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Botanical contributions to improve the assessment of soil bioengineering works

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To my daughter Lucia

Abstract

The research described in this PhD dissertation was conducted during the period 2014-2016 at the Centre of Applied Studies for the Sustainable Management and Protection of Mountain Areas (Ge.S.Di.Mont. - University of Milan) in Edolo (BS) with the objective of devising botanical methods/tools that could be useful for improving the evaluation of soil bioengineering works in mountain areas. This research has led to the formulation of two floristic-vegetational indices: the Ecological Index of Maturity (EIM) and the Index of Ecological Success (IES). These indices were applied to three study areas located in mountainous areas of Lombardy (Northern Italy) affected by landslides which were followed by soil stabilization works using soil bioengineering techniques. On the basis of the results obtained, the indices have proven practical and functional for the evaluation of soil bioengineering works for slope stabilization as they consider their effectiveness in stabilizing soil as well as their efficiency in minimizing human impact on the ecosystem and landscape.

This thesis also reports research to assess the effectiveness of the chromatographic fingerprinting of the extracts of fine roots for the identification of plant species. The results obtained showed that the analysis of chromatographic fingerprints is an effective method for the identification of some of the species studied whereas it is less useful for the identification of other species. This suggested ideas for further research on this topic and application both in soil bioengineering (e.g.: the evaluation of the success of the fine roots of plants used for soil stabilization) and in other sectors dealing with the study of the rhizosphere and plant (root)-soil interaction.

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INTRODUCTION

This thesis presents the results of scientific research conducted during the three years (2014 - 2016) of the PhD course in Environmental Sciences at the Centre of Applied Studies for the Sustainable Management and Protection of Mountain Areas (Ge.S.Di.Mont. - University of Milan) in Edolo (Brescia). This research was conducted with the aim of providing botanical tools and/or methods to improve the assessment of soil bioengineering works, with reference to landslide stabilization. Soil bioengineering is a relatively recent discipline (BISCHETTI et al. 2012) that uses low-impact measures which utilize live plants (or parts thereof) as building materials in combination with other materials (such as stones, soil, timber, steel, etc.) for soil stabilization. Although these techniques are increasing in popularity worldwide, appropriate tools for evaluating the success of such measures are still lacking because it is difficult to assess the efficiency of soil stabilization over time and, even more challenging to evaluate its efficiency in minimizing human impact on the ecosystem and landscape.

In the first part of this thesis (Section I) two indices, that have been developed and applied in three areas of the Val Camonica affected by soil bioengineering works, are presented: Ecological Index of Maturity (EIM) (GIUPPONI et al. 2015a)

and Index of Ecological Success (IES) (GIUPPONI et al. 2017b). The EIM measures the disturbance affecting plant communities considering the phytosociological class, chorotype and coverage of each species present, while the IES is an index for evaluating the effectiveness of soil bioengineering works in mountain areas with particular reference to slope stabilization. The IES compares the EIM values of the vegetation of an area affected by soil stabilization work with the expected EIM value at a precise time after completion of soil stabilization work. To perform this comparison it was necessary to analyze EIM trends over time after the end of soil stabilization work and to develop a model that would allow the expected EIM value to be estimated. So the EIM was applied to vegetation of various chronosequences (chapter 2), one of which was analyzed in detail and used as a model to estimate the expected EIM and therefore to calculate the EIS (chapter 3).

In the second part of this thesis (Section II) the results of the first application of a new method to identify the fine roots of plants based on the analysis of the chromatographic fingerprint of root extracts are provided (GIUPPONI et al. 2017c). This method was developed because the identification of plants through the analysis of their fine roots is currently extremely difficult (or well-nigh impossible) when using traditional identification tools such as dichotomous keys

and/or illustrated atlases. Although this problem can be overcome by molecular analysis (BOBOWSKI et al. 1999), there are other analytical methods, such as chromatographic analysis, which could be useful for the identification of the fine roots of plants and which could provide interesting data for the evaluation of the success of the roots of plants used for soil stabilization and, more in general, for the study of the complex plant(root)-soil relationship. Hence, this method was applied to several samples of roots (belonging to six plant species) collected in the Alps and results were analyzed statistically and commented.

Some of the results contained in this dissertation have been published in scientific journals and presented at national and international conferences; other data are in articles in print or under review.

SECTION I

Application of floristic-vegetational indices for the analysis of soil stabilization
works conducted in Val Camonica (Northern Italy)

1. Ecological Index of Maturity (EIM) to evaluate the vegetation disturbance of areas affected by soil bioengineering works

1.1 Introduction

The environmental restoration of areas disturbed by anthropogenic or natural factors is currently a topic of fundamental importance for global sustainable development (ARONSON et al. 2006; ARONSON & ALEXANDER 2013). Thirty years after the founding of Ecological Restoration as a scientific discipline, there are still many sectors in which knowledge needs to be widened in order to improve future intervention. This dissertation presents a new index, the Ecological Index of Maturity (EIM) (GIUPPONI et al. 2015a), to evaluate the level of disturbance of the vegetation of areas affected by anthropic interventions, defining disturbance as the intensity/frequency of phenomena that cause destruction of plant biomass due to biotic or abiotic factors (GRIME 2001) as well as anthropogenic activities. The index provides information as to whether the vegetation, and therefore the ecosystem, is or has been subject to disturbance (natural or anthropogenic) or alteration, and hence whether or not anthropogenic activities (restoration and/or

other work) has been successful. This index is based on the floristic-vegetational indices system devised by TAFFETANI & RISMONDO (2009), updated by RISMONDO et al. (2011), for the evaluation of the environmental functionality of agro-ecosystems. The literature includes various indices that measure the naturalness or the degree of human disturbance of an area starting from the study of plants or plant communities (JALAS 1955; GÉHU & GÉHU-FRANK 1988; LOIDI 1994; EDERRA 1997; INGEGNOLI 1999, 2011; HILL et al. 2002; KIM et al. 2002; STEINHARDT et al. 2009) but the index we present takes into account the modern notions of dynamic-catenal phytosociology (RIVAS-MARTÍNEZ 2005) which are based on the concept of dynamic vegetation, i.e. the spontaneous replacement of plant communities, at a particular observation point, over time. Succession occurs due to changes (type and/or intensity) in environmental factors caused by natural (landslides, fires, climate change etc.) or anthropogenic disturbance (mowing, cutting of forests, scrub clearance etc.). In the absence of disturbance the succession process determines a stable plant community defined climax or potential vegetation, although recently such terms have been replaced by "current potential vegetation" (BIONDI 2011), indicating the vegetation that represents the most advanced stage in the serial succession within a given biogeographic area, obviously excluding major and sudden climate change

events. The vegetation series (*sigmetum*) is the base unit of dynamic phytosociology (synphytosociology) and consists of all the associations (plant communities) linked by dynamic relationships found within an area with the same potentiality of vegetation (RIVAS-MARTÍNEZ 2005; BIONDI 2011) and in which each plant community represents a specific dynamic stage of the series. The EIM is therefore based on the study of plant communities according to the traditional and integrated phytosociological approach (BRAUN-BLANQUET 1964, 1979; BIONDI 1994, 2011; BLASI & FRONDONI 2011; POTT 2011; CRISTEA et al. 2015), a scientific method used worldwide and adopted by the Habitats Directive 92/43 EEC, which is the most important European legislation for nature protection. The recent system devised by TAFFETANI & RISMONDO (2009) and RISMONDO et al. (2011) has found various applications in work aimed at assessing the environmental quality of a territory (TAFFETANI et al. 2011; LANCIONI & TAFFETANI 2012; GIUPPONI et al. 2013; GIUPPONI & GIORGI 2016). However, it is of limited practical use since it consists in a series of independent indices, each of which provides the measurement of a single ecological factor. The EIM, instead, by fitting together the information given by some of these indices (index of maturity, index of exotic component and index of endemic component), provides a single value that is indicative of the overall disturbance (biotic and/or abiotic) affecting the

vegetation of an area. This can be useful to assess the effectiveness of environmental restoration or soil bioengineering work comparing, for example, the EIM value of post-intervention vegetation with that of pre-intervention vegetation or with that of the current potential vegetation.

The EIM has been applied to the vegetation of three mountain areas of Val Camonica (Lombardy, Italy) affected by soil stabilization work using soil bioengineering techniques, in order to test its validity.

1.2 Ecological Index of Maturity

The Ecological Index of Maturity (EIM) measures the level of disturbance affecting a plant community considering: phytosociological class, chorotype and coverage of each species present. It is calculated using the following formula developed by GIUPPONI et al. (2015a):

$$EIM = \frac{IM \cdot \left[\left(1 - \frac{IE}{100} \right) + \frac{IL}{100} \right]}{1 + \frac{IL}{100}}$$

where EIM is the ecological index of maturity, IM is the index of maturity, IE is the index of the exotic component and IL is the index of the endemic component. The EIM is therefore the result of the union of three separate indices, developed by TAFFETANI & RISMONDO (2009) and RISMONDO et al. (2011), each measuring a separate ecological characteristic of the vegetation.

The index of maturity (IM) measures the actual dynamic stage of succession in relation to the coverage and phytosociological class to which each species of a plant community belongs. In phytosociology the class represents the broadest rank of syntaxonomy (a science that studies vegetation systematics) grouping together plants that have similar ecological, physiognomic-structural and phytogeographical characteristics. IM is expressed by the following formula developed by TAFFETANI & RISMONDO (2009):

$$IM = \frac{\sum_{i=1}^n (c_i \cdot m)}{C}$$

where IM is the index of maturity, c_i is the coverage value of each single species, i ($i = 1, 2, \dots, n$) is the number of species, m is the coefficient of maturity of the phytosociological class to which each species belongs, C is the total coverage

value obtained by summing the values of c for all the species present. The coefficient of maturity (m) is the value assigned by TAFFETANI & RISMONDO (2009) and RISMONDO et al. (2011) to the main phytosociological classes of European vegetation according to the physiognomic-structural, synecological characteristics and the syndynamic role of the vegetation of each class (Table 1.1). It varies from 0 (cultivated, ornamental or exotic species that have no evolutionary significance and are therefore not attributable to specific syntaxonomic class) to 9 (species of the mature forest vegetation classes).

The index of exotic component (IE) provides the percentage of exotic species (cultivated, casual adventitious, naturalized, hemi-hemerophyte) of a plant community considering exotic species coverage compared to total coverage. It measures the degree of exotic contamination and artificiality of the vegetal coenoses in relation to human pressure (human disturbance) in the territory and is calculated as follows:

$$IE = \frac{\sum_{i=1}^n [c_{(e)}]_i}{C} \cdot 100$$

where IE is the index of exotic component, $c_{(e)}$ is the coverage value of each

exotic species, i ($i = 1, 2, \dots, n$) is the number of exotic species and C is the total coverage value obtained by summing the values of c for all the species present (TAFFETANI & RISMONDO 2009). This index does not take into consideration the number of exotic species of a community but only their total coverage as it measures the loss of identity of the landscape due to the presence of these species that subtract space from autochthonous species.

The index of endemic component (IL) gives the percentage of endemic species of a community considering their coverage compared to that of all the species present. The index of endemic component is calculated according to the following formula:

$$IL = \frac{\sum_{i=1}^n [c_{(i)}]_i}{C} \cdot 100$$

where IL is the index of endemic component, $c_{(i)}$ is the coverage value of each endemic species, i ($i = 1, 2, \dots, n$) is the number of endemic species and C is the total coverage value obtained by summing the values of c for all the species present (TAFFETANI & RISMONDO 2009). IL take into consideration the total coverage, not the number, of endemic species of a plant community.

EIM values can range from 0 (high vegetation disturbance) to 9 (undisturbed vegetation) as for the IM. In particular the EIM value corresponds to that of the IM when the plant community does not present alien species, whereas it tends to decrease much more markedly the higher the IE and the lower the IL. If there are no exotic species the EIM is the same as the IM independently of whether there are endemic species since a territory may not have an endemic component for historical and / or biogeographic reasons. The appearance of endemic species (if these are present in a given area) following restoration (or other work) is however an indicator of environmental quality and of the ecological success of work, which is why the EIM compensates, with the IL, the lowering of the value due to the IE.

Phytosociological class	m
* cultivated or exotic species	0
STELLARIETEA MEDIAE	1
ORYZETEA SATIVAE	1
POLYGONO ARENASTRI-POETEA ANNUAE	2
CHARETEA FRAGILIS	2
LEMNETEA MINORIS	2
POTAMETEA PECTINATI	2
TUBERARIETEA GUTTATAE	2
CAKILETEA MARITIMAE	2
SAGINETEA MARITIMAE	2
THERO-SUAEDETEA SPLENDENTIS	2
ARTEMISIETEA VULGARIS	3
MOLINIO-ARRHENATHERETEA	4
BIDENTEAE TRIPARTITAE	4
ISOËTO-NANOJUNCETEA	4
FESTUCO VALESIAEAE-BROMETEA ERECTI	5
KOELERIO GLAUCAE-CORYNEPHORETEA CANESCENTIS	5
LYGEO SPARTI-STIPETEA TENACISSIMAE	5
NARDETEA STRICTAE	5
POETEA BULBOSAE	5
ASPLENIETEA TRICHOMANIS	5
FESTUCO-SESLERIETEA	5
CARICETEA CURVULAE	5
SEDO ALBI-SCLERANTHETEA BIENNIS	5
THLASPIETEA ROTUNDIFOLII	5
EUPHORBIO PARALIAE-AMMOPHILETEA AUSTRALIS	5
CRITHMO MARITIMI-STATICETEA	5
JUNCETEA MARITIMI	5
PEGANO HARMALAE-SALSOLETEA VERMICULATAE	5
SARCOCORNIETEA FRUTICOSAE	5
CARICETEA CURVULAE	5
SPARTINETEA GLABRAE	5
CARDAMINETEA HIRSUTAE	6
GALIO APARINES-URTICETEA DIOICAE	6
PARIETARIETEA JUDAICAE	6
ADIANTETEA CAPILLI-VENERIS	6
LITTORELLETEA UNIFLORAE	6
MONTIO FONTANAE-CARDAMINETEA AMARAE	6
OXYCOCCO PALUSTRIS-SPHAGNETEA MAGELLANICI	6
PHRAGMITO AUSTRALIS-MAGNOCARICETEA ELATAE	6
SCHEUCHZERIO PALUSTRIS-CARICETEA NIGRAE	6
EPILOBIETEA ANGUSTIFOLII	7
MULGEDIO ALPINI-ACONITETEA VARIEGATI	7
TRIFOLIO MEDII-GERANIETEA SANGUINEI	7
BETULO CRPATICAE-ALNETEA VIRIDIS	7
CALLUNO VULGARIS-ULICETEA	8
CISTO LADANIFERI-LAVANDULETEA STOECHADIS	8
CISTO CRETICI-MICROMERIETEA JULIANAE	8
CYTISETEA SCOPARIO-STRIATI	8
RHAMNO CATHARTICAE-PRUNETEA SPINOSAE	8
ROSMARINETEA OFFICINALIS	8
NERIO OLEANDRI-TAMARICETEA AFRICANAE	8
ROBINIETEA	8
ERICO CARNEAE-PINETEA SYLVESTRIS	9
JUNIPERO SABINAE – PINETEA SYLVESTRIS	9
QUERCO ROBORIS-FAGETEA SYLVATICAE	9
QUERCETEA ILICIS	9
VACCINIO MYRTILLI-PICEETEA ABIETIS	9
ALNETEA GLUTINOSAE	9

Table 1.1 - Coefficient of maturity (m) of each phytosociological class (modified from RISSONDO et al. 2011). Syntaxa nomenclature follows BIONDI et al. (2014). *, cultivated or exotic species that haven't evolutionary significance (not attributable to specific phytosociological class). The description of the phytosociological classes is reported in Supporting Material I (page 14).

Supporting Material I

Definition and description of the phytosociological classes of Table 1.1 (BIONDI et al. 2014):

STELLARIETEA MEDIAE Tüxen, Lohmeyer & Preising ex Von Rochow 1951

Annual, ephemeral, weed ruderal nitrophilous and sub-nitrophilous vegetation found throughout the world with the exception of warm tropical regions.

ORYZETEA SATIVAE Miyawaki 1960

Weed vegetation of rice fields comprising vascular phanerogams and cryptogams, particularly algae.

POLYGONO ARENASTRI-POETEA ANNUAE Rivas-Martínez 1975 corr. Rivas-Martínez, Bascónes, T.E. Díaz, Fernández-González & Loidi 1991

Nitrophilous pioneer vegetation of small therophytes and hemicryptophytes that grows on tamped and nitrified soils subjected to trampling: paths, roadsides, crevices of paved roads.

CHARETEA FRAGILIS F. Fukarek ex Krausch 1964

Pioneer, rooted seagrass beds that grow in calm, clear, oligotrophic to mesotrophic, brackish to fresh waters, on underwater soils of ponds, lakes and surface waterways.

LEMNETEA MINORIS O. Bolòs & Masclans 1955

Pleustophytic vegetation that colonizes fresh to brackish waters.

POTAMETEA PECTINATI Klika in Klika & Novák 1941

Perennial macrophytic communities of fresh, occasionally brackish, mesotrophic to eutrophic, running or standing, waters.

TUBERARIETEA GUTTATAE (Br.-Bl. in Br.-Bl., Roussine & Nègre 1952) Rivas Goday & Rivas-Martínez 1963 nom. mut. propos. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002

Annual, ephemeral, xerophytic and thermophilous non-nitrophilous vegetation, with a short winter-spring vegetative cycle. It grows mainly in the Mediterranean macrobioclimate, extending into the Temperate macrobioclimate, mostly in the Submediterranean variant of the mesotemperate thermotype, and is indifferent to the chemical nature of the substratum.

CAKILETEA MARITIMAE Tüxen & Preising ex Br.-Bl. & Tüxen 1952

Therophytic halo-nitrophilous pioneer vegetation from the beach front of emerged coasts, following the surf zone, on sandy and fine-pebbly beaches, where organic material, carried by the sea, accumulates and decomposes.

SAGINETEA MARITIMAE Westhoff, Leeuwen & Adriani 1962

Pioneer vegetation characterized by small therophytic, xerophilous, halophilous and sub-halophilous, occasionally sub-nitrophilous species, on sandy-loamy or rocky substrates of the

Atlantic and Mediterranean littorals.

THERO-SUAEDETEA SPLENDENTIS Rivas-Martínez 1972

Pioneer coastal or continental vegetation of annual species of the genus *Salicornia* found in brackish marshes that are temporarily inundated and in salt pans.

ARTEMISIETEA VULGARIS Lohmeyer, Preising & Tüxen ex Von Rochow 1951

Perennial pioneer synanthropic ruderal and nitrophilous herbaceous vegetation that grows on soils rich in organic matter, in Eurosiberian and Mediterranean regions.

MOLINIO-ARRHENATHERETEA Tüxen 1937

Hygrophilous, meso-hygrophilous or mesophilous meadows that occur from the coastal to montane and high-montane areas, mainly distributed in the Temperate macrobioclimate, and to a lesser extent in the Mediterranean macrobioclimate. These communities grow on mineral to more or less rich in organic matter soils. The syntaxon includes strongly manured to un-manured meadows.

BIDENTETEA TRIPARTITAE Tüxen, Lohmeyer & Preising ex Von Rochow 1951

Pioneer annual nitrophilous and hygrophilous vegetation that grows on nitrogen-rich, muddy or silty-pebbly substrates.

ISOËTO-NANOJUNCETEA Br.-Bl. & Tüxen ex Westhoff, Dijk & Passchier 1946

Ephemeral amphibious vegetation from temporary ponds, mainly characterized by therophytes and occasionally accompanied by hemicryptophytes and dwarf geophytes, that grow on soils periodically flooded by oligotrophic, eutrophic or, rarely, sub-salt waters.

FESTUCO VALESIIACAE-BROMETEA ERECTI Br.-Bl. & Tüxen ex Br.-Bl. 1949

Primary and secondary pastures dominated by xerophilous and mesophilous hemicryptophytes that grow in the hilly and mountainous areas of Europe and of the western sector of Siberia. They grow mostly on calcareous and alkaline or subacidic substrata.

KOELERIO GLAUCAE-CORYNEPHORETEA CANESCENTIS Klika in Klika & V. Novák 1941

Perennial, pioneer vegetation dominated by hemicryptophytes, with varying numbers of annual species, that grows on sandy oligotrophic soils whose stability may vary.

LYGEO SPARTI-STIPETEA TENACISSIMAE Rivas-Martínez 1978 nom. conserv. propos. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002

Mediterranean, perennial, thermo-xerophilous, steppe grasslands dominated by tufted Gramineae, found throughout the Mediterranean region, though with a western Mediterranean optimum; they grow on deep calcareous soils in the thermo- to supra-Mediterranean thermotypes, and in the semiarid to subhumid ombrotypes.

NARDETEA STRICTAE Rivas Goday in Rivas Goday & Rivas-Martínez 1963

Dense grassland vegetation that grows on acidic, decalcified, deep soils. It is found in the

Atlantic, central European and Mediterranean regions, in the bioclimatic belts with oro- and supratemperate and supra- oro- and cryoro-Mediterranean thermotypes.

POETEA BULBOSAE Rivas Goday & Rivas-Martínez in Rivas-Martínez 1978

Highly productive, perennial, mainly hemicryptophytic, Mediterranean pastures, with many therophytes, dominated by small Graminaceae and Fabaceae. They grow on both oligotrophic and eutrophic soils, from the thermo- to supra-Mediterranean thermotypes, with an ombrotype ranging from sub-arid to humid. These communities are distributed prevalently in the western Mediterranean and in Italy are widespread in Sardinia, though they may also be found in other areas with a Mediterranean macrobioclimate.

ASPLENIETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdorfer 1977

Non-nitrophilous, perennial vegetation dominated by chasmophytes and occasionally by chomophytes, that grows in the crevices of rocks, cliffs and walls. Communities with a holarctic distribution.

FESTUCO-SESLERIETEA Barbéro-Bonin 1969

Basophilous primary grasslands (in some cases also secondary grasslands) consisting of graminoids and dwarf chamaephytes that grow on cryoturbate soils covered in snow for long periods of time. These communities range from the supratemperate thermotype to the cryorotemperate thermotype, where they are most widespread.

SEDO ALBI-SCLERANTHETEA BIENNIS Br.-Bl. 1955

Pioneer, open vegetation consisting of perennial (often succulent), dwarf chamaephytes and geophytes, accompanied by ephemeral therophytes, with a Eurosiberian and Mediterranean distribution. It develops on siliceous and calcareous rock surfaces, from the meso- to lower orotemperate and from the thermo- to supra- Mediterranean thermotypes, with an ombrotype ranging from semiarid to hyperhumid.

THLASPIETEA ROTUNDIFOLII Br.-Bl. 1948

Perennial vegetation that grows on screes, loose debris substrata, slope deposits, moraines and stream terraces in the Boreal, Temperate and Mediterranean macrobioclimates.

EUPHORBIO PARALIAE-AMMOPHILETEA AUSTRALIS Géhu & Rivas-Martínez in Rivas-Martínez, Asensi, Díaz-Garretas, Molero, Valle, Cano, Costa & Díaz 2011

Psammophilous perennial vegetation from coastal sandy and fine-pebbly dunes with a Mediterranean, Atlantic and Macaronesian littoral distribution, that is important in dune construction and stabilization processes.

CRITHMO MARITIMI-STATICETEA Br.-Bl. in Br.-Bl., Roussine & Nègre 1952 em. Biondi 2007

Halo-chasmophytic and halo-tolerant vegetation with varying habitus that develops on rocky marine sites exposed to marine waters or subjected to marine salt spray.

JUNCETEA MARITIMI Br.-Bl. in Br.-Bl., Roussine & Nègre 1952

Mediterranean perennial salty and brackish grasslands.

PEGANO HARMALAE-SALSOLETEA VERMICULATAE Br.-Bl. & O. Bolòs 1958

Vegetation of halo-nitrophilous, heliophilous chamaephytes and nanophanerophytes dominated by succulent *Chenopodiaceae* and entomophilous *Asteraceae*.

SARCOCORNIETEA FRUTICOSAE Br.-Bl. & Tüxen ex A. Bolòs & O. Bolòs in A. Bolòs 1950
em. Biondi, Casavecchia, Estrelles & Soriano, 2013

Pioneer, perennial, hyperhalophilous, succulent, woody and semi-woody vegetation mainly spread in the salt basins of the Mediterranean and thermo-Atlantic coasts also represented into the Sinai Peninsula.

CARICETEA CURVULAE Br.-Bl. 1948 nom. cons. propos. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002

Acidophilous grasslands with an arctic-alpine distribution.

SPARTINETEA GLABRAE Tüxen in Beeftink 1962

Pioneer ampho-Atlantic and Mediterranean coastal vegetation consisting of perennial formations that grow on muddy brackish soils that are inundated for long periods of time; in the Mediterranean area, this vegetation only occurs in the northern Adriatic sector.

CARDAMINETEA HIRSUTAE Géhu 1999

Annual ephemeral vegetation, with a spring and summer life cycle, that develops in nitrified and semi-shaded habitats and that forms internal and external wood and shrub fringe communities.

GALIO APARINES-URTICETEA DIOICAE Passarge ex Kopecký 1969

Nitrophilous, mainly perennial or therophytic vegetation that is either anthropogenic or natural, and grows in mesophilous to hygrophilous habitats, depending on the degree of edaphic humidity and/or shade.

PARIETARIETEA JUDAICAE Oberdorfer 1977

Nitrophilous and synanthropic perennial vegetation dominated by hemicryptophytes. Chasmochomopytic communities that develop in cliff and wall crevices.

ADIANTETEA CAPILLI-VENERIS Br.-Bl. in Br.-Bl., Roussine & Nègre 1952

Chasmophytic vegetation that is rich in bryophytes and pteridophytes and grows in the water dripping crevices of calcareous rocks or of Tufa deposits. Mainly present in the Mediterranean macrobioclimate, though also in the Temperate macrobioclimate.

LITTORELLETEA UNIFLORAE Br.-Bl. & Tüxen ex Westhoff, Dijk & Passchier 1946

Perennial, pioneer, dwarf, amphibious vegetation that colonizes the shore of oligo-mesotrophic lakes and ponds.

MONTIO FONTANAE-CARDAMINETEA AMARAE Br.-Bl. & Tüxen ex Klika & Hadac 1944

Bryophyte-rich communities that colonize cold springs, small streams and dripping or wet walls, in an acidic to neutral-alkaline environment, found in the supra- to cryorotemperate bioclimatic belts, rarely in the lowland belt.

OXYCOCCO PALUSTRIS-SPHAGNETEA MAGELLANICI Br.-Bl. & Tüxen ex Westhoff, Dijk & Paschier 1946

Boreal vegetation of acid, oligotrophic, peat bogs with perennial vegetation dominated by Sphagnum and the presence of herbaceous phanerogams (mostly sedges), nano-phanerorophytes and, sometimes, of phanerophytes.

PHRAGMITO AUSTRALIS-MAGNOCARICETEA ELATAE Klika in Klika & Novák 1941

Perennial helophytic communities that colonize marsh and lacustrine environments as well as fluvial areas, on eutrophic to meso-oligotrophic soils of brackish and fresh waters.

SCHEUCHZERIO PALUSTRIS-CARICETEA NIGRAE nom. mut. propos. ex Steiner 1992

Hygrophilous fen vegetation dominated by sedges, with the possible presence of various mosses including Sphagnum, that grows from the lowland (glacial relicts) to the cryorotemperate thermotype belts, on peaty, para-peaty and mineral soils characterized by oligotrophic to mesotrophic nutrient conditions, neutral- alkaline to moderately acid acidity, and no dry-periods.

EPILOBIETEA ANGUSTIFOLII Tüxen & Preising ex Von Rochow 1951

Pioneer perennial herbaceous vegetation consisting of large macrophytes that grow on deep damp forest soils that are rich in organic matter; it is found in forest clearings or on the edges of forests in the Temperate macrobioclimate.

MULGEDIO ALPINI-ACONITETEA VARIEGATI Hada7 & Klika in Klika & Hada7 1944

Perennial vegetation of megaforbs and tall grasses found on deep humid soils that are rich in organic matter, from the supra-Mediterranean to the cryorotemperate thermotypes.

TRIFOLIO MEDII-GERANIETEA SANGUINEI Müller 1962

Herbaceous linear vegetation that occurs directly on woodland edges or in contact with their mantle communities.

BETULO CARPATICAE-ALNETEA VIRIDIS Rejmánek in Huml, Lepš, Prach & Rejmánek 1979

Meso-hygrophilous thickets or microforests of deciduous bushes or small trees that grow in ravines and on slopes, where avalanches released, and with abundant winter snow-cover, mainly in the subalpine areas but also in the high-montane ones.

CALLUNO VULGARIS-ULICETEA MINORIS Br.-Bl. & Tüxen ex Klika in Klika & Hada7 1944

Atlantic and sub-Atlantic small-shrub vegetation, dominated by chamaephytes and nano-phanerophytes (Ericaceae and Genisteae species), that grows on acidic substrata and poor soils.

CISTO LADANIFERI-LAVANDULETEA STOECHADIS Br.-Bl. in Br.-Bl., Molinier & Wagner 1940

Mediterranean, heliophilous, xerophytic vegetation, dominated by nano-phanerophytes and chamaephytes, that grows on eroded siliceous soils.

CISTO CRETICI-MICROMERIETEA JULIANAE Oberdorfer ex Horvat 1958

Chamaephytic and nano-phanerophytic communities, often rich in pulvinate and thorny species, that grow throughout the central and eastern Mediterranean on various substrata. In the Italian Peninsula, the syntaxon is found on the Adriatic side of the central-southern Apennines and in the Ionian sectors.

CYTISETEA SCOPARIO-STRIATI Rivas-Martínez 1975

Mantle vegetation and communities of fruticose species dominated by *Fabaceae* that grow on acidic soils in Atlantic, sub-Atlantic and west-Mediterranean areas.

RHAMNO CATHARTICAE-PRUNETEA SPINOSAE Rivas Goday & Borja ex Tüxen 1962

Shrubland and mantle communities that are dynamically related to the deciduous forests of the *Quercus-Fagetea* class.

ROSMARINETEA OFFICINALIS Rivas-Martínez, T.E. Díaz, F.Prieto, Loidi & Penas 2002

Mediterranean calcicole vegetation dominated by chamaephytes and nanophanerophytes that grows on immature or eroded soils. These communities typically occur in the Mediterranean macrobioclimate, where the thermotype is mesomediterranean to oromediterranean, though they can also be found in the mesotemperate thermotype (sub-Mediterranean variant of the temperate macrobioclimate), in the western and central Mediterranean.

NERIO OLEANDRI-TAMARICETEA AFRICANAE Br.-Bl. & O. Bolòs 1958

Shrub and tall-grass communities that grow on the beds and banks of intermittent rivers and creeks in the Mediterranean, Saharo-sindic and Irano-Turanian regions, in the infra- to meso-Mediterranean thermotype.

ROBINIETEA Jurko ex Hadac & Sofron 1980

Anthropogenic, neophytic forest vegetation dominated by *Robina pseudoacacia*, with a herb layer rich in nitrophilous species, often referred to the classes *Artemisietea vulgaris* and *Galio aparines-Urticetea dioicae*.

ERICO CARNEAE-PINETEA SYLVESTRIS Horvat 1959

This *syntaxon* includes western-central Alpine communities, such as xerophilous pine forests dominated by *Pinus sylvestris*, thermophilous pine forests dominated by *Pinus sylvestris* and *Ostrya carpinifolia*, as well as most of the *Pinus mugo* communities.

JUNIPERO SABINAE-PINETEA SYLVESTRIS Rivas-Martínez 1965 nom. inv. propos. Rivas-Martínez, Díaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002

Coniferous woodland communities and brushwoods dominated by juniper and co-occurring

shrubs that grow in the meso- to oro-Mediterranean and in the supra- to lower orotemperate thermotypes and are semi- continental and often relict.

QUERCO ROBORIS-FAGETEA SYLVATICAE Br.-Bl. & Vlieger in Vlieger 1937

Mesophilous and thermophilous forest vegetation typical of the Temperate macrobioclimate that grows in the mesotemperate and supratemperate thermotypes, occasionally extending as far as the Mediterranean macrobioclimate.

QUERCETEA ILICIS Br.-Bl. in Br.-Bl., Roussine & Nègre 1952

Vegetation communities that include prevalently evergreen and sclerophyllous forests, maquis and garrigues. They are found throughout the Mediterranean macrobioclimate, as well as in the temperate macrobioclimate but only in the mesotemperate thermotype. They have no particular soil requirements.

VACCINIO MYRTILLI-PICEETEA ABIETIS Br.-Bl. in Br.-Bl., Sissingh & Vlieger 1939

Forest acidophilous communities, dominated by conifers, that are distributed in circumboreal areas and are linked to regions with a cold to cold-temperate climate. In Italy they are found in the Alps as well as in some regions in the northern Apennines.

ALNETEA GLUTINOSAE Br.-Bl. & Tüxen ex Westhoff, Dijk & Passchier 1946

Eurosiberian and Mediterranean swamp forest and shrubland vegetation; it grows on the top of the gley soils that are usually rich in undecomposed organic matter up to peat.

SALICI PURPUREAE-POPULETEA NIGRAE Rivas-Martínez & Cantó ex Rivas-Martínez, Báscones, T.E. Díaz, Fernández-González & Loidi 2001

Meso-hygrophilous, riparian, deciduous forests that grow along alluvial plains in the Mediterranean, Thermo Atlantic and sub-Mediterranean Regions.

1.3 Study areas

The EIM was applied to three cases of soil stabilization works located on mountain slopes of three lateral valleys of Val Camonica (Lombardy, northern Italy, Southern Alps) (Figure 1.1):

- a) Azzone (Scalve Valley, BG; latitude: $45^{\circ} 58' 34.1''$ N, longitude: $10^{\circ} 07' 15.5''$ E)
- b) Val Dorena (Monno, BS; latitude: $46^{\circ} 12' 49.06''$ N, longitude: $10^{\circ} 17' 50.57''$ E)
- c) Val Palot (Pisogne, BS; latitude: $45^{\circ} 48' 25.44''$ N, longitude: $10^{\circ} 10' 00.25''$ E)

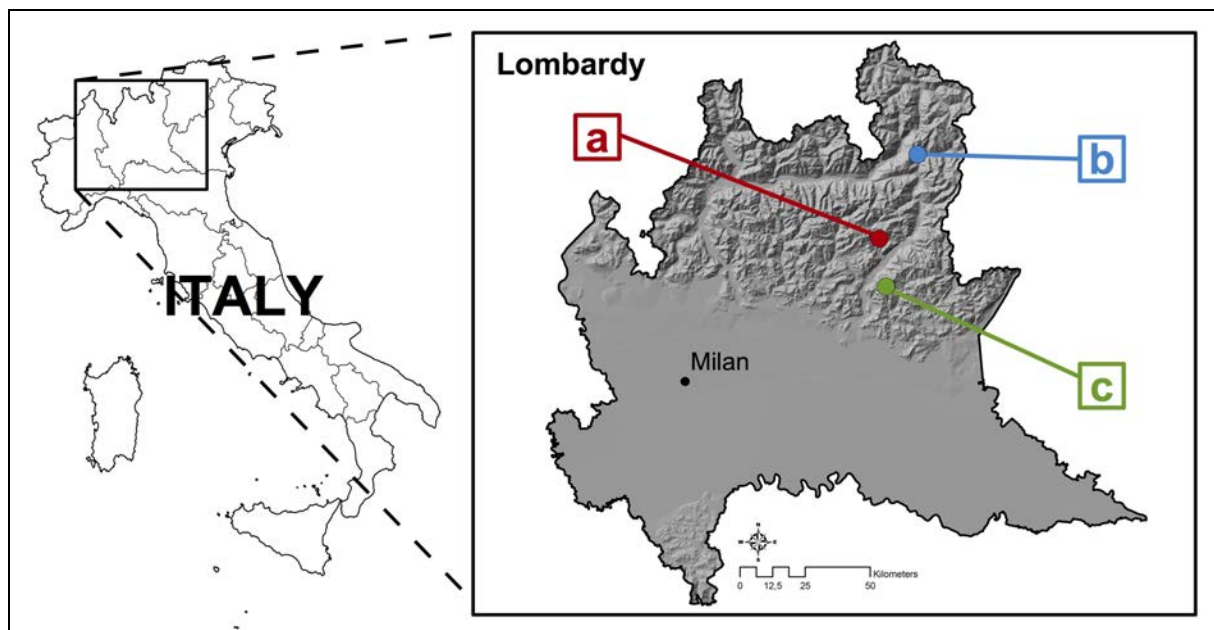


Figure 1.1 - Location of the study areas: Azzone (Val di Scalve) (a), Val Dorena (b) and Val Palot (c).

The three areas are part of the North-eastern ecoregional Subsection (Central and Eastern Alps Section, Alpine provinces, Temperate Division) (BLASI et al. 2014) of the Temperate Oceanic bioclimate zone (RIVAS-MARTÍNEZ & RIVAS-SÁENZ 2009). The three sites were affected by landslides and subject to slope stabilization works carried out with soil bioengineering techniques. In Val Dorena, further landslides occurred (and still occur), after soil stabilization and environmental restoration work, in Azzone exotic species were sown including *Lupinus polyphyllus* (currently present), while in Val Palot no exotic species were sown and no further landslides have occurred after the environmental restoration works.

1.3.1 Azzone

The study area is located in a catchment basin of the municipality of Azzone (Scalve Valley, Lombardy, Northern Italy; coordinates: 45° 58'34.1" N, 10° 07'15.5" E), has an area of 13.000 m² and is localized at an altitude which ranges from 1.000 to 1.150 m. It is a natural area that is within the Site of Community Importance "Alta Val di Scalve" (SIC 2060004) and has undergone stabilization work using soil bioengineering (BISCHETTI et al. 2012) techniques, following a landslide. The landslide occurred in April 1992 and involved debris-colluvial material sliding on underlying bedrock consisting of Mesozoic rocks with low permeability belonging to the formation of "Argillite of Lozio". The landslide material, in the form of mud and debris, flowed down a small catchment basin removing vegetation, soil and part of the forest road located below. Work to stabilize the mountainside was performed in 1995 under the direction of the Regional Forestry Administration. Figure 1.2 shows the map of the work carried out to stabilize the slope.

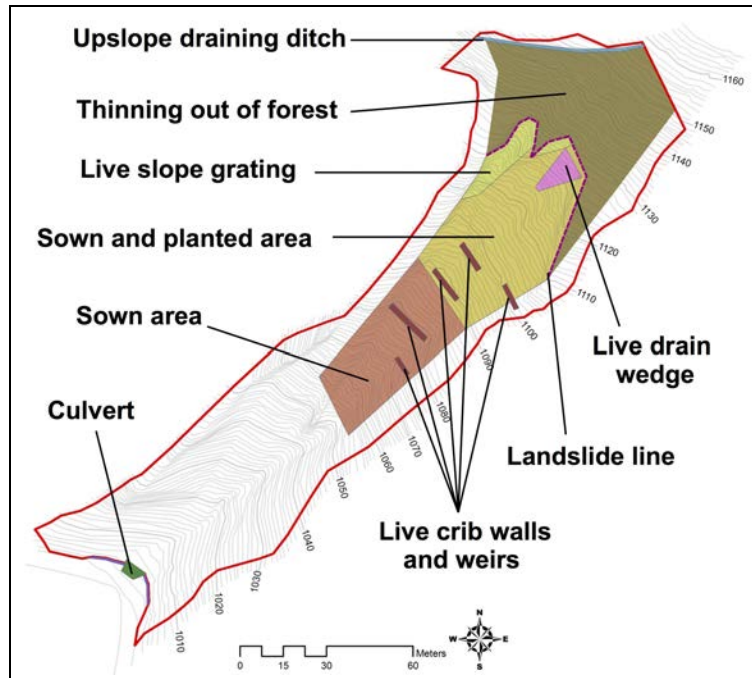


Figure 1.2 - Map of soil stabilization works of Azzone (modified from MAURO 2000)

Upon completion of soil bioengineering works, vegetation was restored by planting some tree species (*Acer pseudoplatanus*, *Acer campestre*, *Fraxinus excelsior*, *Fraxinus ornus*, *Salix appendiculata*, *Salix caprea*, *Salix purpurea*, *Sorbus aucuparia* and *Fagus sylvatica*) and sowing various grass species (*Festuca rubra*, *Festuca cinerea*, *Trifolium repens*, *Trifolium pratense*, *Lotus corniculatus*, *Poa pratensis*, *Lolium perenne*, *Dactylis glomerata*, *Achillea millefolium*, *Medicago lupulina*, *Onobrychis viciifolia*, *Phleum pratense*, *Sanguisorba minor*, *Anthyllis vulneraria*, *Lathyrus pratensis* and *Lupinus polyphyllus*). Sowing was performed by broadcast sowing a generic commercial seed mixture (Table 1.2) without taking into consideration its floristic composition, while the choice of tree

species was more meticulous: broad-leaved trees typical of woods in the study area, obtained from the nearby forest nursery in Borno (BS), were planted. The soil stabilization project did not provide for maintenance work. Nevertheless, no further landslides have occurred since work finished.

Species	%
<i>Festuca rubra</i> L.	32
<i>Festuca cinerea</i> Vill.	28
<i>Trifolium repens</i> L.	7
<i>Lotus corniculatus</i> L.	6
<i>Poa pratensis</i> L.	5
<i>Lolium perenne</i> L.	4
<i>Dactylis glomerata</i> L.	3
<i>Achillea millefolium</i> L.	2
<i>Medicago lupulina</i> L.	2
<i>Onobrychis viciifolia</i> Scop.	2
<i>Phleum pratense</i> L.	2
<i>Sanguisorba minor</i> Scop.	2
<i>Trifolium pratense</i> L.	2
<i>Anthyllis vulneraria</i> group	1
<i>Lathyrus pratensis</i> L.	1
<i>Lupinus polyphyllus</i> Lindl.	1

Table 1.2 - Floristic composition and percentage of species making up the seed mixture used for sowing.

The study area has a pre-alpine suboceanic climate (Figure 1.3), and belongs to the South-Orobic Geobotanical District (ANDREIS 2002) within the Central Alpine sector of the Eurosiberian biogeographical region (RIVAS-MARTÍNEZ et al. 2004). According to the recent classification of the ecoregions of Italy proposed by BLASI et al. (2014) it is part of the North-eastern Alps ecoregional subsection (Central and Eastern Alps section, Alpine Province, Temperate Division). It is part of the Central-western Alpine neutro-basophilic series of silver fir and spruce (*Abieti-*

Piceion) (VERDE et al. 2010) in which the mature vegetation stage (current potential vegetation) consists of montane coniferous forests (*Picea abies* and *Abies alba*) with *Fagus sylvatica* (DEL FAVERO 2002, 2004) while the intermediate stages are made up, in dynamic-evolutive order, of communities of *Adenostylion alliariae/Calamagrostion villosae* (fringe) and of the community of *Sambuco racemosae-Salicion capreae* (forest mantle). A comparison of the past five years of weather data with the historical series provided by MAURO (2000), shows no significant variations that could have modified the vegetation dynamics; this suggests that the current potential vegetation is the same as that of twenty years ago.

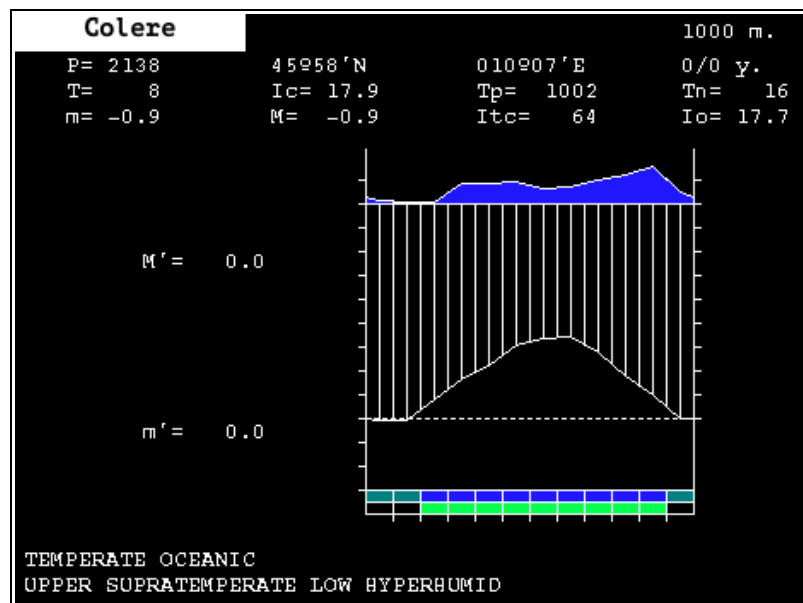


Figure 1.3 - Ombrothermic diagram (WALTER & LIETH 1960) of the weather station of Colere (Scalve Valley). Data source: weather station of Piantoni M., 2010-2014. The diagram has been created using the software developed by RIVAS-MARTÍNEZ & RIVAS-SÁENZ (2009).

The soils outside the landslide area are shallow or moderately deep soils of Calcaric Cambisols type (PREVITALI et al. 1992) while the landslide area presents scarcely evolved and extremely thin soil that developed on the landslide debris. A thin layer of soil, sourced locally, was added during restoration work but only in less steep areas where trees were planted.

1.3.2 Val Dorena

Val Dorena (b) is located within the municipality of Monno (high Val Camonica; altitude: 1600 m a.s.l.) and has a catchment area of about 2.5 km² of which 0.12 km² still under active erosion. Average annual rainfall ranges from 900 mm to 1000 mm (Figure 1.4) and the geological substratum is represented by micaschists, gneisses and phyllites of the Archeozoic era above which loose fluvioglacial debris of the Quaternary period are present.

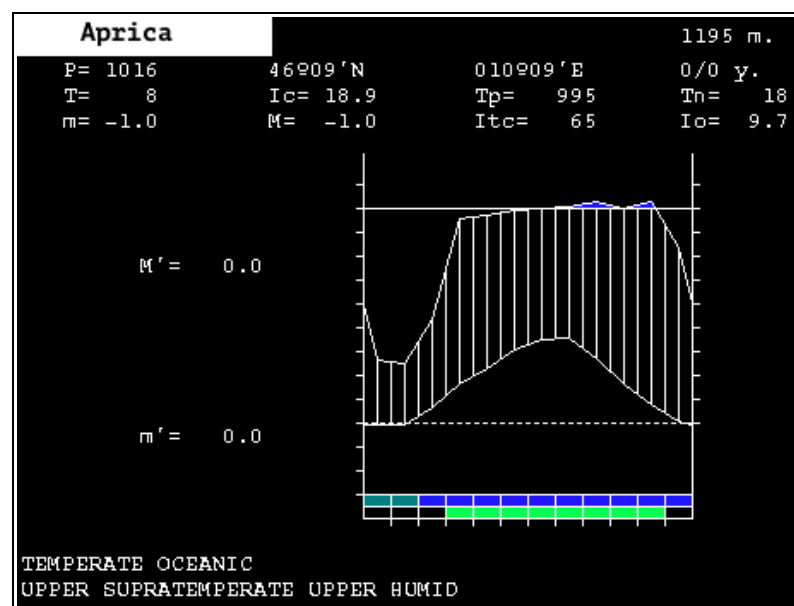


Figure 1.4 - Ombrothermic diagram (WALTER & LIETH 1960) of the weather station of Aprica (SO). Data source: Centro Meteorologico Lombardo, 2009-2013. The diagram has been created using the software developed by RIVAS-MARTÍNEZ & RIVAS-SÁENZ (2009).

The area is subject to instability, already reported at the beginning of the 20th century and still active, caused by the erosive activity of the Dorena stream at the

base of the slopes which has gradually laid bare the bedrock and destabilized the fluvioglacial deposits. The erosive activity of the stream combined with the runoff action of surface water shape the landslide's scarp; the resulting steepness contributes to slope instability (Figure 1.5). In the past (from the '60s to the '80s), several slope stabilization works were performed using traditional techniques in order to limit soil erosion. The area considered in this study, however, includes only a part of the whole area of instability – the area in which the most recent works were carried out in 2000 according to soil bioengineering criteria (Figure 1.6). Val Dorena belongs to the High Camuno Geobotanical District (ANDREIS 2002) and is part of the Central Alpine acidophilus series of silver fir and spruce (VERDE et al. 2010) in which the mature vegetation stage consists of forest dominated by *Picea abies* (DEL FAVERO 2002, 2004; VERDE et al. 2010) established on coarse and moderately deep acid reaction soils (Spodi-Distric Cambisols) (PREVITALI et al. 1992).

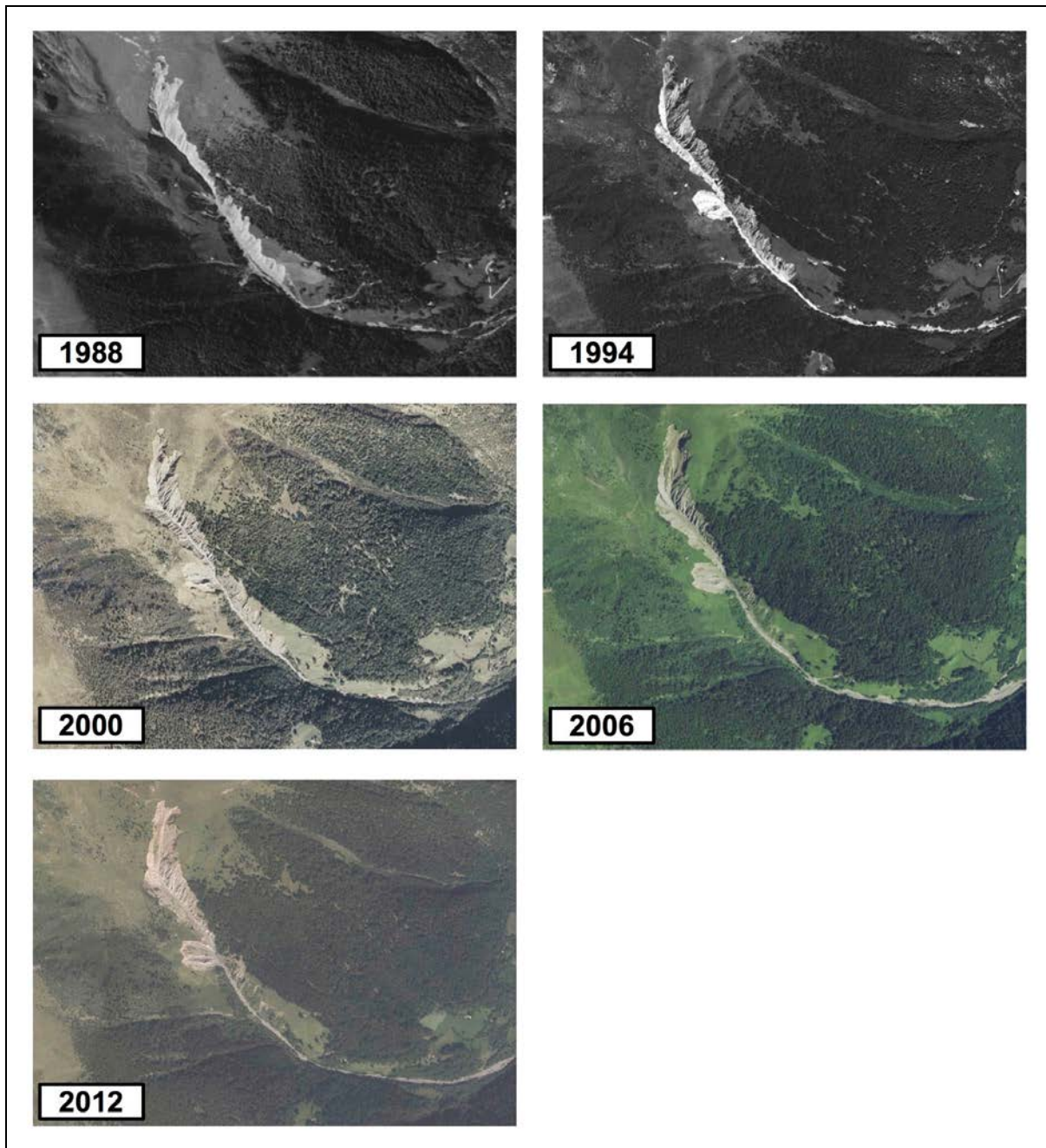


Figure 1.5 - Study area in the year 1988, 1994, 2000, 2006 and 2012. Data source: Geoportale Nazionale (www.pcn.minambiente.it/GN/)

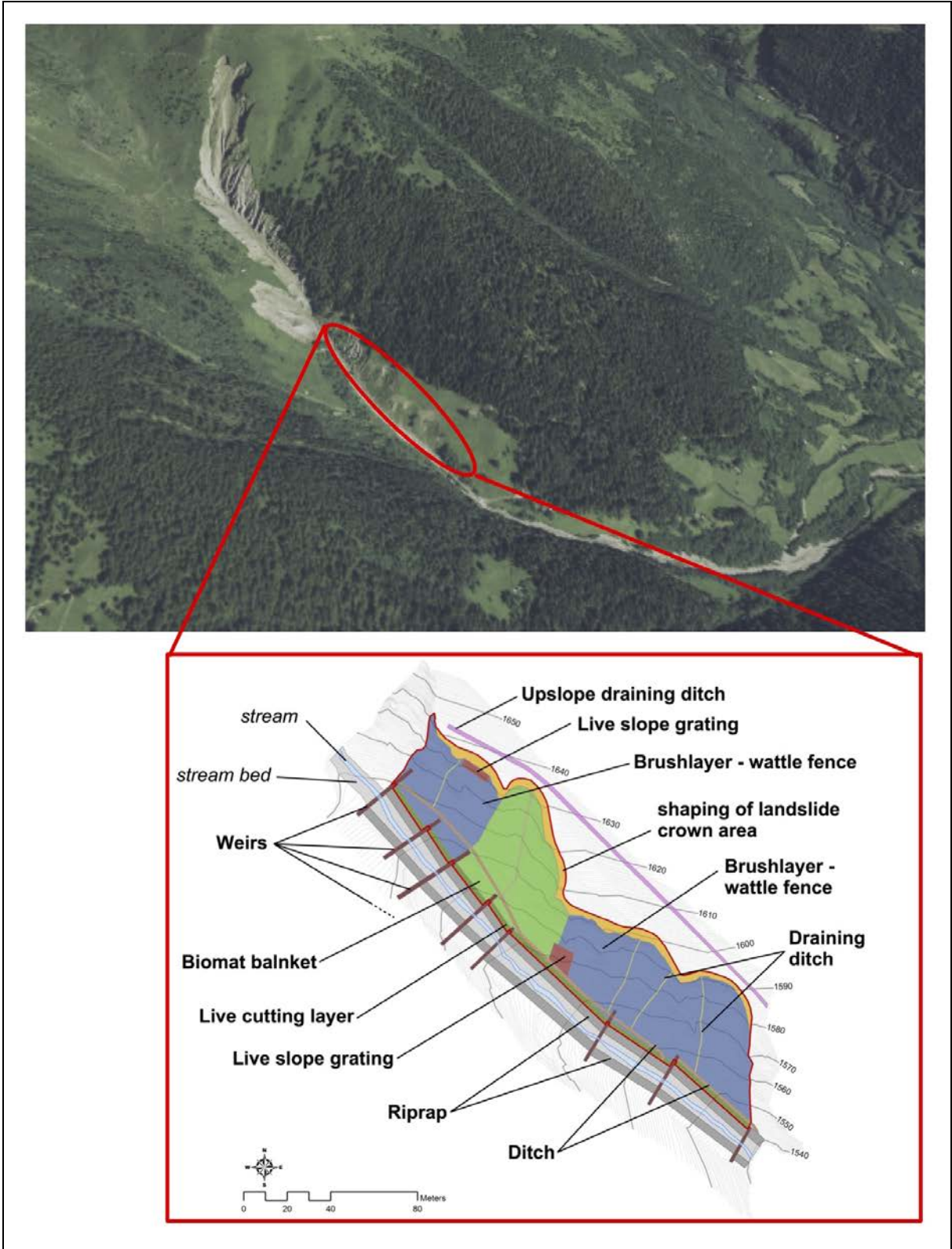


Figure 1.6 - Map of soil stabilization work performed in Val Dorena in 2000.

1.3.3 Val Palot

Val Palot (c) is located in the Municipality of Pisogne in the Prealps of the province of Brescia (altitude: 1000 m a.s.l.) and has a suboceanic climate where average annual rainfall totals about 1500 mm (Figure 1.7).

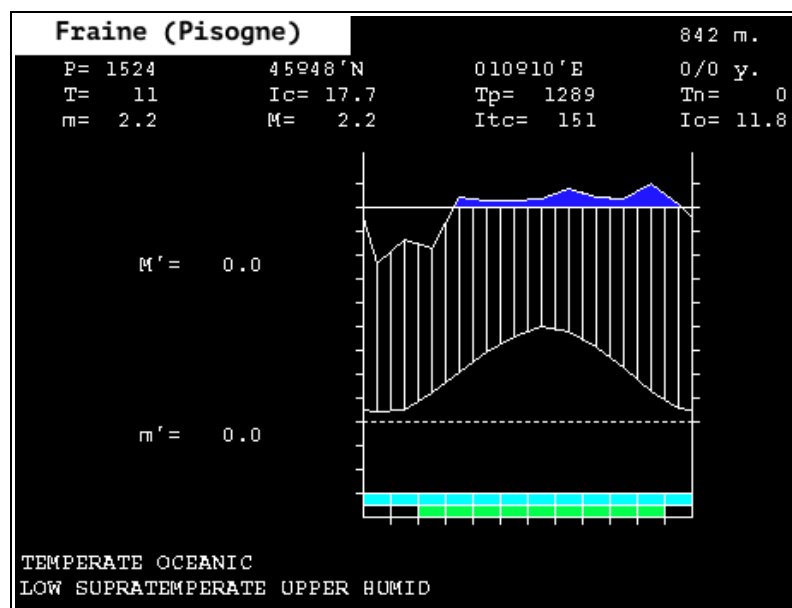


Figure 1.7 - Ombrothermic diagram (WALTER & LIETH 1960) of the weather station of Fraine (Pisogne, Val Palot). Data source: ARPA Lombardia, 2005-2014. The diagram has been created using the software developed by RIVAS-MARTÍNEZ & RIVAS-SÁENZ (2009).

In 1993 it was affected by extensive instability over an area of approximately 4000 m² which is located at the escarpment of a glacial terrace near Palotto. This terrace, consisting of glacial and fluvioglacial deposits lying on a substrate consisting of metamorphic rocks part of the formation "Maniva micaschists", was subject to landslides of a certain importance even before 1993 and, hence, metal

cages were installed to stabilize the slope in the 1960s but did not produce the desired results. After the 1993 landslide, slope stabilization was performed in 1996 using soil bioengineering techniques (Figure 1.8), after which there were no further landslides.

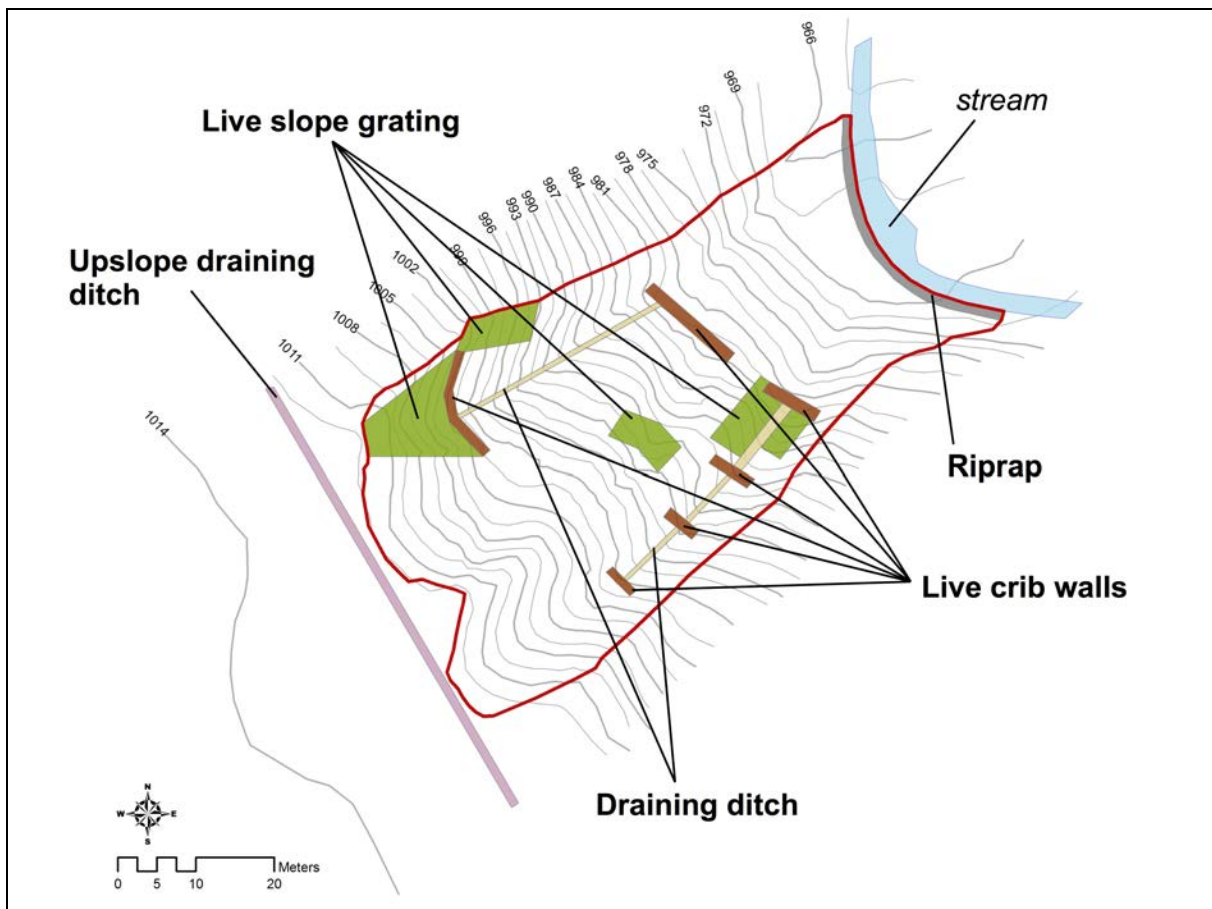


Figure 1.8 - Map of soil stabilization work performed in Val Palot in 1996.

The study area is part of the Camuno-Caffarese Geobotanical District (ANDREIS

2002) and the Central Alpine acidophilus series of silver fir and spruce (VERDE et al. 2010). The mature vegetation stage consists of spruce forest (*Picea abies*) together with beech (*Fagus sylvatica*) (DEL FAVERO 2002, 2004) on moderately deep soil (Spodi-Distric Cambisols) (PREVITALI et al. 1992).

1.4 Materials and methods

Data on the vegetation of the three study areas were collected by performing some phytosociological relevés (fifteen in Azzone, sixteen in Val Dorena and twelve in Val Palot) in accordance with the method of the Zurigo-Montpellier Sigmatist school (BRAUN-BLANQUET 1964) and using the conventional Braun-Blanquet abundance/dominance scale (r = rare species in the relevé; + = coverage < 1%; 1 = coverage 1-5%; 2 = coverage > 5-25%; 3 = coverage > 25-50%; 4 = coverage > 50-75%; 5 = coverage > 75-100%) to estimate species coverage.

In general the relevés of the forest and shrubland areas were performed on a plot of 100 m² (10 x 10 m) while the relevés of the herbaceous vegetation were performed on an area of 25 m² (5 x 5 m). The relevés were performed in spring and summer 2014 (Azzone) and 2015 (Val Dorena and Val Palot). Each relevé was georeferenced with a GPS (Global Positioning System). Species were determined using the floristic keys of PIGNATTI (1982) and TUTIN et al. (1968) while the chorological type (chorotype) of each species was extrapolated from "Flora of Italy" (PIGNATTI 1982). OBERDORFER (1992), MUCINA et al. (1993), DEL FAVERO (2002), AESCHIMANN et al. (2004), UBALDI (2008a, 2008b), LANDOLT et al. (2010) and BIONDI & BLASI (2015), were consulted for phytosociological data. The

scientific names of species follow the recent flora of Central and Eastern Lombardy (MARTINI et al. 2012) while syntaxa nomenclature follows that of the Vegetation Prodrome of the Italy (BIONDI et al. 2014; BIONDI & BLASI 2015).

Vegetation data were analysed statistically using cluster analysis in order to highlight the floristic and physiognomic similarities of the relevés after ordinal values of abundance/dominance had been converted into percentage of coverage as proposed by CANULLO et al. (2012): r = 0,01 %; + = 0,5 %; 1 = 3,0 %; 2 = 15,0 %; 3 = 37,5 %; 4 = 62,5 %; 5 = 87,5 %. Cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and the chord distance coefficient (LEGENDRE & GALLAGHER 2001). The relevés were also sorted according to principal coordinates analysis (PCoA). Statistical analysis was performed using R 3.2.1 (R CORE TEAM 2015).

Combining the results of data analysis, field observations and georeferenced points collected, a vegetation map of the study areas was also drawn up using ArcGIS 10 software (©Esri, Redlands, CA, USA). The IE, IL, IM and EIM were calculated for each vegetation group using the above-mentioned formulas. GIS (Geographic Information System) software was used to calculate the average EIM value of the entire areas subject to soil stabilization work considering the extent of each type of vegetation.

1.5 Results

The results provided by the floristic-vegetational analysis conducted in the three study areas are shown below.

1.5.1 Azzone

152 species were identified in the study area of Azzone, most of which are common in central-eastern Lombardy (MARTINI et al. 2012). *Festuca cinerea*, an endemic species of the Western Alps sown once soil stabilization work had finished, was considered an allochthonous species since it does not belong to the flora of the district in which the study area is situated.

The results provided by the statistical analysis (cluster analysis and PCoA) of the 15 relevés conducted in this study area identified six types of vegetation (Figure 1.10):

- Montane *Picea abies* forest (Aa): vegetation characterized by *Picea abies* (dominant tree) with some species of *Fagetalia sylvaticae* (*Quercus robur*-*Fagetea sylvaticae*) including *Fagus sylvatica*, *Hedera helix*, *Hepatica*

nobilis, *Lathyrus vernus*, *Helleborus niger*, *Cyclamen purpurascens*,
Veronica urticifolia, *Luzula nivea* and *Galium sylvaticum*.

- *Festuca cinerea* grassland (Ba): made up of *Festuca cinerea* (dominant grass sown when soil stabilization works had finished) and some species of *Bromion erecti* (*Origanum vulgare*, *Sanguisorba minor* and *Calamagrostis varia*).
- Vegetation dominated by shrubs and broadleaf trees (Ca): plant community made up of shrubs of *Sambuco racemosae-Salicion capreae* (*Corylus avellana*, *Salix caprea*, *Rosa canina*, *Rubus idaeus* and *Rubus ulmifolius*) and of *Mulgedio alpini-Aconitetea variegati* (*Rubus caesius* and *Salix appendiculata*) with some young trees of *Fagetalia sylvaticae* (*Fraxinus excelsior*, *Acer pseudoplatanus* and *Laburnum alpinum*). The herbaceous layer consists in various plants of *Quercu roboris-Fagetea sylvaticae*.
- *Calamagrostis varia* grassland (Da): vegetation made up of *Calamagrostis varia* (dominant species) and other species including *Carex flacca*, *Sanguisorba minor*, *Origanum vulgare*, *Eupatorium cannabinum*, *Clematis*

vitalba (*Bromion erecti*), *Petasites albus* and few species of *Sambuco racemosae-Salicion capreae* and *Seslerietalia ceruleae*.

- *Hieracium tenuiflorum-Origanum vulgare* community (Ea): made up of several species of *Trifolio medii-Geranietea sanguinei* (*Hieracium tenuiflorum*, *Galium mollugo*, *Hypericum montanum*, *Hypericum perforatum*, *Veronica chamaedrys* and *Clinopodium vulgare*) and some species of *Bromion erecti* (such as *Origanum vulgare*, *Sanguisorba minor*, *Anthyllis vulneraria*, *Briza media* and *Carex caryophyllea*).
- *Lupinus polyphyllus-Fraxinus excelsior* community (Fa): vegetation with *Lupinus polyphyllus* (dominant exotic species sown at the end of the soil stabilization works), other species of *Atropetalia belladonnae* (*Fragaria vesca*, *Bromus ramosus*, *Myosotis sylvatica*, *Eupatorium cannabinum* and *Digitalis lutea*) and some trees of *Querco-Fagetalia sylvaticae* (*Fraxinus excelsior* and *Acer pseudoplatanus*). More details on the ecology of this plant community are reported in GIUPPONI et al. (2015b) (Supporting Material II, page 45).

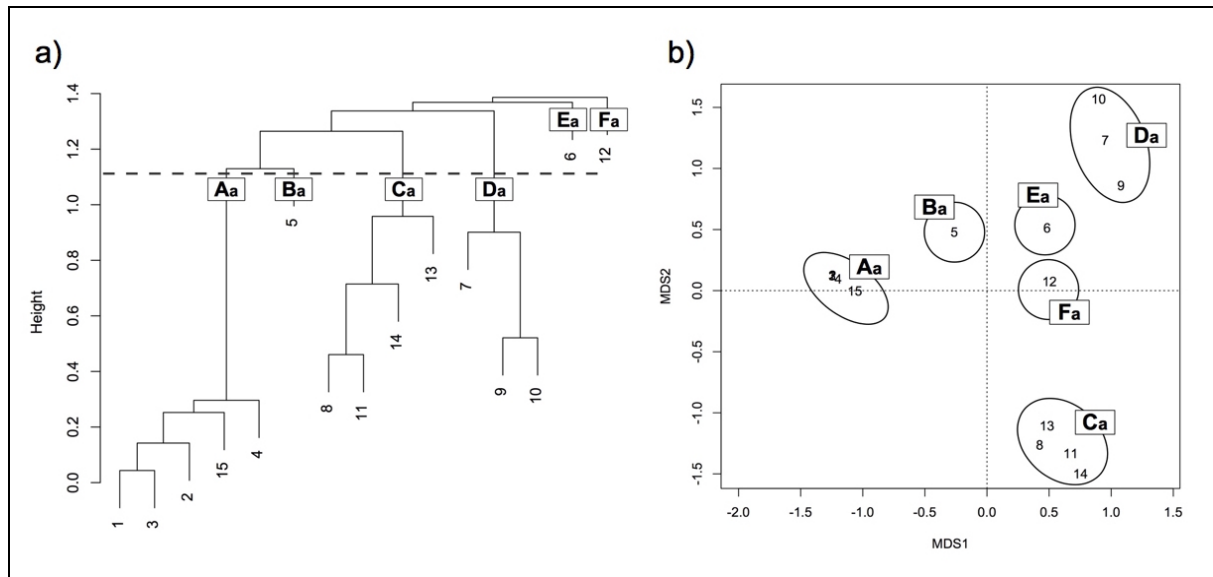


Figure 1.10 - Dendrogram of relevés (a) performed in the study area of Azzone provided by cluster analysis and ordering of the relevés according to PCoA (b). The numbers indicate the codes of each relevé and the letters in the boxes identify the groups of vegetation (clusters). Variance justified by the PCoA axes: MDS1 = 33,53%; MDS2 = 20,66%. The data provided by the PCoA confirm the subdivision of the relevés into five groups according to the results of cluster analysis.

For further details on the composition of the six types of vegetation see the phytosociological table of the relevés (Supplemental Data I).

The vegetation map (Figure 1.11) shows that shrubland (Ca) is the dominant type of vegetation in the restored area, extending along the entire edge of the conifer forest (Aa). *Calamagrostis varia* grassland (Da) and *Hieracium tenuiflorum-Origanum vulgare* community are located in the centre of the valley where the soil is not completely stabilized while *Festuca cinerea* grassland (Ba) and *Lupinus polyphyllus-Fraxinus excelsior* community (Fa) are present in patches. The vegetation of cluster Aa represents the forest outside the area in

which the soil bioengineering work took place, and which should therefore have been present in the study area before the landslide and subsequent environmental restoration. It would seem reasonable to presume that this forest represents the current potential vegetation (BIONDI 2011) of the study area.

Table 1.3 lists the values of IE, IL, IM and EIM of the six vegetation types of the study area of Azzone.

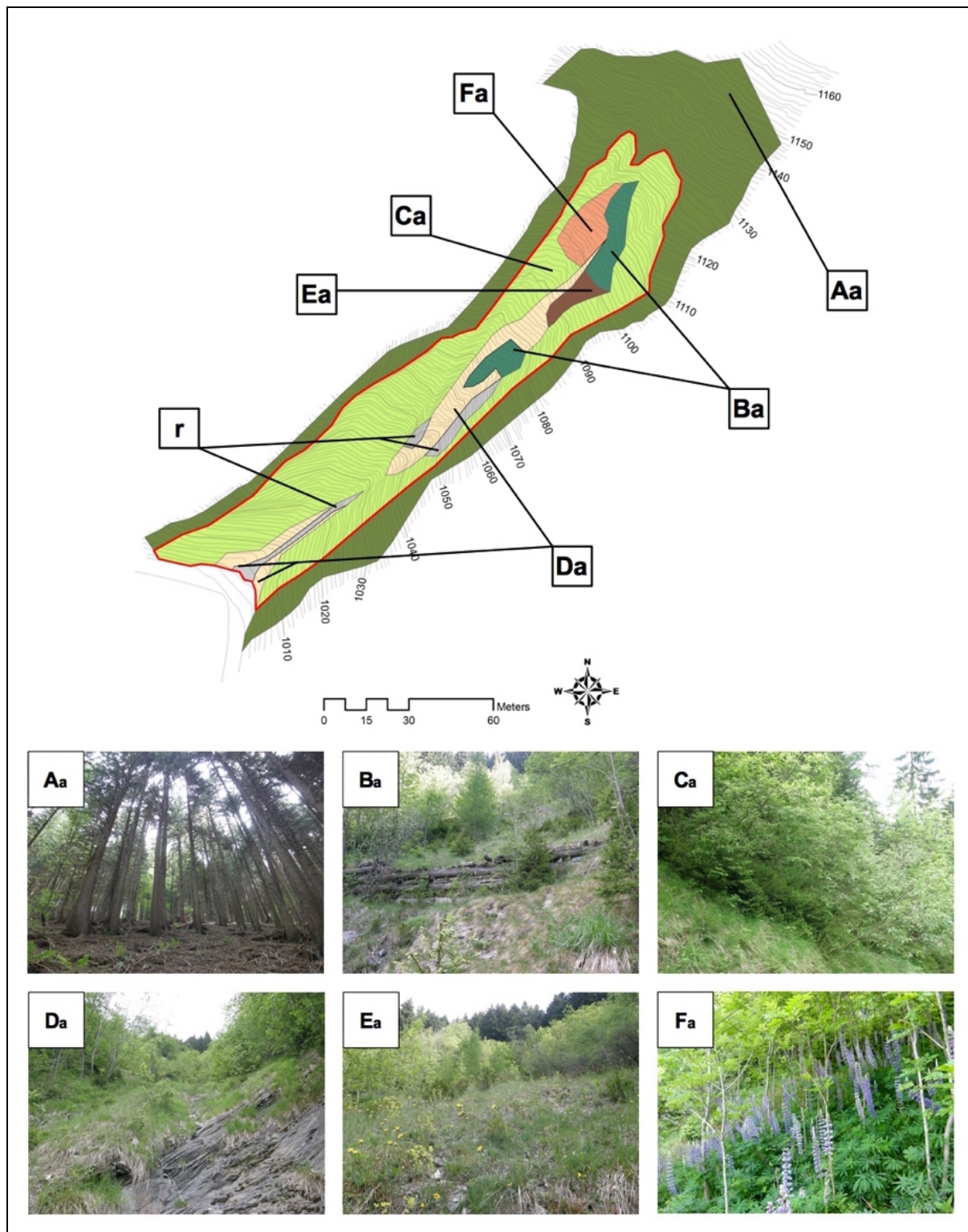


Figure 1.11 - Map of current vegetation of the study area of Azzone and images of the six types of vegetation. The letters in the boxes identify the types of vegetation: A_a, montane *Picea abies* forest; B_a, *Festuca cinerea* grassland; C_a, vegetation dominated by shrubs and broadleaf trees; D_a, *Calamagrostis varia* grassland; E_a, *Hieracium tenuiflorum-Origanum vulgare* community; F_a, *Lupinus polyphyllus-Fraxinus excelsior* community; r, rocks). The red line marks the area affected by soil bioengineering works.

Vegetation	IE	IL	IM	EIM
Aa	0,80	0,00	8,86	8,79
Ba	52,43	0,00	6,44	3,06
Ca	0,19	0,00	7,90	7,89
Da	0,40	0,38	6,35	6,33
Ea	1,11	1,13	6,93	6,86
Fa	53,59	0,00	7,49	3,56

Table 1.3 - Values of IE, IL, IM and EIM of the six vegetation types of Azzone (A_a, montane *Picea abies* forest; B_a, *Festuca cinerea* grassland; C_a, vegetation dominated by shrubs and broadleaf trees; D_a, *Calamagrostis varia* grassland; E_a, *Hieracium tenuiflorum-Origanum vulgare* community; F_a, *Lupinus polyphyllus-Fraxinus excelsior* community).

Particularly high values of IE are associated with vegetation clusters Fa and Ba which have extensive cover of two allochthonous species: *Lupinus polyphyllus* and *Festuca cinerea* respectively. Alien species are much less abundant in the other clusters. The calculation of the IL shows that the endemic component is present only in clusters Da and Ea and is represented by a few species with low coverage values. Conifer forest (Aa) is the vegetation with the highest value of IM, followed by shrubland (Ca), *Lupinus polyphyllus-Fraxinus excelsior* community (Fa), *Hieracium tenuiflorum-Origanum vulgare* community (Ea), *Festuca cinerea* grassland (Ba) and *Calamagrostis varia* grassland (Da). The highest value of EIM is that of conifer forest (Aa) followed by shrubland (Ca), *Hieracium tenuiflorum-Origanum vulgare* community (Ea), *Calamagrostis varia* grassland (Da), *Lupinus polyphyllus-Fraxinus excelsior* community (Fa) and

Festuca cinerea grassland (Ba). The EIM value of clusters Ba and Fa is much lower than the IM value due to the greater presence of alien species and the absence of endemic species in these vegetation groups. EIM values for clusters Ca, Da and Ea are also lower than IM values but less markedly than in clusters Ba and Fa, due to a reduced exotic component and the presence of some endemic species.

Supporting Material II

Lupinus polyphyllus-*Fraxinus excelsior* community: floristic, vegetational and ecological characteristics

The *Lupinus polyphyllus*-*Fraxinus excelsior* plant community (Fa) has some unique characteristics due to the abundant presence of *Lupinus polyphyllus* (GIUPPONI et al. 2015b). *Lupinus polyphyllus* Lindl. (large-leaved lupine, big-leaved lupine, many-leaved lupine or garden lupine) is a legume native to North-west America which was introduced to Britain in 1846 and quickly spread throughout Europe as an ornamental plant or for forage (TUTIN et al., 1968; FREMSTAD, 2010).

It is a species reported in the inventory of European alien species "Delivering Alien Invasive Species Inventories for Europe" (DAISIE, 2009) and is now spontaneous throughout the Alps (AESCHIMANN et al., 2004; FOEN, 2006; CELESTI-GRAPPOW et al., 2009, 2010; LANDOLT et al., 2010). In Lombardy it was observed for the first time in Monno and in Temù (Valcamonica) by Silvio Frattini in 2006. However, this observation, reported by CONTI et al. (2007) and BANFI & GALASSO (2010), was not included in the flora of Central-eastern Lombardy by MARTINI et al. (2012). *Lupinus polyphyllus* is a mesophilic perennial herbaceous species (hemicryptophyte) living in areas with an oceanic climate and soils with a neutral acid reaction, moderately rich in nutrients and humus (LANDOLT et al., 2010). In the Alps it prefers hilly and mountain areas (AESCHIMANN et al., 2004) contributing to the formation of acidophilus plant communities with megaphorbs that grow at the edges and in the clearings of deciduous or coniferous forests. These communities belong to *Epilobion angustifolii* Tüxen ex Eggler 1952 (*Atropetalia belladonnae* Vlieger 1937; *Epilobietea angustifolii* Tüxen & Preising ex Von Rochow 1951) (AESCHIMANN et al, 2004; LANDOLT et al., 2010).

GIUPPONI et al. (2015b) analyzed the floristic, vegetational and ecological characteristics of a community of *Lupinus polyphyllus*-*Fraxinus excelsior* present in the study area in Azzone (Figure II.1). The main results provided by this analysis are shown below.



Figure II.1 - *Lupinus polyphyllus*-*Fraxinus excelsior* plant community.

The phytosociological relevé of the vegetation (Supplemental Data I, relevè n. 12) identified the 34 species making up the community. Most of these species are common in the territory of Central-eastern Lombardy (MARTINI et al., 2012). *Lupinus polyphyllus* is the species dominating the herbaceous layer, accompanied by other species typical of the edges and clearings of mountain forests (*Epilobietea angustifolii*; *Trifolio medii-Geranietea sanguinei*; *Mulgedio alpini-Aconitetea variegati*) together with those of meadows (*Molinio-Arrhenatheretea*), sown at the end of the soil stabilization work. The shrub layer consists of some species of *Rhamno catharticae-Prunetea spinosae* while tree cover is provided mainly by maples and ashes (*Acer pseudoplatanus* and *Fraxinus excelsior*), some of which were planted during environmental restoration work.

Figure II.2 shows the biological spectrum and the chorological spectrum of the station's flora. The biological spectrum highlights the fact that the most abundant category is that of the hemicryptophytes (61.8%) followed by phanerophytes (11.8%) and geophytes (8.8%). Chorological analysis shows that paleotemperate, Euro-Siberian and Eurasian species together account for 41.2% of the total, followed by orophytes, European-Caucasian and circumboreal species. Exotic species account for 2.9%, while no endemic species were found. *Festuca cinerea* has been introduced when seed was sown (MAURO, 2000).

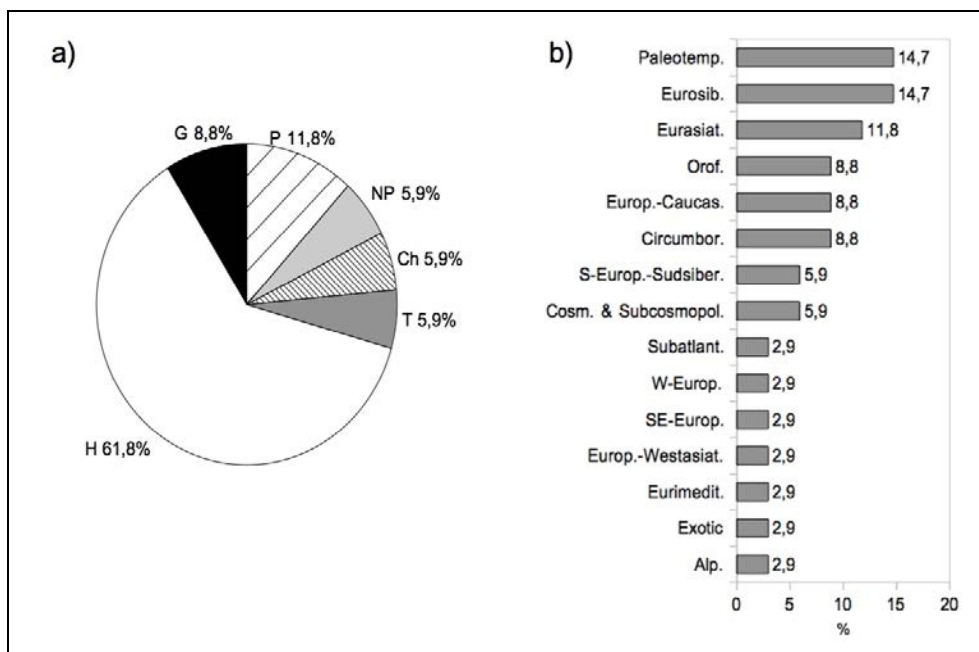


Figure II.2 - Biological spectrum (a) and chorological spectrum (b) of *Lupinus polyphyllus-Fraxinus excelsior* plant community (T, therophytes; H, hemicryptophytes; Ch, chamaephytes; G, geophytes, P, phanerophytes; NP, nanophanerophytes).

Analysis of the graphs regarding the ecological indices of LANDOLT et al. (2010) (Fig. II.3) shows that plant communities in which *Lupinus polyphyllus* is present are made up of species requiring the moderately moist soils with a neutral-acid reaction, fairly rich in nutrients and poorly aerated, of semi-shaded mountain stations with sub-oceanic climate.

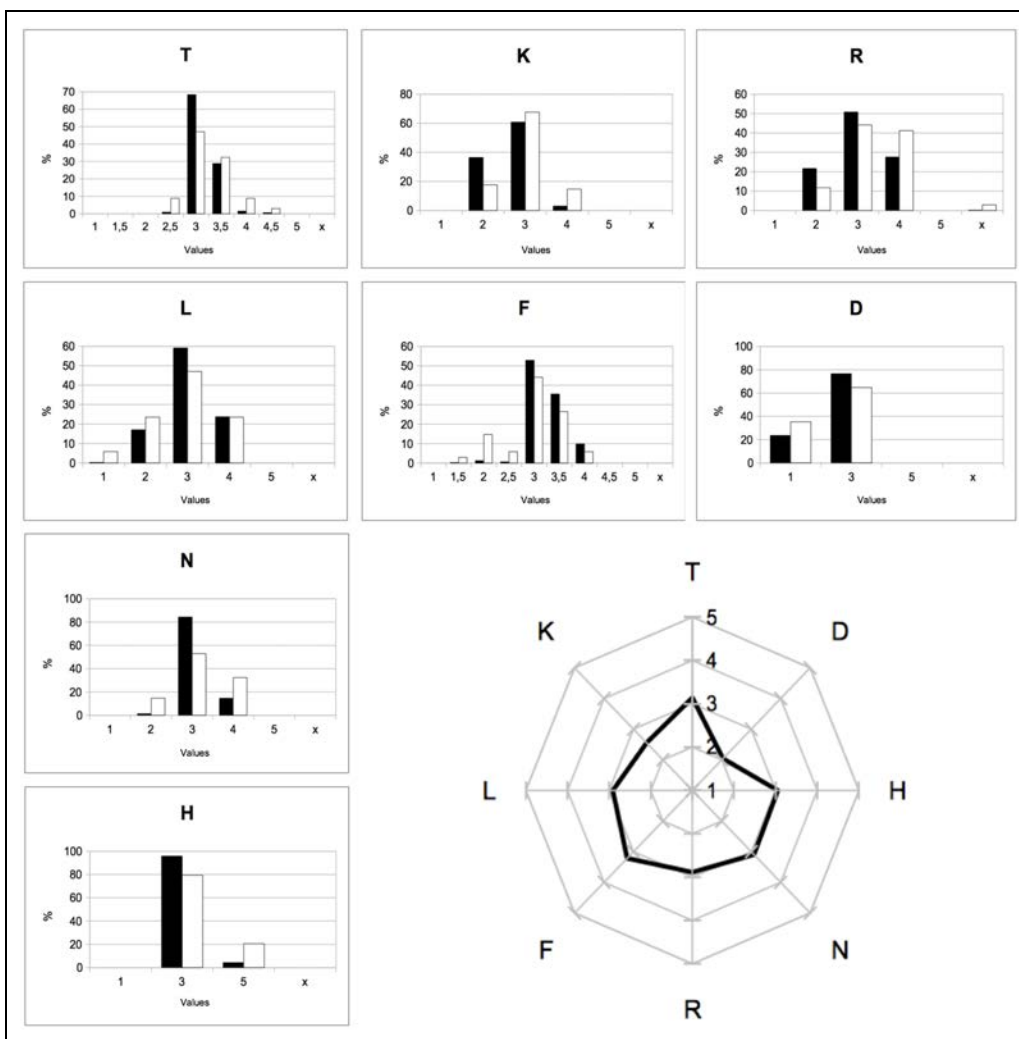


Figure II.3 - Histograms of the ecological indices of LANDOLT et al. (2010) (the white bars refer to species frequency while the black bars refer to species coverage) and radar graph (the average value of each index were weighted for species coverage). Legend: T, temperature; K, continentality; L, light; F, moisture; R, reaction; N, nutrients; H, humus; D, aeration.

In the area where the relevé was performed there is a forest of *Acer pseudoplatanus* and *Fraxinus excelsior* trees in which herbaceous layer mainly consists in species sown at the end of the slope stabilization work (dominated by *Lupinus polyphyllus*), accompanied by other species typical of more mature dynamic stages. The environmental and ecological characteristics (provided by the analysis of the vegetation) of the study area highlighted similarities with the requirements of ornamental lupine (soil with a neutral-acid reaction, moderately rich in nutrients and humus; sub-oceanic climate; montane belt), hence justifying the fact that the plant took root and survived in the area. There are several species of lupine in the Alps (*Lupinus albus* L., *Lupinus angustifolius* L., *Lupinus reticulatus* Desv., *Lupinus luteus* L. and *Lupinus perennis* L.) (AESCHIMANN et al., 2004; LANDOLT et al., 2010) but the thermal requirements of *Lupinus polyphyllus* make it the only species able to establish itself in the montane, and sometimes in the subalpine, belt (AESCHIMANN et al., 2004). The requirements in terms of light (L = 3) and moisture (F = 3), restrict the ornamental lupine to environments that are not too bright and to soils with at least moderate water availability.

It is reasonable to presume that *Lupinus polyphyllus* has been stably present in the restored area since it was sown, since the ecological and environmental conditions of the territory do not seem to have changed significantly over this period and to date. It was definitely present in 2000 since it was observed by MAURO (2000) during floristic-physiognomic monitoring of the vegetation, associated with environmental engineering work carried out in the area, and reported as *Lupinus perennis*. The stable presence of the lupine seems linked to an equilibrium based on the fact that young maples and ashes ensure good light to the undergrowth (which allows the lupine to survive) while the legume contributes to enriching the soil with nutrients and nitrogen thus favouring demanding species such as *Acer pseudoplatanus* and *Fraxinus excelsior*.

According to the terminology referring to alien species proposed by PYŠEK et al. (2004), *Lupinus polyphyllus* can be considered a naturalized species in this area as a stable population has been present for a period of over ten years during which there has been no additional contribution of propagules by humans. Observation of areas adjacent to the one which underwent restoration established that the ornamental lupine has not spread beyond the area in which it was introduced and therefore this species is not an invasive alien; nevertheless it would be appropriate to monitor its future propagation as all naturalized species are potentially invasive species (RICHARDSON & PYŠEK, 2012). The fact that the species has not spread outside the area in which it was introduced would appear to be due to ecological-environmental reasons. The dense spruce forest surrounding the restored area represents too closed an environment for the light requirements of the lupine while, conversely, the meadows and pastures which in some places interrupt the woods would constitute an overly bright habitat for its survival.

1.5.2 Val Dorena

The results provided by the statistical analysis (cluster analysis and PCoA) of the 16 relevés conducted in Val Dorena identified five types of vegetation (Figure 1.12):

- *Trifolium repens-Festuca laevigata* community (A_b): made up of some elements of mowed meadows of the *Molinio-Arrhenatheretea* class (*Trifolium repens*, *Trifolium pratense*, *Achillea millefolium*, *Lotus corniculatus*), *Festuca laevigata* (*Festuco-Seslerietea*), and some species of screes (*Thlaspietea rotundifolii*) such as *Rumex scutatus*, *Tolpis staticifolia* and *Tussilago farfara*. Some willows (*Salix purpurea*), included in the bioengineering work, were also observed, most of them in poor condition and some dead.
- *Tolpis staticifolia-Poa glauca* community (B_b): made up of various species of screes (*Thlaspietea rotundifolii*) such as *Tolpis staticifolia*, *Poa glauca*, *Epilobium collinum* and *Tussilago farfara*, together with some species of *Bromion erecti* (pastures dominated by xerophilous and mesophilous

hemicryptophytes).

- Willow shrubland (*Salix purpurea*, *Salix caprea* and *Salix alba*) (C_b): vegetation characterized by willows (introduced during restoration work), *Epilobium angustifolium*, *Festuca rubra*, *Alnus alnobetula*, *Alnus incana*, *Betula pendula* and two trees of *Vaccinio myrtilli-Piceetea abietis* class (forest acidophilous communities, dominated by conifers): *Larix decidua* and *Picea abies*.
- Mountain meadow (D_b): plant community made up almost entirely of herbaceous species of *Trisetum flavescens-Polygonum bistorta* most of which were present only in this cluster: *Anthriscus sylvestris*, *Geranium sylvaticum*, *Persicaria bistorta*, *Anthoxanthum odoratum*, *Colchicum autumnale*, *Phyteuma ovatum*, *Potentilla erecta*, *Ranunculus acris*, *Trollius europaeus*.
- Montane *Picea abies* forest (E_b): conifer forest dominated by *Picea abies* together with other elements of *Piceion excelsae* such as *Larix decidua*, *Sorbus aucuparia*, *Vaccinium myrtillus*, and some herbaceous species of

Quercus robur-*Fagetum sylvaticae* including *Luzula nivea*, *Hieracium murorum*, *Maianthemum bifolium*, *Athyrium filix-femina* and *Oxalis acetosella*.

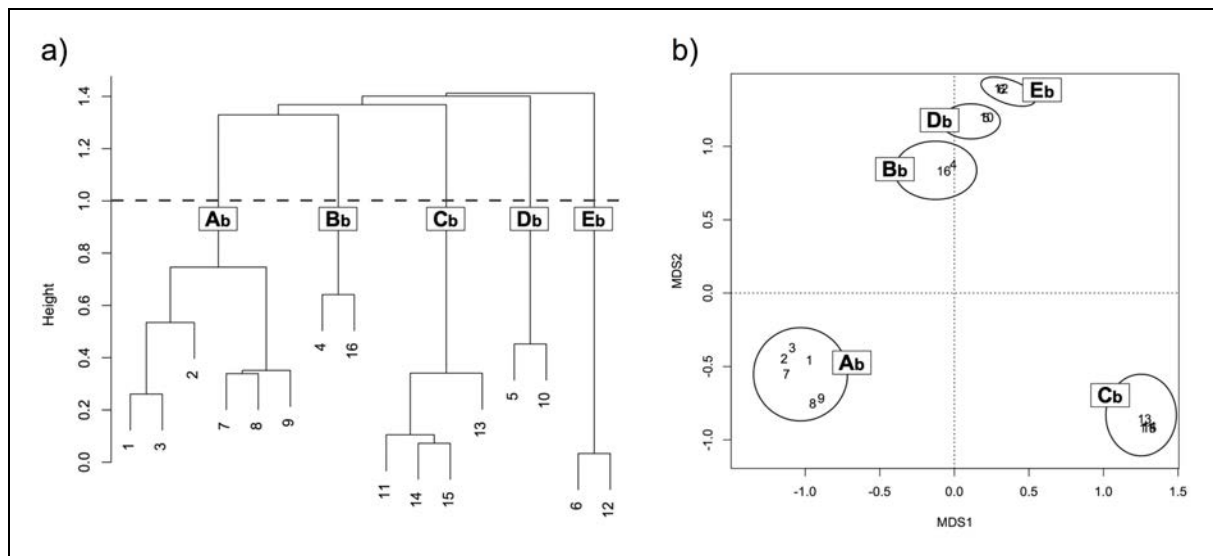


Figure 1.12 - Dendrogram of relevés (a) performed in Val Dorena provided by cluster analysis and ordering of the relevés according to PCoA (b). The numbers indicate the codes of each relevé and the letters in the boxes identify the groups of vegetation (clusters). Variance justified by the PCoA axes: MDS1 = 33,51%; MDS2 = 23,83%. The data provided by the PCoA confirm the subdivision of the relevés into five groups according to the results of cluster analysis.

For further details on the composition of the five types of vegetation see the phytosociological table of the relevés (Supplemental Data II).

Figure 1.13 shows the map of the current vegetation of the Val Dorena and images related to the types of vegetation identified while Table 1.4 lists the values of IE, IL, IM and EIM.

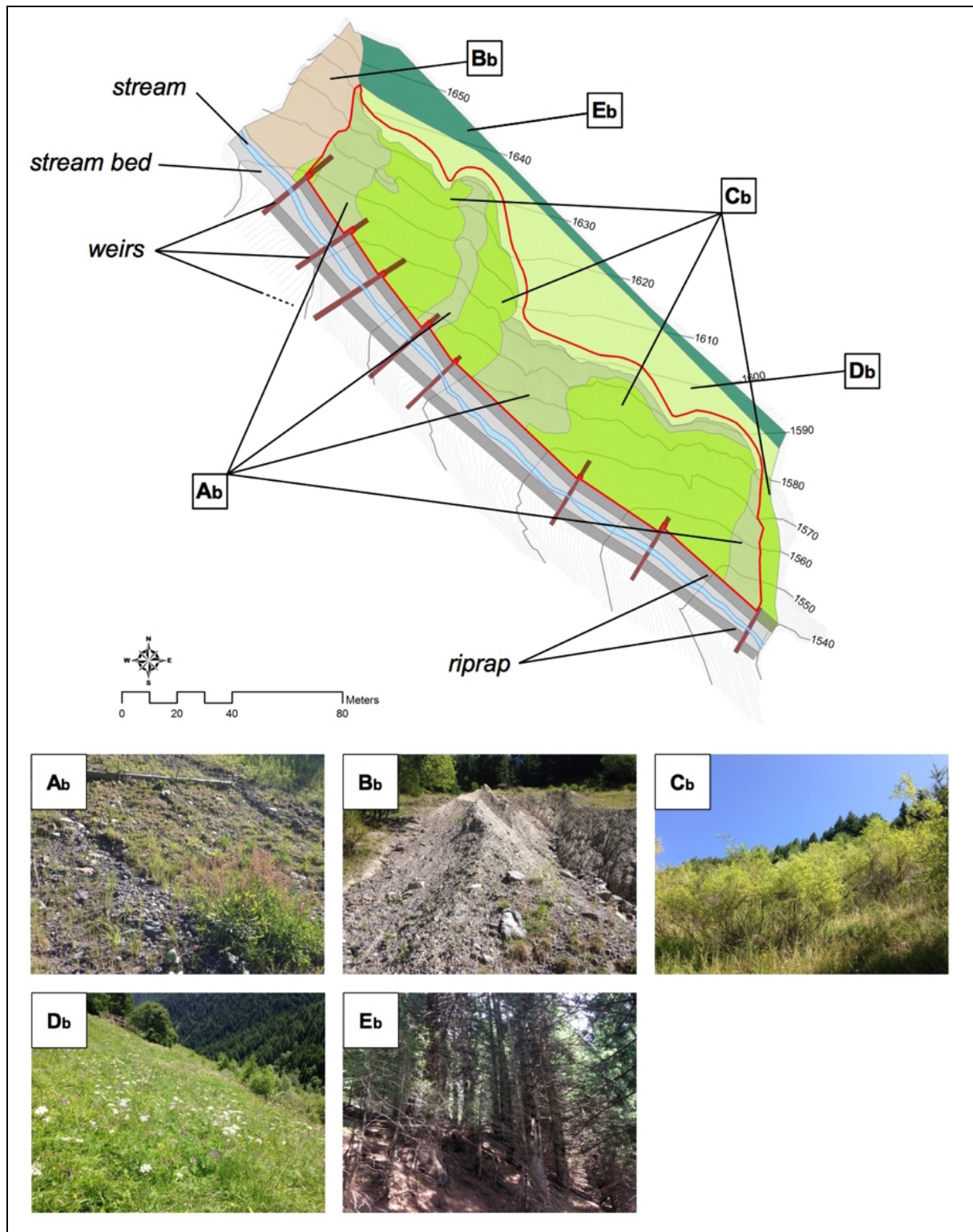


Figure 1.13 - Map of current vegetation of Val Dorena and images of the five types of vegetation. The letters in the boxes identify the five types of vegetation: *A_b*, *Trifolium repens-Festuca laevigata* community; *B_b*, *Tolpis staticifolia-Poa glauca* community; *C_b*, willow shrubland; *D_a*, mountain meadow; *E_b*, montane *Picea abies* forest. The red line marks the area affected by soil bioengineering works.

Vegetation	IE	IL	IM	EIM
Ab	0,00	6,51	4,49	4,49
Bb	0,00	39,60	4,90	4,90
Cb	0,41	0,66	8,15	8,12
Db	0,00	0,92	4,40	4,40
Eb	0,00	0,55	8,99	8,99

Table 1.4 - Values of IE, IL, IM and EIM of the five vegetation types identified in Val Dorena (A_b , *Trifolium repens-Festuca laevigata* community; B_b , *Tolpis staticifolia-Poa glauca* community; C_b , willow shrubland; D_b , mountain meadow; E_b , montane *Picea abies* forest).

The vegetation A_b is located inside the restored area where landslides still occur (the presence of this disturbance is evident from the low EIM value); vegetation type B_b is to be found in the areas outside the restored area where small landsliding phenomena still occur (also in this case such disturbance is expressed by a low EIM value); the willow shrubland (C_b) is present where soil stabilization work was carried out and where there have been no further landslides or other disturbance (as confirmed by the EIM value); the meadow (D_b) is located above the landslide area and has a low EIM value due to human disturbance (mowing) of the area; the spruce forest (E_b), which is most probably the current potential vegetation, has the highest EIM value (quite close to the maximum value) that is indicative of the absence of disturbance.

Except for *Erigeron canadensis* (present in cluster C_b) no exotic species were found while there are three endemic species of the Alps: *Centaurea nigrescens* subsp. *transalpina* (in clusters C_b and D_b), *Phyteuma scheuchzeri* (in cluster E_b) and *Tolpis staticifolia* (in clusters A_b , B_b and C_b).

1.5.3 Val Palot

The results provided by the statistical analysis (cluster analysis and PCoA) of the 12 relevés conducted in Val Palot identified seven types of vegetation (Figure 1.14):

- Montane *Picea abies* forest (A_c): made up of spruce (dominant tree species), *Larix decidua*, *Sorbus aucuparia*, *Polypodium vulgare* and *Vaccinium myrtillus* (*Vaccinio myrtilli-Piceetea abietis*) with various species of *Fagetalia sylvaticae* including *Fagus sylvatica*, *Polygonatum multiflorum*, *Polygonatum verticillatum*, *Prenanthes purpurea* and *Dryopteris affinis*.
- *Matteuccia struthiopteris*-*Cirsium montanum* community (B_c): mainly made up of two plants found exclusively in this cluster: *Matteuccia struthiopteris* and *Cirsium montanum*, together with *Aegopodium podagraria*, *Fraxinus excelsior* (planted), and some elements of *Artemisietea vulgaris* such as *Urtica dioica*, *Geranium robertianum* and *Geum urbanum*.

- *Fraxinus excelsior*-*Aruncus dioicus* community (C₂): forest vegetation made up of *Fraxinus excelsior* (planted during soil stabilization work) and some young spruces. The herbaceous layer consists in various plants of *Fagetalia sylvaticae* including *Aruncus dioicus*, *Carex digitata* and *Leucojum vernum*.
- *Petasites albus*-*Impatiens noli-tangere* community (D₂): vegetation of hygrophilic megaforbs of which the most abundant is *Petasites albus*. Moreover, the herbaceous layer is characterized by the presence of *Impatiens noli-tangere* and *Caltha palustris*, in the shrub layer there are brambles (*Rubus idaeus* and *Rubus* spp.) whereas the tree layer presents broad-leaved trees such as *Fraxinus excelsior*, *Alnus incana* and *Acer pseudoplatanus*.
- Young broad-leaved wood (E₂): made up of various herbaceous species of the *Fagetalia sylvaticae* and *Vaccinio myrtilli-Piceetea abietis* (*Acer pseudoplatanus*, *Fraxinus excelsior*, *Picea excelsa*, *Sorbus aucuparia*) with some elements of *Sambuco racemosae-Salicion capreae* including *Sambucus nigra*, *Corylus avellana* and *Salix caprea*.

- Bramble shrubland (F_c): made up of various species of *Sambuco racemosae-Salicion capreae* including *Rubus idaeus*, *Rubus* spp., *Salix caprea*, *Corylus avellana* and *Sambucus nigra*, with others of *Quercus robur-Fagetum sylvaticae* (*Athyrium filix-femina*, *Dryopteris filix-mas*, *Fraxinus excelsior*, *Petasites albus*) and of *Epilobietum angustifolii* (*Senecio ovatus*, *Fragaria vesca*, *Epilobium montanum*).
- Mountain meadow (G_c): community of herbaceous plants of *Trisetum flavescens-Polygonum bistortae* of which the most abundant species are: *Dactylis glomerata*, *Persicaria bistorta*, *Trisetum flavescens*, *Anthoxanthum odoratum*, *Holcus lanatus*, *Phyteuma ovatum* and *Trifolium pratense*.

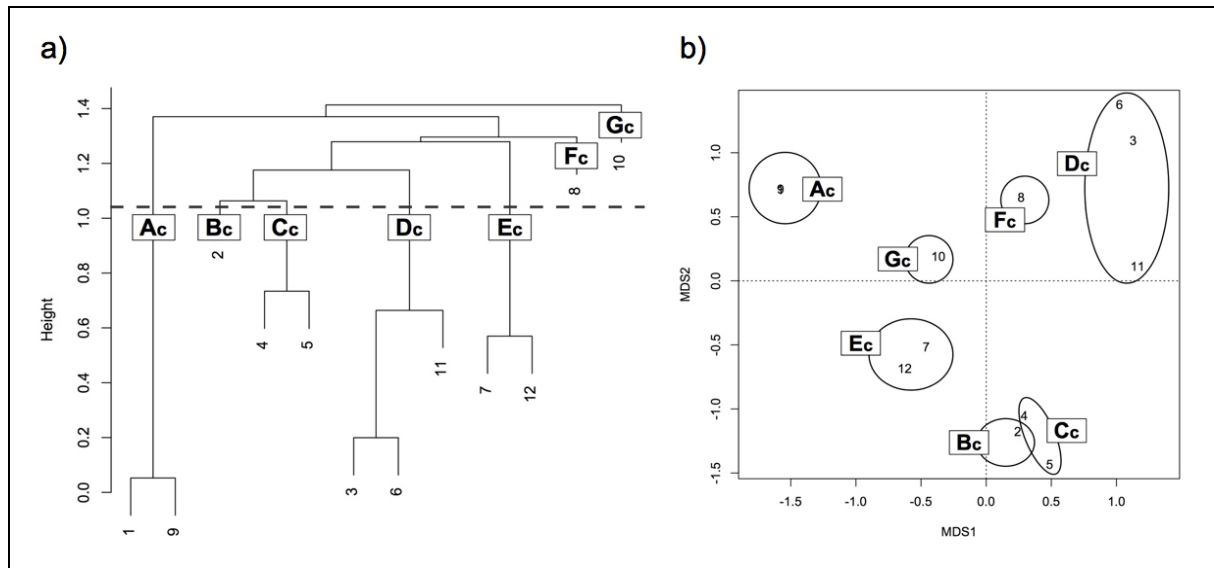


Figure 1.14 - Dendrogram of relevés (a) performed in Val Palot provided by cluster analysis and ordering of the relevés according to PCoA (b). The numbers indicate the codes of each relevé and the letters in the boxes identify the groups of vegetation (clusters). Variance justified by the PCoA axes: MDS1 = 27,16%; MDS2 = 19,26%. The data provided by the PCoA confirm the subdivision of the relevés into seven groups according to the results of cluster analysis.

For further details on the composition of the five types of vegetation see the phytosociological table of the relevés (Supplemental Data III). Figure 1.15 shows the map of the vegetation of the Val Dorena and images related to the types of vegetation identified while Table 1.5 lists the values of the various indexes.

Vegetation	IE	IL	IM	EIM
Ac	0,00	0,00	8,98	8,98
Bc	0,00	0,00	8,73	8,73
Cc	0,00	0,00	8,73	8,73
Dc	0,00	0,00	8,77	8,77
Ec	0,00	0,00	8,56	8,56
Fc	0,00	0,00	7,94	7,94
Gc	0,00	1,59	4,05	4,05

Table 1.5 - Values of IE, IL, IM and EIM of the seven vegetation types identified in Val Palot (Ac, montane *Picea abies* forest; Bc, *Matteuccia struthiopteris*-*Cirsium montanum* community; Cc, *Fraxinus excelsior*-*Aruncus dioicus* community; Dc, *Petasites albus*-*Impatiens noli-tangere* community; Ec, young broad-leaved wood; Fc, bramble shrubland; Gc, mountain meadow).

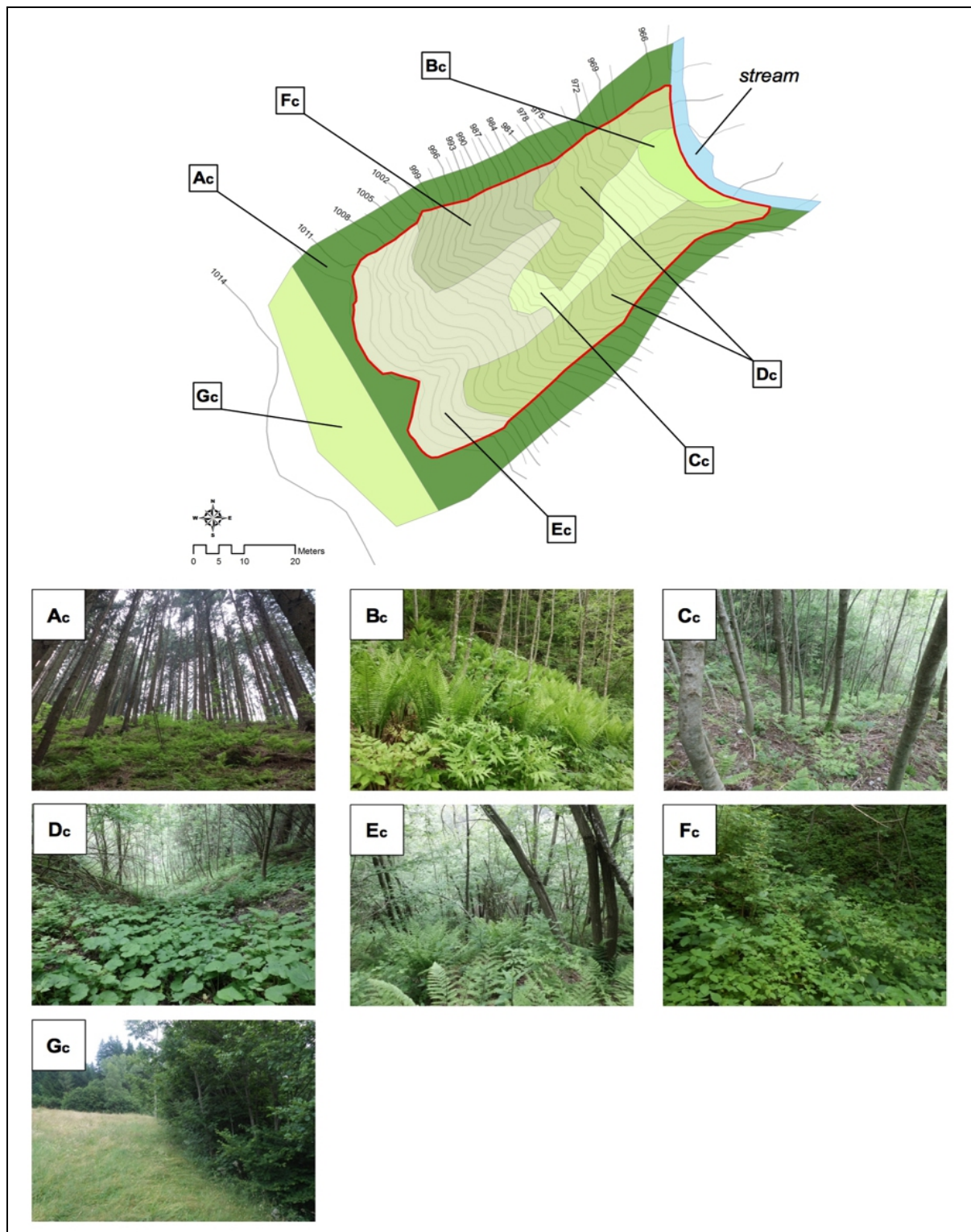


Figure 1.15 - Map of current vegetation of Val Palot and images of the seven types of vegetation. The letters in the boxes identify the types of vegetation: A_c , montane *Picea abies* forest; B_c , *Matteuccia struthiopteris*-*Cirsium montanum* community; C_c , *Fraxinus excelsior*-*Aruncus dioicus* community; D_c , *Petasites albus*-*Impatiens noli-tangere* community; E_c , young broad-leaved wood; F_c , bramble shrubland; G_c , mountain meadow. The red line marks the area affected by soil bioengineering works.

Most of the vegetation types identified in Val Palot have a high EIM value (EIM > 7.5) which indicates a low disturbance level; an exception is the meadow (G_c), located at the top of the landslide area, which lower EIM value reflects the simple fact that this community is subject to frequent disturbance from hay-cutting.

A_c is the vegetation type with the highest EIM since it represents the mature forest (current potential vegetation) located at the edge of the landslide; D_c is located where the water flows; B_c is located at the base of the slope where the gradient is lower; C_c on a ridge and E_c in the upper part of the landslide which presumably was subject to less disturbance. The bramble shrubland (F_c) is located where overthrown trees have reduced tree cover and encouraged the development of more heliophilous plants such as brambles (disturbance caused by overthrowing is indicated by the EIM value of this type of vegetation which is 7.94, just below the other forest communities). In this study area no exotic species were identified while there are two endemic species of the Alps (PIGNATTI 1982): *Centaurea nigrescens* subsp. *transalpina* and *Avenula praeusta*, both in the meadow (G_c).

1.6 Discussion

The EIM can be applied in any environmental and geographical context as long as comprehensive information regarding the flora and phytosociological and synphytosociological aspects of the territory and therefore the coefficient of maturity of the vegetation classes present is available. Its key features are that it considers vegetation as a "super-indicator" of environmental quality, and its absence of methodologies and tools particularly difficult to use. The method based on the estimated percentage of coverage according to the scale of BRAUN-BLANQUET (1964) was used for measuring the coverage/abundance of species making up plant communities in the example given. There are, however, other scales to estimate species coverage, just as there are many methods to measure the abundance of species (KENT 2012; CRISTEA et al. 2015). While estimates give values that may be slightly different from one researcher to another, frequency and density measurements are more precise but require recognition of individuals of a species. This may be a serious problem in the case of herbaceous vegetation considering that such vegetation frequently consists in perennial plants with complex growth forms (for example cespitose, rhizomatous and stoloniferous). The EIM can be applied using abundance values obtained from estimates and

those provided by measurements although, in our view, the use of the ordinal Braun-Blanquet scale is the most practical, widespread and, therefore, appropriate method of ascertaining this information.

As regards the values of the coefficient of maturity (m) of the various phytosociological classes (Table 1.1) on which the IM, and therefore the EIM, depend, it should be noted that the authors (TAFETANI & RISMONDO 2009; RISMONDO et al. 2011) have proposed a scale of ten numbers in an attempt to categorize the dynamic stages of succession in dynamic-evolutive order according to current knowledge of vegetation dynamics. Since the values of m are discrete numbers (scores), attention must be paid when manipulating such scores mathematically. In plant ecology, as well as in landscape ecology, it is common to make even complex calculations using discrete numbers, for example when ascertaining the averages of the indicator values of ELLENBERG (1979), LANDOLT (1977) and PIGNATTI (2005) in order to deduce the environmental characteristics of a station on the basis of the floristic composition of the plant community. Although studies using such indices may have some methodological limits, results are highly indicative of the environmental conditions of an area, so much so that the ecological indices of ELLENBERG (1979), LANDOLT (1977) and PIGNATTI (2005) are still widely used today in central Europe, in the Alps and in

Italy respectively.

JALAS (1955) proposed a method for measuring the anthropogenic impact and the unnaturalness of vegetation (hemeroby) based on a four-point scale that was later extended to a ten-point scale by HILL et al. (2002). This index of unnaturalness was mainly used to categorize plant species and plant communities and has been applied in Central Europe and Asia (GRABHERR et al. 1995; KIM et al. 2002). A value of hemeroby was assigned to many plants of the flora of Berlin (LINDACHER 1995) and these values were then treated in the same manner as the indices of ELLEMBERG (1979), resulting in errors. INGEGNOLI (1999, 2011) developed similar score scales for vegetation in order to calculate the "biological territorial capacity" (BTC) of a landscape and its "fittest vegetation" while MACHADO (2004) proposed a series of values that, attributed to various features of a landscape (biotic elements, artificial elements, physical alteration, level of fragmentation etc.) and processed mathematically, give the degree of naturalness of an area (index of naturalness). Moreover, TOMASELLA et al. (2007) proposed some scores that, once attributed to species, habitats and plant communities, and inserted into a complex mathematical formula, allow the "environmental ecological value" (VEA) to be calculated in order to evaluate the quality of habitats. Logically, results given by mathematical indices that process numbers

provided by estimates and/or scores, including the EIM and others mentioned above, may present some errors, and due attention should be paid in their analysis and interpretation. It should be noted that the EIM does not provide an exact measurement, but rather an indication of the level of disturbance affecting vegetation. It is therefore inopportune to perform statistics or comparisons between numbers that differ only slightly, perhaps merely in a few decimal places. For example, if there are two types of vegetation, one with an EIM of 2.25 and the other with an EIM of 2.50, it could only be inferred that in both cases the vegetation is highly disturbed; no further conclusions may be drawn until the types of disturbance (that may well differ) have been investigated in each case. If, instead, two types of vegetation have EIMs respectively of 2.25 and 8.45, it is clear that the former has a high level of disturbance and the second a lesser degree, enabling it to reach a dynamic stage of succession close to the final stage.

As regards the EIM values calculated for the plant communities present in the Azzone study area, it can be concluded that the vegetation of cluster A_a represents the final stage, undisturbed and unaltered, of the dynamic series (current potential vegetation) while the vegetation of cluster C_a is indicative of slight disturbance; disturbance is greater in cluster D_a and E_a , and extremely high

in B_a and F_a. The difference in the EIM between cluster C_a and cluster D_a is due to the different level and type of vegetation disturbance. Vegetation disturbance in cluster D_a consists in continuous water and debris runoff that removes soil and vegetation in the center of the catchment basin, blocking the dynamic succession. Instead, the disturbance affecting cluster C_a is less intense and due to the after-effects of the restoration work that, twenty years later, still has repercussions on the vegetation. In this case the disturbance is not a slightly destructive phenomenon in progress at the time when the relevé was carried out but a phenomenon occurring with such low frequency that the vegetation has almost been able to reach a final dynamic stage. The vegetation of cluster E_a presents disturbances which are very similar to those of cluster D_a (and similar EIM values) since in this case the rock is disintegrating and therefore there is slight soil movement due to the slope and the flow of water.

The EIM values of clusters B_a and F_a, much lower than those of the other clusters, are due to the high coverage of exotic plants introduced by human intervention. In this case disturbance is again due to the restoration work carried out in 1995 but, unlike the vegetation of clusters C_a and D_a, in this case disturbance is still intense and is represented by exotic species (*Lupinus polyphyllus* and *Festuca cinerea*) which prevent the growth of less competitive autochthonous plants.

Essentially, the disturbance that affects vegetation clusters B_a and F_a , rather than consisting in a direct agent of biomass destruction (GRIME 2001), is due to the competitive exclusion of autochthonous species (by exotic species) that can be seen as an alteration (destruction) of natural vegetation that causes a loss of identity for the landscape. The introduction of exotic species should be avoided in the context of environmental restoration since it can cause severe ecological impact on ecosystems (VILÀ et al. 2011) and may result in economic damage. In this case, both *Lupinus polyphyllus* and *Festuca cinerea* have prevented various shrubs of the forest mantle (*Rhamno catharticae-Prunetea spinosae* class) from establishing themselves, caused interference with the vegetation dynamics and slowed down succession, hindering the re-establishment of the pre-landslide forest. In particular, the lack of heliophyle shrubs in the vegetation of cluster E could be due to the fact that the broader leaves of *Lupinus polyphyllus* intercept most of the light radiation, leaving the surface of the ground in the shade to a height of about 50 cm. In these conditions the heliophyle shrubs of the mantle, which grow more slowly than *Lupinus polyphyllus*, would not be able to develop. Similarly, the dense turf consisting of *Festuca cinerea* (cluster B_a) could prevent the seeds of shrubs and trees from coming into contact with the ground and germinating.

The application of the EIM in the restored area of the Val Dorena highlighted the presence of an undisturbed spruce forest (cluster E_b), present outside the area affected by works, which most probably represents the current potential vegetation of this area (the EIM value is close to the maximum value). The vegetation with the lowest EIM value was that of cluster D_b - the mountain meadow adjacent to the restored area. In this case the intense disturbance that affected this type of vegetation is due to the periodic grass-mowing performed by man for decades. Although the EIM value of cluster D_b is very similar to those of clusters A_b and B_b, the type of disturbance that affects the vegetation of the latter two clusters is very different. The disturbance that affects vegetation clusters A_b and B_b is, in fact, due to continuous debris falls which block vegetation dynamics and favour the growth mainly of species of the class *Thlaspietea rotundifolii* and of the class *Festuco valesiacae-Brometea erecti*. Although the disturbance that affects the vegetation of clusters A_b and B_b is very similar, the two plant communities differ from a floristic point of view, since B_b is located within the area affected by restoration and presents some species of the class *Molinio-Arrhenatheretea*, presumably sown, (such as *Trifolium repens*, *Trifolium pratense*, *Dactylis glomerata* and *Lolium perenne*), while the vegetation of cluster A_b, lying outside the area affected by the works, has no such species.

The vegetation of cluster C_b , that has a fairly high EIM value due to the presence of planted trees (which survived) and those which have grown spontaneously, is located in the restored area where gradients are lower and landslides are therefore absent or less frequent. In Val Dorena, differently from at Azzone, only one exotic species was found (*Erigeron canadensis*), with low coverage value and only in cluster C_b . In this case, therefore, the EIM and IM values coincide for the vegetation of clusters that do not have exotic species.

In the Val Palot study area, where there was no further instability after soil stabilization work was carried out, the vegetation has high EIM values. The vegetation of cluster G_c is an exception, and represents the mountain meadow located in contact with the area affected by the works. This vegetation has an EIM value very similar to that of the mountain meadow of Val Dorena (D_b) since both meadows are mainly made up of species of *Molinio-Arrhenatheretea* and present the same disturbances (regular mowing) that, obviously, characterize meadows. Also in Val Palot the current potential vegetation is represented by the mountain forest of spruce and beech which is described by cluster A_c and that has an EIM value close to the index maximum. The mature forest (A_c) is located on the edge of the landslide area, while inside the area there are the other types of vegetation (B_c , C_c , D_c , E_c and F_c) whose EIM values do not deviate significantly

from that of the mature forest. This confirms the low level of disturbance to which the vegetation of this area is subject. Among the plant communities present in the restored area, the one with the lowest EIM is, however, that of cluster F_c that describes a type of vegetation which developed after some trees had fallen, therefore after natural disturbance.

In general, as regards the moment in which the EIM should be applied to vegetation in a restored area it should be noted that there is no "best" time although it is advisable to use the EIM at least one year after restoration work so that, where tree or shrub species have been planted, only those which have actually taken root are considered. It would be interesting to apply the EIM in the same area at different times to assess the trend of EIM values over time and to calculate and compare the EIM values of restoration work performed to date in order to identify the most successful environmental restoration projects and thus improve future intervention.

2. Vegetation analysis of a chronosequence and EIM trends

This chapter presents the floristic-vegetational data collected by GIUPPONI et al. (2017a) at various sites of upper Val Camonica in which soil stabilization works was carried out and the same mixture of seeds was sown in different years (chronosequence). These data were used to analyze the trend in EIM values over time and compared with the EIM values of vegetation of other chronosequences whose data were available in the literature.

2.1 Introduction

The environmental restoration of areas affected by destructive phenomena (anthropogenic and/or natural) is currently a priority issue for those involved in land management (ARONSON & ALEXANDER 2013), in particular in mountain protected areas. Many mountain ecosystems with high biodiversity are in fact vulnerable to soil erosion which, in some cases, may evolve into disasters that endanger human activity and the environment. Different methods are, therefore, used to protect the land from soil erosion and mitigate damage, including

hydraulic-forestry intervention. In the past such projects were carried out without considering their impact on the ecosystem, whereas techniques and materials with a low environmental impact typical of soil bioengineering have been used since the second half of the 20th century (BISCHETTI et al. 2012). The choice of plant species and procedures is of great importance in the restoration of the vegetation and, in recent years, studies have been carried out on various techniques for creating semi-natural grassland in restoration projects (ÖAG 2000; KIEHL et al. 2010; HAGEN et al. 2014), and seed mixtures suitable for varied environments have been marketed. Although these mixtures allow the creation of turf with a satisfactory aesthetic appearance in the first years after sowing, there are no in-depth studies regarding the vegetation dynamics of the areas in which they are used, and therefore data to aid understanding as to whether these seed mixtures are effective for the reconstitution of structurally more complex and more advanced plant communities (such as forest), present before the disturbance, are lacking. Knowledge of what may happen in a vegetation system after sowing a seed mixture is of great importance to those involved in land management and, even more so, for those involved in the environmental restoration and/or soil bioengineering.

GIUPPONI et al. (2017a) conducted a study of the floristic, ecological and dynamic

characteristics of the vegetation that developed following sowing of a mixture of commercial seeds, in various sites (chronosequence) of two protected areas of the Val Camonica (Southern Alps, Lombardy) so as to understand the effects of sowing the mixture and to evaluate its use in future soil bioengineering works to be conducted in the two protected areas. The data collected by GIUPPONI et al. (2017a) were used to devise a model of the trends in EIM values over time and compared with data from three other chronosequences of vegetation studied in the Alps for which data was available in the literature (BARNI & SINISCALCO 1999; D'AMICO et al. 2014; GILARDELLI et al. 2016).

2.2 Materials and methods

2.2.1 Study areas

The study was conducted by analyzing the vegetation of five sites situated in upper Val Camonica (Lombardy, Northern Italy) in the municipalities of Saviore dell'Adamello, Corteno Golgi, Sonico and Cevo, four of which are situated within the Adamello Park and one in the Sant'Antonio Valleys Nature Reserve (SIC IT2070017; CASALE et al. 2008) (Figure 2.1).

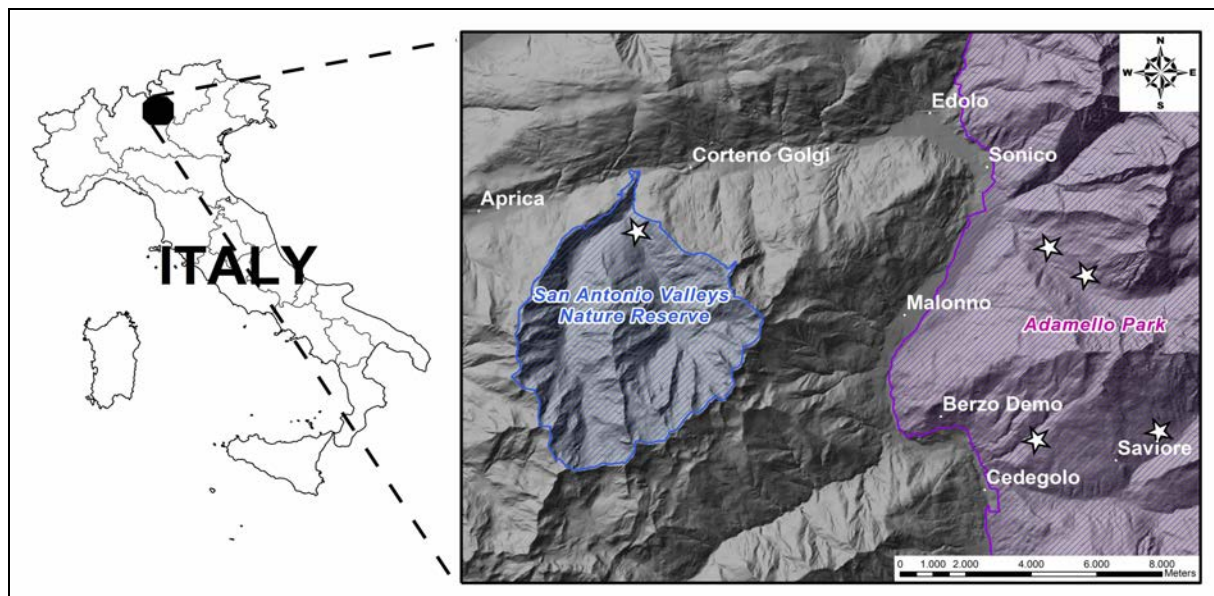


Figure 2.1 - Study area (latitude: 46° 07'48,87"N, longitude: 10° 18'38,59"E). The stars indicate the five sites where the phytosociological relevés were performed.

These sites belong to the same land units, have similar environmental

characteristics and were subject to land stabilization work using traditional and soil bioengineering techniques. In particular, crib walls, to protect roads, and weirs and channels, to control the waters of streams and stabilize banks, were constructed in the five areas. Work was carried out in different periods (from 2000 to 2012) by the "Consorzio Forestale Alta Val Camonica" which used the same mixture of seeds for sowing. The five sites represented a chronosequence of year of sowing (2012, 2011, 2009, 2005, 2000), they are small (50-150 m²) but they are the only ones with similar altitude, exposure, slope and substratum.

The area studied has a climate with rainfall over 1000 mm per year mainly concentrated in the spring and the autumn; the annual average temperature is about 8,5 ° C, minimum temperatures and precipitation are during the winter months (Figure 2.2). The three areas are part of the Temperate Oceanic bioclimate zone (RIVAS-MARTÍNEZ & RIVAS-SÁENZ 2009).

According to the recent classification of the ecoregions of Italy (BLASI et al. 2014), it is part of the "Central and Eastern Alps" section (Alpine Province, Temperate Division). The vegetation sampling sites are situated in the montane belt (1190 m -1310 m) where potential vegetation consists of dense spruce forests belonging to the *Piceion excelsae* alliance (VERDE et al. 2010) on soils with an acid to very acid reaction (Cambic Podzols) due to the crystalline geological

substratum (PREVITALI et al. 1992).

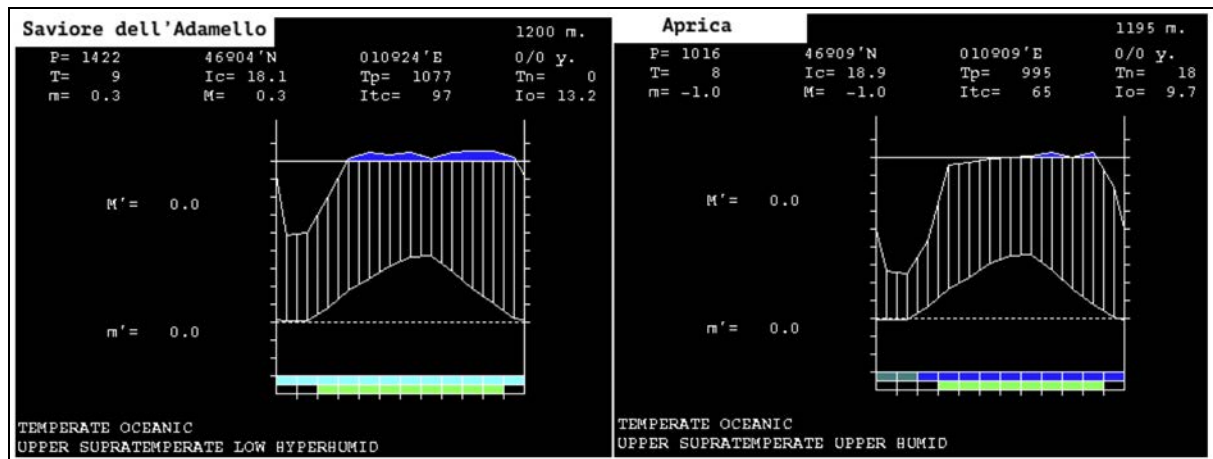


Figure 2.2 - Ombrothermic diagrams (WALTER & LIETH 1960) of Savio dell'Adamello (BS) and Aprica (SO) for the period 2009-2013. Data source: Centro Meteorologico Lombardo. The diagram has been created using the software developed by RIVAS-MARTÍNEZ & RIVAS-SÁENZ (2009).

Table 2.1 shows the chemo-physical characteristics of a Cambic Podzol typical of upper Val Camonica.

Soil horizon	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH	Organic matter (%)
Ah	0 – 7,5	77,8	14,8	7,4	4,0	32,35
E	7,5 – 10	51,8	37,3	10,9	3,7	7,40
Bs1	10 – 20	77,4	17,4	5,2	4,0	8,75
Bs2	20 – 40	83,1	12,9	4,0	3,9	8,26
CB	40 – 45	66,5	23,3	10,2	4,2	3,81

Table 2.1 - Physical-chemical properties of a typical Cambic Podzol soil of the upper Val Camonica. Data source: PREVITALI et al. (1992).

In the five sites where the relevés were performed the soil was highly disturbed and altered due to the work done which, in some cases, exposed the bedrock.

Nevertheless, no allochthonous soil was added; the soil removed during excavation activities was used, together with fine soil retrieved locally, in order to reconstitute a layer of fertile soil (about 30 cm deep) suitable for sowing.

The sites belong to the alpine acidophilus dynamic series of silver fir and spruce (*Calamagrostio arundinaceae-Piceo excelsae sigmetum*) whose dynamic stages consist of: fringe of *Calamagrostion arundinaceae*, mantle of *Sambuco racemosae-Salicion capreae* (*Rubetum idaei*, *Piceo-Sorbetum aucupariae*) and wood of *Piceion excelsae* (VERDE et al. 2010). Each of the five areas borders with mountain forest of spruce (*Piceion excelsae*) that is characterized almost exclusively by *Picea excelsa*, in the tree layer, while the shrub and herbaceous layer presents *Rubus idaeus*, *Vaccinium myrtillus*, *Oxalis acetosella*, *Hieracium murorum*, *Luzula nivea*, *Athyrium filix-femina*, *Saxifraga cuneifolia*, *Maianthemum bifolium* and *Viola biflora*. This forest, present in the five areas before the land stabilization work, represents the current potential vegetation.

2.2.2 Seed mixture

The mixture of seeds used for sowing is a commercial product; both floristic composition and seed provenance are known (Table 2.2). The mixture consists solely of perennial herbaceous species typical of mowed meadows (*Molinio-Arrhenatheretea*) of which graminaceous species are 94.8% by weight. The seeds come from several states in Northern Europe, mainly from Denmark. This mixture was designed to be used in mountainous areas of the Alps and Apennines, mostly to grass ski slopes and areas affected by human activities and/or environmental restoration work. In the areas studied, grassing was performed with hydroseeding (dosage: 30-35 g/m²).

Species	%	Country
<i>Festuca rubra</i>	59,8	DK
<i>Lolium perenne</i>	10	DK
<i>Festuca ovina</i>	7	DK
<i>Poa pratensis</i>	6	DK
<i>Dactylis glomerata</i>	5	DK
<i>Phleum pratense</i>	3	DE
<i>Festuca pratensis</i>	3	DK
<i>Trifolium repens</i>	2	NZ
<i>Trifolium pratense</i>	2	DE
<i>Agrostis capillaris</i>	1	NL
<i>Trifolium hybridum</i>	1	CDN
<i>Achillea millefolium</i>	0,2	A

Table 2.2 - Seed mixture composition and provenance of seeds (DK = Denmark; DE = Germany; NZ = New Zealand; NL = Netherlands; CDN = Canada; A = Austria).

2.2.3 Vegetation sampling and data analysis

Five phytosociological relevés were performed (one for each site indicated in Figure 2.1), according to the method of the Zurich-Montpellier Sigmatis School (BRAUN-BLANQUET 1964), and checked periodically from May to September 2014 (the maximal cover of the season for each species was used). The size of sampling plots was 25 m² (5 x 5 m) and the relevés were carried out in plots that are indicative of the vegetation of the five sites. Species were determined using the dichotomous keys of PIGNATTI (1982) and cover indices were assigned using the abundance-dominance scale of PIGNATTI & MENGARDA (1962). Cluster analysis was performed (using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), and chord distance coefficient) in order to highlight the floristic-physiognomic similarities of the relevés.

The ecological indices of LANDOLT et al. (2010), were used to analyze the ecological requirements of the vegetation. The following indices of the life forms proposed by TAFFETANI & RISSONDO (2009) were calculated: IT = index of therophytic component; IH = index of hemicryptophytic component; IF = index of perennial non-hemicryptophytic component. The indices of life forms express the proportion of species cover belonging to one group. IF is useful to determine the

evolution of the vegetation towards stable coenoses with low disturbance levels (such as the coenoses of forests), this including geophytes, chamaephytes, nanophanerophytes and phanerophytes.

The ecological index of maturity (EIM), according to GIUPPONI et al. (2015a) (chapter 1.2) was calculated for each relevé in order to analyse EIM trends over time. Various materials (manuals, scientific papers and internet sites) were consulted as regards the phytosociological classification. Statistical analyses were performed using the software R 3.2.1 (R CORE TEAM 2015). Scientific names of species are according to MARTINI et al. (2012); names of phytosociological classes follow BIONDI et al. (2014).

2.2.4 Comparison with data from other chronosequences

The trend in EIM values related to the chronosequence under study was compared with the trend in EIM values of three other vegetation chronosequences (available in the literature) referring to alpine environments each with very different environmental and ecological characteristics:

- chronosequence of abandoned limestone quarries in the Pre-alps of the

province of Brescia (GILARDELLI et al. 2016)

- proglacial chronosequence of Lys Glacier (north-western Italian Alps)
(D'AMICO et al. 2014)
- chronosequence of abandoned fields of the western Italian Alps (BARNI & SINISCALCO 1999)

To do this, the EIM value of the vegetation described in each chronosequence was calculated and a model to describe EIM trends over time was devised. In order to calculate the EIM of the vegetation described in GILARDELLI et al. (2016) and in BARNI & SINISCALCO (1999) the presence of the dominant/characterizing species (as indicated by the authors) was used, while the calculation of the EIM of the vegetation described in D'AMICO et al. (2014) was based on the percentage of coverage of species constituting communities.

2.3 Results

In the five areas sampled in Val Camonica, 63 plant species were identified, the majority of which are common or very common in the central and eastern Lombardy area (MARTINI et al. 2012). *Avenula praeusta* and *Hypericum perforatum* are the only two uncommon species identified. Figure 2.3 presents the dendrogram given by cluster analysis showing the relevés (and the mixture of seeds) separated into three main clusters (A, B and C). Cluster A is made up of seed mixture and relevés in areas where seed was sown more recently (2-3 years ago); cluster B corresponds to relevé 4; cluster C groups the relevés carried out in sites where seeds were sown less recently (9-14 years ago).

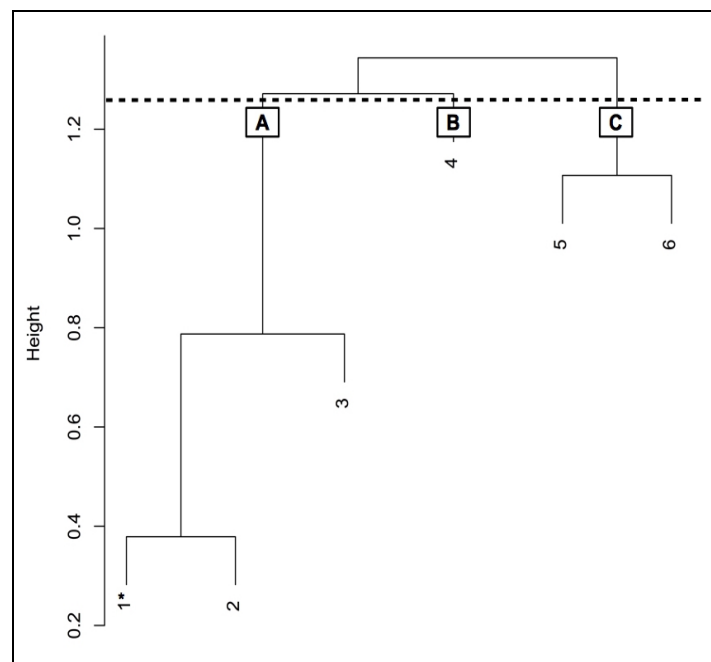


Figure 2.3 - Dendrogram of relevés (1* = seed mixture) divided into three clusters (A, B and C).

The phytosociological table of relevés (Supplemental Data IV) shows the floristic-physiognomic differences between the three clusters. Cluster A is mainly composed of herbaceous species of the classes *Molinio-Arrhenatheretea* and *Artemisietea vulgaris* while cluster C has a number of shrubs of the class *Rhamno catharticae-Prunetea spinosae* (*Sambuco racemosae-Salicion capreae*) and species (grasses, shrubs and trees) typical of the mature woods of the class *Quercu roboris-Fagetea sylvatica* (*Fagetalia sylvatica*) and, to a lesser extent, of the class *Vaccinio myrtilli-Piceetea abietis* (*Piceion excelsae*). Cluster B has characteristics midway between the other two clusters since compared to cluster A it has no species of *Artemisietea vulgaris* and is richer in species of *Rhamno catharticae-Prunetea spinosae* and *Quercu roboris-Fagetea sylvatica*.

The different physiognomy of the three groupings of vegetation is clear in the graph showing the indices of biological form (Figure 2.4); cluster A has a high percentage of hemicryptophytes (which account for the entire mixture) and negligible amounts of IF (phanerophytes, nano-phanerophytes, chamaephytes and geophytes), cluster B presents a moderate percentage of IF while cluster C has a higher percentage of IF and the IH value tends to be lower.

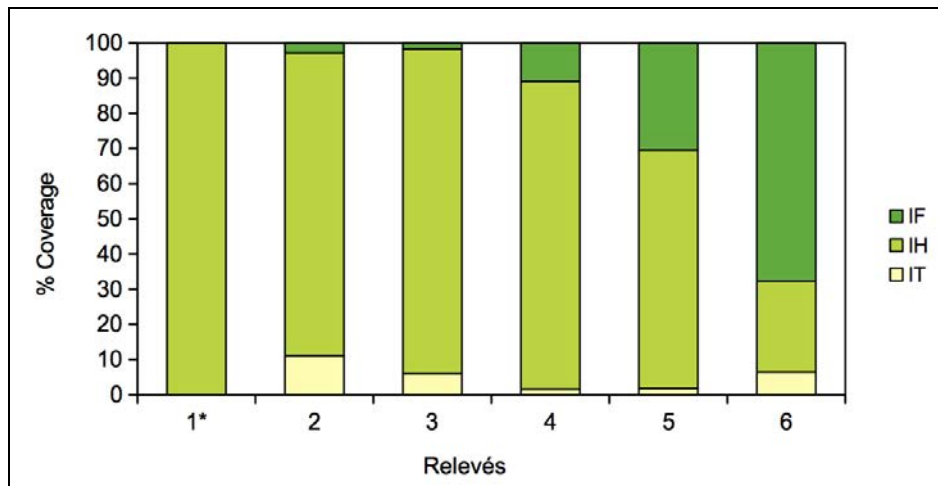


Figure 2.4 - Histogram of life forms indices of relevés and seed mixture (1*). IT = index of therophytic component; IH = index of hemicryptophytic component; IF = index of perennial non-hemicryptophytic component (geophytes, chamaephytes, nano-phanerophytes and phanerophytes).

Moreover, there is a moderate percentage of therophytes (IT index) in relevés 2 and 3 (cluster A) while in the relevés of clusters B and C such values are almost nil, except for relevé 6. This is due to the fact that while the therophyte component of relevés 2 and 3 is made up of species belonging to the ruderal, nitrophilous vegetation of the classes *Stellarietea mediae* and *Artemisietea vulgaris*, that of relevé 6 is represented only by *Melampyrum pratense* (with moderate coverage value) which is an annual species of mature broad-leaved forests (*Quercus robur*-*Fagetum sylvaticae*) and is not an indicator of disturbance.

The three groupings of vegetation also differ substantially in their chorological

features (Supplemental Data IV). Cluster A has a high presence of species with widespread geographic distribution (cosmopolitan and subcosmopolitan) and an exotic species from North America (*Erigeron canadensis*) quite common in Italy in uncultivated areas (CELESTI-GRAPPOW 2009). Clusters B and C differ from cluster A due to the presence, albeit slight, of a contingent of endemic species of the Alps (PIGNATTI 1982) consisting of: *Phyteuma scheuchzeri*, *Phyteuma betonicifolium* and *Avenula praeusta*. From the ecological requirements of the coenoses synthesized by the average values of the indices of LANDOLT et al. (2010) shown in Supplemental Data IV, it is clear that cluster A consists of heliophilous species of soils rich in nutrients and moderately poor in humus while cluster C has more sciaphilous species preferring oligotrophic soils with medium-high humus content. The vegetation of cluster B presents ecological characteristics which are midway between clusters A and C.

The graph in Figure 2.5 compares the time elapsed after sowing with the EIM values of the five phytocoenoses, and shows the model which describes the trend of the EIM according to time. The graph shows a gradual increase in EIM values from the site in which sowing was performed in 2012 (relevé 2) to the site sown in 2000 (relevé 6). In this case the two variables are linked by a relationship ($EIM = 2,263 \ln(x) + 1,991$) that accurately describes ($R^2 = 0,957$) the trend of the EIM

over time (x). The model allows the time necessary for the system to reach the maximum EIM value (mature forest vegetation), which in this case is about 25 years, to be identified.

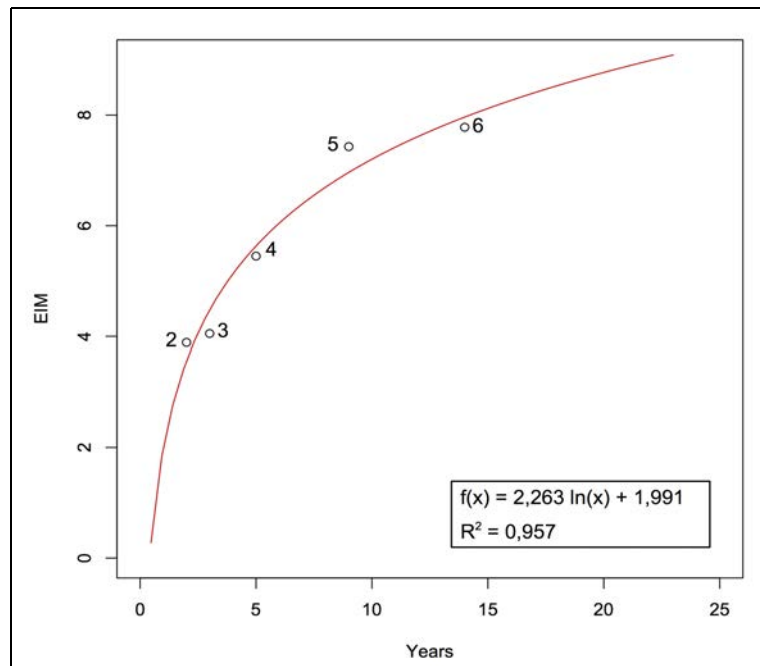


Figure 2.5 - Trend of the ecological index of maturity (EIM) over time (x) (years elapsed after sowing) of the chronosequence of Val Camonica. Shown: relationship between the two variables, coefficient of determination (R^2) and function trend line. The numbers refer to the relevés.

Figure 2.6 shows the graphs of the EIM trends over time, referring to the other three chronosequences considered in this thesis. A comparison of the graphs shows that the amount of time elapsing before the systems reach the maximum EIM value varies considerably in the different cases: about 25 years for chronosequence a (areas of Valcamonica), about 120 years for chronosequence b (abandoned limestone quarries), about 200 years for chronosequence c

(proglacial areas), and about 60 years for chronosequence d (abandoned fields).

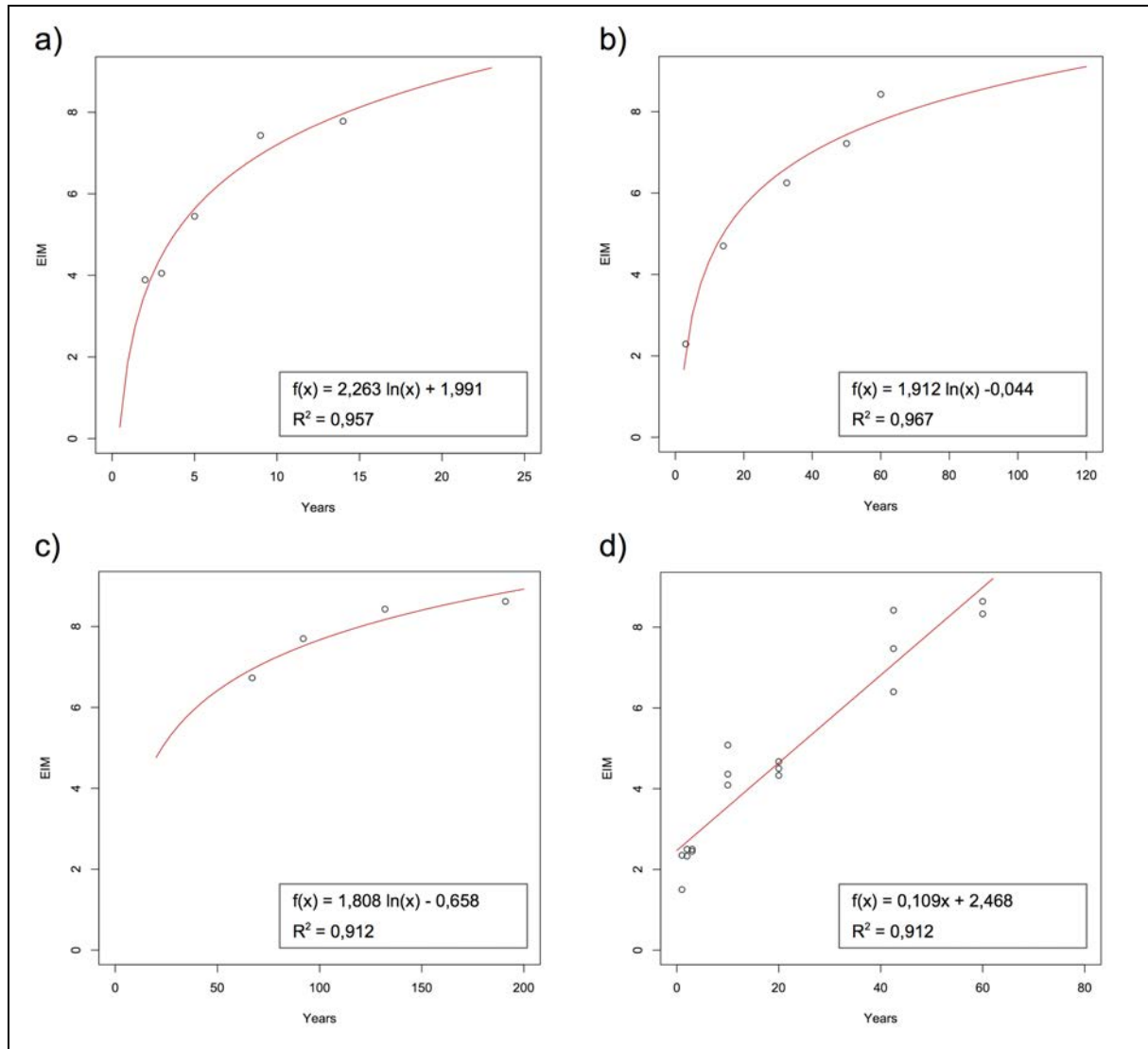


Figure 2.6 - Trend of the ecological index of maturity (EIM) over time (x) of the four chronosequences: a, Val Camonica chronosequence (GIUPPONI et al. 2017a); b, limestone quarries chronosequence (GILARDELLI et al. 2016); c, proglacial chronosequence (D'AMICO et al. 2014); d, abandoned fields chronosequence (BARNI & SINISCALCO 1999). The graphs show: relationship between the two variables, coefficient of determination (R^2) and function trend line.

2.4 Discussion

The results obtained from the analyses have yielded a range of information on the characteristics of the vegetation of the five sites of Val Camonica and have highlighted the main changes (floristic, physiognomic and ecological) that occur over time in areas where seed mixture has been used. Cluster analysis divided the relevés into three groups: those in cluster A which represent the early dynamic stages of the series and those in clusters B and C which describe more mature stages of the series. They are characterized by phytocenoses with different floristic composition, structure and ecological requirements. The decrease of heliophilous species over time elapsed after sowing is explained by the variation in the structure of the vegetation, with a clear predominance of herbaceous species in the early stages gradually replaced by shrubs and trees, the shape of which allows most of the light energy to be captured, producing a selective pressure that leads to the appearance of sciaphilous nemoral species. Five years after sowing, species of the classes *Artemisietea vulgaris* and *Stellarietea mediae*, which are indicators of environmental disturbance, disappear, while some endemic elements appear. Over a number of years the ruderal species decrease while the competitive species increase, in agreement with the model of GRIME

(2001) for the early stages of secondary succession in deforested areas on moderately fertile soils with a temperate climate, and as observed by GÜSEWELL & KLÖTZLI (2012) in sown areas on roadsides in the Swiss National Park.

The mixture, whilst having the advantage of not containing exotic species, has the disadvantage of being poor in species and has seeds from populations other than those in the area of study. The use of commercial seed in restoration can in fact alter the genetic diversity and structure of local populations (MCKAY et al. 2005; AAVIK et al. 2012; THOMAS et al. 2014); it would therefore be opportune to use autochthonous seeds better suited to the ecological conditions of the site hence avoiding (or reducing) genetic pollution. Improvements in the composition of the mixture (type of species and abundance), by using locally sourced seed, could implement ecological functionality and speed up succession (PRACH et al. 2014). In general, the composition of the species of the mixture should reflect that of the communities that make up the early stages of the spontaneous dynamic series, knowledge of which is of paramount importance to enhance environmental restoration activities (WALKER & DEL MORAL 2008). In this specific case species of *Calamagrostion arundinaceae* (e.g.: *Calamagrostis arundinacea*, *Calamagrostis villosa*, *Lilium martagon* and *Molopospermum peloponnesiacum*) and some elements of *Sambuco racemosae-Salicion capreae* (e.g.: *Salix caprea*,

Corylus avellana e *Rubus idaeus*) should be included. In fact, these species make up the communities of the initial stages of the dynamic series, i.e. of the fringe, (*Calamagrostion arundinaceae*) followed by the mantle (*Sambuco racemosae-Salicion capreae*) and then the climax community (*Piceion excelsae*) (VERDE et al. 2010). Promoting this dynamic series of vegetation would probably help the system to achieve the mature forest stage more quickly, reducing interference due to the presence of species that are not relevant to the stages of the series.

As regards the analysis of the ecological index of maturity (EIM) of the Val Camonica chronosequence, results showed that EIM values increase according to time elapsed after sowing (Figure 2.5). The identification of the relationship between EIM and time elapsed after sowing has allowed the EIM trend of dynamic series to be expressed mathematically and has provided an estimation of the time required for forest reconstitution which, in this case, was about twenty five years. Unfortunately, few data were collected in this study (only five relevés), but the seed mixture was used in a few (and small) areas and of these we chose only areas which could actually be compared with one other (those with similar exposure, slope, altitude substratum and environment) and where the mixture was sown in different years in order to analyze the changes in vegetation over time.

By analyzing the EIM trend regarding the other chronosequences reported in the literature (Figure 2.6) it is clear that in all cases the EIM tends to increase over time, but not uniformly in the various sites, most probably due to the different characteristics of each site, and in particular, to soil characteristics. In fact, in the cases in which the vegetation dynamics start from less evolved soils (or even from rocks or screes), such as those of the chronosequence of the abandoned limestone quarries (GILARDELLI et al. 2016) and of the proglacial chronosequence (D'AMICO et al. 2014), much greater time spans are required (120 - 200 years) to reach the maximum EIM value (if indeed this value can be reached). Instead, when vegetation dynamics start from a situation in which the soil is more evolved (for example that of abandoned fields or areas affected by restoration/soil bioengineering works) the system reaches the maximum EIM value much more quickly (25 - 60 years). Indeed in abandoned fields and meadows where the soil is rich in fine particles and nutrients, some demanding forest species such as *Fraxinus excelsior* and *Acer pseudoplatanus* are quickly able to re-colonize areas abandoned by agriculture (DEL FAVERO 2004). Instead, in areas affected by soil stabilization intervention the vegetation dynamics should take place in a relatively short time due to the fact that fertile soil is often added, plants are sown and trees planted. Although the analysis of EIM trends would require

further case studies, the results of this study could be used to facilitate the assessment of the success of soil bioengineering (or restoration) works. In fact, by calculating the expected EIM value of an area at a given time after work has been completed, a comparison can be made with the real EIM value (calculated after analyzing the vegetation of the area), enabling appropriate considerations on the work carried out to be drawn.

3. Index of Ecological Success (IES) to assess the success of soil bioengineering works

3.1 Introduction

The evaluation of the effectiveness of soil bioengineering work performed for soil stabilization and/or for ecological restoration is of great importance in order to identify and replicate the most successful approaches and techniques as well as to analyse the causes of less successful ones. This point is especially relevant in view of the increasing awareness, throughout the world, regarding soil stabilization, nature conservation and ecosystem restoration issues (ARONSON et al. 2006; ARONSON & ALEXANDER 2013). The scientific community, as a consequence, is called, today more than ever, to sustain land managers, planners and practitioners, providing them with new techniques and new analytical tools in order to counter the continuing loss of soil, habitats and biodiversity, associated both with natural processes and man-made technical work.

In terms of techniques adopted for soil stabilization, in the last decades low-impact measures which use live plants (or parts thereof) as building materials, in

combination with other materials (such as stones, soil, timber, steel, etc.), have been developed and used. These techniques are based on the principles of soil bioengineering (SCHIECHTL 1973, 1980, 1991; BISCHETTI et al. 2012; STUDER & ZEH 2014) and are increasing in popularity worldwide (LEWIS 2000; LI & EDDLEMAN 2002; LI et al. 2006; WU & FENG 2006; HOLANDA et al. 2008; PETRONE & PRETI 2008, 2010; SINGH 2010; STOKES et al. 2010; DHITAL & TANG 2015). Unfortunately appropriate tools for evaluating the success of such measures are still lacking, both because soil bioengineering is a relatively recent discipline (BISCHETTI et al. 2012), and because it is difficult to assess the efficiency of soil stabilization over time and, even more, its efficiency in minimizing human impact on ecosystems and on the landscape. Measuring the success of soil bioengineering work presents problems similar to those that affect the evaluation of the success of environmental restoration work - there are still great difficulties in identifying valid evaluation criteria as well as very little information for monitoring sites once work has finished due to a lack of funds. Some methods to evaluate the success of restoration work have been proposed (HOBBS & NORTON 1996; SERI 2004; PALMER et al. 2005; RUIZ-JAÉN & AIDE 2005a, 2005b; DUFOUR & PIEGAY 2009; SUDING 2011; GONZÁLEZ et al. 2015), but some of them are too descriptive, while others are specific for a single field (in particular river environments).

The lack of quantitative methods to assess the success of restoration and of soil bioengineering work is particularly relevant for mountain environments, which are at the same time extremely rich in terms of biodiversity and the presence of endemic species (AESCHIMANN 2004; CAMARERO et al. 2006; VAN GILS et al. 2012; FERNÁNDEZ CALZADO et al. 2012; ELUMEEVA et al. 2014), and fragile and vulnerable to natural and anthropogenic disturbances (PALOMBO et al. 2014). One of the major causes of such vulnerability, which determines soil erosion and loss of environments and biodiversity, is landslides susceptibility, which requires stabilizing works that are frequently based on soil bioengineering principles. CHUANG et al. (2011) have recently developed a method that evaluates the reestablishment of the vegetation in landslide areas by integrating remote sensing (RS), geographic information system (GIS), image classification (aerial photograph) and the Markov chain model, that is one of the simplest mathematical models for simulating the dynamics of ecological succession (USHER 1979, 1981; LEPŠ 1988). On the one hand, the method fulfils the objective of evaluating and simulating the reestablishment of the vegetation (in terms of coverage) in an area subject to landslide, but on the other it provides only an indication of the physiognomy of the vegetation (e.g. forest, grassland, shrubland) without providing data on the floristic composition of the plant

communities and on disturbances (natural or anthropogenic) affecting these communities. RUIZ-JAÉN & AIDE (2005b) have instead developed a method to assess restoration success by examining the vegetation structure, species diversity and ecosystem processes. However, this method again does not provide data concerning the floristic composition and ecology of the plant community. In mountain environments this leads to a gap in information which, in turn, hinders a correct evaluation of the success of soil bioengineering work (or restoration work), especially in terms of its impact on ecosystems and the landscape.

To address the issue this thesis presents a new tool for evaluating the effectiveness of soil bioengineering work in mountain areas with particular reference to landslide stabilization: the index of ecological success (IES) (GIUPPONI et al. 2017b). The IES is based on the ecological index of maturity (EIM), developed by GIUPPONI et al. (2015a), which provides a measure of the disturbance level to which a particular type of vegetation of a given area is subject. The EIM considers the phytosociological class, the degree of coverage and the chorotype of each species of a plant community. The EIM (and therefore the IES) is based on the phytosociological study of the vegetation, a qualitative and quantitative scientific approach (BIONDI 2011; POTT 2011; CRISTEA et al. 2015) widely used in geobotany and adopted by the Habitats Directive 92/43/EEC, the

most important European legislation for nature conservation (BIONDI 2013). The IES, in addition to considering the degree of disturbance (natural and/or anthropogenic) to which vegetation is subject, takes into consideration the coverage of exotic species present in the plant communities of the study area and the time elapsed since the end of the soil bioengineering work. In this sense the IES measures the functionality of the soil bioengineering work in slope stabilization (absence of landslides and/or other disturbance to the system) and the impact on the ecosystem and the landscape (presence/absence of exotic species and physiognomy of the vegetation) considering the time elapsed after the completion of the work (vegetation dynamics).

The application of the IES to three study cases located in the Southern Alps (Italy) where slope stabilising works performed with soil bioengineering techniques were carried out in 1995, 1996 and 2000, are reported and commented; guidelines for its application are provided as well.

3.2 Index of Ecological Success (IES)

The index of ecological success (IES) evaluates soil bioengineering work by measuring the intensity of vegetation disturbance. It is based on the concept that a certain type of vegetation is an indicator of a certain type of environment (and ecosystem). The IES is defined as follows (GIUPPONI et al. 2017b):

$$IES = \frac{EIMc_{(t)}}{EIMe_{(t)}}$$

where IES is the index of ecological success, EIMc is the value provided by the calculation of the EIM (ecological index of maturity) (GIUPPONI et al. 2015a) referring to the vegetation present in the study area at a certain time t (years since the end of the soil bioengineering works) and EIMe is the value of EIM expected for the vegetation of that area at the time t . IES values of around 1 mean that the calculated EIM (EIMc) is similar to the expected EIM (EIMe) and therefore that intervention has been successful. If the IES values are between 0 and 1 intervention was less successful, while IES values higher than 1 mean that effects were better than expected.

The EIMe can be estimated or, better still, calculated, considering the expected

vegetation (at time t) that can be identified considering:

- the pre-disturbance vegetation, if floristic-vegetation data collected in the area before the disturbance occurred are available;
- the vegetation on the edge of the area affected by soil stabilization works (which often coincides with pre-disturbance vegetation);
- the "current potential vegetation" (BIONDI 2011), that is the vegetation that represents the most advanced stage in the serial succession within a given biogeographic area, excluding major and sudden climate change events;
- the vegetation of the stage of succession at time t if comprehensive data on the vegetation dynamics of the territory where the study case area is situated are available in the literature. This is the most accurate method as it considers the floristic changes to communities over time (succession), although data regarding the timing with which these changes occur are scarce in the literature.

The EIM is calculated using the formula developed by GIUPPONI et al. (2015a) that was already mentioned in the chapter 1.2.

3.3 Application to three study cases

3.3.1 Study areas

The IES was applied to three cases of slope stabilization works located on mountain slopes of three lateral valleys of Val Camonica (Lombardy, northern Italy, Southern Alps): Val di Scalve (a), Val Dorena (b) and Val Palot (c). For a description of the three study areas please see chapter 1.3.

3.3.2 Materials and methods

Data regarding the vegetation currently present in the three study areas were collected performing some phytosociological relevés in accordance with the method of the Zurigo-Montpellier Sigmatis school (BRAUN-BLANQUET 1964). For details regarding the collection of floristic and vegetation data and their analysis please see chapter 1.4.

The EIM of each each type of vegetation identified, the average EIM value of the

three study areas (considering the coverage of all types of vegetation present within the areas affected by the works), and the IES of the three sites (using the above-mentioned formula) were calculated. In this case the EIMe value (expected EIM) was calculated using data published by GIUPPONI et al. (2017a) for the estimation of forest reconstitution time in areas of Val Camonica where a commercial mixture of seeds was sown at the end of soil stabilization works (chapter 2): $EIMe = 2,263 \ln(t) + 1,991$, where EIMe is the value of EIM expected over time (t) (years elapsed after soil stabilization work).

3.3.3 Results

The vegetation types of the three study areas and the values of EIMc of each plant community have already been presented in chapter 1.5.

Table 3.1, Table 3.2 and Table 3.3 show the IES value of the soil stabilization work in Val di Scalve, Val Dorena and Val Palot calculated considering the coverage of the vegetation types present in the areas affected by soil bioengineering works.

	vegetation type					
	B _a	C _a	D _a	E _a	F _a	r*
area (m ²)	439	5114	845	141	301	259
EIMc	3,06	7,89	6,33	6,86	3,56	0,00
average value of EIMc	6,91					
years after the end of the work	19					
EIMe	8,65					
IES	0,80					

Table 3.1 - Value of EIMc (calculated EIM), value of EIMe (expected EIM) and IES (index of ecological success) value of the area affected by soil bioengineering work in Azzone (Val di Scalve). Vegetation type: B_a, *Festuca cinerea* grassland; C_a, vegetation dominated by shrubs and broadleaf trees; D_a, *Calamagrostis varia* grassland; E_a, *Hieracium tenuiflorum-Origanum vulgare* community; F_a, *Lupinus polyphyllus-Fraxinus excelsior* community; r*, rocks.

	vegetation type		
	A _b	C _b	D _b
area (m ²)	2790	5350	725
EIMc	4,49	8,12	4,40
average value of EIMc	6,67		
years after the end of work	15		
EIMe	8,12		
IES	0,82		

Table 3.2 - Value of EIMc (calculated EIM), value of EIMe (expected EIM) and IES (index of ecological success) value of the area affected by soil bioengineering work in Val Dorena. Vegetation type: A_b, *Trifolium repens-Festuca laevigata* community; C_b, willow shrubland; D_b, mountain meadow.

	vegetation type				
	B _c	C _c	D _c	E _c	F _c
area (m ²)	133	307	1054	977	377
EIMc	8,73	8,73	8,77	8,56	7,94
average value of EIMc	8,58				
years after the end of the work	19				
EIMe	8,65				
IES	0,99				

Table 3.3 - Value of EIMc (calculated EIM), value of EIMe (expected EIM) and IES (index of ecological success) value of the area affected by soil bioengineering work in Val Palot. Vegetation type: B_c, *Matteuccia struthiopteris-Cirsium montanum* community; C_c, *Fraxinus excelsior-Aruncus dioicus* community; D_c, *Petasites albus-Impatiens noli-tangere* community; E_c, young broad-leaved wood; F_c, bramble shrubland.

Comparing the IES values of the three areas shows that while the IES value of Val Palot (Table 3.3) is close to 1, that of the Val Dorena (Table 3.2) and that of Azzone (Table 3.1) are lower. The low IES value of the Val Dorena is due to the fact that the soil stabilization works carried out were not as effective as in Val Palot and that post-intervention landslides have partially prevented the reestablishment of forest vegetation and hence impeded the complete success of the work. The photos in Figure 3.1, in addition to corroborating the above, show that in the three cases the soil bioengineering works (live crib walls) have had a different impact on the landscape of the three areas.

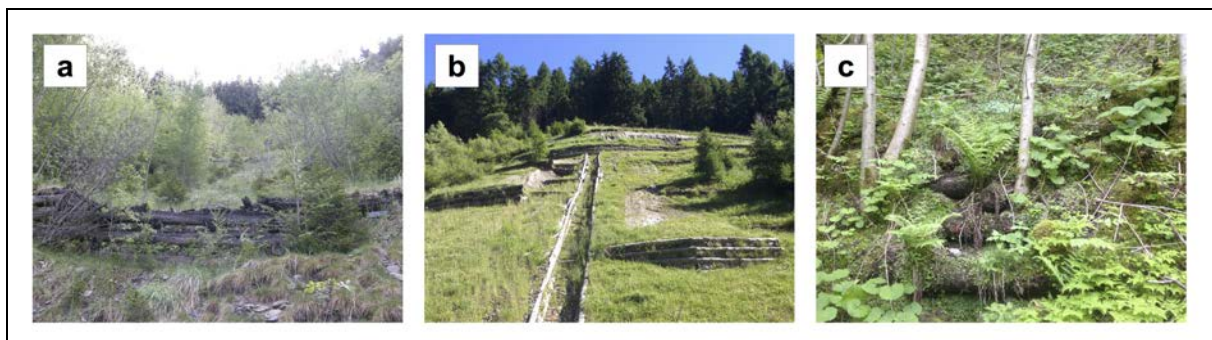


Figure 3.1 - Some live crib walls realized in the two study areas (a, Azzone; b, Val Dorena; c, Val Palot) photographed when the vegetation relevés were carried out. In the study area of Azzone the crib walls are partially covered by vegetation. In Val Dorena the live crib walls are still visible (because not covered by vegetation) and there are signs of small landslides. The live crib wall in Val Palot is covered by vegetation and perfectly integrated into the environment so that it was difficult to spot.

In fact, in the area of Val Palot, where there are no signs of recent landslides (visible in Val Dorena), the soil bioengineering structures are fully integrated into

the system and masked by vegetation while in Val Dorena the structures realized are still easily visible and poorly integrated into the surrounding landscape. In Azzone the structures are partially covered by vegetation but the low IES value (compared to that of the Val Palot) is due to the presence of exotic species (*Lupinus polyphyllus* and *Festuca cinerea*) that, at the very least, detract from the identity of the ecosystem and landscape.

3.4 Discussion

The practical examples reported seem to confirm the effectiveness of the IES in evaluating the success of soil bioengineering techniques conducted in mountain environments, with particular reference to slope stabilizing work. The IES value of the area where soil stabilization work was successful (Val Palot) and forest vegetation reestablished is, in fact, close to 1 (the expected EIM value is very similar to the calculated EIM), while where soil stabilization work has only been partially successful (Val Dorena) the IES value was lower as was the case where exotic species were sown (Azzone). This index, based on the application of the phytosociological method, addresses the gap in quantitative and qualitative knowledge regarding plant communities after slope stabilization work and can therefore integrate the method of CHUANG et al. (2011) and of RUIZ-JAÉN & AIDE (2005b). Moreover, the IES, by providing a numeric value indicative of the level of success of the soil bioengineering work, is a method which allows the mathematical treatment of data and hence statistical analysis. Other evaluation methods proposed to date do not provide a numerical value and indicate the degree of success only in terms of judging whether work was a success/failure based on the fact that an area undergoing soil bioengineering or environmental

restoration presents a number of characteristics which, very often, are described only vaguely, with no indication of what should be measured and how measurements should be made. The Society for Ecological Restoration International (SERI) proposed a list of nine attributes that a restored ecosystem should have in order to be considered successful (SERI 2004) but no indications are provided about the way to measure them by a standardized method (for example: the ecosystem must be resilient to a variety of disturbances and self-sustainable; it must have an adequate structure of coenoses; it should function in a similar way to the reference ecosystem). At the same time, however, SERI provides a robust conceptual and methodological framework for a correct evaluation of restoration works by comparing the characteristics of the restored environment with those of environments that serve as reference models for the planning of restoration projects (SERI 2004). This concept, as well as being considered in restoration work, should also be applied to soil bioengineering work. It was in fact incorporated into the IES through the comparison between the value of EIMc (calculated EIM) and that of the EIMe (expected EIM).

The calculation of EIMe at a certain period of time after soil stabilization works, in a specific environment and geographical area, requires the knowledge of vegetation dynamics. Although this subject, appertaining to integrated

phytosociology or synphytosociology (RIVAS-MARTÍNEZ 2005; BIONDI 2011; CRISTEA et al. 2015), has developed only in recent decades, important and useful contributions have been published, such as the recent work by BLASI (2010a) on the vegetation series of Italy with a map of the series (BLASI 2010b) or other similar papers (RIVAS-MARTÍNEZ 1987; ORSOMANDO 1993; GAFTA & PEDROTTI 1994; LORITE et al. 1997). These contributions are very useful for technicians who deal with vegetation (and who intend to apply the IES) as they provide an idea of the vegetation to refer to in order to calculate the EIME and evaluate the success of soil bioengineering work.

Hugo Meinhard Schiechtl, one of the leading exponents of soil bioengineering, emphasizes the importance of studying vegetation dynamics (using the phytosociological method) and, indeed, in his publication "*Sicherungsarbeiten im Landschaftsbau*" (SCHIECHTL 1973) highlights important aspects deriving from knowledge of the spontaneous recolonization of the vegetation (and the final plant community that is to be achieved) for a correct evaluation of future soil bioengineering work (including plant species to be used), so as to maximize the success of interventions. SCHIECHTL (1973, 1991) reports several models of plant succession in areas subject to landslides (valid for the territory of the Eastern Alps) as a support tool for those planning soil bioengineering intervention for

slope stabilization. These models, similar to those reported in phytosociological studies, provide excellent guidance on plant community succession in a given environment but, very often, do not provide data on the timing of vegetation dynamics. This creates considerable difficulty in determining at what dynamic stage the vegetation of an area affected by soil stabilization works should be at a certain point in time after the end of the work. These difficulties will inevitably affect the evaluation of the success of the works. The studies that record the timing of plant community succession up to the final stage (climax or, more correctly, "current potential vegetation" (BIONDI 2011)) are few and the data reported are often not generalizable. This is why many methods that evaluate the success of restoration work (or soil stabilization) recommend using the pre-intervention situation as a comparison model (HOBBS & NORTON 1996; SERI 2004; HOBBS & HERRIS 2001; RUIZ-JAÉN & AIDE 2005a) although this leads to the mistake of neglecting the timing of vegetation dynamics. In our view, in agreement with SCHIECHTL (1973), if a sufficiently clear picture of the vegetation succession of a given study area is not available, a thorough study of vegetation dynamics should be carried out before applying the IES (in theory this study should be performed even before planning intervention but, unfortunately, only rarely is this the case). Only if a study of the vegetation dynamics cannot be performed should the

situation affected by the works (at a certain time after their conclusion) be compared with the pre-disturbance situation. In the present case, since a detailed study of vegetation dynamics conducted in areas of Val Camonica affected by soil stabilization works followed by sowing to facilitate the restoration of the vegetation was available (GIUPPONI et al. 2017a), the EIS was calculated with a good level of accuracy.

It must also be noted that the values obtained with the IES, as well as those of the EIM, must be properly interpreted by specialized technicians (botanists) taking into consideration the phytosociological tables of the relevés (which describe the phytocoenoses of an environment in detail) in addition to the mere values provided by the indices (which are extremely synthetic), since different vegetation types may provide very similar values of EIM (GIUPPONI et al. 2015a).

In conclusion, the IES is a tool which seems to meet the expectations for which it was devised, although further studies (not only in mountain environments affected by landslides) could improve knowledge of the index and suggest possible improvements. In addition, it is a method which responds to three fundamental features in an "era" in which the demand for natural environment and ecosystem restoration by the public and policy makers has never been greater (SUDING 2011): 1) it is relatively easy to apply (albeit by competent

technicians), 2) it is economical, and 3) it is based on a consolidated approach (the phytosociological method) widely applied in the world in accordance with the principles of soil bioengineering (SCHIECHTL 1973, 1991).

SECTION II

Chromatographic analysis of fine roots extracts

4. Effectiveness of fine root fingerprinting as a tool to identify plants of the Alps: results of a preliminary study

Article in press (GIUPPONI et al 2017c).

4.1 Introduction

Plant identification is a routine procedure for botanists as it is the basis for conducting floristic and vegetation research. There are about 4,500 plant species in the Alps (AESCHIMANN et al. 2004) and, over the years, various tools that enable them to be identified, such as dichotomous keys and illustrated atlases, have been developed (AESCHIMANN et al. 2004; AESCHIMANN & BURDET 1989; DALLA FIOR 1974; FENAROLI 1998; LAUBER et al. 2012; PIGNATTI 1982; TUTIN et al. 1968). Although most of these tools take into account the flora of individual states that make up the Alps (rather than the flora of the entire Alpine region), identification keys and atlases remain the tools used most frequently by botanists. However, identification methods based on molecular analyzes such as DNA barcoding, a modern technology to provide rapid and accurate species identifications using short DNA sequences (ALI et al. 2014; BHARGAVA & SHARMA 2015; STOECKLE 2003), are becoming increasingly popular.

Traditional plant keys (and atlases) are based on the identification of species through the analysis of the morphological (i.e. Linnean) characteristics of the plants, especially those regarding reproductive structures (flowers and sporanges), but also those of sterile structures (leaves and stems in particular). These characteristics refer almost exclusively to epigeal organs and only for the identification of particular taxa (e.g. genera and species of the Gramineae and of the Orchidaceae families) is it necessary to analyze the morphological characteristics of underground structures that, in most cases, relate to underground stems (rhizomes, bulbs and tubers). Plant identification keys rarely deal with or describe the characteristics of the roots except for those of specific taxa, such as the *Cuscuta* genus that includes species with modified roots (haustoria) (TUTIN et al. 1968). CULTER et al. (1987) developed a specific manual for identifying the roots of trees and shrubs according to their root anatomy in order to address this gap, but this handbook, which only deals with some woody plants widespread in Britain and Northern Europe, is difficult to use for the identification of the fine roots (\varnothing 2 mm in diameter) of plants in the Alps (and plants in general) because they do not show secondary growth. Lore Kutschera and his study group analyzed *in situ* root systems of various plants (grasses, shrubs and trees) trying to relate the structure and function of belowground plant

parts to their structure and function aboveground. Kutschera's studies have resulted in publications which also include illustrations of the roots of various plants (both agricultural and those occurring in the natural environment), many of which are widespread throughout the Alps (KUTSCHERA & LICHTENEGGER 1982a,b; KUTSCHERA et al. 1997). Unfortunately, however, such research projects were not designed with the specific objective of creating a tool that allows plants to be identified through the morphological analysis of their roots (or the analysis of root system architecture) and therefore are not useful for this purpose.

It is therefore difficult (or well-nigh impossible) to quickly and easily recognize the fine roots of plant species in the Alps using the traditional keys and atlases currently available. This is an obstacle to the study of the rhizosphere and thus to the development of research to investigate the complex plant(root)-soil relationships which may have practical implications of considerable importance in mountain environments, for example in the evaluation of the success of the roots of plants used in soil bioengineering works (BISCHETTI et al. 2012) for soil stabilization, increasingly common throughout the Alps (and mountain ranges in general) in order to counter the loss of biodiversity (PALOMBO et al. 2014) and soil.

If the recognition of alpine plants by morphological/anatomical analysis of the

roots is not easily feasible, modern genetic analysis allows this problem to be overcome (BOBOWSKI et al. 1999; BRUNNER et al., 2001; JACKSON et al. 1999; LINDER et al., 2000) but is time-consuming and expensive due to the DNA purification steps (LINDER et al. 2000). In addition to DNA analysis there are, however, other analytical methods, such as chromatographic analysis, which could be useful for the identification of the fine roots of plants. Chromatographic analysis is a technique that separates the different chemical substances present in solution and that, when applied to biological fluids such as root extracts, could allow the identification of individual plant species according to the composition of such extracts, i.e. according to their chromatographic fingerprint.

Unlike DNA analysis, chromatographic analysis also allows the responses of plants to the environment to be evaluated since the extracts of live tissues contain substances produced by the metabolism of organisms (which also depends on various environmental factors). Hence, this analysis may provide important information on the responses of plants to the environment in which they live. Chromatographic analysis, and more specifically High Performance Liquid Chromatography (HPLC), is a technique employed in various fields of analysis which, broadly speaking, relate to botany (BARDAROV et al. 2015; CUI et al. 2016; EKIN et al. 2016; PAN et al. 2016; SALEM et al. 2014) but to date has

never been used in studies designed to identify plant species by analyzing their root extract fingerprint. This work therefore has the objective of analyzing the similarities/differences in the chromatographic fingerprints provided by HPLC analyses of the root extracts of six trees species widespread in the Alps (*Betula pendula* Roth, *Picea abies* (L.) H. Karst., *Fagus sylvatica* L., *Larix decidua* Mill., *Fraxinus excelsior* L. and *Corylus avellana* L.) in order to determine whether plants in the Alps (or at least those studied in this paper) can be identified by the analysis of the chromatographic fingerprints of the extracts of their fine roots. The results of the analyses are reported and discussed in order to evaluate the potential of this method and its possible future applications in the main areas of botany that are of greatest interest in the Alpine region (GIUPPONI et al. 2017c).

4.2 Materials and methods

4.2.1 Sample collection

One hundred and sixty-two samples of fine roots belonging to six different tree species common in the Alps (*Betula pendula*, *Picea abies*, *Fagus sylvatica*, *Larix*

decidua, *Fraxinus excelsior* and *Corylus avellana*) were collected during summer 2015. The root samples were collected in nine large sampling areas distributed throughout the Alps and, in each area, three root samples, taken from different individuals of different sizes (and presumably different ages), were collected for each species. Figure 4.1 shows the location of the nine sampling areas.

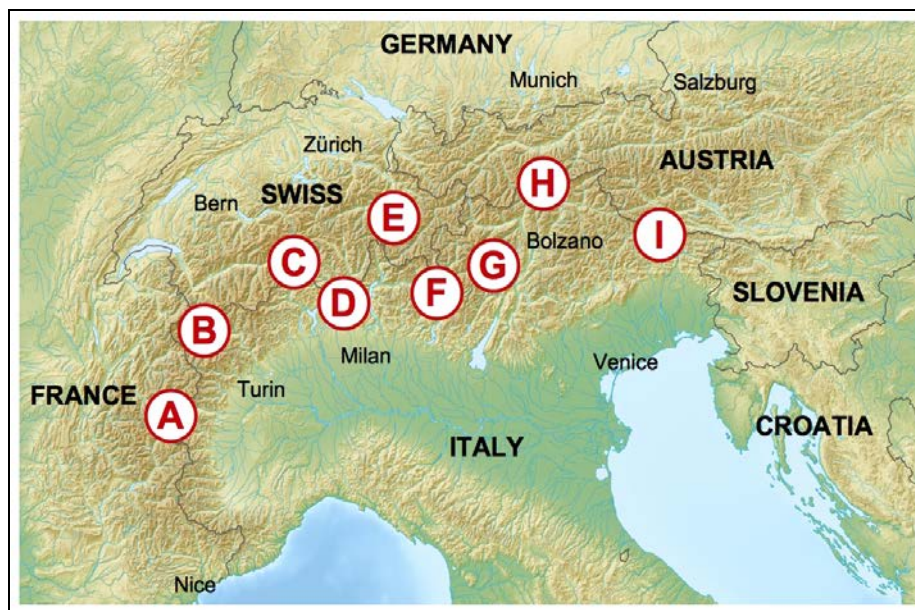


Figure 4.1 - Localization of the nine sampling areas: A, Cottian Alps (lat.: 44° 45'50"N; long.: 7° 03'10"E); B, Graian Alps (lat.: 45° 42'50"N; long.: 7° 05'50"E); C, Lepontine Alps (lat.: 46° 15'30"N; long.: 8° 20'50"E); D, Lugano pre-Alps (lat.: 46° 05'15"N; long.: 9° 00'40"E); E, Western Rhaetian Alps (lat.: 46° 51'00"N; long.: 9° 30'25"E); F, Adamello-Presanella Alps (lat.: 46° 05'10"N; long.: 10° 25'30"E); G, Garda pre-Alps (lat.: 45° 42'50"N; long.: 7° 05'50"E); H, Western Tauren Alps (lat.: 47° 03'00"N; long.: 11° 32'00"E); I, Carnic Alps (lat.: 46° 30'40"N; long.: 12° 57'00"E). The names of the subdivision of the Alps are according to MARAZZI (2005).

Each sample consisted in about 10 g of fine roots taken from the same plant organism by cutting and removing the apical part of the roots for a length of about 10 cm. The roots were collected at a depth of between 20 and 60 cm after

digging holes in the soil in the vicinity of the trunk and of the primary roots of the chosen plant. Data regarding the localization (latitude and longitude) and the altitude of the place where each sample was collected were recorded using GPS (Garmin eTrex® 30).

4.2.2 Sample preparation and extraction

Each root sample was washed with distilled water to remove soil particles and then dried in an oven at 60 ° C for 24 h. In order to obtain a representative root sample a superfine powder was prepared from roots using mechanical grinding-activation in an energy intensive vibrational mill MM 400 (Retsch. Haan, Germany). The mill was vibrating at a frequency of 30 Hz for 1 minute using two 50 ml jars with 20 mm stainless steel balls.

In detail, 2 g of each root sample were homogenized with 2 g of diatomaceous earth (Dionex ASE Prep DE, Thermo-Fisher Scientific™, Waltham, MA, USA) and transferred into a 22 ml stainless steel cell (Dionex™ ASE™ 350, Thermo Scientific™, Waltham, MA, USA). The remaining empty part of the cell was filled with diatomaceous earth and then inserted in the cell tray of the Accelerated

Solvent Extractor (Dionex™ ASE™ 350, Thermo-Fisher Scientific™, Waltham, MA, USA). Samples were extracted with 100 % methanol (Sigma-Aldrich, St. Louis, MO, USA). Conditions of extraction were: 3 cycles of 5 minutes each, temperature off and rinse volume of 90 ml. Final root extract in methanol was collected and dried by using a rotary evaporator and subsequently reconstituted in 12 ml of methanol.

4.2.3 Sample clean-up

Aliquot of each root extract (3 ml) was purified through Supelclean LC-8 SPE 3 ml (Supelco Analytical, Bellefonte, PA, USA) after a column conditioning with 5 ml methanol and 5 ml Millipore Water. Final elution of analytes (2 ml) was performed with methanol.

4.2.4 HPLC analysis

Separation of chemical substances was carried out by injection of 50 µl of each

extract into a reverse phase high-performance liquid chromatography (HPLC Jasco 2089 quaternary pump, AS 2057 autosampler; Jasco, Ishikawa-cho, Japan) with a mobile phase of methanol (A) and H₂O 0.05% Formic acid (B). The separation was carried out by gradient elution (0-3 min: A20%-B80%; 3-20 min: A45%-B55%; 20-30 min: A80%-B20%; 30-50 min: A100%; 50-65 min: A20%-B80%) on a reverse-phase column (Spherisorb ODS-2; 5 μ m, 125 \times 4 mm; Waters Corporation, Milford, MA, USA) at a flow rate of 1 ml min⁻¹ using a UV/VIS 2075 detector. Analysis time was 65 minutes and analytes were detected at 280 nm. The processing and instrument control software program (Jasco Chrom Pass, Chromatography Data System, version 1.8.6.1) were used for data analysis and reporting.

4.2.5 Statistical analysis

Results provided by HPLC analysis were statistically analyzed using various multivariate analysis techniques. In particular, cluster analysis was performed to determine the level of similarity between the chromatographic fingerprints of the various samples and to group those showing the greatest similarity. Cluster

analysis was performed using Jaccard index and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, for each cluster p -values were calculated via multiscale bootstrap resampling (EFRON & TIBSHIRANI 1993). The samples were ordered using PCoA (Principal Coordinates Analysis) and analyzed by CCA (Canonical Correspondence Analysis) to highlight any factors that determined sample order.

The data provided by chromatographic analysis were arranged in a matrix (HPLC matrix) containing peak presence/absence at various retention times for each sample in order to perform multivariate analysis. Information for peak presence/absence was coded numerically as follows: 1, present; 0, absent. CCA was performed using HPLC matrix and sampling localization matrix (containing data related to latitude, longitude and altitude of each sample). Statistical analysis was performed using the "vegan" (DIXON 2003) and the "pvclust" (SUZUKI & SHIMODAIRA 2006) packages of the R 3.3.2 (R CORE TEAM 2015) software.

4.3 Results

HPLC analysis of the root extracts provided 162 chromatograms (the number of samples collected and analyzed), six of which (one for each species) are shown in Figure 4.2.

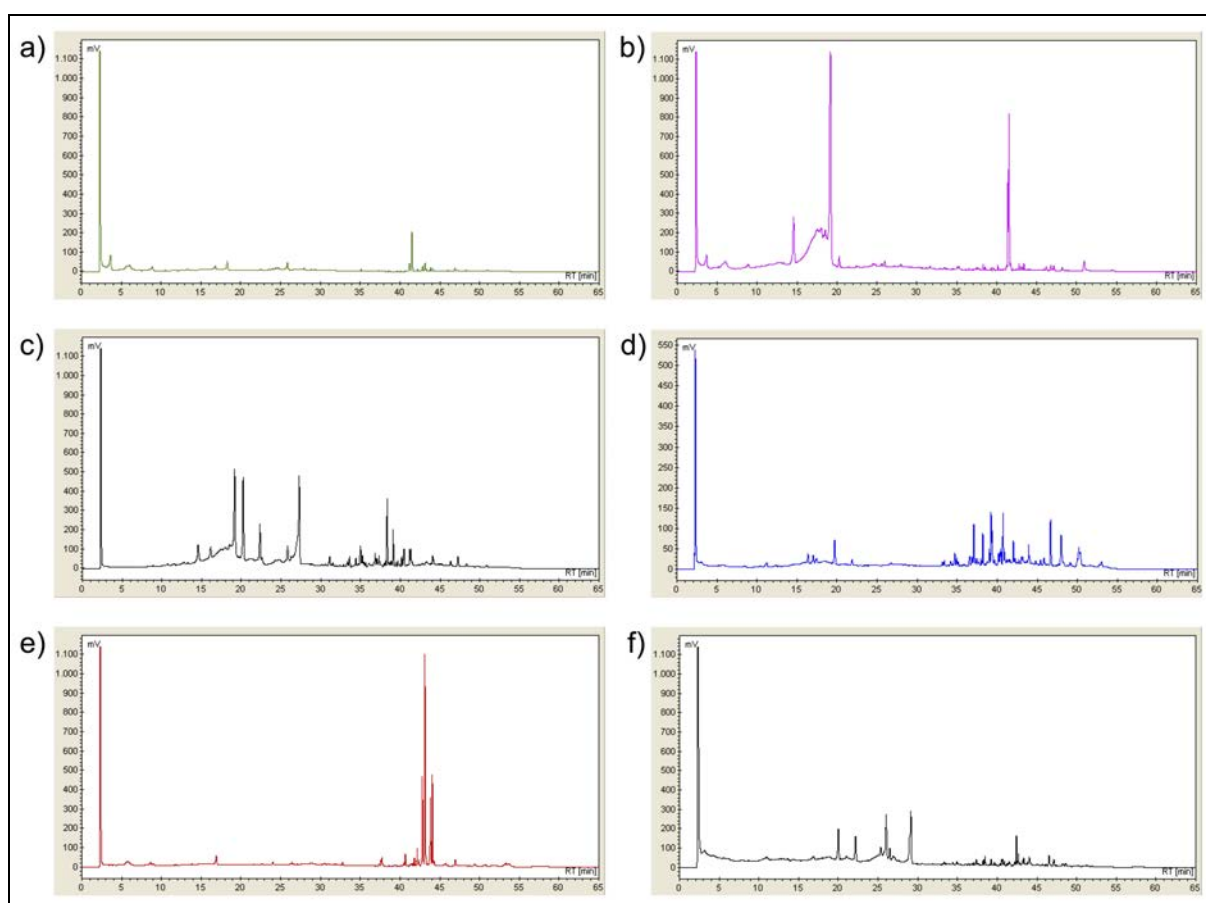


Figure 4.2 - Chromatograms of the six samples of different species studied: a, *Larix decidua* (sample code: Larix_E1); b, *Picea abies* (sample code: Picea_H1); c, *Fraxinus excelsior* (sample code: Fraxinus_A2); d, *Fagus sylvatica* (sample code: Fagus_G2); e, *Betula pendula* (sample code: Betula_I3); f, *Corylus avellana* (sample code: Corylus_E2).

The chromatograms of the various samples differ both as regards the number of peaks (the number of chemical substances present in the extracts), as well as for the retention times at which peaks develop (type of substances present in the extracts). Thus, the various samples have a specific chromatographic fingerprint that identifies them.

Figure 4.3 shows the dendrogram yielded by cluster analysis of the samples and Table 4.1 shows the p -values of each cluster highlighted by the dendrogram. In this case most of the clusters are well supported by data, in fact many Approximately Unbiased (AU) p -values are greater than 90%. The dendrogram shows that samples of the same species have a chromatographic fingerprint more similar to each other than to that of samples of other species.

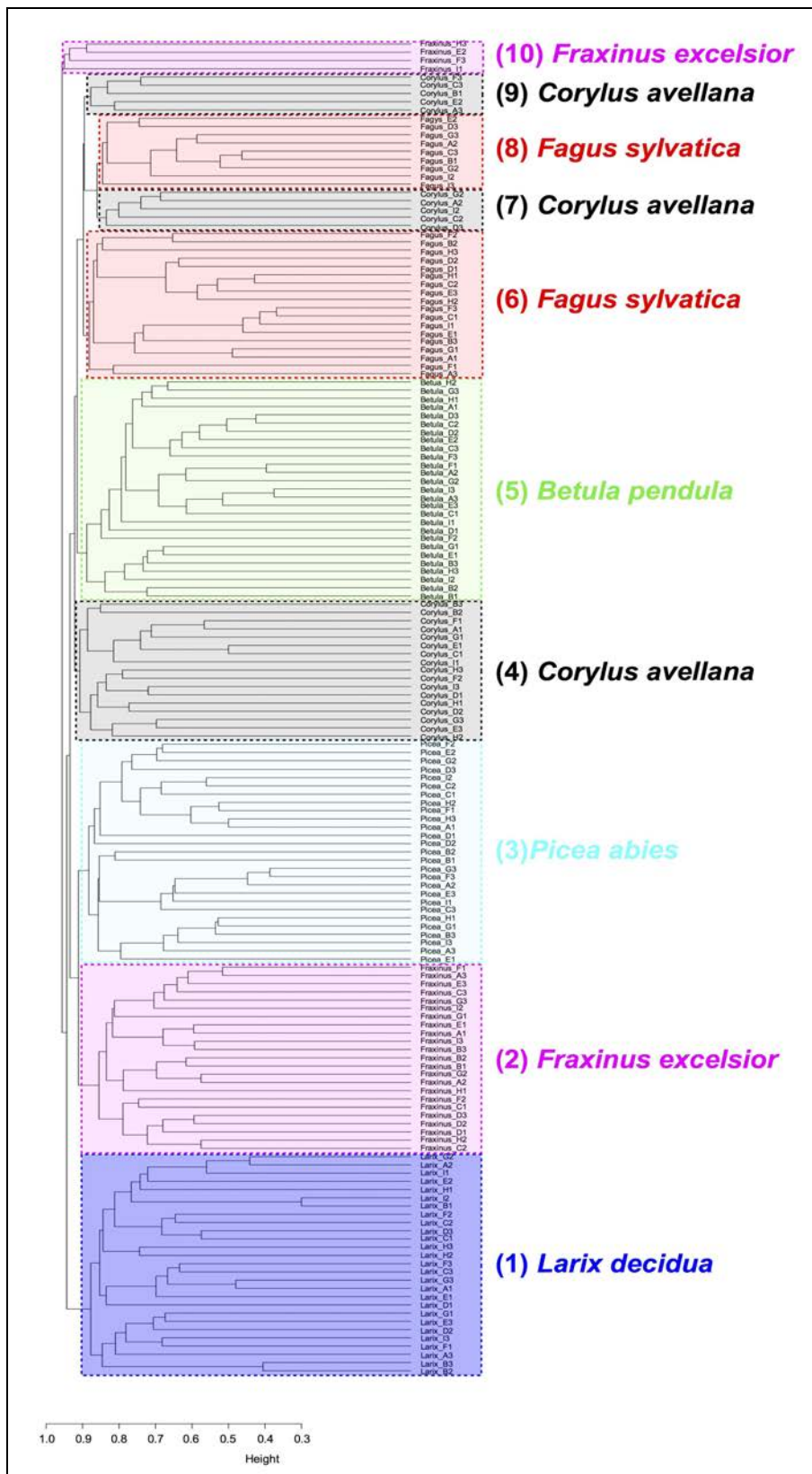


Figure 4.3 - Dendrogram of samples returned by cluster analysis. The numbers in brackets indicate the ten monospecific clusters.

	AU <i>p</i> -values (%)	BP values (%)
Cluster 1	98	13
Cluster 2	93	35
Cluster 3	99	4
Cluster 4	46	0
Cluster 5	98	6
Cluster 6	99	0
Cluster 7	90	5
Cluster 8	77	1
Cluster 9	67	0
Cluster 10	87	2

Table 4.1 - *P*-values of the ten clusters highlighted in the dendrogram (Figure 3): AU (Approximately Unbiased) *p*-value and BP (Bootstrap Probability) value.

Moreover, the samples of *Larix decidua*, *Picea abies* and *Betula pendula* are all grouped into three clusters (cluster 1, cluster 3 and cluster 5) while the samples of *Fraxinus excelsior*, *Corylus avellana* and *Fagus sylvatica* are divided into several clusters. The 27 samples of *Corylus avellana* samples were in fact divided into three clusters (clusters 4, 7 and 8), those of *Fagus sylvatica* into two clusters (clusters 6 and 8) while those of *Fraxinus excelsior* make up two separate clusters (clusters 2 and 10). This indicates that, in the case of larches, birches and spruce trees, the chromatographic fingerprints of the root extracts are similar within the same species and do not vary greatly, despite differences in the environmental conditions of the plants from which root samples were taken. However, the fingerprints of hazels, beeches and ash trees are not so similar within each species

and have therefore been divided into several groups (although these groups are all monospecific). These clusters also group samples collected at different sampling areas which would seem to exclude the fact that the fingerprint of samples of the same species collected in the same sampling area are more similar to each other than to those collected in a different area.

The biplot of PCoA analysis (Figure 4.4) confirms the results of cluster analysis. Samples analyzed, in fact, tend to be grouped according to the species to which they belong, especially the larch and spruce samples that are located respectively in the first and fourth quadrant of the graph.

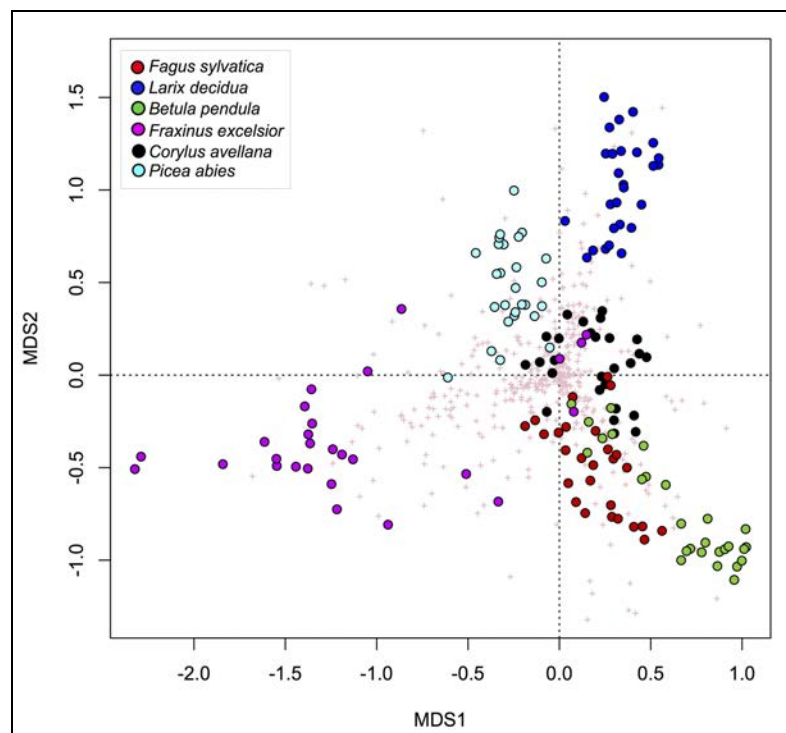


Figure 4.4 - Biplot of PCoA (Multidimensional Scaling, MDS). Total variance justified by the first and second axis: 11,03% (MDS1 = 6,04%; MDS2 = 4,99%).

The graph resulting from CCA (Figure 4.5) shows the presence of a main ecological gradient along the first axis (CCA1) which corresponds to the increase in altitude of the site where the samples were collected. As the altitude factor increases so, gradually, does the number of chromatographic fingerprints of the spruces and larches which are all located on the right half of the biplot. This trend is justified by the ecology of the two conifers, since *Picea abies* grows preferably in the mountain belt and in the subalpine belt of the Alps (where the samples were collected), while *Larix decidua* is found mostly at high altitudes (in the subalpine belt), frequently in mixed forests with spruce (DEL FAVERO 2004; PIGNATTI & PIGNATTI 2014).

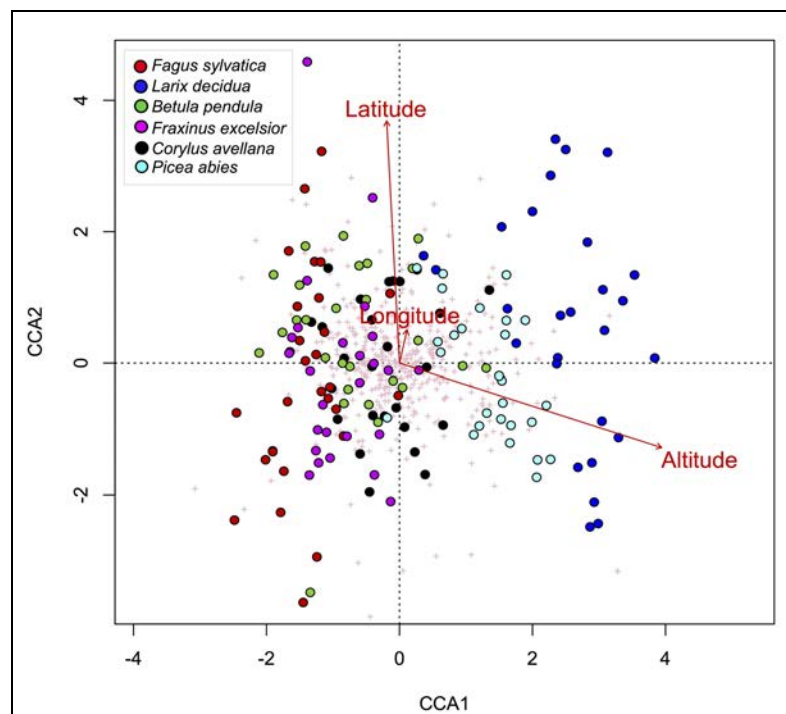


Figure 4.5 - CCA ordination biplot of samples (points) associated with latitude, longitude and altitude (arrows).

4.4 Discussion

HPLC analysis of the root extracts of the samples collected and their statistical analysis yielded interesting results relating to the objectives of this work. The dendrogram given by cluster analysis (Figure 4.3) showed that the chromatographic fingerprint of birch samples, as well as those of spruce and larch, are all similar within the same species, and can therefore identify the species. This means that the analysis of the chromatographic fingerprints of the extracts of larch, birch and spruce roots (performed according to the methodology reported in this paper) can be a valuable method to identify these species. Hence, if just a few grams of a sample of an unknown root (belonging to one of these three species) are available, its chromatographic fingerprint can be compared with that of other identified samples (at least one for each of the six species considered in this work) to identify it. However, in the case of the other three species studied (hazel, beech and common ash) the matter is more complicated. The hazel samples, as well as those of beech and ash, were not in fact grouped into a single cluster but were divided into different groupings (Figure 4.3). Nevertheless, each of these clusters consists of samples of a single species which indicates that the chromatographic fingerprint of a hazel sample

(and the same is true for beech and ash) is anyway more similar to another sample of the same species than to one of a different species.

The subdivision of samples of beech, ash and hazel into multiple clusters may be due to various reasons. These plants are probably more sensitive to differences in the environmental conditions in which they live in the Alps and hence their roots produce different chemicals in response to the different environmental factors.

The root samples collected may also belong to populations of plants which have adapted to living in different environments (ecotypes), since beech, hazel and ash are three species that grow in the Alps in ecological conditions which can vary considerably as regards the type and the chemical reaction of the soil (LANDOLT et al. 2010, DEL FAVERO 2004). Nevertheless, the identification of the roots of a hazel, a beech or an ash through the analysis of their chromatographic fingerprints (according to the method reported in this paper) may well not be particularly straightforward. In order to reduce the risk of making mistakes in their identification it would be appropriate to compare their fingerprints with that of several known samples of each species.

The results obtained in this study can partially confirm the statement in the title of this paper because, as mentioned above, only for some of the species considered (larch, spruce and birch) the chromatographic fingerprints of fine root

extracts (according to the methodology suggested here) seem to be a good method of recognition. Nevertheless, this conclusion has also led to further ideas for scientific research that could increase current botanical knowledge and facilitate the development of methodologies for studying the rhizosphere:

1) Firstly, it would be interesting to perform the same analysis on other species in the Alps, especially on shrubs and herbaceous plants, to determine whether other species have a chromatographic fingerprint that identifies them. This paper studied the roots only of very common woody plants in the Alps whose recognition might seem unimportant if it were possible to observe the plants' epigeal structures. However, for those who study the root apparatuses of plants in their natural environment, to have a tool/methodology which allows the identification of species (even of the most common trees) when only the thin roots are available would be very useful in practical terms. In fact, in natural environments, trees often make up plant communities (pluri-specific groupings) where it is very difficult to determine to which plant a fine root found in a section of soil belongs, without long and arduous excavation to uncover the primary roots and/or the trunk from which the roots branch off. In the case of herbaceous plants the problem is even more complicated. To identify the roots without conducting laborious excavations would facilitate the study of root-soil

relationships, and therefore the architecture of root systems, the stability of trees, the contribution of roots to stabilizing mountain slopes and the physical properties of various species in various environments, issues addressed extensively in recent years (CHIATANTE et al. 2002; BISCHETTI et al. 2009, 2016; DUPUY et al. 2005; REUBENS et al. 2007; DI IORIO et al. 2011; MONTAGNOLI et al. 2012; SCIPPA et al. 2012; TRUPIANO et al. 2012) due to the potential consequences for land conservation and the reduction of environmental risks due to natural disasters.

2) In addition to expanding the study to other species in the Alps, the study area could be widened in order to verify if, for example, the chromatograms of the roots of larches in the Alps are similar to those of larches in other areas, and therefore to determine whether the identification of species through the analysis of the chromatographic fingerprints of root extracts can also be applied to areas beyond the Alps.

3) An analysis of chromatographic fingerprints compared with DNA analysis could yield interesting information about whether chromatograms can vary according to genetic changes in populations. This would determine whether fingerprint analysis is able to highlight such differences, and hence whether it can also be used for the identification of subspecies, varieties, ecotypes and

populations.

4) From a chemical point of view it would be interesting to identify the substances contained in the root extracts (highlighted in the chromatograms); this could be achieved by mass spectrometry which is an analytical technique used for the identification of unknown substances that is also often used with separation techniques such as HPLC (MAHER et al. 2015). The identification of the substances present in the root extracts would also allow to perform quantitative, as well as qualitative, analyses.

5) The analysis of the chromatographic fingerprints of root extracts could also be compared with the trends of environmental variables to determine if the presence of one or more peaks expressed in the chromatograms can be attributed to a response of the plant to one or more environmental factors (due to the soil, climate or stress of various origin). DI MICHELE et al. (2006) noted that the roots of plants of *Spartium junceum* grown in steep slope conditions have different protein profiles compared to plants grown in a level position; similar observations might be possible using chromatographic analysis. This study did not collect data concerning the environmental and site characteristics of the locations where the samples were taken apart from those related to localization (latitude and longitude) and altitude. However, it is well known that, in the Alps, an increase in

altitude corresponds to a decrease in atmospheric temperature of about 0.6 ° C per 100 m (KÖRNER 2003) and, in fact, the graph provided by CCA (Figure 5) showed that as altitude increases so does the number of fingerprints of plants that generally live in areas with colder temperatures (spruce and larch). It would be interesting, from an ecological and ecophysiological point of view, to perform a similar study taking into consideration a greater number of environmental variables (for example, soil reaction, slope, depth, precipitation, temperature, exposure, etc.) in order to analyze whether any changes in chromatographic fingerprints can be due to one (or combinations) of these variables.

In conclusion, this research (GIUPPONI et al 2017c), which developed a method to obtain and analyze the chromatographic fingerprints of the root extracts of six plant species widespread throughout the Alps, has shown that some species have a chromatographic fingerprint that clearly identifies them while others have less characterizing fingerprints thus making their identification more difficult. Despite the identification of the plants of the Alps by the analysis of chromatographic fingerprints of fine roots is not as effective as other methods (for example DNA analysis) the results of this study suggested new research ideas that deserve to be addressed in order to widen knowledge of plant biology and identify tools and methodologies to facilitate the study of plants in the Alps and their interactions

with the environment.

CONCLUSION

The research carried out during the PhD course allowed information of botanical interest to be acquired and methods/tools to aid in the assessment of soil bioengineering works targeted at soil stabilization in mountain areas to be developed. In particular, the floristic-vegetational indices presented (EIM and IES) were shown to be efficient for assessing the success of the works completed in the three study areas analyzed, although it would be useful to apply them to further case studies and in different geographical and environmental contexts. This would provide a more complete picture of their validity in assessing the success of soil bioengineering works. Unfortunately, the application of such indices is not immediate since it depends on the time required to perform an analysis of the vegetation; therefore to apply them to a large number of case studies would require far greater timespans than those of a PhD course. The EIM and IES could have been applied to many more case studies if, for soil bioengineering projects completed in the past, monitoring projects, including that of the vegetation, had been provided for (and performed). Unfortunately, monitoring of vegetation following the implementation of soil stabilization works is almost never provided for due to the simple fact that it represents an additional

cost. Nevertheless, monitoring is of paramount importance to assess and improve the techniques used (especially those that affect the environment) and to acquire information about the characteristics and functioning of systems altered by human activities. This research, as well as testing the two indices, has therefore provided floristic-vegetational data which, although referring to only three study cases, seek to compensate for the lack of information regarding the vegetation found in areas affected by soil bioengineering work for the stabilization of slopes. Given the importance (both theoretical and applicative) of results which could be provided by further floristic-vegetational studies similar to this, it would be opportune that future soil bioengineering projects provide for vegetation monitoring at various times after the completion of the works and that such monitoring be conducted according to the phytosociological method which is a scientific method that can make an important contribution in choosing the species to be used in soil bioengineering interventions (SCHIECHTL 1991) and elsewhere. In fact, monitoring the vegetation according to phytosociological criteria would allow the indices presented in this thesis to be applied and, therefore, an evaluation of the work performed and a comparison with the data presented in this thesis. This would also provide the opportunity to modify/supplement the indices if/as required so as to make them as effective as possible for the

evaluation of soil bioengineering works, but without making them too complex and difficult to apply. One of the merits of these indices is in fact represented by the considerable amount of information that they provide considering the ease with which they can be applied by (botanical) technicians.

As regards the study conducted to evaluate the effectiveness of the analysis of the chromatographic fingerprints of fine roots for the identification of plants, results showed that this analysis can be an effective method of identification, at least for some species. In fact, this study found that, among the species studied, some have chromatograms which clearly distinguish them while others have fingerprints which do not allow the species to be easily identified. It must be noted that this methodology for the identification of plants has been devised and applied for the first time in this work; further studies that can perfect the method and extend its application to a larger number of species would therefore be required. To be able to identify plants through the analysis of the chromatographic fingerprints of their fine roots would be very useful both in soil bioengineering (for example, to assess the contribution of the roots of the various species present in an area of study to soil stabilization) and in all those subjects that deal with the study of the rhizosphere and plant(root)-soil relationships.

While the application of the EIM and IES mostly requires field skills (vegetation

analysis) and analysis tools which are relatively simple and inexpensive, the method developed for the identification of plants through the analysis of the chromatographic fingerprints of the extracts of their roots requires chemistry-biology laboratory skills and sophisticated analytical tools. During the PhD course, it was decided to undertake research which could contribute to improving the evaluation of soil bioengineering works that required manual dexterity in the field as well as studies that required skills in the use of laboratory instruments so as to learn different methodologies and have a complete picture of the levels of study on which scientific research regarding environmental sciences can be performed nowadays.

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SUPPLEMENTAL DATA

- Supplemental Data I - Phytosociological table of relevés conducted in
Azzone
- Supplemental Data II - Phytosociological table of relevés conducted in
Val Dorena
- Supplemental Data III - Phytosociological table of relevés conducted in
Val Palot
- Supplemental Data IV - Phytosociological table of relevés conducted in
five areas (chronosequence) of Val Camonica

Supplemental Data I

Phytosociological table of relevés conducted in Azzone

The relevés are listed according to the sequence given by the cluster analysis. The boxes highlight the groups of species which characterize the five clusters. The symbols referring to the life form are according to RAUNKIAER (1934): H, hemicryptophytes; T, therophytes; G, geophytes; P, phanerophytes; NP, nanophanerophytes. m, coefficient of maturity; pres., presence in table.

		1	3	2	15	4	5	8	11	14	13	7	9	10	6	12		
Relevé code		Aa	Aa	Aa	Aa	Aa	Ba	Ca	Ca	Ca	Ca	Da	Da	Da	Ea	Fa		
Cluster		Aa	Aa	Aa	Aa	Aa	Ba	Ca	Ca	Ca	Ca	Da	Da	Da	Ea	Fa		
Surface (m²)		100	100	100	100	100	25	100	100	100	100	100	100	25	25	100		
Inclination (°)		30	38	34	34	30	26	30	34	34	32	26	36	40	32	32		
Exposure (°)		240	185	215	185	275	225	250	235	190	160	225	230	230	225	220		
Altitude (m)		1085	1090	1165	1040	1038	1085	1032	1120	1046	1100	1008	1060	1089	1123	1130		
Trees coverage (%)		95	95	100	80	90	5	90	90	80	95	1	20	5	1	80		
Shrubs coverage (%)		20	5	5	10	20	10	70	5	10	5	5	40	20	5	5		
Grass coverage (%)		1	5	5	40	50	80	30	90	90	80	40	50	70	70	95		
Mosses coverage (%)		1	5	1	10	10	1	0	1	0	0	0	0	1	0	0		
Litter (%)		90	90	95	40	30	0	0	0	5	15	0	0	0	0	0		
Rock (%)		0	0	0	5	0	20	0	0	1	5	30	10	20	30	0		
Max height of trees (m)		25	25	25	15	30	3	5	7	10	10	3,5	4	3	3	7		
Max height of shrubs (m)		3	3	1,5	1	2	1	1,5	0,5	1,5	0,5	1,5	1	1	1	0,6		
Max height of grass (m)		0,3	0,3	0,5	0,8	0,2	1	1	0,5	0,5	0,8	0,6	0,6	1	0,8	1,5		
Life form	Chorotype	Number of species															Pres. %	m
STELLARIETEA MEDIAE Tüxen, Lohmeyer & Preising ex Von Rochow 1951																		1
T	Eurasiat.	<i>Lamium purpureum</i> L.															7	
ARTEMISIETEA VULGARIS Lohmeyer, Preising & Tüxen ex Von Rochow 1951																		3
T	Subcosmop.	<i>Geranium robertianum</i> L.															27	
H	Paleotemp.	<i>Cirsium vulgare</i> (Savi) Ten.															7	
H	Eurasiat.	<i>Cruciata laevipes</i> Opiz															7	
MOLINIO-ARRHENATHERETEA Tüxen 1937																		4
H	Paleotemp.	<i>Arrhenatherum elatius</i> (L.) P. Beauv. Ex J. Presl & C. Presl															27	
H	Europ.-Caucas.	<i>Inula salicina</i> L.															27	
H	Eurasiat.	<i>Plantago lanceolata</i> L.															27	
H	Eurosib.	<i>Achillea millefolium</i> L.															20	
H	Europ.-Caucas.	<i>Ajuga reptans</i> L.															13	
H	Paleotemp.	<i>Dactylis glomerata</i> L.															13	
H	Circumbor.	<i>Holcus lanatus</i> L.															13	
H	Eurasiat.	<i>Poa trivialis</i> L.															13	
H	Subcosmop.	<i>Trifolium pratense</i> L.															13	
H	Paleotemp.	<i>Trifolium repens</i> L.															13	
H	Eurosib.	<i>Leucanthemum vulgare</i> Lam.															7	
H	Paleotemp.	<i>Lotus corniculatus</i> L.															7	
H	Europ.	<i>Centaurea nigrescens</i> Willd.															7	
H	Circumbor.	<i>Festuca rubra</i> L.															7	
H	Paleotemp.	<i>Lathyrus pratensis</i> L.															7	
H	Circumbor.	<i>Phleum pratense</i> L.															7	
ASPLENIETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdorfer 1977																		5
H	Orof. S-Europ.	<i>Valeriana tripteris</i> L.															27	
H	Endem.	<i>Campanula carnica</i> Mert. & W.D.J. Koch															13	
H	Cosmop. Temp.	<i>Asplenium trichomanes</i> L.															7	
H	Endem. Alpica	<i>Phyteuma scheuchzeri</i> All.															7	
FESTUCO-SESLERIETEA Barbéro & Bonin 1969																		5
H	Orof. Centro-Europ.	<i>Sesleria caerulea</i> (L.) Ard.															33	
H	Endem. Alpica	<i>Carduus defloratus</i> subsp. <i>summanus</i> (Pollini) Arc.															7	
Ch	Endem. Alpica	<i>Globularia cordifolia</i> L.															7	
FESTUCO VALESIIACAE-BROMETEA ERECTI Br.-Bl. & Tüxen ex Br.-Bl. 1949																		5
H	Eurasiat.	<i>Origanum vulgare</i> L.															47	
H	Eurasiat.	<i>Calamagrostis varia</i> (Schrad.) Host															40	
H	Paleotemp.	<i>Sanguisorba minor</i> Scop.															33	
H	Exotic	<i>Festuca cinerea</i> Vill.															27	
H	N e Centro-Europ.	<i>Anthyllis vulneraria</i> subsp. <i>carpatica</i> (Pant.) Nyman															13	
H	Eurosib.	<i>Briza media</i> L.															13	
H	Eurasiat.	<i>Carex caryophyllea</i> Latourr.															13	
G	Europ.	<i>Carex flacca</i> Schreb.															13	
H	Endem. Alpica	<i>Avenula praeusta</i> (Rchb.) Holub															7	
T	Paleotemp.	<i>Medicago lupulina</i> L.															7	
H	Medit.-Mont.	<i>Onobrychis viciifolia</i> Scop.															7	
H	Orof. S-Europ.	<i>Prunella grandiflora</i> (L.) Scholler															7	
KOELERIO GLAUCAE-CORYNEPHORETEA CANESCENTIS Klika in Klika & V. Novák 1941																		5
Ch	Europ.-Caucas.	<i>Sedum acre</i> L.															13	
THLASPIETEA ROTUNDIFOLII Br.-Bl. 1948																		5
H	SE-Europ.	<i>Laserpitium krapfii</i> subsp. <i>gaudinii</i> (Moretti) Thell.															13	
EPILOBIETEA ANGUSTIFOLII Tüxen & Preising ex Von Rochow 1951																		7
H	Eurosib.	<i>Fragaria vesca</i> L.															53	
H	Paleotemp.	<i>Eupatorium cannabinum</i> L.															33	
H	W-Europ.	<i>Digitalis lutea</i> L.															20	
H	Eurasiat.	<i>Bromus ramosus</i> Huds.															13	
H	Paleotemp.	<i>Myosotis sylvatica</i> Ehrh. ex Hoffm.															13	
H	Orof. S-Europ.	<i>Stachys alpina</i> L.															13	
H	Exotic	<i>Lupinus polyphyllus</i> Lindl.															7	
H	Centro-Europ.	<i>Senecio ovatus</i> (P. Gaertn., B. Mey. & Scherb.) Willd.															7	
MULGEDIO ALPINI-ACONITETEA VARIEGATI Hada7 & Klika in Klika & Hada7 1944																		7
NP	Eurasiat.	<i>Rubus caesius</i> L.															60	
P	Centro-Europ.	<i>Salix appendiculata</i> Vill.															47	
H	Orof. S-Europ.	<i>Cirsium erisithales</i> (Jacq.) Scop.															20	
H	Eurasiat.	<i>Geranium sylvaticum</i> L.															7	

Supplemental Data II

Phytosociological table of relevés conducted in Val Dorena

The relevés are listed according to the sequence given by the cluster analysis. The boxes highlight the groups of species which characterize the five clusters. The symbols referring to the life form are according to RAUNKIAER (1934): H, hemicryptophytes; T, therophytes; G, geophytes; P, phanerophytes; NP, nanophanerophytes. m, coefficient of maturity; pres., presence in table.

Relevé code	1	3	2	7	8	9	4	16	11	14	15	13	5	10	6	12		
Cluster	Ab	Ab	Ab	Ab	Ab	Ab	Bb	Bb	Cb	Cb	Cb	Cb	Db	Db	Eb	Eb		
Surface (m²)	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	100		
Inclination (°)	40	34	30	36	45	38	43	30	36	34	24	24	24	22	24	32		
Exposure (°)	193	214	212	200	202	198	210	194	208	196	196	190	196	194	216	155		
Altitude (m)	1600	1640	1620	1580	1590	1605	1630	1630	1614	1585	1620	1580	1652	1620	1685	1550		
Trees coverage (%)	0	0	0	0	0	0	0	0	10	10	1	10	0	0	95	100		
Shrubs coverage (%)	1	1	1	1	1	1	1	1	90	40	70	60	0	0	1	1		
Grass coverage (%)	70	85	95	95	20	50	50	10	80	50	30	30	100	100	30	1		
Mosses coverage (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1		
Litter (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70	50	
Rock (%)	30	15	5	5	80	50	50	90	0	0	0	0	0	0	0	95		
Max height of trees (m)	-	-	-	-	-	-	-	-	8	7	6	8	-	-	25	20		
Max height of shrubs (m)	0,5	0,1	0,2	0,2	0,3	0,3	0,15	0,1	3	2	2	2	-	-	1,5	0,3		
Max height of grass (m)	0,7	1,1	1	1,5	0,6	0,6	0,5	0,5	1	1	1,1	1,3	0,9	1	0,4	0,5		
Life form																		
Chorotype																		
Number of species	16	22	18	19	7	12	15	15	31	32	36	33	36	42	16	16	Pres %	m

STELLARIEA MEDIAE Tüxen, Lohmeyer & Preising ex Von Rochow 1951																		1
T	Avv.	<i>Erigeron canadensis</i> L.	13
ARTEMISIEA VULGARIS Lohmeyer, Preising & Tüxen ex Von Rochow 1951																		3
H	Circumbor.	<i>Artemisia vulgaris</i> L.	6
T	Eurasiat.	<i>Melilotus albus</i> Medik.	6
MOLINIO-ARRHENATHEREAE Tüxen 1937																		4
H	Eurosib.	<i>Achillea millefolium</i> L.	+	+	+	+	+	+	+	r	+	+	+	+	+	.	88	
H	Paleotemp.	<i>Lotus corniculatus</i> L.	.	+	+	+	.	+	.	+	+	+	+	+	+	.	69	
H	Subcosmop.	<i>Trifolium pratense</i> L.	+	r	+	1	.	r	.	.	+	+	+	+	+	.	69	
H	Circumbor.	<i>Phleum pratense</i> L.	r	.	r	+	.	+	r	.	+	+	+	+	.	r	63	
H	Paleotemp.	<i>Trifolium repens</i> L.	1	1	1	2	1	1	.	.	+	+	+	.	.	.	63	
H	Eurasiat.	<i>Vicia cracca</i> L.	.	+	+	+	.	+	.	.	+	+	+	r	r	.	63	
H	Paleotemp.	<i>Dactylis glomerata</i> L.	+	+	+	+	+	+	+	+	+	.	56	
H	Subcosmop.	<i>Silene vulgaris</i> (Moench) Garcke	.	+	r	r	+	+	+	+	+	.	56	
H	Eurosib.	<i>Leucanthemum vulgare</i> Lam.	.	.	.	r	.	r	.	.	+	+	r	+	+	.	50	
H	Eurasiat.	<i>Festuca pratensis</i> Huds.	+	r	.	+	r	+	+	+	.	.	44	
H	Circumbor.	<i>Agrostis capillaris</i> L.	+	+	r	+	+	.	31	
H	Eurasiat.	<i>Galium album</i> Mill.	+	+	+	+	+	.	31	
H	Paleotemp.	<i>Lathyrus pratensis</i> L.	.	.	.	r	.	r	+	+	+	.	31	
H	Circumbor.	<i>Lolium perenne</i> L.	+	+	+	+	+	31	
H	Circumbor.	<i>Festuca rubra</i> L.	1	1	1	1	.	.	25	
T	Paleotemp.	<i>Medicago lupulina</i> L.	.	.	.	r	.	r	+	+	.	.	25	
H	Circumbor.	<i>Taraxacum officinale</i> agg.	r	+	+	r	.	.	.	25	
H	Paleotemp.	<i>Anthriscus sylvestris</i> (L.) Hoffm.	r	r	.	1	1	25	
H	Eurasiat.	<i>Alchemilla vulgaris</i> L. agg.	.	r	+	+	19	
H	Eurasiat.	<i>Geranium sylvaticum</i> L.	.	r	1	1	19	
H	Eurasiat.	<i>Plantago lanceolata</i> L.	+	.	.	.	r	+	19	
H	Eurasiat.	<i>Poa trivialis</i> L.	+	+	19	
G	Circumbor.	<i>Persicaria bistorta</i> (L.) Samp.	.	r	1	1	19	
H	Circumbor.	<i>Rumex acetosa</i> L.	.	.	r	+	+	19	
H	Eurasiat.	<i>Anthoxanthum odoratum</i> L.	+	+	13	
G	Centro-Europ.	<i>Colchicum autumnale</i> L.	+	+	13	
H	Orof. S-Europ.	<i>Phyteuma ovatum</i> Honck.	+	+	13	
H	Eurasiat.	<i>Potentilla erecta</i> (L.) Raeusch.	+	+	13	
H	Subcosmop.	<i>Ranunculus acris</i> L.	+	+	13	
H	Art.-Alp.	<i>Trollius europaeus</i> L.	+	+	13	
T	Eurasiat.	<i>Viola tricolor</i> L.	r	.	.	+	.	13	
H	Circumbor.	<i>Holcus lanatus</i> L.	+	.	.	.	+	.	13	
H	Europ.-Caucas.	<i>Pimpinella major</i> (L.) Huds.	+	.	13	
H	Endem. Alpica	<i>Centaurea nigrescens</i> subsp. <i>transalpina</i> (Schleich. ex DC.) Nyman	r	.	+	.	13	
H	Paleotemp.	<i>Heracleum sphondylium</i> L.	r	.	6	
H	Europ.-Caucas.	<i>Leontodon hispidus</i> L.	6	
H	Eurasiat.	<i>Trisetum flavescens</i> (L.) P. Beauv.	r	.	6	
ASPLENETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdorfer 1977																		5
H	Endem. Alpica	<i>Phyteuma scheuchzeri</i> All.	r	6
CARICETEA CURVULAE Br.-Bl. 1948 nom. cons. propos. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002																		5
F	Orof. S-Europ.	<i>Phyteuma orbiculare</i> L.	+	+	r	25
G	Orof. SW-Europ.	<i>Paradisea liliastrum</i> (L.) Bertol.	.	r	r	.	.	13
H	Orof. S-Europ.	<i>Campanula scheuchzeri</i> Vill.	+	.	6
H	S-Europ.-Sudsiber.	<i>Nardus stricta</i> L.	r	.	6
FESTUCA VALESIIAE-BROMETEA ERECTI Br.-Bl. & Tüxen ex Br.-Bl. 1949																		5
H	N e Centro-Europ.	<i>Anthyllis vulneraria</i> subsp. <i>carpatica</i> (Pant.) Nyman	.	.	+	+	r	r	r	+	.	.	38	
T	Centro-Europ.	<i>Rhinanthus alectorolophus</i> (Scop.) Pollich	.	r	+	+	r	.	38	
H	Eurimedit.	<i>Salvia pratensis</i> L.	.	r	r	+	+	31	
H	Subatlant.	<i>Brachypodium rupestre</i> (Host) Roem. & Shult.	r	+	1	19
H	Eurosib.	<i>Briza media</i> L.	r	+	+	19	
H	Centro-E-S-Europ.	<i>Hippocrepis comosa</i> L.	.	.	r	r	19	
H	Europ.-Caucas.	<i>Pimpinella saxifraga</i> L.	r	r	13	
H	Orof. S-Europ.	<i>Prunella grandiflora</i> (L.) Scholler	r	r	13	
H	Paleotemp.	<i>Silene nutans</i> L.	+	r	13	
Ch	Eurimedit.	<i>Thymus longicaulis</i> C. Presl	+	+	13	
H	Europ.	<i>Arabis hirsuta</i> (L.) Scop.	6	
H	Orof. Europ.	<i>Linum catharticum</i> subsp. <i>suecicum</i> (Murb. Ex Hayek) Hayek	6	
T	Europ.-Caucas.	<i>Galium verum</i> L.	r	.	.	.	6	

Supplemental Data III

Phytosociological table of relevés conducted in Val Palot

The relevés are listed according to the sequence given by the cluster analysis. The boxes highlight the groups of species which characterize the five clusters. The symbols referring to the life form are according to RAUNKIAER (1934): H, hemicryptophytes; T, therophytes; G, geophytes; P, phanerophytes; NP, nanophanerophytes. m, coefficient of maturity; pres., presence in table.

Relevé code	1	9	2	4	5	3	6	11	7	12	8	10	
Cluster	Ac	Ac	Bc	Cc	Cc	Dc	Dc	Dc	Ec	Ec	Fc	Gc	
Surface (m²)	100	100	25	100	25	25	25	25	25	25	25	25	
Inclination (°)	33	32	24	36	38	34	32	36	32	32	34	4	
Exposure (°)	95	35	60	28	32	30	30	24	38	38	28	45	
Altitude (m)	980	990	975	995	1005	995	1010	978	1005	1005	992	1020	
Trees coverage (%)	95	90	50	40	70	50	40	85	70	70	30	0	
Shrubs coverage (%)	5	10	10	30	5	10	20	20	50	50	80	0	
Grass coverage (%)	50	90	90	95	90	95	90	60	90	90	20	100	
Mosses coverage (%)	10	50	40	20	10	70	20	1	5	5	1	0	
Litter (%)	20	10	0	1	1	0	0	0	5	5	0	0	
Rock (%)	0	0	0	0	0	0	0	0	0	0	0	0	
Max height of trees (m)	25	25	12	16	15	12	10	12	15	15	10	-	
Max height of shrubs (m)	4	3	1	2	1	1	0,9	1,5	3	3	1,3	-	
Max height of grass (m)	0,8	1	1,3	1,3	1	1	1	1,2	1,5	1,5	0,8	1,1	
Life form													Pres. %
Chorotype													m

ARTEMISIETEA VULGARIS Lohmeyer, Preising & Tüxen ex Von Rochow 1951

H	Circumbor.	<i>Geum urbanum</i> L.	.	.	r	+	r	+	r	r	+	.	.	67
H	Subcosmop.	<i>Urtica dioica</i> L.	.	.	+	.	.	+	+	.	+	.	.	42
T	Subcosmop.	<i>Geranium robertianum</i> L.	.	.	+	.	.	.	+	+	.	.	1	33
G	Circumbor.	<i>Elymus repens</i> (L.) Gould	r	8

MOLINIO-ARRHENATHERETEA Tüxen 1937

H	Paleotemp.	<i>Dactylis glomerata</i> L.	.	.	r	r	.	+	.	r	.	.	.	2	42
H	Orof. S-Europ.	<i>Phyteuma ovatum</i> Honck.	.	r	.	.	r	r	+	33
G	Circumbor.	<i>Pericaria bistorta</i> (L.) Samp.	+	r	2	25
H	Europ.-Caucas.	<i>Primula vulgaris</i> Huds.	+	.	.	+	+	.	.	25
H	Circumbor.	<i>Caltha palustris</i> L.	+	+	17
H	Orof. S-Europ.	<i>Geranium phaeum</i> L.	.	.	+	+	17
H	Eurasiat.	<i>Poa trivialis</i> L.	r	+	17
H	Paleotemp.	<i>Silene dioica</i> (L.) Clairv.	r	r	.	17
H	Eurosib.	<i>Achillea millefolium</i> L.	+	8
H	Circumbor.	<i>Agrostis capillaris</i> L.	+	8
H	Eurasiat.	<i>Alchemilla vulgaris</i> L.	+	8
H	Eurasiat.	<i>Anthoxanthum odoratum</i> L.	1	8
H	Paleotemp.	<i>Anthriscus sylvestris</i> (L.) Hoffm.	.	.	.	+	8
H	Paleotemp.	<i>Arrhenatherum elatius</i> (L.) P. Beauv. Ex J. Presl & C. Presl	+	8
H	Endem. Alpica	<i>Centaurea nigrescens</i> subsp. <i>transalpina</i> Schleich. ex DC.) Nyman	+	8
H	Europ.-Caucas.	<i>Cynosurus cristatus</i> L.	+	8
H	Circumbor.	<i>Holcus lanatus</i> L.	2	8
H	Europ.-Caucas.	<i>Leontodon hispidus</i> L.	+	8
H	Circumbor.	<i>Lolium perenne</i> L.	+	8
H	Paleotemp.	<i>Lotus corniculatus</i> L.	+	8
H	Paleotemp.	<i>Myosotis sylvatica</i> Ehrh. ex Hoffm.	r	.	8
H	Eurasiat.	<i>Plantago lanceolata</i> L.	+	8
H	Circumbor.	<i>Poa pratensis</i> L.	+	8
H	Eurasiat.	<i>Potentilla erecta</i> (L.) Raeusch.	+	8
H	Subcosmop.	<i>Ranunculus acris</i> L.	+	8
G	Eurasiat.	<i>Ranunculus ficaria</i> L.	+	8
H	Circumbor.	<i>Rumex acetosa</i> L.	+	8
H	Subcosmop.	<i>Silene vulgaris</i> (Moench) Garcke	+	8
H	Circumbor.	<i>Taraxacum officinale</i> agg.	+	8
H	Subcosmop.	<i>Trifolium pratense</i> L.	1	8
H	Paleotemp.	<i>Trifolium repens</i> L.	+	8
H	Eurasiat.	<i>Trisetum flavescens</i> (L.) P. Beauv.	+	8
H	Eurasiat.	<i>Vicia cracca</i> L.	+	8

FESTUCO VALESIAEAE-BROMETEA ERECTI Br.-Bl. & Tüxen ex Br.-Bl. 1949

H	Endem. Alpica	<i>Avenula praeusta</i> (Rchb.) Holub	+	8
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GALIO APARINES-URTICETEA DIOICAE Passarge ex Kopecký 1969

G	Eurosib.	<i>Aegopodium podagraria</i> L.	.	.	1	+	+	+	+	+	50
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MONTIO FONTANAE-CARDAMINETEA AMARAE Br.-Bl. & Tüxen ex Klika & Hadac 1944

H	Circumbor.	<i>Cardamine flexuosa</i> With.	.	.	+	.	.	+	+	.	.	.	r	.	33
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EPILOBIETEA ANGUSTIFOLII Tüxen & Preising ex Von Rochow 1951

H	Eurosib.	<i>Fragaria vesca</i> L.	.	.	+	+	+	1	+	+	+	+	1	.	75
H	Centro-Europ.	<i>Senecio ovatus</i> (P. Gaertn., B. Mey. & Scherb.) Willd.	+	+	+	.	.	+	+	.	+	+	+	.	67
H	Eurasiat.	<i>Epilobium montanum</i> L.	+	r	.	.	+	.	25
H	Orof. S-Europ.	<i>Stachys alpina</i> L.	.	.	r	8

MULGEDIO ALPINI-ACONITETEA VARIEGATI Hada7 & Klika in Klika & Hada7 1944

P	Centro-Europ.	<i>Salix appendiculata</i> Vill.	.	.	.	+	+	.	.	+	+	+	+	.	50
H	Art.-Alp.	<i>Myosotis decumbens</i> Host	.	.	+	.	r	+	.	.	+	.	r	.	42
H	Orof. S-Europ.	<i>Saxifraga rotundifolia</i> L.	.	.	+	.	.	+	+	25

Supplemental Data IV

Phytosociological table of relevés conducted in five areas (chronosequence) of

Val Camonica

The relevés are listed according to the sequence given by the cluster analysis. Percentages of seeds are listed for the mixture (code = 1*). Syntaxonomical classes are listed according to the coefficient of maturity (m). The groups of species that characterize the three clusters are highlighted by boxes. The symbols related to life forms are those proposed by RAUNKIAER (1934): H, hemicryptophytes; T, therophytes; G, geophytes; NP, nano-phanerophytes; P, phanerophytes; Ch, chamaephytes. ** = sown species.

		Relevé code	1*	2	3	4	5	6			
		Cluster	A	A	A	B	C	C			
		Year of sowing	-	2012	2011	2009	2005	2000			
		Protected Area	-	Adamello Park	S. Antonio Valleys	Adamello Park	Adamello Park	Adamello Park			
		Latitude N	-	46° 05'18,6"	46° 08'51,5"	46° 08'28,8"	46° 07'59,5"	46° 05'11,8"			
		Longitude E	-	10° 25'07,1"	10° 12'50,2"	10° 22'35,4"	10° 23'27,2"	10° 22'12,8"			
		Altitude (m)	-	1310	1240	1190	1290	1220			
		Investigated area (m ²)	-	25	25	25	25	25			
		Slope (°)	-	36	26	45	45	6			
		Exposure (°)	-	100	90	200	180	210			
		Trees cover (%)	-	0	0	0	0	35			
		Shrubs cover (%)	-	0	0	5	20	15			
		Herbaceous cover (%)	-	65	85	60	45	30			
		Max height of trees (m)	-	0	0	0	0	5			
		Max height of shrubs (m)	-	0	0	1	1,5	1,5			
		Max height of grass (m)	-	1,5	1,2	1	1,5	0,8			
		Landolt indices:									
		T – temperature	3,20	3,24	3,42	3,06	2,98	3,08			
		K – continentality	3,07	3,03	2,99	3,06	3,30	3,17			
		L – light intensity	3,32	3,28	3,59	3,05	2,89	3,15			
		F – soil moisture	2,96	3,01	2,95	2,69	2,87	2,89			
		R – substrate reaction	2,93	2,97	3,00	2,47	2,89	2,93			
		N – nutrients	3,22	3,51	3,37	2,47	2,86	2,97			
		H – humus	3,16	3,08	3,05	4,09	3,50	3,25			
		D – aeration	1,34	1,61	2,32	1,63	2,54	1,90			
		CSR strategy:									
		C – competitors (%)	36,40	37,50	35,70	38,80	45,80	67,40			
		S – stress-tolerators (%)	30,70	25,70	29,40	33,30	30,70	17,90			
		R – ruderals (%)	32,90	36,80	34,90	27,90	23,50	14,70			
Life form	Chorotype	Provenance of seeds	N. of species	12	23	21	25	36	29	Pres. %	m
STELLARIETEA MEDIAE Tüxen, Lohmeyer & Preisling ex Von Rochow 1951											
T	Avv.	-		Erigeron canadensis L.				.	.	.	33
ARTEMISIETEA VULGARIS Lohmeyer, Preisling & Tüxen ex Von Rochow 1951											
T	Subcosmop.	-		Geranium robertianum L.				.	.	.	33
H	Europ.-Caucas.	-		Rumex obtusifolius L.				.	.	.	33
H	Subcosmop.	-		Urtica dioica L.				.	.	.	33
T	Eurasiat.	-		Galium aparine L.				.	.	.	17
H	Europ.-Caucas.	-		Verbascum thapsus L.				.	.	.	17
MOLINIO-ARRHENATHERETEA Tüxen 1937											
H	Eurosib.	A	** Achillea millefolium L.	0,2	+	1	+	+	+	100	
H	Circumbor.	NL	** Agrostis capillaris L.	1,0	+	+	2	+	+	100	
H	Paleotemp.	DK	** Dactylis glomerata L.	5,0	1	2	+	+	+	100	
H	Paleotemp.	DE	** Trifolium repens L.	2,0	+	+	+	+	+	100	
H	Circumbor.	DK	** Festuca rubra L.	59,8	2	2	1	+	.	83	
H	Circumbor.	DE	** Phleum pratense L.	3,0	+	+	+	.	.	67	
H	Subcosmop.	NZ	** Trifolium pratense L.	2,0	1	2	.	+	+	83	
H	Eurasiat.	DK	** Festuca pratensis Huds.	3,0	+	+	.	.	.	50	
H	Circumbor.	DK	** Lolium perenne L.	10,0	+	+	.	.	.	50	
H	Circumbor.	DK	** Poa pratensis L.	6,0	+	+	.	.	.	50	
H	Paleotemp.	-	Lotus corniculatus L.	.	+	.	.	+	.	33	
H	Paleotemp.	-	Silene dioica (L.) Clairv.	.	+	17	
H	Subcosmop.	-	Silene vulgaris (Moench) Garcke	.	+	17	
H	Circumbor.	-	Taraxacum officinale Weber	+	17	
H	Eurimedit.-Subatl.	CDN	** Trifolium hybridum L.	1,0	17	
KOELERIO GLAUCAE-CORYNEPHORETEA CANESCENTIS Klika in Klika & V. Novák 1941											
H	Centro- Nordeurop.	DK	** Festuca ovina agg.	7,0	+	+	+	+	+	100	
H	Art.Alp.	-	Silene rupestris L.	.	.	.	+	+	.	33	
FESTUCO-SESLERIETEA Barbéro & Bonin 1969											
H	Orof. S-Europ.	-	Myosotis alpestris F.W. Schmidt	.	.	+	.	+	.	33	
ASPLENIETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdorfer 1977											
H	Endem. Alp.	-	Phyteuma scheuchzeri All.	+	.	17	
FESTUCO VALESIIAE-BROMETEA RECTI Br.-Bl. & Tüxen ex Br.-Bl. 1949											
H	Eurasiat.	-	Ranunculus bulbosus L.	.	.	+	.	.	.	17	
H	Endem. Alp.	-	Avenula praeusta (Rchb.) Holub	+	17	
TRIFOLIO MEDII-GERANIETEA SANGUINEI Müller 1962											
H	Europ.-Caucas.	-	Hypericum montanum L.	.	.	.	+	+	.	33	
H	Paleotemp.	-	Hypericum perforatum L.	.	.	.	+	.	+	33	
H	S-Europ.-Sudsiber.	-	Veronica chamaedrys L.	+	.	17	

			MULGEDIO ALPINI-ACONITETEA VARIEGATI Hada7 & Klika in Klika & Hada7 1944			7
H	Orof. S-Europ.	-	<i>Saxifraga rotundifolia</i> L.	.	.	17
			EPILOBIETEA ANGUSTIFOLII Tüxen & Preising ex Von Rochow 1951			7
H	Circumbor.	-	<i>Epilobium angustifolium</i> L.	.	+	50
H	Eurosib.	-	<i>Fragaria vesca</i> L.	.	.	50
			RHAMNO CATHARTICAE-PRUNETEA SPINOSAE Rivas Goday & Borja ex Tüxen 1962			8
P	Europ.-Caucas.	-	<i>Corylus avellana</i> L.	.	.	50
P	Eurasiat.	-	<i>Salix caprea</i> L.	.	.	50
P	Eurosib.	-	<i>Populus tremula</i> L.	.	.	33
NP	Circumbor.	-	<i>Rubus idaeus</i> L.	.	.	33
NP	Eurimedit.	-	<i>Rubus ulmifolius</i> Schott	.	.	17
P	Europ.	-	<i>Sorbus aucuparia</i> L.	.	.	17
			QUERCO ROBORIS-FAGETEA SYLVATICAE Br.-Bl. & Vlieger in Vlieger 1937			9
G	Circumbor.	-	<i>Oxalis acetosella</i> L.	.	.	83
G	Orof. Centro-Europ.	-	<i>Petasites albus</i> (L.) Gaertn.	.	.	67
H	Europ.-Caucas.	-	<i>Chaerophyllum hirsutum</i> subsp. <i>villarsii</i> (W.D.J. Koch) Briq.	.	.	50
P	Europ.-Caucas.	-	<i>Fraxinus excelsior</i> L.	.	.	50
P	Orof. S-Europ.	-	<i>Laburnum alpinum</i> (Mill.) Bercht. & J. Presl	.	.	50
H	Orof. SW-Europ.	-	<i>Luzula nivea</i> (L.) D.C.	.	.	50
T	Eurosib.	-	<i>Melampyrum pratense</i> L.	.	.	50
H	Eurosib.	-	<i>Hieracium mororum</i> L.	.	.	33
H	SE-Europ.	-	<i>Knautia drymeia</i> Heuff.	.	.	33
H	Endem. Alp.	-	<i>Phyteuma betonicifolium</i> Vill.	.	.	33
H	Europ.-Caucas.	-	<i>Lamium flavidum</i> F. Herm.	.	.	17
G	Paleotemp.	-	<i>Platanthera bifolia</i> (L.) Rich.	.	.	17
H	S-Europ.-Sudsiber.	-	<i>Ranunculus nemorosus</i> DC.	.	.	17
G	Subcosmop.	-	<i>Dryopteris filix-mas</i> (L.) Schott	.	.	17
H	Circumbor.	-	<i>Solidago virgaurea</i> L.	.	.	17
H	Eurasiat.	-	<i>Veronica officinalis</i> L.	.	.	17
H	Paleotemp.	-	<i>Brachypodium sylvaticum</i> (Huds.) Beauv.	.	.	17
NP	Paleotemp.	-	<i>Solanum dulcamara</i> L.	.	.	17
NP	Centro-Europ.	-	<i>Hippocrepis emerus</i> (L.) Lassen	.	.	17
P	Eurosib.	-	<i>Betula pendula</i> Roth	.	.	17
H	Europ.-Caucas.	-	<i>Stellaria nemorum</i> subsp. <i>nemorum</i> L.	.	.	17
			VACCINIO MYRTILLI-PICEETEA ABIETIS Br.-Bl. in Br.-Bl., Sissingh & Vlieger 1939			9
G	Paleotemp.	-	<i>Dactylorhiza fuchsii</i> (Druce) Soó	.	.	33
P	Eurosib.	-	<i>Picea abies</i> (L.) H. Karst.	.	.	33
P	Orof. Centro-Europ.	-	<i>Larix decidua</i> Mill.	.	.	17

APPENDIX I

Syntaxonomic scheme

Nomenclature and numerical code of syntaxa follow BIONDI et al. (2014)

15. Cl.: *MONTIO FONTANAE-CARDAMINETEA AMARAE* Br.-Bl. & Tüxen ex Klika & Hadac
1944

30 Cl.: *ASPLENIETEA TRICHOMANIS* (Br.-Bl. in Meier & Br.Bl. 1934) Oberdorfer 1977

33 Cl.: *THLASPIETEA ROTUNDIFOLII* Br.-Bl. 1948

34 Cl.: *ARTEMISIETEA VULGARIS* Lohmeyer, Preising & Tüxen ex Von Rochow 1951

35 Cl.: *EPILOBIETEA ANGUSTIFOLII* Tüxen & Preising ex Von Rochow 1951

35.1 Ord.: *ATROPETALIA BELLADONNAE* Vlieger 1937

35.1.2 All.: *Epilobion angustifolii* Tüxen ex Egger 1952

39 Cl.: *STELLARIETEA MEDIAE* Tüxen, Lohmeyer & Preising ex Von Rochow 1951

40 Cl.: *GALIO APARINES-URTICETEA DIOICAE* Passarge ex Kopecký

43 Cl.: *MULGEDIO ALPINI-ACONITETEA VARIEGATI* Hadac & Klika in Klika & Hadac 1944

44 Cl.: *TRIFOLIO MEDII-GERANIETEA SANGUINEI* Müller 1962

46 Cl.: *FESTUCO-SESLERIETEA* Barbéro & Bonin 1969

46.2 Ord.: *SESLERIETALIA CAERULEAE* Br.-Bl. in Br.-Bl. & Jenny 1926

47 Cl.: *CARICETEA CURVULAE* Br.-Bl. 1948 *nom. cons. propos.* Rivas-Martínez, Diaz,
Fernández-González, Izco, Loidi, Lousa & Penas 2002

51 Cl.: *FESTUCO VALESIIACAE-BROMETEA ERECTI* Br.-Bl. & Tüxen ex Br.-Bl. 1949

51.2 Ord.: *BROMETALIA ERECTI* Koch 1926

51.2a Subord.: *LEUCANTHEMO VULGARIS-BROMENALIA ERECTI* Biondi, Ballelli, Allegrezza & Zuccarello

51.2a.1 All.: *Bromion erecti* Koch 1926

52 Cl.: KOELERIO GLAUCAE-CORYNEPHORETEA CANESCENTIS Klika in Klika & V.
Novák 1941

56 Cl.: MOLINIO-ARRHENATHERETHEA Tüxen 1937

56.2 Ord.: *ARRHENATHERETALIA ELATIORIS* Tüxen 1931

56.2.3 All.: *Trisetum flavescens*-*Polygonum bistorta* Br.-Bl. & Tüxen ex Marschall 1947

64 Cl.: RHAMNO CATHARTICAE-PRUNETEA SPINOSAE Rivas Goday & Borja ex Tüxen
1962

64.4 Ord.: *SAMBUCETALIA RACEMOSAE* Oberdorfer ex Passarge in Scamoni 1963

64.4.1 All.: *Sambucus racemosa*-*Salix caprea* Tüxen & Neumann 1950

69 Cl.: SALICETEA PURPUREAE Moor 1958

71 Cl.: QUERCO ROBORIS-FAGETEA SYLVATICAE Br.-Bl & Vlieger in Vlieger 1937

71.1 Ord.: *FAGETALIA SYLVATICAE* Pawlowski in Pawlowski & Wallisch 1928

72 Cl.: ERICO CARNEAE-PINETEA SYLVESTRIS Horvat 1959

74 Cl.: VACCINIO MYRTILLI-PICEETEA ABIETIS Br.-Bl. In Br.-Bl., Sissingh & Vlieger 1939

74.1 Ord.: *PICEETALIA EXCELSAE* Pawlowski in Pawlowski & Wallisch 1928

74.1.1 All.: *Picea excelsa* Pawlowski in Pawlowski & Wallisch 1928

75 Cl.: ROBINIETEA Jurco ex Hadac & Sofron 1980