# D3.1 Preliminary dataset on available carbon and biodiversity across the network

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## DISCLAIMER

# Table of content:

# Executive Summary

This deliverable is associated with the ongoing work of Task 3.1 and has the following objectives: it outlines the methodology used to collect the required data (i.e., aboveground dendrometric data and plant diversity data), explains how High Intensity Sites (HIS) were identified for further remote sensing analysis, and describes the processes used to compute carbon stocks and assess plant diversity. Finally, it provides a comprehensive description of the preliminary dataset on available carbon stocks and plant diversity across the network. This dataset, which is stored in a password-protected folder (link), is accessible to WILDCARD project partners, the Project Officer, and reviewers. It includes data from 13 and 6 chronosequences available within WILDACARD consortium for C stocks and plant diversity, respectively.

# Keywords

Abandoned agricultural land; above ground carbon; chronosequence; natural regeneration, plant diversity; rewilding; spontaneous succession.

# Acronyms

AGB: Aboveground Biomass

DBH: Diameter at breast height (1.30 m)

GA: Grant Agreement

INBO: Research Institute for Nature and Forest (Belgium)

IBER-BAS: Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences

UNITO: University of Torino (Italy)

UNIUD: University of Udine (Italy)

USB: University of South Bohemia (Czech Republic)

VUKOZ: Silva Tarouca Research Institute for Landscape and Ornamental Gardening (Czech Republic)

# Definitions

The definitions of the terms used in this Deliverable are sourced from the WILDCARD Project Glossary v. 1.0 (link):

**Basic wood density** (σ)**:** the weight of a dried wood sample divided by the fresh wood sample volume, expressed in g $cm^{-3}$ (Bitunjac et al. 2023).

**Carbon Stock:** the absolute quantity of carbon held within a pool at a specified time (MgC $ha^{-1}$) (IPCC, 2000).

**Chronosequence:** a series of sites that differ in age or time since abandonment, but otherwise occur on similar soil types and environmental conditions within the same climatic zone (Chazdon, 2013; De Palma et al., 2018). Chronosequences, by assuming space-for-time substitution, are used to infer temporal dynamics from measurements at sites of different ages, but similar land-use histories.

**Forest Stand:** a community of trees, including aboveground and below-ground biomass and soils, sufficiently uniform in species composition, age, horizontal and vertical structure, and environmental conditions to be managed as a unit (IPCC, 2003, 2006).

**Forest structure:** horizontal and vertical distribution of layers and attributes in a forest including the trees, shrubs, and ground cover (Gadow et al., 2012).

**Forest type:** a category of forest defined by its composition and/or site factors (locality), as categorised by each country in a system suitable to its situation (EEA, 2007).

**Land abandonment:** process whereby human control over land (e.g., agriculture) is given up and the land is left to nature.

**Plant biodiversity**: variety and variability of plant life within a specific area or ecosystem.

**Plot:** portion of a site within which stand and/or soil data are collected and that is considered representative of that specific site.

**Sampling unit:** sampling element or point within a plot (i.e. a tree, a soil sampling point, etc.).

**Secondary forests:** forest or woodland area which has regenerated through largely natural processes after human-caused disturbances or equivalently disruptive natural phenomena (Chokkalingam and De Jong, 2001).

**Site:** homogenous area in terms of environmental conditions, land use (forest or agriculture) and time since abandonment. For the purposes of the Wildcard project, it includes one or more plots.

**Time since abandonment:** time in years since forest management has stopped (WP2) or agricultural use has been abandoned (WP3).

# 1. Scope of the deliverable

WILDCARD WP3 ("Carbon and biodiversity development following natural rewilding of abandoned agricultural land") aims, for the first time, to assemble harmonized and open-access datasets on carbon (C) stocks, C sequestration rates, and biodiversity changes resulting from the natural reforestation of abandoned lands across Europe.

Specifically, Task 3.1 focuses on collecting, quality-checking, and validating aboveground dendrometric data and plant species composition information from existing or newly established chronosequences from at least 26 locations across Europe (Table 3, Part B of the GA). Each location may include one or more chronosequences (replicates) representing different stages of development following agricultural abandonment (i.e., time since abandonment, TSA). The collected aboveground data - such as single tree measurements and lying deadwood - are used by WILDCARD partners to calculate C stocks in various ecosystem pools at the site level (Task 3.1). When combined with soil C stock data (Task 3.2), this information helps track changes in total C over time (Task 3.3). Additionally, plant biodiversity changes are assessed by evaluating species richness, the Shannon diversity index, and quantifying functional diversity at each site within each chronosequence (Task 3.1).

This Deliverable is related to the ongoing work of Task 3.1 and has the following objectives:

1. **Methodology description**: to outline the methodology used to collect the required data (i.e., aboveground dendrometric and plant diversity data) by three complementary approaches:
    - i) A literature review of existing chronosequences across Europe that report aboveground C stocks and/or plant diversity.
    - ii) The use of unpublished chronosequences from WILDCARD partners.
    - iii) The establishment of new chronosequences to collect data according to standardized protocols ('WP3 Aboveground biomass protocol' and 'WP3 Vegetation sampling protocol').
2. **Identification of data gaps:** to identify data gaps in relation to the ecoregions and ecological gradients outlined in Milestone 1, and to develop a strategy to address them.
3. **Identification of High-Intensity Sites (HIS):** to describe the methodology used to identify High-Intensity Sites Aboveground (HIS-above), where biodiversity data are available and LiDAR and/or photogrammetry will be applied to capture high-resolution structural data for habitat characterization.
4. **Assessment of carbon stock and biodiversity:** to describe the methodology used for computing C stocks in the aboveground ecosystem pools and for assessing plant diversity at the site level.
5. **Preliminary dataset:** to present the preliminary dataset on available C stocks and plant diversity across the network. This dataset, which is stored in a password-protected folder (link), is accessible to WILDCARD project partners, the Project Officer, and reviewers. It includes data from 13 and 6 internal chronosequences for C stocks and plant diversity, respectively.

# 2. Gathering existing data on carbon and biodiversity

## 2.1 Aboveground carbon

### 2.1.1 Literature data

***Literature search strategy***

We searched the scientific literature in May 2024, focusing on studies that assess aboveground biomass and C in the context of natural afforestation processes on land previously used for agriculture (i.e., meadows, pastures, or cropland). Relevant publications were identified by searching *Scopus online* using a combination of search terms, Boolean operators (AND & OR), and truncation (*) to construct the following search query:

> **TITLE-ABS-KEY** ("aboveground biomass" OR "tree biomass" OR "aboveground carbon" OR "carbon stock") AND **TITLE-ABS-KEY** ("secondary succession" OR "forest expansion" OR "abandonment" OR "chronosequence" OR "natural afforestation") AND **PUBYEAR** > 1994 AND **PUBYEAR** < 2025.

The search was limited to the past 30 years (1994-2024). To further refine the search, we restricted the fields to Title, Abstract, and Keywords, and included only articles in English. Moreover, only peer-reviewed original research articles and review papers were considered to ensure the reliability of the sources. The search yielded 737 articles. However, most of them were conducted outside Europe and thus were excluded from the review. The final list of relevant papers amounted to 157.

***Literature screening***

Titles and abstracts were reviewed to exclude irrelevant studies. The following criteria were established to determine which article should be retained:

(i) Focus on forests established on land previously used for agriculture, including grasslands, pastures, meadows, or cropland.
(ii) Natural afforestation (i.e., no plantations established on former agricultural land).
(iii) Assessment of biomass and/or C stock of aboveground components (i.e., trees and/or shrubs) at various stages since abandonment.
(iv) Provide at least aggregated data on aboveground carbon stocks and/or biomass.

After screening the titles and abstracts, 61 articles were used for a full-text review. Following the full-text screening, using the same criteria again, 15 papers remained. Due to the paucity of papers, the list was expanded by examining their references for additional relevant publications. After this refinement, the final list included 19 studies.

***Results of the literature review***

The review (Table 1) revealed that only a small number of studies report aboveground biomass or C data along secondary succession on former agricultural land. From the initial set of articles retrieved via the Scopus search, most were excluded because the chronosequences were based on artificially reforested areas, rather than investigating the process of natural recolonization. Additionally, many studies that describe sites along secondary succession did not report data on aboveground C storage at the site/plot or individual tree level.

Regarding the temporal distribution of studies, no clear trend was evident, as no consistent increase or decrease in studies over time was observed. Over 75% of the studies were published in the last 10 years. However, data collection (when specified) mostly took place prior to this period, with most studies collecting data more than 10 years ago. Concerning time since abandonment (TSA), more than two thirds of the studies covered a period of >50 years, while three studies (conducted in Poland) focused on time spans of <20 years.

Geographically, the distribution of studies across Europe was uneven. The majority of studies were conducted in Italy (7) and Poland (3), with a higher density in the Continental and Alpine ecoregions. Only one study each was conducted in the Atlantic and Boreal ecoregions (Figure 1), highlighting a significant gap in coverage for Western and Northern European countries.

*Table 1. Key information about the studies reporting data on aboveground biomass and/or carbon in secondary successions on land previously used for agriculture (e.g., cropland, grassland, meadows, pastures). TSA = time since abandonment.*

| ID | Year | Country | TSA | Number of time steps | Sampling year | Reference |
|---|---|---|---|---|---|---|
| 1 | 2006 | Italy | 0 - 212 | 4 - 6 | 1999 - 2001 | Thuille and Schulze, 2006 |
| 2 | 2007 | Denmark, Sweden, Netherlands | 1 - 90 | NA | NA | Vesterdal et al., 2007 |
| 3 | 2008 | Italy | 0 - 75 | 6 | NA | Alberti et al., 2008 |
| 4 | 2008 | Switzerland | 0 - 223 | 5 | 2001 | Risch et al., 2008 |
| 5 | 2014 | Italy | 15 - 35 | 2 | 2012 | Novara et al., 2014 |
| 6 | 2014 | Netherlands | 10 - 94 | 10 | 2001 - 2005 | Bose et al., 2014 |
| 7 | 2014 | Sweden | 100 | NA | 2011 | Freschet et al., 2014 |
| 8 | 2014 | Italy | 0 - 63+ | 4 | NA | Guidi et al., 2014 |
| 9 | 2014 | Poland | 1 - 19 | 4 | NA | Zasada et al., 2014 |
| 10 | 2017 | Poland | 1 - 19 | NA | 2011 - 2012 | Jagodziński et al., 2017 |
| 11 | 2017 | Germany | 0 - 100 | 3 | 2017 | Mazinanian, 2018 |
| 12 | 2018 | Poland | 7 - 120 | 12 | 2016 - 2017 | Jagodziński et al., 2018 |
| 13 | 2019 | Italy | 45 - 100 | 5 | 2016 | Badalamenti et al., 2019 |
| 14 | 2019 | Italy | NA | NA | NA | Facioni et al., 2019 |
| 15 | 2019 | Italy | 0 - 70 | 3 | NA | Pellis et al., 2019 |
| 16 | 2021 | Slovakia | NA | 2 | 2018 - 2019 | Bucha et al., 2021 |
| 17 | 2021 | Poland | 2 - 17 | 4 | NA | Gawęda et al., 2021 |
| 18 | 2023 | Spain | 12 - 56 | 3 | 2008 | Velázquez et al., 2023 |
| 19 | 2024 | France | 0 - 74 | 5 | 2020 | Weissgerber et al., 2024 |

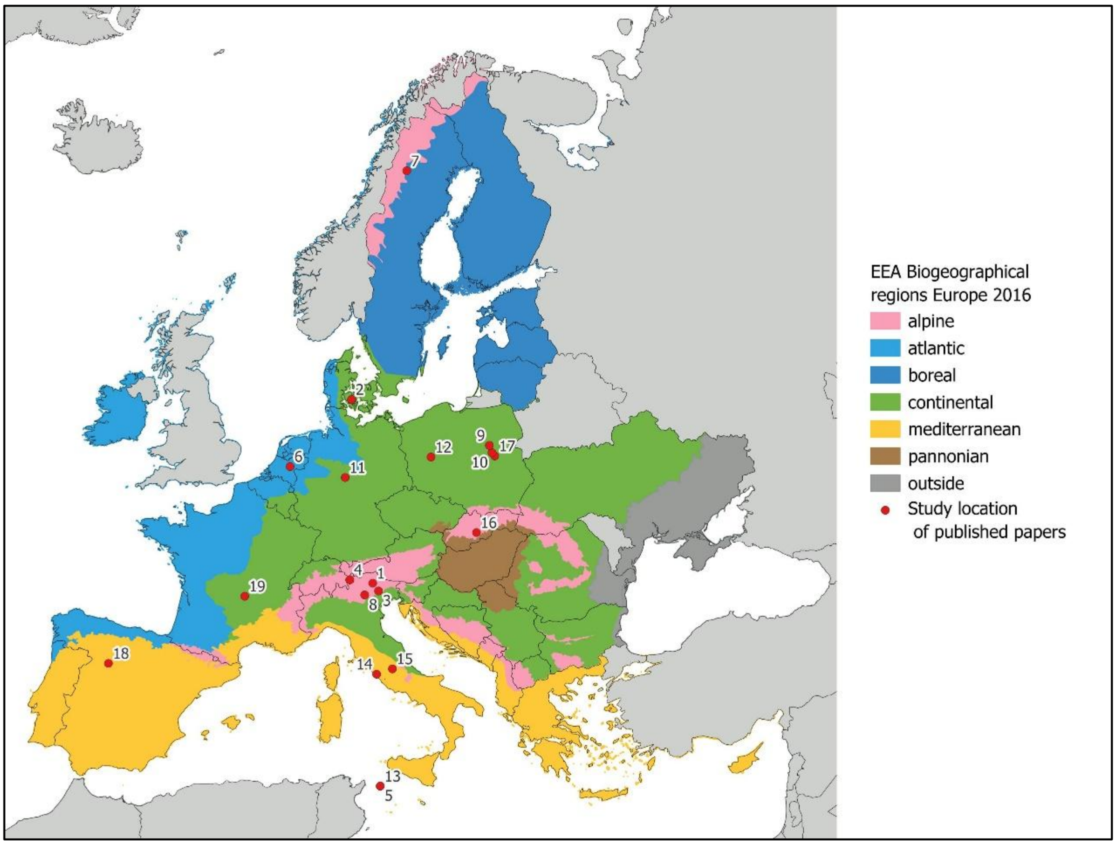


*Figure 1. Spatial distribution of the selected studies across European ecoregions.*

### 2.1.2 WILDCARD-internal chronosequences for C stocks assessment

The current number of internal chronosequences for aboveground dendrometric data (already existing or newly established) that are available within the WILDCARD consortium amounts to 19, compared to the 26 foreseen according to the GA (see Table 3 of Part B). For each chronosequence, meta-data have been collected and verified, including information on the ecoregion, the number of sites according to WILDCARD criteria, geographic coordinates, forest type, and land use prior to secondary succession (Table 2 and Figure 2). Dendrometric data for all listed chronosequences (257 sites in total) will be available by Month 18 (Milestone 5). Chronosequences for which individual tree data are unavailable will be sampled according to standard project protocols (link). Note that TSA for each site within a chronosequence was primarily determined using historical aerial photographs for the specific site along with local information. For some sites, wood cores were or will be collected to determine tree age (dendrochronological approach). This activity will be completed by Month 18 (Milestone 5), and therefore some information in the preliminary dataset presented here may be modified.

*Table 2. List of WILDCARD- internal chronosequences with indication of the number of sites where dendrometric and plant diversity data are available. Chrono_id = id of the chronosequence.*

| N | Ecoregion | Country | Region | Chrono_id | Age range | N. of sites with den-drometric data | N. of sites with plant diver-sity data | Funding |
|---|---|---|---|---|---|---|---|---|
| 1 | Continental | Czech Republic | Bohemian Karst | VUKOZ_1 | 20-88 | 15 | 46 | WILDCARD |
| 2 | Continental | Czech Republic | Šumava Mts.-wet | VUKOZ_2 | 20-79 | 11 | 11 | WILDCARD |
| 3 | Continental | Czech Republic | Šumava Mts. - dry | VUKOZ_3 | 20-79 | 5 | 5 | WILDCARD |
| 4 | Continental | Czech Republic | Beskydy Mts. | VUKOZ_4 | 20-80 | 4 | 4 | WILDCARD |
| 5 | Pannonian | Hungary | Kiskunsag NP | VUKOZ_5 | 25-58 | 16 | 16 | WILDCARD |
| 6 | Atlantic | Belgium | Everbeekse bossen | INBO_1 | 0-30 | 3 | 3 | WILDCARD |
| 7 | Atlantic | Belgium | Everbeekse bossen | INBO_2 | 0-20 | 3 | 3 | WILDCARD |
| 8 | Alpine | Bulgaria | Western Rhodopes | IBER-BAS_1 | 10-80 | 15 | 15 | WILDCARD |
| 9 | Alpine | Bulgaria | Rila moun-tain | IBER-BAS_2 | 10-100 | 15 | 15 | WILDCARD |
| 10 | Alpine | Bulgaria | Pirin moun-tain | IBER-BAS_3 | 10-80 | 15 | 15 | WILDCARD |
| 11 | Alpine | Italy | Friuli Vene-zia Giulia (Taipana) | UNIUD_1 | 0-75 | 20 | 20 | PNRR NBFC |
| 12 | Continental | Italy | Toscana | UNIUD_2 | 0-75 | 20 | 20 | PNRR NBFC |
| 13 | Continental | Italy | Abruzzo | UNIUD_3 | 0-75 | 20 | 20 | PNRR NBFC |
| 14 | Mediterranean | Italy | Basilicata | UNIUD_4 | 0-75 | 20 | 20 | PNRR NBFC |
| 15 | Alpine | Italy | Friuli Vene-zia Giulia (Faedis) | UNIUD_5 | 0-75 | 15 | - | PRIN |
| 16 | Alpine | Italy | Friuli Vene-zia Giulia (Ampezzo) | UNIUD_6 | 0-75 | 15 | 15 | PRIN |
| 17 | Alpine | Italy | Friuli Vene-zia Giulia (Ampezzo) | UNIUD_7 | 0-75 | 15 | - | PRIN |
| 18 | Alpine | Italy | Piemonte | UNITO_1 | 0-75 | 15 | 15 | PRIN |
| 19 | Alpine | Italy | Piemonte | UNITO_2 | 0-75 | 15 | 15 | PRIN |
| | | | | | **TOTAL** | **257** | **258** | |

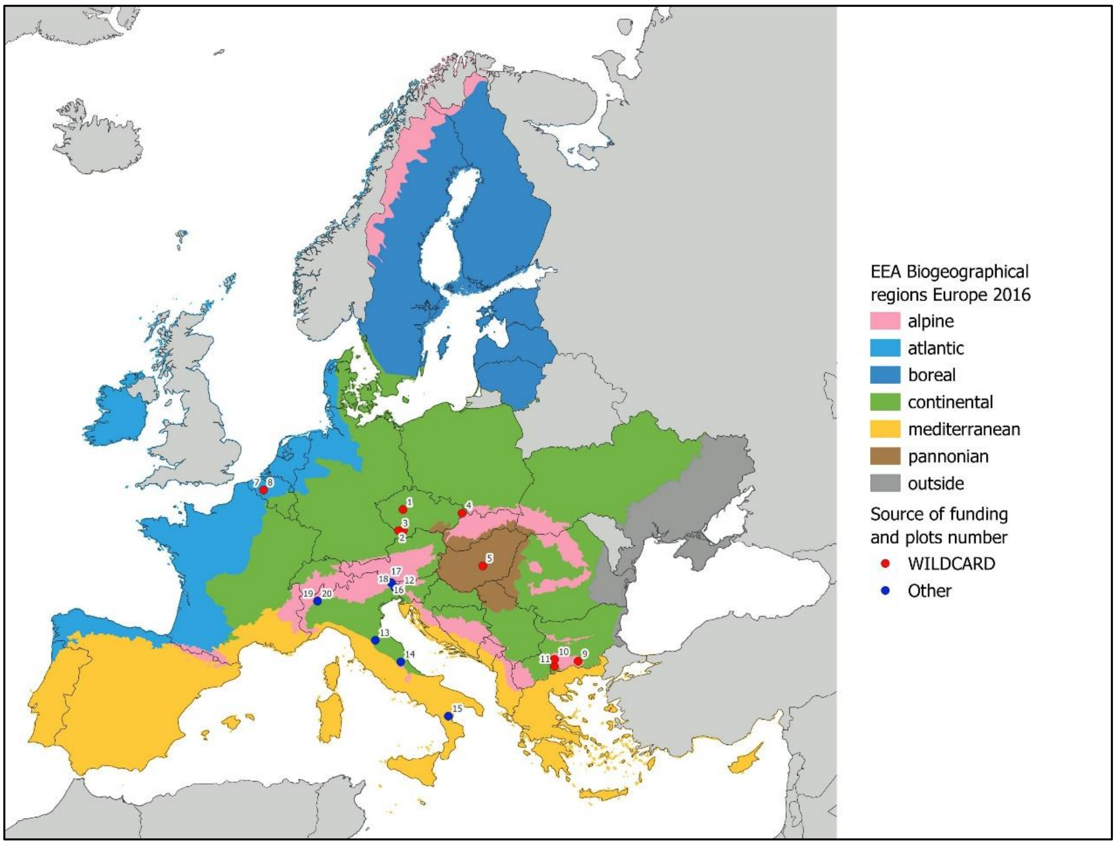


*Figure 2. Location of the WILDCARD internal chronosequences. Red dots represent chronosequences funded by WILDCARD, while blue dots indicate chronosequences established with other funding sources. The numbers correspond to the chronosequence number reported in Table 2.*

### 2.1.3 Filling the gap: involvement of external institutions

In order to fill the remaining gaps in those ecoregions that are poorly or not represented, external research groups have been contacted to explore their willingness to share dendrometric data for estimating aboveground C stocks through the service contracts foreseen in WP3. The selection of these groups was based on the review of the existing literature as reported in 2.1.1 (i.e. chronosequences sampled less than 5-10 years ago), prioritizing sites for which species diversity data were also available. The selection was further augmented with research groups directly recommended by WILDCARD partners, which had not been evident from the literature review. More specifically, potential external data providers were identified for the Mediterranean (Sicily and Central Spain), the Atlantic (France, Northern Spain and Portugal), the Continental (Poland, Denmark, Western Ukraine), Pannonian (Hungary) and Boreal (Baltic countries, Scandinavia) ecoregions. This should bring the total number of chronosequences well above the target of 26 set in the GA.

*Table 3. List of potential external chronosequences based on the review of the existing literature (i.e. chronosequences sampled less than 5-10 years ago), prioritizing sites for which species diversity data were also available. The selection was further supplemented with research groups directly recommended by WILDCARD partners, which were not highlighted in the literature review.*

| N | Ecoregion | Country | Region | Institution | N. chronosequences | N. of sites per chrono |
|---|---|---|---|---|---|---|
| 20 | Continental | Ukraine | Pop Ivan, Rachiv | Mendel Univesity in Brno | 1 | 6 |
| 21 | Alpine | Slovakia | Veporské Hills, Hriňová | Technical University in Zvolen | 1 | 4 |
| 22 | Alpine | Slovakia | Skorušické Hills, Zabiedovo | Technical University in Zvolen | 1 | 5 |
| 23 | Pannonian | Slovakia | Slovak Karst, Kečovo | Technical University in Zvolen | 1 | 4 |
| 24 | Mediterranean | Italy | Sicily | Unversity of Palermo | 1 | 5 |
| 25 | Atlantic | Denmark | Djursland | Rasmus Ejrnæs, Aarhus University | 1 | 4 |
| 26 | Atlantic | Denmark | Mid-Jytland | Rasmus Ejrnæs, Aarhus University | 1 | 4 |
| 27 | Continental | Slovenia | | Research Centre of the Slovenian Academy of Science and Art | 1 | 3 |
| | | | | | **TOTAL** | **35** |

## 2.2 Plant biodiversity

### 2.2.1 Literature data and gaps

We reviewed the literature on plant biodiversity changes in spontaneous vegetation succession on abandoned agricultural land, with a particular focus on long-term studies using a chronosequence approach. We selected scientific literature through a search conducted in February 2024, focusing on studies assessing plant biodiversity in the context of natural regeneration on land previously used for agriculture (i.e., cropland, but also meadows and pastures). Relevant publications were identified using *Scopus online* and a search query constructed with Boolean operators (AND, OR) and truncation (*). The search was limited to the past 30 years (1994-2024):

> **TITLE-ABS-KEY** ("Regener*" OR "succession" OR "passive restoration" OR "forest expansion") AND **TITLE-ABS-KEY** ("abandon" OR "chronosequence" OR "old field*" OR "fallow" OR "set aside") AND **TITLE-ABS-KEY** ("abundance" OR "assemblage" OR "biodiversity" OR "community" OR "composition" OR "cover" OR "diversity" OR "richness" OR "vegetation" OR "plant traits" OR "species").

This search yielded 3319 hits, reduced to 3175 after excluding unrelated research fields (e.g. psychology), and further to 1299 by filtering for European countries. Titles and abstracts were reviewed to exclude irrelevant studies based on the following criteria:

(i) Focus on spontaneous succession on former abandoned agricultural land.
(ii) No management of succession (e.g., no plantations, seeding, burning or fertilization of former agricultural land). Only mild grazing was allowed in some cases.
(iii) Assessment of plant biodiversity changes through measurement of species richness, cover, diversity indices, plant traits or other relevant variables at various stages since abandonment.

After screening titles and abstracts, 63 studies were identified as relevant, which were then reduced to 30 after discarding those that did not meet the specified criteria. This number increased to 68 through a 'snowball approach,' which involved screening references within the identified studies based on their reference list, and incorporating additional studies known to the authors. The selected studies span six European ecoregions (Figure 3 and Table 4): Alpine, Atlantic, Boreal, Continental, Mediterranean, and Pannonian. They cover 18 countries (with two studies involving multiple countries), reflecting a broad geographic scope across various climates and ecosystems in Europe (Figure 4 and Figure 5). The selected literature primarily examined the transition of abandoned agricultural land into forests, grasslands, and shrublands. In most cases, the studies tracked succession over several decades, ranging from the initial years after abandonment to >100 years. The studies aimed to capture ecological shifts in plant biodiversity, structure, and ecosystem function during long-term succession.

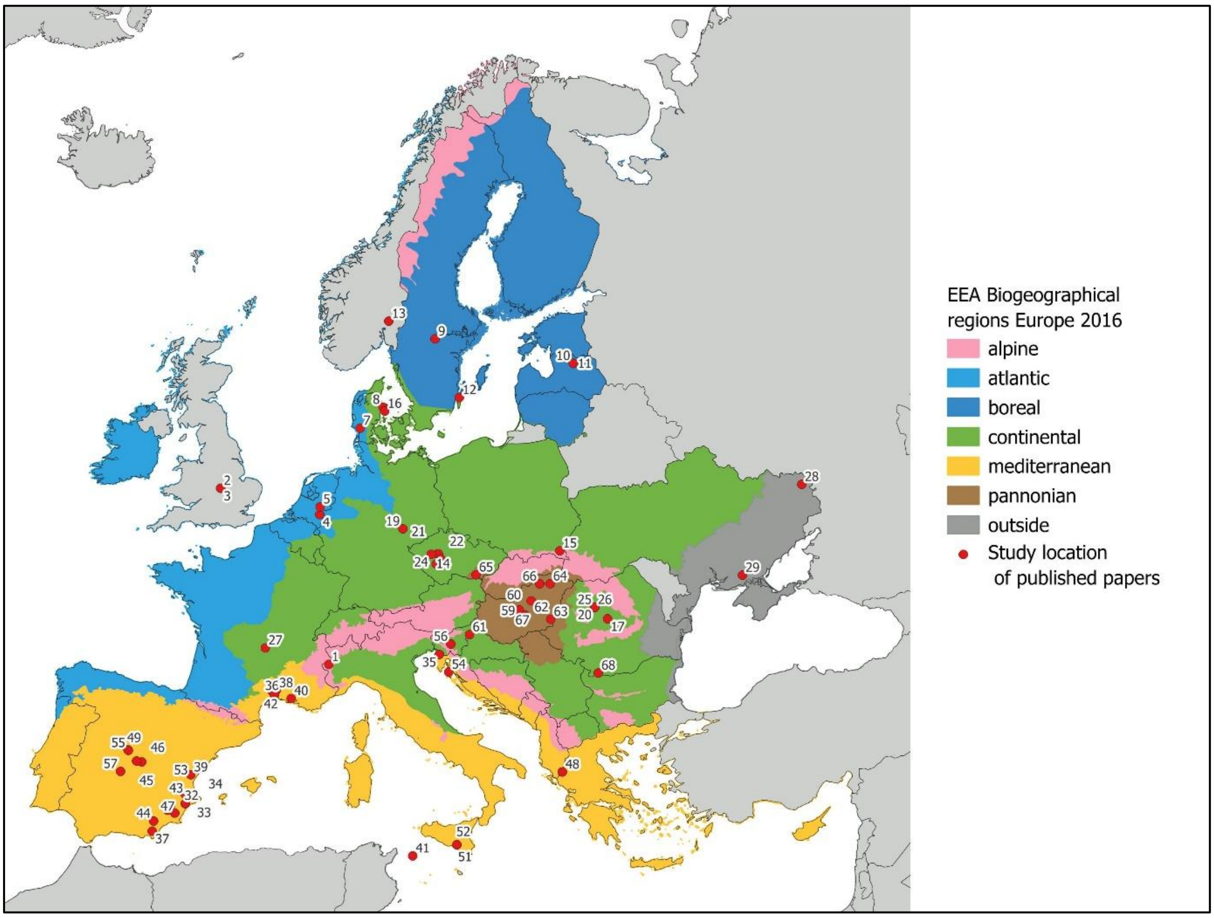


*Figure 3. Map showing the locations where plant biodiversity changes were studied in abandoned agricultural land. The numbers correspond to those reported in Table 4.*

*Table 4. Key information about the studies reporting data on biodiversity in secondary successions on land previously used for agriculture (e.g., mainly croplands).*

| | Ecoregion | Country | Time since abandonment (TSA) | Year | References |
|---|---|---|---|---|---|
| 1 | Alpine | Italy | 0, 1, 3, 10, 20, 40, >60 | 2000 | Barni & Siniscalco 2000 |
| 2 | Atlantic | UK | 1, 2-3, 4-5, 6-9, >10 | 2011 | Boatman et al. 2011 |
| 3 | Atlantic | UK | 9 | 2000 | Critchley & Fowbert 2000 |
| 4 | Atlantic | Netherlands | 1-34 | 2005 | Kardol et al. 2005 |
| 5 | Atlantic | Netherlands | 20, 38, 54 | 1998 | Smit & Olff 1998 |
| 6 | Atlantic | Netherlands | 0-25 | 2012 | van de Voorde et al. 2012 |
| 7 | Atlantic - Continental | Denmark | 1-28, almost yearly | 2023 | Pedersen et al. 2023 |
| 8 | Boreal | Sweden | 43-57, 86-102 | 2010 | Dahlström et al. 2010 |
| 9 | Boreal | Latvia | 3, 6, 12, 13, 19 | 2012 | Ruskule et al. 2012 |
| 10 | Boreal | Latvia | 10 fields abandoned (1988, 1991, 1992, 1993, 1995, 1996, 2000) | 2016 | Ruskule et al. 2016 |
| 11 | Boreal | Sweden - island | 5–14, 15–49, 50–279, ≥280 | 2017 | Schmid et al. 2017 |

| | | | | | |
|---|---|---|---|---|---|
| 12 | Boreal | Norway | ca. 20, 30, 40, 50 | 1998 | Staaland et al. 1998 |
| 13 | Atlantic - Continental | Denmark | compiles several studies, TSA not specified but >80 years. | 2008 | Ejrnaes et al. 2008 |
| 14 | Continental | Czech | 2, 8, 55 | 2021 | Backhaus et al. 2021 |
| 15 | Continental | Poland | ca. 30 | 2016 | Barabasz-Krasny, B. 2016 |
| 16 | Continental | Denmark | 15, 8, 25, 25, 30, 44, 45 (+14 and 1, 3, 5, 8, 13, 15, 18, 30, 32, 40, 50 and 5, 35 | 2003 | Ejrnaes et al. 2003 |
| 17 | Continental | Romania | 1- 20 | 2015 | Fenesi et al. 2015 |
| 18 | Continental | Czech Republic | 34-89 | 2012 | Jirova et al. 2012 |
| 19 | Continental | Germany | 20-28 | 2016 | Knapp et al. 2016 |
| 20 | Continental | Romania | 1, 8, 16, 30 | 2016 | Nemet et al. 2016 |
| 21 | Continental | Czech Republic | 13-67 | 1994 | Prach & Pyšek 1994 |
| 22 | Continental | Czech Republic | 12-60 | 1999 | Prach & Pyšek 1999 |
| 23 | Continental | Czech Republic | 1-91 | 2014 | Prach et al. 2014 |
| 24 | Continental | Czech Republic | 1-99 | 2024 | Řehounková et al. 2024 |
| 25 | Continental | Romania | 40 | 2005 | Ruprecht 2005 |
| 26 | Continental | Romania | 40 | 2006 | Ruprecht 2006 |
| 27 | Continental | France | Several stages up to 74 yrs | 2024 | Weissgerber et al. 2024 |
| 28 | Continental-Pannonian | Hungary & Czech Republic | 1-15 | 2009 | Szabó & Prach 2009 |
| 29 | Continental- Stepic | Ukraine | 5-15 in Reserve and 15-27 in buffer zone (1-2,3-9, 10-15, 15-20, 20-25 | 2020 | Borovyk 2020 |
| 30 | Continental- Stepic | Ukraine | 6, 15, 31, 50 and ca. 97 years | 2023 | Dembicz et al. 2023 |
| 31 | Mediterranean | Spain | 2-70 years (50% between 17-36 | 2024 | Amodeo et al. 2024 |
| 32 | Mediterranean | Spain | 0–2, 3–8, 9–15, 15–32, >32 | 2004 | Bonet 2004 |
| 33 | Mediterranean | Spain | 0-60 | 2004 | Bonet, A., & Pausas, J. G. 2004 |
| 34 | Mediterranean | Spain | 1, 10, 25, 50 | 2001 | Bonet et al. 2001 |
| 35 | Mediterranean | Slovenia | 1, 3, 6, 9, 13, 15 and 100 | 2021 | Čarni et al. 2021 |
| 36 | Mediterranean | France | 1-ca. 150 | 1996 | Debussche et al. 1996 |
| 37 | Mediterranean | Spain | 100 | 2018 | Estruch et al. 2018 |
| 38 | Mediterranean | France | 2, 7, 8, 11, 12, 26, 29, 35, 40, 42 | 2004 | Garnier et al. 2004. |
| 39 | Mediterranean | Spain | >20 | 2016 | Hernández Martínez 2016 |
| 40 | Mediterranean | France | 2, 35, 150 | 2012 | Jaunatre 2012 |
| 41 | Mediterranean | Italy -Sicily | 1-2, 3-6, 7-15, 16-30, >30 | 2008 | La Mantia et al. 2008 |
| 42 | Mediterranean | France | 1,7, 15 | 1994 | Lavorel et al. 1994. |
| 43 | Mediterranean | Spain | 6, 9, 25, 40, >50 | 2008 | Lesschen et al. 2008 |

| | | | | | |
|---|---|---|---|---|---|
| 44 | Mediterranean | Spain | 3, 12, 56, 63, 84 | 2014 | Lozano et al. 2014 |
| 45 | Mediterranean | Spain | 1-60 (categories not stated) | 2010 | Martinez-Duro et al. 2010 |
| 46 | Mediterranean | Spain | 1-60 (categories not stated) | 2012 | Martinez-Duro et al. 2012 |
| 47 | Mediterranean | Spain | 1, 4, 10, 20,30 | 1996 | Martinez-Fernandez, et al. 1996 |
| 48 | Mediterranean | Greece | 1945, 1970, 1996 and 2015, >70 years (so 5, 24, 50 and 75 | 2023 | Mastrogianni et al. 2023 |
| 49 | Mediterranean | Spain | 1, 3, 7, 11, 25, 40 | 2023 | Molina et al. 2023 |
| 50 | Mediterranean | Spain | 0, 2, 5, 10, 20, 30, 60, 100 | 2017 | Romero-Díaz et al. 2017 |
| 51 | Mediterranean | Italy -Sicily | >50 | 2007 | Ruhl 2007 |
| 52 | Mediterranean | Italy -Sicily | >50 | 2007 | Rühl, J. 2007 |
| 53 | Mediterranean | Spain | 50 and 100 | 2010 | Santana et al. 2010 |
| 54 | Mediterranean | Croatia- Is-land | 1910, 1959, 1986, 2006 | 2018 | Sedlar et al. 2018 |
| 55 | Mediterranean | Spain | 40->60 | 2020 | Valverde-Asenjo et al. 2020 |
| 56 | Mediterranean | Slovenia | 0-3, 5-10, 25, 50 | 2017 | Van Hall et al. 2017 |
| 57 | Mediterranean | Spain | 1-9, 10-18, 19-33, >33 | 2020 | Vaquero Perea 2020 |
| 58 | Pannonian | Hungary | <10, 10-20, 20-40 | 2014 | Albert et al. 2014 |
| 59 | Pannonian | Hungary | 1 to 69, (most between 15 and 60) | 2014 | Bartha et al. 2014 |
| 60 | Pannonian | Hungary | wet 0-19, 20-39, 40-137, and 0-19, 20-39, 40-62, and > 150 non ploughed stand as reference | 2015 | Boecker et al. 2015 |
| 61 | Pannonian | Slovenia | 20 years (1985, 2005, 2015) | 2010 | Cojzer & Brus 2010 |
| 62 | Pannonian | Hungary | 1–7, 8–20, and 21–57 | 2011 | Csecserits et al. 2011 |
| 63 | Pannonian | Hungary | 1, 4, 11, 25, 38 | 1998 | Molnar & Botta-Dukat 1998) |
| 64 | Pannonian | Hungary | 14, 39, 63, 101, 142, 193 | 2014 | Novák et al. 2014 |
| 65 | Pannonian | Czech Re-public | Pavlov Hills (4–12, 13–39 and 40–69); Pouzd rany Steppe (5–18, 19–47 and 48–70); Duna-jovice Hills (6–19, 20–56 and 57–71) | 2015 | Sojnekova & Chytry 2015 |
| 66 | Pannonian | Hungary | 1-5, 6-10, 11-20, 21-30 | 2022 | Szirmai et al. 2022 |
| 67 | Pannonian | Hungary | >10, 10-20, 20-40, >40 | 2018 | Török et al. 2018 |
| 68 | Pannonian | Romania | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 20 | 2019 | Veselý et al. 2019 |

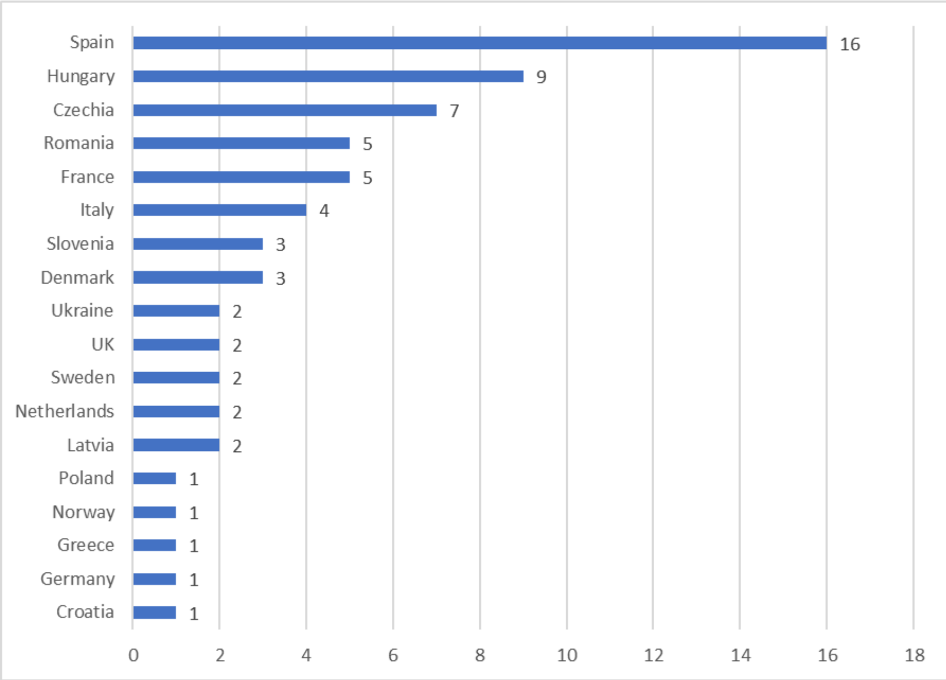


*Figure 4. Distribution of studies on biodiversity changes in abandoned agricultural fields, categorized by country.*

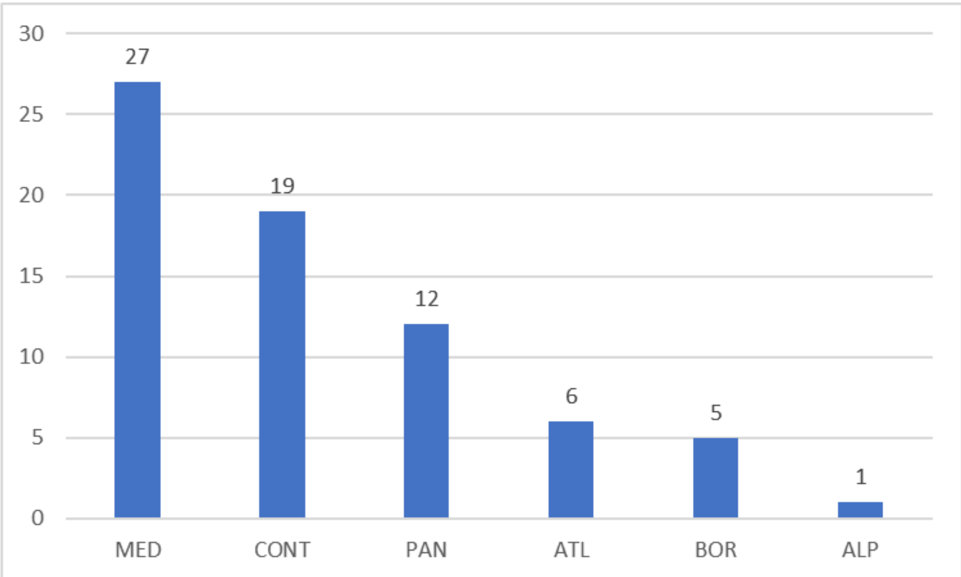


*Figure 5. Distribution of studies on plant biodiversity changes in abandoned agricultural fields, organized by ecoregion. Three studies share two regions, so they are represented in two bars.*

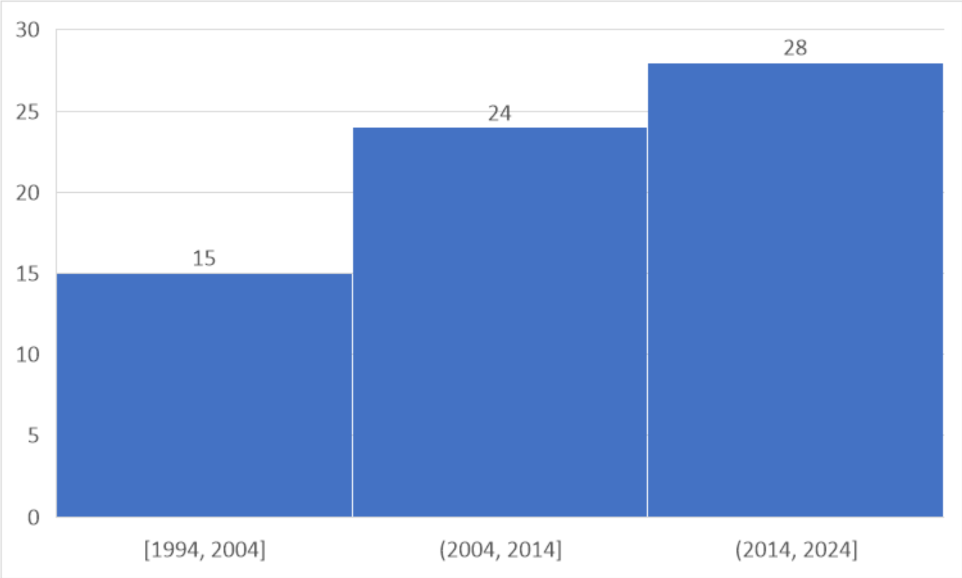


*Figure 6. Distribution of studies by publication date on changes in plant biodiversity in abandoned agricultural fields.*

The current set includes publications from 1994 to 2024 on spontaneous succession, natural regeneration, and rewilding, with a slight increase in the number of studies from the 1990s onward (Figure 6). Notable research hubs were identified in the Czech Republic, Spain, Poland, and Hungary, which have made significant contributions to the literature in the Continental, Mediterranean, and Pannonian regions. In contrast, the Boreal, Atlantic, and Alpine regions are underrepresented, highlighting the need for further research to address knowledge gaps regarding the successional dynamics of abandoned land in Europe. For some studies by the same authors, it was not possible to determine whether they had used the same chronosequence or had considered new sample sites.

Most of these studies focused on land previously used for cereal crops such as wheat, barley, and maize, as well as vineyards, olive and almond groves, chestnut orchards, and other crops like alfalfa, tomatoes, and potatoes. Some sites were plowed or left with crop remnants after harvest. Several studies emphasized that the type of former crops and the final management practices before abandonment are critical for setting the initial conditions for succession, influencing species composition and vegetation trajectories. Additionally, the size of the agricultural area and surrounding land uses were identified in some studies as factors affecting successional trends. The literature typically measured species richness, plant cover, diversity indices, and plant traits, providing valuable insights into biodiversity trends and environmental changes during succession. Environmental variables such as latitude, longitude, altitude, mean annual temperature, and precipitation were either reported or inferred in the studies.

All data gathered from previously published papers will be assessed, and an overview of trends in plant biodiversity changes on abandoned agricultural land is currently being developed. Principal investigators of chronosequences in regions not included in the project have been contacted to share their original datasets for a more detailed and consistent analysis at the European scale (see section 2.2.3).

### 2.2.2 WILDCARD-internal chronosequences for plant diversity assessment

An assessment of internal chronosequences (Table 2) from which plant diversity data are available or will be collected during the lifespan of the WILDCARD project was conducted. A total of 17 chronosequences were identified (Table 5) for 258 sites, most of them including HIS-above (see Chapter 3).

*Table 5. List of WILDCARD-internal chronosequences with plant diversity data. Chrono_id = id of the chronosequence.*

| N | Ecoregion | Country | Region | Chrono_id | Age range | Data avaliability | N. of sites |
|---|---|---|---|---|---|---|---|
| 1 | Continental | Czech Republic | Bohemian Karst | VUKOZ_1 | 20-88 | Available | 46 |
| 2 | Continental | Czech Republic | Šumava Mts.-wet | VUKOZ_2 | 20-79 | Available | 11 |
| 3 | Continental | Czech Republic | Šumava Mts. - dry | VUKOZ_3 | 20-79 | Available | 5 |
| 4 | Continental | Czech Republic | Beskydy Mts. | VUKOZ_4 | 20-80 | Spring 2025 | 4 |
| 5 | Pannonian | Hungary | Kiskunsag NP | VUKOZ_5 | 25-58 | Spring 2025 | 16 |
| 6 | Atlantic | Belgium | Everbeekse bossen | INBO_1 | 0-30 | Spring 2025 | 3 |
| 7 | Atlantic | Belgium | Everbeekse bossen | INBO_2 | 0-20 | Spring 2025 | 3 |
| 8 | Alpine | Bulgaria | Western Rhodopes | IBER-BAS_1 | 10-80 | Spring 2025 | 15 |
| 9 | Alpine | Bulgaria | Rila mountain | IBER-BAS_2 | 10-100 | Spring 2025 | 15 |
| 10 | Alpine | Bulgaria | Pirin mountain | IBER-BAS_3 | 10-80 | Spring 2025 | 15 |
| 11 | Alpine | Italy | Friuli Venezia Giulia (Taipana) | UNIUD_1 | 0-75 | Available | 20 |
| 12 | Continental | Italy | Toscana | UNIUD_2 | 0-75 | Available | 20 |
| 13 | Continental | Italy | Abruzzo | UNIUD_3 | 0-75 | Available | 20 |
| 14 | Mediterranean | Italy | Basilicata | UNIUD_4 | 0-75 | Spring 2025 | 20 |
| 16 | Alpine | Italy | Friuli Venezia Giulia (Ampezzo) | UNIUD_6 | 0-75 | Spring 2025 | 15 |
| 18 | Alpine | Italy | Piemonte | UNITO_1 | 0-75 | Spring 2025 | 15 |
| 19 | Alpine | Italy | Piemonte | UNITO_2 | 0-75 | Spring 2025 | 15 |
| | | | | | | **TOTAL** | **258** |

### 2.2.3 Filling the gap: involvement of external institutions

To address the gaps in underrepresented or poorly represented ecoregions, external research groups have been contacted to assess their willingness to share plant diversity data through service contracts outlined in WP3. The selection of these groups was based on the review of existing literature (section 2.2.1), prioritizing sites where plant diversity data were already available. The selection was further augmented with research groups directly recommended by WILDCARD partners, which were not highlighted in the literature review. For external outreach, potential collaborators and data providers were identified across various ecoregions, including the Mediterranean (France, Italy-Sicily, and Spain), Alpine (Slovakia), Atlantic (Denmark, Portugal, France, and the Netherlands), Pannonian (Hungary and Slovenia), Continental (Hungary, Germany, Poland, Slovenia, Romania, and Ukraine), and Boreal (Finland, Norway, Sweden, and the Baltic countries). The willingness of these collaborators to contribute varies. Some are unable to contribute immediately, while others have already established chronosequences and primary datasets. Additionally, some collaborators are willing to establish new sites, collect primary data, or engage in both activities.

# 3. Selection of High-Intensity Sites Aboveground (HIS-above)

In WILDCARD, High-Intensity Sites (HIS-above and HIS-below) are designated locations where additional sampling activities are carried out. Specifically, at HIS-above sites the focus is on characterizing forest structure using terrestrial laser scanning (TLS) and/or unoccupied aerial laser scanning (ULS), and examining its links to biodiversity (Task 3.1). At HIS-below sites, the sampling includes chemical and physical soil analysis, as well as eDNA sampling from the mineral soil and forest floor (Task 3.2; Milestone 3). To optimize resource use and ensure as complete datasets as possible for the same sites, WP3 has aimed to align the two types of HIS wherever feasible.

Starting from the list of WILDCARD-internal chronosequences (Table 2), HIS sites were selected through an expert-driven approach. Each research team leveraged their detailed knowledge of local land use history, ecosystem dynamics, structure and geographic conditions to identify HIS within each single chronosequence. Sites where plant diversity data were available were included in HIS-above (see also Table 5). As most of those sites occur in the country and ecoregion where the WILDCARD partner is located (Figure 2), additional HIS will be selected among those within the external chronosequences reported in Table 3.

Following this approach, of all sites within the WILDCARD-internal chronosequences, 137 were designated as HIS-above and 95 as HIS-below (Table 6, both values well above the goals set in Part B of the GA). Regarding external chronosequences, an additional 35 HIS-above and HIS-below were identified. Sampling of HIS-above will be completed by the end of Month 30 (Milestone 13), while HIS-below will be sampled by the end of Month 24 (Milestone 7).

*Table 6. Number of HIS-above and HIS-below sites, for each WILDCARD internal chronosequence.*

| N | Chrono id | N. of HIS-above | N. of HIS-below |
|---|---|---|---|
| 1 | VUKOZ_1 | 15 | 15 |
| 2 | VUKOZ_2 | 11 | 8 |
| 3 | VUKOZ_3 | 5 | 5 |
| 4 | VUKOZ_4 | 4 | 4 |
| 5 | VUKOZ_5 | 16 | 7 |
| 6 | INBO_1 | 3 | 3 |
| 7 | INBO_2 | 3 | 3 |
| 8 | IBER-BAS_1 | 15 | 5 |
| 9 | IBER-BAS_2 | 15 | 5 |
| 10 | IBER-BAS_3 | 15 | 5 |

| | | | |
|---|---|---|---|
| 11 | UNIUD_1 | 5 | 5 |
| 12 | UNIUD_2 | 5 | 5 |
| 13 | UNIUD_3 | 5 | 5 |
| 14 | UNIUD_4 | 5 | 5 |
| 15 | UNIUD_5 | 0 | 0 |
| 16 | UNIUD_6 | 5 | 5 |
| 17 | UNIUD_7 | 0 | 0 |
| 18 | UNITO_1 | 5 | 5 |
| 19 | UNITO_2 | 5 | 5 |
| **TOTAL** | | **137** | **95** |

*Table 7. Total number of sites, along with the number of HIS-above and HIS-below sites, for each WILDCARD external chronosequence.*

| N | Ecoregion | Country | N. of HIS-above | N. of HIS-below |
|---|---|---|---|---|
| 20 | Continental | Ukraine | 6 | 3 |
| 21 | Alpine | Slovakia | 4 | 4 |
| 22 | Alpine | Slovakia | 5 | 5 |
| 23 | Pannonian | Slovakia | 4 | 4 |
| 24 | Mediterranean | Italy | 5 | 5 |
| 25 | Atlantic | Denmark | 4 | 4 |
| 26 | Atlantic | Denmark | 4 | 4 |
| 27 | Continental | Slovenia | 3 | 3 |
| | | **TOTAL** | **35** | **35** |

# 4. Assessment of aboveground carbon stock

## 4.1 Material and methods

For the purposes of WILDCARD, the following C pools according to the IPCC guidelines are considered (IPCC, 2006):

1. **Aboveground biomass** comprises all living standing trees (at least 2.5 cm in diameter), including their stems, branches, bark, seeds, and foliage. Aboveground biomass also includes living understory plants.
2. **Belowground biomass** includes all living root biomass of trees and understory plants, for roots with a diameter >2 mm.
3. **Deadwood** includes all dead woody biomass either standing (snags, stumps) or coarse and fine woody debris lying on the ground.
4. **Forest floor litter** includes leaves, needles, twigs, and all other dead biomass with a diameter less than 2.5 cm, lying on the ground. This includes small-sized dead biomass that is decomposed but has not yet become part of the soil (OL horizon).
5. **Organic soil carbon** includes all organic C in soil. In the absence of specific information upon which to select an alternative depth interval, it is good practice to compare stock change factors in the topsoil (0-30 cm, the depth used for IPCC Tier 1 calculations). The following horizons are distinguished:

- **Soil organic horizon:** horizon dominated by organic material. It is constituted by the OL (fresh litter), OF (fragmented and/or altered organic matter) and OH (dark, well-decomposed, amorphous organic matter) (ICP Forests, 2020). An O layer may be at the surface of a mineral soil or at any depth beneath the surface if it is buried (ICP Forests, 2020);
- **Soil mineral horizon:** a mineral horizon formed at the surface or below an O buried horizon (ICP Forests, 2020).

This Deliverable is focusing on the first three items from the list above. Forest floor litter and soil carbon (items 4 & 5) will be considered in Deliverable 3.2 (Month 36).

### 4.1.1 Standing living trees

Biomass of living trees ($B_{trees}$; kg) was estimated using the R package *allodb* starting from the individual dendrometric data collected in the field at the plot level (for more details on variables collected in the field by WILDCARD, cf. 'WP3 Aboveground biomass protocol'). This package contains a wide list of published allometric equations and functions proposed to compute aboveground biomass (Gonzalez-Akre et al., 2022). The data component of the package is based on 701 woody species identified at 24 large Global Earth Observatory (ForestGEO) plots representing a wide diversity of extratropical forests. The *allodb* package includes 570 allometric equations to estimate individual-tree biomass. The authors checked and combined the equations using a weighting function designed to ensure optimal equation selection over the full tree size range with smooth transitions across equations. If needed, the dataset of allometric equations can be customized with built-in functions that subset the original dataset and add new equations. Although equations were curated based on a limited set of forest communities and number of species, this resource is appropriate for portions of the global extra-tropics. Missing equations for the species surveyed in the field were retrieved from literature or local information, where possible.

To calculate the biomass of living trees using *allodb* (Figure 7), a table (i.e. dataframe *sensu* R) with DBH (cm), parsed species Latin names (genus and species names separately), and site(s) coordinates (reference system: WGS84) was needed. The species were then identified among the 701 woody species already embedded in the database of the package. In case no valid allometric equation was available for the species, the existence of an equation for the genus was checked. If none was available for the genus either, the most general equation at the family level was used. For example, there was no equation for *Salix caprea*, and hence an equation for the genus *Salix* was used. No equation for *Sorbus aucuparia* was present in the package and neither for the genus *Sorbus*; thus, a generic equation for the family *Rosaceae* was chosen to calculate the biomass.

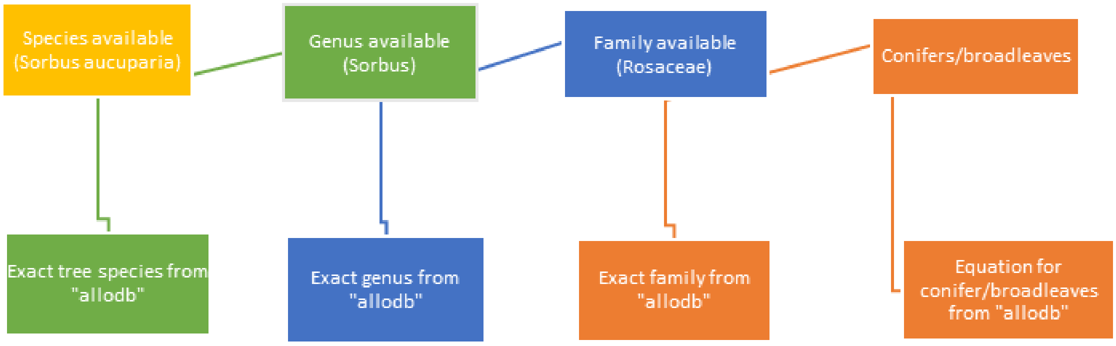


*Figure 7. Workflow to associate the allometric equation to compute aboveground biomass of an individual tree knowing its species and DBH.*

Total aboveground biomass at the plot level was then calculated as the sum of all surveyed trees and expressed as tons per hectare (Mg $ha^{-1}$), considering the size of the plot on which the trees were surveyed.

### 4.1.2 Root biomass of standing living trees

The root biomass per hectare of standing trees at the plot or site level ($B_{roots}$; Mg $ha^{-1}$) was estimated using the following equation:

$$B_{roots} = RS \times B_{trees}$$

where RS is the root-to-shoot ratio reported by IPCC (2006) and $B_{trees}$ is the total tree above-ground biomass (t $ha^{-1}$) (Table 8).

*Table 8. Values of the root-to-shoot ratio (RS) according to IPCC (2006).*

| Aboveground biomass (AGB) | Root-to-shoot ratio (RS) |
|---|---|
| AGB < 75 t $ha^{-1}$ | 0.43 |
| 75 t $ha^{-1}$ ≤ AGB < 150 t $ha^{-1}$ | 0.26 |
| AGB ≥ 150 t $ha^{-1}$ | 0.24 |

### 4.1.3 Deadwood biomass

**Standing dead trees**

For unbroken standing dead trees, biomass was estimated using the same procedure as in the case of living trees (i.e. *allodb* package). A biomass reduction factor was then applied to correct the potential overestimation resulting from lower wood density in different decay stages. The reduction factors (RF) were calculated from the data by Bitunjac et al. (2023) for broadleaves and conifers according to the equation:

$$RF = \frac{\sigma_{decay}}{\sigma_{base}}$$

where $\sigma_{decay}$ is the basic density of a specific decay class and $\sigma_{base}$ is the basic density of living trees (0.596 and 0.468 g cm$^{-3}$ for broadleaves and conifers, respectively; Bitunjac et al 2023). The specific reduction factors for broadleaves and conifers by decay stage are reported in Table 9.

*Table 9. Reduction factors for broadleaves and conifers by decay stage*

| Taxonomic group | Decay class | Reduction factor |
|---|---|---|
| Broadleaves | 1 | 0.91 |
| | 2 | 0.76 |
| | 3 | 0.59 |
| | 4 | 0.42 |
| | 5 | 0.26 |
| Conifers | 1 | 0.80 |
| | 2 | 0.71 |
| | 3 | 0.58 |
| | 4 | 0.42 |
| | 5 | 0.34 |

**Broken standing dead trees (snags)**

The biomass of broken standing dead trees ($B_{snag}$; kg), was quantified according to the equation:

$$B_{snag} = \frac{\pi}{4} \times \left(\frac{dbh}{100}\right)^2 \times h_{snag} \times FF \times \sigma_b$$

where $h_{snag}$ is the height of the snag (m). FF is an average form factor (i.e., FF = 0.5) used to consider stem taper; $\sigma_b$ is the basic density (kg m$^{-3}$) of the deadwood for the specific decay class (see below).

Standing dead biomass (unbroken dead trees and broken snags) at the plot level was finally expressed per hectare, taking into account the size of the plot (Mg ha$^{-1}$).

Root biomass for the unbroken dead trees and broken snags was computed following the procedure adopted for standing living trees described above.

**Coarse woody debris**

The biomass of coarse woody debris ($B_{CWD}$; kg), i.e. lying logs with a diameter >10 cm was quantified using the formula below, based on the equation of a truncated cone:

$$B_{CWD} = \frac{\pi}{4}\left(D_b^2 + D_t^2 + D_b D_t\right)\frac{l_{log}}{3} \times \sigma_b$$

where $D_b$ and $D_t$ are the diameters at the base and at the top of the lying deadwood (log)(all are expressed in m), respectively. $l_{log}$ is the length of lying deadwood piece (m); $\sigma_b$ is the basic density of lying deadwood decay class (see below).

The total biomass of the coarse woody debris found in each plot was finally scaled to hectare taking into account the size of the plot (Mg $ha^{-1}$).

**Fine woody debris**

The biomass of fine woody debris ($B_{CWD}$; Mg $ha^{-1}$) was calculated according to the following equation (Harmon et al., 1996):

$$B_{FWD} = \frac{9.859}{8} \times \sum_{i=1}^{n} \frac{d_i^2}{L} \times \sigma_i$$

where $d_i$ is the diameter of the piece of fine deadwood *i* (cm) intercepted by a linear transect in the field, $\sigma_i$ is the basic density of the decay class (see below) and L is the length of the transect (m).

**Stumps**

The biomass of the stumps (kg) was determined according to the equation:

$$B_{stump} = \frac{\pi}{4} \times \left(\frac{d_{stump}}{100}\right)^2 \times h_{stump} \times \sigma_b$$

where $d_{stump}$ and $h_{stump}$ are the average diameter (cm) and average height (m) of the stump, respectively; $\sigma_b$ is the basic density of the stump decay class (see below). The biomass of the stump's roots was determined according to the equation:

$$B_{root\_stump} = B_{stump\_as\_a\_tree} \times RS$$

where $B_{stump\_as\_a\_tree}$ is the biomass of a tree of DBH equal to the diameter of the stump calculated using the *allodb* R package and RS is the root-to-shoot ratio. Also in this case, all data were scaled to the hectare, taking into account the size of the plot.

### 4.1.4 Basic density of deadwood by decay stage

Standard basic density of deadwood by decay class reported in the literature for main European tree species were used to calculate deadwood biomass. In particular, density values provided by Bitunjac et al. (2023) for five broadleaf (*Quercus, Carpinus, Alnus, Fraxinus, Fagus*) and three conifer genera (*Abies, Picea, Pinus*) were considered. If the species of the deadwood piece was not included in the dataset or was not identified, the basic density of broader categories (i.e., broadleaves and conifers) was applied (Bitunjac et al. 2023; Table 10).

*Table 10. Dead wood basic density (DWBD) by decay classes retrieved from Bitunjac et al. (2023) and obtained using pooled data from the literature survey and data from an experimental study conducted by the authors, for tree genera separately, and combined into Broadleaves and Conifers groups.*

| Genus | Decay class | Dead wood basic density (g cm$^{-3}$) |
|---|---|---|
| *Alnus* | 1 | 0.422 |
| | 2 | 0.359 |
| | 3 | 0.286 |
| | 4 | 0.197 |
| | 5 | 0.120 |
| *Carpinus* | 1 | 0.392 |
| | 2 | 0.428 |
| | 3 | 0.339 |
| | 4 | 0.211 |
| | 5 | 0.140 |
| *Fagus* | 1 | 0.555 |
| | 2 | 0.388 |
| | 3 | 0.264 |
| | 4 | 0.248 |
| | 5 | 0.220 |
| *Fraxinus* | 1 | 0.527 |
| | 2 | 0.431 |
| | 3 | 0.392 |
| | 4 | 0.265 |
| | 5 | 0.151 |
| *Quercus* | 1 | 0.617 |
| | 2 | 0.519 |
| | 3 | 0.397 |
| | 4 | 0.299 |
| | 5 | 0.196 |
| *Abies* | 1 | 0.343 |
| | 2 | 0.305 |
| | 3 | 0.247 |
| | 4 | 0.174 |
| | 5 | 0.149 |
| *Picea* | 1 | 0.381 |
| | 2 | 0.34 |
| | 3 | 0.27 |
| | 4 | 0.19 |

| | | |
|---|---|---|
| | 5 | 0.157 |
| *Pinus* | 1 | 0.379 |
| | 2 | 0.334 |
| | 3 | 0.277 |
| | 4 | 0.214 |
| | 5 | 0.165 |
| Broadleaves | 1 | 0.542 |
| | 2 | 0.450 |
| | 3 | 0.349 |
| | 4 | 0.251 |
| | 5 | 0.153 |
| Conifers | 1 | 0.375 |
| | 2 | 0.334 |
| | 3 | 0.271 |
| | 4 | 0.198 |
| | 5 | 0.160 |

### 4.1.5 Grass and shrub biomass

The biomass per hectare of the herbaceous layer ($B_{grass}$; Mg ha$^{-1}$) and shrubs ($B_{shrub}$; Mg ha$^{-1}$) were calculated using the allometric equations in Table 11.

*Table 11. Allometric equations for calculating the biomass per hectare of the herbaceous and shrub layer. C = coverage expressed in %; H = depth in cm.*

| Grass | | |
|---|---|---|
| **Height** | **Equations** | **Source** |
| < 20 cm | = (3,003*LN(Co)+1,207)/10 | Charles B. Halpern and Eric A. Millet, 1996 |
| > 20 cm | = Co/100 * (-0.0038 * H^2 + 0.25 * H) | Bovio e Ascoli, 2013 |
| > 40 cm | = 0,1778 *H * Co/100 - 0,052 | Mou G, 2015 |
| **Shrubs** | | |
| **Species** | **Equations** | **Source** |
| Ferns | = 0,0010 * H^1,6430 * ARCSEN(RADQ(Co/100)))^0,3705 | Vega et al., 2022 |
| *Erica* | =0,0294*H^0,9054*(ARCSEN(RADQ(Co)))^0,5545 | Vega et al., 2022 |
| *Ulex* | = 0,1111*H^0,7087*(ARCSEN(RADQ(Co)))^0,2868 | Vega et al., 2022 |
| *Calluna* | = 0,09*(Co/100)*(10000*(H/100)^1,06) | Gonzalez et al. 2013 |
| *Rubus* | = (0,2508*(Co)^0,7885)/100) | Smith and Brand, 1983 |
| *Genista* | = Co/100 * (937+5,99*H)/100 | Castagneri et al., 2013 |
| *Myrtillus* | = (0,1496*Co)^0,934)/100 | Smith and Brand, 1983 |
| *Juniperus* | = 0,1*(Co/100)^2*((H/2)*0,405) | Riccardi et al., 2007 |

For those species or genera that were not included in Table 11, total biomass ($W_{total}$ in t ha$^{-1}$) was estimated using the general equations for macro-phanerophytes by De Cáceres et al. (2019):

$$CA_m = 5.8458 \times H_m^{1.4944} \qquad [cm^{-2}]$$

$$B_{total,m} = 0.7856 \times V_{total}^{0.8101} = 0.7856 \times (\frac{H_m}{100} \times \frac{CA_m}{10000})^{0.8101} \qquad [kg\ individual^{-1}]$$

$$N = \frac{C}{100} \times \frac{10000}{CA_m} = \frac{C \times 100}{CA_m} \qquad [plants\ m^{-2}]$$

$$W_{total} = \frac{N \times B_{total,m}}{1000} \times 10000 \qquad [t\ ha^{-1}]$$

where $CA_m$ is the estimated projected crown area of an average individual (cm$^2$) derived from the measured mean height ($H_m$ in cm); $B_{total,m}$ is the biomass of an average individual (kg individual$^{-1}$); $V_{total}$ is the apparent shrub volume (m$^3$); N is estimated shrub density (individuals m$^{-2}$) and C is the measured cover (in %).

Grass root biomass was calculated using a root-to-shoot ratio of 4, as suggested by IPCC (2006) for boreal (Dry & Wet), cold temperate wet and warm temperate wet grasslands. Shrub root biomass was calculated using the same approach as for the roots of standing living trees.

### 4.1.6 Carbon content

**Living biomass:** the conversion from biomass to C stock (MgC ha$^{-1}$) was obtained using an average C content of 0.47 (IPCC 2006).

**Dead biomass:** the conversion of biomass into C stock was carried out considering a carbon content (%) retrieved from the literature. According to Bitunjac et al. (2023), the C fraction remains largely constant across decay stages, and thus for this study an average of 0.475 for the broadleaves and 0.505 for conifers is being used (Bitunjac et al. 2023).

### 4.1.7 Data elaboration and preliminary dataset creation

Existing and new data collected by the WILDCARD partners through 2024 (the preliminary dataset) were stored as Excel files in the project's MS Teams platform. The data were then processed using the R statistical package (R version 4.4.0, R CoreTeam, Austria). The final output table (i.e. 'Preliminary dataset on available carbon data across the network') reporting aggregated information on carbon stocks in the different ecosystem pools is accessible in a password protected project folder (link). In the same folder, the R scripts for data processing are also available.

## 4.2 Results

### 4.2.1 Description of the preliminary dataset

The preliminary dataset on aboveground C stocks consists of 13 chronosequences (215 sites) for which all dendrometric data at the single tree (10.507 in total) or single deadwood piece level (2.959 in total) were already available (Table 12). For grassland sites (TSA=0), the estimation of aboveground biomass was always done according to the WILDCARD field protocol (link). Cropland sites (TSA=0) were assumed to not have standing biomass for most of the year.

*Table 12. List of the chronosequences constituting the preliminary dataset of this deliverable. Chrono id = id of the chronosequence; land use = land use before the secondary succession took place (G=grassland; C=cropland).*

| N | Ecoregion | Country | Region | Chrono id | Age range | N. of sites | Land use | Funding |
|---|---|---|---|---|---|---|---|---|
| 1 | Continental | Czech Republic | Bohemian Karst | VUKOZ_1 | 20-88 | 15 | C | WILDCARD |
| 8 | Alpine | Bulgaria | Western Rhodopes | IBER-BAS_1 | 10-80 | 15 | | WILDCARD |
| 9 | Mediterranean | Bulgaria | Rila mountain | IBER-BAS_2 | 10-100 | 15 | | WILDCARD |
| 10 | Alpine | Bulgaria | Pirin mountain | IBER-BAS_3 | 10-80 | 15 | | WILDCARD |
| 11 | Alpine | Italy | Friuli Venezia Giulia (Taipana) | UNIUD_1 | 0-75 | 20 | G | PNRR NBFC |
| 12 | Continental | Italy | Toscana | UNIUD_2 | 0-75 | 20 | G | PNRR NBFC |
| 13 | Continental | Italy | Abruzzo | UNIUD_3 | 0-75 | 20 | G | PNRR NBFC |
| 14 | Mediterranean | Italy | Basilicata | UNIUD_4 | 0-75 | 20 | G | PNRR NBFC |
| 15 | Alpine | Italy | Friuli Venezia Giulia (Faedis) | UNIUD_5 | 0-75 | 15 | C | PRIN |
| 16 | Alpine | Italy | Friuli Venezia Giulia (Ampezzo) | UNIUD_6 | 0-75 | 15 | G | PRIN |
| 17 | Alpine | Italy | Friuli Venezia Giulia (Ampezzo) | UNIUD_7 | 0-75 | 15 | C | PRIN |
| 18 | Alpine | Italy | Piemonte | UNITO_1 | 0-75 | 15 | G | PRIN |
| 19 | Alpine | Italy | Piemonte | UNITO_2 | 0-75 | 15 | C | PRIN |
| | | | | | **TOTAL** | **215** | **-** | **-** |

The calculated C stocks at the site level, along with site and plot metadata, were compiled into a single table containing the following fields:

**A) SITE AND PLOT DESCRIPTION:**

**CODE_ID:** unique code identifier

**INSTITUTION:** name of the institution

**CHRONO_ID:** chronosequence ID

**SITE_ID:** site ID within the chronosequence

**PLOT_ID:** unique plot ID within the site

**TSA:** time since agricultural abandonment, years

**SURVEY_YEAR:** year of the survey

**FOREST_TYPE:** forest type according to the European classification

**VERTICAL_STRUCTURE:** vertical structure (single-layer, two-layered, multi-layered)

**HORIZONTAL_STRUCTURE:** horizontal structure (random, regular, grouped)

**COVERAGE:** coverage (regular, full, incomplete, poor)

**MANAGEMENT TYPE:** management type (high forest, coppice, temporary high forest [i.e. conversion from coppice to high forest], not determinable)

**RECENT_MANAGEMENT:** signs of recent silvicultural practices (yes, no)

**ASPECT:** aspect in degrees

**SLOPE:** slope in degrees

**COORDINATES_N:** latitude

**COORDINATES_E:** longitude

B) **STAND DENDROMETRIC SUMMARY:**

**N_trees:** number of standing trees per ha (alive + dead) (N $ha^{-1}$)

**G_tot:** total basal area ($m^2$ $ha^{-1}$)

**d_avg:** mean diameter (i.e. diameter of the tree with mean basal area) (cm)

C) **BIOMASS:**

**AGB_tree_alive:** aboveground biomass - alive trees (Mg $ha^{-1}$)

**BGB_tree_alive:** belowground (root) biomass - alive trees (Mg $ha^{-1}$)

**AGB_tree_dead:** aboveground biomass - unbroken and broken dead trees + stumps (Mg $ha^{-1}$)

**BGB_tree_dead:** belowground (root) biomass - unbroken and broken dead trees + stumps (t $ha^{-1}$)

**V_CWD:** coarse woody debris volume, $m^3$ $ha^{-1}$

**B_CWD:** coarse woody debris biomass (Mg $ha^{-1}$)

**V_FWD:** fine woody debris volume ($m^3$ $ha^{-1}$)

**B_FWD:** fine woody debris biomass (Mg $ha^{-1}$)

**AGB_shrubs:** aboveground biomass - shrubs (Mg $ha^{-1}$)

**BGB_shrubs:** belowground (root) biomass - shrubs (Mg $ha^{-1}$)

**AGB_grass:** aboveground grass dry biomass (Mg $ha^{-1}$)

**BGB_grass:** belowground (root) grass biomass (Mg $ha^{-1}$)

**D) CARBON STOCKS (all in MgC $ha^{-1}$):**

**AGC_tree_alive:** aboveground carbon stock - alive trees

**BGC_tree_alive:** belowground (root) carbon stock - alive trees

**AGC_tree_dead:** aboveground carbon stock - unbroken and broken dead trees + stumps

**BGC_tree_dead:** belowground (root) carbon stock - unbroken and broken dead trees + stumps

**C_CWD:** carbon stock in coarse woody debris

**C_FWD:** carbon stock in fine woody debris

**AGC shrubs:** aboveground carbon stock - shrubs

**BGC shrubs:** belowground (root) carbon stock - shrubs

**AGC grass:** aboveground carbon stock - grass

**BGC grass:** belowground (root) carbon stock - grass

### 4.2.2 Next steps

The collection of aboveground dendrometric data for the missing chronosequences (both internal and external) is ongoing and will be completed by Month 18 (Milestone 5). The data will be processed according to the methodology described above, probably with some adjustments/updates to the allometric relationships for shrub biomass estimation (Table 11).

The output data on C stocks by ecosystem pool at site level will finally be made available on the project website through a webGIS portal (Result 1 - “FAIR data on C stocks and biodiversity following rewilding”). This portal will allow the users to select individual sites or groups of sites to view and download the data.

The final dataset will be also merged with belowground C stock estimates in Deliverable D3.2 “Final dataset on carbon sequestration and biodiversity following rewilding of abandoned agricultural land”.

# 5. Biodiversity assessment

## 5.1 Material and methods

The project aims to gather data on changes in vegetation cover of vascular plant species across different successional stages. Vascular plants are used as an indicator of biodiversity changes following agricultural abandonment. The series span over 20 years to capture successional dynamics, including shifts in species composition. Data collection begins no later than three years after the last disturbance event, such as agricultural abandonment or the cessation of management practices. The study is limited to sites that have developed through spontaneous succession, excluding any additional disturbances or management practices such as mowing, burning, or fertilization (Table 5). Mild grazing is permitted only in sites where exclusion is not possible. Data collection follows a chronosequence over a continuous period, using standardized 5x5 $m^2$ plots.

**Site Selection:** sampling sites and dates were selected in collaboration with the WP3 coordinator and local teams. To effectively summarize vegetation trends, portions of each site representing different successional stages were chosen for sampling. The selection was based on a preliminary assessment of species variation within the area. Samples were then established in homogeneous and representative sections of each site to record species composition and cover.

**Time Since Abandonment (TSA):** for each sampled vegetation plot , the time elapsed since agricultural abandonment was recorded to ensure consistency in successional series data. On each vegetation sample of 5x5 $m^2$, all plant species were recorded along with their estimated cover. The vegetation cover within each vegetation sample is assessed across different vertical layers.  For each vascular plant species within a vegetation sample, cover is visually estimated and recorded as a percentage of the area it occupies.

**Sampling Cover:** using a phytosociological approach, each vascular plant species in the sample is recorded with its visually estimated cover, expressed as a percentage of the area occupied. The ideal plot size is 5m x 5m, where each 1% cover represents 50 cm x 50 cm. Plots can be either square or rectangular, depending on the site's characteristics. Species cover is reported in increments such as 1%, 2%, 3%, 5%, 10%, etc., up to 100%. Species with cover below 1% are indicated with a "+" (e.g., 0.1%), while those with negligible cover are marked as "r" (e.g., one or two small individuals, ~0.02%). The total cover in the plot, calculated as the sum of all species, may exceed 100% due to overlap. For further details, see Kent (2011).

**Vertical Structure:** vegetation cover estimates include several layers: E0 – moss layer (including mosses and lichens), E1 – herb layer (all herbs and trees and shrubs shorter than 1 m), E2 – shrub layer (shrubs and trees generally shorter than 3–5 m), and E3 – tree layer (woody plants taller than 3–5 m). This structured approach provides detailed insights into the layering and height distribution of vegetation in each successional stage. Species identification was preliminarily done by cross-referencing against (species name matching – gbif.org) to maintain consistency across project partners. For taxonomically challenging groups, such as *Rubus spp.*, *Alchemilla spp.*, and *Taraxacum spp.*, identification was limited to the genus level. Herbarium sheets and photographs were prepared when off-site species identification was required.

## 5.2 Results

### 5.2.1 Description of the preliminary dataset

The preliminary dataset on plant diversity consists of 6 of the 17 WILDCARD-internal chronosequences (86 sites; see Table 5 for the complete list). Similarly to what was done for the C stock assessment, meta-data for each chronosequence were collected and verified, including information of the species and metadata, including the ecoregion, the number of sites according to WILDCARD criteria, geographic coordinates, forest type, land use prior to secondary succession, environmental variables (e.g., elevation, precipitation, temperature), and other factors. The number of species and the vegetation cover per sample were also provided.

The plant diversity data (Species sheet) at the site level, along with site and plot metadata (Environmental variables sheet), were compiled into a single file. PLOT_ID has an identical code for associated data in the Species and Environmental Variables sheets. Species sheet list the species, indicating the vegetation layer where it occurs (i.e., E3, E2, E1). If vertical structure was not sampled, the total cover of each species is provided. The metadata include fields as described in section 4.2.1 for INSTITUTION, CHRONO_ID, SITE_ID, PLOT_ID, TSA, SURVEY_DATE, ASPECT, SLOPE, COORDINATES_N, COORDINATES_E, and additionally the following fields:

**ECOREGION:** Alpine, Atlantic, Boreal, Continental, Mediterranean, Pannonian

**COUNTRY:** Country where the data are collected

**REGION**: name for the geographical region (e.g., Bohemian Karst)

**LOCALITY:** Name of the locality or identifying site name

**ALTITUDE:** elevation in m a.s.l.

**TYPE OF SERE:** Character of the sere, e.g., cropland, pastures, meadows, forest, or other contexts considered in relation to the cessation of management.

**REFERENCE SITE:** Is there data for the reference site?

**TARGET ECOSYSTEM:** Undisturbed community on the site if it was not disturbed, e. g. type of grassland, wetland, riparian, scrubland, shrubland, forest (conifer, broadleaf, mixed), or other.

**RELEVE AREA:** Size of the vegetation sample (i.e., generally 5 x 5 $m^2$, but other sizes may be acceptable e.g., 2x2 – 20x20 $m^2$).

**PERMANENT_CHRONOSEQUENCE:** Whether data are from permanent plots or chronosequences

**PRECIPITATION:** Average annual precipitation in mm.

**TEMPERATURE:** Average annual temperature in in °C (Celsius scale).

**pH:** Measured pH value at the locality/site or at least a rough estimation based on geology for the whole locality (acid, neutral, basic).

**HABITAT MOISTURE:** Empirical measure or at least a rough estimate of site moisture (Flooded, wet, mesic, dry).

**E3: Cover of tree layer (%)**

**E2: Cover of shrub layer (%)**

**E1: Cover of herb layer (%)**

**E0: Cover of moss layer (%)**

**OVERALL COVER:** Overall cover considering all layers in the vegetation sample.

**NUMBER OF SPECIES:** Number of species per vegetation sample.

**PUBLISHED:** Whether there is an associated publication of the data contributed for further information.

**CONTRIBUTORS:** Data providers, sampling team or people associated with the data.

### 5.2.2 Next steps

**Increasing chronosequences' number**

The total number of WILDCARD-internal chronosequences with plant diversity data is 17 (Table 5), 11 of which will be sampled in spring and early summer 2025. As mentioned above, to address the gaps in underrepresented or poorly represented ecoregions external research groups have been contacted to assess their willingness to share plant diversity data through service contracts outlined in WP3. All this information will eventually be merged with the data extracted from the literature review (cf. section 2.2.1). All acquired information will then be used for further analysis of plant diversity changes during successional dynamics and the factors influencing such trends.

**Plant Functional Diversity and Ecosystem Functioning**

To investigate how plant functional diversity influences ecosystem functioning — particularly in relation to carbon dynamics and storage — we will focus on a core set of functional traits known to affect productivity, decomposition, and biomass accumulation. These include Specific Leaf Area, Leaf Nitrogen and Carbon Content, Leaf Dry Matter Content, and Vegetative Height.

Trait data will be compiled from the TRY Plant Trait Database (Kattge et al., 2020), targeting species that together represent at least 80% of the relative abundance within each vegetation layer. This ensures that the most dominant and functionally influential species are captured, following established community-weighted trait protocols (Díaz et al., 2016).

To ensure data quality and comparability across sites, the analytical workflow will include:

- Data cleaning, standardizing values to remove outliers and improve reliability.
- Imputation of missing data: missing trait values will be estimated using a phylogenetically informed imputation approach, allowing estimation of trait data based on evolutionary relationships among species. Suitable R packages are *V.PhyloMaker* (Jin & Qian, 2019) and *funspace* (Carmona et al., 2024).
- Dimensionality reduction using Principal Component Analysis to summarize key trait variation.

- Computation of functional diversity indices. Based on the PCA trait space, we will compute three widely used indices of functional diversity: Functional Richness (FRic), reflecting the volume of functional space occupied by the community; Functional Evenness (FEve), measuring the regularity of species distributions in trait space; Functional Divergence (FDiv), quantifying the degree of trait differentiation among species, often associated with niche partitioning.

These indices will be calculated using the Trait Probability Density (TPD) method (Carmona et al., 2019), a robust and flexible approach that is particularly well-suited for handling incomplete or imputed trait datasets, and has been highlighted as one of the most reliable methods for quantifying functional diversity (Stewart et al., 2023). This standardized yet flexible pipeline will be applicable across different sites within the WILDCARD project, ensuring methodological consistency while allowing adaptation to local vegetation characteristics and data availability.

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