upgraded into a long-term project.	• The interventions studied will be replicated across the entire Intervention site, ensuring that the experimental variables of interest are properly isolated, as outlined in Section 7.1.2 of this document. • Local-scale interventions must focus on implementing best agricultural practices, livestock management practices, or restoration actions suited to the agrarian context under study. • Interventions must not negatively impact surrounding areas or endangered species and should be aligned with biodiversity conservation while maintaining the representativeness of agrarian habitats in the region. • Project interventions will be designed at both the plot and landscape scales. • Landscape-scale interventions should emphasize sustainable landscape management principles, with a primary focus on enhancing ecological connectivity.

6. Biodiversity project's roadmap

How should a biodiversity project develop? What are the necessary steps? Below is a summary of the key steps involved in the development of a biodiversity project.

Figure 01. Biodiversity project roadmap. Each step outlines the key actors involved: Biodiversity Partner (BP) and the Promoter (PRO).



STEP 1: Identify your intervention area

Begin by defining the area where your company, association, or stakeholders intend to take action. Whether the goal is land restoration, reducing the environmental footprint or risk in a sourcing region, or ensuring compliance with environmental policies, the BP can assist in evaluating and prioritizing the most critical areas for intervention.

STEP 2: Co-design biodiversity project interventions

Once the Intervention site is selected, discussions should focus on defining the scope of practices, surface area, and monitoring period. If necessary, a comprehensive plan can be co-designed to ensure that restoration goals are achieved while remaining compatible with agricultural production.

STEP 3: Experimental design for the monitoring scheme

After determining the scope and biodiversity-enhancing actions, FGN can assist in designing a practical and statistically sound monitoring scheme, as detailed in Section 7.1. For monitoring site selection, the BP will identify comparable plots with similar soil, climate, and management conditions and define the monitoring schedule in consultation with all stakeholders, including farmers.

STEP 4: In-field monitoring

Throughout the project duration, the BP staff or other qualified experts will conduct monitoring at least twice a year, following the methodologies outlined in Sections 7.3 and 7.4. Monitoring will take place during peak biological activity seasons or during periods of significant differences caused by management interventions.

STEP 5: Annual and milestone reporting

Yearly reports will be produced, including biodiversity results, raw and processed data, and calculated metrics using the formulae in Section 7.6. These reports will provide biodiversity performance metrics at the plot, site, and management (thesis) levels.

STEP 6: Claim your biodiversity gains

With each yearly biodiversity gain, the biodiversity benefits resulting from implemented interventions can be claimed as Biodiversity Units (BU) in all projects. In long-term projects (20+ years), these Biodiversity Units may also be monetized.

7. Methodology

7.1. Experimental design

7.1.1. Challenges in agrarian ecosystems

Biodiversity in agrarian ecosystems is influenced by a wide range of dynamic factors. To ensure a reliable assessment of biodiversity gains and/or losses, it is essential to distinguish experimental variables under study from non-experimental variables.

When designing an experimental approach in an agrarian ecosystem, the following challenges must be addressed:

- **Landscape and Plot Scales**: due to the intensive management and relatively small surface areas of agricultural land, biodiversity metrics must include biodiversity groups that respond at both plot and landscape scales.

- **Avoiding a biased definition of biodiversity**: the basket of metrics used in this approach has been pre-established for agrarian ecosystems. Some interventions may have a greater positive impact on certain biodiversity groups than others. By using a fixed basket of metrics, this methodology ensures objectivity—measuring biodiversity as a whole, rather than selectively assessing only species that are likely to benefit from interventions.

- **Inter-annual and Intra-annual comparisons**: in agrarian ecosystems, measuring biodiversity in year 0 does not provide a reliable baseline. Crop rotations, seasonal land management changes, and climate variability significantly affect species abundance and richness in any given year. Inter-annual comparisons are only reliable with 10+ years of data series (Andrade et al., 2021), while intra-annual comparisons are essential for assessing early-stage project impacts. This approach incorporates both types of comparisons.

- **Short-term outputs**: approaches based on long-term data series (10+ years) are often disconnected from land managers' needs, delaying decision-making, funding, reporting, and regulatory compliance. This limits the scalability of biodiversity interventions. This methodology offers an initial biodiversity impact assessment within just one year, which can be used for early biodiversity gain claims and refined through subsequent monitoring campaigns. The impact guidance for interventions will be reviewed every five years, incorporating new biodiversity data and detected trends from ongoing monitoring.

- **No absolute thresholds**: in many ecosystems, absolute thresholds can be used to define acceptable species abundance and richness levels. However, this is not the case in agrarian ecosystems, unless long-term data series (10+ years) are available (Andrade et al., 2021). The same species count or individual count may be high or low depending on monitoring timing, crop type, or climatic conditions of previous months. This approach establishes a relative approach based on the gains and lossess of each particular indicator within a metric, adjusting for seasonal and annual variability and allowing for shorter-term assessments (within one year).

- **Incorporating *Interest* as a key variable**: relying solely on species abundance, richness, and distribution as biodiversity indicators can be misleading in agrarian ecosystems. For example, a sample containing 10 individuals from five different species of aphids (pests) should not be valued the same as a sample with 10 individuals from five different species of solitary bees (pollinators). The latter provides more meaningful insights into landscape quality, even if both samples have identical abundance, richness, or species evenness. This approach integrates *Interest* as a key variable in the biodiversity equation to account for ecological function and sensitivity to management practices.

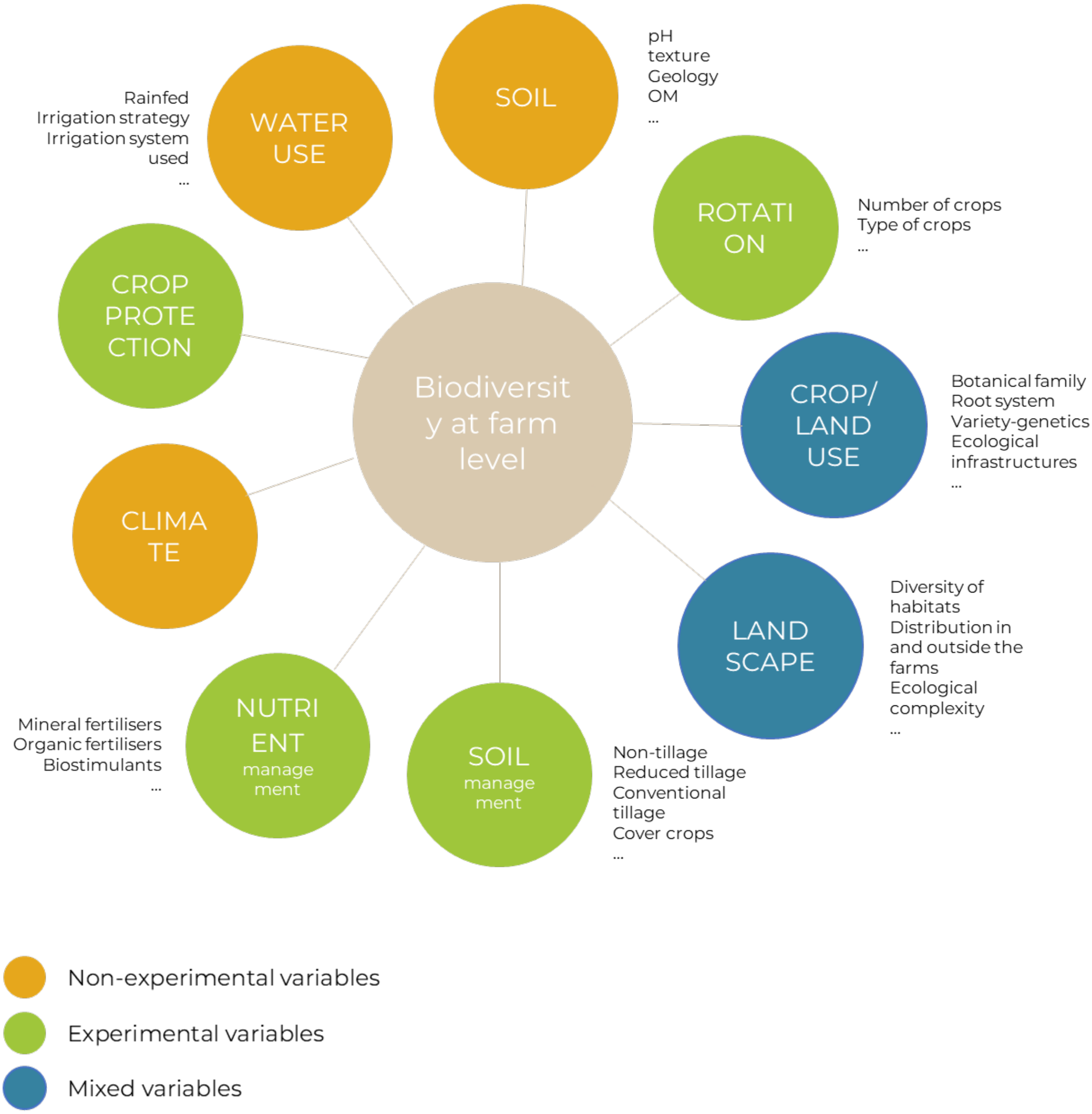
7.1.2. Comparable situations

Unlike natural ecosystems, where population dynamics are generally not influenced by human intervention and rarely change over short time scales, agricultural ecosystems experience intense environmental modifications that affect a wide range of organisms annually or even within shorter periods.

In Figure 02, variables inherent to land use and/or land management are classified as experimental variables, while those independent of land use changes are categorized as non-experimental variables. Some variables may be either experimental or independent, depending on the type of interventions included in the project.

A clear understanding of all variables and the ability to isolate experimental variables—to prevent interference from external factors—are essential for designing meaningful trials that accurately assess biodiversity gains.

Figure 02. Types of variables influencing agrarian biodiversity.



CROP/ LAND USE

Reference and Intervention sites must have the same crop and/or land use, unless the objective of the study is to assess the substitution of the crop and/or land use itself. In such cases, this variable would be considered an experimental variable. Biodiversity is highly influenced by plant structure, pest presence, plant tissue, and substances secreted by plants.

LAND SCAPE

Reference and Intervention sites must be located within a landscape of similar quality as per Landscape metric, unless the study aims to assess how an improved landscape (hedgerows, buffer strips, afforested areas...) influences biodiversity gains. In such cases, this variable would be considered an experimental variable. Comparable sites must have a score difference of ≤ 1.5 units.

SOIL

Reference and Intervention sites must have similar soil characteristics. Biodiversity is strongly influenced by the physical and chemical propperties of the soil. At least, the following thresholds/categories for pH and soil texture will be considered. pH thresholds are: <5.5, 5.5-6.5, 6.6-7.5, 7.6-8.5, >8.5. Soil texture categories are: light soil, medium soil, heavy soil. In farming practice light soil is a term used to describe sands and sandy loams; medium soils include sandy silt loam and silt loam; while heavy soils are the clay soils.

CLIMA TE

Reference and Intervention sites must have similar climatic conditions. Biodiversity is influenced by factors such as temperature, evapotranspiration, and precipitation—not only of the monitoring day but in the preceding months. Comparable sites must be located within a 20 km radius, have a difference in elevation ≤100 m, and share a similar orientation and slope.

WATER USE

Reference and Intervention sites must have similar water management practices. Biodiversity is strongly influenced by water availability. Rain-fed areas cannot be compared to irrigated areas due to their fundamental differences in water dynamics. Comparable conditions must have the same type of water use and irrigation system (if irrigated).

7.1.3. Capturing agrarian dynamism

Due to the dynamic nature of agrarian ecosystems, at least two monitoring events (repetitions) must be conducted each monitoring year. These events should take place at different stages of the agricultural cycle, capturing the most significant land management changes between Reference and Intervention sites. Each repetition must account for the following conditions:

- Peak biodiversity activity for both Intervention and Reference sites (as exemplified in Box 2).
- Acute differences in land management between Intervention and Reference sites (as exemplified in Box 2).
- Measurements are taken for the three types of sites in each monitoring repetition (Intervention and Reference sites)

Box 2: Examples of timing for the two annual monitoring events:

- Substitution of agricultural crops with natural or semi-natural habitats: first repetition during crop flowering phase, second repetition after harvest/before sowing.
- Substitution of cereal crops with legume crops: first repetition during the development phase of both crops (March/April in the Northern Hemisphere), second repetition during the flowering phase of both crops (May/June in the Northern Hemisphere).
- Substitution of cereal crops with lavender crops: first repetition during the flowering phase of the cereal crop (May/June in the Northern Hemisphere), second repetition during the flowering phase of the lavender crop (July in the Northern Hemisphere).
- Implementation of winter cover crops in summer crops: first repetition when the winter cover crop is in place (March in the Northern Hemisphere), second repetition during the flowering phase of the summer crop (July in the Northern Hemisphere).
- Implementation of summer cover crops in winter/spring crops: first repetition during the flowering phase of the winter/spring crop (May/June in the Northern Hemisphere), second repetition when the summer cover crop is fully developed (September in the Northern Hemisphere).
- Conventional agriculture Vs. Organic/Regenerative Agriculture of any given crop: first repetition during the crop development phase, second repetition during the crop flowering phase.
- Rotational grazing Vs Conventional grazing in permanent pastures: first repetition during the growth season in Spring (March/April in the Northern Hemisphere), second repetition during the growth season in Autumn (October/November in the Northern Hemisphere).

7.1.4. Gathering sufficient data

A minimum number of comparable data sets must be collected for all interventions under assessment. The required data collection criteria vary based on the intended use of this methodology. The following guidelines apply:

(1) Short-term projects (1-5 years):
Assessing biodiversity gains resulting from specific interventions

All metrics are treated as plot-level metrics and are subject to the following criteria:

- A minimum of three monitoring points per type of land use/land management under assessment (three for each Reference site and three for each intervention type in a Intervention site), selected through stratified random sampling. In the case of metric Birds, just one monitoring location is needed given the wider area covered by the data extracted in each monitoring point. The location of this single monitoring point is pre-selected under the criteria of being in a homogeneous representative area of the plot under study and as close to its centre as possible.

- An equal number of monitoring points for each type of land use/land management under assessment.

- At least two monitoring repetitions per year, as specified in Section 7.1.3.

Assessments of potential biodiversity gains (or losses) can be used for reporting on a maximum area of 1,000 hectares within the same region over a five-year period. After the initial five years, a comparable effort is required for renewal or for the inclusion of additional 1,000-hectare lots.

(2) Long-term projects (20+ years):
Consolidating and potentially monetizing biodiversity gains linked to sustainable land management practices

In long-term projects, the metric Birds becomes a landscape-level metric. For plot-level metrics, the same criteria as in the short-term projects applies. For landscape metrics (e.i., birds), the following criteria must be followed:

- A minimum of one monitoring point per 30 hectares of Intervention site and Reference site needs to be established for plot metrics. In the case of landscape metrics (i.e. Birds), a minimum of one monitoring point per 100 hectares needs to be established not only in the Reference and the Intervention sites but also in every land use/land management covering more than 5% of the Project site.

- A minimum of two repetitions per monitoring year, as specified in Section 6.1.3.

- Landscape metrics monitoring must be conducted annually.

7.2. Defining a Basket of biodiversity metrics

To calculate Biodiversity Gains (BG, %) and express them as Biodiversity Units (BU), this method establishes a system based on a Basket of biodiversity metrics. This concept is inspired by the Consumer Price Index (CPI), which measures inflation or price variation over time by tracking the cost of a representative set of goods and services typically consumed by households. For the purposes of this methodology, the metrics are specifically selected to quantify biodiversity in agricultural areas (see section 7.4. Non-structural metrics), with the following characteristics:

- Metrics must consist of higher-level taxa rather than individual species (Operational Taxonomic Units, OTU). In eDNA sampling, species level is treated as a molecular OTU (Kestel et al., 2022). In arthropod and flora monitoring, individual organisms are identified to species level whenever possible, though often only genera or families are distinguished at the morphospecies level (Derraik et al., 2010; Barratt et al., 2003).

- Metrics must reflect the overarching goals of conservation, restoration, or specific policies/regulatory measures of the agroecosystems in which the Intervention site is located.

- Metrics must address all ecosystem services impacted, such as water quality, soil quality, and pollination services, among others.

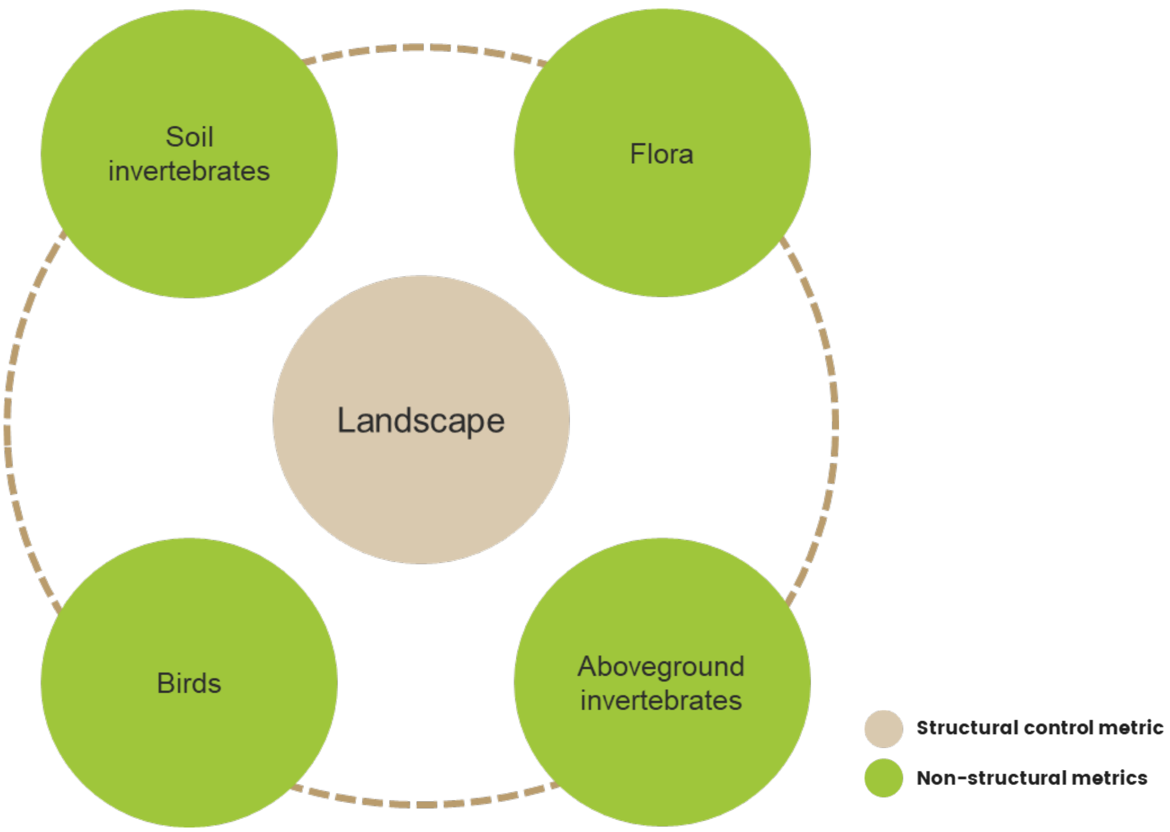
Additionally, the Basket of biodiversity metrics must:

- Contain a minimum of five metrics, as per section 7.4.

- Include at least one structural control metric, as per section 7.3.

The configuration of the Basket of biodiversity metrics is shown in **Figure 03**.

Figure 03. Configuration of the Basket of biodiversity metrics.



7.3. Structural metric - Landscape

Agrarian landscape refers to the areas of land that are used for crop production and also provide additional services such as climate regulation, water regulation, and biodiversity conservation (Bommarco & Potts, 2013). These landscapes play a crucial role in delivering benefits at regional or global scales and they represent a wide variety of habitats including crops, noncrop vegetation patches, woodlands, wetlands, grasslands, and forests (Philpott, 2013). This natural and others semi-natural habitats (hedgerows, live fences and other linear habitats) are the indispensable covers in agro-landscape, which maintain higher biodiversity because they provide refuge, foods and breeding place (Song et al. 2016; Harvey et al., 2005). The presence of noncrop vegetation, the distance to natural areas, and the complexity of the landscape can all affect the capability of the landscape for hosting biodiversity.

This metric is not used for the calculation of Biodiversity gains (BG, %) or Biodiversity Units (BU). It is meant to ensure comparability between Intevention and Reference sites for the correlation of plot-level metrics, but also as insurance for project developers in cases in which biodiversity gains transform into losses due to important pressures outside the Project site. It includes a dual mechanism: (1) it records changes in the landscape at Project site caused by the project developer; and (2) it tracks changes in the landscape within a 1 km buffer zone from the center of the Intervention site and Project site itself.

The landscape measurement methodology is developed in three steps: (1) a desk assessment supported by GIS tools; (2) an on-site assessment supported by field work; and (3) calculation of landscape metrics and the Landscape Structural Suitability Index (LSSI).

(1) Step 1: Desk assessment supported by GIS tools

Using GIS tools, a map of land uses and ecological infrastructures is generated. This map, in addition to showing land uses, must include:

- elements with a positive impact on biodiversity at the landscape level, such as hedgerows, woodlands, isolated trees, or ponds;
- elements with a negative impact on biodiversity, such as roads or similar infrastructure.

These features should be mapped in a 1 km buffer area from the center of the target plot. For European projects, the Level 3 classification of the CORINE Land Cover is recommended as the base map for land use, but more precise cartographic products are used whenever available for the study area.

(2) Step 2: On-Site Landscape Assessment

A field survey is conducted after creating the land use map to verify on-site that the quality of landscape elements and land uses within the study area corresponds to the GIS layers. If any discrepancies are found, the GIS layers are updated accordingly.

(3) Step 3: Calculation of landscape metrics and the LSSI

The land uses polygon map created in Step 1 is converted into a raster with a resolution of 10m². Within the project site, five landscape metrics are calculated: (1) Percentage of Landscape (PLAND), (2) Perimenter-area fractal dimension (PAFRAC), (3) Patch Density (PD), (4) Patch Cohesion Index (COHESION), and (5) Core Area Percentage of Landscape (CPLAND). Open-access tools such as FRAGSTATS or the lsm_c_cpland function from the landscapemetrics package in R (Hesselbarth et al., 2019; Team, 2024) are recommended for this analysis.

These five metrics are then standardized using values from 1 to 5 and combined using a specific formula to compute the **"Landscape Structural Suitability Index" (LSSI).** This formula is inspired by the "Landscape Biodiversity Index", proposed by the Word Resources Institute in their report "Sustainability Index for Landscape Restoration" (**Formula 01**), wich proposes the creation of a landscape index based on the aggregation of five landscape metrics used in landscape ecology, which allow for the comparison between different landscapes or changes within the same landscape over time (Cristales et al., 2020). Here, the landscape metrics agreggated report on five main aspects of the landscape: composition, configuration, fragmentation, structural connectivity and habitat quality that are crucial to evaluate that influence the functioning and ecological health of the landscape (**Table 04**). To sum up, the LSSI provides a comprehensive view of the structure and potencial capacity of the landscape to support biodiversity.

Formula 01: *Landscape Structural Suitability Index (LSSI)*

$$LSSI = \frac{PLAND + PAFRAC + PD + COHESION + CPLAND}{5}$$

LSSI: Landscape Structural Suitability Index (1-5)

PLAND: Percentage of Landscape

PAFRAC: Perimeter-area fractal dimension

PD: Patch Density

COHESION: Patch Cohesion Index

CPLAND: Core Area Percentage of Landscape

For a project to be elegible, Landscape scores of Reference and Intervention sites will not differ in more than 1.5 units of value as per the LSSI, ensuring comparability. In Long-term projects, total Landscape score of the project site must always increase due to project interventions.

Table 04: *Description of the Landscape metrics that make up the Landscape Structural Suitability Index (LSSI)*

Landscape aspect	Landscape metric	Type of metric	Range and Units	Interpretation
Composition	Percentage of Landscape (PLAND)	Class level- Area and edge metric	0 < PLAND ≦ 100 Units: Percent	PLAND measures the proportion of the total landscape occupied by a specific class or type of land cover. PLAND quantifies the relative abundance of a particular land cover type within a given landscape, expressed as a percentage. It provides insight into the dominance and distribution of different habitat types. PLAND is useful in land-use planning, biodiversity conservation, and assessing environmental changes over time.
Configuration	Perimeter-Area Fractal Dimension (PAFRAC)	Landscape level- Shape metric	1 ≦ PAFRAC ≦ 2 Units: None	PAFRAC describes the complexity of patch shapes within a landscape. This index measures the relationship between the perimeter and area of patches, providing information on the irregularity and complexity of their edges: – Values close to 1 → Indicate simple and compact shapes, such as circles or squares. – Values close to 2 → Indicate more irregular and complex shapes, with more sinuous and fragmented edges. Landscapes with high PAFRAC values may indicate greater fragmentation and more irregular edges, influencing ecological

				processes such as species dispersal and interaction with the environment. Landscapes with low PAFRAC values tend to be more homogeneous and have geometrically simpler shapes. This metric is essential in biodiversity studies, habitat management, and land-use change analysis.
Fragmentation	Patch Density (PD)	Class level-Aggregation metric	PD > 0, constrained by cell size. Units--> Number per 100 hectares	PD is a simple measure of the extent of subdivision or fragmentation of the patch type. PD is ultimately constrained by the grain size of the raster image, because the maximum PD is attained when every cell is a separate patch. Patch density is a limited, but fundamental, aspect of landscape pattern. Patch density has the same basic utility as number of patches as an index, except that it expresses number of patches on a per unit area basis that facilitates comparisons among landscapes of varying size.
Structural connectivity	Patch Cohesion Index (COHESION)	Class level-Aggregation metric	0 < COHESION < 100 Units-->Percent	COHESION measures the degree of connectivity between patches of the same class within a landscape. This index evaluates the spatial continuity of patches within the same category, considering their size and distribution. A high cohesion value indicates that the patches are well connected and form a more homogeneous landscape, while a low value suggests a more fragmented landscape.

Habitat quality	Core Area Percentage of Landscape (CPLAND)	Class level-Core Area metric	0 ≦ CPLAND < 100 Units-->Percent	CPLAND measures the proportion of the total landscape occupied by the core areas of patches. This index quantifies the percentage of the landscape that consists of core areas, which are the interior portions of patches that are not affected by edge effects. It helps assess habitat quality and landscape connectivity (indirectly): – High values → Indicate a landscape with large, well-connected core areas, which are crucial for species that require interior habitat. – Low values → Suggest a fragmented landscape with limited core habitat, potentially increasing exposure to edge effects and reducing biodiversity.

7.4. Non-structural metrics

For the purposes of this methodology, biodiversity metrics are indicator taxa specifically selected to quantify biodiversity in agricultural areas. These taxa must be identified at the OTU level (e.g., soil invertebrates, breeding birds), and all species within those taxa should be surveyed.

7.4.1. Soil invertebrates

Macroinvertebrates represent millions of species that are vital for soil functioning. This includes the so-called soil engineers—termites, ants, and earthworms—as well as certain species of millipedes and beetles that modify soil structure and the distribution of resources (particularly organic matter) within the soil for other organisms. They also influence microbial communities responsible for mineralization and humification. These organisms consume all types of organic residues in association with soil microflora (Lavelle et al., 1997).

Soil invertebrates are sampled by using a hoe to dig a 30 × 30 × 30 cm hole in the ground, processing the excavated material (including any surface vegetation or debris) through a litter reducer with a 4 × 4 mm screen. One hole is dug at each sampling point. After the three perforations at each site (one perforation per sampling point, with three sampling points per site), the hoe and litter reducer are cleaned and sterilized with a 70% ethanol solution. The soil from these perforations is combined and homogenized in a container, and a final 300-gram sample is prepared. Each sample is stored in a labeled, refrigerated zip-lock bag and sent to the laboratory within five days, where a specialized company conducts environmental DNA (eDNA) analysis using quantitative PCR (qPCR) and next-generation sequencing (NGS), following standardized methodologies. This process identifies the diversity of soil invertebrates present in the sample.

The QBS-ar (Soil Biological Quality-arthropod) index is calculated (Menta et al., 2018). This index, which does not require complex taxonomic identification at the species level (Parisi et al., 2005), is based on the principle that high-quality soils harbor a greater number of microarthropod groups that are morphologically well-adapted to soil conditions than low-quality soils. Each group is assigned a value between 5 and 20 according to the EMI (Eco-Morphological Index) and the value of each group present in the sample is summed up to calculate the final score according to Formula 02:

Formula 02: *Soil Biodiversity Quality Index (QBS-ar)*

$$QBSar = \sum_{i=1}^{n} EMI(i)$$

QBSar: QBS-ar index (5-375)

EMI(i): EMI value assigned to the i^{th} invertebrate group in the sample

n: number of different groups present in the sample

The QBS-ar index combines two essential aspects of soil microarthropods: (1) their presence, representing biodiversity, and (2) their ability to adapt to soil conditions, reflecting vulnerability (Menta et al., 2018). The QBS-ar index, introduced over a decade ago, has been widely applied to various ecosystems, including agricultural lands and grasslands, to effectively assess soil biological quality.

Menta et al. (2018) describes how to perform a visual analysis of the soil samples without involving eDNA. If preferred, this option can substitute for eDNA analysis.

7.4.2. Flora

The diversity of wild flora associated with agricultural systems is at risk due to intensification, which includes practices such as the widespread use of mineral fertilizers, pesticides, herbicides, intensive soil management, and habitat destruction, among others (Storkey et al., 2011). Wild flora provides a wide range of beneficial ecosystem services, including protection against erosion, improvement of soil structure, provision of food resources for a variety of beneficial organisms, and nitrogen fixation, among others (Isbell et al., 2017). The flora metric must be measured following these two methodologies, depending on the type of agoecosystem surveyed: agricultural crops or herbaceous communities associated with meadows and pastures (in a livestock context).

- Agricultural crops

For flora measurement in agricultural crops, five consecutive 4-square-meter quadrats are set up at each sampling point along a straight line, and all specimens are identified to the most specific taxonomic level possible. When identification at the species level is not feasible, distinct morphotypes within the same genus or family are distinguished.

Within each quadrat, species richness is recorded, and the approximate percentage cover for each taxon is estimated (Stobbe et al., 2013; Veldhoen et al., 2016; Whalley & Hardy, 2000). Percentage cover is used as a proxy of abundance instead of number of individuals due to the challenge of distinguishing individual plants of the same species, particularly in vegetative structures such as tussocks or herbaceous plants.

For calculating abundance, the independent data from the five consecutive quadrats are averaged to assign values to the transect itself, and the data of all the transects in any given thesis is averaged to calculate the mean soil coverage for the thesis. Species richness is calculated for the transect as a whole (considering the number of different species within all five quadrats), and the richness of all transects in any given thesis is averaged to calculate the mean richness of the thesis.

- Herbaceous communities associated with meadows and pastures (livestock context)

For flora measurement in herbaceous communities associated with meadows and pastures (in a livestock context), five 0.5 × 0.5 m ubquadrats are placed by aligning their centers with the centers of five 2 × 2 m grid cells located within the 2 × 10 m quadrant (transect), where all specimens are identified to the most specific taxonomic level possible. When identification at the species level is not feasible, distinct morphotypes within the same genus or family are distinguished. In studies on herbaceous pastures in Spain, the prevalent sampling unit has traditionally been 0.25 m² (0.5 × 0.5 m), as used by researchers such as Puerto (1976), Navascués et al. (1986), Rico et al. (1985), Zuazúa et al. (1985), García-Rodríguez et al. (1986), Pérez-Corona et al. (1996), and Rivero and Puerto (1996), among others. However, the size of the quadrat can be adjusted according to the type of vegetation, starting from a minimum quadrat size of 0.5 meters per side.

Within each subquadrant, species richness is recorded, and the approximate percentage cover for each taxon is estimated (Stobbe et al., 2013; Veldhoen et al., 2016; Whalley & Hardy, 2000). This allows relative species dominance to be assessed and extrapolated to the entire 2 × 10 meter quadrant (transect), assuming subquadrat data are representative. Percentage cover is used as a proxy of abundance instead of number of individuals due to the challenge of distinguishing individual plants of the same species, particularly in vegetative structures such as tussocks or herbaceous plants.

For calculating abundance, the independent data from the three 2 x 10m quadrats (transect) is averaged to calculate the mean soil coverage for the thesis. Species richness is calculated for the transect as a whole (considering the number of different species within all four subquadrants), and the richness of all transects in any given thesis is averaged to calculate the mean richness of the thesis.

7.4.3. Above-ground invertebrates

Above-ground invertebrates play a fundamental role in terrestrial ecosystems due to their position at the base of the food chain and the wide range of ecological functions they perform (Saunders, 2018). These functions significantly contribute to maintaining a healthy and resilient ecosystem. Among their primary functions are pollination, nutrient recycling, organic matter decomposition, as well as pest predation (Culliney, 2013).

Pollinators, for instance, are experiencing a rapid decline (Dicks et al., 2021). In agricultural ecosystems, pollinator diversity is linked to increases in both the quality and quantity of crop yields (Aizen et al., 2009). Furthermore, studies indicate that many pollinator groups are useful for monitoring environmental pollution, aiding in pest and disease control, and providing cultural and aesthetic value (Katumo et al., 2022).

Above-ground invertebrates are sampled combining two different sampling techniques on the field and one common method for analysis on the laboratory:

- **Use of a garden vacuum in the field:**
  A vacuum with at least 36V and 2.5Ah is used, fitted with a dress stocking to capture arthropods. Sampling is conducted on sunny days with minimal wind and temperatures between 10°C and 30°C to optimize arthropod capture. Vacuuming is performed for 2 minutes per point, covering a straight transect of approximately 10 meters (aligned with the flora transect). Both the soil surface and vegetation are sampled to target potential arthropod refuges.

After vacuuming, the contents from the three transects conducted per land use/management type are placed in a zip-lock bag, refrigerated, and sent to the laboratory within 5 days. This vacuuming method, widely used in various studies, is particularly effective for sampling arthropods located both on the ground and within vegetation (McCravy, 2018; Sunderland et al., 1995).

- **Collection of flower and plant clippings in the field:**
  After vacuuming, flowers and plant clippings from each transect are collected and placed into the same zip-lock bag in proportions reflecting the plant species' surface coverage. The samples are then refrigerated and sent to the laboratory within 5 days. For example, if a land use/management area is covered by 70% wheat, 10% poppies, 10% *Lolium* sp., and 10% bare soil, then approximately 70% of the remaining space in the bag would be filled with wheat, 10% with poppies, 10% with *Lolium* sp., and 10% left unfilled.

  This technique ensures that larger or faster arthropods that may escape the vacuum (e.g., butterflies, solitary bees, honeybees, bumblebees) are still detectable in the laboratory through DNA traces left on flowers and vegetation.

- **Environmental DNA (eDNA) in the laboratory**:
  A specialized company performs environmental DNA (eDNA) analysis using quantitative PCR (qPCR) and next-generation sequencing (NGS), following standardized methodologies. A “spike” mechanism is also employed to determine the quantity of DNA present. As a result, both species richness (number of species) and relative abundance (relative amount of DNA per species) in the sample can be determined.

Relative abundance per species (relative amount of DNA) varies across a wide range of values, with large deviations sometimes caused by unexpected circumstances such as the presence of a nest or a pest outbreak near the monitoring point. Therefore, the use of logarithms was introduced to help tame the data according to Formula 03.

Formula 03: *Calculation of abundance for Aboveground invertebrates.*

$$A = \sum_{i=1}^{n} \log{(ai + 0.1)}$$

A: abundance

n: total number of species

ai: relative amount of DNA of species i

7.4.4. Birds

Birds play a vital role in agricultural landscapes, contributing significantly to the sustainability of agroecosystems. For example, insectivorous species serve as natural allies in biological pest control, reducing crop infestations without the need for chemical interventions (Wenny et al., 2011). Similarly, certain passerine birds are essential for pollination, supporting the reproduction of both cultivated and wild plants (Sekercioglu, 2012). Additionally, their role as seed dispersers promotes habitat regeneration and enhances biodiversity (Garcia et al., 2010).

However, agricultural intensification practices—such as the widespread use of agrochemicals, monocropping, loss of crop rotations, elimination of stubble fields and fallows, and removal of natural vegetation remnants—pose significant threats to bird populations dependent on farmland ecosystems (Tscharntke et al., 2005).

Finally, birds also serve as critical bioindicators; their diversity and abundance reflect the overall health of ecosystems, making them valuable for detecting the impacts of intensive agricultural practices (Gregory & van Strien, 2010).

For bird sampling, one recorder able to identify birds species and number of cues per species is placed at each monitoring point (listening station). An example of such type of recorders would be the PUC BirdWeather. Recordering devices are installed during 24 hours in each monitoring repetition and then something similar to the Vocal Activity Rate (VAR) per species is calculated, but taming the number of calls per species using the logarithm of number of calls + 0.1 instead of the actual number of calls. The VAR index is an index of relative abundance that measures the number of calls of each species per unit of recording time (Pérez-Granados et al., 2019). By using the logartithm proposed, large deviations in the number of calls of any given species are tamed according to the following **Formula 04**:

Formula 04: *Vocal Activity Rate Index (VAR) tamed with logarithm of number of calls + 0.1.*

$$A = VAR_{\log(calls+0.1)} = \frac{\sum_{i=1}^{n} \log\ (ci + 0.1)}{Total\ recording\ time\ (minutes)}$$

A: abundance

$VAR_{\log(calls+0.1)}$: *Vocal Activity Rate Index (VAR) tamed with logarithm*

n: total number of species

ci: number of calls of species i

However, before calculating the VAR, it is necessary to filter the bird species recorded by the BirdWeather PUC device or similar devices, in order to exclude records of species not associated with the agrarian habitat under study and whose presence may be due to the existence of nearby habitats adjacent to the monitored plot, as a result of the device’s large detection range

Depending on the scope of the project, bird measurements will be performed in two different ways:

(a) **Short-term projects**: Birds are treated as a plot-level metric. At least one listening station will be established for each thesis. At each station, one recorder will be placed during 24 hours. The listening stations do not need to be part of a fixed route because they are measured at the level of isolated plots in the thesis of interest.

(b) **Long-term projects**: Birds are treated as a landscape-scale metric. At least one listening station will be established per 100 hectares of Intervention site, Reference site, and every land use/land management covering more than 5% of the Project site. Listening stations must be part of a fixed route and monitored together during each monitoring event with recorders placed during 24 hours.

7.5. Climatic conditions for measuring biodiversity

All biodiversity groups must be measured under specific climatic conditions, as shown in **Table 0**5.

Table 05: *Time and Weather Conditions for Measuring Biodiversity Metrics*

Biodiversity metric	Climatic conditions
Flora	No climatic restrictions.
Above-ground invertebrates	Light wind conditions or absence of wind (< 10 km/h). Measured on the Beaufort scale, values below 5 (fresh breeze), when branches of moderate size move and small trees begin to sway. Absence of rain or water-logged soil Good weather conditions: sunny and warm, coinciding with peak activity periods. Temperature equal to or greater than 13ºC. Between 13ºC and 17ºC, it is important for it to be sunny with cloud cover of 50% or less. Temperature not exceeding 30ºC.
Soil invertebrates	Absence of rain, water-logged or frozen soils to ensure the collection and delivery of samples to the laboratory under optimal conditions or proper soil sampling. For the QBS-ar (Soil Biological Quality-arthropod):

	Light wind conditions or absence of wind (< 10 km/h). Sunny conditions, coinciding with peak activity periods. The best time to take soil samples is outside of dry periods as this condition causes vertical migration, immobilization, and aestivation of soil microarthropods.
Birds	Light wind conditions or absence of wind (< 10 km/h). Absence of rain. Light rain may be acceptable if birds are active, and visibility is good. Avoid moderate and heavy rain. Good visibility conditions (absence of fog and extreme backlighting). Conduct surveys during the early hours of the morning until no later than 11 a.m., when temperatures are more moderate, and birds are most active. Avoid surveys before heavy storms or during unstable weather conditions.

7.6. Calculation process

The calculation process is based on aggregating the aforementioned sets of bioindicator data, resulting in Biodiversity Gains (BG), measured as a percentage change (%) and expressed in Biodiversity Units (BU). Each Biodiversity Unit per hectare per year ($BU \cdot ha^{-1} \cdot year^{-1}$) represents a 1% increase or decrease in the aggregated biodiversity metrics included in the selected set. The calculation is performed using the Biodiversity Matrix shown in **Figure 04**.

The Biodiversity Matrix, developed by FGN, calculates the percentage of change needed to go from the value of an indicator gathered in a Reference site (Ref) to the value of the same indicator gathered in an Intervention site (Int) of any given monitoring repetition, averaging the percentage of change of all indicators within a metric to come up with the final percentage of change. The values of the indicators QBS-ar, Abundance (A), Richness (R) and Interest (I) used in the Biodiversity Matrix are the average of all the monitoring points measured under the Reference or Intervention thesis. Details on the calculation formulas for the indicators QBS-ar and Abundance (A) are provided in Section 7.4., while details on the calculation formulas for the indicator Interest (I) is provided in Section 7.6.1.

The maximum percentage of change is capped to 500%, coinciding with the maximum amount of change achievable in the indicator Interest. Biodiversity Units ($BU \cdot ha^{-1} \cdot year^{-1}$) can then be calculated, with each BU defined as a 1% increase in biodiversity per hectare per year.

Figure 04: *The Biodiversity Matrix*

Indicator/ Gain	SoilInv_Ref	SoilInv_Int
QBS-ar	QBS-ar_Ref	QBS-ar_Int
Gain_QBS (%)	Min(5;QBS_Int/QBS_Ref-1) (%)	
Gain_Metric (%)	μ Gain (QBS) (%)	

Indicator/ Gain	Flora_Ref	Flora_Int	AboveInv_Ref	AboveInv_Int	Birds_Ref	Birds_Int
Abundance (A)	μ % soil coverage per monitoring point	μ % soil coverage per monitoring point	μ Abundance per monitoring point	μ Abundance per monitoring point	μ Abundance per monitoring point	μ Abundance per monitoring point
Gain_A (%)	Min(5;μ%coverage_Int/μ%coverage_Ref-1) (%)		Min(5;μ Abunudance_Int/μ Abundance_Ref-1) (%)		Min(5;μ Abunudance_Int/μ Abundance_Ref-1) (%)	
Richness (R)	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point	μ nb. Species per monitoing point
Gain_R (%)	Min(5;μ nb. species_Int/μ nb. Species _Ref-1) (%)		Min(5;μ nb. species_Int/μ nb. Species _Ref-1) (%)		Min(5;μ nb. species_Int/μ nb. Species _Ref-1) (%)	
Interest (I)	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point	μ Interest per monitoring point
Gain_I (%)	Min(5;μ Interest_Int/μ Interest_Ref-1) (%)		Min(5;μ Interest_Int/μ Interest_Ref-1) (%)		Min(5;μ Interest_Int/μ Interest_Ref-1) (%)	
Gain_Metric (%)	μ Gain (A, R, I) (%)		μ Gain (A, R, I) (%)		μ Gain (A, R, I) (%)	

Biodiversity Gain	
(BU ha^{-1} year^{-1})	**μ Gain (Soil invertebrates, Flora, Aboveground invertebrates, Birds) * 100 (BU ha^{-1} year^{-1})**

7.6.1. Calculation process for the indicator Interest

To assign a value for Interest (I), a discrete number between 0 and 5 is assigned to each species according to five compatible criteria: (1) conservation status of the species or habitats assessed based on the IUCN lists, (2) invasive concern, (3) biodiversity relevance (ecosystem services performed/favored, associated species), (4) indicators of disturbance (soil, nutrients), (5) environmental significance of an species due to its higher sensibility the quality of the overall ecosystem (Birkhofer et al., 2018). Criteria (1) and (2) have priority over the rest whenever available. These criteria are in accordance with the Ellenberg values related to biodiversity in the case of Flora (Tyler et al., 2021). The categories considered are defined in Tables 06-08.

Table 06: *Thresholds of Interest for Flora.*

Flora — Thresholds of Interest (I):
0 --> Alien invasive species/Bare soil/Mulch.
1 --> Annual herbaceous species without nectar, linked to disturbed mismanaged soils (artificially N-enriched soils, annual/biannual soil disturbance, hydro morphism, heavy metals, etc.)
2 --> Annual herbaceous species with nectar and biannual (or longer) species without nectar
3 --> Biannual (or more) herbaceous species with nectar, herbaceous species representative of low agrarian disturbance (e.g. mesiculous), woody shrubs (<3m)
4 --> Trees (>3 m), plants included in habitats of the EU Habitats Directive, or red listed under the category of Near threatened (NT)
5 --> Plants included in priority habitats of the EU Habitats Directive or red listed under the categories of Vulnerable (VU), Endangered (EN) or Critical risk (CR)

Table 07: *Thresholds of Interest for Aboveground invertebrates.*

Aboveground invertebrates—Thresholds of Interest (I):
0 --> Alien invasive species.
1 --> Non-pollinating phytophagous species
2 --> Generalists and hematophagous
3 --> Specialists (e.g. detritivores, xylophagous, fungivores, saprophagous, necrophagous) and Araneae.
4 --> Coprophagous, natural enemies, pollinators and species red listed under the category of Near threatened (NT).
5 --> Species red listed under the categories of Vulnerable (VU), Endangered (EN) or Critical risk (CR).

Table 08: *Thresholds of Interest for Birds.*

Birds — Thresholds of Interest (I):
0 --> Alien invasive species
1 --> LC/L (least concern/listed)
2 --> NT (Nearly threatened)
3 --> VU (vulnerable)
4 --> EN (endangered)
5 --> CR (critical risk)

Once each species in the sample has a value assigned, the Interest of the complete sample is calculated weighing the Interest of each species according to its representativeness in the total abundance of the sample using the following **Formula 05**

Formula 05: *Interest*

$$\mathrm{I} = \sum_{i=1}^{n} \frac{I(i)x \log(\mathrm{ai} + 0.1)}{\sum_{i=1}^{n} \log(\mathrm{ai} + 0.1)}$$

I: interest value of the sample (1-5)

I(i): interest value assigned to species i (0 - 5)

ai: abundance (number of individuals, number of calls or relative amount of DNA) of species i in the sample

n: total number of species in the sample

Log (ai + 0.1): it's used as a weight

7.6.2. Calculation process for determining the Biodiversity gain of a Short-term project (1-5 years)

The Biodiversity Matrix is calculated independently for each monitoring repetition, being the actual biodiversity gain (in number of BU per hectare per year, BU ha^{-1} $year^{-1}$) the average of Repetition 1 and Repetition 2 of each year for the thesis under assessment. All metrics within the Biodiversity Matrix are used for calculating the Biodiversity gain.

One Biodiversity Unit per hectare per year (BU $ha^{-1} \cdot year^{-1}$) corresponds to a 1% increase or decrease in the biodiversity indicators of the Biodiversity Matrix.

Although biodiversity measurements are only performed in year 1 in short-term projects, BU can be claimed every year (up until year 5) according to the development state of the project. Each year, the following **Formula 06** will be used for claims and reporting:

Formula 06: *Biodiversity units in Short-term projects*

$$BU = BG \; x \; Ai \; x \; n$$

BU: number of Biodiversity Units per Intervention site and year.

BG: Biodiversity Gain (BU $ha^{-1} \cdot year^{-1}$)

Ai: area of intervention (ha)

n: number of years (it will be 1 if claims are made annually)

7.6.3. Calculation process for determining the Biodiversity gain of a Long-term project (20+ years)

In these types of projects, biodiversity gains are calculated independently for plot-level (**Formula 07**) and landscape-level metrics (**Formula 08**). A combination of the two is necessary to calculate actual BU (BU ha^{-1} $year^{-1}$). Although plot metrics are only measured every 5 years, BU corresponding to them will be claimed every year according to the development state of the project. Although landscape-level metrics (i.e. Birds) are measured every year, BU corresponding to it will be claimed every 5 years (starting in year 5) according to the percentage of change registered of the trendlines defined for each indicator between year 1 and years 5, 10, 15 and 20, respectively. The value of the indicator of the trendline in year 1 is considered the Reference (Ref) in the Biodiversity Matrix, and that of the years 5, 10, 15 and 20 is considered the intervention (Int).

For plot-level metrics, only the metrics Soil invertebrates, Flora, and Aboveground invertebrates of the Biodiversity Matrix are used for calculating the Biodiversity gain of any given year. For landscape-level metrics (Birds), only the metric Birds of the Biodiversity Matrix is used.

***Formula 07:** Annual biodiversity units in years 1-4, 6-9, 11-14 and 16-20 of Long-term projects*

$$BU = BG\ (i)\ x\ Ai\ x\ n\ x\ R1$$

BU: number of Biodiversity Units per Project site and year

*BG (i): Biodiversity Gain (BU ha $^{-1}$·year $^{-1}$) calculated in the last monitoring year (i), considering only metrics Soil invertebrates, Flora, and Aboveground invertebrates of the Biodiversity Matrix (Figure 04)**

*Ai: area of intervention**

n: number of years (it will be 1 if claims are made annually)

R1: ratio 4/5, considering plot-level metrics only 4 of the 5 metrics

**There can be different biodiversity gains for different areas of intervention, in which case the BU produced in each type of intervention will be summed up.*

***Formula 08:** Biodiversity units in years 5, 10, 15 and 20 of Long-term projects*

$$BU = (BG\ (i)\ x\ Ai\ x\ n\ x\ R1) + \left((BG_{Birds(i)} - BG_{Birds(i-1)} \right) x\ Ai\ x\ R2)$$

BU: number of Biodiversity Units per Project site and year

*BG (i): Biodiversity Gain (BU ha $^{-1}$·year $^{-1}$) calculated in the last monitoring year (i), considering only the metrics Soil invertebrates, Flora, and Aboveground invertebrates of the Biodiversity Matrix (Figure 04)**

*Ai: area of intervention**

n: number of years (it will be 1 if claims are made annually)

R1: ratio 4/5, considering plot-level metrics only 4 of the 5 metrics

BG_Birds (i): Biodiversity Gain (BU ha $^{-1}$) for metric Birds of the current monitoring year (i), considering only the metric Birds and based on the percentage of change of the trendline registered for each indicator between year 1 and current year (Figure 04)

BG_Birds (i-1): Biodiversity Gain (BU ha $^{-1}$) for metric Birds of the previous calculated year (Figure 04)

R2: ratio 1/5, considering landscape-level metrics only 1 of the 5 metrics

**There can be different plot-level biodiversity gains for different areas of intervention, in which case the BU produced in each type of intervention will be summed up.*

8. Verification system

Although a framework for calculating biodiversity gain as objectively and systematically as possible is proposed, the process remains highly sensitive to the quality of the field data collected. There is increasing pressure from regulations and scientific organizations to ensure that such information, especially when used in specific contexts (such as corporate claims), is verifiable and transparent.

To address this, two mechanisms are proposed to ensure the quality of the data used in the calculations:

1. **Verification of biodiversity monitoring events** to ensure that data is collected in a standardized and accurate manner.

2. **Verification of biodiversity interventions** to ensure that their quality is acceptable and consistent throughout the project.

The instruments for both verification systems include:

- A mobile application for real-time image uploads, which records GPS coordinates, date, time, weather conditions, staff details, the metric under evaluation, the monitoring point code, and photographs for each protocol within a dedicated data collection application.

- GIS software to analyze land use cartography and track changes at the landscape scale.

- Annual Farm Register Books documenting all agrarian plots included in both the Intervention and Reference sites.

8.1. Data traceability and quality

The type of materials required to ensure data traceability and quality depends on the biodiversity metric under assessment.

Landscape Metric

A GIS-assisted verification of land use cartography for the Intervention site is conducted and updated annually.

As outlined in the monitoring protocols in Section 7.3, at the beginning of the project and every five years, all land use/management types covering more than 5% of the total Intervention site area, along with all types of project interventions, are assessed on-site. The objective of this assessment is to verify that the land uses and/or land management shown in the mapping accurately correspond to the land uses present in the study area.

This assessment takes place at a minimum of two monitoring points per land use/management types, selected through stratified random sampling. One picture per monitoring point will be uploaded to the data collection application, along with GPS coordinates, date, time, staff details, and the monitoring point code.

Soil Invertebrates Metric

Two pictures per monitoring point will be uploaded to the mobile application, along with GPS coordinates, date, time, weather conditions (temperature, percentage of cloud cover, percentage of moisture content and wind speed according to Beaufort scale), staff details, type of metric, and monitoring point code.

The first picture will capture the materials used for sample collection alongside the raw soil sample. The second picture will provide a general view of all invertebrates identified during the process.

Flora Metric

Eight pictures per transect will be uploaded to the data collection application, along with GPS coordinates, date, time, staff details, type of metric, and monitoring point code.

The first picture will capture a general view of the transect from the starting point. The next five pictures will provide vertical foreground views of each 2×2m square along the transect. The seventh picture will capture a general view of the transect from the endpoint. The eighth picture will display all recorded species and coverage on a white sheet of paper, accompanied by a sample of each species. Only in cases where the vegetation cover consists of a homogeneous crop, photos three to six may be replaced with a single photo that clearly shows the crop being assessed.

Aboveground Invertebrates Metric

Two pictures per monitoring point will be uploaded to the data collection application, along with GPS coordinates, date, time, weather conditions (temperature, percentage of cloud cover, percentage of moisture content and wind speed according to Beaufort scale), staff details, type of metric, and monitoring point code.

The first picture will show the materials used for sample collection and a general view of the transect from the starting point. The second picture will capture the sample and a general view of the transect from the endpoint. The third picture will display all identified invertebrates during the identification process.

Birds Metric

Two pictures per monitoring point will be uploaded to the mobile application, along with GPS coordinates, date, time, weather conditions (temperature, percentage of cloud cover, percentage of moisture content and wind speed according to Beaufort scale), staff details, type of metric, and monitoring point code.

The first picture will show the recording device installed at the beginning, while the second picture will show the same device once the 24 hours of recording have passed.

8.2. Verification of biodiversity interventions

In both short-term and long-term projects, plot-levelmetrics are monitored only once every five years. While this frequency is sufficient for estimating potential biodiversity gain per year, it does not provide enough evidence to ensure that project interventions are being carried out with the same level of rigor and quality during the years without plot metric monitoring. Therefore, an additional layer of verification is necessary to assess the project interventions themselves.

In this verification system, all land use and land management types covering more than 5% of the total Project site area—as well as all Intervention sites andReference sites—are assessed on-site. This assessment includes at least one monitoring point per 30 hectares, with a minimum of two monitoring points per surface type. Monitoring points are selected through stratified random sampling.

For each monitoring point, four pictures will be uploaded to the data collection application, along with GPS coordinates, date, time, staff details, and the monitoring point code.

Additionally, annual Farm Register Books for all agrarian plots within the Project and Reference sites will be maintained to ensure that project interventions and land management practices remain consistent with the project's requirements.

9. References

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