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# Trees as indicators of climate change, geomorphological processes and anthropogenic impact in northern Italy

PhD Thesis
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I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person.

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## CONTENTS

# 1. ABSTRACT

Climate is changing due to both natural and anthropogenic causes. Among the several natural “sentinels” of climate change, trees potentially represent excellent indicators for reconstructing the changing climatic conditions in the recent and remote past and for monitoring the impacts of the current global warming and the related environmental conditions. Trees respond to changes in climate (air temperature, precipitation rate, water availability, etc.) and environment (soil nutrients, pathogen infections, mechanical wounding, etc.) both rapidly, modifying their tree-ring growth rate and physiological processes, and slowly, modifying their distribution. Chemical elements and pollutants deriving from human activity can mask the climatic signal but, at the same time, trees become precious collectors of data that can be used for multidisciplinary research.

In this Ph.D. project, I aimed at testing the potential use of trees as indicators of climate, environmental and human impacts in different morphoclimatic conditions, and to investigate if natural and anthropogenic conditions can mask the climatic signal recorded in trees. For this purpose, I selected different key sites in northern Italy.

I applied remote sensing techniques, dendrochronological and dendroisotopical approach and the investigation of Volatile Organic Compounds both in tree leaves and in tree rings at the Miage Glacier (AO), the widest debris-covered glacier in Italy. This site has been chosen because of its uniqueness: the Miage Glacier is the only glacier, in the southern side of the Alps, characterized by the presence of abundant supraglacial vegetation that confers an important ecological value to the site. The Miage Glacier has been recognised as a geomorphosite for its scientific, scenic, cultural, economic and educational values.

The results show that high-resolution satellite images allow the rapid detection of supraglacial trees whenever their density is high and that tree establishment is driven by supraglacial slope, debris thickness, glacier thickness and surface velocity. Supraglacial trees are characterized by tree-ring width, stable carbon and oxygen isotopes and needle volatile organic compounds that are significantly different compared to trees located outside the glacier.

The dendrochronological approach also resulted to be successful for defining areas affected by glacial melting water and, as a consequence, for assessing geomorphological hazards in glacial environments. Tree-ring width and terpenes in annual tree rings were found to be valuable indicators of fungal infection in mountain environments.

I also performed magnetic and mineralogical analyses of tree bark samples both at the head of a sample Alpine valley, in Santa Caterina Valfurva (SO), near one of the widest Alpine glacier, the Forni Glacier, and in an urban polluted context, the city of Milan, in order to identify the accumulation rate of magnetic particles and compare different morphoclimatic environments. The

results show that outer tree bark can be useful for monitoring the distribution of pollutants with magnetic properties and suggest the role of trees as PM sinks in urban areas.

Overall, the results presented in this thesis represent a contribution for a better understanding of the potential use of trees both in high mountain environments and in urban areas for monitoring the impacts of the current climatic and environmental changes.

Some of the proposed approaches represent scientific novelties because were never applied in extreme environments or were never considered in the context of the current climate change.

**RIASSUNTO**

Il cambiamento climatico in atto a livello globale è dovuto sia a cause di origine naturale sia all'azione antropica. Esistono numerose "sentinelle" del cambiamento climatico; tra queste, le piante arboree costituiscono potenzialmente degli ottimi indicatori sia per ricostruire le condizioni climatico-ambientali del passato recente e remoto, sia per monitorare gli impatti del riscaldamento climatico in atto. Le piante legnose infatti sono in grado di rispondere a variazioni del clima (temperatura dell'aria, tasso delle precipitazioni, disponibilità di acqua, etc.) e dell'ambiente (disponibilità di nutrienti, infezioni di organismi patogeni, ferita da impatto, etc.) sia rapidamente, modificando la velocità di crescita, le caratteristiche degli anelli di accrescimento annuali e la fisiologia, sia più lentamente attraverso variazioni della loro distribuzione spaziale. Elementi chimici e inquinanti possono mascherare il segnale climatico ma, allo stesso tempo, le piante diventano preziosi registratori di dati chimico-ambientali quali, ad esempio, il particolato atmosferico derivante da attività antropiche, utilizzabili per ricerche multidisciplinari.

L'obiettivo di questo progetto di dottorato è stato quello di testare il potenziale utilizzo degli alberi quali indicatori degli impatti climatici, ambientali e antropici in diverse condizioni morfo-climatiche e di identificare eventuali condizioni naturali o antropiche in grado di mascherare il segnale climatico stesso. A questi scopi, ho selezionato alcuni siti chiave nel nord Italia.

Ho applicato tecniche di remote sensing, studi dendrocronologici e dendroisotopici e analisi dei composti organici volatili nelle foglie e negli anelli di accrescimento annuali al Ghiacciaio del Miage (AO), il più esteso ghiacciaio italiano coperto da detrito (debris-covered glacier). Il sito rappresenta un unicum in quanto presenta un'estesa copertura arborea epiglaciale ubicata sulla porzione terminale della lingua di ablazione. Il ghiacciaio è stato riconosciuto come geomorfosito, sulla base dell'importante valore ecologico che si aggiunge alle valenze scientifiche, culturali, sceniche, economiche e didattiche già note.

I risultati di questo lavoro mostrano che le immagini satellitari ad alta risoluzione possono essere usate per localizzare rapidamente gli alberi epiglaciali, quando la densità arborea è elevata, e che la colonizzazione della superficie di un ghiacciaio nero da parte degli alberi è correlata ai parametri di pendenza, spessore del detrito epiglaciale, spessore del ghiacciaio e velocità superficiale del ghiacciaio. L'ampiezza anulare, gli isotopi stabili del carbonio e dell'ossigeno negli anelli di accrescimento e i composti organici volatili nelle foglie delle piante epiglaciali si differenziano significativamente da quelli che caratterizzano gli alberi situati sulle morene e nelle aree limitrofe al ghiacciaio.

La dendrocronologia è risultata inoltre essere un metodo valido per definire le aree influenzate dall'acqua di fusione glaciale e pertanto per studiare le pericolosità geomorfologiche negli ambienti

glaciali. Le variazioni dell'ampiezza anulare e i terpeni presenti negli anelli di accrescimento sono inoltre risultati essere due efficaci indicatori di infezioni ad opera di funghi in ambienti montani.

In contesto vallivo e antropizzato ho utilizzato anche analisi magnetiche e mineralogiche su campioni di corteccia arborea. I siti selezionati sono stati la città di Milano e un sito più remoto, il paese di Santa Caterina Valfurva (SO), ubicato alla testata della Valtellina, poco a valle del Ghiacciaio dei Forni, uno tra i più estesi ghiacciai vallivi composti italiani. I risultati di questa analisi mostrano che la distribuzione del particolato magnetico di origine antropica può essere monitorata utilizzando la corteccia esterna degli alberi, e che gli alberi rappresentano trappole di particolato atmosferico in aree urbane, contribuendo al generale miglioramento della qualità dell'aria.

Nel complesso, i risultati presentati in questa tesi rappresentano un contributo per la miglior comprensione del ruolo degli alberi, sia in ambienti di alta montagna sia in aree urbane, per il monitoraggio degli impatti del cambiamento climatico e delle variazioni ambientali ad esso correlate. Alcuni degli approcci proposti rappresentano delle novità dal punto di vista scientifico, in quanto alcune metodologie, pur essendo già note, non sono mai state applicate in ambienti estremi o non sono mai state prese in considerazione nell'ambito delle risposte al cambiamento climatico in atto.

## 2. INTRODUCTION

Climate change impacts and trees

The impacts of climate change have been particularly pronounced in the Italian Alps in the last two centuries. In fact, the Alpine environment is more vulnerable to the impacts of global warming because of its rough topographic features, climatic borderline equilibrium and increasing tourism (Beniston, 2005). Temperatures in the Alpine area have risen at a much higher rate compared to the average values of the northern hemisphere (Auer et al., 2007) and a change in the precipitation rate, consisting in a general decrease in the number of rainy days and an increase in frequency of intense rainy events, was detected (Castellari et al., 2014). As a consequence, Alpine regions are undergoing evident evolutions related to the cryosphere, hydrosystems and geomorphological dynamics, determining changes in natural hazards and induced risks (Einhorn et al., 2015).

Overall, there is large consensus in the scientific community concerning the threat that climate change may pose to environmental, social and economic systems (IPCC, 2014).

Among the impacts of climate change in the Italian Alps, the most evident consequences are the retreat and fragmentation of glaciers (Smiraglia et al., 2015) and the increase in thickness and extension of supraglacial debris coverage (Diolaiuti et al., 2003). Recently deglaciated areas and the surface of debris-covered glaciers (DCGs) have thus become new habitats for biological forms including bacteria, animals and both herbaceous and arboreal plants (Cannone et al., 2008; Franzetti et al., 2013; Tampucci et al., 2016). The distribution and characteristics of trees (including their physiology and growth patterns), in particular, represent a source of information for reconstructing the Alpine environment and climate in the recent past (Pelfini et al., 2012).

Tree distribution is strictly influenced by the position of the altitudinal treeline, which is known to be related to climatic and environmental conditions (e.g., Compostella and Caccianiga, 2016; Leonelli et al., 2016). However, even below the treeline, trees are not homogeneously distributed, due to biotic and abiotic factors that influence their establishment and germination. In particular, in recently deglaciated terrains, vegetation establishment follows a specific trend, related to a gradual shift in the dominant processes leading to vegetation establishment; from abiotic to biotic. Colonization begins with pioneer species, that are adapted to dominant abiotic processes (sediment properties, hydrology, slope, exposure, moisture) and grow where there is no, or low, competition for resources. Then, biotic parameters (competition with other species, tolerance, inhibition) gradually become more important in the establishment of vegetation. In these areas, dendrochronology represents an important tool for reconstructing past glacial activity (Garavaglia et al., 2010).

At temperate latitudes, distinct seasonal climate drives plants into periodic dormancy, resulting in the formation of annual tree rings (Fritts, 1976). Tree-ring growth is influenced by several factors, including climate, topography, nutrient availability and competition for resources with other trees. Thus, trees growing at the same site, in very similar climatic and environmental conditions, should develop similar year-to-year growth variability. After dating tree rings and measuring ring-width applying standard dendrochronological techniques, the comparison of ring-width patterns of different trees allows the reconstruction, at yearly resolution, of past climatic and environmental conditions that influenced tree-ring development at a specific site (Stokes and Smiley, 1968). For this reason, dendrochronology is widely applied in the reconstruction of past climate and environment. Climate reconstruction can be performed using trees growing near the extremities of their ecological amplitude, where they may be subject to climatic stresses: in these conditions, it is likely that tree-growth is limited by climate variables (Fritts, 1971).

Geomorphological events can also impact tree growth and can be dated applying dendrochronological methods. Landslides, rockfall events, seismic events, volcanic eruptions, floods, avalanches, debris flows, glacier retreat and advance are among the most common events that can be dated successfully using dendrochronology (Torbenson, 2015; Pelfini and Santilli, 2008).

Stable isotope techniques can be very useful in environmental reconstructions as the stable carbon and oxygen isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) of tree rings can provide long-term records of plant physiological processes. $\delta^{13}C$ is a good proxy of leaf-level intrinsic water use efficiency (WUEi), which is given by the ratio between leaf net photosynthetic rate and stomatal conductance (Dawson et al, 2002). Plant $\delta^{18}O$ is influenced by source water $\delta^{18}O$, but it is also inversely related to the ratio of atmospheric to leaf intercellular water vapour pressure, and can thus provide a time-integrated indication of leaf stomatal conductance during the growing season (Farquhar et al., 2007).

Trees respond to the external stresses also by modifying the production of volatile organic compounds (VOCs). These compounds play a central role in the plant-environment interactions by affecting key life processes such as defense and communication (Guerrieri and Digilio, 2008). They are produced in normal metabolic processes as well as in response to biotic and abiotic stresses (Mello and Silva-Filho, 2002). Plants growing at high altitude are characterized by several ecological, morphological, physiological and phytochemical adaptations due to limiting climatic conditions. In particular, VOC emission rates are related to temperature (Räisänen & *alii*, 2009), light (Staudt and Seufert, 1995) and humidity (Janson 1993).

Despite the wide knowledge already existing about changes in tree distribution (e.g., Theurillat and Guisan, 2001; Harsch et al., 2009) and about tree growth and physiology (e.g., Constable et al., 1999; Leonelli and Pelfini, 2008) as a response to the current climate warming, there is a lack of information about the evolution of tree distribution on the surface of debris-covered glaciers and about the impact of climate change, including both biotic and abiotic stresses, on tree-ring growth and physiological processes in high-mountain environments.

Trees are also useful indicators for monitoring in detail the distribution of air pollutants, whose concentration has been increasing globally following the intense urbanization characterizing the past decades (Jacob and Winner, 2009). Magnetic properties of plant organs are related to vehicular and industrial pollution (Flanders, 1994). Abrasion products from asphalt and from vehicle brake systems can also cause the emission of particulate matter (PM) with measurable magnetic properties (Sagnotti et al., 2006). Trams and trains also emit iron particles that are generated from the friction between wheel and brake interfaces (Kam et al., 2011), and the emissions generated from industrial metallurgical processes have been shown to contain magnetic components (Hunt et al., 1984). Trees act as passive collectors of atmospheric particulate matter (Matzka and Maher, 1999) and heavy metals (Orlandi et al., 2002). Iron, among other pollutants, has been proven to mask the climatic signal recorded in tree rings, thus preventing the reconstruction of accurate environmental and climatic information stored in them (Leonelli et al., 2012). Even though the relationship between air pollution and magnetic parameters is well understood for tree leaves (Rai, 2013), only a very limited number of studies have investigated the potential of measuring magnetic parameters of tree bark for monitoring air pollution (Kletetschka et al., 2003; Zhang et al., 2008; Kletetschka, 2011), thus the possibility of using tree bark for monitoring air quality in urban and extra urban sites has not been sufficiently investigated yet. The advantage in using tree bark instead of tree leaves for environmental analyses is that most of the tree species located in big cities at low altitudes are deciduous. Monitoring of the air quality using tree leaves is not possible during the winter months, which are typically more polluted, whereas bark can be sampled during every season.

In this Ph.D. project, I aimed at filling several gaps existing in the knowledge of the interactions between a changing climate/human influence and trees in northern Italy, applying a multi-disciplinary approach involving both methods that are usually performed in other contexts and new techniques. For each topic independent objectives were defined, but they all concur to a better understanding of the impacts of climate change and human pressure, especially in high-mountain environments that are particularly sensitive to biotic and abiotic stress.

<u>Tree distribution on the surface of debris-covered glaciers</u>

I analysed tree distribution and characteristics on debris-covered glaciers (DCGs), in order to investigate whether supraglacial arboreal vegetation in the Alpine environment can be considered a valuable indicator of microclimatic and local environmental changes. Among the Italian DCGs, the Miage Glacier (Mont Blanc Massif, Western Italian Alps) represents the ideal one to analyse supraglacial trees. The Miage Glacier is the widest DCG in Italy, with a well-developed supraglacial debris cover reaching at most 2 m thickness. Thick debris (exceeding a few centimetres) allows the glacier to reach very low altitudes, in fact the glacier front is located at about 1730 m a.s.l., much below the climatic treeline in the area, which is at about 2100 m a.s.l. (Leonelli and Pelfini, 2013). As a result, nowadays the Miage Glacier is characterized by the presence of abundant supraglacial trees and it represents a unique situation in the European Alps. The distribution of trees is related to several ecological and environmental factors, and can indirectly give information about the characteristics of supraglacial debris cover and on the recent glacial dynamics (Pelfini et al., 2007). For this reason, not only I tested a novel approach to directly detect supraglacial trees from satellite images (**chapter 3.1**), but I also analysed a selection of glacier parameters in order to detect which ones mainly influence tree germination and growth on the supraglacial debris (**chapter 3.2**).

<u>Trees for geomorphological investigations and geomorphosite assessment</u>

The current climate warming causes remarkable changes of the Alpine landscapes and the related geodiversity and biodiversity. Vegetation, and trees in particular, represents a valuable indicator of both climatic and environmental changes. I summarized the advantages in using vegetation for geomorphological investigations in mountain environments, i.e., for characterizing the processes occurring at the surface of debris-covered glaciers and on recently deglaciated surfaces (**chapter 4.1**).

Then I reported a peculiar case study in which I applied the typical dendrochronological techniques in order to analyse the impact of melting water fluctuations on trees located on the shore of an ice-contact lake named Lago Verde located at the Miage Glacier (**chapter 4.2**).

Finally, I investigated the role of vegetation in the geomorphosite assessment of debris-covered glaciers, choosing the Miage Glacier as an ideal study area in the Italian Alps. In fact, nowadays debris-covered glaciers represent a distinctive category of glacial geomorphosites, as they represent one of the main responses of the glacial environment to the current climate change (**chapter 4.3**).

<u>Trees for investigating environmental stress in glacial and periglacial environments</u>

Environmental stress can be defined as any environmental change that acts to reduce the fitness of organisms (Koehn and Bayne, 1989). Environmental stress can be due both to changes in abiotic factors such as temperature, water supplies, chemical components and to biotic stresses e.g. parasitism and competition (Bijlsma and Loeschcke, 2005). Tree responses to climate change and related environmental stress in high-mountain environments include the modification of tree-ring patterns and growth rate, the modification of carbon and oxygen stable isotope ratio in the annual tree rings, the formation of tree-ring anomalies and the emission of different rates of Volatile Organic Compounds (VOCs).

I applied dendrochronological techniques, the analysis of carbon and oxygen isotopes in tree rings and the investigation of VOCs in tree leaves in order to test if these approaches are useful for the identification of environmental stresses in trees located on the surface of the Miage debris-covered Glacier (**chapter 5.1**).

Then I tested a novel approach with the aim of performing an early diagnosis of tree disease in high-mountain environments by analysing the terpene content within annual tree rings of trees putatively infected by a common pathogen fungus in Italy, comparing the results with putatively not infected trees. The current climate change determines an increase in average air temperature also during the winter months, thus causing the modification of the habitat of fungal pathogens, that are also colonizing areas located at higher elevation, where they were previously absent (La Porta et al., 2008) (**chapter 5.2**).

<u>Trees for describing the distribution of air pollutants</u>

Trees act as bioindicators allowing the detailed monitoring of the distribution of atmospheric PM. In particular, magnetic properties of several tree tissues are related to vehicular and industrial pollution. In order to investigate PM distribution, the comparison between polluted areas and “control”, less polluted, sites, is necessary.

I applied magnetic analyses on tree bark of trees located in one of the most polluted areas of Italy, the city of Milano and I compared the results with data gained in a site located at a higher altitude where air pollution is less pronounced, i.e. in the Upper Valtellina (**chapter 6.1**).

Summarizing, the objectives of this Ph.D. project were to:

i) Identify a rapid and accurate method for detecting trees growing on the surface of debris-covered glaciers using data obtained from satellite images and field surveys;
ii) Define and deepen the knowledge about the utility of vegetation, and in particular of trees, in geomorphological investigations and geomorphosite assessment in the Alpine environment;
iii) Evaluate if tree-ring patterns, stable isotopes and Volatile Organic Compounds produced and emitted by trees in different tissues can be useful for detecting environmental stress and pathogen infection at high altitudes and their relationship with the ongoing climatic trend;

iv) Describe the spatial distribution of air pollutants through the analysis of magnetic particles deposited on tree bark in a highly polluted area in the Po plain and in a less disturbed site at higher elevations.

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# 3.

# TREE DISTRIBUTION ON DEBRIS-COVERED GLACIER SURFACE

**3.1.**

# A first attempt to detect supraglacial trees on debris-covered glaciers by means of high-resolution satellite images

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**Abstract**

Tree cover is spreading on the surface of debris-covered glaciers following the ongoing increasing in debris-covered area and thickness. Since the presence of supraglacial trees is related to several parameters, detailed analysis of its occurrence, distribution and features may contribute to describe the behavior and evolution of debris-covered glaciers and their changes over time.

In this paper, we present the results from a pilot study aimed at identifying the distribution and density of supraglacial trees on the widest Italian debris-covered glacier, the Miage Glacier (Mont Blanc Massif), applying remote sensing techniques.

The Normalized Difference Vegetation Index (NDVI) was calculated on high-resolution satellite images. The results of this analysis were integrated with altitude and slope data in order to better define the areas characterized by the presence of trees. The results underline that the NDVI calculated on high-resolution satellite images allows the detection of supraglacial trees with some sensor-specific differences due to the resolution and wavelengths of the satellite images used.

**Keywords**: supraglacial trees, debris-covered glaciers, remote sensing, Pleiades, SPOT 7, Miage Glacier.

## Introduction

Supraglacial vegetation is becoming a common feature on debris-covered glaciers (DCGs). These glaciers are widespread in the mountain chains of Asia and New Zealand, but their number is increasing in the European Alps as well (Diolaiuti, D'Agata, & Smiraglia, 2003). On the Southern side of the Alps supraglacial vegetation is present on some debris-covered glaciers, e.g., the Miage Glacier (Mount Blanc Massif), characterized by abundant shrubs and trees, the Brenva Glacier (Mount Blanc Massif), with a scattered but widespread vegetation cover on the detached debris-covered tongue, the Belvedere Glacier (Monte Rosa Group), where small supraglacial shrubs and trees are present, and the Calderone Glacier (which is now divided in two partially debris-covered *glacierets* and represents the only ice body in the Apennines) (Pelfini, & Leonelli, 2014).

Recent literature (see Oerlemans, Giesen, & Van den Broeke, 2009) reports darkening phenomena affecting several mountain glaciers, thus suggesting that an increase in the number and extent of debris-covered ice will occur in the next future. This leads to a noticeable modification in the Alpine landscape. The debris cover also represents a new habitat for living organisms, such as microorganisms (Franzetti et al., 2013), animals (Gobbi, Isaia, & De Bernardi, 2011), yeasts (Turchetti et al., 2013), and vegetation (Gentili et al., 2015). Even if supraglacial debris covers in a continuous and uninterrupted way the largest part of the ablation zone of actual DCGs (Benn & Evans, 2010), other glaciers show wide buried ice areas on their melting tongues as well. Although these latter glaciers cannot be considered actual debris-covered ice bodies, herbaceous vegetation is beginning to colonize their rock mantle. Examples are the tongue of Forni Glacier (Ortles-Cevedale Group) where herbaceous plants are found on the medial moraine, the Ciamousseretto Glacier (Gran Paradiso Group), the Capra Glacier (Levante Group) and the Amola Glacier (Adamello-Presanella Group) (Pelfini & Leonelli, 2014).

The Miage Glacier can be considered the only debris-covered glacier of the Italian Alps characterized by the presence of an actual supraglacial forest: grass, shrubs and trees colonize the lower sector of the ablation tongue and they move downvalley according to the glacier surface velocity (Leonelli & Pelfini, 2013). The velocity, slope, thickness, debris grain size, porosity and moisture of the supraglacial debris layer are the main elements controlling both germination and growth of vegetation, especially of trees (Caccianiga et al., 2011). In particular, trees can be found on the lower portion of the glacier tongue, where the debris is thick enough, the glacier velocity is low and the slope gentle (Leonelli, Pelfini, & Morra di Cella, 2009; Pelfini, Santilli, Leonelli, & Bozzoni, 2007; Pelfini et al., 2012; Vezzola, Diolaiuti, D'Agata, Smiraglia, & Pelfini, 2016). Trees are a precious source of climatic and environmental information (e.g., Fritts, 1976) and, in the case of debris-covered glaciers, tree-ring chronologies from supraglacial trees and growth anomalies in tree rings can considerably increase the current knowledge related to the glacier dynamics (Pelfini et al., 2007). The analysis of the distribution of supraglacial trees represents the first step in the selection of appropriate areas for the collection of samples from trees and the analysis of tree-ring characteristics (Leonelli et al., 2014). However, detection and analysis of supraglacial trees is still a challenge, because field investigations are expensive and, moreover, not always possible in remote areas.

Satellite images represent a new source of data in the analysis of vegetation in glacial environments (Danby & Hik, 2007; Vescovo & Gianelle, 2006) but they have not so far been applied in the investigation of the occurrence and distribution of trees, and more in general of vegetation, on the surface of debris-covered glaciers. High resolution multispectral satellite images can be used to detect areas characterized by vegetation, also in the case of low-density vegetation. For instance, vegetation indexes were applied to identify mosses and lichens in polar regions (e.g. Shin, Kim H., Kim S., & Hong, 2014), in the classification of grasslands (Schuster, Schmidt, Conrad, Kleinschmit, & Forster, 2015) and in the study of vegetation in mountain environments (Zhang Z.M., Zhang Z.K., Guo, Tao, & Ou, 2013).

This research aimed at testing a remote sensing approach to identify the occurrence, distribution and density of trees on the surface of the widest Italian debris-covered glacier, the Miage Glacier (Mont Blanc Massif, Italy). In this paper, we present the first results obtained by calculating the most commonly used vegetation index, the Normalized Difference Vegetation Index (NDVI, Rouse, Haas, Schell, & Deering, 1974) based on high-resolution satellite images.

## Study area

The Miage Glacier (45°47’ N, 6°52’ E) is the widest debris-covered glacier in the Italian Alps, with an area of 10.47 km$^2$ (Smiraglia et al., 2015; Fig.1).

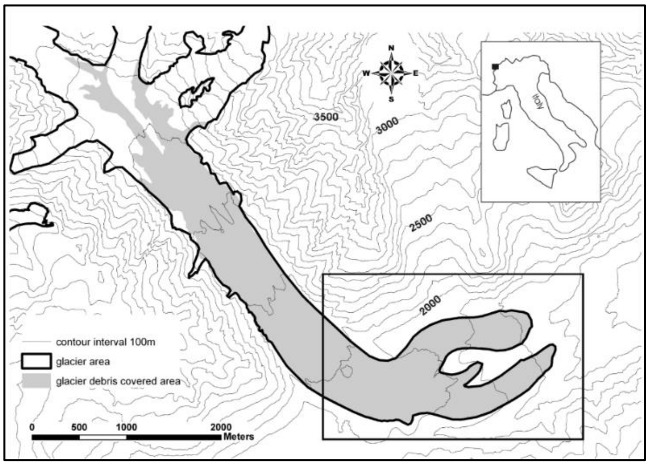


***Figure 1.*** *Location map (Miage Glacier, Aosta Valley, Italy). The area investigated in this study is represented with a black box.*

The glacier drains the southwest slope of Mont Blanc in the Aosta Valley (Western Italian Alps) and shows a continuous debris cover on the ablation tongue. Its shape and morphology resemble the huge Asian debris-covered glaciers. The glacier snout terminates in two main lobes (the southern and northern lobes) and a smaller intermediary one. Until the last decade the most-known and best developed debris-covered glaciers of the Alps were all located on the Italian side of the Alpine chain (the Miage and the Brenva glaciers in the Mont Blanc Massif and the Belvedere glacier in the Monte Rosa Group). The Miage Glacier now represents the best example of an active debris-covered glacier in Italy and it has been the subject of many investigations. It was first explored in the 18th century by De Saussure and since then many studies of its geomorphologic and glaciological features have been carried out (Baretti, 1880; Capello, 1959; Cunietti, 1961; Deline, 1999; Deline & Orombelli, 2005; Lesca, 1974; Sacco, 1917; Smiraglia, Diolaiuti, Casati, & Kirkbride, 2000; Thomson, Kirkbride, & Brock, 2000). Some studies also addressed the development of supraglacial debris (Deline, 2005), others focused on the calving phenomena occurring at its ice-contact lake and on the lake abrupt drainage events (Deline et al., 2004; Diolaiuti et al., 2006; Masetti, Diolaiuti, D'Agata, & Smiraglia, 2010), on the thermal properties of the debris (Mihalcea et al., 2008) and on the presence of vegetation supported by the occurrence of a debris cover (Caccianiga et al., 2011; Garavaglia, Pelfini, & Motta, 2010; Pelfini et al., 2012; Richter, Fichter, & Grüninger, 2004). Abundant vegetation located on the debris surface confers a high value to the Miage Glacier when it is considered as a geomorphosite (Bollati, Smiraglia, & Pelfini, 2013; Bollati, Leonelli, Vezzola, & Pelfini, 2015; Garavaglia, Pelfini, & Bollati, 2010; Pelfini & Bollati, 2014; Pelfini, Bollati, Pellegrini, & Zucali, 2016). In fact, the debris cover is colonized by vegetation, particularly on the lowermost part, where tree species (*Larix decidua* Mill. and *Picea abies* Karst) occur (Fig. 2). In recent investigations, microfauna and bacteria were also sampled and analyzed on the glacier surface (Franzetti et al., 2013).

On the Miage Glacier, high rates of debris are supplied by rock falls and avalanches from the surrounding rock walls and have enabled the development of the present debris-covered glacier tongue, effectively slowing down the glacier retreat (Diolaiuti, D'Agata, Meazza, Zanutta, & Smiraglia, 2009). The debris shows thicknesses ranging from a few centimeters (in the upper glacier sector) up to 1.5 meters (close to the glacier terminus), mainly depending on the surface slope and the glacier flow magnitude. The grain sizes range from rock boulders to fine pebbles and sand and mainly consist of crystalline rocks: gneiss, micaschist and granite (Deline, 2005).

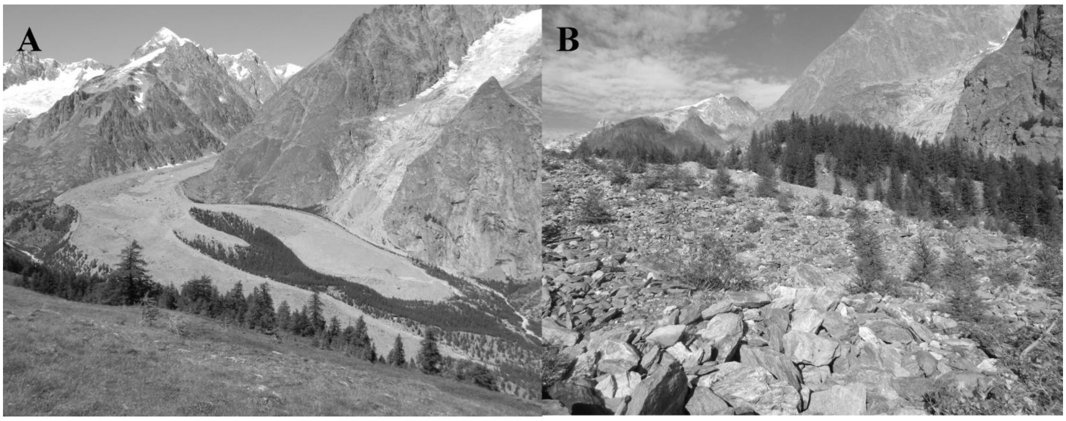


***Figure 2.*** *The ablation tongue of the Miage Glacier in the Aosta Valley, characterized by two main lobes and a smaller one in between (A) (photo: courtesy of D. Zannetti, August 2011). Supraglacial vegetation has colonized the terminal part of the glacier tongue (B) (photo: L.C. Vezzola, August 2012).*

**Materials and methods**

Two high-resolution satellite images were obtained from Airbus Defence and Space (www.geostore.com). The first one was acquired by the Pleiades 1B satellite, launched on December 2, 2012, and the second one by the SPOT 7 satellite, launched on June 30, 2014; both satellites were launched by CNES (Centre National d'Études Spatiales), the space agency of France. These systems deliver an optical high-resolution panchromatic image and a four bands multispectral image (see Table 1), orthorectified via a Digital Terrain Model. For the Pleiades image, pixel size is 0.5 m x 0.5 m for the orthorectified panchromatic channel and 2 m x 2 m for the multispectral bands (Gleyzes, Perret, & Kubik, 2012). The Pleiades image used in this study was acquired on 21$^{st}$ August 2013 under clear sky conditions and with 0% cloud cover. The SPOT image was acquired on September 25, 2015 under clear sky conditions and cloud cover was absent. Pixel size is 1.5 m x 1.5 m for the orthorectified panchromatic channel and 6 m x 6 m for the multispectral bands (Prost, 2014).

***Table 1.*** *Characteristic wavelengths of the four bands for the Pleiades and SPOT 7 satellite images.*

| Satellite image | Spectral Band | Wavelengths |
|---|---|---|
| Pleiades 1B | Blue | 430-550 nm |
| | Green | 490-610 nm |
| | Red | 600-720 nm |
| | Near Infrared | 750-950 nm |
| SPOT 7 | Blue | 455-525 nm |
| | Green | 530-590 nm |
| | Red | 625-695 nm |
| | Near Infrared | 760-890 nm |

Beside the high spatial resolution, the main advantage of the sensors on board Pleiades and SPOT 7 is the availability of a NIR channel that allows a clear distinction of vegetation (see Fig. 3) and calculation of vegetation indexes. To further improve the resolution of the multispectral bands, both images were individually pan-sharpened using the panchromatic channel and co-registered to avoid pixel misalignment. The NDVI was calculated on each scene according to the formula:

$$NDVI = \frac{B_{NIR} - B_R}{B_{NIR} + B_R}$$

where the NDVI is unitless and ranges between 0 and 1. Detection of supraglacial trees was further based on thresholding the NDVI, using a threshold of 0.2 which is a value commonly used in literature (Bayramov, Buchroithner, & Bayramov, 2016; Maxwell & Sylvester, 2012).

To further restrain pixels and avoid misclassification due to areas in shadow with a high NDVI, two additional criteria were adopted (see Fig. 4): 1) pixels should be below the altitude of the glacier vegetation and tree limit, which in the study area was detected between 2000 m and 2100 m in 2006 a.s.l.; Caccianiga et al., 2011), where trees can actually grow, and 2) terrain slope should be less than 10°. In fact, slope is one of the main parameters influencing tree establishment on the Miage Glacier (Vezzola et al., 2016). Altitude and slope were calculated by Diolaiuti et al. (2009) from a DEM (Digital Elevation Model) with a 10 m x 10 m spatial resolution that these authors derived from high-resolution aerial photographs (a stereo pair). Both altitude and slope from the DEM were oversampled via bilinear interpolation to 0.5 m spatial resolution, as well as the SPOT-derived NDVI, to set a common spatial resolution for all the datasets. Finally, the classification results were clipped using the glacier outlines obtained from Smiraglia et al. (2015) and the classification images from the Pleiades and SPOT images were inter-compared to assess common areas of supraglacial trees.

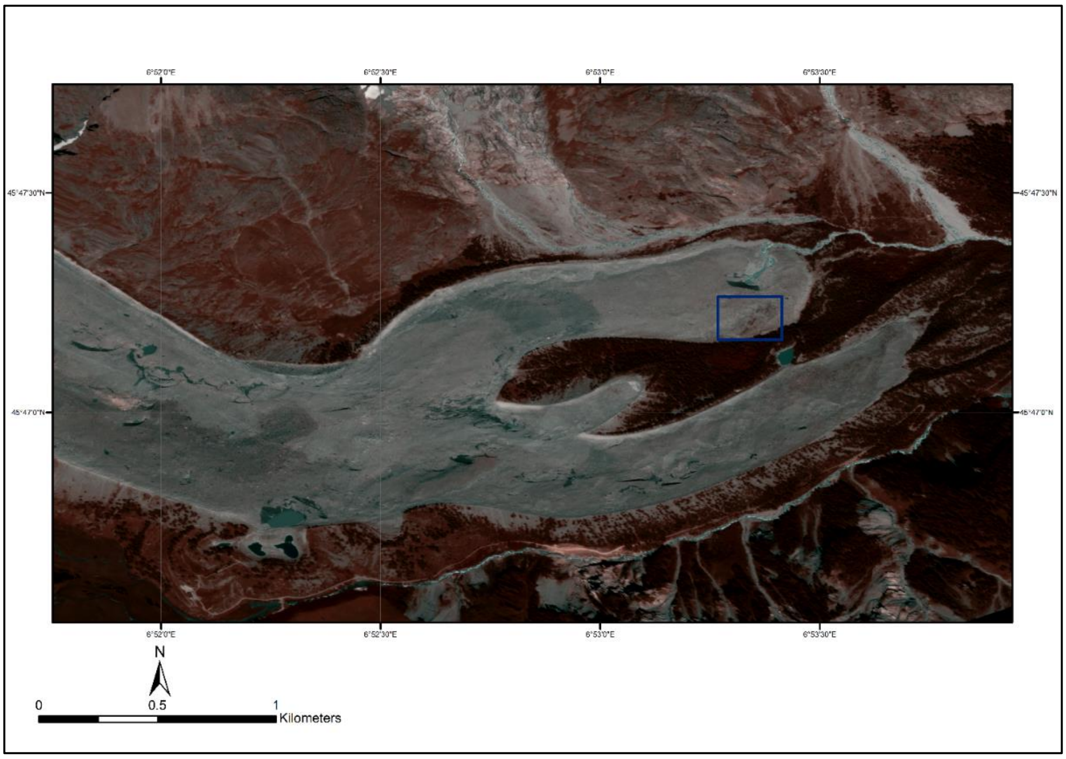


***Figure 3.*** *False colour composite using bands 4-3-2 (NIR, RED, GREEN) of the Pleiades image. Trees appear red. Note the forest between the two main lobes and abundant supraglacial vegetation on the northern lobe, within the blue box.*

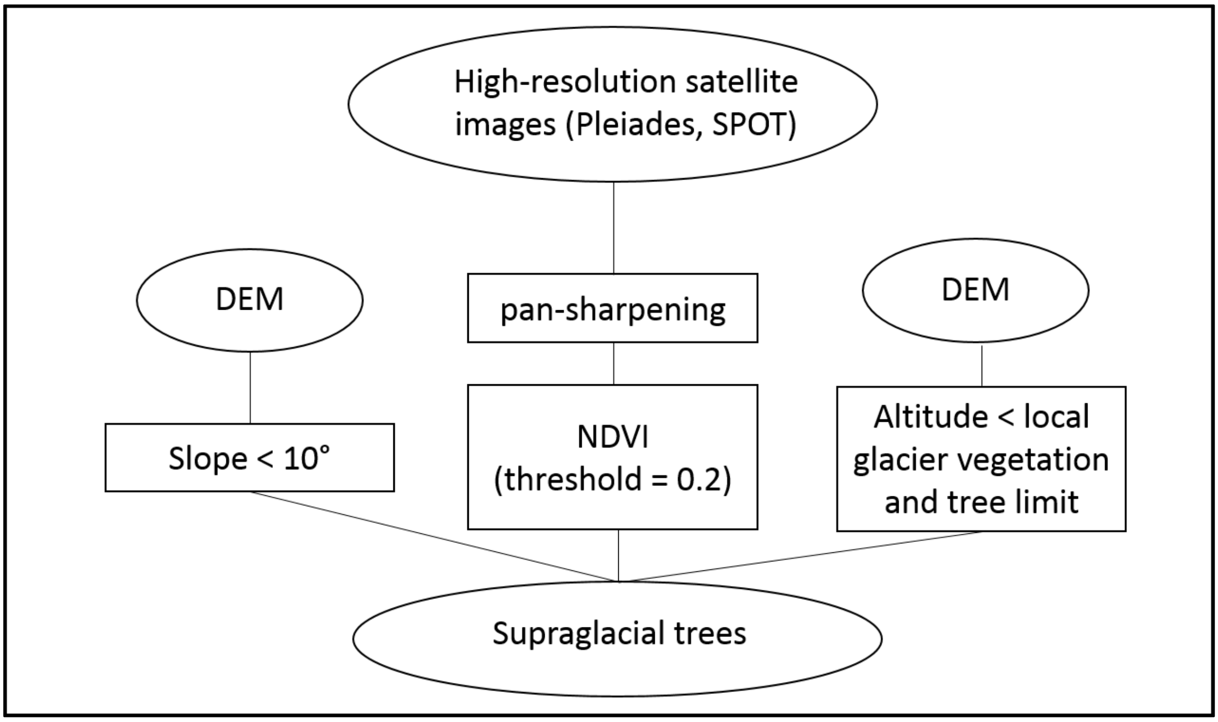


***Figure 2.*** *Flow chart of the method applied for the identification of supraglacial trees through the remote sensing approach.*

## Results

The classification based on the NDVI and the slope and altitude criteria allowed the detection of a total tree cover of 5905 $m^2$ on the Pleiades image (Fig. 5A) and of 11,578 $m^2$ on the SPOT 7 image (Fig. 5B). Pixels with values supporting the potential presence of trees were detected between 1700 m and 2100 m a.s.l. and, in particular, over 50% of them (51.84% on the Pleiades image and 50.30% on SPOT) are located between 1800 m and 1850 m a.s.l., near the internal margin of both the north and south lobes. The applied method suggests that most abundant tree cover is located between 1750 and 1900 m a.s.l. on both images, with a larger area always detected on the SPOT image compared to the Pleiades image (8348 $m^2$ and 4074 $m^2$, respectively). Only in the altitude belts 1900 – 1950 m a.s.l. and 2000 – 2050 m a.s.l., more trees were detected on the Pleaides image compared to SPOT (Fig. 6).

If only the two main lobes of the glacier tongue are considered, from 1700 up to about 2050 m a.s.l., 2573 $m^2$ of tree cover were detected on the north lobe on the Pleiades image and 1966 $m^2$ on the south lobe, whereas for SPOT, 4121 $m^2$ of trees were detected on the north lobe and 4541 $m^2$ on the south lobe. In spite of the difference in the area of supraglacial tree cover detected on the SPOT vs Pleiades image, the trend that can be observed looking at the two lobes is similar, i.e. when a larger area is detected on the Pleiades image on the north lobe of the Miage Glacier compared to the south lobe, more trees can be seen on the SPOT image on the north lobe compared to the south one (Fig. 7).

The area characterized by supraglacial trees that was detected on both the Pleiades and SPOT images was 4050 $m^2$, and if only the two main lobes are considered, 1981 $m^2$ were detected on both images on the north lobe and 1382 $m^2$ on the south lobe (Fig. 8).

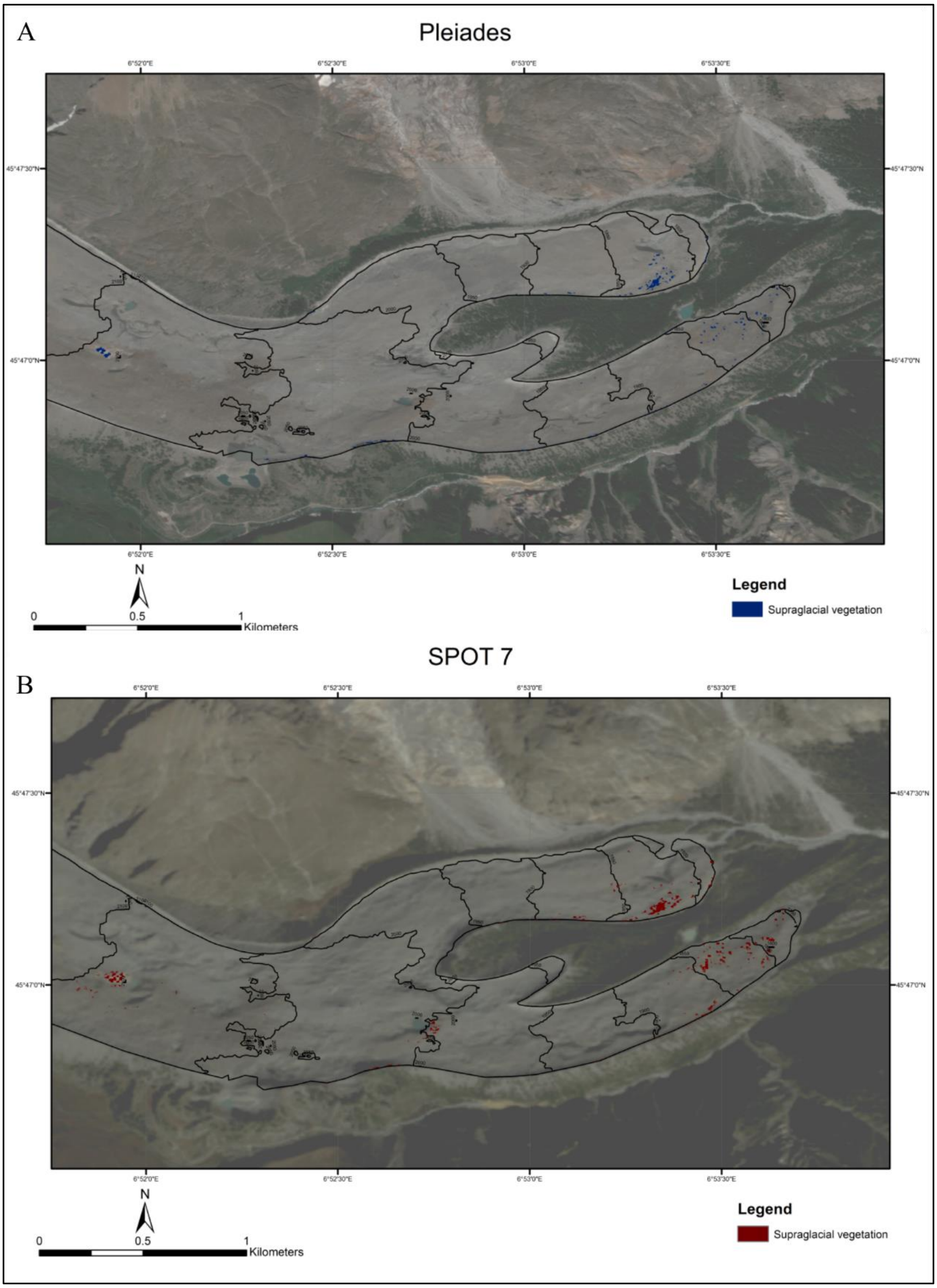


***Figure 5.*** *Classification based on NDVI, altitude and slope criteria performed on A) the Pleiades image and B) the SPOT image. Only the areas detected as characterized by supraglacial trees are reported.*

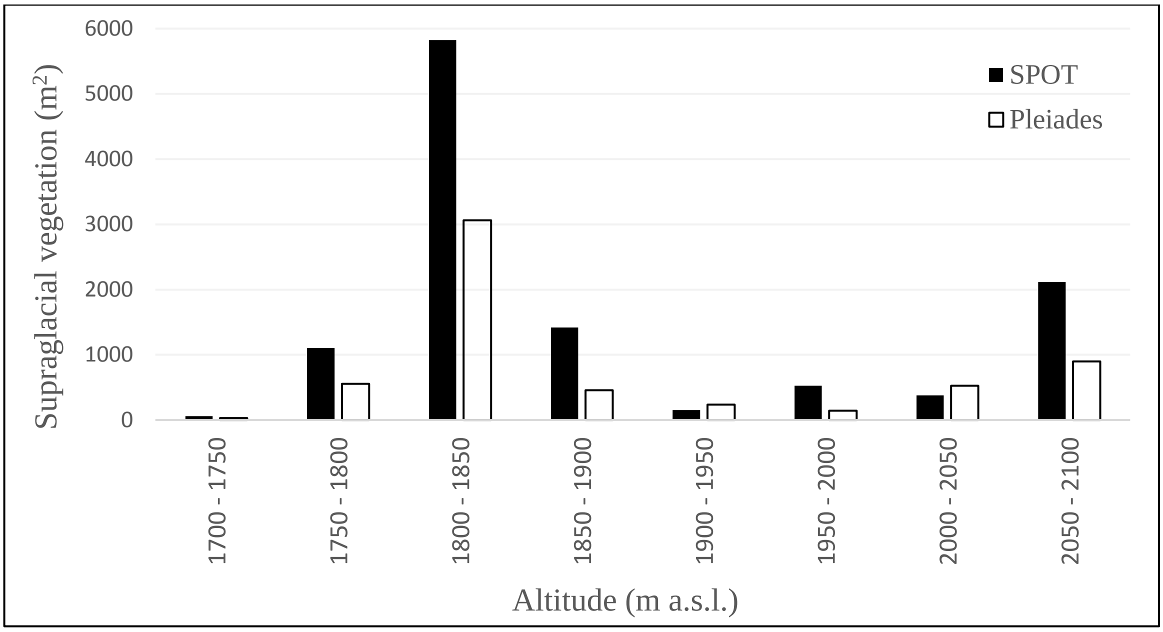


***Figure 6.*** *Hypsographic curve of supraglacial trees on the Miage Glacier, detected on the SPOT (black bars) and on the Pleiades (white bars) images.*

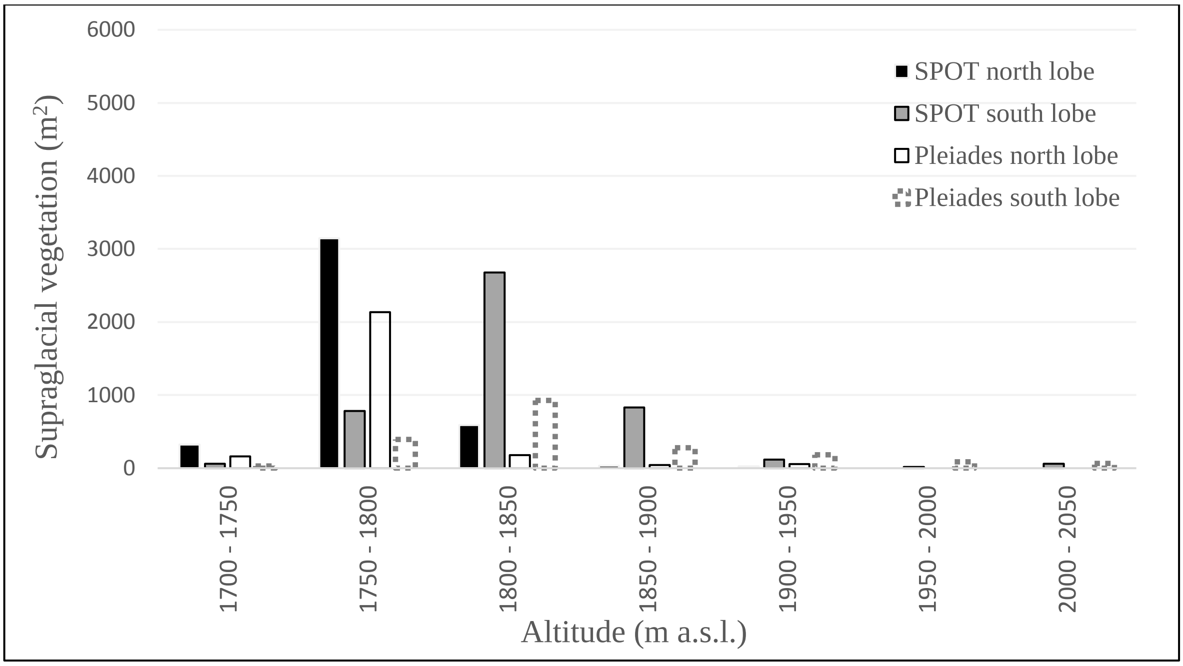


***Figure 7.*** *Hypsographic curve of the north and south lobes of the Miage Glacier detected on the SPOT (black bars) and Pleiades (white bars) images.*

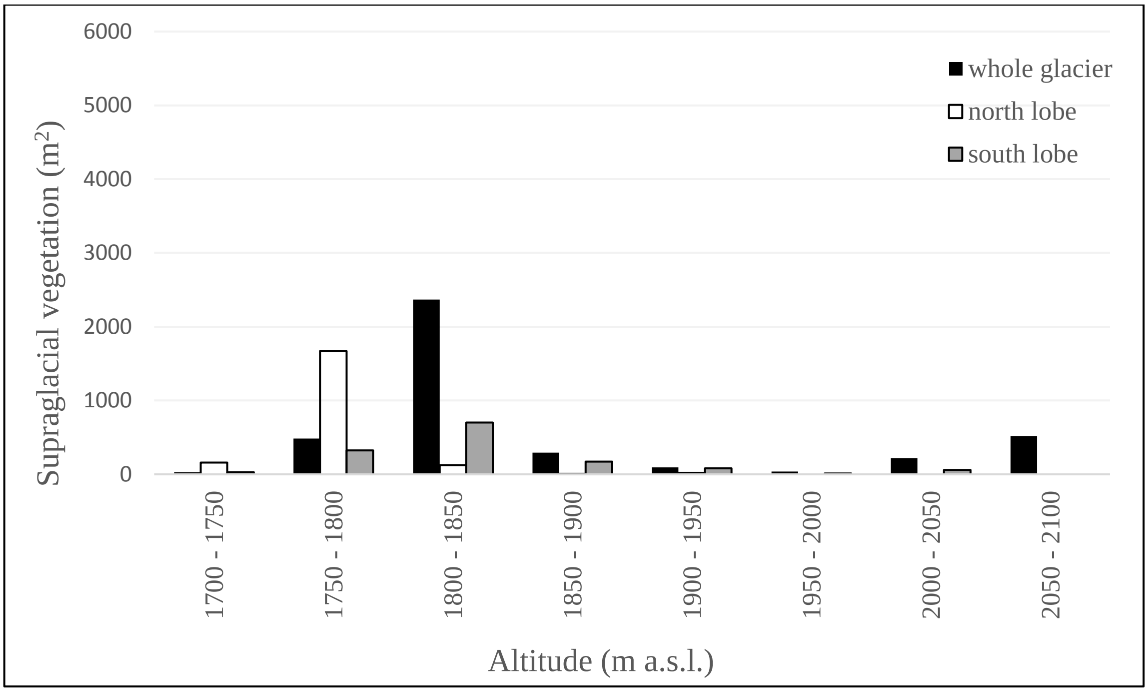


***Figure 8.*** *Hypsographic curve of supraglacial trees common to both the Pleiades and SPOT images, considering the whole glacier (black bars), only the north lobe (white bars) and only the south lobe (grey bars).*

**Discussion and conclusion**

This study represents a first attempt to map supraglacial tree occurrence on the surface of debris-covered glaciers through a remote sensing approach based on high-resolution satellite images. The proposed method was applied to the Miage Glacier (Mont Blanc Massif, Italian Alps), which is characterized by abundant supraglacial vegetation, including both herbaceous and arboreal plants, on the glacier tongue.

We calculated the NDVI on high-resolution Pleiades and SPOT 7 images. The NDVI is a powerful index also capable of detecting vegetation cover in the case of sparse vegetation, i.e. in arid (Weiss, Gutzler, Allred Coonrod, & Dahm, 2004) and Arctic (Laidler, Treitz, & Atkinson, 2007) environments. The applied approach allowed the detection of pixels featuring values that are compatible with the occurrence of supraglacial trees on both the Pleiades and SPOT 7 images, although some differences were detected between them.

In particular, even though the two satellite images could find similar patterns of distribution of supraglacial trees, a greater vegetation cover was detected on the SPOT image, about twice the value detected on the Pleiades image. This difference can be only partially explained considering that the acquisition year of the two images was different. Although the density of supraglacial trees is increasing on the Miage Glacier (Leonelli, Pelfini, Morra di Cella, & Garavaglia, 2011), an increase as pronounced as the one detected analyzing the satellite images is very unlikely. In fact, the number of supraglacial trees does not increase at the observed rate, due to the instability of glacier surface and the formation of ice cliffs (the latter ones caused by differential ablation processes), that determine tree uprooting and death over time (Pelfini et al., 2012). Ablation is more intense on ice cliffs, and both processes of down-wasting and back-wasting (*sensu* Benn & Evans, 2010) occur and influence supraglacial trees located in their proximity (Bollati et al., 2015).

Thus, the differences might instead be due to specific characteristics of the two sensors that could influence the NDVI calculation. In fact, NDVI variations are strictly related to the near infrared and red spectral bands, and several factors such as atmospheric conditions, solar illumination, lack of bandwidth correspondence and spatial and radiometric resolution of sensors can come into play. As concerns spatial resolution, previous studies showed that sensors with a finer spatial resolution produce lower NDVI values compared with sensors characterized by coarser spatial resolution (e.g., Abuzar, Sheffield, Whitfield, O'Connell, & McAllister, 2014; Soudani, François, le Maire, le Dantec, & Dufrêne, 2006).

A further problem is represented by the properties of tree cover on the Miage Glacier. In fact, the distribution of supraglacial plants is usually extremely fragmented and sparse, but also the characteristics of the most abundant tree species do not allow the formation of uniform areas on the glacier surface that can be easily detected through the analysis of satellite images. In particular, the main arboreal species detected on the Miage Glacier are *Larix decidua* Mill. and *Picea abies* Karst, whose leaves are very small. Moreover, even though mature trees have been identified through field surveys on the glacier, most of them are quite young (i.e., they are less than 30 years old) and suffer stress conditions due to the glacier flow and movements inducing surface instability (Leonelli & Pelfini, 2013), thus they do not form a well-developed canopy and tend to be smaller than 30 cm.

In summary, our results suggest that NDVI calculation on high-resolution satellite images (i.e., Pleiades and SPOT 7), together with slope and altitude thresholds, allows the detection of supraglacial trees, although what sensor yields the best accuracy is still uncertain. However, we cannot safely assume that some herbaceous plants are not included in the results obtained from the applied method. For this reason, accurate field surveys should be performed in order to define whether the areas detected with this approach are actually characterized by trees or if herbaceous vegetation is also identified.

Future investigations will aim at performing detailed field surveys on the Miage Glacier surface (possibly coupled with a satellite scheduled acquisition), in order to better assess the accuracy of the remote sensing approach and improve the understanding of sensor-related differences. Field surveys using Unmanned Aerial Vehicles (UAVs or drones) equipped with both an optic and NIR camera could also be performed to further improve the spatial resolution of the data and the DEM. The same method could be applied i) to study other alpine debris-covered glaciers, in order to select areas where dendrochronological sampling can be performed; ii) to acquire multi-temporal qualitative information where field surveys are particularly difficult or not possible and iii) to contribute to the study of glacial geo(morpho)sites by assessing their ecological value also based on the occurrence and abundance of supraglacial trees.

**Acknowledgments**

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## 3.2

**Assessing glacier features supporting supraglacial trees: a case study of the Miage debris-covered Glacier (Italian Alps)**

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**Abstract**

The number of debris-covered glaciers featuring supraglacial trees is increasing worldwide, as a response of high mountain environments to climate warming. Generally, their distribution on the glacier surface is not homogeneous, thus suggesting that some glacier parameters influence germination and growth of trees. In this study, we focused our attention on the widest Italian debris-covered glacier, the Miage Glacier (Mont Blanc massif). We analyzed the ablation area in the range from 1730 m to 2400 m a.s.l. where continuous debris coverage is present and trees are found. Using data obtained by remote sensing investigations and field surveys we defined a record of glacier parameters to be analyzed with respect to the presence and abundance of trees.

We found that supraglacial trees are present at the Miage Glacier: i) whenever exceeding a debris thickness threshold (≥19 cm); ii) with a gentle slope (≤ 10°); iii) with a low glacier surface velocity (≤ 7.0 m/y); and iv) where the vertical changes due to glacier dynamics are positive (i.e. prevalent increase due to both slow debris accumulation and preservation of ice flow inputs that we found ranging between +7 m and +28 m over 28 years). The statistical analysis supports our findings.

The analysis of the same parameters might be conducted on other debris-covered glaciers featuring supraglacial trees, in order to evaluate if such conditions are local ones or if they are general factors driving germination and growth of trees.

By identifying the features supporting the presence and growth of trees in these environments, and their thresholds, a contribution is given for a better understanding of the importance of debris-covered glaciers and, in general, of debris-covered ice, as a refuge for trees during glacial and warm intervals of the Holocene.

**KEYWORDS:** debris-covered glacier, Italian Alps, Miage Glacier, remote sensing, supraglacial debris, supraglacial trees

## Introduction

Debris-covered glaciers (DCGs) are common features worldwide, as they have been observed in Europe, New Zealand, Asia and South America (e.g. Diolaiuti et al., 2003; Brook et al., 2013; Ghosh et al., 2014; Emmer et al., 2015). The recent literature (see Oerlemans et al., 2009) reports darkening phenomena affecting several mountain glaciers thus contributing to change their surface conditions and supporting their transformation into actual debris-covered glaciers. This leads to a noticeable modification in the alpine landscape, also giving new sites of scientific and cultural interest (Bollati et al., 2014). Moreover, the debris coverage represents a new habitat for living organisms, such as microorganisms (Franzetti et al., 2013), animals (Gobbi et al., 2011), yeasts (Turchetti et al., 2013) and plants (Gentili et al., 2015). Arboreal vegetation may also be present, whenever the glacier tongue is located below the treeline. The distribution of supraglacial trees is generally not homogeneous, thus suggesting that some glacier parameters influence germination and growth of plants. In the recent literature some authors reported as possible driving factors, for both herbaceous and arboreal vegetation: thick debris mantle, fine grain size (i.e.: from sand to pebbles), and slow surface glacier velocity; these factors also affect debris stability and then the suitability for supraglacial areas to support vegetation growth (Caccianiga et al., 2011; Leonelli and Pelfini, 2013). Arboreal vegetation is also a precious source of information to describe the behavior of DCGs. The strong sensitivity of arboreal vegetation to changes in site stability and in surface velocity was analyzed by Pelfini et al. (2007), who used tree rings cored from supraglacial trees to reconstruct glacier dynamics and evolution. In fact, accelerated ice flow determines disturbances in the normal growth of supraglacial trees and these perturbations are recorded by the plants as scars, compression wood and tree-ring eccentricity.

Thus, years characterized by intense glacier flow can be detected studying tree rings of supraglacial arboreal vegetation.

In spite of these previous studies, which underlined that supraglacial tree vegetation reflects peculiar environmental conditions (Pelfini et al., 2007) and gave a list of possible factors driving the colonization of the buried ice (Caccianiga et al., 2011), no research was found dealing with the exact role played by each one of the glacier features in allowing and supporting or preventing tree germination and growth. Thus, dedicated studies on selected locations embracing both the analysis of glacier features and arboreal vegetation characteristics (i.e. abundance of trees) are needed.

Among the existing methods allowing the extraction of geomorphological and glaciological data, for describing the supraglacial environment and detecting the most suitable sites to permit and support tree germination and growth, remote sensing is the most valuable one. In fact, this technique not only allows a wide area to be covered, and in this way to collect distributed data, but it also permits the survey to be repeated over several images and sources, thus giving a multi-temporal analysis.

Aerial and satellite images have been applied in the recent past in order to analyze DCGs, with the aims to detect their boundaries and to estimate volume and area changes (e.g. Ranzi et al., 2004; Smiraglia et al., 2005; D'Agata et al., 2005; Diolaiuti et al., 2009; Gjermundsen et al., 2011), to describe supraglacial debris distribution and its changes over time (e.g. Stokes et al., 2007; Mihalcea et al., 2008a; Minora et al., 2015), to identify changes in the velocity of the glacier (e.g. Luckman et al., 2007) and to detect and model their ablation rate (e.g. Nicholson and Benn, 2006; Mihalcea et al., 2008b; Reid and Brock, 2010; Fyffe et al., 2014). Orthophotos, satellite and aerial images were also used in order to monitor changes in the distribution of alpine and subalpine vegetation (e.g. Müllerová, 2004; Vescovo and Gianelle, 2008), to analyze the recent *ecesis* in the glacier forefield (Garavaglia et al., 2010) and to detect variations in the treeline (e.g. Danby and Hik, 2007; Leonelli et al., 2009). Vezzola (unpublished data) have recently tested a method based on the analysis of color orthophotos to rapidly detect and map supraglacial trees on DCGs, finding how the discontinuous distribution, low density and small canopy featured by trees represent the main limits in detecting them from remotely sensed data. Moreover, color orthophotos are not always available, in particular at high altitudes, and high resolution satellite images are often still cost prohibitive.

Since the germination and growth of supraglacial vegetation is controlled not only by climate conditions but also by glacio-related features (Pelfini et al., 2012), in this work we aimed at i) describing and quantifying the overall glacier features (i.e. debris occurrence and thickness, debris-surface temperature, debris moisture, surface slope, aspect, surface velocity and ablation rates) dominating a representative alpine DCG where an actual supraglacial forest is found and ii) assessing the role and weight played by each glacier feature in driving tree vegetation presence, growth and distribution.

For these purposes we focused our analyses on the most representative Italian DCG, the Miage Glacier (Mont Blanc massif), the unique glacier on the southern side of the Alps featuring an actual supraglacial forest in the lower portion of its ablation tongue. In addition, this glacier underwent a long sequence of both direct and remote sensed investigations over the last decades (see details reported in the "Study area" section) thus offering a robust and wide database to look for relations, if any, among tree occurrence and glacier features. Last but not least, the authors of this contribution are also analyzing vegetation of Alpine glacier forelands (increasing their extent due to the ongoing cryosphere degradation) thus supporting comparisons among tree occurrence, growth and distribution in these newly exposed zones and on DCGs.

Since supraglacial debris was also present during past glacial periods, this study does not only describe the phenomenon of current colonization of the supraglacial environment performed by trees, but it also promotes the analysis of the role played by the supraglacial debris in supporting the establishment and growth of arboreal species in the past. By identifying the features supporting the presence of trees in these environments, and their thresholds, a contribution is given for a better understanding of the importance of debris-covered glaciers and, in general, of debris-covered ice, as a refuge for trees during both glacial intervals and warm periods occurred in the Holocene, as already suggested for plants (Ravazzi et al., 1999; Fickert et al., 2007; Caccianiga et al., 2011).

**Study area**

The Miage Glacier in the Aosta Valley Autonomous Region (45° 47' N, 6° 52' E) is the widest debris-covered glacier in the Italian Alps (Fig.1). It is located in the Val Veny, on the southwest slope of the Mont Blanc massif, it features an area of about 10 $km^2$ (Diolaiuti et al., 2012) and its ablation tongue is characterized by two main lobes and a smaller one in between. The ablation tongue of the glacier shows quite continuous debris coverage from 1730 m a.s.l. up to 2400 m a.s.l., which thickness varies from a few centimeters up to 2 m: increasing debris thickness is generally detected with decreasing elevation (Mihalcea et al., 2008a). The Miage-debris cover is mainly composed by igneous and metamorphic rocks (Brock et al., 2010). The debris grain size largely varies from rock boulders to fine pebbles, sand and clay. Debris thickness and lithology influence albedo and consequently the ablation rate. In particular, the main lithology is given by gneiss and micaschists followed by granites. These latter are composed by milky white quartz, white plagioclase feldspars and pink potassium feldspars; moreover, also green biotitic and epidotic rocks are present. Gneiss is made from the same minerals as granites. In addition, chloroschists, amphiboles and epidotic rocks and schists like ardesia featuring a black color and with intrusions of graphite are present. A red patina is present at the rock surface, it is constituted by minerals from clays, manganese and iron oxides and it derived by deep hydrothermal circulation affecting rock cracks (Franzetti et al., 2013). Ice cliffs and supraglacial lakes are present on the largest part of the glacier ablation tongue, where ablation rate is higher, as a consequence of the exposure of bare ice.

Scientific research has been conducted on this glacier since the 18$^{th}$ century. It mostly concerned its geomorphologic and glaciological features and their changes over time (e.g. Baretti, 1880; Capello, 1959; Cunietti, 1961; Deline, 1999; Smiraglia et al., 2000; Diolaiuti et al., 2009; Fyffe et al., 2014), but also the calving phenomena and drainage events occurring at its ice-contact lake, the Miage Lake (Deline et al., 2004; Diolaiuti et al., 2005; Masetti et al., 2010), and its educational and touristic values and the role of the glacier in the frame of geo-heritage (Bollati et al., 2013 and Bollati et al., 2014).

The characteristics of the Miage Glacier as a new habitat for plants have also been studied. For what concerns trees, the most abundant species in the supraglacial debris are *Larix decidua* Mill. and *Picea abies* Karst, but other vascular plants are present. Trees taller than 1 m are mainly located at the southern lobe and the oldest living trees are about 60 years old as they move down-valley together with the supraglacial debris according to the local surface velocity of the glacier and, once they reach the glacier terminus, they fall (Pelfini et al., 2012). Tree density is higher on the northern lobe, but the oldest and tallest trees are found on the southern lobe. At the Miage Glacier, trees represent a valid source of glaciological and climatic data as they archive much information in tree-ring morphology, but also tree-ring stable isotopes and volatile organic compounds emitted from leaves allow the key factors influencing tree development on supraglacial debris to be investigated (Leonelli et al., 2014).

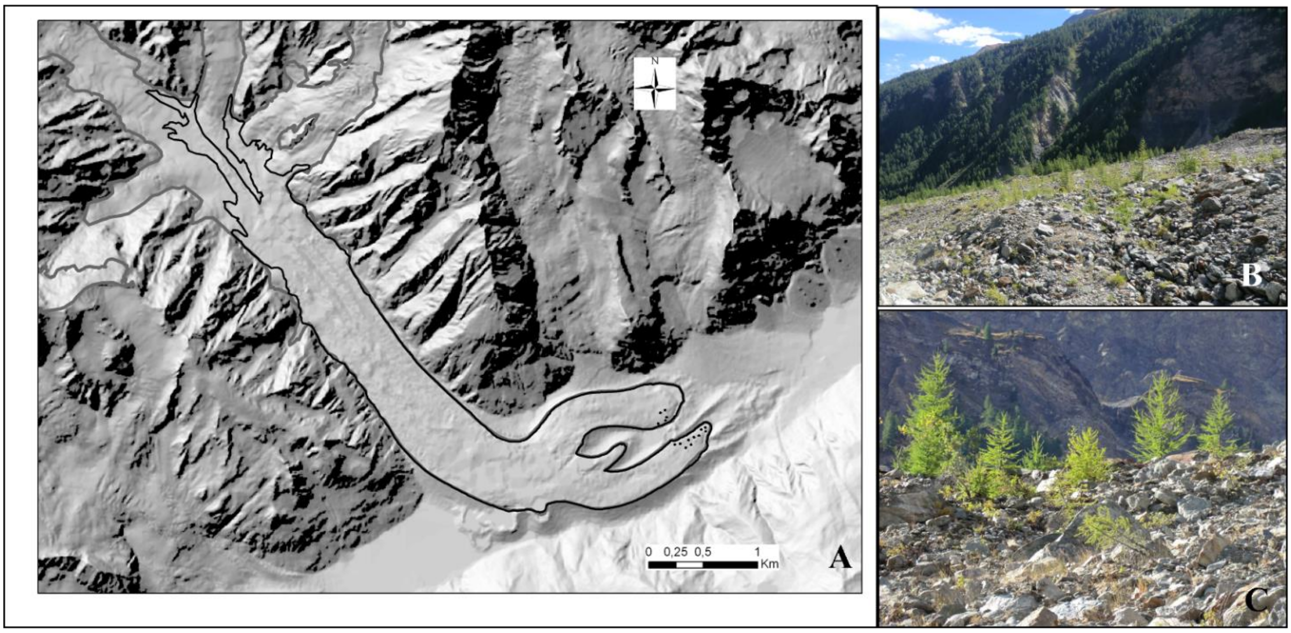


***Figure 1.*** *(A) The Miage debris-covered Glacier (45° 47' N, 6° 52' E) in the Val Veny, Mont Blanc Massif, is characterized in its snout part by the presence of (B, C) vegetation on the supraglacial debris, including trees. The 15 selected plots characterized by the presence of trees selected for this study are reported in fig. 1A.*

**Materials and methods**

<u>Extraction and analysis of the main features characterizing the whole ablation tongue of the Miage Glacier</u>

Firstly, a database including all the main features of the Miage debris-covered ablation tongue (from 1730 m to 2400 m a.s.l. ca.) was developed. Then the data was analyzed in order to describe the average conditions of a wide and representative alpine debris-covered glacier. In particular, the database included the following glacier parameters:

- Debris surface temperature (°C);

- Debris thickness (m);
- Normalized Difference Moisture Index (NDMI, absolute value);
- Slope (°);
- Ablation rate (cm w.e./day);
- Variation in glacier thickness over 28 years (1975-2003) (m);
- Aspect (N, S, E, W);
- Surface velocity measured in the past (by Lesca et al., 1974) and in recent time (by Diolaiuti et al., 2005) on some selected points at the glacier surface (m/year).

The point surface debris temperature was measured by Mihalcea et al. (2008a) every 5 minutes by thermistors and data loggers, close to the ablation stakes (see below). The sensor tips were attached to flat rock surface (2 cm thick, 10 cm x 10 cm), 2 cm below the debris surface. The data recorded at this depth are normally considered the indicative of point surface temperature and used within several international protocols to study permafrost and frozen ground (see Osterkamp, 2003; Guglielmin et al., 2008). For describing the mean debris thermal conditions, we analyzed the temperature data recorded on a day with clear sky conditions.

Moreover, we also used surface temperature estimated from satellite images acquired at the same time of the point debris temperatures. We analyzed ASTER kinetic surface temperatures derived from an ASTER image acquired on 01–08–2005 at 10:40 am UTC +1:00. These data feature a resolution of 90 m x 90 m and were fully described by Mihalcea et al. (2008a).

Debris thickness was obtained by Mihalcea et al. (2008a) from the ASTER image acquired on August 2005 on a day with clear sky conditions. Debris thickness variability over the whole debris covered tongue was estimated through an empirical relation developed by these authors coupling measured debris depth at some selected glacier sites with the surface temperature data of the same sites extracted from the thermal level of the ASTER image. This approach, previously tested by Taschner and Ranzi (2002), was found to be a good method to describe the supraglacial debris of debris-covered glaciers and was also applied more recently by other authors (Minora et al., 2015) on wide and representative DCG areas.

NDMI on the Miage Glacier was derived from a Landsat image acquired on July 2002 on a day with clear sky conditions (image code: LE71950282002230EDC00). No liquid precipitation occurred in the study area in the six days preceding the Landsat image acquisition (according to the meteorological data from the network managed by the Regione Autonoma Valle d'Aosta, RAVA). NDMI was calculated according to Equation (1), with NIR being Landsat TM Band 4 and MIR being Landsat T band 5 (Wilson and Sader, 2002).

$$\mathrm{NDMI} = \frac{\mathrm{NIR} - \mathrm{MIR}}{\mathrm{NIR} + \mathrm{MIR}} \quad (1)$$

Slope and Aspect were calculated by Diolaiuti et al. (2009) from a DEM (Digital Elevation Model) featuring a 10 m x 10 m spatial resolution. The DEM was obtained from 2003 aerial photos (RAVA flight).

The ablation rate values were obtained from a network of ablation stakes installed in summer 2005 (at the same sites where also point surface temperature was measured) and maintained up to the end of summer 2006. More than 20 ablation stakes were drilled into the ice to evaluate ice melt with varying debris thickness and altitude (i.e. from 1800 m to 2400 m a.s.l.).

The stakes were distributed according to one longitudinal and two cross profiles on the debris-covered area. They were monitored from June to October 2005 and 2006.

Variation in glacier thickness over the period 1975-2003 was calculated by Diolaiuti et al. (2009), who compared digital elevation models (DEMs), derived from historical records, in particular maps (1975; scale 1: 10,000) and stereo pairs of aerial photos (1991 and 2003; scale 1: 15,000).

Annual surface velocity of the Miage Glacier was measured by the Differential Global Positioning System method (Diolaiuti et al., 2005; Caccianiga et al., 2011; Franzetti et al., 2013) in the period 2002-2009. Moreover, historic data were already published, thus permitting an analysis of the variability of glacier flow over time.

Analysis of the glacier features on areas characterized by tree presence

With the aim of identifying the role and weight of environmental parameters driving and supporting tree occurrence and growth at the glacier surface we analyzed the field data partially published by Pelfini et al. (2012) and collected in the summer seasons 2006 and 2007 in the snout part of the Miage Glacier. More precisely, we selected the field plots where these authors found well established tree vegetation (i.e.: all the plots we selected were characterized by tree abundance exceeding 25 trees/plot) and on these sites we looked for the dominant glaciological features.

The information gained in the field included, in particular, the number of trees (both species *Larix decidua* Mill. and *Picea abies* Karst) in each plots.

In this study, 15 plots (plot size: 15 m x 15 m) were considered (they are reported in fig.1) and a categorical value (25, 50, 75 and 100) was assigned to each plot, depending on the abundance of trees:

- 25 = number of trees ranging between 1 and 25;
- 50 = number of trees ranging between 25 and 50;
- 75 = number of trees ranging between 50 and 75;

- 100 = number of trees above 75.

For the selected plots, the following parameters were extracted from our glaciological database:

- Altitude (m a.s.l.);
- Debris surface temperature (°C);
- Debris thickness (m);
- Normalized Difference Moisture Index (NDMI, absolute value);
- Slope (°);
- Ablation rate (m water equivalent /year);
- Variation in glacier thickness over 28 years (1975-2003) (m).
- Aspect (N, S, E, W);
- Surface velocity at the glacier surface (m/year);
- Distance from the closest vegetation source area (m).

Distance from the closest vegetation source area was calculated using the 2005 color orthophotos (RAVA), measuring the distance between each selected plot and the closest forested area located outside the glacier tongue.

Then we analysed the environmental conditions dominating the 15 plots we selected to find threshold of the parameters suitable to divide areas with abundant tree vegetation from areas with scarce presence of supraglacial trees. For this purpose, diagrams coupling tree abundance at each plot with the environmental parameter values at the same plot were developed.

Moreover, a one-way ANOVA was performed in order to compare 15 supraglacial plots located above the treeline, where trees are surely absent (tree abundance = 0), against the 15 plots previously selected characterized by the presence of trees (tree abundance = 50, 75, 100) located at altitudes below the local treeline. This statistical analysis was performed to evaluate the parameters (slope, debris thickness, debris-surface temperature, ablation rate, variation in ice thickness over 28 years (1975-2003), aspect and NDMI) more meaningfully related to tree presence and abundance.

**Results**

<u>The Miage debris-covered Glacier: main features and characteristics</u>

The parameters indicating the characteristics of the Miage Glacier are reported in Fig.2.

Point debris-surface temperature shows a general decreasing trend with increasing altitude, in particular the average surface temperature at the glacier terminus is close to 30°C and at 2400 m a.s.l. it is reduced to 17°C. Peculiar values are observed between 2050 and 2150 m a.s.l., where both the minimum, maximum and average values increase (Fig.2.1). Considering a daily cycle 24 hours long it resulted that ground-measured temperatures of debris cover during a sunny summer day exceed 30°C, and are about 1-4°C during night. Daily temperature excursion is therefore 28-33°C. Close to the terminus, debris temperature is positive throughout the vertical profile during almost the entire ablation season, and a continuous ice melt occurs at the bottom.

ASTER kinetic surface temperatures resulted to be similar to ground measured temperatures: mean surface temperature over areas 90 m × 90 m wide varies between 25°C at 2059 m and 32°C close to the terminus. The Pearson's correlation coefficient between ASTER and the field surface temperature data is 0.94 over the analyzed area.

Calculated debris thickness and ASTER temperatures are not collinear (r = 0.146) and can therefore be entered simultaneously in statistical analyses. The calculated debris thickness varies between 18 cm up to 55 cm (average value over 90 m × 90 m areas). Overall, debris thickness increases downwards, but is thinner in crevassed areas. The lowest thickness was measured at 1956 m a.s.l. in a crevassed area where the glacier divides into three lobes. Thickest debris corresponds to areas close to the terminus (Fig.2.2).

NDMI on the Miage Glacier shows a minimum value of 16 (corresponding to maximal moisture) between 1951 and 2050 m a.s.l. and a maximum value of 50 (corresponding to drier environments) above 2350 m a.s.l. (Fig.2.3). No big difference in the NDMI values is detected between the glacier terminus and 2050 m a.s.l., while maximum NDMI decreases starting from 1950 m to 2250 m a.s.l., where its value passes from 49 to 42, and above 2250 m it raises up to 50.

The minimum value of slope detected is 0°, while the maximum value is 45°. The average varies between 8° and 13° and it does not seem to follow a specific trend related to altitude (Fig.2.4).

The ablation rate is influenced by debris distribution with rates varying from -0.3 m/y, where debris exceeds 55 cm thickness, to -5.5 m/y, where the debris is very thin or absent (Fig.2.5). The ablation rate generally decreases from the higher to the lower altitudes, with higher values in June-July and lower values in August-September.

The changes in ice thickness over the period 1975-2003 show a general glacier volume loss (–16.640 x $10^6$ $m^3$) from 1975 to 2003. Nevertheless, focusing on the two time sub-windows (i.e.: 1975-1991 and 1991-2003), opposite trends are evident: in the period 1975-1991 the volume variation of the Miage Glacier was about +19.25 x $10^6$ $m^3$, while in the period 1991-2003 a volume decrease of about –36.2 x $10^6$ $m^3$ occurred. The thickness changes resulted positive (i.e.: depth increase) in the lower glacier sector where debris mantle exceeds the *critical value* (this latter is the debris thickness driving a buried ice ablation rate equal to the one of bare ice at the same elevation, see also Mattson et al., 1993, once this value is exceeded ablation rates are found diminishing) with values up to + 18 m at 1730 m a.s.l.; the thickness variation was found to be negative (i.e.: depth decrease) in the upper glacier zones (from 2250 m a.s.l.) where the debris layer is thinner, here the changes locally exceed -30 m (Fig.2.6).

As regards the aspect, 74% of the Miage Glacier surface ranges between 0° and 180° (predominantly East) from 1730 to 2400 m a.s.l. (Fig.2.7).

Annual surface velocity of the Miage Glacier measured by the Differential Global Positioning System method ranged between 0.3 m/y and 90 m/y, and it shows a clear vertical gradient (Fig.3); the surface velocity values diminishing with glacier elevation and with the decreasing slope was also found by Pelfini et al. (2012) who described the thicker debris mantle where compressing flow is occurring, instead thinner and sparse debris where extending flow is dominant thus driving crevasses development and evolution.

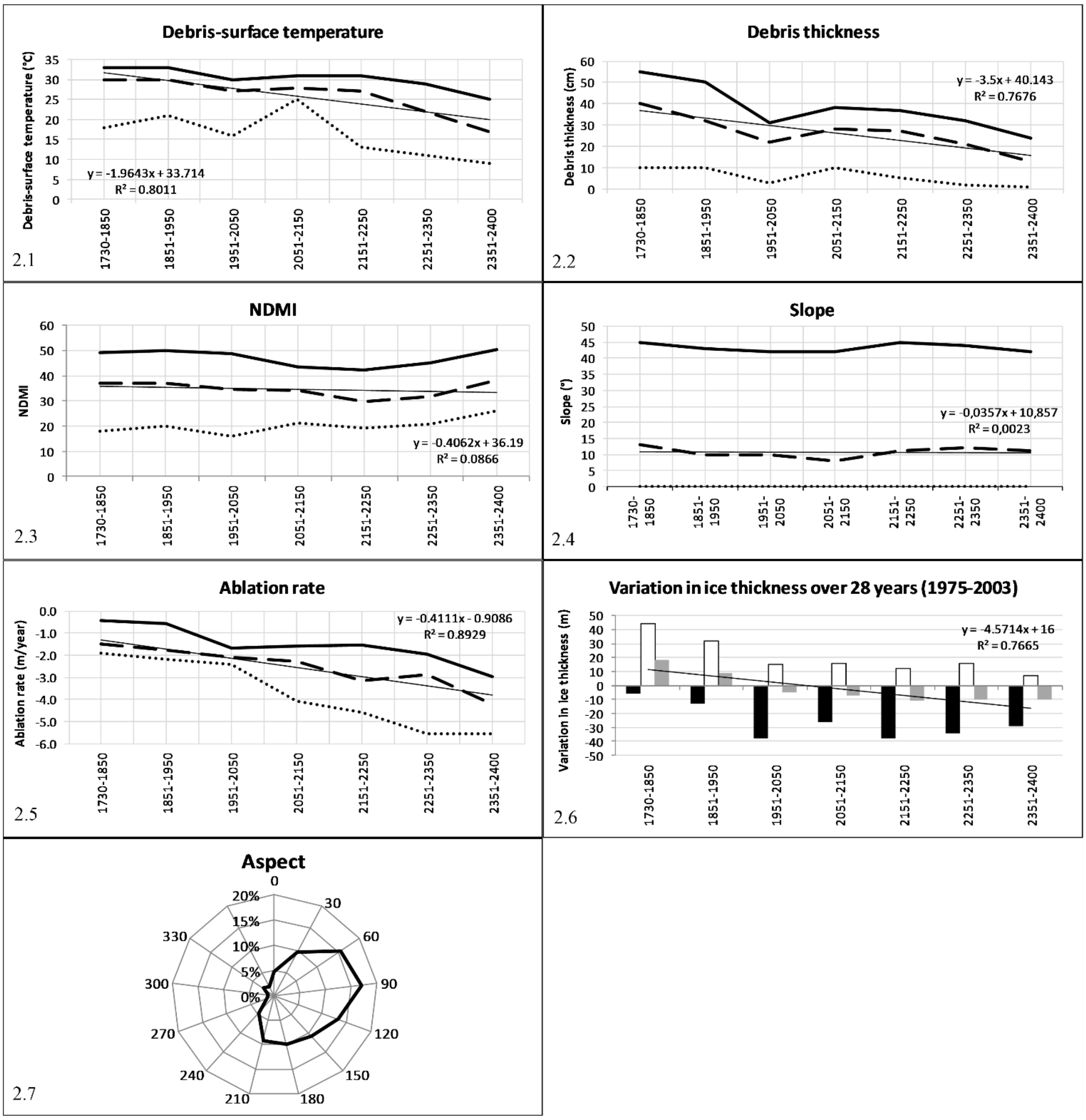


*__Figure 2.__ Characteristics of the Miage Glacier between 1730 and 2400 m a.s.l.. Altitude ranges are reported on the X axis. Maximum values are indicated with a continuous line, minimum values with a discontinuous line with small segments, average value with a discontinuous line with larger segments.*

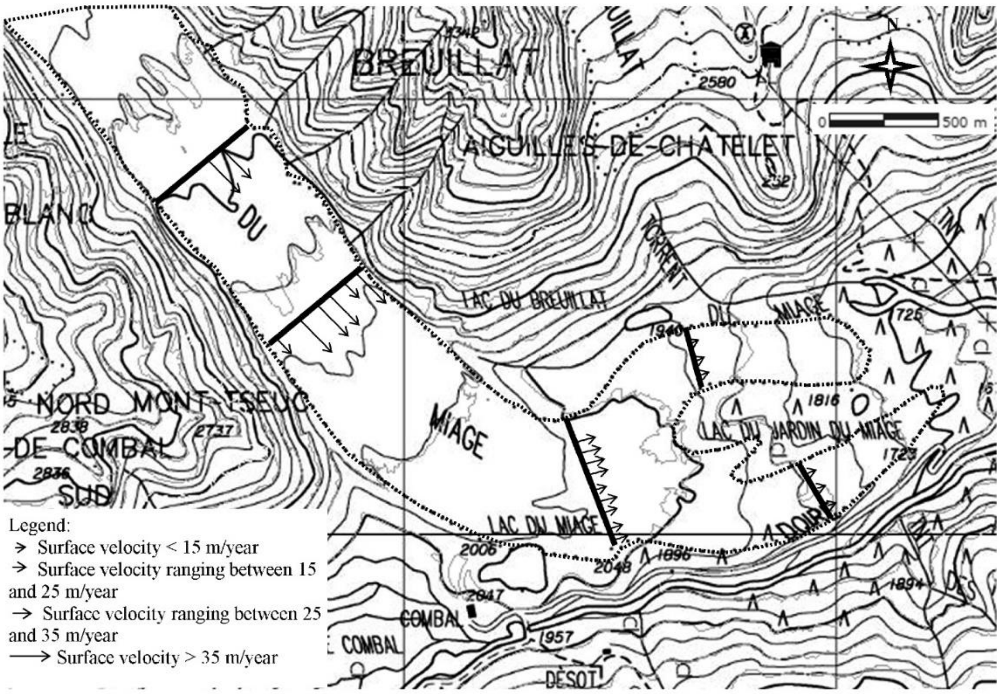


***Figure 3***. *Ice flow at the surface of the Miage Glacier at different altitudes. The arrows represent the velocity vectors measured in 2006 (average velocity calculated from July to November), from 1950 m to 2250 m a.s.l.*

<u>Glacier features on the areas showing tree vegetation presence</u>

The characteristics of the Miage Glacier in the 15 selected plots are reported in Fig.4.

All the plots we selected were characterized by tree abundance exceeding 25 trees/plot.

In the selected plots debris surface temperature is found ranging between 19°C and 33°C where trees are present and, in particular, tree vegetation with lower density is only present where temperature has a value between 29°C and 33°C (Fig.4.1).

Trees are present where debris thickness ranges between 19 cm and 55 cm. In particular, more than 90% of the plots are characterized by thickness ranging between 32 cm and 55 cm (Fig.4.2).

Values of NDMI where arboreal vegetation is present range between 19 and 44 (Fig.4.3).

Slope ranges between 2° and 10° where trees are present (Fig.4.4).

Ablation rate where tree vegetation is present ranges between -0.6 and -1.8 m/y (Fig.4.5).

The average variation in glacier thickness over 28 years (1975-2003) never shows negative values where trees are present, and it ranges between +7 m and +28 m (Fig.4.6).

More than 85% of the Miage Glacier is characterized by an aspect ranging between 0° and 180° where tree vegetation is present and, in particular, 60% shows a NE aspect (between 0° and 90°) (Fig.4.7).

Annual surface speed in the selected plots ranges between 0.8 m/y and 7.0 m/y (Fig. 4.8).
Distance from the closest source area (the nearest trees outside the glacier margins) for the selected plots ranges between 33.8 m and 177.4 m. A clear trend is detectable: where the density of tree vegetation is lower, the distance from the source area is higher (between 177.38 m and 79.25 m), and it gradually decreases when the density of trees increases (the plots with the highest density are located at a distance ranging between 94.0 m and 33.8 m from the closest source area) (Fig. 5).
The results of the statistical analysis highlight that there is a statistically significant correlation at the $p<0.05$ level between all the considered parameters and the presence/absence of trees, except for the aspect and NDMI factors that were deemed as not statistically significant. The results of the analysis are reported in Table 1.

***Table 1.*** *Results of the statistical analysis highlighting a statistically significant correlation at the $p<0.05$ level between all the considered parameters (but aspect and NDMI) and the presence/absence of trees*

| **Parameter** | **ANOVA test** |
|---|---|
| Ablation rate | $F(3,26) = 28.78$; $p<0.000$ |
| Debris-surface temperature | $F(3,26) = 8.95$; $p<0.000$ |
| Variation in ice-thickness over 28 years | $F(3,26) = 65.42$; $p<0.000$ |
| Slope | $F(3,26) = 50.33$; $p<0.000$ |
| Debris thickness | $F(3,26) = 43.47$; $p<0.000$ |
| Aspect | $F(3,26) = 1.29$; $p<0.29$ |
| NDMI | $F(3,26) = 1.91$; $p<0.15$ |

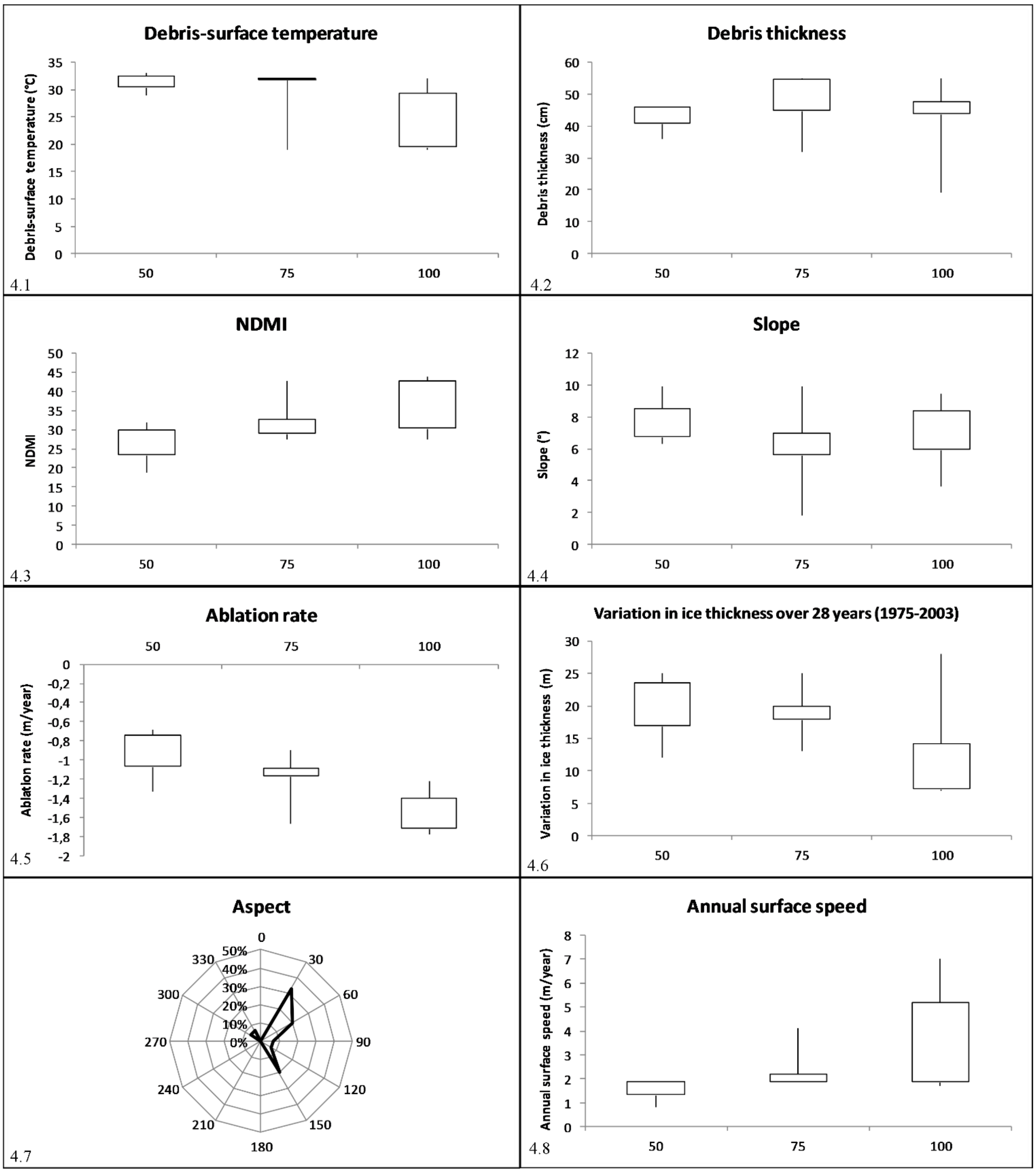


***Figure 4.*** *Characteristics of the Miage Glacier in the 15 selected plots characterized by the presence of supraglacial trees. Tree vegetation abundance is reported in the X axis, respectively at low, medium and high-density (50, 75, 100).*

## Discussion

The Miage Glacier is known to be one of the few glaciers worldwide (and the only one in Italy) characterized by the presence of abundant supraglacial vegetation, including well developed trees, that can also be detected using color orthophotos with a pixel size of 0.5 m x 0.5 m. However, supraglacial vegetation can be detected in this way only when its density is very high (Vezzola et al., unpublished data) thus leading to the identification of vegetation on the Miage Glacier only where altitude ranges between 1730 m and 1850 m a.s.l.. This altitudinal range only represents 5%

of the whole glacier area occupied by continuous supraglacial debris (Fig. 6). Nevertheless, the treeline in the Val Veny for some species reaches an altitude of 2250 m a.s.l. (Leonelli and Pelfini, 2013). Even though the distance between the 15 plots selected in this study and the closest proglacial forested area is an important factor in the establishment of supraglacial trees (Fig. 5), their reduced density at an altitude above 1850 m a.s.l. suggests that one or more glacier parameters influence germination and development of supraglacial trees.

Thus, the selection of glacier parameters here presented has been done in order to describe the main morphological and environmental conditions at the surface of an alpine debris-covered glacier. The selection was oriented to the features derivable from remote sensing sources to be able i) to cover the whole debris-covered surface and ii) to ensure repeatability of the methods to other DCGs on the Alps and elsewhere. We analyzed the variability of the same parameters on selected glacier areas where supraglacial arboreal vegetation has been observed. The tree presence and features were detected and described through field surveys, thus giving high resolution data and assuring that we had selected areas with an actual presence of trees. Even if our sample was restricted (overall, the 15 supraglacial selected plots characterized by the presence of trees featured an area of 3375 $m^2$), this study allowed for the first time the identification of the glacial features, and their thresholds, permitting supraglacial tree germination and growth. In fact, trees are only present in areas featuring higher stability (i.e.: slow surface velocity, < 7 m/year), thick debris cover (deeper than 19 cm), gentle slope (≤ 10°) and positive changes in ice thickness (ranging between +7 m and +28 m over 28 years). These conditions seem depicting a supraglacial stable environment favorable to tree germination and growth.

More precisely, even though in the lower portion of the ablation tongue debris thickness ranges between 10 cm and 55 cm, trees are only present where debris is at least 19 cm thick (observed in only 1 of the 15 considered plots), and more than 90% of the selected plots are located where debris thickness exceeds 30 cm. On a debris-covered glacier, debris thickness plays a key role in root frost occurrence during summer; in fact at ice-debris interface the temperature is always at the melting point (Brock et al., 2010), thus, a thinner debris causes cooler (and in some cases also frozen) root conditions, while thicker layer allows warmer and more favorable root conditions. In fact, cold drives stress conditions having a negative impact on forest ecosystems, as underlined by Groffman et al. (2001) who found that more frequent soil freezing events could cause changes in root and microbial mortality and losses of nitrogen. The length of the yearly cold period is also an important factor in determining the stress conditions influencing trees: overwinter climate can cause loss of nutrients and, as a consequence, it also represents a disturbance to the development of supraglacial trees (Tierney et al., 2001). The duration of the favorable period for growth in the European Alps, characterized by daily mean root-zone temperature of about 7°C, has to be at least of three months

(Körner and Paulsen, 2004). For this reason, dedicated experiments are needed, in order to define what are the actual root conditions for both supraglacial trees and trees of the same species and age outside the glacier area at the same altitude, on stable lateral moraines (not ice-cored and not showing permafrost occurrence); in this way it will be possible to describe the microclimatic conditions influencing the roots of supraglacial conifers and in particular during summer, when the conditions in the supraglacial area and outside the glacier are deeply different, thus probably requiring a higher root frost tolerance for the supraglacial trees.

Moreover, stable isotopes in tree rings studied by Leonelli et al. (2014) showed that supraglacial trees are mainly fed by water from liquid precipitation, thus suggesting that tree roots are not so close to buried ice to absorb the derived melting water.

Jones et al. (2005) detected supraglacial vegetation on the Matanuska Glacier (Alaska) only where debris exceeds 25 cm thickness. Our findings and the recent literature seem suggesting a thickness threshold allowing germination and growth of supraglacial trees, that is probably linked to root frost tolerance of tree species.

Debris thickness is linked to glacier surface velocity (Gilcrist et al., 2003), another parameter influencing tree establishment. Thick debris cover presents approximately 3-4 layers: a fine layer at the bottom with melting water along the first centimetres followed by a mixed layer of fine and coarse debris, a layer of coarse debris with clasts of 1-10 cm, and a final layer at the surface with clasts larger than 10 cm. The rock debris layer is found generally thicker than the “critical value” (sensu Mattson et al., 1993). This latter is a depth threshold value which has to be locally evaluated and on the Alps is in the range between 4 and 6 cm (Franzetti et al., 2013, see the supporting information). On the Miage it was found equal to 3 cm (Mihalcea et al., 2008a). The debris mantle on the lower sectors is thicker than this threshold and it actually reduces magnitude and rates of buried ice melt (Brock et al., 2010) thus allowing the glacier to maintain its ablation tongue at low altitudes as well.

The 15 selected plots featuring tree vegetation are located where slope does not exceed 10° and glacier surface velocity is lower than 7.0 m/y, thus highlighting the importance of debris stability in the establishment of supraglacial trees. Glacier surface velocity and slope are key factors also in the establishment of herbaceous vegetation on the Miage Glacier (see Caccianiga et al., 2011).

Another environmental variable influencing tree germination on the glacier surface is vertical disturbance due to ice thickness loss. This disturbance can be evaluated through multiannual ice thickness variations as the ones we evaluated by comparing 2003 and 1975 DEMs. On the Miage Glacier tongue, supraglacial debris coverage modulates the magnitude and rates of buried ice ablation (see Diolaiuti et al., 2009): variation in ice thickness from 1730 m to 2400 m a.s.l. over 28 years has been observed as ranging from -30 m to +44 m, the latter where supraglacial debris exceeds 30 cm thickness, but no tree vegetation was detected on the glacier surface where variation in ice thickness shows negative values over 28 years, thus suggesting that the areas characterized by intense reduction in ice thickness are less favourable to tree germination and growth.

Even if ablation rate does not seem to directly play a role in the establishment of supraglacial trees, several studies, carried out both in the European Alps and in the Himalayan glaciers, show that ablation rate is usually reduced where debris coverage exceeds a few centimetres thickness (D'Agata and Zanutta, 2007; Juen et al., 2014; Pratap et al., 2015). In the snout part of the Miage Glacier, where supraglacial debris coverage is thicker, ablation rate is particularly reduced, thus increasing glacier surface stability and, as a consequence, tree establishment.

Debris-surface temperature, aspect and NDMI do not seem to directly influence tree colonization on the glacier surface. The values of these parameters in the selected plots reflect the characteristics of the Miage Glacier between 1730 m and 1850 m a.s.l. also where tree vegetation is not present. Debris-surface temperature on the Miage Glacier was already identified as not being directly linked with plant colonization (Caccianiga et al., 2011). The values observed in the snout part of the glacier do not seem to neither limit nor support tree growth, even though a very wide day/night temperature range could represent a stress factor for the supraglacial arboreal species. The results concerning the aspect in the 15 selected plots reflect the aspect of the whole glacier terminus, thus suggesting that this environmental variable does not directly influence the establishment of trees. NDMI does not show high variability across altitude range on the glacier, especially in its terminal part. Since this index contrasts the near-infrared band (which is sensitive to albedo of leaf chlorophyll) to the mid-infrared band (which is sensitive to absorbance of leaf moisture), it should be directly linked to the presence of vegetation, both herbaceous and arboreal. We suggest that the resolution of the Landsat images used to calculate NDMI does not allow the detection of differences in moisture related to the discontinuous distribution of trees on the supraglacial debris. This index was already used to study changes in moisture in different environments (e.g. Lin et al., 2009; Brom et al., 2012), but we could not find scientific literature about its use on debris-covered glaciers. Further analysis of moisture characterizing the supraglacial debris will provide a better understanding of the role of this variable in tree establishment.

The statistical analysis, even if restricted to a limited number of plots both below and above the treeline, supports our observations: all the parameters that seem to play a role in tree establishment and growth on the surface of a debris-covered glacier, actually show a statistically significant correlation with tree presence. Ablation rate and debris-surface temperature also appear to be significant in tree establishment, even though we could not identify specific thresholds related to the presence of trees.

The statistical ANOVA analysis was not performed for glacier velocity since it was locally evaluated (through DGPS point measurements) and at some plots above the treeline we have no measurements.

Debris-covered glaciers are suitable sites where to observe the growth of conifers in alpine soils with cooler root conditions than trees located outside glaciers, and these conditions may become more frequent in a changing climate. In fact, in a warmer climate, colder soils are expected to occur, due to a thinner and less persistent snow winter coverage also outside glacier areas (Groffman et al., 2001), thus negatively affecting plants, especially because fine roots are more easily damaged in a situation of colder soils, and as a consequence nutrient uptake is compromised (Tierney et al., 2001). On the other hand, in a warming climate, earlier snow disappearance causes warmer soil temperature in spring (Luetschg and Haeberli, 2005). For this reason, tree species that are able to tolerate colder soils during winter and higher temperature amplitude cycles in summer, as the ones in glacial environments, will probably be the ones with a major occurrence in a context of warmer climate, while trees showing a low root frost tolerance will have more difficulties in surviving these changes.

The study of tree ecesis and germination year in the glacier foreland of a currently retreating debris-free glacier of the Italian Alps, the Forni Glacier (Stelvio National Park, Italy), determined by means of dendrochronological approach and whorls branch counting, shows an acceleration of the ecesis in the last few years, with an average value of 7 years (Leonelli et al., submitted). The glacier foreland that underwent deglaciation about 50-70 years ago is characterized by a much higher tree density compared to what we observe even in the plots characterized by the highest number of trees on the surface of the Miage Glacier, and since tree colonization on this DCG started about 100 years ago (Deline and Orombelli, 2005), we suppose that, even where the supraglacial conditions are favorable to tree colonization, the presence of ice under the debris and the glacier velocity (also if reduced) limit their establishment.

Of course many other conditions outside the glacier where trees are present need to be better investigated and compared to the ones in the supraglacial environment, in particular substrate temperature and moisture, in order to better understand the differences between the surface of DCGs and the areas surrounding the glacier as habitats for trees.

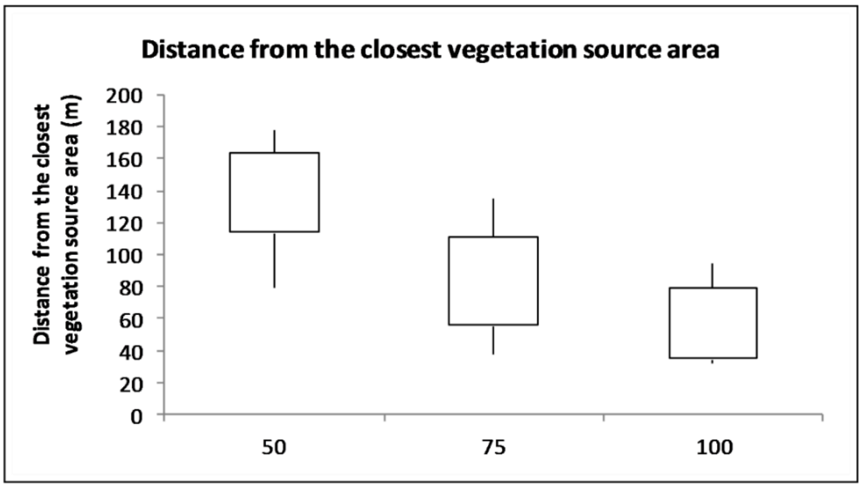


***Figure 5.*** *The relationship between vegetation abundance (selected plots) and the distance between the plots and the closest spot featuring vegetation outside the glacier. Tree abundance increases (100) at decreasing distances between the plot and the vegetation source area.*

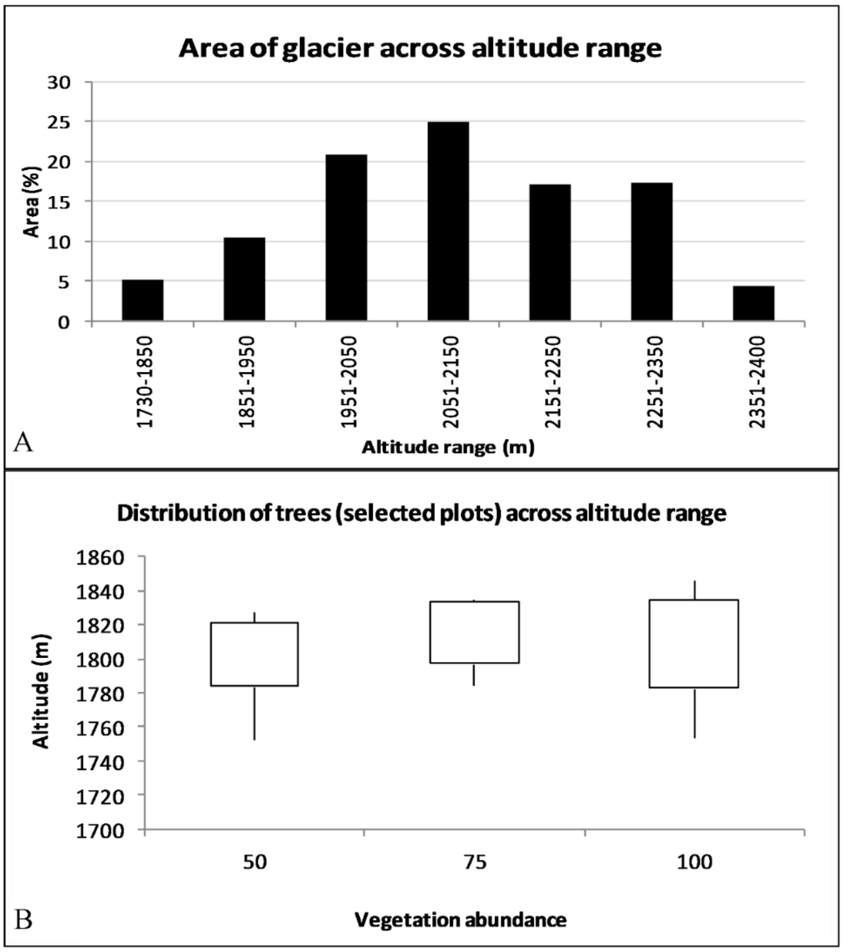


***Figure 6.*** *(A) Area of the Miage Glacier tongue per altitude belts (from glacier terminus at about 1730 m to 2400 m a.s.l.); (B) supraglacial tree distribution over the ablation tongue is only detectable between 1730 m and 1850 m a.s.l. using color orthophotos with a pixel size of 0.5 m x 0.5 m, an altitude range representing 5% of the area characterized by the presence of supraglacial debris.*

## Conclusion

The originality of this research lies in the comparison between environmental parameters characterizing supraglacial debris and tree location, thus allowing the identification of well-defined intervals for several variables that characterize the spots where arboreal vegetation is present and well established. By knowing what are the values of a set of glacier parameters allowing tree establishment on a DCG, the actual or potential presence of trees on the surface of other DCGs may be predicted.

The methodology here presented represents a possible approach for the investigation of remote glacial areas and for the assessment of supraglacial tree presence at the regional scale. Further studies may then be conducted in the field in order to analyze dendroclimatic and dendroglaciological signals (Pelfini, 1999; Leonelli et al., 2011; Coppola et al., 2013), if morphological and environmental conditions suggest arboreal vegetation presence in the study area.

With this study, the already known link between glacier dynamics and supraglacial trees is even more emphasized and, in particular, our results suggest that glacier surface stability is the main factor influencing tree vegetation establishment on the supraglacial debris, with debris thickness, slope, variation in ice thickness and glacier velocity being the environmental variables mainly driving tree colonization. Our results suggest that, whenever the described parameters would show values supporting tree establishment and growth, debris-covered glaciers may have acted as refugia for tree species during the colder and warmer periods of the Holocene.

Future investigations will aim at i) analyzing the same glacier parameters on other debris-covered glaciers featuring supraglacial trees, in order to evaluate if such conditions are local to the current study area or if they are general factors driving establishment of trees, ii) investigating debris lithology where trees are present, in order to assess the role played by the lithological properties of supraglacial debris in driving tree presence, iii) investigating the actual conditions along the debris layer where tree roots develop and iv) describing tree root physiology on the supraglacial debris and outside (comparative analysis).

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# 4.

# USING TREES FOR GEOMORPHOLOGICAL INVESTIGATIONS AND GEOMORPHOSITE ASSESSMENT IN HIGH MOUNTAIN ENVIRONMENTS

## 4.1.

# Biogeomorphology: sampling and analysis of proglacial and supraglacial vegetation

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## Abstract

Herbaceous and arboreal vegetation are useful tools in the study of the landscape transformations affecting glacial areas. In glacier forelands, the study of vegetation succession enables the identification of the age of the deglaciated terrain and it contributes to investigation of the distribution of the main geomorphological processes occurring in the area, such as gravitative and glaciofluvial processes. On the surface of debris-covered glaciers, the analysis of the distribution and characteristics of vegetation provides information on the glacier dynamics, such as its velocity and the stability of the supraglacial debris. In this chapter, two methodologies for the study of the distribution and characteristics of vegetation in glacial environments are proposed. The first one enables a rapid and preliminary investigation of the study area (both in the glacier foreland and at the surface of a debris-covered glacier) through remote sensing analysis, by performing a supervised classification on colour orthophotos. The second method involves detailed field surveys to describe species composition and, when applied to recently deglaciated areas, enables the estimation of terrain age. On the other hand, on debris-covered glaciers the analysis of vegetation, in particular arboreal species, allows the investigation of the past and current glacier dynamics.

**KEYWORDS**: glacier retreat, vegetation succession, supraglacial vegetation, debris-covered glacier, glacier foreland

## Introduction

Glacier retreat and the increase in number of debris-covered glaciers are amongst the main consequences of the ongoing climate change in high mountain environments. Geomorphic processes affecting recently deglaciated areas, including stream activity, frost action, debris fall and mass movement, cause rapid variation of the glacier foreland (Ballantyne, 2002a). On the other hand, the supraglacial debris coverage is characterized by frequent changes, due to ice flow, differential ablation rate and surface velocity, causing continuous downvalley debris displacement (Pelfini *et al*., 2012).

Even though these landscape transformations create unstable surfaces, glacier forelands and the surface of debris-covered glaciers represent new habitats for biological forms, such as bacteria, animals and plants (e.g. Cannone *et al*., 2008; Nakatsubo *et al*., 2010; Zumsteg *et al*., 2012; Franzetti *et al*., 2013; Arroniz-Crespo *et al.*, 2014). The characteristics of vegetation in particular, including both herbaceous (herbs, characterized by no woody stem above ground) and arboreal (trees) species, can provide detailed data about the terrain age of glacier forelands. Moreover, vegetation is not homogeneously distributed in these areas, since its growth is negatively influenced by the occurrence of gravitative and glaciofluvial processes affecting the area after deglaciation. For this reason, the analysis of the distribution and age of vegetation represents a contribution to the identification and mapping of past and current processes occurring in the area. The arboreal vegetation colonizing the supraglacial environment of a debris-covered glacier can provide information about the glacier dynamics, in particular its velocity, debris stability and debris thickness (Caccianiga *et al*., 2011; Leonelli and Pelfini, 2013). In fact, vegetation establishment and growth in such areas are related to several climatic and environmental parameters, as well as to glacier dynamics and the frequency and intensity of geomorphological processes. Thus making vegetation a valuable tool for the study of changing glacial environments (Gentili *et al.*, 2015).

In recently deglaciated terrains vegetation establishment follows a specific trend, related to a gradual shift in the dominant processes leading to vegetation establishment, from abiotic to biotic. Colonization begins with pioneer species, that are adapted to dominant abiotic processes (sediment properties, hydrology, slope, exposure, moisture) and grow where there is no, or low, competition for resources. Then, biotic parameters (competition with other species, tolerance, inhibition) gradually become more important in the establishment of vegetation. This variation in process dominance, from abiotic to biotic, results in a gradient in species composition and vegetation cover with increasing terrain age (for more details see Matthews, 1992; Rossi *et al*., 2014; Suvanto *et al*., 2014). Vegetation succession is influenced not only by climatic conditions, but also by geomorphic processes. In particular, paraglacial processes are dominant in the preliminary phases of colonization. When the ecosystem is at a more developed stage (when late successional vegetation such as arboreal species occur), then vegetation has a stabilizing effect on the deglaciated terrain (Eichel *et al*., 2013; Figure 1).

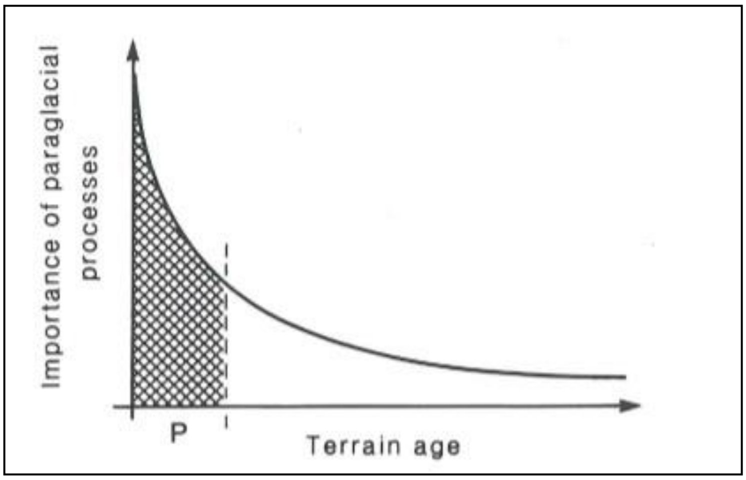


***Figure 1.*** *Decline of the importance of paraglacial processes with increasing distance and time from the retreating glacier. The darker area represents the phase characterized by dominance of paraglacial processes (from Matthews, 1992).*

Several factors influence the colonization of vegetation and, in particular, arboreal vegetation on the surface of debris-covered glaciers. The distance between the closest proglacial forested area and the glacier itself is certainly important, but other glacier parameters play a role in the establishment of arboreal vegetation, such as glacier surface velocity, slope, debris thickness and grain size of the substrate (Leonelli and Pelfini, 2013). Therefore, investigating the distribution and characteristics of supraglacial arboreal species contributes to the study of debris-covered glaciers and can be investigated using dendrochronological techniques.

Dendrochronology is a dating method based on the analysis of tree rings (Fritts, 1976). Tree-ring width and characteristics are influenced by several factors including climate and environmental disturbances. For this reason, a dendrochronological study performed on supraglacial vegetation enables the reconstruction of the evolution of debris-covered glaciers in the recent past. In fact, growth disturbances and compression wood are produced as a response to a dynamic glacier surface (Pelfini *et al*., 2007; Garavaglia *et al*., 2010), thus making dendrochronology a valid approach to determine the years characterized by high surface instability and to reconstruct past glacier dynamics.

An approach for analyzing the dynamics of glacial environments through the investigation of vegetation is outlined in the remainder of this chapter.

**Vegetation distribution using remote sensing**

Remote sensing techniques enable the identification of herbaceous and arboreal vegetation in glacier forelands and on debris-covered glaciers. There are only a limited number of studies using remote sensing techniques in these environments, for example Klaar *et al*. (2014) successfully applied it in the study of vegetation succession in glacier forelands in Alaska (USA), with the aim of analyzing the interactions between physical and biological processes in recently deglaciated terrains. Until now, only Vezzola *et al*. (submitted) mapped the distribution of vegetation on the surface of a debris-covered glacier, using colour images to investigate the largest debris-covered glacier in the Italian Alps, the Miage Glacier (Aosta Valley, Western Italian Alps). Both these studies found that remote sensing is certainly a useful approach in an initial mapping of vegetation in these areas, however full understanding can only be gained by complementing it with field surveys to obtain detailed information about vegetation distribution. By using remote sensing, it is possible to cover wide areas and collect distributed data, as well as enabling repeat analysis over different years. However, the small canopy, reduced height and discontinuous distribution of the vegetation growing in these areas cannot be easily identified by remote sensing techniques, since it will not be visible in the image as a consistent green patch. For this reason, using solely remote sensing for this research would result in an underestimation of vegetation coverage.

High resolution is an essential requirement to detect vegetation in glacial environments. Images featuring a pixel size of at least 0.5 m x 0.5 m are appropriate for the investigation of herbaceous and arboreal species; resolutions any lower make it impractical to distinguish the vegetation (see the example in Figure 2).

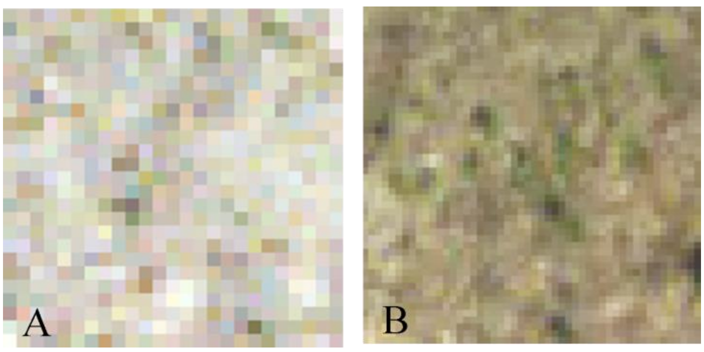


***Figure 2.*** *The same 25 m$^2$ area on the surface of the debris-covered Miage Glacier in the Italian Alps, in a colour orthophoto acquired in A) 1999 with pixel size 1m x 1m and B) 2010 with pixel size of 0.5m x 0.5m. Green pixels represent vegetated areas. As can be seen, a pixel size of 0.5m x 0.5m allows a more accurate analysis, compared to the image featuring pixel size of 1m x 1m.*

Colour orthophotos are aerial photographs that have been geometrically corrected, so that the scale in the image is uniform and they are authentic representations of the surface of the planet. Colour orthophotos acquired in the last 10 years generally have sufficient resolution to be suitable for the analysis of vegetation in glacial environments. For research purposes, colour orthophotos are often available for free or at discounted prices from the local and regional administrative offices or mapping agencies. For instance, in Italy colour orthophotos may be found on the website of the Geoportale Nazionale (www.pcn.minambiente.it), while in the United Kingdom the Ordnance Survey (www.ordnancesurvey.co.uk) can be contacted.

Both in the case of recently deglaciated areas and debris-covered glaciers, a semi-automatic approach can be employed, by performing a supervised classification using Maximum Likelihood algorithm. The study area must be extracted from the orthophotos and, using a GIS software such as ENVI ("ENvironment for Visualizing Images", a software commonly used for image analysis; for more information and purchasing see the website www.exelisvis.com/ProductsServices/ENVIProducts/ENVI.aspx), the classifier must be trained to discriminate between classes through selection of appropriate Regions Of Interest (ROIs). These can be either polygons or individual pixels, and must include at least one ROI corresponding to vegetation. To do this in ENVI, after uploading the image, the "Roi Tool" must be selected (Basic Tools > Region of Interest > Roi Tool), and the pixels in the "Zoom window" manually selected where vegetation is present (Figure 3). The ROIs must then be saved on the computer.

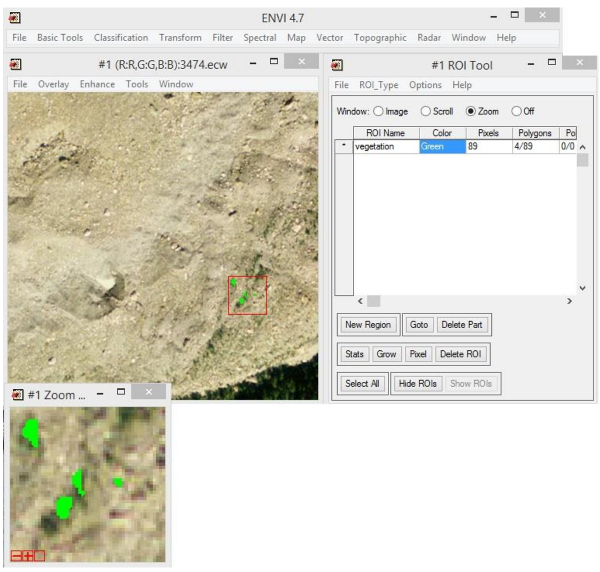


***Figure 3.*** *The selection of appropriate pixels for the ROI in ENVI called "vegetation" in a colour orthophotos representing a debris-covered glacier.*

To accurately classify the images, it is best to select a similar number of pixels for each ROI. The number of selected pixels is related to the size and resolution of the image and it can vary greatly. As a general rule, for images featuring pixel size of 0.5m x 0.5m, at least 2000 pixels should be selected for each ROI. The number of ROIs can also vary, in some studies the selection of only one ROI, corresponding to vegetation, can be enough; all the other features will be automatically classified as belonging to another ROI. The final result, in this case, will be a two-colour image, defining the distribution of vegetation and of all the other features that are classified as "different from vegetation". However, more ROIs can also be selected, to describe other features in the image. In this latter case, the probability of classifying some pixels incorrectly is reduced.

After defining the ROIs, an automatic classification of the image must be performed, using the Maximum Likelihood algorithm. In ENVI, the option "Maximum Likelihood" must be selected (Classification > Supervised > Maximum Likelihood). A new window will appear, in which the saved ROIs must be selected and the Probability Threshold must be chosen (for this study I suggest a value of 0.9, that usually enables a correct classification of the image pixels). This will lead to an automatic classification of every pixel of the image (Figure 4).

After this step, visual comparison of the resulting masks against the colour orthophotos is highly recommended to validate the results: in fact, manual correction of the image is often necessary, since the automatic approach in some cases detects vegetation in areas where there is no vegetation (due, for instance, to shadows or debris that sometimes feature a colour that is similar to some species of vegetation). After performing this procedure, the area occupied by vegetation (both herbaceous and arboreal species) can be easily calculated, by multiplying the number of pixels featuring vegetation by the area of each pixel.

Overall, this method provides an initial approach to quickly identify the more stable areas on debris-covered glaciers, characterized by the presence of abundant vegetation. It also enables the identification of the areas characterized by geomorphic activity affecting the distribution of vegetation in recently deglaciated areas; no or low vegetation is present where glaciofluvial and gravitative processes influence the area.

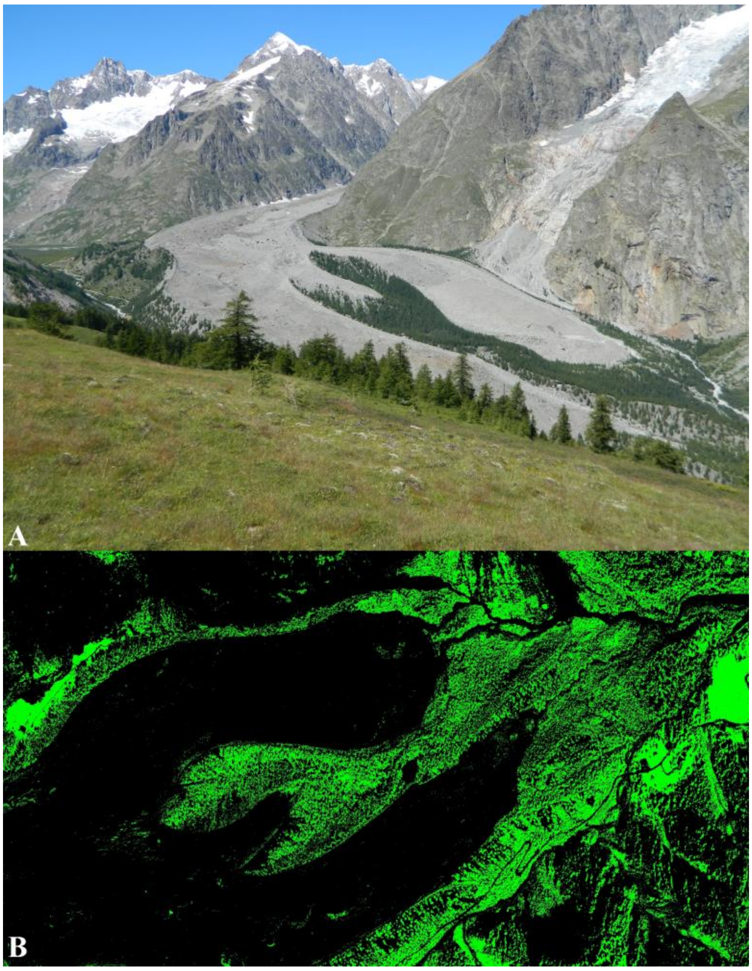

***Figure 4****. (A) The snout part of the Miage debris-covered glacier (Mont Blanc Massif, Western Italian Alps) (photo: D. Zannetti, August 2011), (B) classified using a semi-automatic approach by choosing two ROIs. The green colour indicates the vegetation; the black colour corresponds to "other features". Vegetation is also detected on the supraglacial debris.*

**Analysis of vegetation: field surveys**

As already established, remote sensing provides preliminary analysis of vegetation presence and distribution. Subsequently, detailed investigation of the characteristics of vegetation should be performed in the field to describe the species established in the area and their relationship with environmental and glacier parameters. This enables the accurate reconstruction of the recent glacial history, through the calculation of the minimum age of the moraines deposited in the glacier foreland, and of the terrain between the moraines. On debris-covered glaciers, the investigation of arboreal vegetation is of particular interest for geomorphological purposes, because tree rings may be dated and consequently used to identify the years characterized by high and low dynamicity of the glacier surface. Moreover, the characteristics of tree rings provide information about the areas affected by glacial melting water on the glacier surface or in its proximity (whenever affecting the growth of arboreal vegetation). For this reason, the analysis of arboreal vegetation represents a contribution to the assessment of the geomorphological hazards related to the dynamics and hydrology of debris-covered glaciers.

Recently deglaciated areas: chronosequences

Field surveys of vegetation in glacier forelands are performed by conducting chronosequences. These are sets of sites formed from the same substrate that differ in the time since they were formed (Walker *et al*., 2010). When planning the vegetation sampling in recently deglaciated areas, it is important to select the distribution, number and size of plots to provide a representative analysis for the whole study area.

When the study of vegetation is very detailed (that is considering both herbaceous and arboreal vegetation) the size of the plots could range between 0.5m x 0.5m and 5m x 5m. The number of plots is related to the size of the study area, however, a minimum of 10 plots homogeneously distributed should always be sampled. The plots should be delimited using quadrats or tape measures (Figure 5). The advantage of using quadrats, when available, is that they are usually characterized by the presence of a grid delimiting smaller squares that allow an easier approximation of vegetation cover.

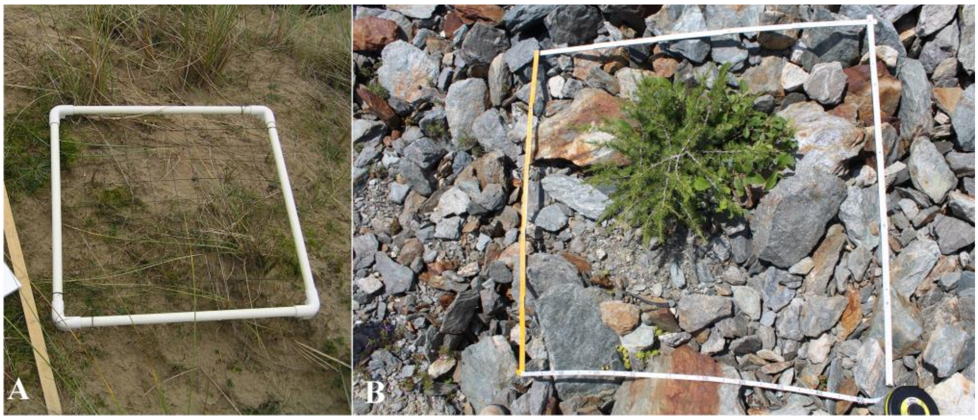


***Figure 5****. A plot delimited by (A) a quadrat and (B) tape measures.*

The characteristics of every plot can then be collected and subsequently organized in one or more tables including the following data:

- List of species;
- Vegetation coverage;
- Age since deglaciation (years);
- Environmental information (site-specific);
- Soil analysis.

In every plot, species and coverage of vegetation must be analyzed. After identifying the species in every plot with the aid of a guide to local flora (e.g. for alpine species: Dalla Fior, 1926) both vegetation cover and the cover of every species detected can be visually estimated using percentage coverage. Usually, relative abundance (the ACFOR scale) rather than absolute is preferred (Table 1).

***Table 1.*** *The ACFOR scale for measuring relative abundance of species in every plot.*

| **Species abundance** | **Letter** |
|---|---|
| Abundant (30% +) | A |
| Common (20 to 29%) | C |
| Frequent (10 to 19%) | F |
| Occasional (5 to 9%) | O |
| Rare (1 to 4%) | R |

Vegetation coverage can also be defined using the Braun-Blanquet scale (Braun-Blanquet, 1932; see example of its application in a glacier foreland in Caccianiga and Andreis, 2004). This associates a symbol, letter or number to each percentage defined in the plot (Table 2).

***Table 2.*** *The Braun-Blanquet scale for determining vegetation coverage.*

| **Percentage cover** | **Braun-Blanquet** |
|---|---|
| Single Individual | r |
| Sporadic | + |
| 0-5% | 1 |
| 5-25% | 2 |
| 25-50% | 3 |
| 50-75% | 4 |
| 75-100% | 5 |

Percentage of cover and vegetation strata are related to the terrain age. For this reason, the variation in species richness in plots located at increasing distances from the actual glacier terminus enables the calculation of the minimum age since deglaciation (see example in Figure 6).

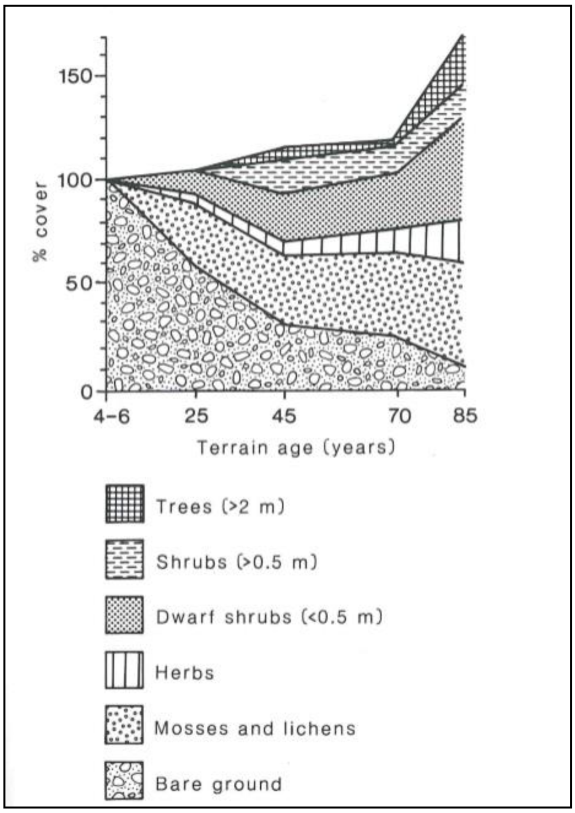


***Figure 6**. Variation of cover percentage and vegetation strata related to terrain age at the Grand Glacier d'Aletsch, in the Swiss Alps (from Lüdi, 1945). Values in parenthesis indicate the height of the strata.*

Environmental parameters must also be evaluated. Slope can be measured using an inclinometer or similar, and aspect determined using a compass. Elevation may be measured using a GPS (Global Positioning System) when available, otherwise it can be calculated using contour lines on a map of the study area. The distance from the current position of the glacier terminus may also be measured using a GPS or a map. If the position of the plot is recorded, distance from the glacier tongue can be easily measured using GIS (using the "measure" and "distance" tools). Other relevant information for the specific study area should be noted during the field surveys, in particular those related to the occurrence of geomorphological processes affecting the location. The most common in glacier forelands are gravitative slope movements, stream flow and drainage.

The representation of the position of each plot and their characteristics in a cartographic map can help in the understanding of the relationship between the position of the glacier terminus and the gradual development of the glacier foreland. In order to do that, the position of the plots can be recorded with a GPS and then reported in GIS for the subsequent creation of a detailed map of the study area.

The analysis of the physical and chemical properties of the soil (horizon identification and designations, grain size analysis, humified organic carbon) represents an additional analysis. It is performed on some homogeneously distributed plots from the study area and it may provide information about the degree of evolution of the terrain. Soil analysis is performed by first identifying the horizons present in a selected profile by digging a plot, as shown in Figure 7 (useful information for distinguishing between different horizons may be found in Chapter 3 of the free online resource "Soil Survey Manual",). Grain size and humified organic carbon can be analyzed using laboratory techniques, so it is important to get samples of soil from each horizon identified in the profiles. Further information on analysing soil properties can be found in numerous articles in Section 1 of *Geomorphological Techniques*.

***Figure 7***. *The soil properties of profiles detected in a plot are investigated. In the image, the identification of the horizons and their depth are being performed (photos: F. Sobacchi; summer 2014).*

The study of the colonization of recently deglaciated terrains can also be focused on arboreal species only. In this case, bigger plots can be considered, still homogeneously distributed in the study area (e.g. plot size 15 m x 15 m). Terrain age is the main factor controlling the colonization of arboreal vegetation, but also herb coverage and altitude (Garbarino *et al*., 2010). The advantage in studying trees lies in the possibility to date the minimum age of the glacier-free surface, through dendrochronological investigations. *Ecesis*, in particular is the lag time between surface exposure and germination of arboreal species (McCarthy and Luckman, 1993). When tree rings are dated, reconstructions of glacier retreat and dynamics can be conducted.

***Figure 8****. A young Norway spruce (species Picea abies Karst) in the Forni Glacier foreland (Italian Alps) during summer 2014. In order to determine its age, whorls branch counting was performed: in this case, three whorls can be easily identified (indicated with red lines), so the tree is three years old.*

Tree size does not always allow the sampling of cores and, for this reason, two different approaches should be applied. For trees taller than about 1 m, standard dendrochronological techniques may be applied (see Torbenson, 2015), to correctly date each tree ring and compare this data to the distance from the glacier front. When trees are smaller than 1 m, and only when the investigated species is a conifer, the age can be determined by whorls branch counting (Figure 8). This technique consists in counting the "layers" of branches, called whorls by botanists, since for tree species producing annual branch whorls, each branch whorl represents a year of life, thus allowing a precise estimation of the age of young trees (e.g. Haire and McGarigal, 2010). In both cases, after defining the tree age, comparison with the distance from the glacier front can be performed. In this way, it is possible to determine the minimum age of the deglaciated terrain, both in very recent times and in further back in time.

Debris-covered glaciers

To analyze detailed glacier dynamics in the recent past, arboreal vegetation can be studied on the supraglacial debris. Tree analysis can be performed using a dendrogeomorphological approach. After selecting one or more species to investigate, the individuals to sample must be selected. Usually, conifers are the most common tree species on the glacier surface (i.e. *Larix decidua* Mill., *Picea abies* Karst). Trees located in different sites on the glacier surface should be chosen, in order to analyze glacier dynamics in different areas. The distribution of trees is usually not homogeneous, due to differences in debris thickness and glacier velocity on the glacier surface, that influence tree establishment and germination (Pelfini *et al*., 2012). For this reason, it is not always possible to get samples from trees homogeneously distributed on the glacier surface. However, if it is possible to select at least ten trees on the glacier surface, the analysis of recent glacier dynamics in the sites surrounding the sampled trees can be successfully conducted.

To analyze tree-ring characteristics, only adult trees should be selected (trees at least 1-2 m high dependent on species), and two cores should be taken from each selected tree, using a Pressler's increment borer (Figure 9). By comparing the sampled cores of each tree, tree rings can be correctly dated. The years characterized by high surface instability are usually characterized by the presence of growth anomalies such as compression wood, abrupt growth changes and eccentricity in the tree rings, that can be observed under a microscope (Figure 10; for details about these specific terms see Torbenson, 2015). By collecting and analyzing samples from trees located at different sites on the glacier surface, distributed data about the glacier past dynamics may be obtained.

In this way, the reconstruction of past events at an annual resolution involving changes in glacier surface stability is possible.

***Figure 9.*** *Tree sampling using a Pressler's increment borer.*

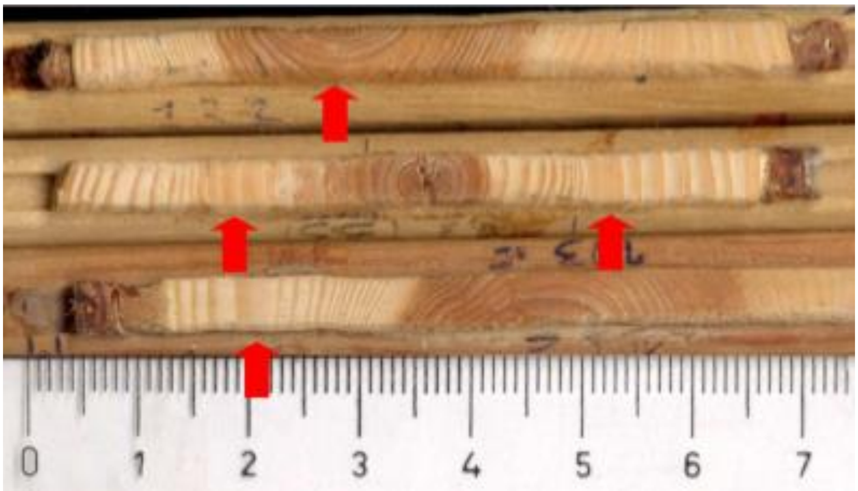


***Figure 10.*** *Three cores sampled from supraglacial trees at the Miage Glacier, presenting typical responses to the substrate movement (indicated by the arrows). The upper core shows stem eccentricity, the central core shows compression wood, the lower core shows an abrupt growth change (from Leonelli and Pelfini, 2013).*

**Conclusion**

The investigation of vegetation in glacier foreland and on the surface of debris-covered glaciers represents a valid contribution in the reconstruction of the recent glacier history and dynamics, especially in the context of climate warming. By applying the methodologies described in this chapter, it is possible to undertake both analyses of vegetation distribution (using remote sensing techniques) and detailed studies of its characteristics using field surveys. In glacier forelands, the study of the distribution and characteristics of vegetation enables the estimation of the age of the deglaciated terrain and it contributes to the investigation of the geomorphological processes occurring after deglaciation, such as gravitative and glaciofluvial processes. On the surface of debris-covered glaciers, the analysis of arboreal vegetation provides information on the glacier past and current dynamics, including glacier velocity and the stability of the supraglacial debris.

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## 4.2.

### The impact of glacial melting water on tree-ring growth at Lago Verde (Italian Alps)

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**Abstract**

Glacial lakes, especially supraglacial, ice contact and proglacial lakes, are becoming common features in the Alpine environment. They are characterized by a high dynamicity with implications in terms of hazard and risk and for the ecological system. With the aim to detect the impact of glacial lake-water on tree-ring growth, a dendrochronological analysis on *Larix decidua* Mill. trees was conducted at three sites located at increasing distances from the shores of the small 'Lago Verde', an ice-contact lake at the Miage Glacier (Western Italian Alps), characterized by frequent changes in its level.

High-water levels of the glacial lake negatively affect tree growth: tree-ring width is generally narrower in trees frequently reached by the lake waters than in trees growing farther from the lake. As the distance from the lake shore increases, the disturbance signals detectable in the tree rings decrease, as evidenced by the average correlation and by the Glk Index calculated between the mean chronologies at the three sites and the respective individual chronologies. Furthermore, the dendroclimatic analysis performed by comparing temperature and precipitation data and the residual chronologies at the Lago Verde supports our assumption: high levels of glacial melting water affect trees, when present, by disturbing growth rates or inducing suffering conditions, thus also altering the correlation with the climatic variables, in particular with the precipitation.

Overall, by evidencing the effects of glacial lake-water on tree-ring growth, this research exposes an approach that might be useful for detecting the areas impacted by glacial melting waters in the past and, as a consequence, for contributing to environmental reconstructions from mid to long time scales and to the assessment of geomorphological hazards related to the dynamics and hydrology of debris-covered glaciers.

**KEYWORDS**: glacial lake, tree rings, debris-covered glacier, melting water, Miage Glacier.

## Introduction

Glaciers are among the most sensitive indicators of climate change (Zemp et al., 2008). The ongoing shrinkage of the criosphere is leading to remarkable glacier tongue retreat (Frezzotti & Orombelli, 2014) and progressive increasing of the supraglacial debris (Deline, 2005), allowing the expansion of glacier forelands and the colonization of the paraglacial and supraglacial environments by vegetation (Cannone et al., 2008; Pelfini and Leonelli, 2014). Thermal changes influence the rate of production and discharge of melting water, controlling the glacial drainage (e.g. Huss et al., 2008) as well as the formation of glacial lakes (e.g. Deline et al., 2004) and the related hazards (Clague and O'Connor, 2014). While debris-free glaciers are characterized by rapid changes of their terminus position and, as a consequence, of their proglacial areas that are progressively modified by geomorphological processes and colonized by plants (e.g., Garavaglia et al., 2010a; Eichel et al., 2013; Leonelli et al., submitted), debris-covered glaciers (DCGs) are characterized by more stable glacier snouts. This is mainly due to the presence of the supraglacial debris that, when exceeding a critical thickness, reduces the ablation rate (Brock et al., 2010). However, glacial drainage systems of DCGs are characterized by rapid and frequent changes of their rate and pattern, inducing changes in glacier streams (Garavaglia et al., 2010b) and lakes, characterized by emptying and refilling phases and water-level fluctuations (Stokes et al., 2007; Benn et al., 2012). A better understanding of such processes is necessary, since debris-covered glaciers are increasing in their number and size, as already observed in Europe (e.g., Diolaiuti et al., 2003), Asia (e.g., Ghosh et al., 2014), New Zealand (e.g., Brook et al., 2013) and South America (e.g., Emmer et al., 2015).

The surface and surroundings of DCGs can be colonized by trees, if the glacier terminus is located below the treeline elevation (Pelfini et al., 2012; Leonelli and Pelfini, 2013). In these areas, trees can be used as indicators of changes in glacier processes as their tree growth is not only affected by climatic conditions, but also by geomorphic processes such as glacier movements (Leonelli et al., 2014) and by soil hydrological processes, influenced by meltwater. In particular, trees living in the proglacial area are strongly influenced by the remobilization of the abundant debris caused by the glacial stream flow and by the glacial melting water itself. In these areas trees are often injured, showing wounds and scars (Pelfini et al., 2007), and present growth anomalies such as compression wood and abrupt growth reductions (Stoffel and Corona, 2014; Garavaglia et al., 2010a).

Dendrochronological analyses can contribute not only to the reconstruction of past glacier fluctuations (e.g. Luckman, 1993; Pelfini, 1999) but also to the reconstruction and dating of past hydrologic fluctuations in floodplains (Tardif and Bergeron, 1997), around lakes (Bergeron et al., 2002) and around rivers (Boucher et al., 2011). The impact of flood events and hydrologic fluctuations on tree-ring growth has been widely documented in several regions of the world (e.g.

Tardif and Bergeron, 1993; Clague et al., 2006; Boucher et al., 2009; Nicault et al., 2014). However, there is a lack of information concerning the impact of rapid, inter- and intra-annual fluctuations of the water-level in glacial lakes on trees in the proglacial and ice-marginal areas. Most dendroglaciological studies have mainly focused on reconstructing past glacier fluctuations and treeline limits (e.g. Pelfini et al., 2014; Leonelli et al., 2016). The impact of melting water on tree vegetation is still unknown.

The main aims of this study are to: i) investigate if trees affected by frequent fluctuations of glacial waters, and growing close to the shores of an Alpine glacial lake, show peculiar tree-ring growth patterns, related to the lake-water fluctuations, ii) compare tree-ring growth at increasing distances from the lake shores, in order to understand how glacial water may affect tree-ring growth and iii) compare the climatic signal recorded in tree-ring chronologies obtained from trees located at increasing distances from the lake, in order to evaluate if glacial lake water may affect the climatic signal in tree-ring records.

**Materials and methods**

Study area

The study area is located in the Veny Valley, on the southern side of the Mont Blanc Massif, in the Western Alps (Valle d'Aosta, Italy). In particular, the site is located inside a special site for the conservation of animals and plants (ZSC “Mont Blanc Glacial Environments”). Among the many glaciers on the steep slopes of the massif, we studied the Miage Glacier, which is the largest DCG in Italy. On the surface of the glacier, a supraglacial forest including both grass, shrubs and trees can be easily observed (Caccianiga et al., 2011; Vezzola et al., 2016). The glacier surface changes are well documented by numerous studies conducted in the area since the 18$^{th}$ century (for a review see Bollati et al., 2014). The glacier hydrology has been investigated, in particular the emptying-filling cycles of the Lago del Miage (Diolaiuti et al, 2006; Masetti et al., 2010) and the implications of hydrological processes in risk mitigation, mainly important because of the high tourist affluence and the dynamics of geomorphological processes affecting the glacier tongue, slopes and three ice-marginal lakes (Lago del Miage, Lago di Breuillard and Lago Verde).

Sampling was conducted at the Lago Verde (coordinates: latitude 45°47'7.83"N, longitude 6°53'24.76"E), a very small glacial lake of about 1500 m$^2$ located at 1823 m a.s.l. between the two main lobes of the Miage Glacier tongue and, more in detail, close to the internal margin of the southern lobe (Fig. 1) The lake is fed by glacier melting waters. Several rapid water-level fluctuations were observed in the recent past, with a strong intra-annual variability of the water level (Fig 2). For example, the difference in level between July and September 2011 are reported in in figure 2C and 2D. The lake water-level fluctuations are presumably related to variations in the subglacial drainage, as already observed at the Lago del Miage (Diolaiuti et al., 2005). As Lesca (1956) reported, there is no certain information about the calendar year corresponding to the Lago Verde formation. The first cartographic evidence of its presence is reported in the topographic I.G.M. (*Italian Military Geographic Institute*, www.igmi.org) map "Monte Bianco", dated 1929. The level of lake water frequently changed also in the past century, e.g. low water-level was recorded in 1952 and 1954 (Lesca, 1956) (Fig. 2A and 2B).

Sparse vegetation is present near the lake shores. The main tree species observed in this area are larch (*Larix decidua* Mill.) and Norway spruce (*Picea abies* (L.) H. Karst). Some trees are flooded by water during several months of the year. Most of the trees around the lake are still alive, even though some dead trees can be found.

The study area is of particular interest because of the peculiar characteristics of the Miage Glacier and Lago Verde. The glacier terminus is located at 1750 m a.s.l., a very low altitude for an alpine glacier, since the average of the glacier fronts in Italy is 2789 m a.s.l. (Smiraglia and Diolaiuti, 2015), it is the only glacier in Italy characterized by a well-developed supraglacial forest and several studies have been conducted for monitoring the subglacial drainage, including dendrochronological investigations (Garavaglia et al., 2010). Moreover, the Lago Verde is the only ice-contact lake in Italy, to our knowledge, characterized by the presence of trees near and inside the lake.

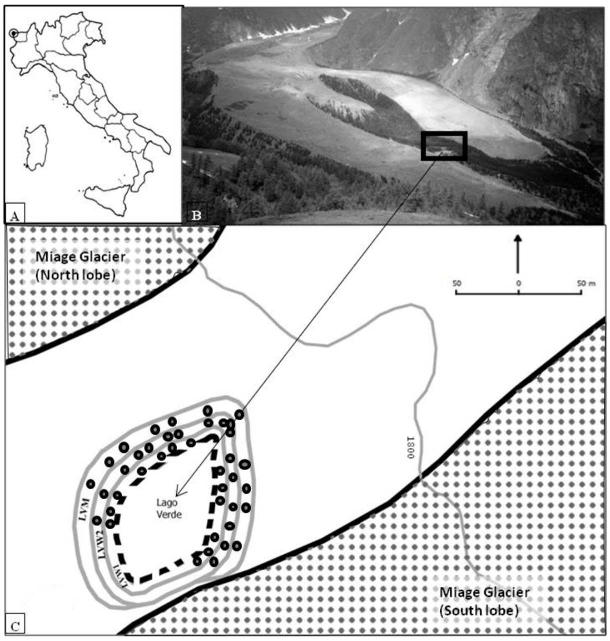


***Figure 1.*** *The study area is reported in the black circle (A). The square refers to the location of the Lago Verde with respect to the two main lobes of the Miage Glacier (B; photograph by D. Brioschi, 2007). In figure C, the lake shore observed in September 2011 is represented by the discontinuous line. The location of the study sites LVW1, LVW2 and LVM is also reported, respectively from the innermost to the outermost, and it is represented by the solid concentric lines around the lake shore. Trees at the LVM site are located on a moraine. The position of the sampled trees is reported with small circles. The dotted areas represent the debris-covered lobes of the glacier.*

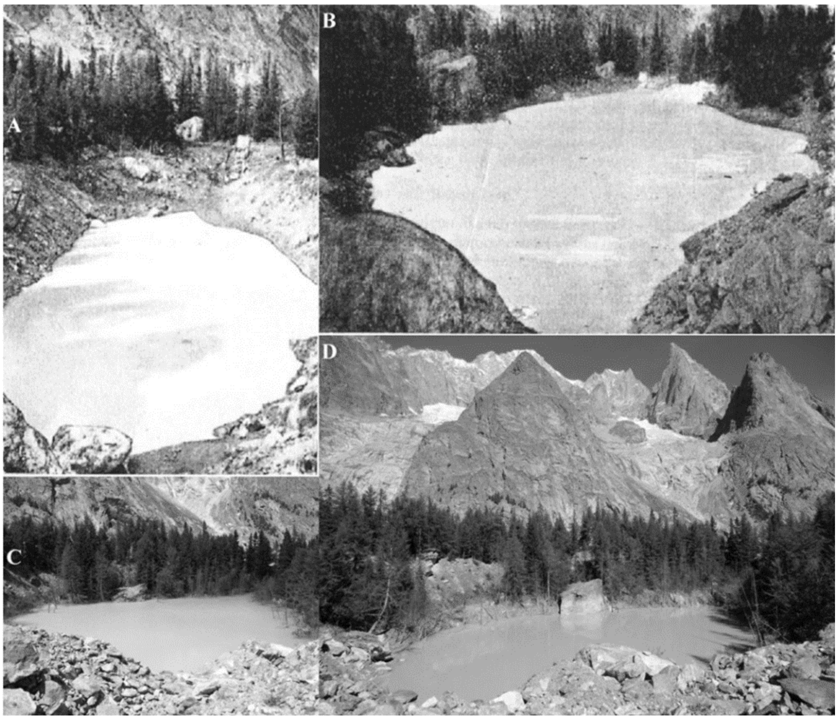


***Figure 2.*** *The Lago Verde in August 1952 (A), in August 1954 (B) (both photographs by Lesca, 1956), in July 2011 (C) and in September 2011 (D).*

Site selection

In the study area three sample sites were selected (Fig. 1C). The first site, named LVW1 ("Lago Verde Water 1") was the innermost accessible at the time of sampling, and it is characterized by trees frequently partially flooded by the lake water and with the stem bases that are often flooded up to approximately 2 m height. The second site, named LVW2, is characterized by trees that may directly take up the lake waters but only when the lake level is high. The third site, named LVM ("Lago Verde Moraine"), is characterized by trees not affected by water-level fluctuations, because of their distance and higher elevation from the lake.

Sampling

Due to the site location inside a ZSC area, we sampled a limited number of trees using a Pressler's increment borer (5 mm diameter): 13 larch trees at LVW1 (including 6 dead trees), 17 trees at LVW2 (including 9 dead trees) and 11 living trees at LVM (Fig. 1C). Samples were taken during three surveys conducted on August 2010 and July and September 2011. In these three times the water level at the Lago Verde was different, the lowest level was observed in September 2011 and it allowed the sampling of trees that were not accessible during the previous surveys. Because of the difficult access to the trees at the LVW1 site, only one core was extracted from the sampled trees at all sites, at about 50 cm above ground, in order to obtain a cambial age as indicative as possible of the real age (Schweingruber, 1988). All trees at LVW1 accessible at the time of the field survey were sampled.

Laboratory analyses

All the samples were glued on wood supports and sanded with progressively finer grade abrasive paper until the wood was polished adequately to enable the annual rings to be detected under the stereoscope. The cores were prepared using standard methods (Stokes and Smiley, 1968) and tree-ring widths were measured to the nearest 0.01 mm, using image analysis i.e., the WinDENDRO software and the LINTAB system with the TSAPWin software (Frank Rinn, Heidelberg, Germany). The tree-ring width series were visually and statistically cross-dated (COFECHA software, Holmes, 1983; Grissino-Mayer, 2001) within and between trees of the same site in order to detect and correct any dating error in the dataset.

The Gleichlaeufigkeit index (Glk), which measures the year-to-year agreement between the interval trends of two chronologies based on the sign of agreement (Eckstein and Bauch, 1969) and the CDI, the Cross Date Index, which combines the information of the Glk and the t-value (Rinn, 2005) were calculated for checking the correct dating of -the raw tree-ring chronologies before constructing the master chronologies at the LVW1, LVW2 and LVM sites.

For the three sites LVW1, LVW2 and LVM and over the common period 1968-1998, the following indices were calculated and their variability among the sites analyzed: average correlation, i.e. the averaged correlation coefficients calculated between the three mean chronologies and the respective individual chronologies, mean sensitivity, which measures the relative change in ring width from one year to the next (Fritts, 1976), and average Glk Index. For this analysis, only the individual series covering the whole common period were considered, and therefore 6 samples at LVW1 and 2 samples at LVW2 were not considered.

A residual chronology for LVW1, LVW2 and LVM was obtained by applying a flexible spline with a 50% frequency cut-off at 30 years to the growth series and then applying a biweight robust mean to the detrended indices. In order to evaluate the climatic signal, a correlation analysis was conducted over the period 1922-2005 between the three residual chronologies and the monthly (January to September) temperature and precipitation. Meteorological data were taken from the HISTALP dataset (Auer et al., 2007).

For both the master chronologies and the residual chronologies at the three sites, the analysis of variance was performed over the common period 1968-1998.

**Results**

The mean ring-width chronologies LVW1, LVW2 and LVM cover the period 1922-2009, 1849-2009 and 1872-2009, respectively (Fig. 3A). They generally show similar growth trends. Tree-ring width at LVW2 is higher compared to LVM between 1958 and 1972, with two relative peaks of maximum growth in 1958-1960 and 1967-1970. Starting from 1980, LVW1 shows lower tree-ring width compared with the other chronologies.

There is a statistically significant difference between groups in the considered common period 1968-1998, as determined by one-way ANOVA ($F(2,90) = 4.856$, $p < 0.05$). A Tukey post-hoc test reveals that tree-ring width at LVW1 is statistically significantly lower (107.9 ± 23.25, $p = 0.015$) compared to LVW2, and that LVW2 is statistically significantly higher (127.94 ± 32.61, $p = 0.034$) compared to LVW3. There is no statistically significant difference between LVW1 and LVW3.

The residual chronologies LVW1, LVW2 and LVM show rather similar tree-ring growth trend, with wide tree-ring width variations along the whole chronologies, particularly between 1930 and 1979 (Fig. 3B). The one-way ANOVA shows that there is no statistically significant difference between groups in the common period 1968-1998 ($F(2,90) = 0.917$, $p = 0.403$).

Average age of trees at LVW1 is 42 years, lower than trees at the other sites, in fact trees at LVW2 show an average of about 68 years and trees at LVM show an average of 108 years.

Individual LVW1 chronologies show generally lower ring widths than at both LVW2 and LVM. There are four exceptions, i.e. the samples LVW04, LVW05, LVW09 and LVW13, which show larger ring-widths than both the LVW2 and LVM mean chronologies during most of the period they cover.

All the LVW1 individual growth curves show a progressive reduction of the tree-ring width in their most recent portions, although some of them grew fast in the last years prior to death: trees LVW02, LVW04, LVW07 and LVW12 were dead at the time of sampling and show some large rings in the outermost rings formed before dying (Fig. 4).

The average correlation coefficient calculated between trees of the same site over the common period 1968-1998 (31 years) shows the highest values at the LVM site ($r = 0.53$), while a lower value is found at the LVW2 ($r = 0.37$) and a very low value, meaning no correlation, at the LVW1 ($r = 0.09$) site. A statistically significant trend between the distance from the lake center and the correlation coefficient value is detected ($r = 0.99$, $p<0.05$) (Fig. 5A).

The mean sensitivity is similar at the three sites, from LVW1 (ms = 0.17) to LVW2 (ms = 0.16) and LVM (ms = 0.16). There is no significant correlation between the distance from the lake center and the mean sensitivity ($r = -0.97$; $p = 0.07$) (Fig. 5B).

The average Glk Index shows higher values in the LVM chronology (Glk = 81) in comparison with the LVW2 (Glk = 74) and LVW1 (Glk = 69) chronologies. There is not a statistically significant trend between the distance from the lake center and the GLK ($r = 0.96$, $p = 0.09$) (Fig. 5C).

The climatic analysis conducted between air temperature and the indexed chronologies shows statistically significant correlation coefficients in the month of June at all the sites: LVW1 ($r = 0.20$; $p<0.05$), LVW2 ($r = 0.26$; $p<0.05$) and LVM ($r = 0.25$; $p<0.05$) sites (Fig. 6A). Significant correlation coefficients between the precipitation variables and the indexed chronologies were detected in the month of June at the LVW2 ($r = -0.25$; $p<0.05$) and LVM ($r = -0.18$; $p<0.05$) sites and not at LVW1 (Fig. 6B).

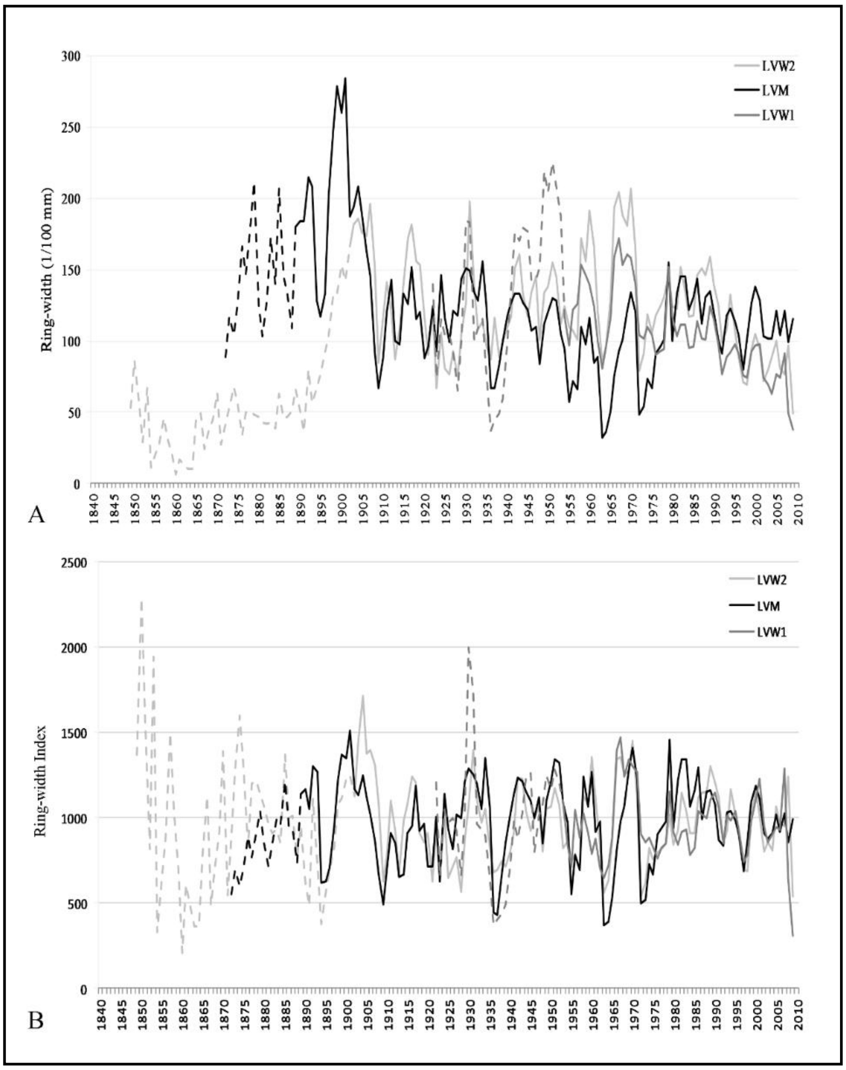


***Figure 3.*** *The ring-width mean chronologies (A) and the residual chronologies (B) obtained at the LVW1, LVW2 and LVM sites. Broken lines show four trees or fewer.*

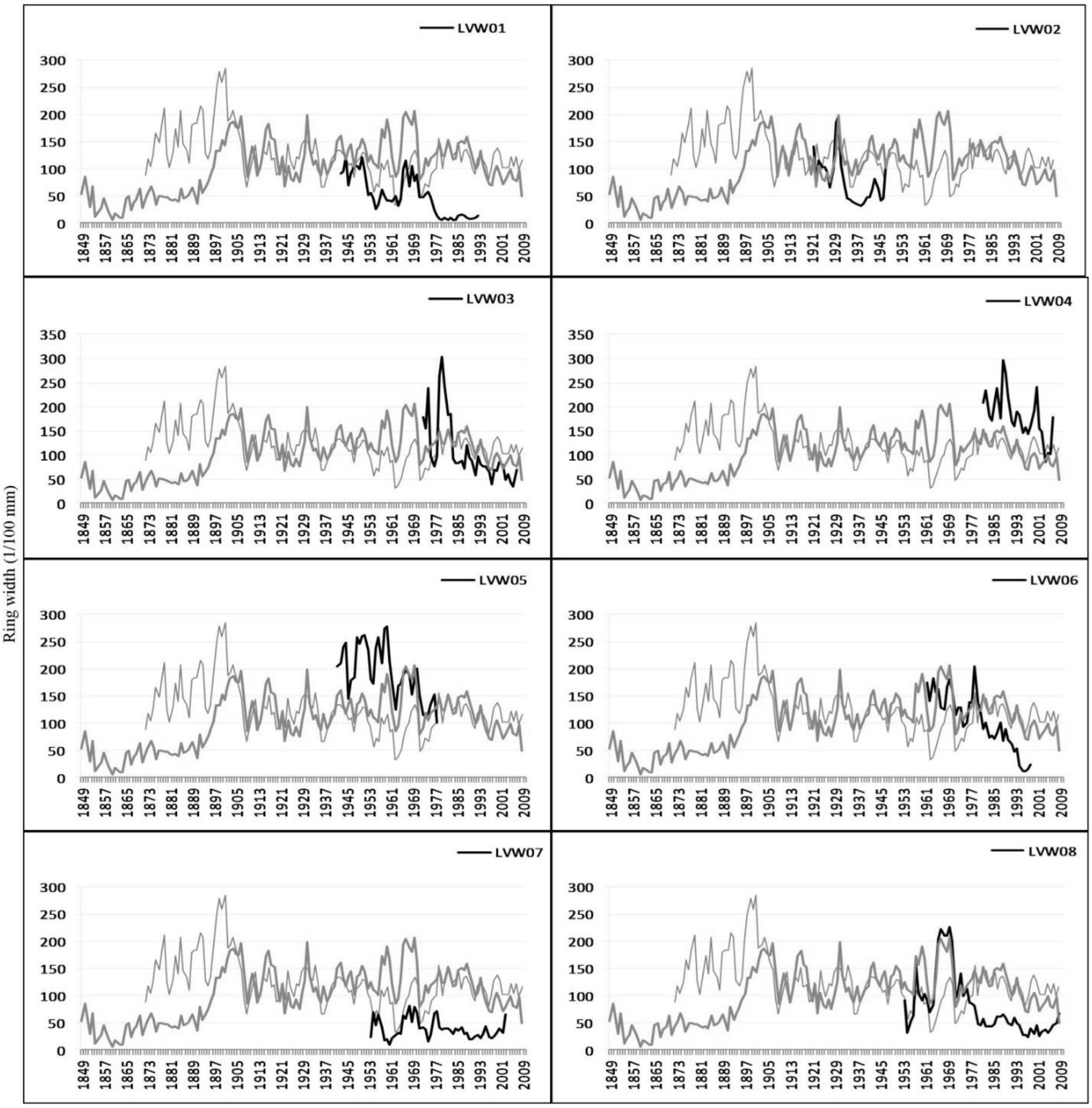


***Figure 4.*** *The ring-width chronologies obtained at the LVW1 site (black lines) compared to the LVW2 and LVM mean chronologies. LVW2 mean chronology is represented with a grey thick line, while LVM mean chronology with a grey thin line (continuing in the next page).*

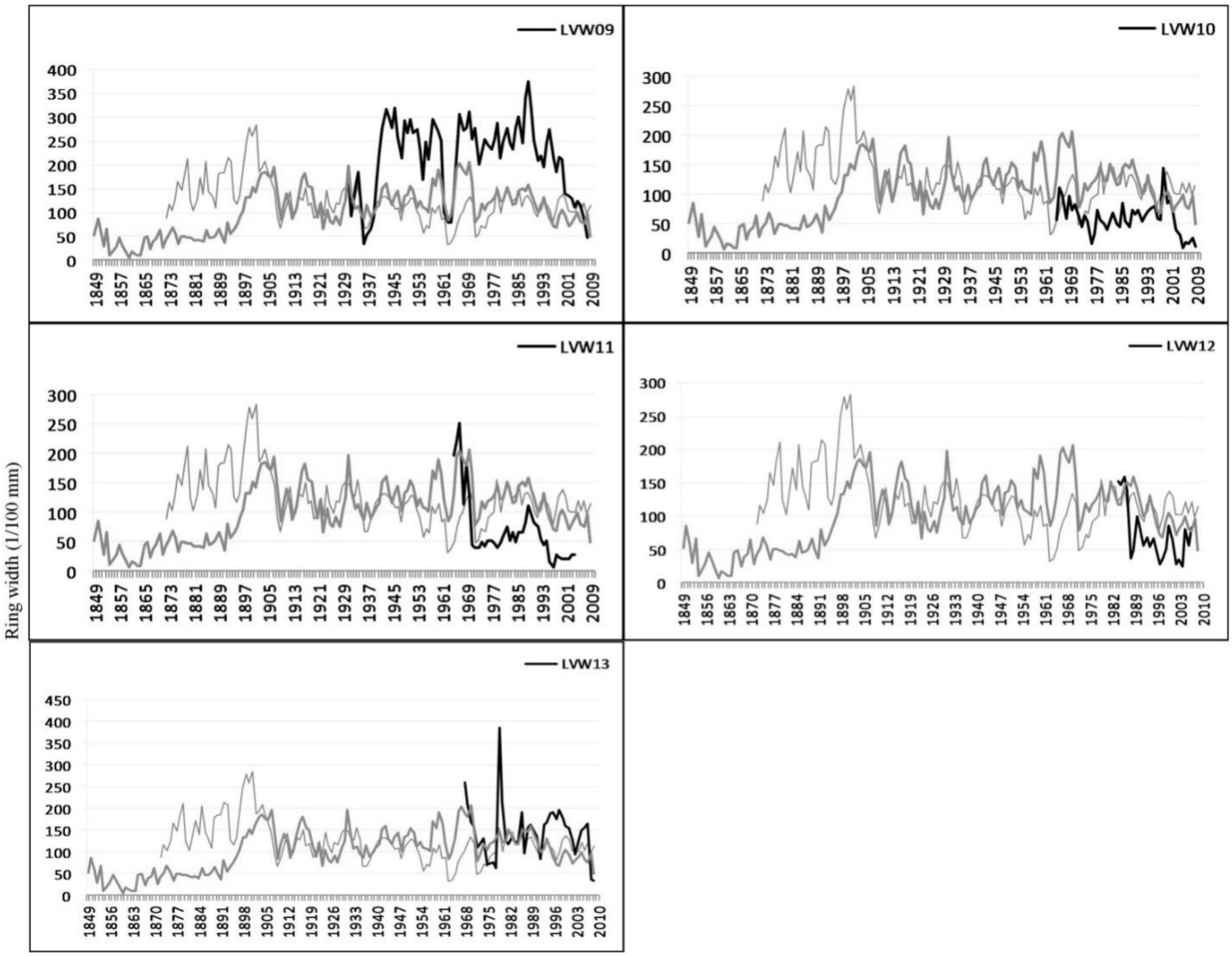


***Figure 4. (continue)*** *The ring-width chronologies obtained at the LVW1 site (black lines) compared to the LVW2 and LVM mean chronologies. LVW2 mean chronology is represented with a grey thick line, while LVM mean chronology with a grey thin line.*

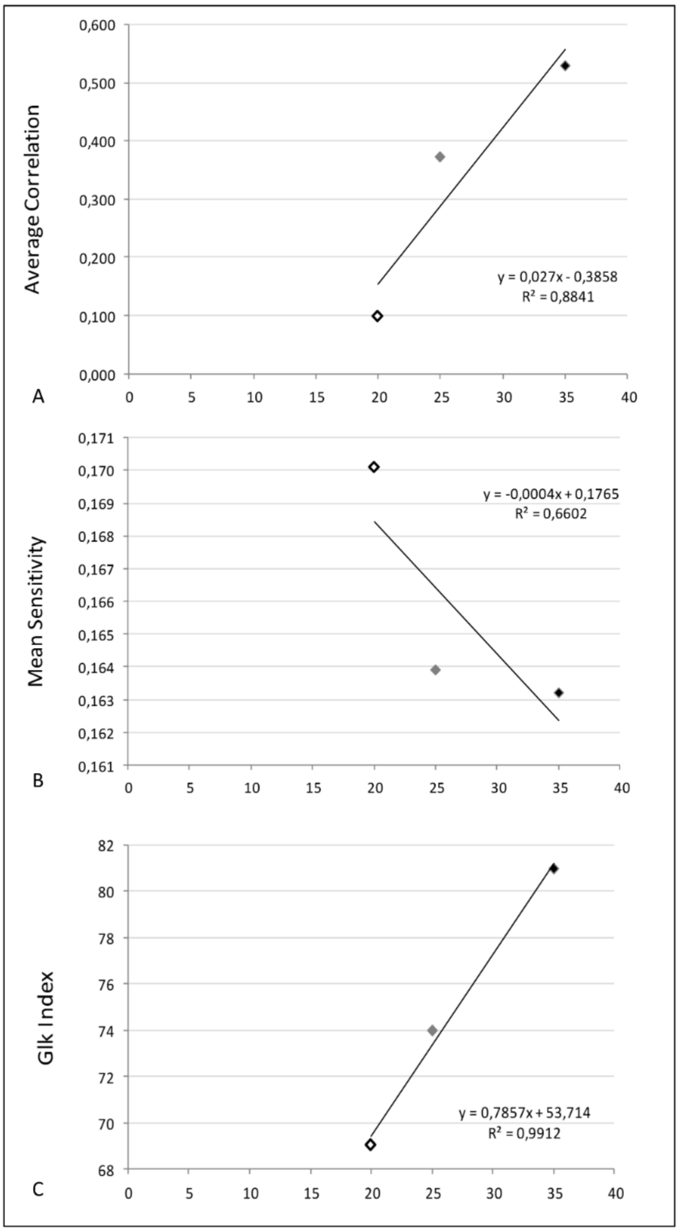


***Figure 5.*** *Correlation coefficient (A), mean sensitivity (B) and Glk Index (C) for the LVW1, LVW2 and LVM sites at increasing distances (in m) from the Lago Verde center.*

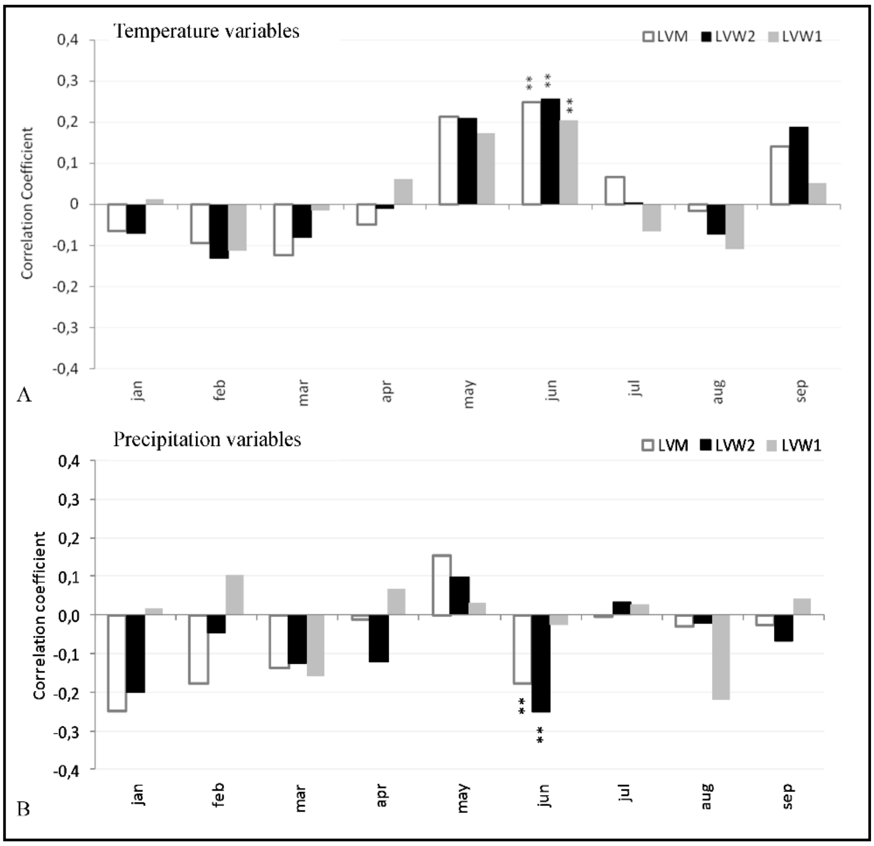


***Figure 6.*** *Climatic analysis performed for the residual chronologies LVW1, LVW2 and LVM over the period 1920-2005 using monthly variables of temperature (A) and precipitation (B). Statistically significant correlation coefficients are marked with two asterisks (p < 0.05).*

## Discussion

Our results show that high levels of glacial lake-water represent a major environmental disturbance for tree growth at the Lago Verde. Two main effects related to the high levels of glacial lake-water were detected: the narrower ring widths characterizing most of the trees at LVW1 when compared with trees at LVW2 and LVM and the high variability of the individual LVW1 chronologies.

Even if the number of sampled trees is limited due to the site location (inside a special area for conservation of plants and animals), our results show that stagnant glacial water represents an important stress factor for trees, and often causes lower growth rates and production of narrower tree rings, especially during the last years of a tree's life. The production of narrower tree rings in the last years of life was observed in another study conducted on trees declining because of other stress factors (Cherubini et al., 2002).

Previous research demonstrated that flood frequency plays a key role for tree survival: areas affected by frequent floods are usually characterized by the presence of young trees (i.e. not older than 40 years) and that the survival rate is reduced, due to anoxic conditions for the roots (e.g.

Gunnarson, 2001; Berthelot et al., 2014). According to these previous findings, we found that glacial water at the Lago Verde determines the same effects on tree growth. All trees at the LVW1 are younger than trees at the other sites, and many of them are dead.

Different tree species have different tolerance to flooding. The Norway spruce, analyzed in this study, is classified as a “low tolerant” species and it does not survive in case of stagnant conditions (Glenz et al., 2006). This could explain the death of numerous trees found at the LVW1 and LVW2 sites, particularly at the LVW1.

Previous studies reported that trees are usually negatively influenced by hydrogeomorphic processes, and show a decrease in tree-ring growth (Stoffel and Bollschweiler, 2009; Stoffel and Wilford, 2012). Our results demonstrate that also intra-annual fluctuations in glacial lake water cause the same growth decrease in trees. However, in four LVW1 trees we found larger tree rings compared to trees at LVW2 and LVM. This can be due to the possibility that trees were less affected by the lake water stagnation through the years, because located in an area that would be flooded only when water level would be very high.

The analysis of tree-growth patterns shows that tree rings have a high potential for environmental studies and reconstruction of landscape changes in glacial environments. In particular, by coupling data derived from the analysis of tree-ring width and data obtained from the analysis of tree-ring stable isotope $\delta^{18}O$, more detailed information related to the effects of glacial water on tree-ring growth, and also about the glacier past hydrology, may be achieved. At the same study site, Leonelli et al. (2014) analyzed tree-ring $\delta^{18}O$ values in the tree-ring cellulose finding that the analysis of oxygen stable isotopes in tree rings allows to distinguish between trees that take up water from the glacial lake and trees that take up water from meteoric precipitation only. With this study we show that also tree-ring width can be used as a preliminary indicator for defining areas impacted by glacial water.

Among the analyzed indexes, we found that the correlation coefficient calculated over the period 1968-1998 is significantly lower in trees at LVW1 compared to trees at LVW2 and LVM sites. This result indicates that tree-ring growth is heavily disturbed in the trees growing closer to the lake shores, as shown by the higher variability in tree growth.

The climatic analysis was conducted to investigate if glacial water influences the climatic signal recorded in the residual chronologies. Even though the accuracy of the dendroclimatic analysis is limited due to the analysis of only one core from each tree, both the sites LVW2 and LVM show similar correlations with the climatic variables and, in particular, statistically significant correlation coefficient values were found in the month of June. On the contrary, the LVW1 site shows statistically significant correlation coefficient with temperature but not with precipitation, thus suggesting that water is not the limiting factor at the site, and that perhaps it might be even negatively influencing, at least in part, the climatic signal in the tree-ring residual chronologies.

**Conclusion**

The selected study area, even if small, represents a unique situation in the Southern side of the Alps, and thus it represents a key site for analyzing the relationship between glacio-related processes and the response of arboreal vegetation.

Since trees are able to "record" the variations of climatic and environmental parameters in their tree rings, dendrochronology is a very useful discipline for monitoring changes in the characteristics of the environment. The lake small dimensions, the difficult access to the flooded trees and the limited number of individuals located in a special site for the conservation of animals and plants, only allowed a relatively limited sampling. However, the obtained results are interesting and allow for a better understanding of the impact of glacial water on tree-ring growth.

Our results show that glacial lake water has a negative effect on the growth of trees periodically flooded. Trees can be used as indicators for defining the areas impacted by glacial water in the past.

The individual chronologies of trees growing at the site LVW1, the closest to the lake, show narrower and more variable tree rings compared to trees growing farther from the lake (LVW2 and LVM sites). The average values of the correlation coefficients calculated for the individual chronologies at each site increase gradually, with LVW1 featuring the lowest value, followed by LVW2 and finally LVM, presenting the highest correlation.

The differences detected in the ring-width chronologies at the three sites at the Lago Verde underline the negative influence of glacial water on tree-ring growth. Since water-level fluctuations at the Lago Verde are frequent and rapid, trees at the LVW1 site show very variable individual growth patterns related to different submersion conditions and tree health conditions.

The results obtained in this study, even if restricted to an individual sample area, demonstrate the capability of tree rings to record environmental information also linked to non-catastrophic events, potentially allowing the detection of topographic surface areas impacted by glacial waters in the past, even when no geomorphological evidence is detected.

Moreover, as changes and expansion of proglacial lakes, and more in general glacial lakes, are leading to hazardous situations for tourists in high mountain environments (e.g. Tinti et al., 1999; Purdie, 2013), an accurate monitoring through tree-ring analysis coupled with remote sensing analysis (e.g. Raj et al., 2014; Emmer et al., 2015) represents the first step for risk mitigation. Information from tree growth anomalies will help to reconstruct past events and lake fluctuations contributing to a better understanding of glacial-related geomorphological hazards.

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## 4.3.

## The role of the ecological value in geomorphosite assessment for the debris-covered Miage Glacier (Western Italian Alps) based on a review of a 2.5 centuries of scientific study

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**Abstract**

Ecological attributes of geomorphosites play a significant role as one of the characteristic components of their scientific value, hence influencing their global value. Ecological attributes can, however, act independently and inform other attributes which characterise scientific and additional values and the sites potential for use. Within the framework of active geomorphosites, environmental changes and recent dynamics can be reconstructed through tree-ring analysis. Glacial geomorphosites are one of the most significant examples of this biotic-abiotic relationship. Among glacial geomorphosites, debris-covered glaciers represent key sites at which to evaluate an ecological attribute. The Miage Glacier, the most representative Italian debris-covered glacier, is an important site for multidisciplinary scientific research, enhancing its global value as a complex geomorphosite. The Miage Glacier has been selected as a study site, firstly to characterise the evolution of scientific research across the last 250 years and then to quantify the ecological support role (ESR) and its influence on the other attributes. The ESR's importance in assessing global geomorphosite values was estimated by studying its variation and the variation of the related attributes as a consequence of an increase in scientific knowledge over time. The ESR variation displays a positive effect on scientific value, in particular, and generates more differentiation between defined sites and visitor trails, thus influencing site selection.

**KEYWORDS**: glacial geomorphosites, ecologic support role, debris covered glaciers, Miage glacier

## Introduction

High mountain geomorphosites (*sensu* Panizza 2001) represent a very interesting subject for both geoheritage assessment and educational purposes (Reynard et al. 2007; Bollati et al. 2011). Their rapid evolution requires attention because, on the one hand, there is the possibility of geomorphosite degradation resulting from changes in the geomorphological processes acting on them (Diolaiuti and Smiraglia 2010; Pelfini et al. 2009), and on the other hand, active geomorphosites (*sensu* Reynard 2004) represent a useful tool for educational purposes (Reynard et al. 2007). In fact, they allow people to experience characteristic geomorphic features just from observing defined areas in the landscape (Reynard et al. 2007; Bollati et al. 2011). The importance of evolving geomorphosites is also associated with the hazards that may derive from geomorphic process changes and their intensification in response to climate change. This is significant, especially in tourist areas where the vulnerability component is present (Brandolini et al. 2006; Pelfini et al. 2009). Hence, the *educational exemplarity* (*sensu* Bollati et al. 2012) of these sites may be considered related to the aforementioned topics. Dissemination of concepts, that are fundamental to both risk scenarios and to the proper way to move through the natural environment, contributes to the educational importance of these types of sites of geomorphological interest (e.g., Bollati et al. 2013).

Moreover, active geomorphosites located in temperature- and precipitation-limited environments may be strongly influenced by climatic variations, because their characteristics are modified by variations in the frequency and intensity of climate-related geomorphological processes. This is in accord with the “narrow definition” of geosite proposed by Grandgirard (1997): “it can be any part of the Earth’s surface that is important for the knowledge of Earth, climate and life history.”

Glacial geomorphosites are among the most significant examples of active geomorphosites (i.e., changing in a “changing climate” (Diolaiuti and Smiraglia 2010)), whose quantitative evaluation should be periodically reassessed as a response to changes in their features (Pelfini 2009; Pelfini et al. 2009; Diolaiuti and Smiraglia 2010). After identifying their attributes during geomorphosite selection (Pelfini and Smiraglia 2003), many glacial geomorphosites were proposed as important because of their high scientific and cultural value (e.g., Pelfini and Gobbi 2005; Pelfini et al. 2005). Particular focus was placed on their ecological and educational attributes (Pelfini et al. 2010a; Garavaglia et al. 2010a).

A distinctive category of glacier geomorphosites is represented by the debris-covered glaciers (DCGs) which arise from a growth in supraglacial debris resulting from climate change-related processes (e.g., rock avalanches from the valley sides due to permafrost degradation and outcropping of endoglacial debris due to increasing ablation rates; Deline 2009). Debris coverage above a certain thickness threshold diminishes the glacial ablation rates (e.g., Mattson et al. 1993;

Mihalcea et al. 2008; Brock et al. 2010), making the DCGs' response to climate changes different to that of debris-free glaciers (DFGs). Hence, DCGs may be considered one of the features in the alpine landscape in which a distinctive response of the natural environment to climate change is evident.

Within the framework of geomorphological heritage assessment, there is agreement among scientists concerning the need for a census of geomorphosites based on objective evidence. However, more often discussed is the quantification of single (*simple*) values and global (*composite*) values (e.g., Reynard et al. 2007). Difficulties may arise in applying rigid evaluation schemes, considering the vast geomorphological differences in different morphoclimatic environments. Another problem is the subjectivity in assessing values (e.g., Bonachea et al. 2005; Bruschi et al. 2011). Considering all these conditions, some proposals were suggested using data base applications (e.g., Giardino et al. 2010; Ghiraldi et al. 2010). For instance, Bollati et al. (2012) proposed a method for assessing and selecting sites of geomorphological interest by employing either the function of the users (e.g., tourists, students of different levels, etc.) or the aim of the project (e.g., valorisation, education, or management). The same application was tested for selecting educational and cultural trails by obtaining global values for single itineraries which also considered natural hazards. Consideration of natural hazards was intended both negatively, to exclude unsuitable itineraries, and positively, as occasions for education about risk and safety conditions, as previously discussed (Bollati et al. 2013).

Many features are considered in geomorphosite selection (e.g., Bollati and Pelfini 2010), and in recent times, new attention has been directed towards their ecological attribute (i.e., the *ecological support role, ESR*; *sensu* Bollati et al. 2012), especially for glacial geomorphosites (e.g., Garavaglia et al. 2010a; Pelfini et al. 2010a). As presented in Table 1, the ecological value changes position and relative importance among groups of attributes depending on the author and the meaning that the author confers to it. Within this framework, the strategic role of vegetation may indeed be considered as influencing transversally the other attributes that are also taken into account when calculating *scientific value*, *additional values* and *potential for use* (Fig. 1). Vegetation's role is especially important in understanding DCGs: when the debris layer is thick enough (i.e., at least 40 cm at the sample site of Miage Glacier; Pelfini et al. 2007), the surface glacier velocity is low, and if the glacier tongue reaches altitudes below the tree line, not only herbaceous and shrub vegetation but also trees can germinate and grow (Pelfini 2009) providing a *rareness* value to these geomorphosites. Moreover, DCGs evolution and dynamics can be studied through the analysis of annual tree rings in supraglacial living trees (e.g., Pelfini et al. 2007; Leonelli and Pelfini 2013a).

The aim of this paper is to quantitatively assess the ecological attribute's contribution to glacier geomorphosites in relation to variation in the composite values (e.g., *global*, *scientific*, and *additional values* and *potential for use* (*sensu* Bollati et al. 2012)) during geomorphosite evaluation. First, the scientific literature concerning the study area was analysed and characterised chronologically. Next, the publications concerning the interaction between vegetation and glacial processes were selected as support for the quantitative re-evaluation of sites. The Miage Glacier, in the Mont Blanc Massif (Western Italian Alps), was selected as the study area because it is a highly representative debris-covered glacier in the Italian Alps for which the scientific literature is broad and varied in terms of the topics investigated.

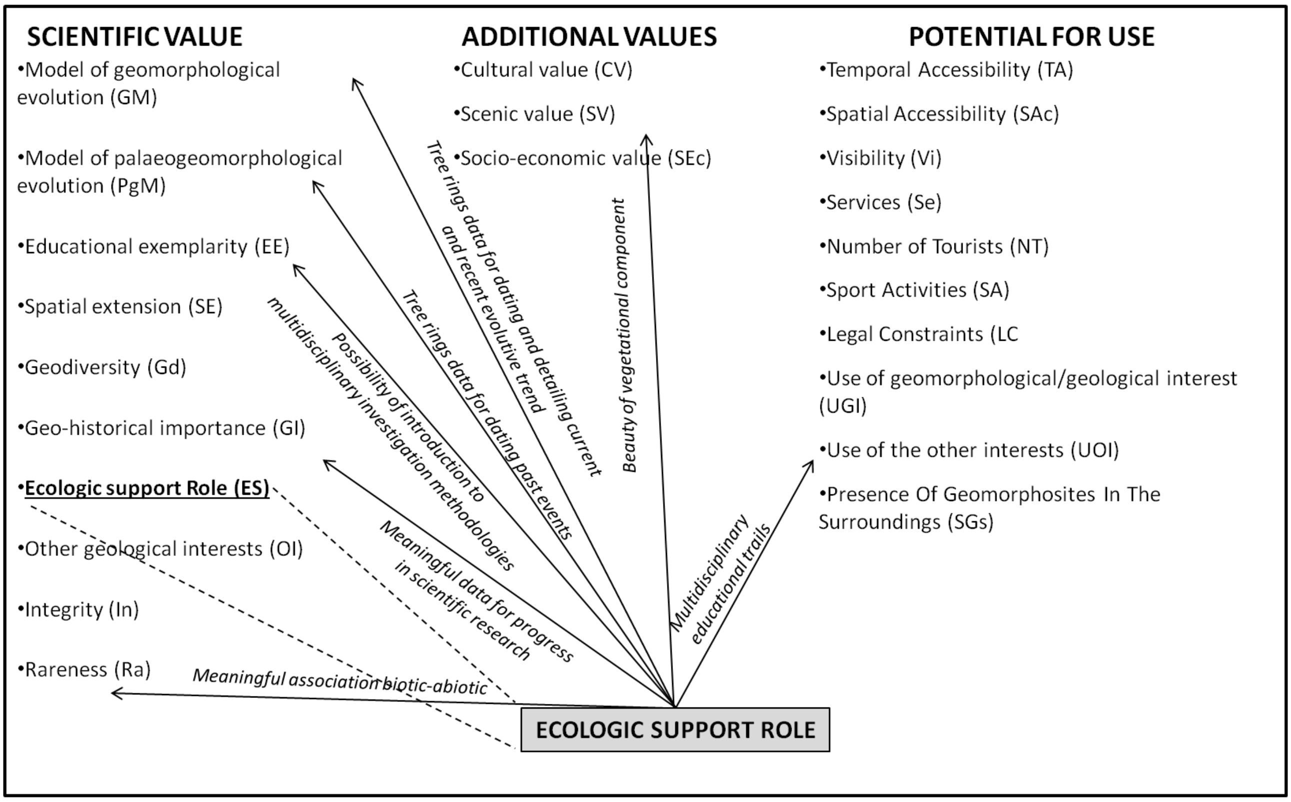


***Figure 1.*** *The transverse influence of the ESR on the other attributes used for geomorphosites evaluation (see criteria in Bollati et al. 2012).*

***Table 1.*** *The different approaches to and considerations of the ecological value (modified from Bollati and Pelfini 2010).*

| *SCIENTIFIC VALUE* | *ADDITIONAL VALUE* |
|---|---|
| Ecological value as support for living nature: (Panizza 2001; Bollati et al. 2012), especially in sensitive contexts (Quaranta 1992; Carton et al. 1994; Rivas et al. 1997; Panizza 2001; Panizza and Piacente 2003; Gray 2004; Pralong 2005; Pralong and Reynard 2005; Pelfini et al. 2010a; Garavaglia et al. 2010).<br>For some Authors the whole landscape may be considered as living organism (Romani 1994).<br>Other terms:<br>Functional value (Gray 2004)<br>Naturalistic value: (Brancucci et al. 1999) | Barca and Di Gregorio (1991); Hooke (1994); Coratza and Giusti (2005);<br><br>Specific category:<br>*Ecological Impact Criterion* - EcI (Reynard et al. 2007; Pereira et al. 2008). |

**Study Area**

The Miage Glacier, located in the Veny Valley (Valle d'Aosta, Italy; Fig. 2a), drains the southwest slope of Mont Blanc. The glacier is approximately 13 km long and shows an ablation tongue, towards the terminus, characterised by two main lobes with a smaller one in between. The valley is included in the "Espace Mont Blanc" area, which is under consideration for inclusion on the UNESCO World Heritage List.

Certainly, the Miage Glacier represents a significant site from an educational viewpoint and due to its accessibility and notoriety (Pelfini et al. 2005; Bollati et al. 2013). Moreover, the entire area is considered a prime example of an open-air laboratory (Pelfini et al. 2009), suitable for research and education on the subject of differential ablation, as demonstrated for other sites by Pelfini et al. (2010b). According to Bollati et al. (2013), the Miage Glacier belongs to the category of "*complex active geomorphosite*" (Pelfini et al. 2009; *sensu* Reynard 2004 and Reynard and Panizza 2005) and its *simple* attributes and *composite* values (especially the scientific value) have been recently discussed (e.g., Pelfini et al. 2005) in terms of risk scenarios (Mortara and Sorzana 1987; Pelfini et al. 2009). Furthermore, the values of geomorphosites, distributed along three sample trails, were quantified for the first time by Bollati et al. (2013), demonstrating quantitatively the important value of the area in terms of geomorphological heritage.

More precisely, the geological, geomorphological and glaciological features enhance the scientific value of the area, as evidenced by extensive scientific research that began during the 18th century when the first papers describing this area were published (De Saussure 1774; Baretti 1880). The Miage Glacier represents the most significant place in the Italian Alps to study the dynamics of a DCG (e.g., Mihalcea et al. 2008; Brock et al. 2010) (i.e., for geomorphosite assessment: *model of* *geomorphological evolution*; *geohistorical importance*; *sensu* Bollati et al. 2012). There are many respects in which the *ESR* of this glacier and the associated processes are important to both vegetation (Fig. 3) (e.g., Pelfini et al. 2010a; Garavaglia et al. 2010a; Caccianiga et al. 2011) and arthropod communities (Gobbi et al. 2011).

The spatial and temporal distributions of the supraglacial tree coverage, that represents a rareness feature for the Miage Glacier as a geomorphosite, were characterised by Pelfini et al. (2007) and Leonelli and Pelfini (2013a). The supraglacial trees are primarily of the species *Larix decidua* Mill. and *Picea abies* Karst. (Pelfini et al. 2007). The investigations regarding the supraglacial trees, in conjunction with glaciological information, allowed the reconstruction of the recent dynamics of the lower portion of the glacier tongue (Fig. 3b) (Pelfini et al. 2007; Pelfini et al. 2012; Leonelli and Pelfini 2013a). In fact, tree rings may record both mechanical stress and climatic signals. For this reason, the trees growing on debris coverage, while being transported by the glacier flow in a manner comparable to a "tapis-roulant" (i.e., "treadmill"; Richter et al. 2004; Pelfini et al. 2005), are precious sources of geomorphological information. For example, the integration of glaciological data for surface velocity over time allowed the tracing of the tree's paths and, subsequently, the determination of the position on the glacier where growth anomalies in the tree rings were recorded (Leonelli and Pelfini 2013a). Moreover, glaciological research indicates that, during the period 1975 - 1988 (Giardino et al. 2001), there was a passage of a kinematic wave which crossed the glacial tongue, modifying the glacier's surface elevation (Thomson et al. 2000). The analysis of tree-ring anomalies, in the supraglacial trees growing on both lobes, allowed the reconstruction of past surface instability and the determination that there was a delay of a number of years in the wave traversing first the southern and then the northern lobe (Pelfini et al. 2007; Leonelli and Pelfini 2013a). An intensification of glacial activity, likely related to the kinematic wave, is also witnessed by trees colonising the proglacial area where the dendrochronological analysis allowed for the collection of information on glacial stream course changes over time (Fig. 3c) (Garavaglia et al. 2010b).

In regards to the portions of the glacier tongue presenting debris-free ice, as in the case of ice cliffs (Fig. 3a) where the ablation is more intense, the processes of down-wasting and back-wasting (*sensu* Benn and Evans 1998) are both present and may differently impact the supraglacial trees. For example, on the northern lobe, the debris cover displacement caused by the glacier flow moves the trees towards the glacier terminus, where they usually die from falling off the front edge of the glacier (Richter et al. 2004; Pelfini et al. 2005; Leonelli and Pelfini 2013a). However, when the trees move down valley along the edge of the ice cliff, they can be involved in the ice wall retreat (back-wasting), ending their life before they reach the glacier front. In contrast, when the ice cliff is buried because of an increase in the debris cover and subsequent lowering of the cliff inclination (as observed on the southern lobe), the trees may continue their movement and colonise the ice cliff slope, too (Pelfini et al. 2012).

Among the significant geomorphosites identified by Bollati et al. (2013) in the Miage Glacier apparatus, there is a well-developed morainic amphitheatre, recognised by different authors as one of the most significant in the European Alps (e.g., "The moraine of the Glacier de Miage is perhaps the most extraordinary in the whole Alps, and has given rise to the Lac de Combal", Murray 1844; Forbes 1843; Baretti 1880) and is referred to in the literature as the Miage Morainic Amphitheatre (Deline and Orombelli 2005; Deline 2009) (Fig. 3f). This amphitheatre was generated by a diversion lobe of the Miage Glacier and colonised by arboreal vegetation. Dendrochronological analysis of the amphitheatre's trees provided data for dating the maximum Holocene expansion in the Western Alps (Deline and Orombelli 2005) (i.e., for geomorphosite assessment: *model of paleogeomorphological evolution*; *geohistorical importance*; Bollati et al. 2012), including the estimated time of formation of Combal Lake (Deline and Orombelli 2005).

The *geodiversity* (i.e., *intrinsic geodiversity*; Panizza 2009) of the Miage Glacier area also benefits from the presence of different lake typologies (Jardin du Miage Lake, Miage Lake, Combal Lake, and Breuillard Lake). Among these lakes, the Jardin du Miage Lake (also known as Lac Vert) is surrounded by arboreal vegetation that is occasionally drowned along the lake edge and impacted by water level changes (Fig. 3d, e). As recently highlighted by Leonelli et al. (2013b), these trees may record these water lake changes by virtue of their growth rates and typical tree-ring isotope signatures related to the low $\delta^{18}O$ of glacier melt waters.

Within the Miage complex geomorphosite, there are several situations where the *ESR*, influenced by outcropping lithology and low drainage conditions deriving from the presence of particular landforms (Prinetti 2010), creates significant features: Breuillard Lake, Combal Lake and the area of Jardin du Miage near the homonymous lake. In particular, the progressive infilling with sediment of Breuillard Lake and Combal Lake allows gradual colonisation by endemic flora, thus increasing the biodiversity of the area (Fig. 3g, h, i) (e.g., Baretti 1880; Prinetti 2010). Concerning these features, some observations are already present on signage along naturalistic trails.

In addition to the glaciological and supporting botanical features, the geological features, that are relevant along the Veny Valley, contribute to *geodiversity*. In fact, the development of the valley along the Pennidic Front, one of the main structural lines of the Alps, lead to the outcrop of different lithologies on the two sides of the valley (Prinetti 2010). The Helvetic crystalline basement of the Mount Blanc Massif on the northern side and the sedimentary coverage of the UltraHelvetic and External Pennidic Domains on the southern side respond differently to gravity (prevailing rock falls on the northern side and debris flow and landslides on the southern side; e.g., Bollati et al. 2013) which generates different geological landscapes (*sensu* Gisotti 1993).

Moreover, the *rareness* attribute is represented not only by the tree coverage but also by a feature of Miage Lake, calving, which is a rarity at level of the Italian Alps (Diolaiuti et al. 2006; Pelfini et al. 2009). In addition, the complete drainage of the lake, which happened in 2004, permitted data collection that allowed for a detailed characterisation of the lake bottom and the hydrological paths (e.g., Deline et al. 2004; Diolaiuti et al. 2005; Masetti et al. 2010).

In general, all the described features contribute to the high *educational exemplarity, additional values* and *potential for use* attributes of the area. The quantitative evaluation of the trails and single sites (see details in Bollati et al. 2013) allowed the creation of a ranking system resulting in the trail 01 to Miage Lake being the most valued.

The Miage complex geomorphosite represents an ideal site to investigate how the vegetative component may transversally influence the composite attributes of *scientific value, additional values* and *potential for use* in geomorphosite value assessment.

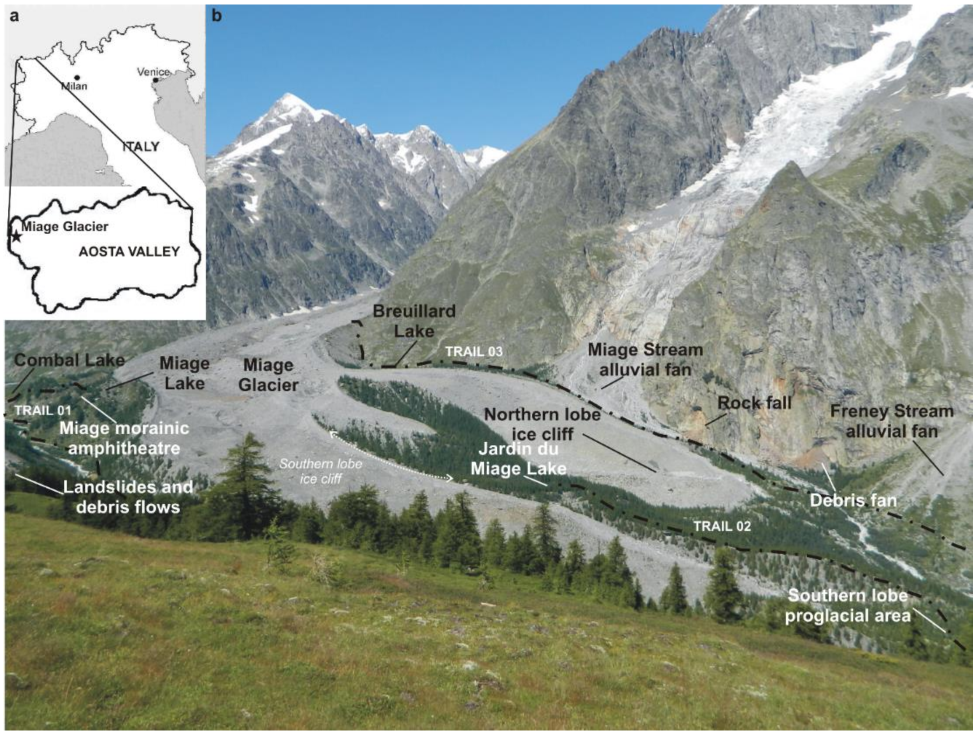


***Figure 2.*** *The Miage Glacier area and the thirteen evaluated sites. a) Geographic location of the Miage Glacier in Veny Valley (Valle d'Aosta); b) panoramic view of the Miage Glacier from La Visaille with the locations of the evaluated geomorphosites and trails; the location and partial extension of the Southern lobe ice cliff is also indicated. The Southern lobe ice cliff is italicised because it was not considered a valuable geomorphosite, because it cannot be reached from any tourist trail and is not completely visible from any tourist trail (Photo by D. Zannetti, 2012).*


***Figure 3.*** *Various significant features for the ESR in the Miage Glacier area. a) Supraglacial vegetation involved mainly in the back-wasting processes at the Northern lobe ice cliff (photo by D. Zannetti, 2012); b) Supraglacial vegetation on the Southern lobe, responding to the debris coverage dynamics (photo by D. Zannetti, 2012); c) Vegetation in the proglacial area of the Southern lobe involved mainly in the glacial stream activity (photo by D. Zannetti, 2011); d-e) Interannual water-level changes at Jardin du Miage Lake involving the drowned trees; the large boulder (white ellipse) allows a comparison between the two photos taken in July 2011 (d; photo by I. Bollati) and September 2011 (e; photo by L. Vezzola). The vegetation present inside the lake basin may be affected in terms of growth rates; f) The Miage Morainic Amphitheatre investigated through dendrochronological, pedological and carbon-14 analysis to determine the age of the different morainic ridges (photo by D. Zannetti, 2012); g-h-i) examples of flora present in the area of Jardin du Miage and Breuillard Lake and typical of the humid environment: Dactylorhiza maculata (L.), a protected species (g; photo by I. Bollati, 2011), Eriophorum scheuchzeri (h; photo by I. Bollati, 2011), Caltha palustris L. (i; photos by I. Bollati, 2011).*

## Methods

The first step of the analysis was the collection and characterisation of the scientific research regarding the Miage Glacier and the Veny Valley area. The collection was focused on the classification of descriptive and scientific papers and on their topic, which has evolved over time. The bibliographic research was performed utilising international databases available online: *Google Scholar* and *Web of Knowledge* (both were consulted in early 2013 and took into account publications through the end of 2012). Information reported by local magazines was not considered. In order not to overemphasise any paper, the maximum value assigned to any single paper was 1. If the paper covered more than one topic, for each topic the value was calculated as follows: $V = \frac{1}{n}$ where n represents the number of topics. Then, for each year, the total number of papers for each topic was calculated.

Hence, starting from the results on the evolution of the scientific knowledge regarding the study area, the scientific research concerning the interaction between arboreal vegetation and glacial processes was used to reconsider the dataset of values (i.e., *scientific*, *additional*, *global*, *potential for use*, *educational index* and *scientific index*), calculated by Bollati et al. (2013), of the Miage geomorphosites and trails. New data had been acquired during 2012 and two sites have been added to the trail 03 to Breuillard Lake (i.e., Debris fan and Rock fall).

All the sites were re-evaluated to highlight the numerical contribution of the arboreal vegetation factor to the other attributes. Six sites were not involved in this re-evaluation (Miage Lake, Miage Stream alluvial fan, Freney Stream alluvial fan, Landslides and debris flows, Debris fan and Rock fall), because either the *ESR* was not meaningful or no scientific data to confirm the *ESR* were available. The assessment was made through the already applied methodology, tested initially along a fluvial valley (Bollati et al. 2012) and then tested in the Miage Glacier area for selecting geo-itineraries in a glacial environment (Bollati et al. 2013). The list of attributes (*single* values), the specific class of the *ESR* and the formula used for calculating the *composite* values are reported in Table 2. In the presented research, the application is used to recalculate the global value of Miage sites by considering the different typology of information available, focusing on the meaningful presence of arboreal vegetation and its transverse influence on the other attributes.

***Table 2.*** *Criteria for the evaluation of geomorphosites and the equations for calculating the parameters of sites and trails according to Bollati et al. (2012) (modified from Bollati et al. 2012; 2013).*

| **A. ATTRIBUTES (SINGLE VALUES)** | | |
|---|---|---|
| **Scientific value (SV)** | **Potential for use (PU)** | |
| Model of geomorphological evolution (representativeness)-GM | Temporal accessibility TA | |
| | Services-Se | |
| Model of palaeogeomorphological evolution-PgM | Visibility-Vi | |
| Educational exemplarity-EE | Number of tourists-NT | |
| Spatial extension-SE | Sport activities-OA | |
| Geodiversity-Gd | Legal constraints-LC | |
| Geo-historical importance-GI | Use of geomorphological/geological interest UGI | |
| Ecologic support role-ESR | Use of the additional interest UAI | |
| Other geological interests-OI | Presence of geomorphosites in the surroundings SGs | |
| Integrity-In | | |
| Rareness-Ra | Spatial accessibility-SA | Calculated Accessibility-CA |
| | | *only for on-foot trails* |
| | | Typology-Ti |
| | | Trend |
| **Additional values (AV)** | | Steepness-St |
| Cultural-Cu | | Sloping-Sl |
| Aesthetic-Ae | | Width-Wi |
| Socio-economic-Ec | | Ground material-GM |
| | | Vegetation on the slope |
| | | Water/Snow along the path-WSP |
| | | Slope Material-SM |
| | | Slope Inclination-SI |
| | | Degree Of Conservation Of The Path-DC |
| | | Human Interventions-HI |
| | | Tourist Information-TI |

| **B. QUANTITATIVE CRITERIA FOR EVALUATION OF ECOLOGIC SUPPORT ROLE** | |
|---|---|
| 0 | Without any connection with the biologic element |
| 0,33 | Presence of interesting flora and fauna |
| 0,67 | The geomorphological features condition/favour the ecosystems |
| 1 | The geomorphological features determine the ecosystems |

**C. FORMULA FOR CALCULATING COMPOSITE VALUES**

| CALCULATED VALUES | EQUATION | Conditions | Ranges |
|---|---|---|---|
| SV | SV = (GM + PgM + EE + SE + Gd + GI + ESR+ OI + In + Ra) | | 0,5-10 |
| AV | AV = (C + Ae + SE) | | 0-3 |
| GV | GV = (SV + AV) | | 0,5-13 |
| Index of use | IU = EE + SE + Ae | | 0-3 |
| PU s.s. | PUss = (TA + Vi + Se + NT + SA + LC + UGI + UAI + SGs) | | 0,25-9 |
| PPU | PPU = (PUss + IU) | | 0,25-12 |
| CA | CA = (Ti + St + Sl + Wi + GM + WSP + SI + SM + DC+ HI + TI) | SAc ≤ 0,4 | 0-11 |
| A_Factor_c | AFc = ((CA/11)+(SAc/0.4))/2 | SAc ≥ 0,6 | 0,25-1 |
| A_Factor_s | AFs = (1+(SAc/1))/2 | | 0,8-1 |
| PU (on-foot trails) | PUc = PPU + AFc | | 0,5-13 |
| PU | PUs = PPU + AFs | | 1,05-13 |
| Scientific Index | SIn= (GM + PgM + GI + OI)/4 | | 0-1 |
| Educational Index | EIn= [EE + Ae + (A_Factor_c/s)]/3 | | 0,083-1 |

| **ITINERARY** | |
|---|---|
| (Σ SCIENTIFICs / n° sites)*MAX | 0-1 |
| (Σ ADDITIONALs / n° sites)*MAX | 0-1 |
| (Σ GLOBALs / n° sites)*MAX | 0-1 |
| (Σ POTENTIAL FOR USEs / n° sites)*MAX | 0-1 |
| (Σ SCIENTIFIC INDEX / n° sites)*MAX | 0-1 |
| (Σ EDUCATIONAL INDEX / n° sites)*MAX | 0-1 |

## Results

The first phase of the scientific publications analysis enhanced the considerable attention paid to the Miage Glacier and its surrounding area since the beginning of the frequentation of this glacial area in the 18th century. The analysis of the two databases brought to the collection a total of 100 scientific works, covering the period 1774-2012 and including the papers belonging to the 18th and 19th centuries, which contain more descriptive glaciological and naturalistic observations (i.e., naturalistic observations and trips, Fig. 4). The investigated subjects are various and the relative percentages are reported in Figure 4.

Figure 5 presents the distribution of the scientific works through time, separated according to the main subject. As evidenced by the data in Figure 5, glaciological and dendrochronological studies have been strongly increasing in recent years, as tools, to quantify the variations in the glacial environment in both space and time, have been developed. This increase may be considered a reflection of the rising interest in climate change.

Moreover, it is possible to see the advance of scientific research, especially in concurrence with the complete drainage event that happened at Miage Lake in 2004, which has allowed the collection of additional data on the hydrological paths and on the shape of the ice cliffs along which the lake develops, which are associated with the entire glacier dynamic (e.g., Diolaiuti et al. 2006).

The second part of the results involves the re-evaluation of thirteen sites, including two new sites not previously evaluated by Bollati et al. (2013). The removal of the *ESR* related to landforms and processes was based on data derived from scientific research developed during the last several years (Fig. 5). At this scope, among the collected papers, special attention was paid to those papers concerning the arboreal vegetation’s response to glacier dynamics and those papers combining these results with attributes of the Miage Glacier as a geomorphosite. In Table 3, the scientific papers on which the re-evaluation of the *ESR* and transversal features were based are summarised and divided according to the circumstances in which the *ESR* is evident as described in the previous paragraphs.

In Figure 6, the re-evaluation results are illustrated for both sites and trails and the variation produced by the *ESR* is evident. The increase in the *scientific value* over time is determined not only by the *ESR* but, according to Figure 1, by all the attributes transversally linked with it. The two sets of values correspond to: i) new composite values (dark grey) obtained not considering the benefit given by the increasing scientific knowledge on the *ESR;* ii) effective composite values (light grey) considering all the data available in the scientific literature regarding these topics. The value increase is evident for most of the parameters. This increase does not involve all the sites, as mentioned above, but all those sites in which the study of vegetation confirms and provides a greater comprehension of the glacial processes and dynamics of this DCG (i.e., Miage Glacier, Miage Morainic Amphitheatre, Jardin du Miage Lake, Southern lobe proglacial area, Northern lobe ice cliff) or where the landforms, processes and outcropping lithology determine the colonisation by the endemic flora, increasing the biodiversity of the area (i.e., Breuillard Lake, Combal Lake; Prinetti 2010).

The trails enjoy the benefits of the value increases as well (Fig. 6). Trail 01 is invariably the most valued one.

According to Bollati et al. (2013), if trail 02 reached the lowest position, the addition of two sites along trail 03 (i.e., Debris fan and Rock fall), not linked with the ecological component, generates an inversion in the ranking (light grey columns in Fig. 6). Alternatively, not considering the ESR (dark grey columns in Fig. 6), trail 02, along which all the sites are affected by the *ESR* variation, undergoes a greater value loss with respect to trail 03 (Table 4).

Percentage variations in the composite attributes for each site and trail are reported in Figure 7, including only the 7 sites in which variations of the *ESR* are confirmed by scientific data. Concerning these sites, *scientific values* variation also reaches values greater than 20% at the Northern lobe ice cliff (i.e., back-wasting processes involving vegetation), the Miage Glacier (i.e., the vegetation response to the dynamics of the debris coverage), the Southern lobe proglacial area and the Miage Morainic Amphitheatre. The variations of *scientific value* at the level of the site are never lower than 12% (Fig. 7).

The *additional values* are responsive to the variation only at Breuillard Lake (16.67%). This is because the *scenic value*, that may be responsive to variation in the *ESR*, is enhanced by the presence of endemic flora that confers a more pleasing aesthetic to the site. The other sites are valuable scenically regardless of the vegetation. Generally, Breuillard Lake is the site which results in more homogeneous response to the variation and which obtained a high average of change (12%) (Fig. 7).

At Breuillard Lake, concerning *potential for use*, the possibility of using the already existing tourist trails, based mainly on the floristic component of the landscape, is promoted by the scientific recognition of the *ESR*. At this site, *potential for use* obtained the maximum percentage of variation (12.32%), whereas this parameter, in general, varies less than the others (2-12%).

The indexes for scientific and educational selection of sites ranged between 7.50 – 24.75% and 11.43-17.68%, respectively (Fig. 7). The Miage Morainic Amphitheatre and Northern lobe ice cliff are particularly favoured by the use of vegetation as an investigative tool for dating glacial advances and retreats in the first case (i.e., scientific index variation: 24.75%) and evolution of back-wasting processes in the second case (i.e., scientific index variation: 16.50%). The biggest variation in the *educational index* was obtained, once again, at Breuillard Lake (17.68%) where *scientific index* did not vary.

Trails present more homogeneous trends, and their value variations are reduced (Fig. 7). The *scientific value* continues as the most favoured one, as seen by the increase in the knowledge of the *ESR*, and its variations result to be significant along trail in which for all the sites the role of glaciers regarding vegetation is fundamental (i.e., 15,85% maximum at trail 02). Trail 03 presents more homogeneous percentage variations.

The percentage variations in the average composite values, not including the sites in which the *ESR* plays no role (see earlier discussion), and the changes in standard deviation are reported in Figure 8. In general, the most variable composite attribute is the *scientific value* (16.43%) (Fig. 8a), as previously discussed. The index used for selection of sites for scientific purposes also increases evidently (Fig. 8a). The standard deviation, which indicates the dispersion of values, especially for the scientific attribute, increases and the sites are more distinct from each other according to this parameter (fig. 8b).

Finally, considering the ranking among all the sites, all the *ESR* affected sites rose in the ranking. However, the only site that maintained its high position is Miage Lake, demonstrating that superb sites such as Miage Lake, in which it is possible to observe active processes such as calving, are highly valued and independent of variation in any other value. Table 4 presents the comparison among the site and trail rankings.

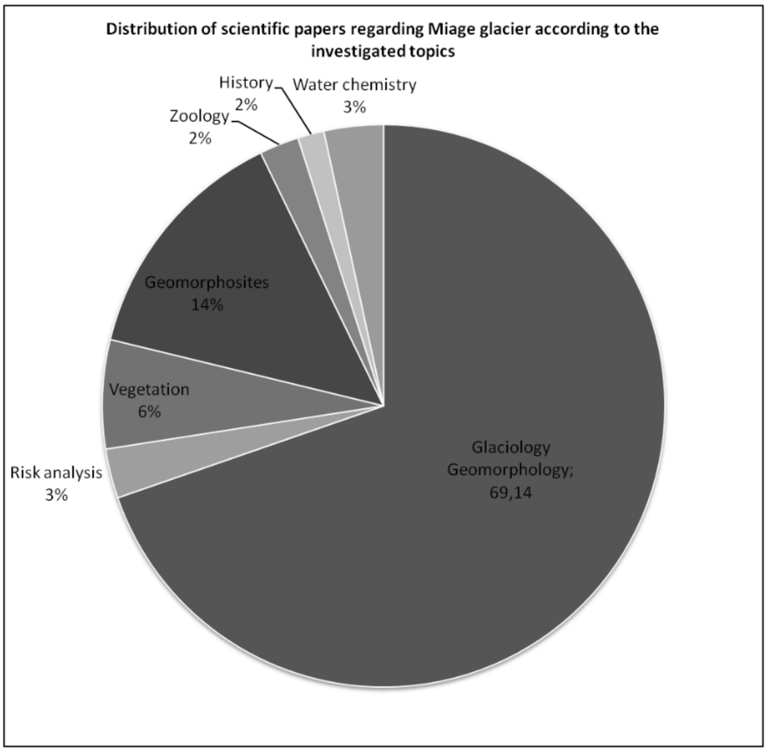


***Figure 4.*** *Percentage of scientific papers according to topic.*

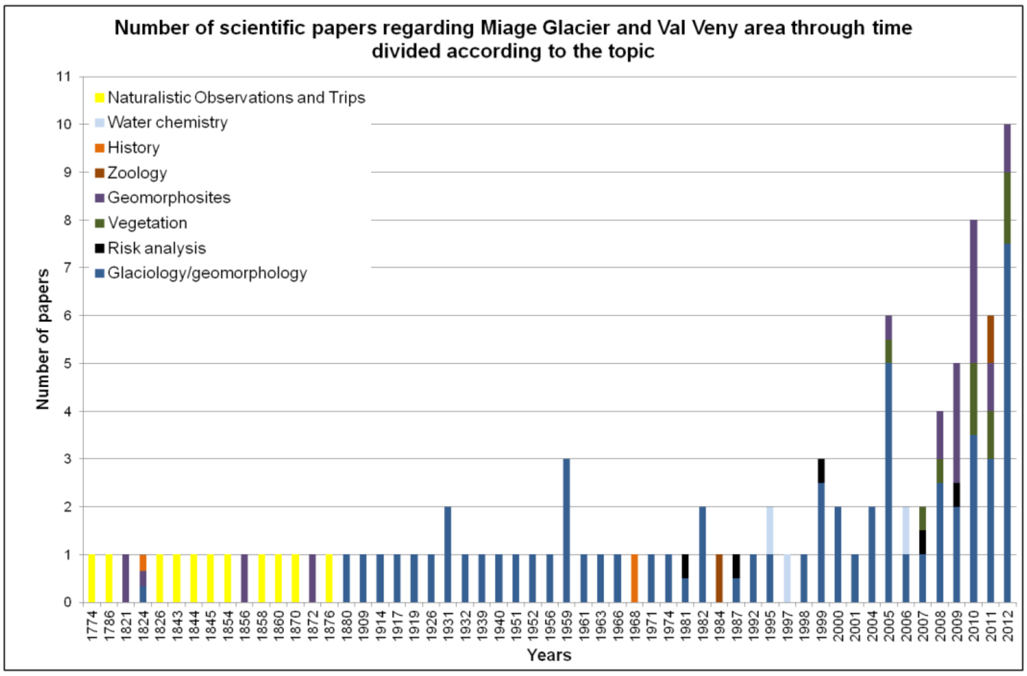


***Figure 5.*** *Scientific research on Miage Glacier. The distribution of scientific papers over time according to the scientific topic studied. The complete drainage of Miage Lake most likely gave a significant boost to scientific research in the area because of the possibility of collecting scientific data.*

***Table 3.*** *Summary of the scientific research concerning ecological interactions involving glacial landforms and vegetation in the Miage Glacier area.*

| SCIENTIFIC PRODUCTION DERIVING FROM INTEGRATION OF BIOLOGICAL AND ABIOLOGICAL DATA | | |
|---|---|---|
| FRAMEWORK | REFERENCES | HIGHLIGHTS |
| Supraglacial vegetation and glaciological data | Pelfini et al., 2007 | Identification and dating of different growth anomalies (e.g. pointer years, compression wood, abrupt growth changes) allowed the individuation of simultaneous presence of different disturbance indicators but not contemporarily on the two glacier lobes. The results fit with glaciological data documenting volume and surface-level variations in the same period. |
| | Leonelli & Pelfini, 2012 | The temporal analysis of abrupt growth changes (AGCs) confirmed a period of higher glacier surface instability, reaching a maximum in the years 1988 (on lobe S) and 1989 (on lobe N), probably related to the passage of a kinematic wave within the glacier tongue. AGCs >+70% and >+40% are suggested as a proxy for substrate instability in spatio-temporal reconstructions in the Alpine environment. |
| | Caccianiga et al., 2011 | Biodiversity on the debris coverage were quantified through observing species assemblages that are comparable with those of subalpine glacier forelands, but with the addition of high-altitude species. |
| Trees at the ice cliffs and glaciological data | Pelfini et al., 2012 | Analysis of tree age and tree distribution patterns on the glacier tongue, especially near at the ice cliffs of northern and southern lobes, suggested that a large number of trees die under conditions of dominating back-wasting on the northern lobe, instead, in the case of prevalence of down-wasting, as on the southern lobe, trees more easily survive and flow downvalley transported by the glacier flux. |
| Proglacial vegetation and glaciological data | Garavaglia et al., 2010b | Dating scars, compression wood and rings width variations allowed the individuation of areas directly affected by glacial discharge or by boulders falling from the glacier front. The concentration in specific years indicated an intensification of glacial activity influencing the forest vegetation. |
| Miage Morainic amphitheatre dating and geomorphological data | Deline, 1999 | Morainic geometry analysis with dating methods (dendrochronology, lichenometry, radio dating, soil analysis) allowed the individuation of a succession of glacier overflowing phases over the right lateral moraine and of heightening phases of the moraines (by superposition) during the Late Holocene, except for the older base of the morainic amphitheatre. The Litte Ice Age contribution was précised. |
| | Deline & Orombelli, 2005 | Integration of data presented by Deline (1999), with digging and coring in intermorainic depressions of the MMA and through a deep core drilling in a dammed-lake infill (Combal), allowed the proposal of The 'Neoglacial model'. It considers the MMA as formed during the whole Neoglacial by a succession of glacier advances and during the LIA, separated by raising phases of the right-lateral moraine by active dumping because of the Miage debris coverage. |
| Drowned vegetation at Lac du Jardin du Miage and glaciological data | Astrade et al. 2012 | Abrupt growth decrease was individuated into the tree rings of the drowned trees at Lac du Jardin du Miage as a response to water level changes. |
| **SCIENTIFIC PRODUCTION LINKING VEGETATION DATA WITH GEOMORPHOSITES CONCEPTS** | | |
| FRAMEWORK | REFERENCES | HIGHLIGHTS |
| Supraglacial vegetation and geomorphosite evaluation | Pelfini et al., 2005 | Dendrochronology analysis on supraglacial vegetation allowed the determination of link with debris coverage that increment the ecological value of Miage as geomorphosite. |
| Supraglacial and proglacial vegetation influencing geomorphosite evaluation | Garavaglia et al., 2010a | Investigating trees which colonize the glacial forefield of debris free glaciers and the debris coverage of DCG allowed to quantify the effects of climate change on tree colonization and to assess the creation of new geomorphosites increasing geodiversity in proglacial areas. |
| Supraglacial vegetation and dating of morainic ridges influencing geomorphosite evaluation | Pelfini et al., 2010 | The dendroglaciological analysis allowed the assessment of the importance of trees in analyzing the present glacier dynamics and, as a consequence, to contribute to the scientific evaluation of a geomorphosite in term of rarity, ecological and educational attributes. |

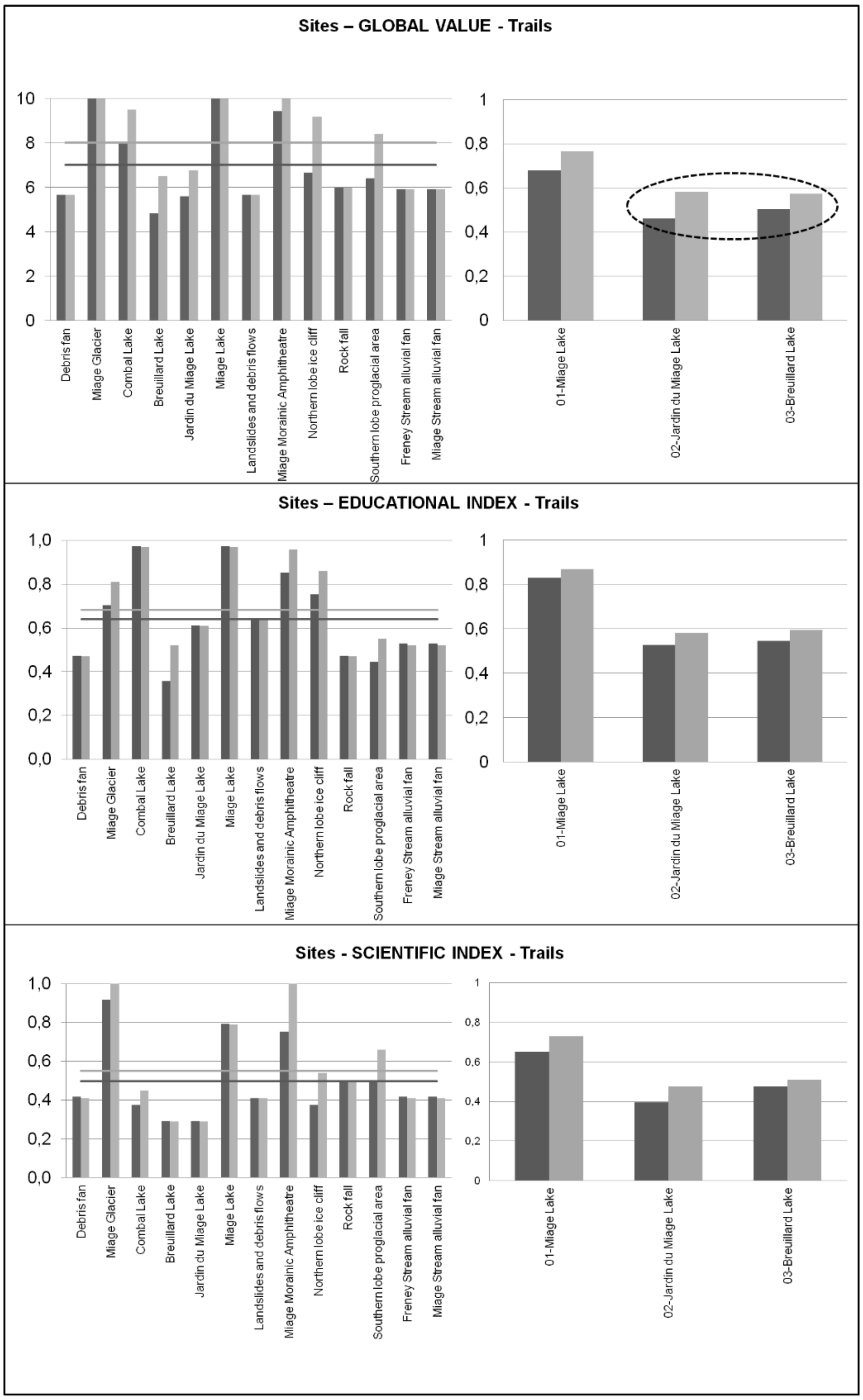


***Figure 6.*** *Variations in the scientific value, scientific index and educational index for sites and trails in the Miage Glacier area. Not all the sites have undergone changes (i.e., Miage Lake, Miage Stream alluvial fan, Freney Stream alluvial fan and Landslides and debris flows, Debris fan and Rock fall). The dark grey columns are the values calculated not considering the data regarding the ecological value of the sites. The light grey columns are the effective values calculated considering all the data coming from the scientific literature regarding the ESR. The horizontal lines in the sites graphs are the respective average values that show the same increment. In the dashed ellipse, the inversion of the final scientific value between trail 02 and trail 03 is highlighted.*

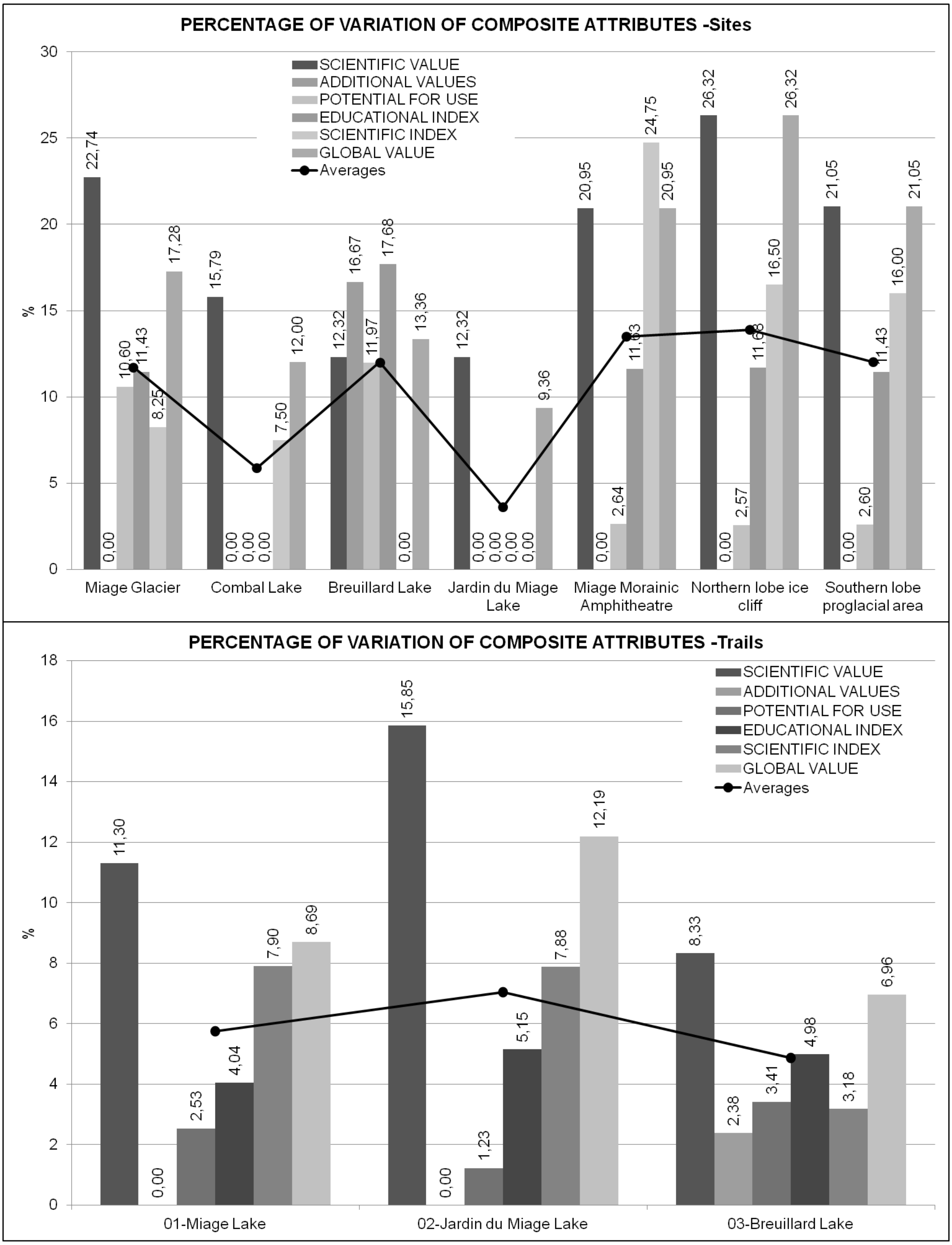


***Figure 7.*** *Percentage variations in the composite attributes in sites and trails including only the sites in which the ESR has a meaning.*

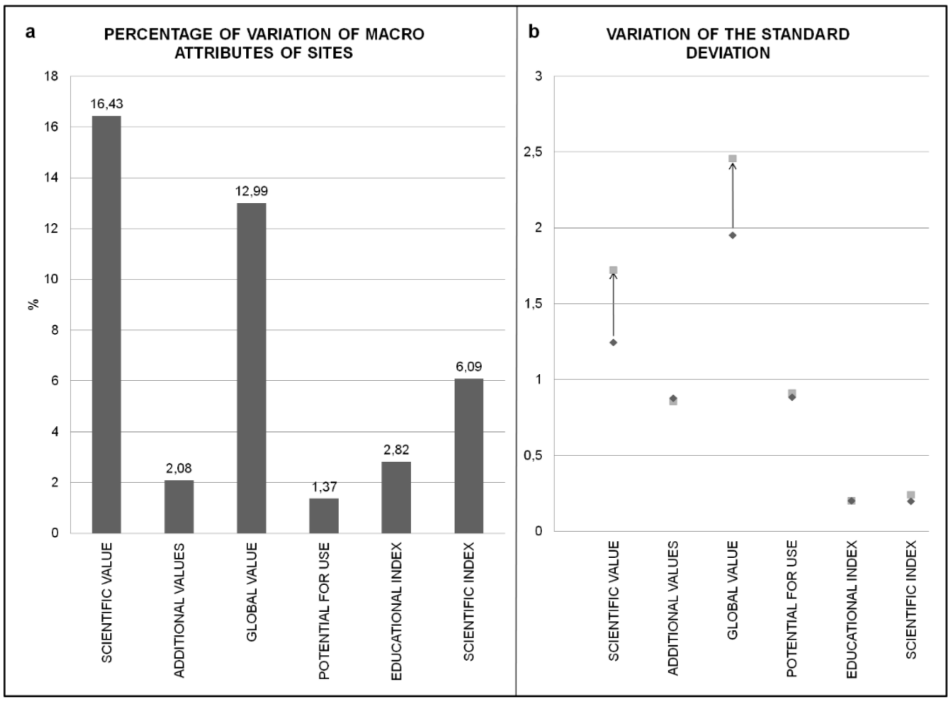


***Figure 8.*** *Variations in the composite attributes: a) Percentage of variation in the composite attributes calculated considering the average in the composite attributes of sites but not considering those in which the ESR has no influence. b) Variation of the standard deviation in the composite attributes of all the sites derived from re-evaluation. The dark grey columns are the values calculated not considering the data on the ecological value of the sites. The light grey columns are the effective values calculated considering all the data coming from the scientific literature regarding the ESR.*

***Table 4.*** *Variation of the ranking of sites according to variation of the ESR data.*

| *Sites rank – Global value* | *Not considering data on ESR* | *Considering data on ESR* |
|---|---|---|
| 01 | Miage Glacier | Miage Glacier |
| 02 | Miage Lake | Miage Morainic Amphitheatre |
| 03 | Miage Morainic Amphitheatre | Miage Lake |
| 04 | Combal Lake | Combal Lake |
| 05 | Northern lobe ice cliff | Northern lobe ice cliff |
| 06 | Southern lobe proglacial area | Southern lobe proglacial area |
| 07 | Rock fall | Jardin du Miage Lake |
| 08 | Freney Stream alluvial fan | Breuillard Lake |
| 09 | Miage Stream alluvial fan | Rock fall |
| 10 | Debris fan | Freney Stream alluvial fan |
| 11 | Landslides and debris flows | Miage Stream alluvial fan |
| 12 | Jardin du Miage Lake | Debris fan |
| 13 | Breuillard Lake | Landslides and debris flows |
| *Trails rank – Global value* | *Not considering data on ESR* | *Considering data on ESR* |
| 01 | 01-Miage Lake | 01-Miage Lake |
| 02 | 03- Breuillard Lake | 02- Jardin du Miage Lake |
| 03 | 02- Jardin du Miage Lake | 03- Breuillard Lake |

**Discussion and Conclusions**

The recognition of glaciers as sites of geomorphological interest is not recent (e.g., Baretti 1880). However, the determination and quantitative description of geomorphosite values for glaciers is a rather new research topic (Pelfini and Smiraglia 2003; Bollati et al. 2013). In particular, great attention has been recently paid to the role of *ESR* in certain settings, such as those of DCGs (Pelfini et al. 2005; Pelfini et al. 2010a; Garavaglia et al. 2010; Gobbi et al. 2011) such as the Miage Glacier, the most important DCG in the Italian Alps, proposed as geomorphosite by Pelfini et al. (2005). The collection of approximately 100 scientific papers, produced over a period of 250 years of scientific research, concerning the Miage Glacier apparatus, initially permitted the understanding of the evolution of scientific interest in this complex geomorphosite. The increasing number of scientific studies on the Miage Glacier, especially during the 21$^{st}$ century, is most likely related to the interest in DCGs as witnesses of climate change (Deline 2009). Additionally, the analysis of the Miage Glacier as a geomorphosite, beyond the first recognition in books of the 18$^{th}$-19$^{th}$ centuries, received a significant boost that coincided with the growth of scientific interest in geoheritage. Among the papers collected, those concerning the use of vegetation as tool for detailing current and past glacial dynamics in different situations (i.e., supraglacial debris coverage, proglacial area, ice cliffs on the lobes, morainic amphitheatre, and glacial lakes with drowned vegetation) have been considered to quantify the contribution of the *ESR* to the composite values (i.e., *scientific*, *additional values* and *potential for use*) of sites and trails (*sensu* Bollati et al. 2012). With the additional data derived from scientific research on vegetation growing within the glacial environment of Miage Glacier, the increase in the *ESR* is evident. Furthermore, the *ESR* influences the other attributes that are closely connected with it in a cascade effect (as shown in Fig. 1): *scientific value* (i.e., *model of geomorphological evolution*, *model of paleogeomorphological evolution*, *educational exemplarity*, *geohistorical importance,* and *rareness*), *additional values* (i.e., *scenic value*) and *potential for use* (i.e., *use of other interest*s). The composite attributes are responsive in different measure to this increase and surely the most involved attribute is the *scientific value*.

In some cases, such as that of Breuillard Lake, the *ESR* exclusively influences the attributes linked with educational purposes, while the attributes strictly used for the calculation of the *scientific index* remain unvaried. In other situations, the opposite occurs (e.g., the Miage Morainic Amphitheatre and Northern lobe ice cliff), and the benefit is obtained when geomorphosites are selected for trails with scientific purposes.

In addition, what emerges is the increase in standard deviation among the single values of sites, once again due to scientific factors. As a consequence, if we do not consider the ecological attribute as a criterion to evaluate sites, there will be less differentiation among the sites and trails which may impede the process of selection among sites for valorisation purposes.

Moreover, the relative importance among trails may change if we exclude the *ESR* attribute. For example, trail 02 is more connected with the biological component and largely benefits from the growth in available scientific data supporting the *ESR* of its sites, allowing it to overtake trail 03 in significance. Hence, the importance of the *ESR* attribute is evident in the selection of sites and trails during the geomorphosite evaluation procedure, especially for the selection of trails for scientific purposes.

In conclusion, the ecological component of the landscape, in relationship with landforms and geomorphic processes, may represent a discriminating factor in geomorphosite value assessment. The results of this study demonstrate that this observation is true, especially in the case of active geomorphosites but also in the case of passive geomorphosites that are currently affected by active processes.

The continuing growth in scientific interest towards this area is expected to result in new data in the future, which will support the necessary periodic revision of geomorphosite assessments.

Moreover, vegetation dynamics, that are related to glaciers activity, represent a consequence of climate change to be enhanced within cultural and educational itineraries, as well as the glacier behaviour itself, since they are poorly known by common people (Garavaglia et al 2012).

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# 5.

# DETECTING ENVIRONMENTAL STRESSES

## 5.1.

# Tree-ring stable isotopes, growth disturbances and needles volatile organic compounds as environmental stress indicators at the debris covered Miage glacier

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## Abstract

First results of an innovative multi-proxy approach applied to glacier-related trees for assessing climatic and substrate influence on tree rings and needle VOCs are reported. Tree-ring stable isotopes, tree-ring growth patterns and needle volatile organic compounds where analysed at two *Larix decidua* Mill. sites in five trees of similar size growing in close areas mainly differentiated by the contrasting geomorphological features: the debris-covered Miage Glacier ('Glacier') and a lateral moraine ('Control'). Over the period 2003-2012, tree rings at the Glacier site showed more enriched $^{13}C$ mean values ($p<0.05$) in the cellulose with respect to the Control site likely due to a lower stomatal conductance induced by low soil water retention, high temperature excursions and high exposure to direct solar radiation. Also $^{18}O$ mean values where higher ($p<0.01$) at the Glacier site, likely due to the assimilation of shallow waters from a superficial root system of supraglacial trees, in contrast to a more developed and stabilized soil at the Control site. The analysis of tree-ring growth patterns of the sampled specimens provided a temporal insight of climatic and geomorphological stress at the Glacier site: here we found higher rates of positive abrupt growth changes (AGCs), but no differences in percent of latewood. Needles volatile organic compounds (VOCs) showed significant differences in some compounds of mono- di- and sesquiterpenes. Those with higher concentrations (b-myrcene and estragole) showed also the largest differences, with higher concentrations at the Glacier site. Tree rings stable isotopes and AGCs, as well as needles VOCs in supraglacial trees may be used as environmental stress indicators in the mid- to short-term, respectively, providing valuable proxies for the assessment of geomorphological and climatic change impacts in the glacial environments of the Alps.

**KEYWORDS**: Tree-ring stable isotopes, Needle VOCs, Debris covered Miage Glacier.

## Introduction

The study of the responses of the Alpine environment to climate change is a critical issue especially in the newly formed habitats of the debris-covered glaciers where a new research frontier is represented by the analysis of supraglacial life forms. The increasing rock weathering on valley slopes and the growing ablation rates favour the debris concentration on the lower portions of glacier tongues (Mihalcea & *alii*, 2008) inducing the progressively transformation from debris-free to debris-covered glaciers. When the debris layer become thicker than the critical value (Mattson and Gardner, 1989), ablation rate is reduced and the glacier shrinkage too. The debris coverage of glaciers may offer new habitats also for yeasts and fungi (Branda & *alii*, 2010; Turchetti & *alii*, 2008), vegetation (Caccianiga & *alii*, 2011) and animals (Gobbi & *alii*, 2011) locally increasing biodiversity (Cannone & *alii*, 2008). When the glacier front is located below the treeline, if the debris mantle is thick enough and if the surface glacier velocity is low, then the supraglacial debris can be colonized by grass and shrubs and also by trees.

The European Alps are a climate sensitive region and are a crucial place for studying the responses of both physical and biological components especially in the fast changing glacial environments. Future scenarios of climate change in the European Alps describe an increase of temperature means and extremes (Beniston & *alii*, 2007) and a general decrease of total precipitations but an increase of summer precipitation events (Brunetti & *alii*, 2004; Christensen and Christensen, 2004) for the next decades. Under these projections, the research of climate change impacts at different spatial scale in the Alpine environment is an important issue for managing the resources of these territories and for understanding how climate-related glaciological and geomorphological processes will change in frequency and intensity and how they will interact with life forms in the next future.

The spatial definition of the climate change impacts in physical and biological components of the Alpine environment is useful for better characterizing the heterogeneous and sometimes contrasting responses that may be induced by the same climatic input (e.g. Jolly & *alii*, 2005). For example, in the year 2003 the summer heat wave that established over Europe and the Alps for about two months induced a marked reduction in glacier mass balances that lasted also in the next years (Braithwaite & *alii*, 2013), and forest productivity decreased at low altitude but not at high altitudes where tree growth was, instead, enhanced (Leonelli and Pelfini, 2008).

The understanding of climatic trends and future impacts of climate change is well supported by the availability of a wealth of meteorological data that on the European Alps last for more than three centuries (e.g. Auer & *alii*, 2007). However, the definition of past natural variability of climate at the century to the millennial scale from remote sites also on the European Alps is usually supported by information derived from climatic proxies like, e.g., tree rings, pollen and lake varves. Tree rings in particular may provide the highest temporal resolution information of past climate at the annual and seasonal scales at least over the period covering the last thousands of years (Fritts, 1976). Several studies have been conducted for reconstructing past climate variability and trends on the Alps, especially for what concerns summer temperatures (e.g. Büntgen & *alii*, 2006; 2011; Coppola & *alii*, 2013). The analysis of tree rings in geomorphological studies has allowed the reconstruction of the frequencies and distribution of climate related past events, like, e.g., debris flows, flood and avalanches (e.g. Strunk, 1997; Pelfini and Santilli, 2008). Moreover, the analysis of growth disturbances in the tree rings could contribute to the definition of the spatial distribution of active processes over time (e.g. Stoffel and Bollschweiler, 2009). Extreme environments for tree growth, like the debris-covered glaciers, and the substrate instability caused by ice flow, differential ablation and glacio-karst phenomena, are responsible of several growth disturbances in supraglacial trees (Richter & *alii*, 2004; Pelfini & *alii*, 2007). After their germination, trees move downvalley according to the surface glacier velocity, yearly recording in the tree rings characteristics (compression wood, stem eccentricity, growth anomalies) the responses to substrate movements (Leonelli and Pelfini, 2013).

Stable isotope techniques can be very useful in environmental reconstructions as the stable carbon and oxygen isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) of tree rings can provide long-term records of plant physiological processes. In $C_3$ plants, $\delta^{13}C$ is a good proxy of leaf-level intrinsic water use efficiency (WUEi), which is given by the ratio between leaf net photosynthetic rate (A) and stomatal conductance (Dawson & *alii* 2002, Farquhar & *alii*, 1989). Plant $\delta^{18}O$ is influenced by source water $\delta^{18}O$, but it is also inversely related to the ratio of atmospheric to leaf intercellular water vapour pressure ($e_a/e_i$), and can thus provide a time-integrated indication of leaf stomatal conductance ($g_s$) during the growing season (Barbour 2007, Farquhar & *alii*, 2007). Measuring plant $\delta^{18}O$ can thus help to separate the independent effects of A and $g_s$ on $\delta^{13}C$ (Scheidegger & *alii* 2000, Moreno-Gutiérrez & *alii* 2012, Roden & Farquhar 2012, Battipaglia et al 2013; 2014). In the glacial environment of the debris-covered Miage Glacier, Leonelli & *alii* (2014) have indeed found that by means of a stable isotope approach it is possible to disentangle precipitation and glacier meltwater-fed trees, thus allowing the possibility to reconstruct past major glacier runoff events. In harsh environment, like the one analysed, trees are expected to respond to the external stresses also by modifying the production of volatile organic compounds (VOCs). Volatile organic compounds emitted by plants, in fact, play a central role in the plant-environment interactions by affecting key life processes such as reproduction, defense and communication (Paré and Tumlinson, 1999; Guerrieri and Digilio, 2008). They are produced in normal metabolic processes as well as in response to biotic and abiotic stresses (Mello and Silva-Filho, 2002; Giorgi & *alii*, 2012a). Plants growing at high altitude as well as in harsh environmental conditions exhibit several ecological, morphological, physiological and phytochemical adaptations. Therefore, in recent years, there has been an increasing interest in the study of VOCs and their implication in many ecophysiological plant processes. Volatile organic compounds (VOCs) emission rates in trees are related to temperature (Räisänen & *alii*, 2009), light (Staudt and Seufert, 1995) and humidity (Janson 1993). Chemically, VOCs emitted by plants belong to several groups of compounds such as terpenoid (isoprene, monoterpenes, diterpenes and sesquiterpenes), acids, alkanes, alkenes, alcohols, esters, ethers, carbonyls, aldehydes and ketones (Maffei, 2010). Isoprene and monoterpenes are the dominant groups in the atmosphere (Kesselmeier and Staudt, 1999): their concentration in the air affects the tropospheric chemistry, the production of air pollutants, aerosols and greenhouse gases (Kesselmeier and Staudt, 1999). Researchers support the idea that climate change may affect the secondary chemicals composition of some plants (Gairola & *alii*, 2010), but the effects of the predominant global change factors (elevated $CO_2$ concentration, $O_3$, UV radiation, temperature) on plants secondary chemistry seems to be plant species-specific (Bidart-Bouzat and Imeh-Nathaniel, 2008). However, the ecosystem's properties, the geographical location and climate have an impact at least on some of the secondary chemicals emissions (Wallis & *alii*, 2011).

The objective of the present research was to identify innovative proxies for the characterization of climate-change impacts on the supraglacial trees of the Miage Glacier. Our hypothesis is that supraglacial trees, growing in the particularly extreme supraglacial environment should hold stress signals with respect to trees growing on stabilized surfaces. In particular, in this paper we investigated in detail tree-ring stable isotopes signals and tree-ring growth patterns, as well as VOCs profile in the needles of supraglacial trees and of trees growing in a control site, in order to analyze and compare their values and assess their role as indicators of the stresses induced by the complex of extreme climate and of supraglacial debris movements.

**Study area**

The Miage Glacier is the third largest Italian glacier. It drains the SW slope of the Mont Blanc Massif in Val Veny, Valle d'Aosta and it is considered the most representative debris-covered glacier in the Italian Alps. The Miage glacier has a surface area of about 11 $km^2$, a length of about 6 km, and shows an ablation tongue characterized by two main lobes plus a small intermediary one (Pelfini & *alii*, 2012). The tongue is covered by supraglacial debris from the altitude of about 2400 m a.s.l. where a medial moraine is present, up to the tongue lower portion and to the glacier front (about 1760 m) where the debris coverage is continuous. The supraglacial morphology is strictly related to differential ablation processes acting in the lower portion of the glacier and to glacier movements, and shows the presence of niches, depression zones and channels. The debris is characterized by different crystalline rock sizes, from boulders to fine pebbles and sand (Deline, 2005), and its thicknesses ranges from few centimetres in the upper tongue sector up to 1.5 m at the glacier terminus (Mihalcea & *alii*, 2008).

Both the glacier tongue lobes are colonized by herbaceous vegetation, shrubs (*Salix* spp.) and trees (mainly European larch, *Larix* decidua Mill., and Norway spruce, *Picea abies* Karst.). Tree density and distribution is different on the two lobes: the north lobe locally shows higher densities of larches along its inner margin with respect to the south lobe, where tree density is lower but where the oldest and tallest trees can be found. The substrate grain size is likely a key factor for plant germination as demonstrated by a higher density of trees smaller than 30 cm height on fine debris areas. Locally (southern side of the north lobe) the high number of very young trees has been interpreted as an increase of colonization rate in recent times (Pelfini & *alii*, 2012).

Tree life, and consequently trees potentiality in recording glacial and climatic information, can be limited by the glacier flow, by the supraglacial morphology evolution and by the ice cliffs retreats due to backwasting processes (ice cliff retreat, due to increased ablation, affects trees causing their death when the cliff edge reaches trees; Pelfini & *alii*, 2012).

Pelfini & *alii* (2007) reconstructed the passage of a kinematic wave by analyzing tree-ring growth disturbances: the glacier growing phase culminated in 1988 (Leonelli and Pelfini, 2013) as also documented by aerial photos (Giardino and others, 2001) and glaciological investigation (Smiraglia & *alii*, 2000). Moreover, Leonelli and Pelfini (2013) by analysing tree-ring Abrupt Growth Changes (AGCs) in supraglacial trees over the 20-year period 1987-2006, found that that the central-lower portion of the south lobe towards the margins was the most unstable demonstrating the possibility of deriving information on the glacier tongue dynamics from the tree rings and the usefulness of growth anomalies as a proxy for glacier surface instability in spatio-temporal reconstructions.

**Methods**

Five supraglacial European larch (*L. decidua*) trees of similar height (about 2 m) on a Glacier site (45º 47’04.08’’ N; 6º 53’ 35.94’’ E) and five larches of the same height in a Control site (45º 47’ 10.13’’ N; 6º 53’ 28.24’’ E) at the same altitude (about 1810 m a.s.l.) on a moraine at about 250 m NW from the first site were selected (fig. 1A). The two sites were both NE-facing and were primarily differentiated by the contrasting geomorphological features and by the forest cover characteristics: the debris-covered Miage Glacier’s south lobe where sparse supraglacial trees growth in rocky substrates (fig. 1B) and the forested moraine between the south and north glacier lobe where old Norway spruce and European larch trees dominate the canopy (fig. 1C). The Glacier site is settled in a glacier area characterized by high surface instability, whereas the Control site is on a developed soil forest. In September 2012 CE, each tree was cored at about 30 cm from the ground by means of a Pressler's increment borer, extracting a passing core from the stems. Moreover, from top branches, about 70 needles per tree were taken and put in close vials and preserved in a frozen environment.

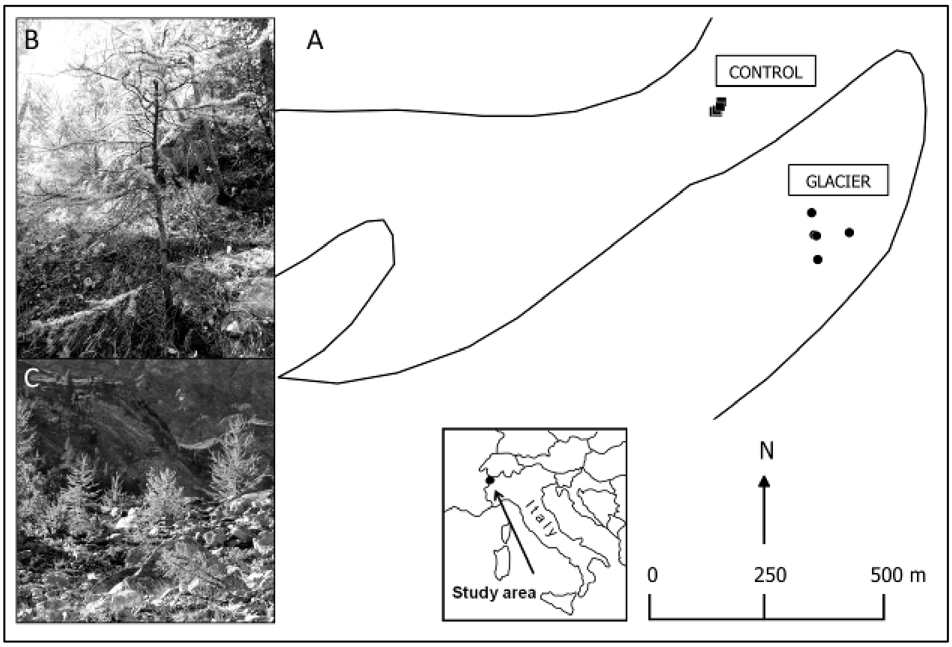


***Figure 1.*** *Sketch map of the study area (A). The points correspond to sampled trees at the Control site on the moraine (photo in B), the squares correspond to the sampled trees at the Glacier site (C).*

<u>Tree-ring methods</u>

Tree rings were analysed for stable isotopes and for characterizing growth patterns of the sampled trees. The passing cores were firstly prepared for ring-width measurements by cutting with a razor blade a transversal surface. Tree rings were then measured with a LINTAB system (Rinntech) at the nearest 0.01 mm, obtaining the total ring width and (where possible) the early/latewood measurements. For the presence of compression wood in Glacier site samples, some of them were measured only for the total ring width. The tree-ring growth series were then visually (TSAPwin software, version 0.53; Rinn, 2005) and statistically (COFECHA; Holmes, 1983; Grissino-Mayer, 2001) cross-dated within trees and between trees (of the same site) for avoiding dating errors in the dataset. For highlighting high-frequency tree-ring growth responses in the rather short time series, a residual chronology for each site was then prepared by applying a flexible spline with a 50%

frequency cut-off at 30 yr to the growth series and then applying a biweight robust mean to the detrended indices, derived from autoregressive modelling (Cook and Briffa 1990).

For the stable isotope analysis, dated tree-rings of the period 2003-2012 were splitted by means of a razor blade and pooled together in small cups, separating them by year and by site. The collected wood samples were then milled in an ultracentrifugal mill (MF 10 basic IKAWERKE) and the resulting powder was put in porous Teflon pockets and the cellulose was extracted following the method of Loader & *alii* (1997). The pockets where processed in different solutions for removing resins, tannins, fats and hemicelluloses (2 hours at 60$^o$C in a solution of 5% NaOH), for removing the lignin (3 baths of 8 hours at 60$^o$C in acetic acid solution containing 7% $NaClO_2$; Battipaglia & *alii*, 2008). The stable carbon and oxygen isotope ratios were measured at the CIRCE laboratory (Center for Isotopic Research on the Cultural and Environmental heritage, Caserta, Italy) by continuous-flow isotope ratio mass spectrometry (Finnigan Mat, Delta S, Bremen, Germany) using 0.03-0.05 mg of dry matter for $^{13}$C measurements and 0.1-0.2 mg for $^{18}$O determinations. We report isotope values in the delta notation for carbon and oxygen, where $\delta^{13}C$ or $\delta^{18}O$ = (Rsample / Rstandard –1) (‰), relative to the international standard, which is VPDB (Vienna Pee Dee Belemnite) for carbon and VSMOW (Vienna Standard Mean Ocean Water) for oxygen. Rsample and Rstandard are the molar fractions of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ for the sample and the standard, respectively. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was better than 0.1‰ for carbon and 0.2‰ for oxygen. The calibration vs VPDB was done by measurement of International Atomic Energy Agency (IAEA) USGS-24 (graphite) and IAEA-CH7 (polyethylene) and vs. VSMOW by measurement of IAEA-CH3 (cellulose) and IAEA-CH6 (sucrose). For the tree-ring $\delta^{13}C$ series, a correction for the decrease in $\delta^{13}C$ of the atmospheric $CO_2$ was applied (Francey & *alii* 1999). The stable isotopes series of the same species where analysed for the differences in mean values at the two sites by means of the Student's t- test.

For each growth series at both sites abrupt growth changes (AGCs) were assessed by calculating the percentage of growth variation in the intervals ±40% with respect to the mean width of the four previous years (Schweingruber & *alii* 1991), as this tree-ring parameter is known to be a good proxy for substrate instability in the Alpine environment (Leonelli and Pelfini, 2013). This analysis was performed only on the subperiod 1991-2012 where at least 4 ring-width series per site were present. Moreover, for the available samples, the percentage of latewood with respect to the total ring was obtained at both sites.

VOCs methods for Larix decidua Mill. needles

All samples were prepared by weighing exactly 3.00 g of *L. decidua* needles (obtained from a representative pool of fresh needles for each tree) in a 20 ml glass vial, fitted with a cap equipped with silicon/PTFE septa (Supelco, Bellefonte, PA, USA), and by adding 1 ml of the internal standard solution (IS) in water (1,4-cineol, 1 µg/ml, CAS 470-67-7) to check the quality of the fibres. At the end of the sample equilibration period (1 h), a conditioned (1.5 h at 280 °C) 50/30 µm Divinylbenzene/Carboxen$^{tm}$/polydimethylsiloxane (CAR/PDMS/DVB) StableFlex$^{tm}$ fiber (Supelco; Bellefonte, PA) was exposed to the headspace of the sample for the extraction (3 h) by CombiPAL system injector autosampler (CTC analytics, Switzerland). 30 ºC was selected as extraction temperature in order to prevent possible matrix alterations (oxidation of some compounds, particularly aldehydes). To keep a constant temperature during analysis the vials were maintained on a heater plate (CTC Analytics, Switzerland).

Headspace solid-phase microextraction (HS-SPME) analysis was performed using a Trace GC Ultra Gas Chromatograph (Thermo-Fisher Scientific; Waltham, MA, USA) coupled to a quadrupole Mass Spectrometer Trace DSQ (Thermo-Fisher Scientific; Waltham, MA, USA) and equipped with an Rtx-Wax column (30 m; 0.25 mm i.d.; 0.25 μm film thickness, Restek, USA). The oven temperature program was: from 35 °C, hold 8 min, to 60 °C at 4 °C/min, then from 60 °C to 160 °C at 6 °C/min and finally from 160 °C to 200 °C at 20 °C /min. Carry over and peaks originating from the fibre were regularly assessed by running blank samples. After each analysis fibres were immediately thermally desorbed in the GC injector for 5 min at 250 °C to prevent contamination. The injections were performed in splitless mode (5 min). The carrier gas was helium at the constant flow of 1 ml $^{-1}$. An *n*-Alkanes mixture ($C_8$-$C_{22}$, Sigma R 8769, Saint Louis, MO, USA) was run under the same chromatographic conditions as the samples to calculate the Kovats Retention Indices (KI) of the detected compounds. The transfer line to the mass spectrometer was maintained at 230 °C, and the ion source temperature was set at 250 °C. The mass spectra were obtained by using a mass selective detector with the electronic impact at 70 eV, a multiplier voltage of 1456 V, and by collecting the data at rate of 1 scan $s^{-1}$ over the *m/z* range of 30-350. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions when available, or by comparing the Kovats retention indices with the literature data. The identification of MS fragmentation patterns was performed either by comparison with those of pure compounds or using the National Institute of Standards and Technology (NIST) MS spectral database.

Volatile compounds measurements from each headspace of the plant extracts were carried out by peak area normalization (expressed in percentage). All analyses were done in duplicate. Data are expressed as mean value and standard deviation.

Analysis of variance (ANOVA) was performed to evaluate differences between VOCs fingerprint, of *L. decidua* samples from the two sampling site. $p < 0.05$ was considered to be significant (SPSS Statistics, 17.0 Inc. Chicago, IL).

**Results**

The tree-ring width mean chronologies obtained at the two sites cover the period 1953-2012 CE (Control site) and 1986-2012 CE (Glacier site); half of the samples are present only since 1989 at the glacier site (which present therefore a median age of 24 years) and since 1986 at the moraine site (median age of 27 years): the median age difference between sites is therefore of 3 years (fig. 2A). At the Glacier site trees showed always higher growth rates than the Control site and some differences in growth patterns between sites are evident (fig. 2A). In particular, trees at the Glacier site, beside containing compression wood in some years (not shown), showed two relative peaks of maximum growth in 1989 and 2007-2009. Moreover, they presented a marked positive growth trend started after 1989. Trees at the Control site showed more homogeneous growth patterns and they presented a less steep positive growth trend.

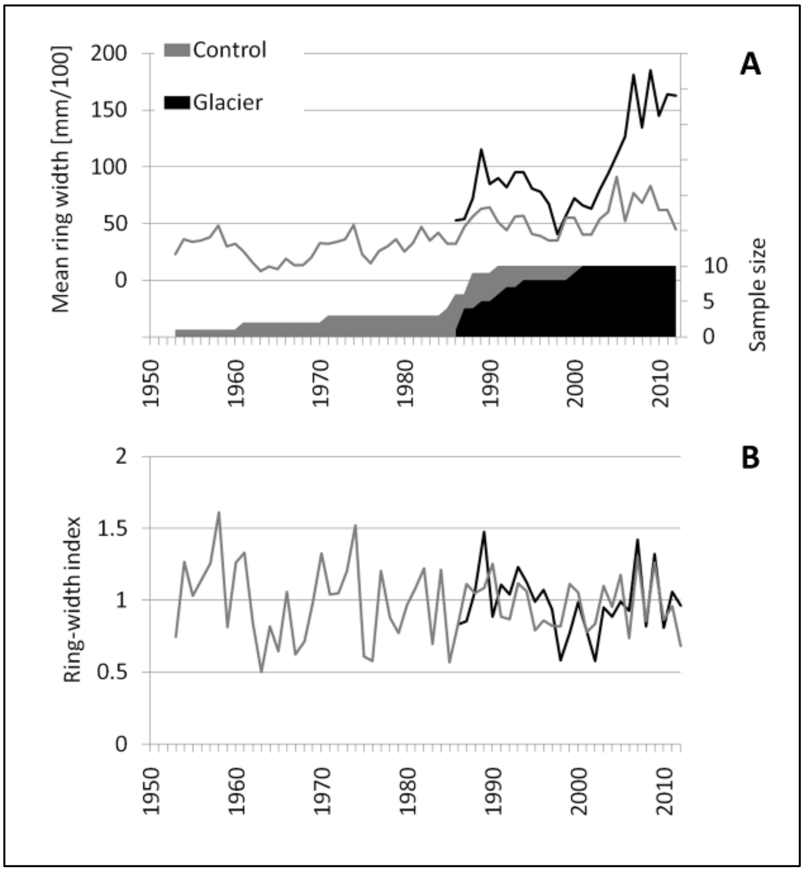


***Figure 2.*** *A) Ring-width mean chronologies from the Control and Glacier sites; sample size is also reported. B) The two residual chronologies derived from tree-ring measurements.*

<u>Tree-ring stable isotopes</u>

The tree-ring stable isotopes showed a lower synchronicity between series in $\delta^{13}C$ than in $\delta^{18}O$ (fig. 3A and 3B). Over the period 2003-2012 at the Glacier site the $\delta^{13}C$ showed nearly always higher values (average: -20.81‰ ±0.66) than the Control site (with the exception only of the year 2010). The difference in mean values (0.91‰) between sites is statistically significant ($p<0.05$). For what concerns the $\delta^{18}O$, the tree-ring cellulose at Glacier site showed always higher values (average: 29.23‰ ±1.77) than at the Control site. The mean difference between sites (1.95‰) is highly significant ($p<0.01$).

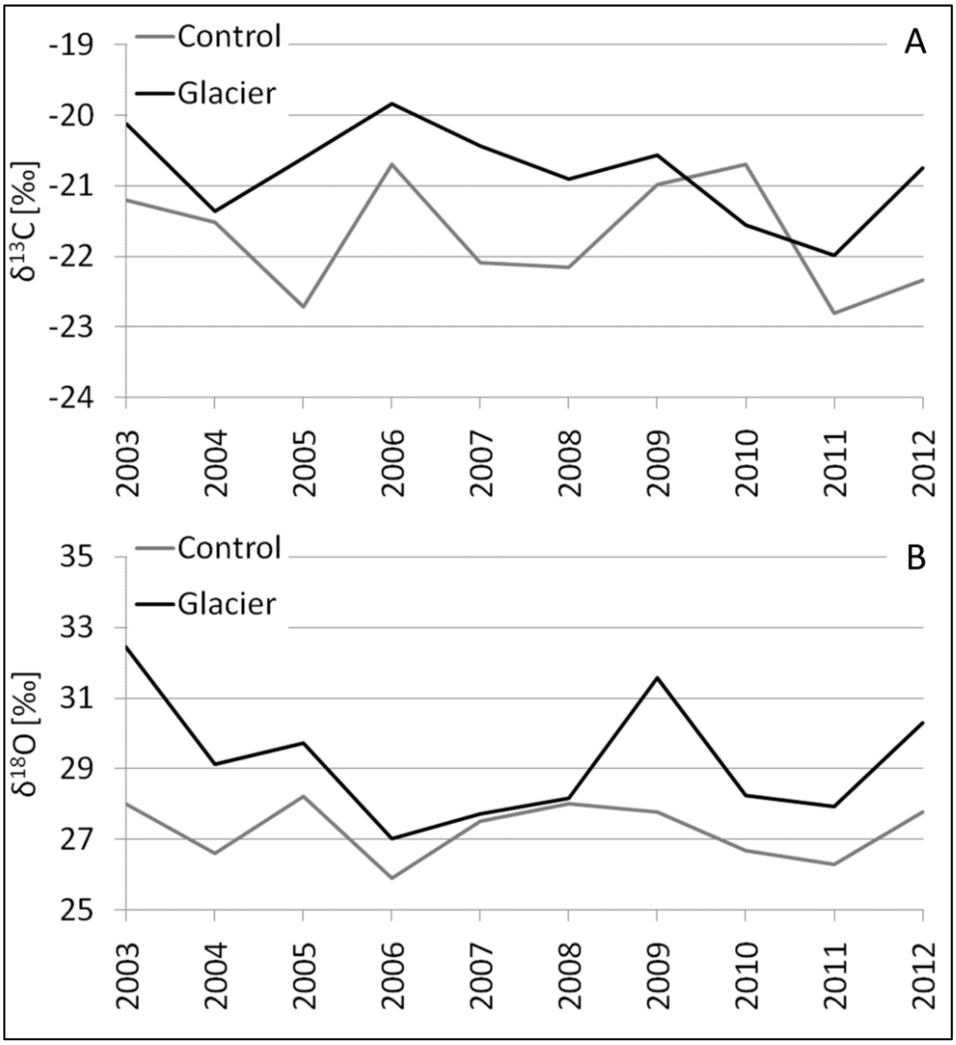


***Figure 3.*** *The stable isotope ratio series constructed at the Control and Glacier sites: tree-ring $\delta^{13}C$ (A) and $\delta^{18}O$ (B) values are reported for the period 2003-2012.*

Tree-ring growth patterns of the sampled specimens

AGCs at the Glacier site were markedly higher than at the Control site (fig. 4): trees at the former site presented rather high percentages of samples with AGCs >+40% in the years 1993 (60%), 2003 (75%), 2007 (60%) and 2009 (50%); additionally, in 1998 they presented high percentages of samples (63%) with AGCs <-40% (fig. 4B). At the Control site the maximum percentage of samples with AGCs >+40% for at least 50% of the samples occurred only in 2005; no years presenting more than 40% of samples with abrupt growth reduction were found at this site (fig. 4A). Considering the indexed chronologies, they show rather similar patterns especially after the year 2003, whereas in the previous portion of the chronologies some minor differences in interval trends are visible (fig. 2B). As regards the percentage of latewood with respect to the total ring width, over the common period covered by data (1997-2012) both sites showed the same mean value (47%), however they showed several years with contrasting values of latewood percentages, like the year 2012 that showed higher percentages of latewood at the Glacier site (fig. 5).

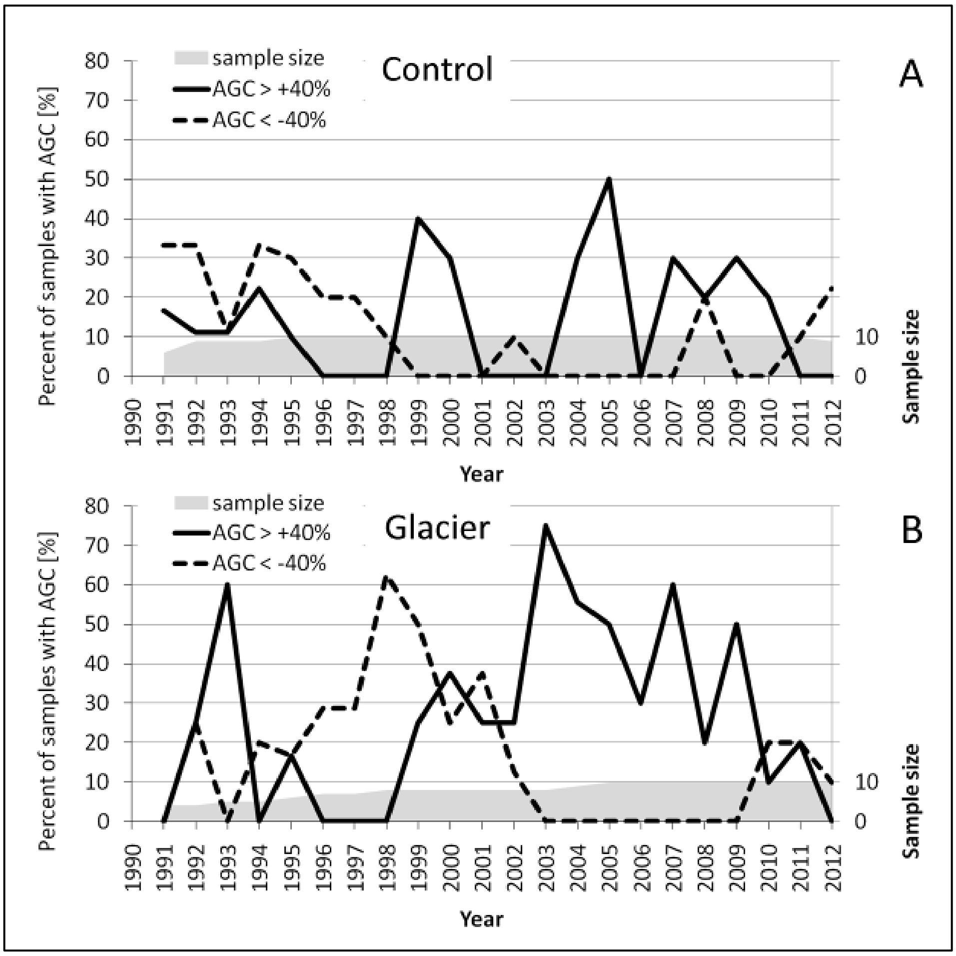


***Figure 4.*** *Percent of samples presenting abrupt growth changes (AGCs) >+40% and <-40% over the common period 1991-2012 where at least 4 samples per site are present in the Control site (A) and on the Glacier site (B); sample size is also reported.*

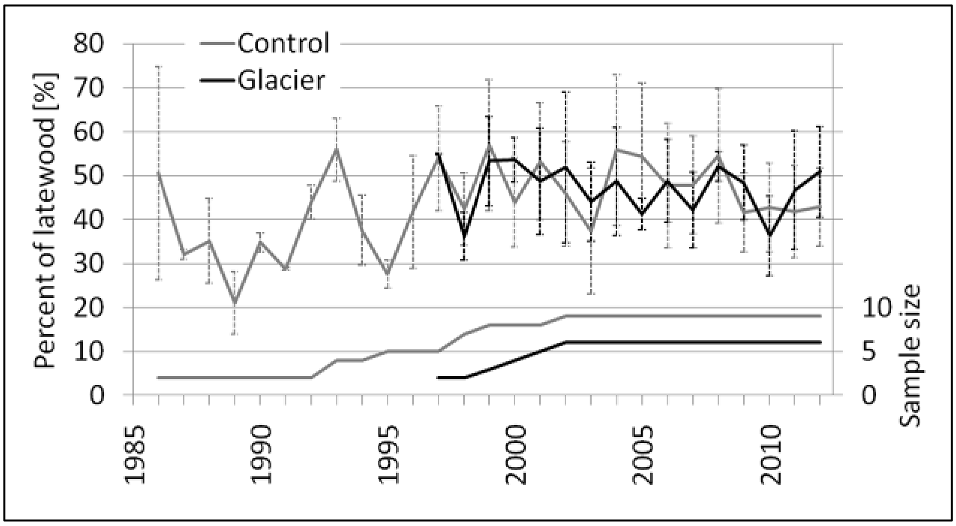


***Figure 5.*** *Percent of latewood calculated with respect to the total ring width both for Control and Glacier sites; error bars indicate ±1 standard deviation. Sample size for the measured cores (see methods) is also reported.*

<u>VOCs in needles</u>

The information derived from the VOC analysis is referred only to the end of the growing season 2012 CE: several and significant differences were observed between the two sampling sites. VOCs in the larches needles showed significant ($p<0.05$) differences in mean concentrations for some of the mono- di- and sesquiterpenes analyzed (fig. 6). In particular, within the terpenes showing significant differences between sites, the largest differences in concentrations at the two sites were observed for those terpenes showing the highest concentrations: namely, the b-myrcene and the estragole, with significantly highest concentrations in the Glacier site. Differently, significantly lower concentrations were always observed in the Glacier site for the other sesquiterpenes (fig. 6B), whereas the other mono- and diterpenes showed higher or lower concentrations at the Glacier site (fig. 6A).

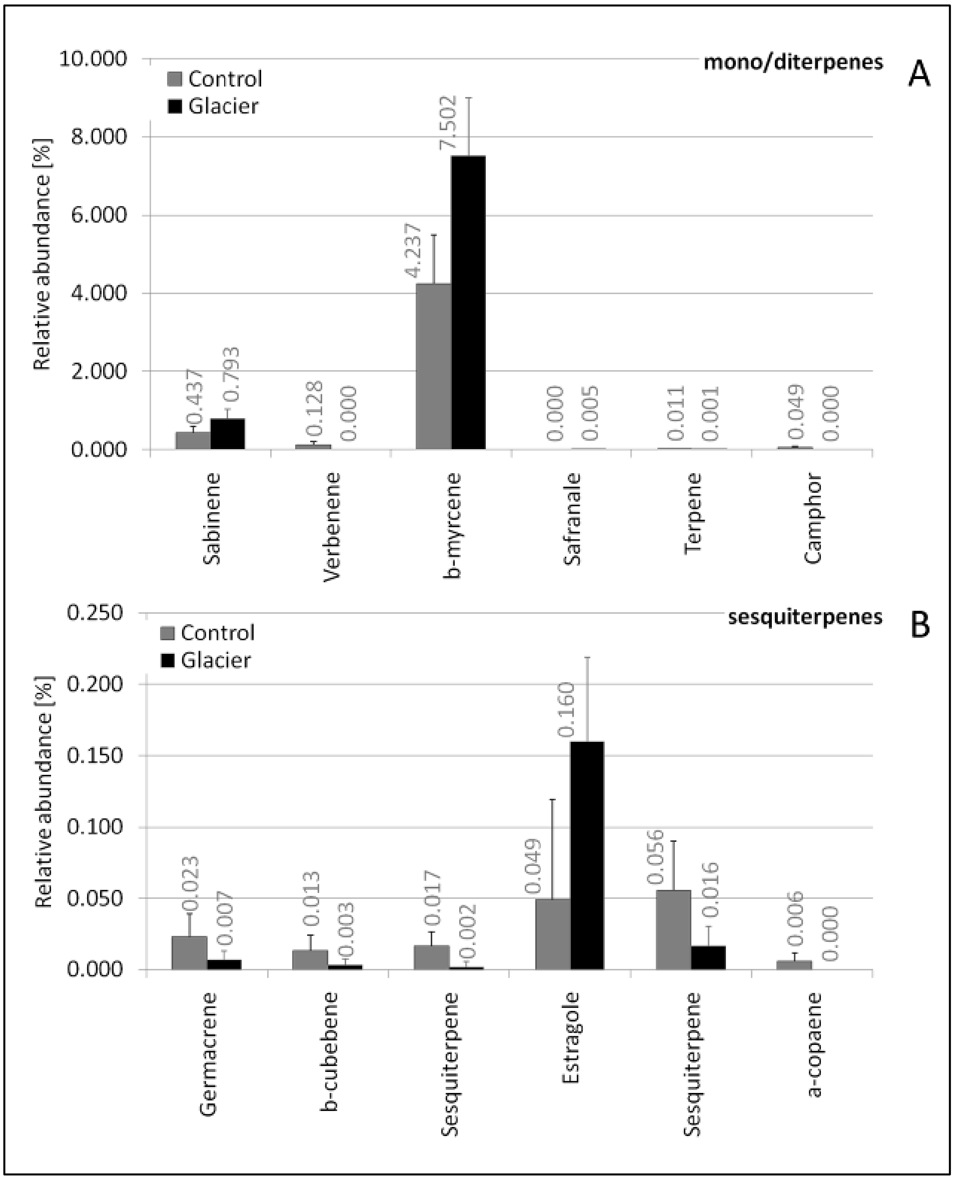


***Figure 6.*** *Concentration of the mono- and diterpenes (A) and olof the sesquiterpenes (B) in the larch needles showing significant differences in mean values between Control and Glacier sites. Error bars indicate +1 standard deviation. The mean values of concentrations are also reported as relative abundance (%) (peak area of volatile compound/total peak area of all volatile compounds).*

**Discussion and conclusion**

This study evidences the possible use of tree-ring proxies for the assessment of mid- to short-term climate change impacts in the glacial environment. In particular, by differentiating the sites primarily by the contrasting geomorphological features (debris-covered glacier and moraine), it was possible to detect how glacier surface movements and climate influence may affect tree growth, therefore potentially opening new approaches for the assessment of climate change impacts over larger areas of vegetated debris-covered glacier surfaces and over longer time periods.

Tree-ring stable isotopes at the Glacier site showed more enriched $^{13}C$ mean values in the cellulose with respect to the Control site. Since the $^{13}C$ value can provide an integrated record of the balance between assimilation rate and stomatal conductance (WUEi), and thus is an indicator of the internal regulation of carbon uptake and water losses (Saurer & *alii*, 2004), our findings suggested that WUEi is improved, at the Glacier site, probably because it is associated with lower stomatal conductance. Indeed, several species have been found to increase WUEi under water limitation (Moreno-Gutiérrez & *alii* 2012, Battipaglia & *alii*, 2009; 2010), and stomatal closure has often been invoked as the main cause (Ogaya and Peñuelas, 2003; Ferrio & *alii* 2007; Ripullone & *alii* 2009). However, photosynthetic activity (A) and stomatal conductance (gs) are strongly coupled and adjustments in both parameters could influence WUEi (Farquhar & *alii*, 1982). Hence, the simultaneous analysis of tree-ring carbon and oxygen isotopes may help discriminate whether changes observed in the carbon isotope values originated from a modification of A or gs because the oxygen isotope composition of the tree rings does not reflect changes in photosynthetic capacity (Dawson & *alii* 2002; Barbour 2007). A positive correlation between $^{13}C$-derived WUEi and $\delta^{18}O$ for trees growing at Glacier site suggests that gs plays a significant role (Scheidegger & *alii*, 2000). Further, those stressed conditions are likely induced by the low soil water retention of the debris cover and by the high temperature excursions that occur daily at the Glacier site (Mihalcea & *alii*, 2008) and to the high exposure to solar radiation of supraglacial trees, in contrast to the forest canopy shading which characterize the environment of the young trees at the Control site.

Tree-ring cellulose was significantly more enriched in $^{18}O$ mean values at the Glacier site than at the Control site. The higher $\delta^{18}O$ values indicate that supraglacial trees are not fed by glacier ice-meltwaters (that would have induced a more depleted cellulose; Leonelli & *alii*, 2013) but only by meteoric precipitations. The similar interval trends found in the $\delta^{18}O$ series at the Control site (where trees are fed only by meteoric precipitation), support the interpretation that trees at both sites are mainly fed by the same, meteoric, waters. The higher values at the Glacier site are likely due to the assimilation of shallow waters from a superficial root system of the supraglacial trees, in contrast to a more developed and stabilized forest soil at the Control site where tree roots may take up water also from deeper soil layers and from the ground where waters are typically more depleted in $\delta^{18}O$ than in the upper layers (Mc Carrol & Loader, 2004).

Even if the trees, sampled at both sites, are young and could be affected to some extent by the so-called "juvenile effect", their physiological responses to external inputs are comparable and the differences between them are due to different environmental settings and not to differences in tree age.

Indeed, the existence of a ‘‘juvenile effect’’ for the first 20–100 years in tree-ring width, density and stable isotope series is well known and the changes over time are the result of morphological and physiological trends characterizing the transition from a juvenile to a mature growth phase (e.g., Lerman and Long, 1979; Schleser, 1992; Buchmann and Ehleringer, 1998). Photosynthesis rates and related physiological attributes differ between juvenile (pre-reproductive plants) and full reproductive specimens (mature plants) (Yoder & *alii*, 1994; Bond, 2000). As a consequence, young trees may present depleted and rising $\delta^{13}C$ with respect to the old ones (Gagen & *alii*, 2007; Loader & *alii*, 2007). As regards the $\delta^{18}O$, contrasting results have been presented: Treydte & *alii* (2006) and Esper & *alii* (2010) has suggested that the juvenile effect (age-related decline trend in juvenile trees) of the tree-ring cellulose $\delta^{18}O$ could exist in young juniper trees in northern Pakistan and in young pine trees in the Spanish Pyrenees, respectively. Nakatsuka & *alii* (2008) also reported that the juvenile effect may influence the decreasing and increasing trends in the tree-ring cellulose $\delta^{18}O$ and $\delta D$, respectively, during the young periods of larch trees in Kamchatka Peninsula, Russia. Conversely, the juvenile effect was not observed in oak cellulose $\delta^{18}O$ from western France, (e.g. Raffalli - Delerce & *alii*, 2004). Leavitt & *alii* (2010) suggested that the juvenile effect was more obvious in stable carbon and hydrogen isotopes, comparing with stable oxygen isotope in tree rings. The physiological mechanism of the juvenile effect of the tree-ring cellulose $\delta^{18}O$ is still unclear by now, and this effect may depend on tree species, stand environments, and other factors (Dorado Linan & *alii*, 2012). Thus more studies concerning the oxygen isotopic juvenile effect are necessary and should include more tree species and more detailed measurements of individual tree ecophysiological conditions before deciding to detrend short chronologies (Li & *alii*, 2011). The juvenile trend problem especially arises when long tree-ring stable isotope series are considered, which especially occurs in dendroclimatic studies (Loader & *alii*, 2013). However, this is not the case of our study, that presents series from sites having trees of very similar age and therefore no detrending was applied to the dendro-isotopic series.

The analysis of tree-ring characteristics was performed for assessing mid-term stress signals in the same supraglacial trees that were selected for the stable isotopes and the VOCs analysis. Tree-ring growth trends and extremes in the sampled specimens at the Glacier site were largely caused by AGCs >+40% that were meanly higher than at the Control site and interested up to 75% of the samples over the study period. AGCs are a good proxy for substrate instability and represent a typical reaction of supraglacial trees, especially for what concerns growth releases (Pelfini & *alii*, 2007; Leonelli and Pelfini, 2013). Considering the indexed chronologies, they show rather similar patterns especially after 2003, thus pointing to a similar influence of climate on tree-ring growth.

However, some minor differences in interval trends are evidence of possible different relationships with climatic factors. This interpretation is also supported by the different interval trends in percentages of latewood noticed at the study sites, even though the mean values are equal (47%) at both sites. Latewood in the tree rings is usually formed when signals of declining growing season, like low day temperatures, are detected by trees, thus determining the transition from the production of early wood to latewood cells (Larson, 1960; Brown, 1970; Antonova and Stasova, 1993; Lebourgeois, 2000). However, a different percentage of latewood at the Glacier site may be also induced by a differential response to climate due to the rocky substrate where supraglacial trees grow. The supraglacial debris cover, beside being related to glacier surface movements, may in fact alter trees' relationships with climate factors since it may alter the water holding capacity and influence the resulting tree-ring growth.

The single measurement of VOCs performed does not allow to make generalizations on tree emissions and physiological responses during the growing season, however an influence of environmental factors, i.e. abiotic stress conditions, on tree secondary metabolites has been found. VOCs at the two sites resulted significantly different in concentration especially for b-myrcene and estragole, the terpenes that showed the highest concentrations and the largest differences between Glacier and Control sites. In particular, at the Glacier site we observed a relative increase of mono/diterpenes compared with sesquiterpenes. Similar findings were observed in other conifer species (Hengxiao & *alii*, 1999; Turtola & *alii*, 2006). More VOCs are expected to be emitted when high temperatures or UV-radiation occur. Monoterpenes and isoprene are thermoprotective molecules, able to stabilize chloroplast membranes when the cells are exposed to high temperatures (Loreto and Schnitzler 2010). A more recent study showed increased of monoterpenes emission rates of subarctic peatlands when irradiated with increasing levels of UV-B radiation, and explained the rising emission as a consequence of oxidative damage to membranes and to the induction of the monoterpene defensive antioxidant pathway (Tiiva & *alii*, 2007). Sesquiterpenes emissions are also correlated with temperature, light and other abiotic (soil moisture, air humidity, plant water stress, fertilization levels) and biotic factors (Duhl & *alii*, 2008; 2012). Duhl & *alii* (2008) found that sesquiterpenes emissions typically increase with temperature but there is considerable variability in emission rates between and within plant species.

Besides being frequently solicited by glacier surface movements as evidenced by the tree-ring analysis, supraglacial trees are also exposed both to the immature substrate of the debris coverage, which is mainly characterized by regolith and finer glacial till, and to the high daily temperature excursions due to rock heating caused by the incident solar radiation. As reported in Mihalcea &

*alii* (2008) debris may experience up to 10ºC of mean daily temperature variations during the growing season (June to August) and in the glacier lower portion the surface temperature is higher of about 10ºC (reaching a mean value of 30ºC) than the temperature of the forested area between the two main lobes (where the Control site is located). Both the scarce presence of nutrients and the high-temperature ranges may be indicated as additionally source of stress for the already solicited supraglacial trees. The differences in the tree-ring stable isotopes and in the AGCs, as well as in the VOCs concentrations in the needles underline the possibility to use these parameters as indicators of the stress generated by the different geomorphological features of the debris covered glacier environment.

Even if based on a restricted area of the Miage Glacier environment, the results of this study underline that a multiproxy approach based on tree-ring stable isotopes and tree-ring growth anomalies, as well as needle VOCs may be used for defining areas of glacio-geomorphological and climatic stress. As demonstrated also by previous studies based on larger datasets (Pelfini & *alii*, 2007; Leonelli and Pelfini, 2013), growth anomalies may allow well defined spatio-temporal reconstructions in the mid-term, whereas VOCs should be monitored year by year or seasonally (e.g. Duhl & *alii*, 2013) to produce track records of climate change impacts. The presented results by identifying stress signals in supraglacial trees allow the opening of new perspectives for reconstructing glaciological and climatic variations in the mid-term by means of the analysis of tree physiological responses. The multiproxy approach proposed in the present study has let the identification of stress indicators (tree-ring $\delta^{13}C$, $\delta^{18}O$ and AGCs, as well as needle VOCs) and can be potentially used for defining the influence of different glaciological conditions (e.g. debris-cover instability, differential ablation and glacio-karst processes) on tree growth and for detecting local climate change impacts over wider glacier areas. This methodological approach may be applied also on different landforms for assessing climate change impacts at the local scale in the heterogeneous Alpine environment.

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## 5.2.

### Volatile terpenes and tree-ring analyses indicate fungal infection in asymptomatic mature Norway spruce trees in the Alps

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**Abstract**

In this manuscript we present the results of the first investigation of volatile monoterpenes content in tree-ring resin, in response to natural infection by *Heterobasidion* spp. in asymptomatic adult Norway spruce [*Picea abies* (L.) Karst.] trees. Twenty-three randomly selected mature trees in Courmayeur (northwestern Italy) were sampled 20 cm aboveground by extracting cores using a Pressler's increment borer. Based on fungal isolations from cores and molecular typing using Taxon-Specific Competitive-Priming (TSCP)-PCR, 12 of these trees were identified to be infected by *Heterobasidion parviporum* Niemelä & Korhonen. Tree-ring growth patterns and volatile monoterpene content in tree rings of trees infected ("pathogen") and not infected ("no pathogen") were determined. Volatile monoterpene analyses and identification were performed by means of Gas Chromatography Mass Spectrometry on a subset of 5 infected and 5 not infected trees. The dendrochronological analysis showed slightly lower tree-ring width in the most recent years of the "pathogen" compared to "no pathogen" mean chronology. Monoterpene analysis highlighted statistically significant differences between "pathogen" and "no pathogen" trees in total absolute amounts of monoterpenes and in relative proportions of terpenes α-pinene, β-pinene, β-phellandrene and γ-terpinene.

This is the first study showing that volatile monoterpenes in tree-ring resin and dendrochronology can be used as an aid to first evaluation of fungal infections, even when these are mostly asymptomatic as usually in the case of *H. parviporum* in Norway spruce. Furthermore, this study also suggests that the presence of pathogenic fungi can represent a disturbing factor for performing dendroclimatic and dendroenvironmental reconstructions.

**KEYWORDS:** Volatile Organic Compounds, tree rings, *Heterobasidion* spp., dendroclimatology.

## Introduction

Inducible Volatile Terpenes (VTs) are abundantly produced and released by different plant organs following abiotic stresses (e.g. Loreto and Schnitzler, 2010; Leonelli et al., 2014) and biotic attacks, including those performed by insects and pathogens (e.g. Holopainen, 2004; Jansen et al., 2011).

In conifers, VTs are produced and stored in several plant structures, including resin ducts. Resin is toxic for most pathogens due to its composition and physical properties (Phillips and Croteau, 1999). In fact, resin contains monoterpenes, diterpenes and sesquiterpenes and some of them, especially when produced and released abundantly, are known to be insecticidal, antimicrobial and fungicidal (Schuck, 1982; Michelozzi, 1999; Trapp and Croteau, 2001). Conifer resin is produced in bark, phloem and xylem by constitutive and inducible secretory structures, emitting primary and secondary resin, respectively.

In Norway spruce [*Picea abies* (L.) Karst], resin accumulates constitutively in axial resin canals in the bark and in stem xylem Traumatic Resin Ducts (TRDs), which appear within the developing xylem after mechanical wounding. The formation of TRDs associated with enhanced level of VTs produced is part of a complex mechanism of defence that is activated in order for the tree to react successfully to the attack of pathogens and to mechanical damage (Franceschi et al., 2000; Nagy et al., 2000; Fäldt et al., 2003; Krokene et al., 2008; Gärtner and Heinrich, 2009; Danielsson et al., 2011; Brauning et al., 2016). TRDs considerably enhance the oleoresin content of Norway spruce, considering that they are larger, and thus their volume is much higher, than constitutive resin ducts. TRDs usually develop in high number in the proximity of the injury caused by mechanical wounding or pathogens, and then their number decreases as the distance from the wound increases (Schmidt et al., 2011). TRDs are commonly used for dating stressing phases in geomorphology, in particular related to mass movements (e.g., Stoffel, 2008; Butler et al., 2010), but their frequency and distribution within tree rings are poorly investigated. In some tree species, most of the vertical resin ducts seem to develop in the latewood (Reid and Watson, 1966) but their distribution is highly variable within the same tree, due to environmental and climatic conditions (Wimmer et al., 1999).

Norway spruce is susceptible of heart rots caused by some fungi included in the *Heterobasidion annosum* (Fr.) Bref. *sensu lato* (*s.l.*) species complex, namely *H. annosum* (Fr.) Bref. and *H. parviporum* Niemelä & Korhonen (Garbelotto and Gonthier, 2013). While the former species is more generalist being able to attack several coniferous tree species, the latter displays a relevant preference for Norway spruce. Regardless of which one of the two species is involved, the disease is virtually asymptomatic in mature trees. In fact, the progressive development of the decay in the heartwood rarely results in the appearance of external symptoms (Garbelotto and Gonthier, 2013). Heart rots caused by *Heterobasidion* spp. are among the major, most destructive and widespread diseases of Norway spruce in Europe, including the Alpine area (Asiegbu et al., 2005; Gonthier et al., 2012; Giordano et al., 2015). Infection occurs through airborne spores (primary infections) colonizing freshly exposed wood surfaces (stumps or wounds in the stem or roots). Subsequently, the fungus can infect uninjured trees by vegetative growth of mycelium through root contacts or grafts (secondary infections) (Garbelotto and Gonthier, 2013).

Due to the current climate change, that causes an increase average air temperature also during the winter months, fungal pathogens are modifying their habitat, also colonizing areas located at higher elevation. The production of spores of *Heterobasidion* spp. is more abundant when air temperature is above 5°C (Gonthier et al., 2005). For this reason, climate warming determines a prolonged time interval favourable for sporulation and infection during the year, and the altitude at which pathogens can be found is shifted at higher elevations (La Porta et al., 2008).

Defensive strategies and VTs production are usually studied in experimental plants obtained from controlled crosses, that are artificially infected by the pathogen (e.g. Cellini et al., 2014; Piesik et al., 2015) or in which the pathogen attack is mimicked by treatment with methyl jasmonate (e.g. Martin et al., 2002; Zeneli et al., 2006). In particular, experiments conducted on Norway spruce reveal that the oleoresin of trees affected by *Heterobasidion* spp. contains significant differences between infected and healthy trees in amounts of (+)-α-pinene, (+)-sabinene, (-)-sabinene, δ-3-carene, (-)-limonene and γ-terpinene (Zamponi et al., 2007). However, we are not aware of any studies conducted on the oleoresin content of mature trees infected by *Heterobasidion* spp. in forest stands. Moreover, little is known about VTs production in asymptomatic trees. A better comprehension of this topic is crucial for developing proper strategies allowing the identification of useful markers enabling early diagnosis of tree diseases.

The main aim of this research was to detect possible differences in volatile monoterpene content in tree-ring resin in response to natural infection by *Heterobasidion* spp. in asymptomatic mature Norway spruce trees. Tree-ring growth was also analysed in trees infected and not infected, in order to investigate if any difference in growth patterns may be ascribed to the presence of the pathogen.

**Materials and methods**

<u>Study area and sampling design</u>

The study site is located at about 1450 m a.s.l. close to the area called Ermitage (45°47’46.11’’N; 6°58’56.39’’E), in the proximity of Courmayeur, in the Autonomous Region of Aosta Valley (northweastern Italy), where *Heterobasidion* spp. was previously detected in a mature mixed

Norway spruce-European larch (*Larix decidua* Mill.) forest and estimated to have infected about 55% of trees (Gonthier et al., 2012).

In the attempt to compare a similar number of infected and putatively not infected trees, 23 randomly selected trees were sampled at the end of June 2015 by extracting four cores at about 90° from one other from the base of each of them (20 cm aboveground) using a Pressler's increment borer. The minimum and mean distance among sampled trees was 25 m and 80 m, respectively. The Diameter at Breast Height (DBH) of sampled trees was comprised between 68 cm and 145 cm (average 99 cm). Cores were transported to the laboratory in plastic straws and stored at 5°C before subsequent analyses.

Due to the high number of cores that was necessary to take from each tree for conducting the analysis of volatile monoterpenes and the pathogen detection, in order to minimize the tree damage only one core was extracted from each tree for the dendrochronological investigation. Thus, two cores were used for pathogen detection and isolation, one for the dendrochronological analyses and one for volatile monoterpene analyses in tree rings (Fig. 1).

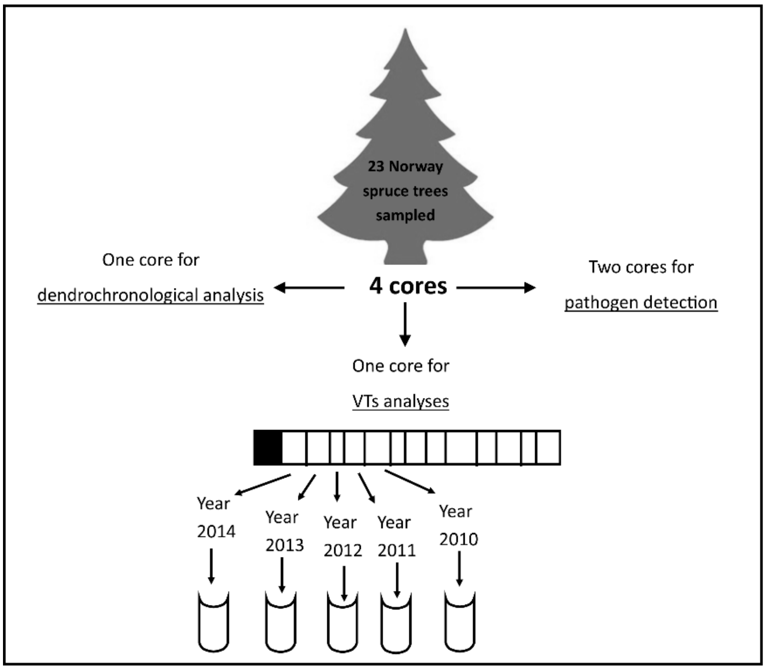


***Figure 1.*** *Experimental design of the analyses.*

Pathogen detection and species identification

Cores were sprayed with a benomyl solution (0.010 g benomyl, 500 µl methanol, 1 l distilled water) and incubated for about 10 days at room temperature (25°C ± 2°C) in a moist chamber as described

by Gonthier et al. (2003). After incubation cores were inspected under a dissecting microscope (x20 magnification) in order to observe emerging colonies of the conidial stage of *Heterobasidion* spp.
Isolations were made by transferring infected wood or fungal hyphae onto 6-cm Petri dishes filled with PCNB-based selective medium (Kuhlman and Hendrix, 1962). All isolates were subsequently subcultured and stored at 5°C on MEA (Malt Extract Agar: 20 g glucose, 20 g malt extract, 2 g peptone, 20 g agar, 1 l distilled water).
DNA from purified fungal isolates was extracted by a hyphal tipping method (Schweigkofler et al., 2004), modified as follows. Fungal mycelium was collected with the tip of a micropipette and suspended in 100 µl of distilled water, frozen on dry ice for 3 min, thawed at 75°C, vortexed for 1 min, and finally centrifuged for 5 min at 19,000 g. Freezing and thawing were repeated three times, with the last thaw extended to 15 min. Samples were then centrifuged for 5 min at 19,000 g and the supernatant was used as template for Polymerase Chain Reactions (PCRs). The species identification of *Heterobasidion* isolates was carried out by DNA-based molecular diagnosis of isolates, resulting in differently sized amplicons depending on the species analyzed. A Taxon-Specific Competitive-Priming (TSCP)-PCR (Garbelotto et al., 1996) combined with a PCR-mediated detection of species-specific DNA insertions in the ML5-ML6 DNA region of the Mitochondrial Large Ribosomal RNA (mt LrRNA) gene was used as described by Gonthier et al. (2001).

Dendrochronological analysis

The cores were prepared for tree-ring dating and ring-width measurements following standard methods (Stokes and Smiley, 1968), usually applied in dendrochronological studies conducted in mountain environments and in the nearest geographical areas (e.g., Garavaglia et al., 2010). Tree-ring widths were measured to the nearest 0.01 mm using the LINTAB system with the TSAPWin software (Frank Rinn, Heidelberg, Germany), and the obtained series were visually and statistically cross-dated using the COFECHA software (Grissino-Mayer, 2001) in order to find and correct any dating error in the dataset. Two main ring-width mean chronologies were built: one, named "pathogen", using the trees infected by *Heterobasidion* spp., and one, named "no pathogen", using trees putatively not infected by the pathogen.
To analyse tree-ring growth trends in the two groups, the raw ring-width series were standardized using the software Arstan (Holmes, 1992) and a residual chronology for each category was prepared applying a negative exponential curve.

Volatile monoterpene analyses in tree rings

Five trees infected and five putatively not infected by *Heterobasidion* spp. were selected for the analysis of volatile monoterpenes. Selection was mainly based on the entirety conditions of the cores: priority was given to the cores with no broken tree rings, at least in the terminal part of the core, and characterized by easily identifiable tree rings.

The last five tree rings of each core (corresponding to the years from 2010 to 2014) were split from each other using a scalpel, for a total of 50 samples (Fig. 1).

The analyses of volatile monoterpene content were performed by means of Gas Chromatography Mass Spectrometry. About 25 mg of cortical and xylem tissues were placed into a sterilised vial. An amount of 200 µl of pentane with tridecane as internal standard was added to each vial and then the vials were put in the ultrasound machine at the temperature of 30°C for 60 minutes.

After this procedure, the vials were left in the shaker for 24 hours. The extracts were then filtered with 0.45 µm PTFE syringe filters and injected (3 µl) in the GC-MS system. An Agilent 7820 GC-chromatograph equipped with a 5977A MSD mass spectrometer with EI ionisation operating at 70 eV was used for analysis. A Chromatographic column J&W Innovax 50 m, 0.20 mm, ID 0.4 µm DF was used. The GC injection temperature was 250°C, splitless mode, and the oven was programmed at 40°C for 1 min, followed by a ramp of 5°C/min to 200°C, and of 10°C/min to 260°C. This high temperature was held for 5 min. Mass spectra were acquired within the 29-350 M/Z interval with an Agilent 5977 MSD spectrometer at three scans $s^{-1}$. Volatile monoterpene identification was done on the basis of both peak matching with library spectral database (NIST 08) and kovats indeces as retrived in literature for the identified compounds.

Total absolute amounts of monoterpenes were expressed as milligrams of monoterpenes in grams of fresh wood (F.W.) and, since data were not normally distributed (Kolmogorov-Smirnov test) they were analysed by non-parametric Mann-Whitney U Test, to test the statistical significance of difference between the two groups “pathogen” and “no pathogen”.

The amount of each monoterpene was expressed as a percentage of total monoterpenes. The average and Standard Error (SE) of the percentage were calculated for each compound and compared between “pathogen” and “no pathogen” trees.

The statistical significance of volatile monoterpene differences for average percentages of monoterpenes was detected by Student’s t-test and, in case of not normally distributed data (Kolmogorov-Smirnov test), by Mann-Whitney U Test. A 0.05 threshold was used as cut-off value in all the statistical tests. Statistical analyses were carried out on SPSS (SPSS software, v.22.0, SPSS Inc., Chicago, USA).

## Results

Pathogen detection and species identification

Out of the 23 sampled trees, 12 were infected by *Heterobasidion* spp. (52%) while the remaining 11 were putatively not infected by the pathogen. None of the cores analysed displayed visible symptoms of wood decay. Based on the molecular diagnostic assay, all infected trees were colonized by *H. parviporum*.

Dendrochronological analysis

The tree-ring width mean chronologies showed synchronous ring-width peaks except than over the past 20 years. The chronologies covered the period for 1902-2015 for “pathogen” trees and 1901-2015 for “no pathogen” trees. Median age was very similar for the two series, i.e., 65 years for “pathogen” trees and 64 years for “no pathogen” trees. The two mean chronologies show similar growth trend, especially after 1970, when more than five trees for both the series contribute to the chronology building (Fig. 2, continuous line).

“Pathogen” trees were characterized by slightly lower tree-ring width in the last 15 years (57 mm/100 on average) compared to “no pathogen” trees.

The two residual chronologies show very similar growth patterns along the whole considered time interval, with the more recent relative peaks of positive growth in 1998 (“pathogen” trees) and 2000 (“no pathogen” trees) (Fig. 3).

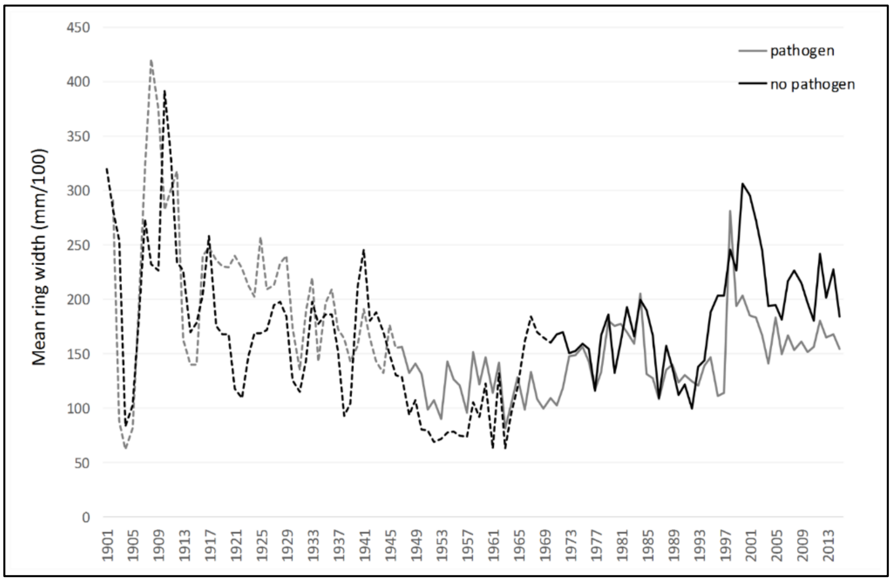


***Figure 2.*** *Ring-width mean chronologies for “pathogen” and “no pathogen” trees. Discontinuous lines characterize the curve built with less than five trees.*

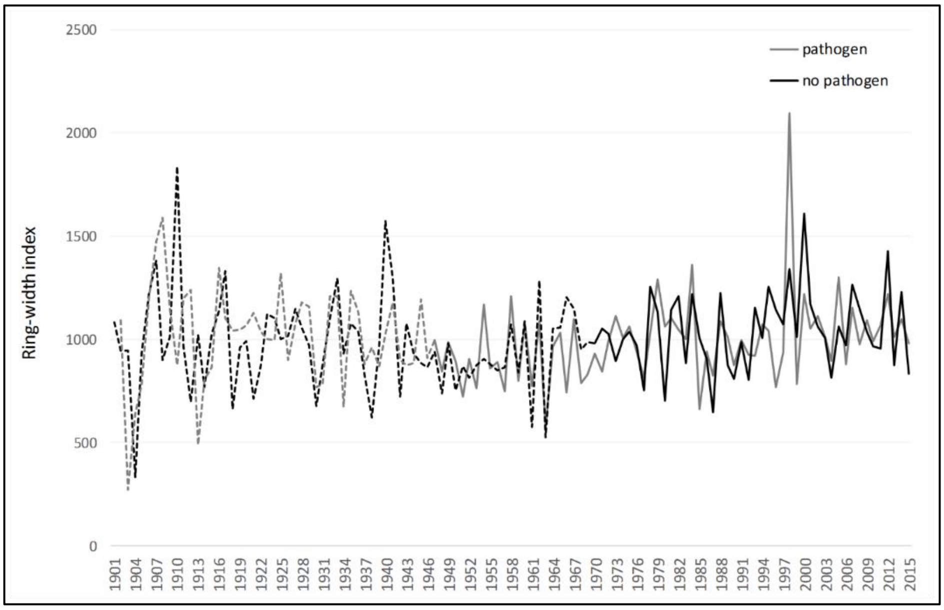


***Figure 3.*** *The two residual chronologies "pathogen" and "no pathogen". Discontinuous lines characterize the curve built with less than five trees.*

Volatile monoterpene analyses in tree rings

Changes in the absolute amounts of terpenes

Mean concentration of total monoterpenes are significantly different (Mann-Whitney U = 168; P < 0.05) between "pathogen" (M = 0.35 mg/g; SE = 0.10) and "no pathogen" (M = 1.86 mg/g; SE = 0.65) trees (Fig. 4).

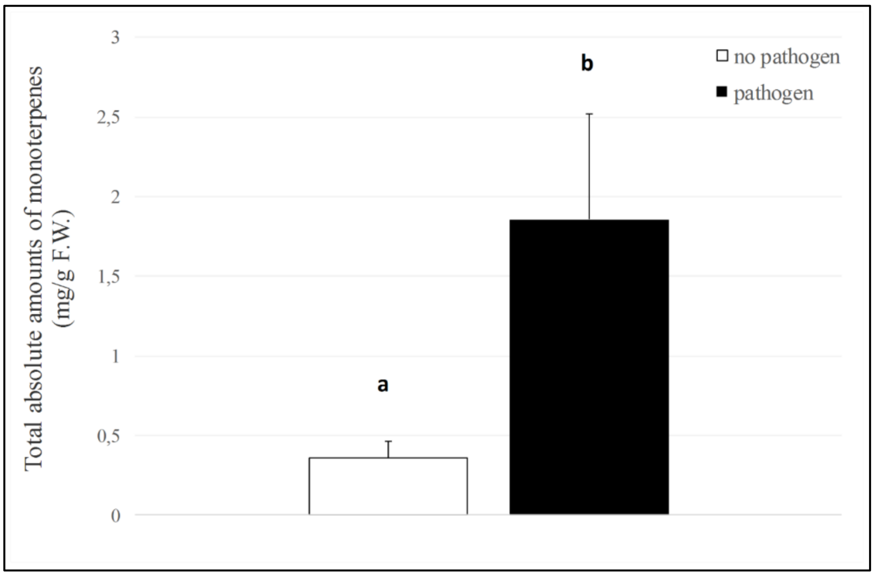


***Figure 4****. Mean (+ SE) values of total absolute amounts of monoterpenes (expressed in mg/g of fresh wood) detected in tree rings of "pathogen" and "no pathogen" trees. Values of columns with different letters differ significantly (P < 0.05).*

Changes in the relative contents of terpenes

Tree rings of “pathogen” and “no pathogen” trees differed significantly for 4 of the 11 considered monoterpenes. The relative content (percentage) of α-pinene and β-pinene were significantly higher in “pathogen” trees compared to “no pathogen” trees (Student’s t test = -2.02; $P < 0.05$ and Mann-Whitney U = 455; $P < 0.05$, respectively), while β-phellandrene (Mann-Whitney U = 164; $P < 0.05$) and γ-terpinene (Mann-Whitney U = 112; $P < 0.05$) were significantly higher in “no pathogen” trees compared to “pathogen” trees. The other monoterpenes also showed higher values in “no pathogen” compared to “pathogen” trees but did not show statistically significant results. These were camphene (Mann-Whitney U = 299; P = 0.79), sabinene (Mann-Whitney U = 291; P = 0.68), 3-carene (Mann-Whitney U = 234; P = 0.13), myrcene (Mann-Whitney U = 302; P = 0.84), limonene (Mann-Whitney U = 218; P = 0.07), cineole (Mann-Whitney U = 222; P = 0.08) and ρ-cimene (Mann-Whitney U = 266; P = 0.37) (Fig. 5).

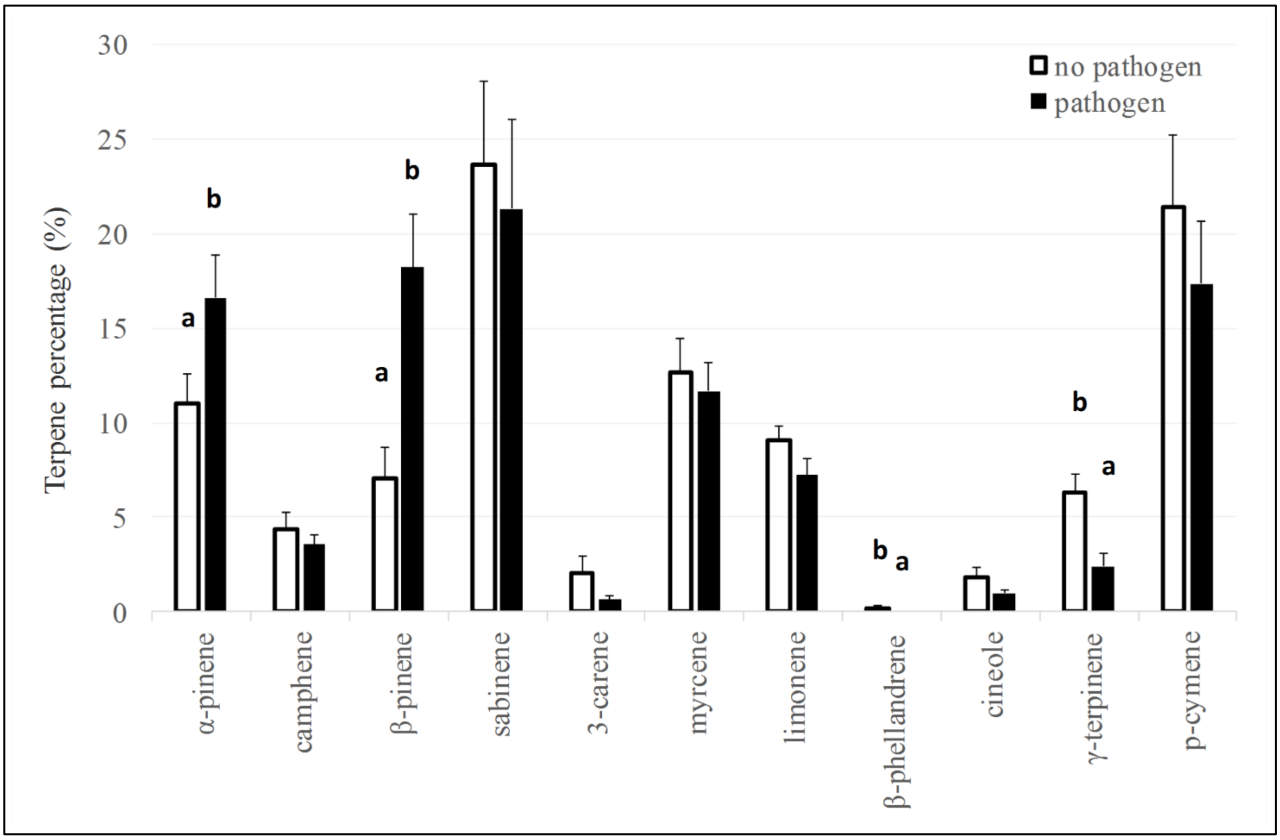


***Figure 5**. Average percentage of monoterpenes in “pathogen” and “no pathogen” tree rings. Statistical difference was determined by t-test and Mann-Whitney U test. Error bars indicate SE. Values of columns with different letters differ significantly (P < 0.05).*

**Discussion**

This study represents the first attempt to detect possible differences in monoterpene content in annual tree rings of adult asymptomatic Norway spruce trees in response to natural infection by a fungal pathogen, i.e., *Heterobasidion* spp.

All *Heterobasidion* infected trees were colonized by *H. parviporum* and none by *H. annosum*, thus confirming that the overwhelming majority of Norway spruce decays in the area are caused by the former species, as previously documented (Gonthier et al., 2003). It should be noted, however, that none of the cores analysed displayed visible symptoms of decay, possibly indicating a recent upward colonization of the fungus from the point of infection in the roots.

The mean ring-width chronology of trees infected by *H. parviporum* showed lower values starting from the late 90's compared to not infected trees, and this would also suggest infection occurred relatively recently. In fact, growth reduction in conifers is common during infection performed by some fungi (Schweingruber, 1996). This pattern was also observed by Cherubini et al. (2002) on *Pinus mugo* Turra trees killed by *H. annosum* and *Armillaria sp*. Although authors found a more remarkable difference in ring-width between infected and not infected trees that we did, it should be noted that pine trees compared to Norway spruce trees are more susceptible to root rot and mortality rather than heart rot (Garbelotto and Gonthier, 2013), and this may explain the higher levels of growth reduction in pines than in Norway spruce trees (Mallett and Volney, 1999). In addition, after pathogen infection, ring width begins to slowly reduce until tree death that can occur after many decades (Vasiliauskas, 1999). All selected trees in this study were still living and asymptomatic, and this can explain the lower difference in ring width between the chronologies. Nevertheless, the progressive reduction in tree-ring width can affect the climatic signal recorded in tree rings, negatively influencing dendroclimatic reconstructions (Trotter III et al., 2002). Our results, even if limited to few trees, support previous investigations conducted on conifers, revealing that Norway spruce infected by *Heterobasidion* spp. shows lower tree-ring width compared to not infected trees (Cherubini et al., 2002).

Total content of monoterpenes is significantly higher in trees infected by *H. parviporum* compared to putatively not infected ones. This result is in agreement with previous studies conducted on Norway spruce treated with methyl jasmonate to simulate terpene synthesis in tree tissues following pathogen infection: total content of terpenes always resulted to be higher in treated compared to control samples, and in particular monoterpene content usually appears to increase much more compared to sesquiterpenes (Martin et al., 2002; Zeneli et al., 2006).

Variations in the terpene profiles and particularly in the proportions of α-pinene, β-pinene, β-phellandrene and γ-terpinene can be a consequence of the defence mechanism activated by the tree following infection by the fungus: the plant reduces the production of the biologically less active compounds and increases the synthesis of the more toxic terpenes. For example, α-pinene and β-pinene were reported as being among the most inhibitory compounds to fungi (e.g., Klepzig et al., 1996). Another interpretation of these results could be that trees characterized by constitutively higher values of some terpenes are less susceptible to death due to fungal infection compared to trees with constitutively lower values of terpenes or that are not able to increase their quantity as a response to infection. For instance, Schiebe et al. (2012) observed significantly lower levels of several monoterpenes in bark of Norway spruce trees that died after treatment with the hormone methyl jasmonate (MeJ), compared with surviving trees, including, among others, some terpenes that we also analysed: camphene, sabinene, myrcene, 3-carene, limonene, cineole.

Concerning the specific compounds analysed, our results are only partially in agreement with the research performed by Zamponi et al. (2007) on branches of Norway spruce trees experimentally inoculated with *H. parviporum*. In that study, α-pinene and γ-terpinene, among others, resulted to be significantly different between infected and not infected trees, as we also found. However, they did not find significant differences between infected and not infected trees for β-pinene and β-phellandrene. Differences between our results and results obtained by Zamponi et al. (2007) could be ascribed to the tissues colonized by the pathogens in the two studies, i.e. heartwood vs sapwood, respectively. In fact, while branches, hence sapwood, was inoculated with *Heterobasidion* spp. by Zamponi et al. (2007), it is likely that our adult Norway spruces were colonized by *H. parviporum* in the heartwood as it occurs as a general rule (Garbelotto and Gonthier, 2013).

## Conclusions

In summary, this study reveals that dendrochronological and monoterpene analyses in tree rings can be used as an aid to first evaluation of Norway spruce health on apparently healthy spruce trees. In particular, the tree-ring mean chronology shows lower values in infected compared to not infected trees in the more recent years and monoterpenes show significant differences between infected and not infected trees.

This is the first study showing that VTs composition in tree rings could be used to reconstruct fungal infection. Both the analysis of total absolute amounts and relative contents of monoterpenes are useful for detecting the presence of the pathogen, and this is particularly important in the case of Norway spruce, where external symptoms of *H. parviporum* are usually poor.

The results here presented allow further considerations about environmental changes under climate changing conditions. Growth rate lowering, evidenced by the development of narrower tree rings, seems to be reduced at the beginning of the pathogen infection and it becomes more evident with the progression of the infection. However, ring-width reduction is also induced by adverse climatic conditions in thermo-limited environments such high mountain areas (Nicolussi et al., 2009; Pelfini et al., 2014) and by several geomorphological surface processes such as landslides and debris flows. Generally, geomorphic events locally destroy or produce serious injuries in trees, consequently affecting their growth rate, but also not disruptive processes like sheet flows can induce the production of narrower rings (Pelfini et al., 2006).

Furthermore, the production of resin ducts, and the related production of volatile terpenes, can be related to mechanical impacts associated with geomorphological processes (Gaertner and Heinrich, 2009).

In this perspective, our results describing the influence of *H. parviporum* infection on tree growth and monoterpenes in tree rings, reveal that dendroclimatic and dendroenvironmental reconstructions can be negatively influenced by fungal diseases.

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[In preparation for submission]

# 6.

# INVESTIGATING THE DISTRIBUTION OF AIR POLLUTION USING MAGNETIC ANALYSIS OF TREE BARK

## 6.1.

## Investigating distribution patterns of airborne magnetic grains trapped in tree barks in Milan, Italy: insights for pollution mitigation strategies

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**Abstract**

High levels of air particulate matter (PM) have been positively correlated with respiratory diseases. In this study we performed a biomonitoring investigation using samples of bark obtained from trees in a selected study area in the city of Milan (northern Italy). Here, we analyze the magnetic and mineralogical properties of the outer and inner bark of 147 trees, finding that magnetite is the prevalent magnetic mineral. The relative concentration of magnetite is estimated in the samples using saturation isothermal remanent magnetization (SIRM) and hysteresis parameters. We also make a first-order estimate of absolute magnetite concentration from the SIRM. The spatial distribution of the measured magnetic parameters is evaluated as a function of the distance to the main sources of magnetic PM in the study area, e.g., roads and tram stops. These results are compared with data from a substantially pollution-free control site in the Central Italian Alps. Magnetic susceptibility, SIRM and magnetite concentration are found to be the highest in the outer tree barks for samples that are closest to roads and especially tram stops. In contrast, the inner bark samples are weakly magnetic and are not correlated to the distance from magnetite PM sources. The results illustrate that trees play an important role acting as a sink for airborne PM in urban areas.

**KEYWORDS**: biomagnetic monitoring, tree bark, Milano, traffic emission, air pollution.

## Introduction

The metropolitan area of Milan in the Po Valley of northern Italy is plagued by high levels of atmospheric particulate matter (PM) due in part to the 'bowl effect' on atmospheric circulation triggered by the surrounding Alps and Apennines mountain chains (e.g., Masetti *et al.* 2015). Recorded levels of PM with grain size ≤ 10 µm ($PM_{10}$) are often above 100 µg/m$^3$ especially during winter months, with a daily yearly average of 50 µg/m$^3$ (Marcazzan *et al.* 2001; Ozgen *et al.* 2016). It is well known that airborne PM is linked with respiratory illness (Donaldson 2003; Faustini *et al.* 2011; Gualtieri *et al.* 2011; Bigi & Ghermandi, 2014; Chiesa *et al.* 2014; Kim *et al.* 2015). Transition metal components such as iron are particularly harmful as they have the potential to produce reactive oxygen species causing inflammation throughout the body (Zhou *et al.* 2003; Birben *et al.* 2012). A recent study by Maher *et al.* (2016) demonstrates that airborne magnetite can enter the brain directly via the olfactory bulb, which can foster Alzheimer disease.

With respect to Milan, Vecchi *et al.* (2008) found high iron PM concentrations, especially during the winter season (average of 0.0086 µg/m$^3$) compared to the summer season (average of 0.0042 µg/m$^3$). They also found that iron concentration in Milan is higher than in other Italian cities (Florence, Genoa). Iron can come from the abrasion of vehicle brake systems (Hoffmann *et al.* 1999, Sagnotti *et al.* 2006) and tram and train rails (Kam *et al.* 2011), as well as from metallurgical processes (Hunt *et al.* 1984). These iron particles bond up with oxygen upon entering the atmosphere to become iron oxides, such as magnetite ($Fe_3O_4$).

The aim of this study is to monitor airborne iron oxides distribution in a test site in Milan. We selected a municipal park surrounded by tram lines/stops and roads characterized by intense daily traffic, which represent obvious sources of iron PM, among other types of pollutants. We opted to sample tree barks as natural repositories (sinks) of these iron PM sources, and to study them with standard rock magnetic techniques to define type and concentration of ferro(i)magnetic iron oxide minerals contained therein. Tree barks are preferred to the more frequently used tree leaves (e.g. Matzka & Maher 1999; Hanesch *et al.* 2003; Moreno *et al.* 2003; Lehndorff *et al.* 2006; McIntosh *et al.* 2007; Szönyi *et al.* 2008; Maher *et al.* 2008), because they accumulate airborne PM the entire year. This is important because PM pollution levels are highest in the winter, when leaves of deciduous species, which are dominant in Milan, are absent. Tree bark is already considered as a valuable indicator for monitoring air quality, for instance by applying INAA and RXRFA (Instrumental Neutron Activation Analysis and Radionuclide X-Ray Fluorescence Analysis) techniques (e.g., Bohm *et al.* 1998; Pacheco *et al.* 2001), and measurements of trace element concentration (e.g., Sawidis *et al.* 2011; Guéguen *et al.* 2012). However, until now only a very limited number of studies have investigated the potential of measuring magnetic parameters of tree bark for monitoring air pollution (Flanders 1994; Kletetschka *et al.* 2003; Zhang *et al.* 2008; Kletetschka 2011). We are not aware of any previous biomonitoring studies conducted in this city, so we present the first results obtained through this approach for the selected study area. The ultimate goal of this study is to quantify on a map the areal dispersion of iron oxides from their sources (e.g., tram stops) to their sinks (tree barks). This could help designing pollution shielding solutions using non-deep tree belts or synthetic bark panels placed at optimal distance and orientation relative to pollution sources.

## Materials and methods

Study Area

This study was conducted in the 25,000 $m^2$ municipal park of Leonardo da Vinci Square (Fig. 1A), located in the eastern part of Milan, at an altitude of about 120 m a.s.l. (centre latitude: 45.478096°N; longitude: 9.22569°E). The area is characterized by intense daily traffic; on a typical working day, the number of cars passing through the study area ranges between 6000 and 7000 (Municipality of Milan, Agenzia Mobilità Ambiente Territorio), and trams circulate with a frequency of one every 3-8 minutes. For the purpose of this study, the area was divided in two portions: an external belt named Area 1, about 20 m wide and located close to the surrounding roads and tram lines/stops, and a more internal Area 2 extending more than 20 m away from the roads (Fig. 1A).

The results obtained in Milan are compared with data from a low-pollution control site. Sampling at the control site was conducted in the village of Santa Caterina Valfurva (hereafter Santa Caterina), belonging to the municipality of Valfurva, characterized by 2600 inhabitants, with very reduced car traffic and absence of trains and trams. The village is located 200 km north of Milan, in Valtellina (central Alps) at an elevation of about 1700 m a.s.l. (Fig. 1B), in a typical Alpine environment shaped by glaciers and mass wasting processes (e.g. Del Ventisette *et al.* 2012; Pelfini *et al.* 2014).

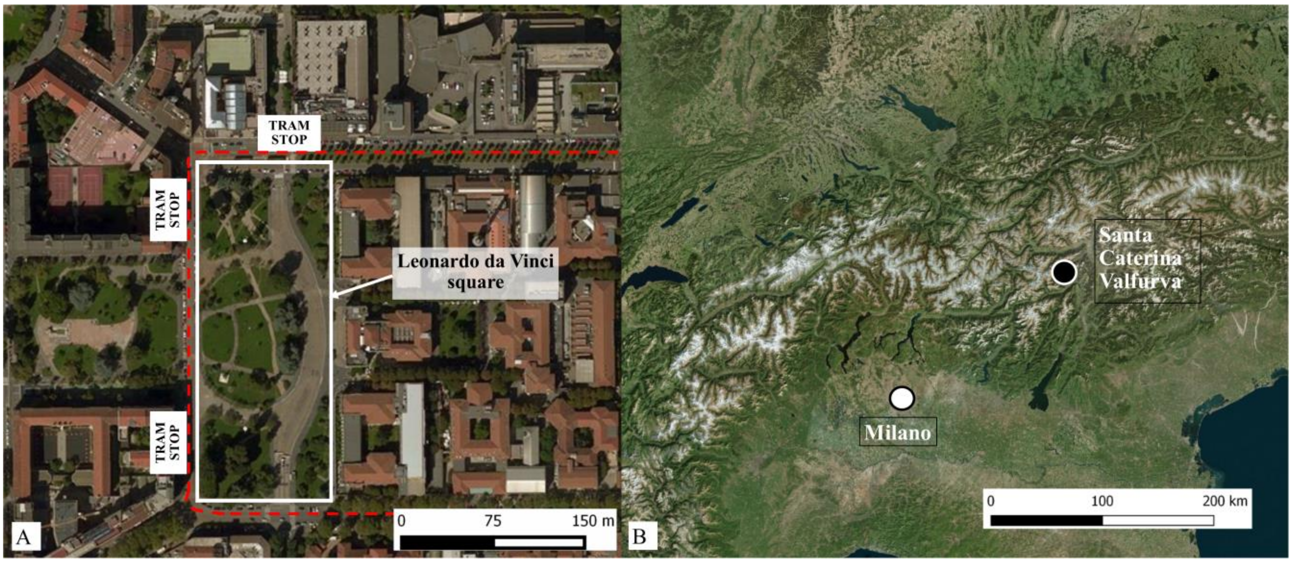


***Figure 1.*** *(A) Leonardo da Vinci square in Milan with the closest tram stops. The red discontinuous lines represent the closest roads. The sampling area was divided in two sections, here named Area 1 and Area 2, respectively closer (distance ≤ 20 m) and further (distance > 20 m) from the surrounding roads. (B) Location map of the study area including Milan and Santa Caterina Valfurva.*

<u>Sampling</u>

Sampling in Milan was conducted on the 22, 24 and 25 November, 2014 on trees belonging to 18 different species including, among others, *Prunus padus*, *Sophora japonica* and *Cedrus atlantica* (see Tab. 1 for a complete list). Sampling at the test site in Santa Caterina was conducted on 27 July 2015 on trees of *Picea abies* species. At both localities, the selected tree species show similar bark textures: fissured, rugose, and coarse-grained (Fig. 2). The absorption properties of these fractal surfaces on airborne micrometric particles are considered analogous, irrespective of taxonomic attribution.

A total of 147 trees were sampled in the Milan test site and 20 trees in the Santa Caterina control site. The position of each tree was recorded using GPS. Each sample consisted of a cylindrical fragment of trunk bark, extracted at approximately 1.5 m above the ground from the tree-side exposed in the direction of the closest street, using a steel extractor tool with a diameter of 2.5 cm. The bark samples were placed in 10-cm$^3$ plastic boxes and closed with plastic tops. For the Milan test site, a total of 99 out of 147 bark samples were cut in the laboratory, using a ceramic knife that we cleaned with ethanol after cutting each sample, into an outer disk sample (i.e., at the contact with the atmosphere) and an inner disk sample, which were labelled "A" and "B", respectively. In 48 particularly thick samples, up to a maximum of four disk samples were obtained ("A", "B", "C", "D" arranged from the outside to the inside; Fig. 3). The remaining 35 samples yielded only an outer "A" sample, i.e., sub-sampling was not possible. In total, 278 samples of trunk bark were obtained from the Milan test site. With regards the control site of Santa Caterina, we extracted one sample of outer bark from each tree for a total of 20 "A" bark samples. All samples were weighed in order to normalize the measurements by sample mass and then refrigerated at 5°C until the day before the analyses.

***Table 1.*** *List of species and the number of trees sampled for each species at the Milan test site.*

| Tree species | Number of sampled trees | Tree species | Number of sampled trees |
|---|---|---|---|
| *Abies alba* | 2 | *Ilex aquifolium* | 11 |
| *Acer negundo* | 5 | *Pinus nigra* | 4 |
| *Acer saccharinum* | 4 | *Populus alba* | 2 |
| *Catalpa bignonioides* | 9 | *Populus nigra* | 11 |
| *Cedrus atlantica* | 16 | *Prunus laurocerasus* | 4 |
| *Cercis siliquastrum* | 3 | *Prunus padus* | 32 |
| *Cupressus arizonica* | 4 | *Sophora japonica* | 21 |
| *Cupressus glabra* | 2 | *Taxus baccata* | 2 |
| *Fagus sylvatica* | 2 | *Tilia cordata* | 13 |

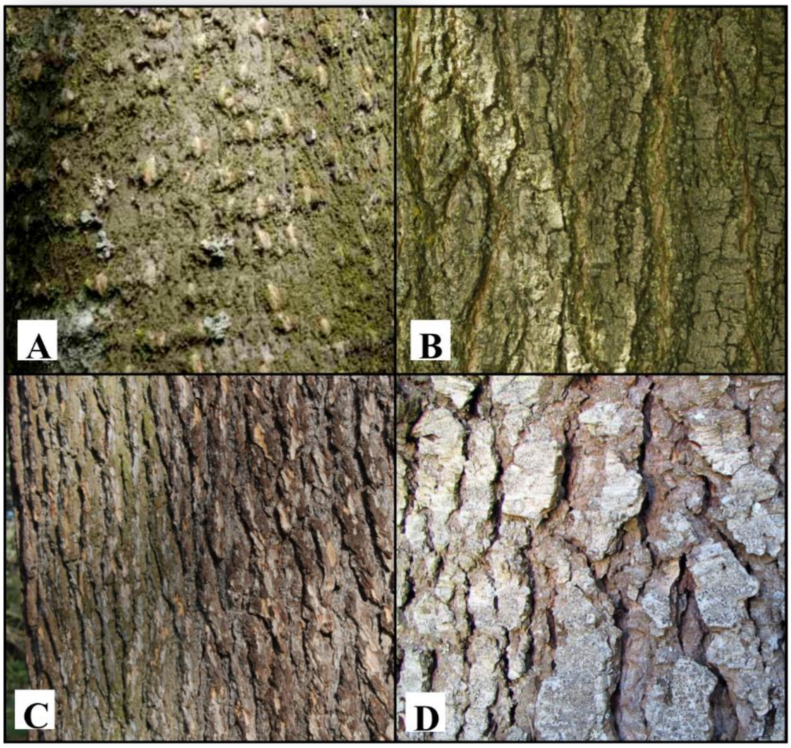


***Figure 2.*** *Tree bark texture of (A) Prunus padus, (B) Sophora japonica, (C) Cedrus atlantica and (D) Picea abies.*

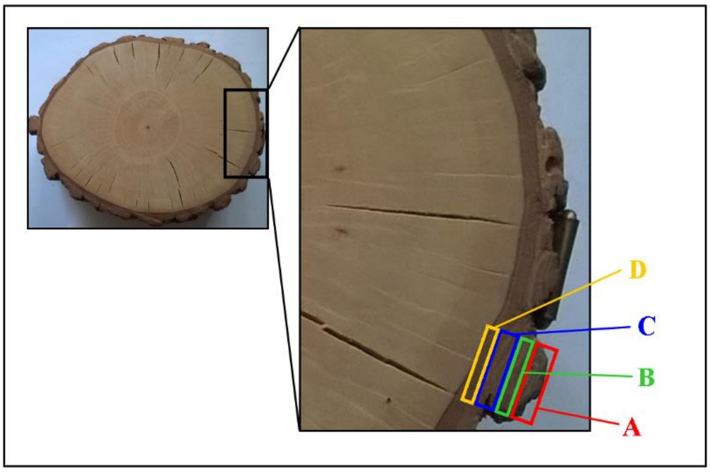


***Figure 3.*** *Explanatory image showing the position of tree bark samples from outer to inner bark, labelled "A", "B", "C", "D".*

Mineralogical analyses

The mineralogy of three “A” bark and two “B” bark samples from Milan was investigated by X-ray diffraction and electron microprobe analysis. X-ray micro-diffraction was performed in transmission geometry on aggregates of mineral particles with 30 to 150 µm size, extracted directly from the tree bark. Measurements were made on an Oxford X’Calibur instrument with Mo X-ray source ($\lambda$= 0.710 Å), polycapillary focusing optics (beam size on the sample apx. 150 µm) and CCD detector. The 2D diffraction data were integrated with the CrysalisRed software and the identification of mineral phases was first achieved by comparison with PDF-2 database, followed by Rietveld refinement. Chemical analysis was performed with an electron microprobe (JEOL JXA 8200), equipped with WDS spectrometers.

2.4 Magnetic analyses

The initial magnetic susceptibility of all samples was measured using an AGICO KLY-2 Kappabridge. A subset of 181 samples were then magnetized at room temperature in incremental fields up to 1 T or occasionally 2.5 T using an ASC Pulse Magnetizer. The resulting isothermal remanent magnetization (IRM) was measured after each step on a 2G Enterprises DC SQUID cryogenic magnetometer located in a magnetically shielded room. Mass-specific susceptibility and SIRM values were calculated. The S-ratio was calculated as $IRM_{300\ mT}$/SIRM (Evans & Heller 2003), where $IRM_{300\ mT}$ is the IRM induced in a field of 0.3 T and the SIRM is the IRM at 2.5 T. Hysteresis loops were measured on a subset of 16 representative “A” bark samples from the Milan test site, using a Micromag 2900 Alternating Gradient Magnetometer, on samples whose saturation IRM was > 5 x $10^{-6}$ $Am^2/Kg$. The obtained hysteresis loops were corrected for high field susceptibility to obtain the saturation magnetization ($M_s$) and saturation remanent magnetization

($M_{rs}$). Measurements were made at the Alpine Laboratory of Paleomagnetism (ALP) of Peveragno (Italy) and at the Laboratory of Natural Magnetism of ETH-Zürich (Switzerland).

2.5 Data mapping

Magnetic susceptibility and SIRM values from the Milan test site were plotted in GIS environment in order to map their areal distribution and distance relative to the surrounding roads and tram lines/stops. Since the sampled trees are relatively evenly distributed in the Milan test site, inverse distance weighted (IDW) interpolation was applied (Johnston *et al.* 2001).

**Results**

Mineralogical analyses

The X-ray diffraction analysis indicates that the most common mineral phase in the analyzed "A" bark samples is quartz. However, "A" bark samples with high susceptibility also contain magnetite ($Fe_3O_4$) and/or iron, and only XRD can detect them in weaker magnetic samples. In one sample, a fragment with metallic lustre was identified as hercynite ($FeAl_2O_4$), as shown by the powder patterns fitted with Rietveld method (Fig. 4).

The analysis on the electron microprobe on "A" bark samples confirms the presence of an iron oxide phase interpreted as magnetite with grain size of about 20 μm (Fig. 5 A). Secondary ion images reveal that some of the magnetite particles are well inserted in the "A" bark samples, and not just deposited on their external surface (Fig. 5 B, D). Moreover, some of the magnetite grains appear have undergone reductive dissolution (Fig. 5 C, D).

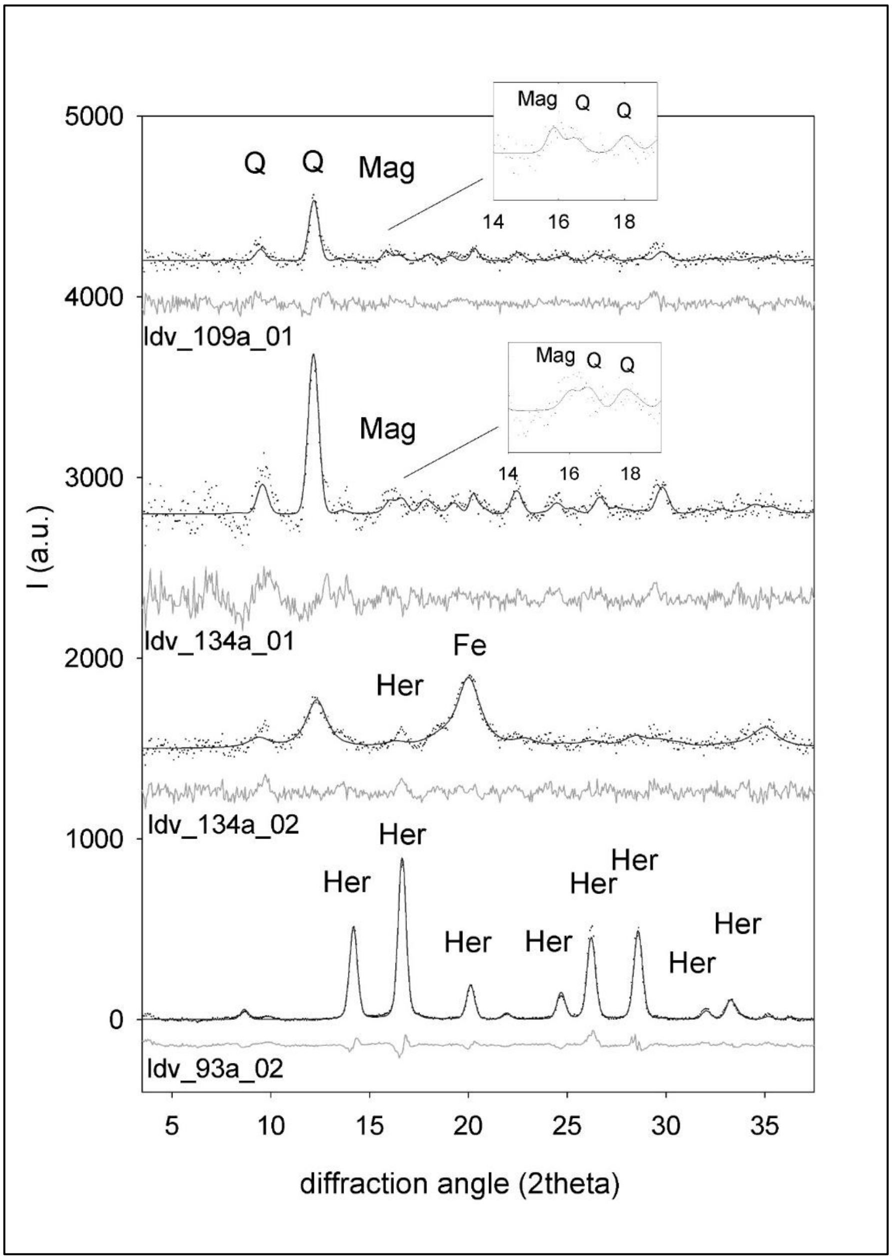


***Figure 4.*** *Experimental laboratory X-ray powder micro-diffraction pattern (dotted), fitted (line) and difference curve computed by Rietveld fit of selected fragments. The samples are labelled in the figure. The main diffraction peaks are indicated: quartz,* $SiO_2$ *(q), metallic iron (Fe), hercynite,* $FeAl_2O_4$ *(Her) and magnetite,* $Fe_3O_4$ *(Mag).*

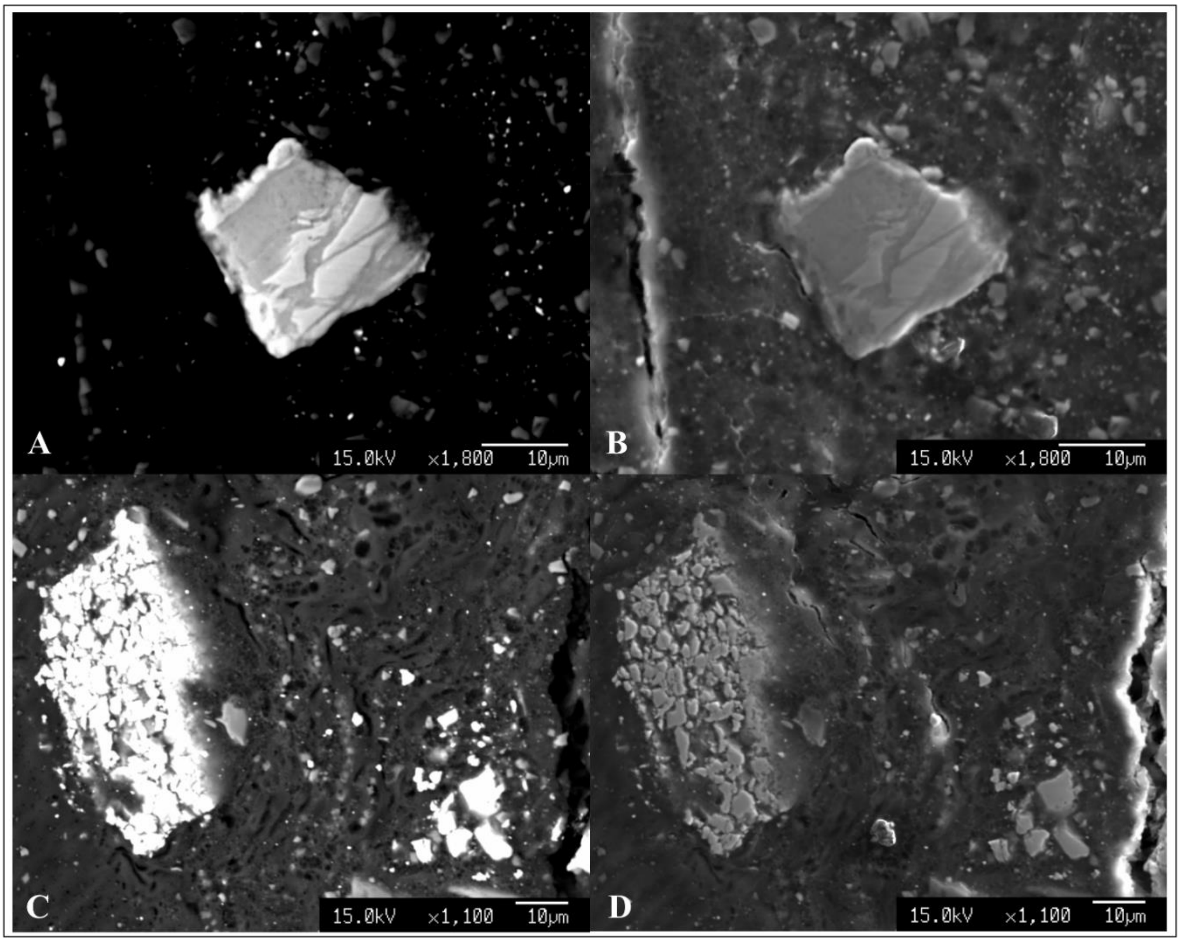


***Figure 5.*** *(A, C) Angular iron oxide particles found in "A" bark sample (outer bark) analyzed at the microprobe and (B, D) secondary ion image showing the integration of the particle in the "A" bark sample. The X-ray and rock-magnetic analyses showed that it consists of magnetite.*

<u>Magnetic analyses</u>

Samples from the Milan test site show a clear trend in the magnitude of magnetic susceptibility, which was found to strongly decrease from the outer "A" bark samples to the inner ("B", "C", "D") samples in the same tree (see Fig. 6 and Tab. 2). The majority of the inner "B", "C" and "D" samples are diamagnetic, which indicates that the organic matter signal dominates the susceptibility. No trend is instead detected within the various inner bark samples, i.e., the susceptibility of the "B" samples is not higher than the "C" or the "D" samples. The outer "A" bark samples from the control site at Santa Caterina show very low values of magnetic susceptibility, comparable to values found in inner "B" bark samples at the Milan test site (Tab. 2).

IRM acquisition curves of representative "A" bark samples from Milan and Santa Caterina show the presence of a low coercitivity magnetic phase that saturates in fields of ~200 mT, which is compatible with magnetite that was identified by X-ray diffraction (Fig. 7). A decrease in the magnitude of the saturation IRM (SIRM) from the outer "A" bark samples to the inner ("B", "C", “D”) bark samples is observed (Fig. 7 A – D; Tab. 2). The S-ratios show values approaching 1 with no differences detectable between “A” and “B” samples, confirming the presence of the low coercivity mineral, magnetite. The outer “A” samples from the control site at Santa Caterina have SIRM magnitudes that are much lower than the “A” samples from the Milan test site (Fig. 7 E, F; Tab. 2).

The magnetic susceptibility and saturation IRM values of “A” samples from the various tree species sampled at the Milan site show a high degree of variability and high standard deviations (Fig. 8 A, B). No statistical tests could be performed due to the very different number of samples available for each species, however, no clear intraspecific trends were observed as well. We stress that the dynamics of passive accumulation of micrometric airborne particles on an absorbing surface (bark) is controlled primarily by physical factors such as air turbulence, moisture, etc., provided the absorbing surfaces are characterized by similar macroscale rugosity and textures irrespective of their taxonomic attributions.

With regards to the hysteresis analyses, most of the 16 "A" samples from Milan showed an open loop with a varying contribution from the high-field paramagnetic components (Fig. 9 A, B, C). These features reflect the presence of a low coercivity ferrimagnetic mineral coexisting with a paramagnetic (positive slope) contribution. Some samples, however, show only a diamagnetic magnetization, due to organic material, i.e., bark (e.g. Fig. 9 D). The $M_{rs}/M_s$ ratios range between 0.07 and 0.14 (mean of 0.09, standard deviation of 0.02), which is compatible with pseudo-single domain (PSD) to multi-domain (MD) magnetite (Dunlop 2002). This agrees with microprobe observation.

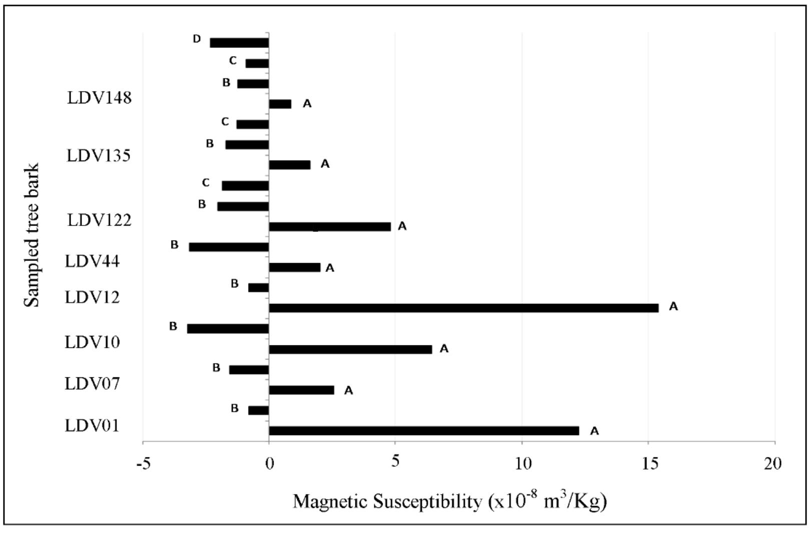


***Figure 6.*** *Mass-specific magnetic susceptibility values on representative samples of tree bark. In the reported examples, outer bark (named "A") always shows higher and positive susceptibility values than the inner bark (named "B", "C" and "D").*

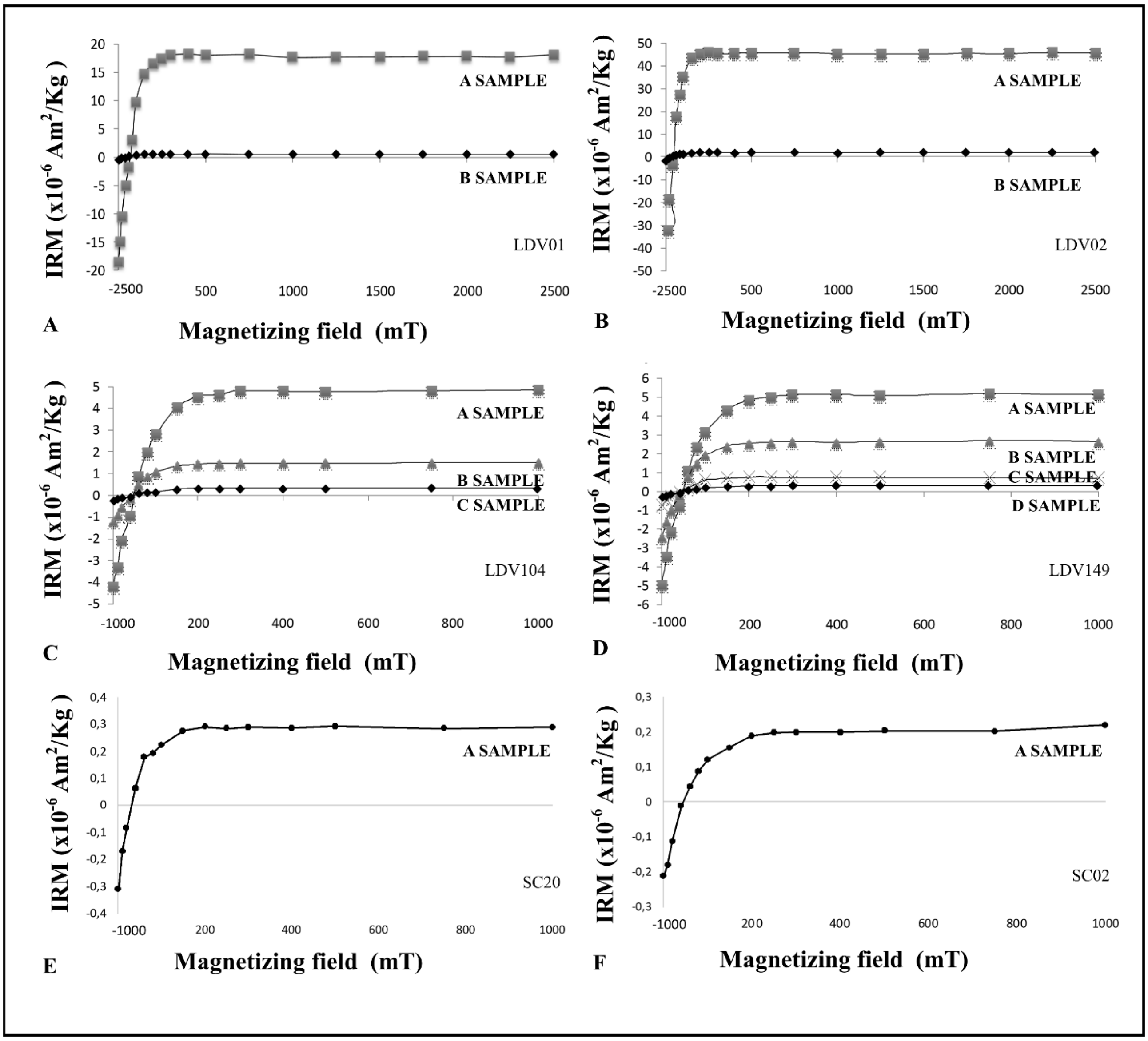


***Figure 7.*** *Mass-specific isothermal remanent magnetization (IRM) backfield acquisition curves on representative bark samples from the Milan test site (panels from A to D), showing the presence of variable amounts of a low coercivity magnetic component interpreted as magnetite. Outer "A" bark samples have always higher IRM values (higher magnetite concentration) than inner "B" or "C" or "D" bark samples. Panels E and F represents IRM acquisitions from two "A" samples from the control site at Santa Caterina.*

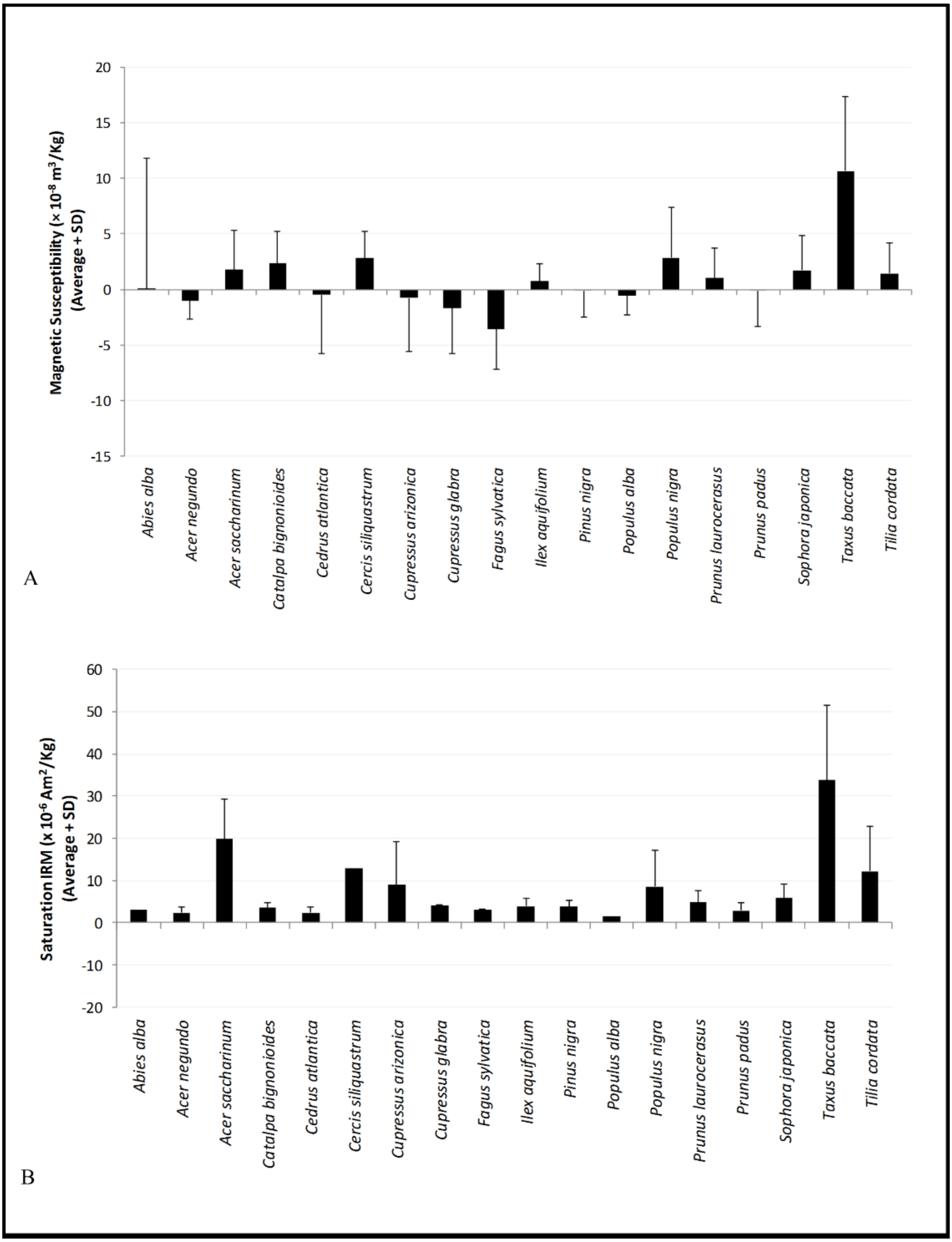


***Figure 8.*** *Magnetic Susceptibility (A) and Saturation IRM (B) for the sampled "A" tree species at the Milan site. High variability and SD can be observed for both parameters. In the case of Saturation IRM, some tree species do not show the SD value due to the analysis of only one sample for that species.*

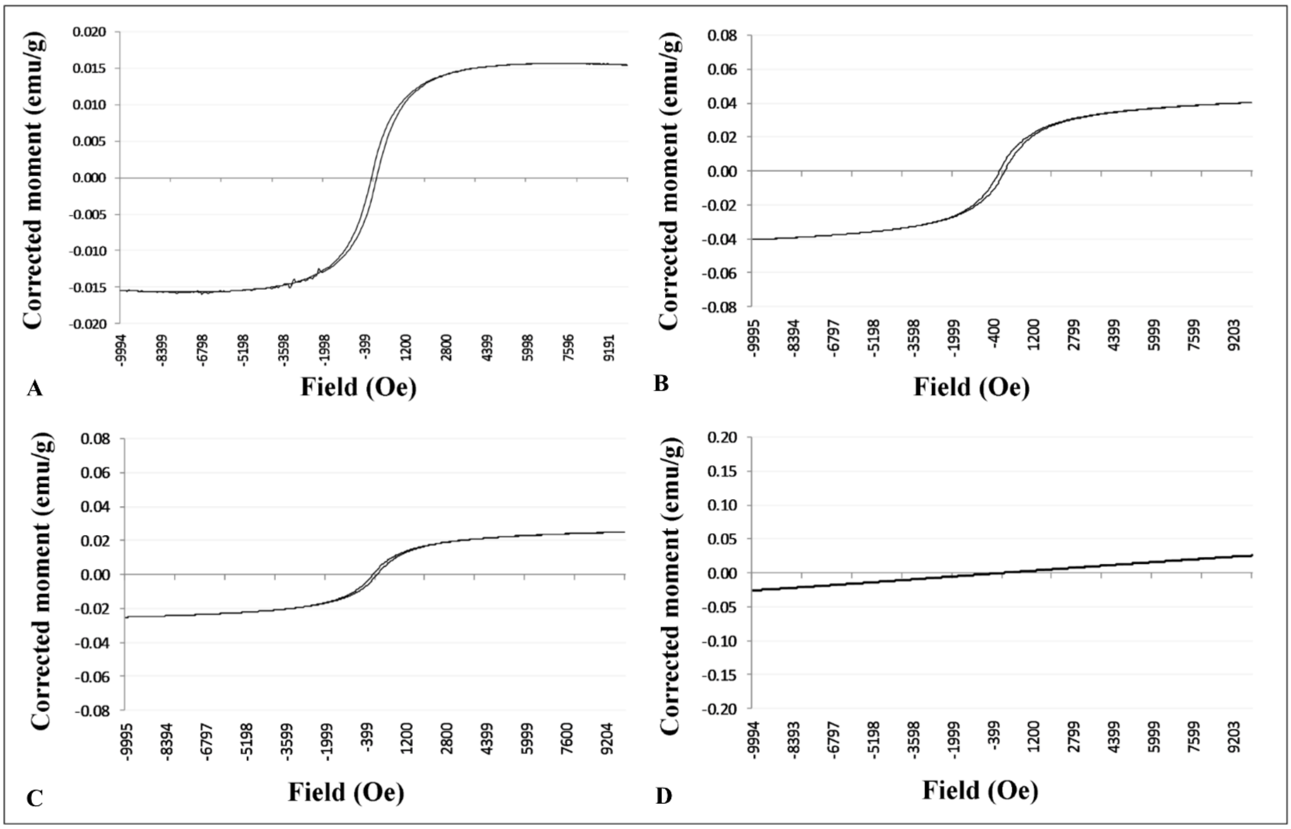


***Figure 9.*** *Hysteresis loops on four representative samples. (A, B, C) Most of the analyzed samples show hysteresis, (D) example of sample that only displays paramagnetic mineralogy.*

***Table 2.*** *Average values of magnetic susceptibility and SIRM measured on the analyzed samples. For bark layers A and B, we distinguished between Area 1 (closer to the roads, i.e. < 20 m) and Area 2 (farer from the roads, i.e. > 20 m).*

| Measured parameter | Bark layer | Area | Average ± SD | Number of samples |
|---|---|---|---|---|
| Magnetic Susceptibility | A | 1 | 1.25 ± 4.2 | 60 |
| (× $10^{-8}$ $m^3$/Kg) | | 2 | 0.52 ± 3.5 | 87 |
| Milan | B | 1 | -1.31 ± 1.9 | 38 |
| | | 2 | -2.26 ± 2.8 | 65 |
| | C | | -1.32 ± 1.7 | 16 |
| | D | | -1.76 ± 0.7 | 3 |
| Magnetic Susceptibility (× $10^{-8}$ $m^3$/Kg) Santa Caterina | A | | -9.87 ± 0.59 | 20 |
| SIRM (x $10^{-6}$ $Am^2$/Kg) | A | 1 | 15.53 ± 18.5 | 42 |
| Milan | | 2 | 9.88 ± 10.2 | 40 |
| | B | 1 | 2.23 ± 2.5 | 42 |
| | | 2 | 2.06 ± 3.4 | 25 |
| | C | | 1.56 ± 2.1 | 10 |
| | D | | 0.45 ± 0.2 | 2 |
| SIRM (x $10^{-6}$ $Am^2$/Kg) Santa Caterina | A | | 0.21 ± 0.16 | 20 |

Mapping of magnetic grains distribution

Distribution maps of magnetic susceptibility and SIRM values for the Milan test site are shown in Figures 10 and 11. The sample locality is subdivided into Area 1, which is within 20 m of the roads and Area 2 with is > 20 m from the roads. The map of magnetic susceptibility for "A" bark samples (Fig. 10 A) shows higher mean values in Area 1 compared to Area 2, with the highest values near tram stops. The low susceptibilities in Area B are comparable to those observed at the control site in Santa Caterina (SC on scale bar in Fig. 10 A). The spatial distribution of susceptibility of the inner "B" samples shows little variability: there is no notable difference between Area 1 and 2, although the “B” samples with highest susceptibility are found at the same location where the “A” sample has high susceptibility (Fig. 10 B).

A similar pattern is also found in the spatial distribution of the SIRM, which shows higher mean values for "A" samples in Area 1, especially near tram stops, while "A" samples from Area 2 display lower values, which are comparable to the control site in Santa Caterina (Fig. 11 A). Inner "B" samples from both Areas 1 and 2 display low SIRM values and no relationship with distance from tram lines/stops (Fig. 11 B).

A positive linear relationship between magnetic susceptibility and SIRM was obtained for "A" samples in Area 1 ($R^2$= 0.89; p = 2.4 x $10^{-4}$; Fig. 12 A). A similar positive linear relationship exists also for "A" samples from Area 2, but at a lower statistical level ($R^2$= 0.18; p = 3.8 x $10^{-5}$; Fig. 12 A). No statistical correlation is found for the "B" samples in both Area 1 and 2 (Fig. 12 B). A positive relationship between susceptibility and SIRM can indicate variation in the volume fraction of the ferro(i)magnetic phase(s), and this variation could be caused by a higher number of particles with same size or by an increase in grain size.

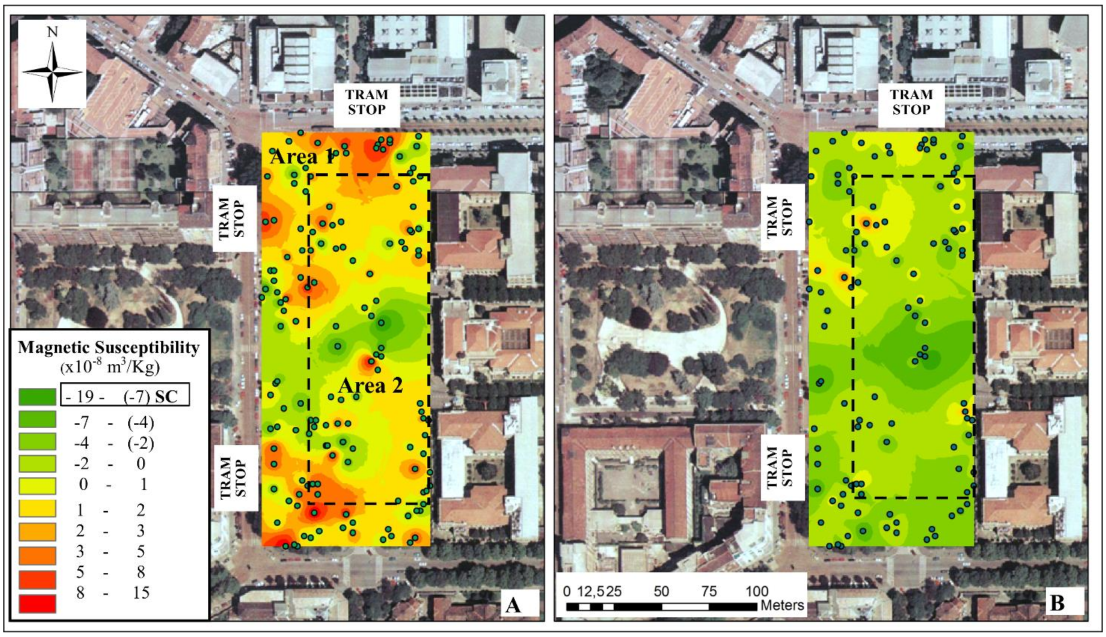


***Figure 10.*** *Spatial distribution (defined using IDW interpolation in GIS environment) of the magnetic susceptibility values of outer "A" bark samples (panel A to the left) and inner "B" bark samples (panel B to the right) recorded at the test site in Milan. The highest values are recorded in "A" bark samples obtained from trees in Area 1, closer to the roads (distance ≤ 20 m) and, in particular, closer to the tram stops. Samples from the control site at Santa Caterina (SC on color scale in panel A) are characterized by low values, belonging to the lowest category detected in the study area in Milan.*

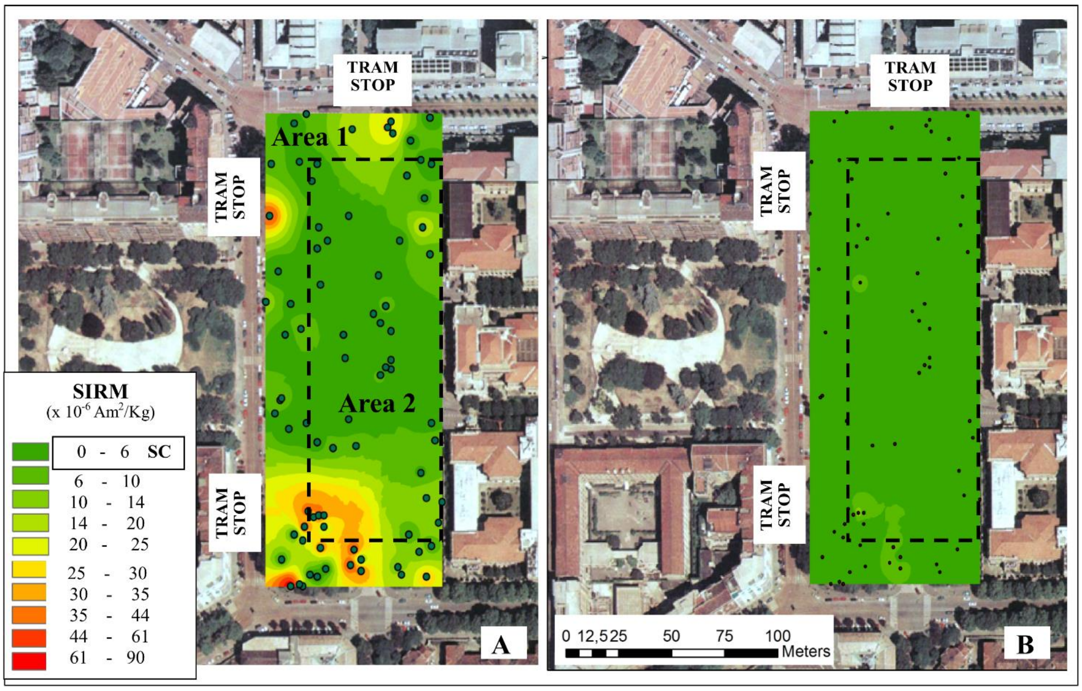


***Figure 11.*** *Spatial distribution (defined using IDW interpolation in GIS environment) of the Saturation IRM (SIRM) values in outer "A" bark samples (panel A to the left) and inner "B" bark samples (panel B to the right) recorded at the test site in Milan. The highest values are recorded in "A" bark samples in Area 1, closer to the roads and tram stops (distance ≤ 20 m). Samples from the control site at Santa Caterina (SC on color scale in panel A) are characterized by values belonging to the lowest category detected in the study area in Milan.*

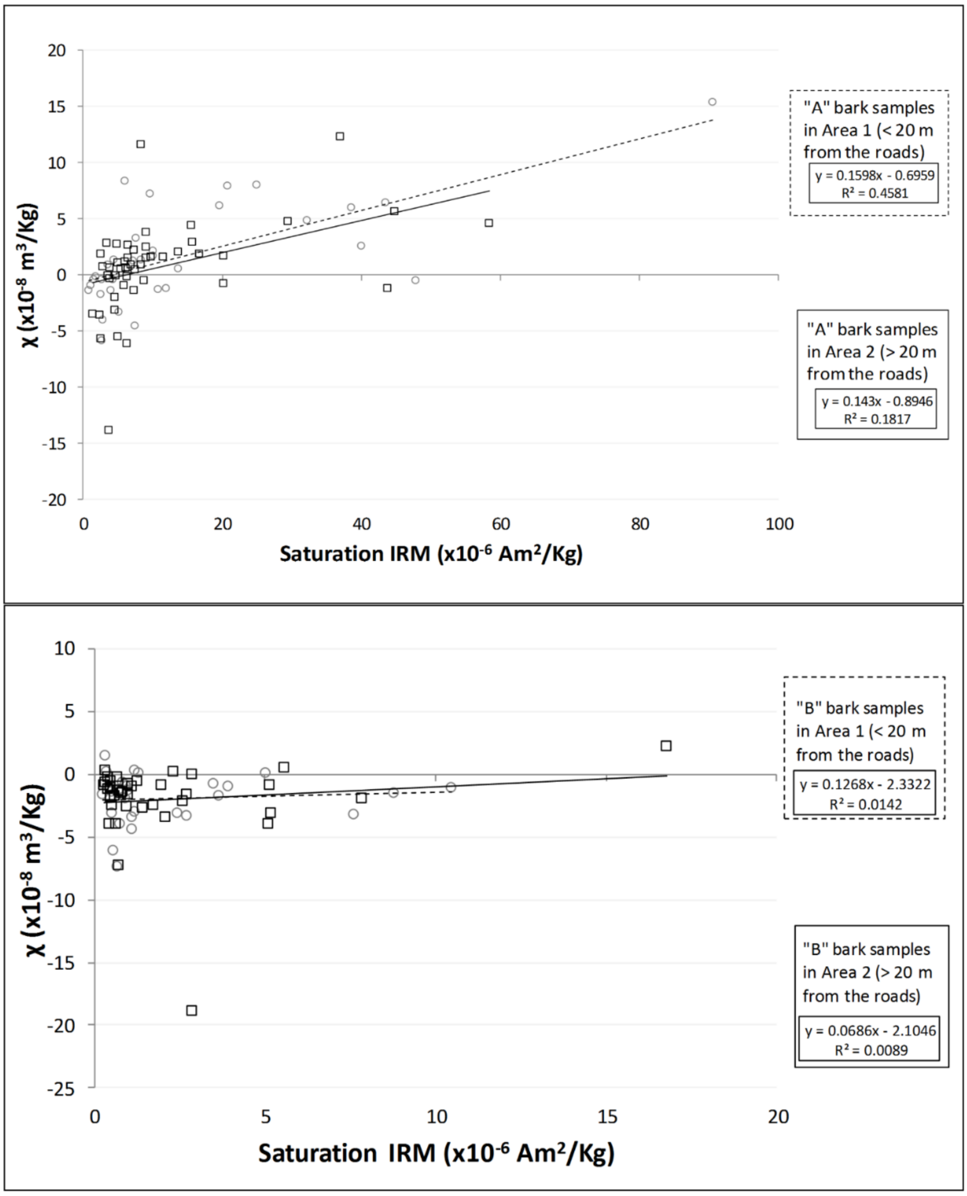


***Figure 12.*** *Plot of saturation IRM versus magnetic susceptibility values, calculated for (A) outer "A" bark and (B) inner "B" samples of trees sampled in Area 1 (discontinuous line) and in Area 2 (continuous line).*

## Discussion and conclusions

X-ray diffraction, microprobe, IRM acquisition, S-ratios and hysteresis measurements all indicate that magnetite is the main magnetic mineral that is deposited at the atmosphere/bark interface and incorporated in outer "A" bark samples. Due to the high number of different tree species and their distribution at the Milan site, a statistical analysis between the SIRM and magnetic susceptibility values found in the different species was not performed. The differences detected between different tree species and within the same species (see Fig. 8) are probably due to the tree location that is very sparse in the selected area. Thus, considering that all the tree species sampled in this study show similar tree bark patterns, the evaluation of the results obtained for the analysed parameters was based on their spatial distribution. The spatial distribution of susceptibility, SIRM and magnetite density shows a strong dependence on distance from roads with traffic and more importantly tram stops. This suggests that the main sources of magnetite in the Milan test site are abrasion of tram braking systems and vehicle combustion processes. Outer "A" bark samples from Area 1 closest to roads and tram stops have the highest susceptibility and SIRM, and therefore highest magnetite concentration. Outer "A" bark samples from Area 2 have values that are comparable to the substantially pollution-free Santa Caterina control site.

Our results are in agreement with previous biomonitoring investigations conducted on tree leaves, which also identified magnetite as the main carrier of the magnetic signal (e.g., Moreno *et al.* 2003; Urbat *et al.* 2004; Mitchell & Maher 2009). They also are compatible with other studies, which identified trams and vehicles as the main sources of the magnetic particulate (e.g., Kardel *et al.* 2012). What it is important to note from our results is that the susceptibility and SIRM values in inner "B" samples are invariably very low (susceptibility being frequently diamagnetic) and close to the susceptibility and SIRM values measured at the Santa Caterina control site. This finding has important implications on how trees serve as sinks for PM. Airborne magnetite particles will be collected through deposition on the exposed outer “A” bark of trees immediate to the PM source. This magnetite is then partially encapsulated into the inner bark ("B", "C" and "D" samples), probably as far in as the suber tissue, where it then undergoes reductive dissolution (Catinon *et al.* 2008; Zhang *et al.* 2008; Catinon *et al.* 2009). Electron microprobe analyses support this hypothesis, showing that at least some of the magnetic particles are not just deposited on the bark external surface but are well integrated in it. Some images show also a partial fragmentation of the magnetite minerals, suggesting that plant physiological processes may dissolve or disintegrate magnetite particles incorporated in the bark, as observed for other types of atmospheric particles (e.g., Freer-Smith *et al.* 2004; Novak *et al.* 2006).

The implication that trees are capable of decomposing magnetite in their bark, emphasizes their role as pollution mitigating organisms. In this respect, this study is of interest for urban planning of green areas and infrastructures (e.g., Tong *et al.* 2016). Our results suggest that placing tree belts near roads with traffic and tram stops would help absorb airborne micrometric magnetite particles thus improving general air quality. Taking this idea further, it may be possible to design panels of synthetic, rugose bark that can be implemented either in lieu of - or together with - tree belts to improve trapping efficiency. Therefore, in conclusion, urban parks, tram stops, and urban railways should always be enclosed in a dense network of tree belts and/or synthetic bark panels. In this way, even highly populated areas could still preserve oases with PM levels not particularly higher than a mountain village, at least relative to the types and sources of PM described here.

**Acknowledgements**

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## 7. GENERAL DISCUSSION AND CONCLUSION

Even if the described approaches were only applied to a limited number of sites, the results of the studies here reported show that trees are valuable indicators of climatic and environmental change, as well as detectors of anthropogenic magnetic particles released in the atmosphere.

In particular, mapping tree distribution through remote sensing techniques, analysing tree-ring width, tree-ring stable isotopes, needle and tree-ring volatile terpenes may contribute to the detection of areas characterized by climatic and geomorphological stress at high altitudes. Magnetic and mineralogical investigations of tree barks can be used to define the distribution of airborne particulate matter in urban areas.

These studies also allowed the detection of disturbing factors that are important to consider in dendroclimatic reconstructions: changes in the water-level of a glacial lake, the surface instability of a debris-covered glacier and fungal infections all determine a variation in tree-ring width that can lead to a wrong interpretation of dendroclimatic reconstructions.

In figure 1 I reported a scheme describing the main results obtained in this Ph.D. project and their implications.

Future research will aim at better address a methodology for detecting supraglacial trees, testing the method that I assessed on other debris-covered glaciers, in Italy and in other countries.

Furthermore, the promising results obtained in the analysis of volatile terpenes in tree rings offer the possibility to investigate other factors damaging tree tissues, e.g. mechanical wounding. The analysis of resin terpenes, together with the dendrochronological approach that is already proven to be effective, could be useful for dating the impact due, for instance, to gravitative events, and for better understanding how trees react to mechanical wounding.

Finally, further research is needed for assessing the use of tree bark for monitoring the distribution of magnetic particles in the air. Choosing a wider urban area, and comparing different arboreal species, could help understanding if there are differences in the collected particles due to the bark texture, and in this way the most efficient tree species could be detected to be used for urban planning purposes.

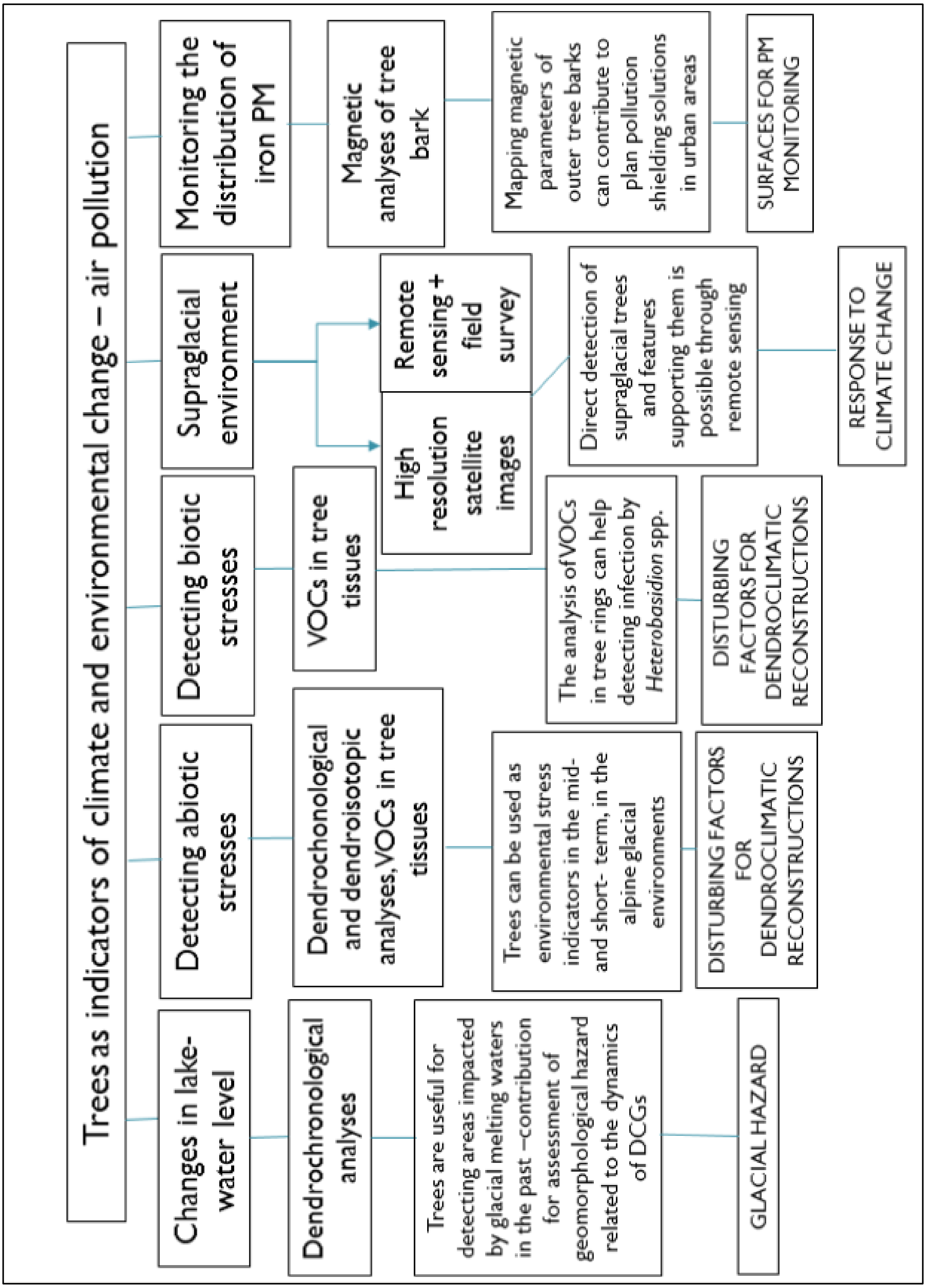


***Figure 1.*** *General scheme reporting the main subjects, methods, results and implications of my Ph.D.*

## 8. CONFERENCE PAPERS

"Giornate della Sostenibilità, Focus Ambiente" – Milano, 21/03/2014

# DOVE "LEGGIAMO" I SEGNALI DEL CAMBIAMENTO CLIMATICO?

**CI SONO INDICATORI EVIDENTI...**



**GHIACCIAI**
Riduzione del volume dei ghiacciai registrata a partire dal 1850

**AREALI OCCUPATI**
Variazione degli areali occupati da animali e piante

**ATMOSFERA**
Incremento delle concentrazioni di anidride carbonica
Incremento delle temperature medie globali

**MARI E OCEANI**
Incremento del livello di mari e oceani

**MA CI SONO ANCHE INDICATORI "NASCOSTI"...**

# ALBERI

## DENDROCRONOLOGIA

Gli alberi crescono formando una serie di ANELLI CONCENTRICI, ciascuno corrispondente a un anno di vita.
Lo studio delle caratteristiche (dimensionali e strutturali) degli anelli di crescita fornisce dati scientifici per effettuare ricostruzioni dell'**ambiente** e del **clima** passati: per esempio, variazioni a scala locale delle condizioni di temperatura e precipitazione, delle proprietà del suolo, della stabilità del substrato su cui l'albero cresce portano a modificazioni dell'ampiezza degli anelli, a variazioni a livello di cellula vegetale e ad anomalie di crescita visibili nelle serie di anelli.
Un clima temperato favorisce la produzione di anelli piuttosto ampi, mentre un clima secco porta alla formazione di anelli più stretti. Mettendo a confronto le serie degli anelli di alberi cresciuti in epoche diverse è così possibile ricostruire l'andamento del clima nel passato.
Grazie alla loro longevità, gli alberi rendono possibile l'ottenimento di informazioni ambientali e climatiche relative a più di mille anni fa e, talvolta, persino a più di 10.000 anni fa.

## COMPOSTI ORGANICI VOLATILI

Tutte le piante emettono componenti chimici detti composti organici volatili (VOCs) sia durante i regolari processi metabolici sia in condizioni di forte stress, legato soprattutto alle elevate temperature, alla scarsità di luce e di umidità. Il monitoraggio delle emissioni di questi composti dalle foglie degli alberi rappresenta un valido contributo all'analisi degli effetti delle variazioni climatiche in atto sugli alberi.

## ISOTOPI STABILI

Gli alberi sono archivi di elementi che ricavano dall'ambiente circostante, quali carbonio, ossigeno, idrogeno e azoto, e che fissano negli anelli di accrescimento annuali. I rapporti degli isotopi stabili variano in relazione alle condizioni climatiche e ambientali, che possono essere così ricostruite per le epoche passate. Per esempio, un valore più elevato del rapporto $^{13}C/^{12}C$ è indice di stress legato all'alta temperatura e alla carenza di acqua per l'albero, mentre il rapporto $^{18}O/^{16}O$ è indicativo del tipo di acqua assimilata dalle radici dell'albero. Elaborate procedure di laboratorio permettono di analizzare i rapporti isotopici per ciascun anello dell'albero e, di conseguenza, per ogni anno a disposizione.

## ECESI E LIMITE DEGLI ALBERI

In ambiente glaciale e su superfici interessate da fenomeni vulcanici, l'ecesi è il processo di colonizzazione e crescita di piante e animali in habitat di nuova formazione. Il cambiamento climatico rende accessibili agli organismi viventi nuovi ambienti, ad esempio la superficie dei ghiacciai per gli alberi.

Recenti monitoraggi rivelano che variazioni del clima sono responsabili di modificazioni della quota massima a cui si possono trovare gli alberi: temperature maggiori favoriscono l'innalzamento del limite degli alberi.

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Vezzola Laura, Pelfini Manuela
Dipartimento di Scienze della Terra "A. Desio"
Università degli Studi di Milano

SGI-SIMP Conference "The Italian geosciences of the future, the future of the Italian geosciences" – Milano, 10/09/2014 – 12/09/2014

**Abstract (Poster Presentation)**

Dendrochronological and dendroisotopic patterns from trees affected by glacier meltwater: the case study of Lago Verde ice-contact lake (Miage Glacier, Italy)

Vezzola L.C. [1], Leonelli G. [1], Pelfini M. [1]
[1] Department of Earth Sciences, University of Milan (Italy)

An increase of the glacier runoff in glacierized mountain catchments has been largely recognized as a consequence of the increasing temperature trends since the last century which induce an intensification of the melting processes. Even if less impacted and with different responses to the climatic inputs, also debris-covered glaciers like e.g. the Miage Glacier (Mt. Blanc Massif, Western Italian Alps), undergo these dynamics. In particular, the debris coverage of the glacier surface is responsible of changes in ablation rates and in meltwater discharge, which typically influence glacier streams, ice-contact and proglacial lakes. When the tongue of debris-covered glaciers reaches altitudes below the treeline, trees may colonize the surroundings of the glacier terminus as well as the glacier surface, thus opening the possibility of assessing, for instance, the influence of lake water-level changes and of past glacier runoff events.
Recently, some researches have been carried out in the proglacial area of the Miage Glacier, on Larix decidua Mill. specimens located close to a small ice-contact lake called Lago Verde, characterized by a great water variability inducing frequent conditions of partial tree submersion. The results show that i) lake water-level changes negatively influence tree-ring growth: trees frequently reached by the lake water show narrower tree rings compared to trees located farther from the lake shores. Moreover, a positive relationship between the residual chronology at the treeline and June temperature was detected, whereas a weaker relationship was found at the Lago Verde, and this pattern may be related to the lower altitude of the Lago Verde, compared to the treeline. ii) Tree-ring cellulose of trees fed by glacial meltwater is significantly more depleted in $\delta^{18}O$ than the one of trees fed only by precipitation (Leonelli et al., 2013).
Overall, the signals of environmental changes detected in the tree rings around the lake opens the possibility to characterize the areas mostly affected by lake water-level changes on a temporal scale and of reconstructing past major glacier runoff events on the medium- to long-term.

References:

Leonelli G., Pelfini M., Battipaglia G., Saurer M., Siegwolf R.T. & Cherubini P. 2013. First detection of glacial meltwater signature in tree-ring $\delta^{18}O$: reconstructing past major glacier runoff events at Lago Verde (Miage Glacier, Italy). Boreas, DOI 10.1111/bor.12055.

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Milano 10-12 Settembre 2014

Vezzola L.C., Leonelli G., Pelfini M.

# DENDROCHRONOLOGICAL AND DENDROISOTOPIC PATTERNS FROM TREES AFFECTED BY GLACIER MELTWATER: THE CASE STUDY OF LAGO VERDE ICE-CONTACT LAKE (MIAGE GLACIER, ITALY)

## BACKGROUND & AIMS

Tree vegetation affected by hydrologic regime changes represents one of the most significative indicators of high mountain environmental changes induced by climate in glacialized areas.
The Lago Verde is located in the internal margin of the southern lobe of the Miage Glacier (Mont Blanc Massif, Aosta Valley, Italy). It is characterized by several and sudden water-level changes throughout the years and during different months of the same year, presumably due to variations in the subglacial drainage of the lake basin.

Tree vegetation is present near the lake shore, including plants submerged by water during several months of the year. Most of the trees around the lake are alive, but some dead trees can also be found. The main tree species observed in this area are *Larix decidua* Mill., *Picea abies* L. Karst and *Salix* spp.

The main **aims** of this study were:

1) To investigate if trees frequently submerged by glacial melting water show characteristic growth trends and typical isotopic signature in the tree-ring cellulose;

2) To analyze the climatic signals in the tree-ring chronologies obtained from trees fed by the lake water and from trees located farther from the lake.

## METHODS

Three sites were selected at the Lago Verde: **LVW1**, where trees are frequently submerged by the lake water, **LVW2**, where trees are less impacted by high water level, and **LVM**, where trees are not affected by the lake water. A fourth site named **VVT** was considered, using data obtained from trees located at the treeline in the Veny Valley, at about 2100 m.

Trees of the species *Larix decidua* Mill. were sampled using a Pressler's increment borer, tree-ring chronologies were then obtained and tree rings were measured and dated.
A residual chronology for LVW2, LVM and VVT was obtained and the climatic signal for these chronologies was evaluated over the period 1920-2005. The correlation with master and the Glk index were calculated for LVW1, LVW2 and LVM.

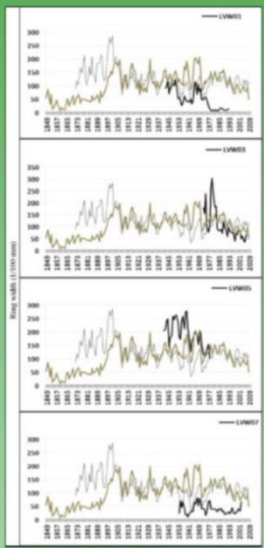
$\delta^{18}O$ and $\delta^{13}C$ isotope analysis for the cellulose of the total rings for LVW1 and LVM was also conducted.

**REFERENCE:**

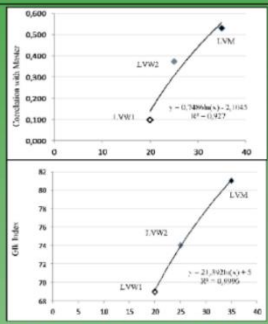
Leonelli G., Pelfini M., Battipaglia G., Saurer M., Siegwolf R.T.W. & Cherubini P. 2013. First detection of glacial melting water signature in tree-ring $\delta^{18}O$: reconstructing past major glacier runoff events at Lago Verde (Miage Glacier, Italy). Boreas, DOI 10.1111/bor.12055.

## RESULTS

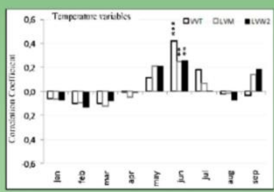
Trees at the LVW1 site generally show narrower tree rings compared to trees at both LVW2 and LVM sites, and most of them are dead.



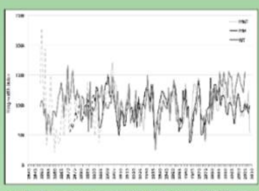

Some examples of tree-ring chronologies obtained at the LVW1 site compared to LVW2 and LVM chronologies.



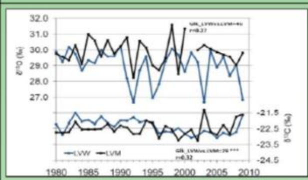

The correlation with master and the Glk index calculated for LVW1, LVW2 and LVM show an increasing trend at increasing distances from the centre of the lake.




There is a positive relationship between the residual chronology at the treeline (VVT) and June temperature, and a weaker relationship at the Lago Verde (LVM, LVW2).



The residual chronologies LVW2, LVM and VVT show similar tree-ring growth trend, with wide tree-ring width variations along the whole chronologies.




LVW1
LVM

Tree-ring cellulose of trees fed by glacial meltwater is significantly more depleted in $\delta^{18}O$ than the one fed only by precipitation.

## CONCLUSIONS

The results show that glacial lake water influences tree-ring growth and tree-ring $\delta^{18}O$ signature and, in particular, **changes in melting water-level represent an environmental disturbance for the trees**.
Trees influenced by the glacial meltwater of the lake show narrower and more variable tree rings compared to trees growing farther from the lake: high levels of glacial melting water represent an important growth stress and cause lower growth rates and production of narrower tree rings, at least during the last years of a tree's life.
Trees fed by glacial meltwater show a lower $\delta^{18}O$ signature with respect to the trees located on the moraine, that are fed only by precipitation waters.
The positive relationship between the VVT chronology and June temperature could be due to the high altitude (2100 m a.s.l.) of trees located at the treeline, in fact trees at the LVW2 and LVM sites, both located at 1800 m a.s.l., show a weaker signal.

Since tree rings register the disturbances due to the presence of glacial melting water, **the reconstruction of the past water-level changes of ice-contact lakes and the detection of past major glacier runoff events** through dendrocronological and dendroisotopic analyses may potentially be conducted, when trees are present.

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Italian Glaciological Committee Conference "The future of the glaciers: from the past to the next 100 years" – Torino, 19/09/2014

**Abstract (Poster Presentation)**

A first approach to detect supraglacial vegetation coverage on debris-covered glaciers using aerial photographs and satellite images: the case study of Miage Glacier

Vezzola L.C.*[1], D'Agata C.[1], Leonelli G.[1], Vagliasindi M.[2], Azzoni R.S.[1], Smiraglia C. [1], Diolaiuti G.[1] and Pelfini M. [1]

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Debris-covered glaciers are becoming a new habitat for vegetation including trees, which germination and growth are controlled by debris layer thickness and grain size, by surface velocity and stability and by the altitude of the glacier tongue. The progressive colonization of debris-covered glacier surface performed by trees is a response to climatic and environmental changes that may be further investigated in trees located in crucial study sites, where the effects of these changes are evident. For this reason, the need for a method that allows the rapid detection of supraglacial trees is increasing.

In this study we present the first results of the identification of supraglacial tree coverage located on the Miage Glacier (Mont Blanc Massif), using aerial photos and satellite images.

Two methods were tested.

1) A semi-automatic method was attempted on aerial images from 2005. Two training classes of pixels were selected on the glacier terminus area, one corresponding to the debris and the other one corresponding to the vegetation, in order to perform a supervised classification using maximum likelihood algorithm.

2) The comparison between the areas characterized by the presence of vegetation, identified through the analysis of aerial images and the direct observation conducted in the study area, and the data of supraglacial temperature, altitude, moisture and thickness of debris, obtained from satellite images, was performed, in order to find a correlation between vegetation presence and these variables.

The main problems concerning the discontinuous distribution and the relatively reduced size of the vegetation (regarding both its height and its canopy) in the identification of supraglacial trees have been discussed in order to identify a rapid but accurate method of investigation.

International Symposium on
**The Future of the Glaciers**
From the past to the next 100 years
Turin, Italy
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Comitato Glaciologico Italiano

UNIVERSITÀ DEGLI STUDI DI MILANO

# A first approach to detect supraglacial vegetation coverage on debris-covered glaciers using aerial photographs and satellite images: the case study of Miage Glacier

**Vezzola L.C.*[1], D'Agata C.[1], Leonelli G.[2], Diolaiuti G.[1], Azzoni R.S.[1], Smiraglia C.[1], Vagliasindi M.[3] and Pelfini M.[1]**

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## INTRODUCTION AND AIMS OF THE STUDY

**Trees located on the surface of debris-covered glaciers represent an important data source for reconstructing the recent dynamics of the glacier surface and of vegetation.** Supraglacial trees may only germinate when debris is thick enough and locally stable.

Plant and tree distribution on debris-covered glacier surface is therefore of great interest in order to further investigate the ongoing responses to climate change and to rapidly monitor the impact of glacier dynamics on vegetation.
The aim of this research is to test rapid methods to detect tree presence in order to better address field work.

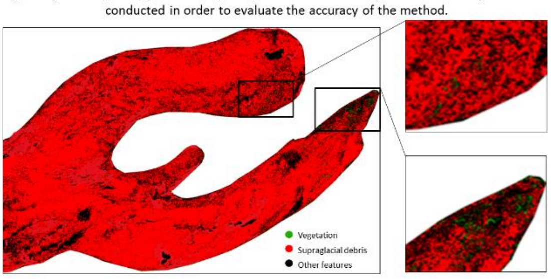
**Two methods have been tested on the Miage Glacier and the first results are here presented**

## STUDY AREA

The Miage Glacier (Mont Blanc Massif) in the Aosta Valley is the largest debris-covered glacier in the Italian Alps. It is about 11 km long and its terminus shows continuous debris cover from 1770 m a.s.l. up to 2400 m a.s.l., with thickness varying from a few centimeters up to 1.5 m: increasing debris thickness is generally detected with decreasing elevation. Trees are mainly located in the lower part of the glacier tongue and the dominant species are *Larix decidua* Mill. and *Picea abies* Karst. Trees taller than 1 m are more present at the glacier termini of the southern lobe. Currently, the oldest supraglacial living trees are 60-70 years old, and different tree density is observed on the glacier tongue (Pelfini *et al.* 2012).

## SEMI-AUTOMATIC METHOD

Colour ortophotos (2005 flight) with a pixel size of 0.5 m were analyzed using the ENVI software. The glacier terminus part of the image was extracted and three Regions Of Interest (ROIs) were selected based on the colour of the pixels, one corresponding to the debris covering the glacier surface, one corresponding to the supraglacial vegetation and the third one corresponding to all the other features, neither recognized as debris nor vegetation.
**A supervised classification was performed using Maximum Likelihood algorithm.**
The comparison between the obtained results and the data already available for the trees growing on the glacier, gained during the years 2006 and 2007 (Pelfini et al. 2012), was then conducted in order to evaluate the accuracy of the method.

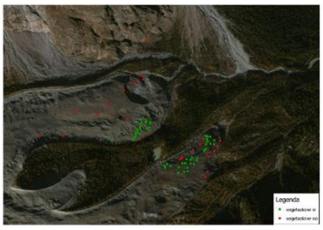



### RESULTS AND DISCUSSION

**A total of 2589 pixels in the terminal part of the Miage Glacier were recognized to be occupied by vegetation**, 386 of which located on the North lobe and 2203 located on the South lobe. By comparing these results with the data available for the same study area, the estimation of the maximum number of trees identified by this method on the North and South lobes was possible.
The semi-automatic method allowed the identification of a **maximum number of 19.3 trees on the North lobe and of 236.8 trees on the South lobe**. In the previous study conducted on the terminal part of the Miage Glacier, 53 squares, of 225 m$^2$ area each, were selected. 1178 trees were observed on the North lobe, 154 of which taller and 1024 smaller than 30 cm. 525 trees were observed on the South lobe, 234 of which taller and 291 smaller than 30 cm.
These preliminary results show that the semi-automatic method considerably underestimates the real number of trees.
**The main problem concerning the study of these trees using aerial photos is their discontinuous distribution.** Supraglacial trees do not form homogeneous forested areas, even though sometimes the presence of trees on the glacier surface is remarkable.
Moreover, aerial photos report **shadows** naturally present in the environment, and they represent a source of misinterpretation in the automatic classification.
From these preliminary results it is reasonable to assume that the supervised classification method, performed using Maximum Likelihood Algorithm, mainly allows the identification of trees taller than 30 cm, despite underestimating the real number of trees.

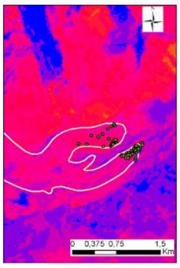
## SATELLITE-DERIVED DATA METHOD

79 points on the surface of the terminal part of the Miage Glacier, 22 of which characterized by the presence of vegetation and 57 characterized by its absence during the summer of 2006 and 2007 (data from aerial photographs) were selected.




The **altitude**, the ASTER-derived **surface temperature** (TIR band, acquired on August 2005), the Landsat-derived **Normalized Difference Moisture Index** (NDMI, acquired on July 2002) and the ASTER-derived **debris thickness** (data from Mihalcea et al. 2008) for those points were obtained.
A value from 0 to 100 was assigned to each point characterized by the presence of vegetation, depending on the abundance of vegetation (0 = no vegetation, 100 = high density vegetation).
Multinomial logistic regression method was then applied to the data, using the software SPSS, selecting "Presence of vegetation" as the predicted output variable.

### RESULTS AND DISCUSSION




Even though no significant results were found in the multinomial logistic regression from the combination of these data, the NDMI provided useful information: in fact, this index is directly related to the presence of vegetation (it contrasts the NIR band 4, which is sensitive to the reflectance of leaf chlorophyll content, to the MIR band 5, which is sensitive to the absorbance of leaf moisture) and even at a 15 m pixel resolution the NDMI results to be lower in areas where no vegetation is detected and higher in areas where vegetation is present.

These first results on a relatively small data sample suggest that **moisture detected through remote sensing analysis is effectively useful in the discrimination between supraglacial areas characterized by the presence of vegetation and by its absence.**

A more detailed analysis of the supraglacial vegetation could be possible through the use of very high resolution satellite data and unmanned aerial vehicles equipped with multispectral cameras.

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**Mihalcea C., Brock B.W., Diolaiuti G., D'Agata C., Citterio M., Kirkbride M.P., Cutler M.E.J., Smiraglia C.**, 2008. Using ASTER satellite and ground-based surface temperature measurements to derive supraglacial debris cover and thickness patterns on Miage Glacier (Mont Blanc Massif, Italy). *Cold regions science and technology* 52, 341-354.
**Pelfini M., Diolaiuti G., Leonelli G., Bozzoni M., Bressan N., Brioschi D., Riccardi A.**, 2012. The influence of glacier surface processes on the short-term evolution of supraglacial tree vegetation: a case study of the Miage Glacier, Italian Alps. *The Holocene*, DOI: 10.1177/0959683611434222.

**Abstract 1 (Poster Presentation)**

Novel indicators of environmental change from trees in the debris-covered glacier foreland: the case study of the Miage Glacier (Mont Blanc Massif, Italian Alps)

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Supraglacial trees are a useful source of data for reconstructing past glacier surface movements and debris-coverage instability. Proglacial trees also represent a useful tool for the identification and dating of changes in the glacial stream discharge and wide-spreading of melting water. Dendroglaciology is currently applied not only for reconstructing glacier fluctuations but also for investigating glacier surface dynamics at decadal scale. Trees and dated tree-ring characteristics such as scars, growth rate and reaction wood may provide information about glacier movements, discharge and hydrology.
The Miage Glacier in the Mont Blanc Massif (Italy), represents a unique situation in the southern side of the Alps, due to the presence of abundant supraglacial vegetation. The density and distribution of trees is strictly linked to glacier surface velocity, thickness of debris-coverage, ablation rate, grain size distribution, slope and ice thickness, as documented by the results obtained during field surveys, data analysis and remote sensing techniques.
The most recent studies show that supraglacial trees can also be considered environmental and climatic stress indicators. Leaf VOC (Volatile Organic Compounds) emissions and tree-ring carbon and oxygen stable isotopes show significant differences in trees located on the supraglacial debris with respect to trees on the lateral moraine, and these results suggest the possibility to apply these techniques in the identification of areas affected by glacio-geomorphological and climatic stress.
Tree-ring characteristics may also be analyzed in order to reconstruct the past hydrology of debris-covered glaciers with annual resolution. In particular, trees fed by glacial meltwater of the Lago Verde (Miage Glacier) show that tree-ring cellulose is more depleted in $\delta^{18}O$ compared to trees fed by other water sources and, moreover, tree-ring width is narrower in trees affected by lake-level fluctuations.

References:

Leonelli G., Pelfini M., Panseri S., Battipaglia G., Vezzola L., Giorgi A. (2014a). Tree-ring stable isotopes, growth disturbances and needles volatile organic compounds as environmental stress indicators at the debris covered Miage Glacier. Geogr. Fis. Dinam. Quat. 37, 101-111.

Leonelli G., Pelfini M., Battipaglia G, Saurer M., Siegwolf R.T.W., Cherubini P. (2014b). First detection of glacial meltwater signature in tree-ring $\delta^{18}O$: Reconstructing past major glacier runoff events at Lago Verde (Miage Glacier, Italy). Boreas 43, 600-607.

Garavaglia V., Pelfini M., Motta E. (2010). Glacier stream activity in the proglacial area of debris covered glacier in Aosta Valley, Italy: an application of dendroglaciology. Geogr. Fis. Dinam. Quat. 33, 15-24.

**Abstract 2 (Oral Presentation)**

Glacier features influencing the presence and abundance of supraglacial trees: the case study of the Miage debris-covered Glacier (Mont Blanc Massif, Italian Alps)

L.C. Vezzola [1], G. Diolaiuti [1], C. D'Agata [1], C. Smiraglia [1] and M. Pelfini [1]
[1] Department of Earth Sciences, University of Milan (Italy)

The number of debris-covered glaciers featuring supraglacial tree vegetation is increasing worldwide, as a response of high mountain environments to the current climate warming. At the debris-covered surface of these glaciers, trees can be found thus giving peculiar landscape and ecosystems. Their distribution is not homogeneous, thus suggesting that some glacier parameters influence germination and growth of trees.

This study was performed on the widest Italian debris-covered glacier, the Miage Glacier in the Mont Blanc massif, where herbaceous and tree vegetation is present at the surface of the glacier tongue. We analyzed the ablation area in the range from 1730 m to 2400 m a.s.l. where a quite continuous debris coverage is present and trees (mainly *Larix decidua* Mill. and *Picea abies* Karst) are present, also reaching an age of 60 years close to the terminus. By remote sensing investigations and through field surveys we obtained a record of glacier parameters (debris thickness, debris-surface temperature, slope, aspect, elevation, ablation rate, surface velocity, debris-NDMI, variation in ice thickness over several years) to be analyzed with respect to the presence and abundance of trees in 15 plots (plot size: 15 m x 15 m).

Our results show that supraglacial trees are present at the Miage Glacier: 1) whenever exceeding a debris thickness threshold (≥19 cm); 2) with a quite gentle slope (≤ 22°), 3) with a low glacier surface velocity (≤ 7.0 m/year), 4) where the ice thinning due to surface ablation is moderate (ranging between -1.8 m/year and -0.7 m/year) and 5) where the vertical changes due to glacier dynamics are positive (i.e. prevalent increase due to both slow debris accumulation and then preservation of ice flow inputs that we found ranging from +7 m and +28 m over a period 28 years long).

The analysis of the same parameters, conducted on other debris-covered glaciers featuring supraglacial trees, may provide new data in order to evaluate if such conditions are local ones or if they are actual and general factors driving germination and growth of trees.

**Abstract 3 (Poster Presentation)**

Widening rate of glacier forelands: the case study of the Forni Valley (Stelvio National Park, Italian Alps)

F. Sobacchi [(1)], L. Vezzola [(1)] and M. Pelfini [(1)]
[1] Department of Earth Sciences, University of Milan (Italy)

Alpine glacier shrinkage is accompanied by a progressive expansion of deglaciated areas, both on the valley slope and in the bottom valley. Glacier retreat provides new habitats for plants and animals, that can colonize the glacier foreland. Chrono-sequences and ecesis have been already studied in order to assess the biological response to climate change in glacial environments, but the expansion of proglacial areas and their evolution over time are also topics of great interest in the context of global change.
The aim of this study is to evaluate the expansion of the proglacial area of the Forni Glacier due to the glacial fluctuations occurred since the Little Ice Age (LIA). This research was conducted in the Forni Valley (Stelvio National Park, Italy), where the Forni Glacier past fluctuations are well documented by at least four moraine ridges, from the LIA until the last advance occurred in the end of the 1970s. Using aerial images, orthophotos and field data, the moraine ridges have been georeferenced, and the expansion rate has been estimated in GIS environment for each time interval defined by the dated moraines.
Assuming an expansion of 100% of glacier foreland between the end of the LIA and the current position of the glacier, our results show that 24,1% of the glacier foreland expanded between 1859 and 1914; 6,7% between 1914 and 1926; 47,3% between 1926 and 1981; 21,9% between 1981 and nowadays. The velocity of expansion was 11.000 $m^2$/year between 1859 and 1914; 14.300 $m^2$/year between 1914 and 1926; 21.700 $m^2$/year between 1926 and 1981; 19.200 $m^2$/year between 1981 and nowadays.
The results obtained in this study show that i) the mean velocity of area expansion of the glacier foreland during the last 88 years was nearly double than the mean velocity during the 1859-1926 time interval, thus featuring an ongoing phase of acceleration starting from the beginning of the last century; ii) the linear glacial retreat (measured by the operators of the Italian Glaciological Committee), instead, calculated for the same four time intervals shows a remarkable acceleration only starting from 1981.
The increasing rate of expansion of the glacier foreland needs to be taken into account not only for a better understanding of the dynamics of biological forms in newly formed proglacial areas, but also for the evaluation of the physical processes involved in the landscape changes, related for instance to the action of melting water on unconsolidated debris.
Future perspective of the research aims at analyzing and comparing different trends of expansion of glacier foreland at the regional scale.

# Widening rate of glacier forelands: the case study of the Forni Glacier (Stelvio National Park, Italian Alps)

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19th Alpine Glaciology Meeting
Milano, Italy
7-8 May 2015

## Introduction

Deglaciated areas are progressively expanding as a consequence of glacier retreat. Proglacial areas are characterized by an elevated geo-ecological significance. The study of glacier foreland expansion represents the first step for investigating the impact of surface processes and re-colonization rate of vegetation in proglacial areas.

## Aim of the study

- To investigate the expansion rate of the proglacial area of the Forni Glacier since the Little Ice Age (LIA) acme
- To evaluate the relationship between the expansion of the proglacial area of the Forni Glacier and the glacier tongue fluctuations

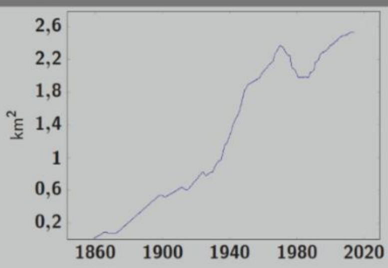
## Methods

- Moraine ridges were identified and georeferenced using aerial photos and orthophotos acquired in different years (1983, 1994, 2000, 2006 and 2010)
- The correct position of the moraines was verified during field surveys
- The expansion rate of glacier foreland during the four time intervals defined by the moraines ridges (1, 2, 3 and 4, see Figure 1) was then estimated in GIS environment

## Results 1


Figure 1: Glacier foreland areas between moraine ridges, since LIA acme

## Results 4




Expansion of proglacial area since LIA acme, calculated using historical data and measurements of glacier retreat (by of the Italian Glaciological Committee)

## Study area

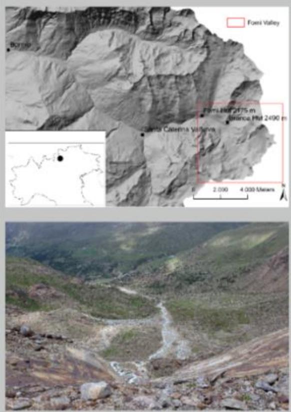


Figure 2: Location of the Forni Valley, Stelvio National Park, Italy (above); the glacier foreland seen from the upper Forni Valley (below)

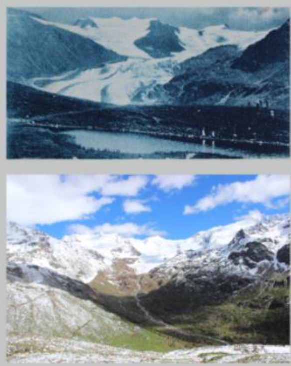
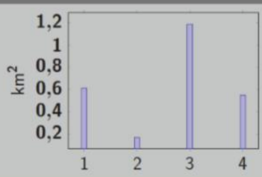


Figure 3: The Forni Valley as it was in the end of '800 (picture above, thanks to Narciso Salvadori for the kind courtesy) and as it is today (below).

## Results 2



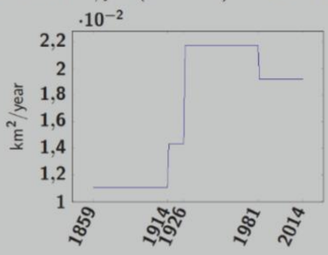

Progressive expansion of the Forni Valley:

1. 24.1 % from 1859 to 1914
2. 6.7 % from 1915 to 1926
3. 47.3 % from 1927 to 1981
4. 21.9 % from 1982 and nowadays

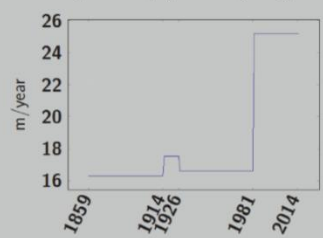
## Results 3

The average expansion rate was not homogeneous in the four time intervals:

1. **0.011** $km^2$/year (1859-1914)
2. **0.0143** $km^2$/year (1915-1926)
3. **0.0217** $km^2$/year (1927-1981)
4. **0.0192** $km^2$/year (1982-2014)




Average velocity of expansion of glacier foreland; the mean velocity during the last 88 years was nearly double than the mean velocity during the 1859-1926 time interval




The linear velocity of retreat of the Forni Glacier shows a notable acceleration starting from 1981

## Conclusions

- These first results shows that the expansion of glacier foreland is not directly proportional to the regression rate of the glacier tongue
- The ongoing studies will allow further analysis about the relationship between the expansion of glacier foreland, surface processes and recolonization rate of vegetation

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Aigeo (Associazione Italiana di Geografia Fisica e Geomorfologia) conference “6th Young Geomorphologists’ Day” – Cagliari, 28/09/2015 – 30/09/2015

**Abstract (Poster Presentation)**

Forni Glacier fluctuations: influence on the biological system in the glacier foreland

L. C. Vezzola [1], F. Sobacchi [1], A. Merlini [1], A. Bonetti [1]
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Glacier retreat is among the most evident impacts of the current climate change. The phases of glacier shrinkage can be reconstructed through geomorphological investigations conducted in the glacier foreland, a suitable site where to evaluate the relationship existing between glacier retreat, geomorphological processes and colonization of newly formed terrains performed by biological forms. The most recent studies conducted in one of the most representative recently deglaciated areas of the Italian Alps are here presented.
The expansion of the proglacial area of the Forni Valley (Stelvio National Park, Italy) was analyzed for the period comprised between the Little Ice Age (LIA) and nowadays, through the use of historical images, orthophotos and field data. In particular, the area and rate of expansion were estimated for the four time intervals defined by the dated moraines, and the results show that the expansion rate was nearly double in the last 88 years compared to the previous years, thus highlighting an acceleration in the expansion rate starting from the beginning of the 20th century.
As the glacier foreland expands, different organisms colonize progressively older terrain, including trees, if the area is located below the treeline. Tree ecesis time and germination year were estimated by means of dendrochronological approach and whorls branch counting, performed on living conifers growing in the most recent deglaciated area, close to the current position of the glacier front. The results of this study show an acceleration of the average ecesis in the last few years, with values ranging between 5 and 11 years, and with an average value of 7 years.
On the other hand, glacier advances destroy forests. The study of buried logs and peat (performed through radiocarbon dating and dendrochronological techniques) contribute to a better understanding of past glacier fluctuations and related climate change. In particular, a buried log found in the Forni Valley revealed information about the Subboreal climatic transition and the related glacier fluctuations.
Overall, these findings evidence some of the complex interactions between abiotic and biotic systems in glacial environments, and the precious contribution of arboreal vegetation in dating glacier changes and monitoring velocity of processes in the glacier foreland over time.

6th YOUNG GEOMORPHOLOGISTS' DAY
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Cagliari, 28-30 September 2015

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE DELLA TERRA "ARDITO DESIO"

# FORNI GLACIER FLUCTUATIONS: INFLUENCE ON THE BIOLOGICAL SYSTEM IN THE GLACIER FORELAND

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Glacier shrinkage is one of the most evident effects of the current climatic trend. The dynamics of glacier retreat gradually expand the area at the glacier front, that is affected by active geomorphic processes and colonization performed by bacteria, animals and plants.


Fig.1. The location of the Forni Valley (in the red circle), in the central Italian Alps.

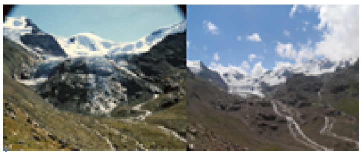

Fig.2. The Forni Glacier in 1988 (on the left) and in 2012 (on the right): in this time interval, the glacier retreated considerably.

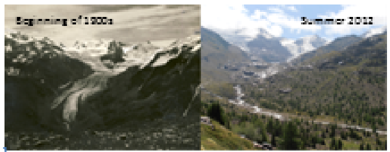


Fig.3. Vegetation gradually colonized the Forni Valley after the glacier retreat started after the LIA.

The **Forni Valley** (Stelvio National Park), where the Forni Glacier is located, is one of the most studied glacier forelands in the central Italian Alps. The main glacier fluctuations occurred since the Little Ice Age are well documented by the dated moraines deposited in the valley. Following the glacier retreat, several plant species, including trees, have progressively colonized the valley.

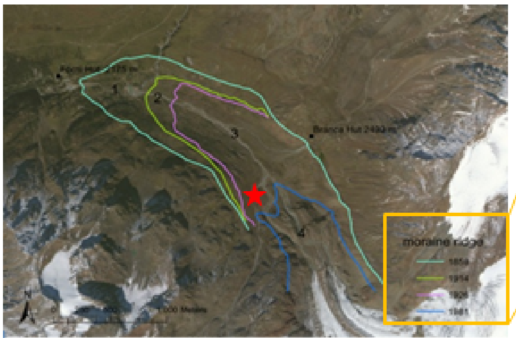


Fig.4. The four areas of the glacier foreland between moraine ridges, since the LIA. The red star indicates the position of the buried log (Fig. 8).

The progressive expansion of the Forni Glacier foreland was investigated in detail for the time interval comprised between the Little Ice Age and 2014, using:
- Historical pictures;
- Colour orthophotos;
- Data obtained during field surveys.

In particular, the area of glacier foreland progressively freed from ice and the rate of expansion were calculated through time.

Progressive expansion of the Forni Valley was obtained in the four time intervals defined by the moraine ridges:
1. 24.1 % from 1859 to 1914
2. 6.7 % from 1915 to 1926
3. 47.3 % from 1927 to 1981
4. 21.9 % from 1982 to 2014

Average rate of expansion of the glacier foreland nearly double in the last 88 years, compared to the 1859-1926 time interval.

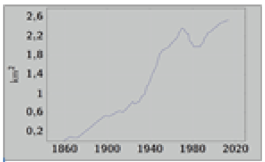


Fig.5. The area of the glacier foreland progressively freed from ice, since the LIA.

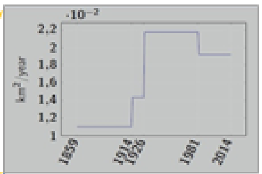


Fig.6. The average rate of expansion of the glacier foreland from the LIA to 2014.

Trees have been studied in order to analyze the dynamics of colonization in the glacier foreland. Tree ecesis is the lag time between surface exposure and germination of arboreal species. In the Forni Valley, tree ecesis has been evaluated using a dendrochronological approach and whorls branch counting, applied on living conifers in the most recent deglaciated areas.

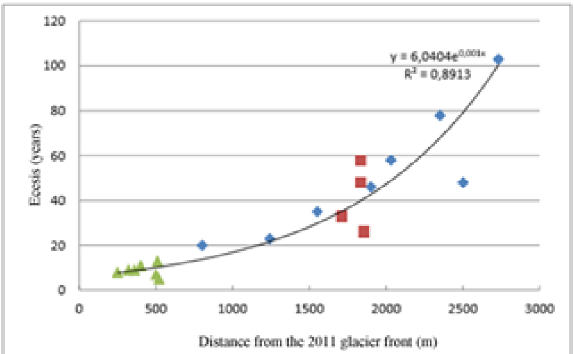


Fig.7. Ecesis intervals at increasing distance from the 2011 glacier front, including data from three studies (Bonetti, 2013 reported in green; Colombi, 2012 in red; Alleri, 2007 in blue).

Tree ecesis changed over time within the Forni Glacier foreland: a valuable acceleration of the average ecesis is evident in the last few years, with values ranging between 3 and 11 years, and with an average of 7 years.

Living trees are not the only useful feature for understanding past glacier changes: during phases of glacier advance, forests are destroyed, but some buried logs may be preserved and then studied in order to obtain information about the glacier behaviour in pre-instrumental times.
A **buried log** was found in the Forni Valley (see Fig. 4), and analyzed through dendrochronological and pedological approach and radiocarbon dating.

Fig.8. Transversal section of the retrieved log. The results of the analyses reveal that it has 285 tree rings and became buried after 4,201-4,052 cal. year BP

The results of the analyses helped the understanding of the glacier fluctuations occurred during the Subboreal climatic transition. In fact, the buried log could belong to a sparse tree coverage or to a developed forest. Thus, the retrieval of a log of 300 years (species *Pinus cembra*) suggests that, about 4,000 years ago, the Forni Glacier was located at an altitude at least higher than 2,300 m.

Leonelli G., Battista A., Pelfini M., submitted. Decreasing ecesis intervals along the Forni Glacier forefield, Italy: an accelerating climate change driven dynamics.
Pelfini M., Leonelli G., Trombino L., Zerboni A., Bollati I., Merlini A., Smiraglia C., Diolaiuti G., 2014. New data on glacier fluctuations during the climatic transition at ~4,000 cal. years BP from a buried log in the Forni Glacier forefield (Italian Alps). Rend. Fis. Acc. Lincei 25, 427-437.
Bonetti A., 2013. Ritiro glaciale recente ed ecesi nella Valle dei Forni (Valtellina, SO): analisi dendroglaciologiche e GIS. Tesi di laurea.
Colombi F., 2012. Ritiro glaciale ed ecesi nell'area proglaciale del ghiacciaio dei Forni (Valtellina, SO). Tesi di laurea.
Alleri M., 2007. Il ritiro del ghiacciaio dei Forni e la ricolonizzazione arborea. Tesi di laurea.

**Abstract (Poster Presentation)**

Assessing glacier features supporting supraglacial trees: the case study of the Miage debris-covered Glacier (Italian Alps)

L.C. Vezzola [1], G.A. Diolaiuti [1], C. D'Agata[1], C. Smiraglia[1], M. Pelfini[1]
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The number of debris-covered glaciers featuring supraglacial trees is increasing worldwide, as a response of high mountain environments to climate warming. Generally, their distribution on the glacier surface is not homogeneous, thus suggesting that some glacier parameters influence germination and growth of trees.

In this study, we focused our attention on the widest Italian debris-covered glacier, the Miage Glacier (Mont Blanc massif). We analyzed the ablation area in the range from 1730 m to 2400 m a.s.l. where continuous debris coverage is present and trees are found. Using data obtained by remote sensing investigations and field surveys we defined a record of glacier parameters to be analyzed with respect to the presence and abundance of trees.

We found that supraglacial trees are present at the Miage Glacier: i) whenever exceeding a debris thickness threshold (≥19 cm); ii) with a gentle slope (≤ 10°); iii) with a low glacier surface velocity (≤ 7.0 m/y); and iv) where the vertical changes due to glacier dynamics are positive (i.e. prevalent increase due to both slow debris accumulation and preservation of ice flow inputs that we found ranging between +7 m and +28 m over 28 years). The statistical analysis supports our findings.

The analysis of the same parameters might be conducted on other debris-covered glaciers featuring supraglacial trees, in order to evaluate if such conditions are local ones or if they are general factors driving germination and growth of trees.

By identifying the features supporting the presence and growth of trees in these environments, and their thresholds, a contribution is given for a better understanding of the importance of debris-covered glaciers and, in general, of debris-covered ice, as a refuge for trees during warm intervals of the Holocene.

# Assessing glacier features supporting supraglacial trees: the case study of the Miage debris-covered Glacier (Italian Alps)

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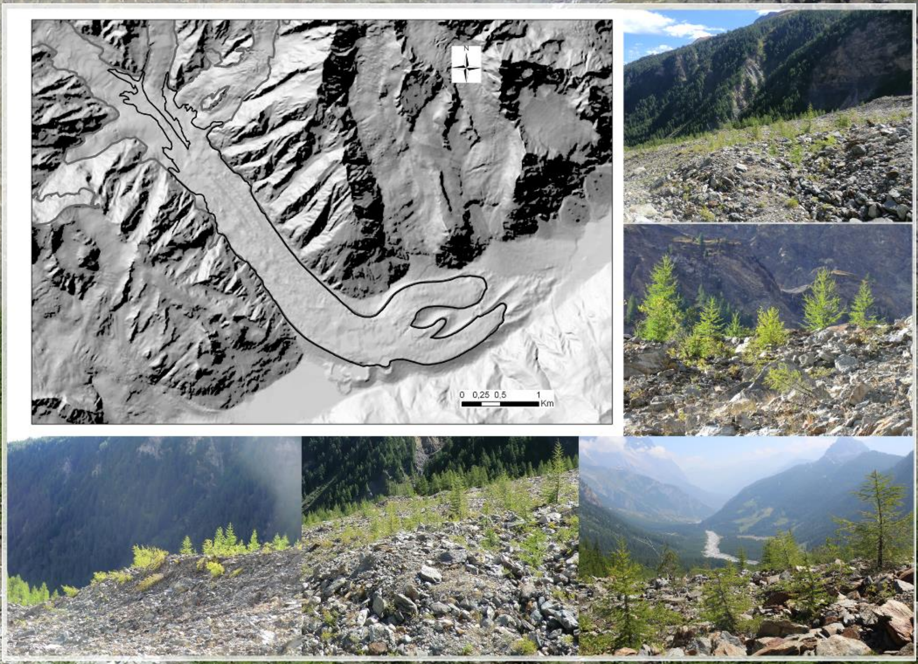
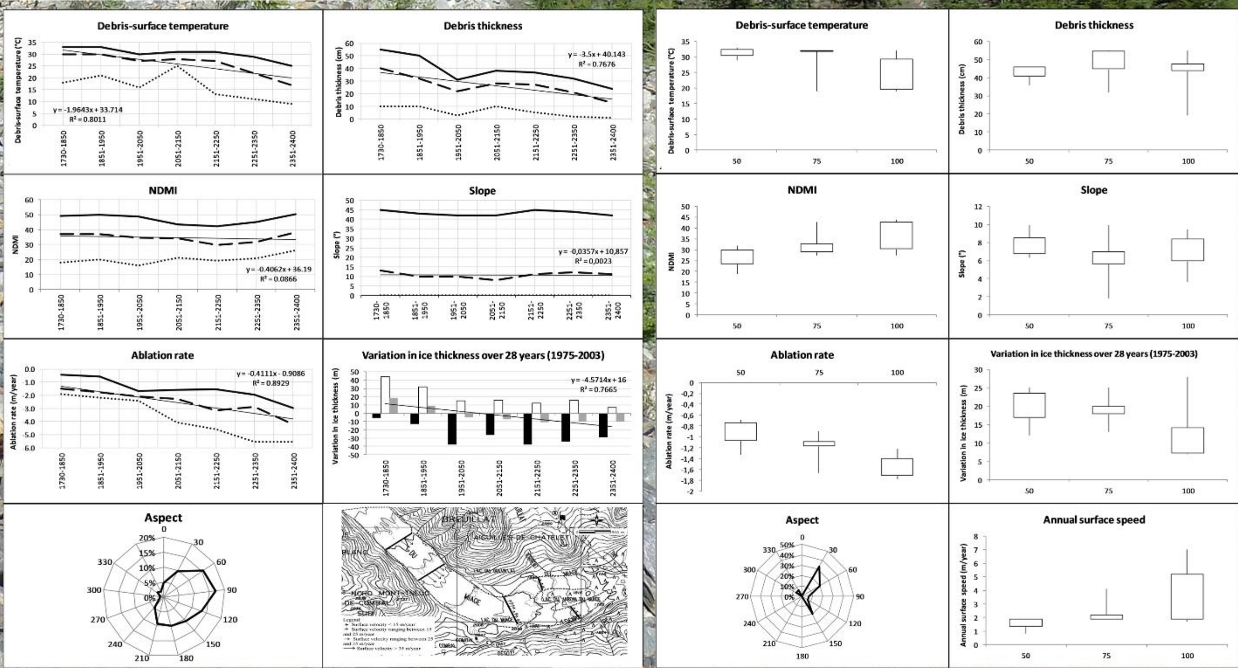
The **Miage Glacier** (Aosta Valley) is the widest debris-covered glacier in the Italian Alps: its ablation tongue shows continuous debris coverage from 1730 m up to 2400 m a.s.l. Arboreal vegetation is present on the supraglacial debris, with *Picea abies* Karst and *Larix decidua* Mill. being the most abundant species.
**Tree distribution is not homogeneous**, suggesting that one or more glacier parameters influence germination and growth of supraglacial trees.

In this study we aimed at **assessing the role and weight played by each glacier feature in driving tree vegetation presence, growth and distribution.**




- **Remote sensing** and **field surveys** were used for building a database including i) the **main features of the debris-covered ablation tongue** and ii) the same glacier parameters in 15 selected plots where **trees** with different abundance are present.
- Moreover, a **one-way ANOVA** was performed in order to compare the glacier parameters of 15 supraglacial plots characterized by the absence of trees against the 15 plots previously selected characterized by the presence of trees.




| Parameter | ANOVA test |
|---|---|
| Ablation rate | F(3,26) = 28.78; p<0.000 |
| Debris-surface temperature | F(3,26) = 8.95; p<0.000 |
| Variation in ice-thickness over 28 years | F(3,26) = 65.42; p<0.000 |
| Slope | F(3,26) = 50.33; p<0.000 |
| Debris thickness | F(3,26) = 43.47; p<0.000 |
| Aspect | F(3,26) = 1.29; p<0.29 |
| NDMI | F(3,26) = 1.91; p<0.15 |

This study allowed for the first time the identification of the glacial features, and their thresholds, permitting supraglacial tree germination and growth. In fact, trees are only present in areas featuring:
- **Slow surface velocity** (< 7 m/year);
- **Thick debris cover** (deeper than 19 cm);
- **Gentle slope** (≤ 10°);
- **Positive changes in ice thickness** (ranging between +7 m and +28 m over 28 years).

The statistical analysis supports our observations.

Accepted for publication in "The Holocene"

Joint Geomorphological Meeting and Journees des Jeunes Geomorphologues – Chambery, France, 27/06/2016 – 30/06/2016

**Abstract 1 (Oral Presentation)**

Recent changes of the ablation tongue and glacier foreland at the Lys Glacier (Italian Alps)

G.A. Diolaiuti [1], L.C. Vezzola [1], A. Senese [1], C. D'Agata [1], D. Fugazza [1], G. Leonelli [2], M. Pelfini [1]
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[2] Department of Earth and Environmental Sciences, University of Milan Bicocca (Italy)

The Lys Glacier (Monte Rosa Group) is the most monitored glacier of the Italian Alps since 1940, with very frequent field measurements of terminus variations and the longest annual reporting of supraglacial debris presence. The recent changes of the ablation tongue and glacier forefield of the Lys Glacier have been evaluated through remote sensing investigations and field surveys. Terminus fluctuations, volume and debris-coverage variations, ablation rates and debris-surface temperatures have been analyzed starting for the last decades. The glacier terminus resulted to be generally retreating (cumulate retreat from 1975 to 2003 of -99 m, with an average of -3.4 m/year), with a unique period of advance occurred between 1976 and 1985. The volume decreased of 15.4 x $10^{-6}$ $m^3$ in the considered time interval (1975-2003) and the supraglacial debris coverage resulted to be strongly increased due to several rock-fall events affecting the rock walls dominating the glacier tongue. The measured surface temperature correlates with debris thicknesses ($r = 0.8$). Supported by this agreement, a map representing the spatial distribution and thickness of debris cover was obtained from the ASTER-derived surface temperature data (TIR band) using the relationship between debris-surface temperature and thickness.
Changes in the proglacial area have been analyzed through geomorphological investigations and dendrochronological dating. As regards the glacier forefield developed from the end of the Little Ice Age to the end of the 20th century, the correlation between dendrochronological and climatic and glaciological data (terminus variations) allowed to investigate the glacier response and ecesis time. The ongoing research on the most recent deglaciated forefield will contribute to a better understanding of tree colonization following the Lys Glacier ongoing retreat that will be compared with data already available for other alpine glaciers.

**Abstract 2 (Poster Presentation)**

Supraglacial trees as environmental stress indicators at the Miage debris-covered Glacier (Italian Alps)

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Supraglacial trees are becoming a common feature on debris-covered glaciers (DCGs). The Miage Glacier (Mont Blanc Massif) is the only DCG on the Southern side of the European Alps characterized by the presence of a supraglacial forest. Trees are negatively affected by glacier surface movements, and growth disturbances are recorded in tree rings. We have analyzed the glacier parameters influencing the establishment and growth of supraglacial trees at the Miage Glacier and we have compared the tree-ring growth patterns and stable isotopes, as well as the needle volatile organic compounds (VOCs), of supraglacial trees ("glacier" site) with respect to trees located on a lateral moraine ("control" site).

We found that supraglacial trees are present where debris is thicker than 19 cm, slope does not exceed 10°, surface velocity is lower than 7.0 m/year and vertical changes due to glacier dynamics are positive.

The analysis of tree-ring growth patterns reveals that trees on the supraglacial debris are affected by a major geomorphological stress compared to trees on the moraine. Tree-ring $\delta^{13}C$ and $\delta^{18}O$ stable isotopes are different in trees at the glacier and control sites, probably due to differences in soil water retention, temperature excursion, time exposure to direct solar radiation and water source. Needle VOCs show significant differences in some terpenes at the two sites, probably due to the extreme temperature conditions on the supraglacial debris.

These results show that supraglacial trees at the Miage Glacier may be used as indicators of environmental stress, and that monitoring their distribution and characteristics can contribute to better assessing the factors controlling the environmental evolution on a typical DCG.

Future research will aim at analyzing the geomorphological features characterizing the Miage Glacier, and at evaluating what are the main hazards on a DCG in the context of a changing climate.

References:

Leonelli et al., 2014. Tree-ring stable isotopes, growth disturbances and needle volatile organic compounds as environmental stress indicators at the debris covered Miage Glacier (Monte Bianco Massif, European Alps). GFDQ 37, 101-111.

Vezzola et al., 2016. Assessing glacier features supporting supraglacial trees: a case study of the Miage debris-covered Glacier (Italian Alps). Holocene, DOI: 10.1177/0959683616632883.

18° Joint Geomorphological Meeting
June 27, 2015 – Chambéry, France

# Supraglacial trees as environmental stress indicators at the Miage debris-cover Glacier (Italian Alps)

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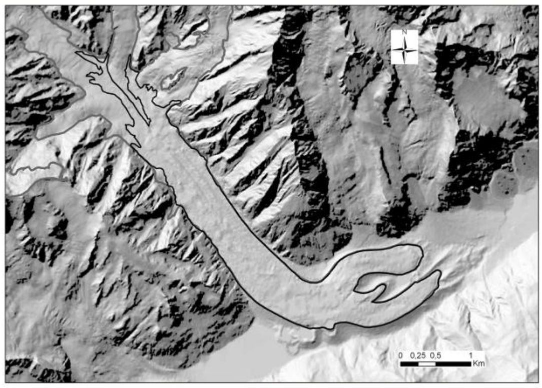
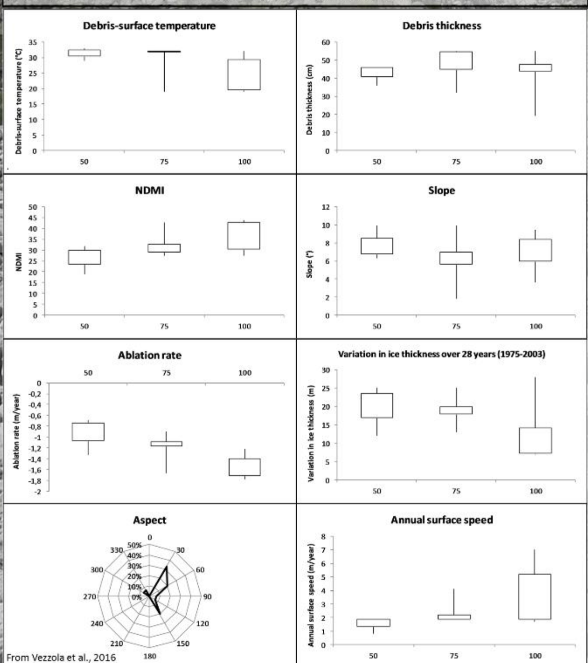
The **Miage Glacier** (Mont Blanc Massif, Western Italian Alps) is the largest debris-covered glacier in Italy.
Its ablation tongue shows quite continuous debris coverage from 1730 m a.s.l. up to 2400 m a.s.l.
The lower portion of the glacier ablation tongue is colonized by vegetation, including well developed trees (with *Picea abies* Karst and *Larix decidua* Mill. being the most abundant species). Trees, and tree rings in particular, are archives of information related to the glacier surface dynamics.

In this study we aimed at i) assessing **the role and weight played by each glacier feature in driving tree vegetation presence**, growth and distribution and ii) comparing **tree-ring growth patterns and stable isotopes, and needle volatile organic compounds (VOCs)** of supraglacial trees ("Glacier" site) with trees on a lateral moraine ("Control" site).




**Remote sensing** and **field surveys** were used for building a database including i) the main features of the debris-covered ablation tongue and ii) the same glacier parameters in 15 selected plots where trees with different abundance are present. In the following graphs the values of the investigated parameters in the plots **where trees are present** are reported (tree abundance on X axis):




From Vezzola et al., 2016

From Vezzola et al., 2016

Supraglacial trees are present where debris is thicker than 19 cm, slope does not exceed 10°, surface velocity is lower than 7.0 m/year and vertical changes due to glacier dynamics are positive. The statistical ANOVA test supports these results.

| Parameter | ANOVA test |
|---|---|
| Ablation rate | F(3,26) = 28.78; p<0.000 |
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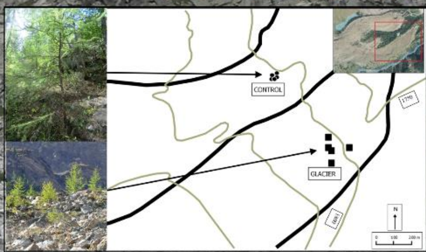
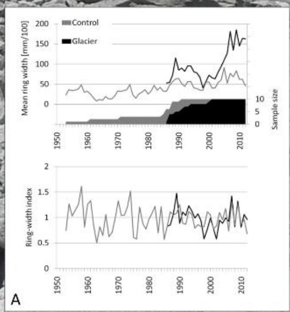
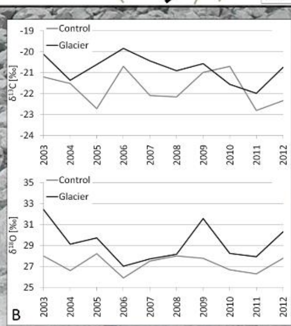
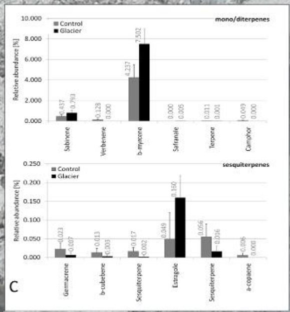
| Aspect | F(3,26) = 1.29; p<0.29 |
| NDMI | F(3,26) = 1.91; p<0.15 |

For the **dendrochronological analysis**, ten European larch trees at about the same altitude (1810 m a.s.l.) were sampled: five at the Glacier site and five at the Control site.




From Leonelli et al., 2014

A) Trees at the Glacier site are always characterized by higher growth rates than trees at the Control site, suggesting that supraglacial trees are affected by major geomorphological stress compared to trees on the lateral moraine.
B) Tree-ring $\delta^{13}C$ and $\delta^{18}O$ stable isotopes are always higher at the Glacier site than at the Control site, due to different conditions of temperature excursion, time exposure to direct solar radiation and water source.
C) Needle VOCs show significant differences in some terpenes at the two sites, probably due to the extreme temperature conditions on the supraglacial debris.

In summary, these results show that supraglacial trees at the Miage Glacier are indicators of environmental stress, and monitoring their distribution and characteristics can contribute to the assessment of the factors influencing the evolution of a typical alpine debris-covered glacier.

Leonelli et al., 2014. Tree-ring stable isotopes, growth disturbances and needle volatile organic compounds as environmental stress indicators at the debris covered Miage Glacier (Monte Bianco Massif, European Alps). GFDQ 37, 101-111
Vezzola et al., 2016. Assessing glacier features supporting supraglacial trees: a case study of the Miage debris-covered Glacier (Italian Alps). Holocene, DOI: 10.1177/0959683616632883...

## 9. PUBLICATIONS

**PUBLISHED MANUSCRIPTS:**

**Vezzola** L.C., Diolaiuti G.A., D'Agata C., Smiraglia C., Pelfini M., 2016. Assessing glacier features supporting supraglacial trees: A case study of the Miage debris-covered Glacier (Italian Alps). The Holocene, 26, 1138-1148

Bollati I., Leonelli G., **Vezzola** L.C., Pelfini M., 2015. The role of Ecological Value in Geomorphosite assessment for the Debris-Covered Miage Glacier (Western Italian Alps) based on a review of 2.5 centuries of scientific study. Geoheritage 7, 119-135.

Leonelli G., Pelfini M., Panseri S., Battipaglia G., **Vezzola** L., Giorgi A., 2014. Tree-ring stable isotopes, growth disturbances and needles volatile organic compounds as environmental stress indicators at the debris covered Miage Glacier (Monte Bianco Massif, European Alps). Geografia Fisica e Dinamica Quaternaria 37, 101-111.

**BOOK CHAPTERS:**

**Vezzola** L.C., 2015. Biogeomorphology: sampling and analysis of proglacial and supraglacial vegetation. In: Geomorphological Techniques (online edition) - ISSN 2047-0371, Chapter: 3.4.10. Biogeomorphology: sampling and analysis of proglacial and supraglacial vegetation, Publisher: British Society for Geomorphology, London, UK, Editors: Clarke LE, Nield JM.

**PROCEEDINGS:**

**Vezzola** L.C., D'Agata C., Leonelli G., Diolaiuti G.A., Azzoni R.S., Smiraglia C., Vagliasindi M., Pelfini M., 2014. Conference paper. A first approach to detect supraglacial vegetation coverage on debris-covered glaciers using aerial photographs and satellite images: the case study of Miage Glacier. International Symposium on the future of the glaciers: from the past to the next 100 years, Torino (TO).

**Vezzola** L.C., Leonelli G., Pelfini M., 2014. Conference paper. Dendrochronological and dendroisotopic patterns from trees affected by glacier meltwater: the case study of Lago Verde ice-contact lake (Miage Glacier, Italy). Congresso Congiunto SGI-SIMP-The future of the Italian Geosciences of the future, Milano (MI).

Bollati I., Leonelli G., **Vezzola** L., Pelfini M., 2015. L'influenza della valenza ecologica sul valore globale dei geomorfositi attivi: il caso del Ghiacciaio del Miage nell'Espace Mont Blanc. Geologia&Turismo 2013. Extended abstract. Volume degli Atti.

Garavaglia V., Leonelli G., Pelfini M., **Vezzola** L., 2011. Tree-ring responses to climate and to seasonal changes in water levels of an ice-contact lake in the Western Italian Alps. Geophysical Research Abstracts Vol. 13, EGU2011-9192-2, 2011, EGU General Assembly 2011. Volume degli Atti.

**MANUSCRIPTS SUBMITTED:**

**Vezzola** L.C., Muttoni G., Merlini M., Rotiroti N., Pagliardini L., Hirt A.M., Pelfini M., submitted to Geophysical Journal International. Investigating distribution patterns of airborne magnetic grains trapped in tree barks in Milan, Italy: insights for pollution mitigation strategies.

**Vezzola** L.C., Fugazza D., Diolaiuti G., D'Agata C., Pelfini M., submitted to Physical Geography. A first attempt to detect supraglacial vegetation on debris-covered glaciers by means of high-resolution satellite images.

**MANUSCRIPTS IN PREPARATION FOR SUBMISSION:**

**Vezzola** L.C., Leonelli G., Garavaglia V., Cherubini P., Pelfini M. The impact of glacial melting water on tree-ring growth at Lago Verde (Italian Alps).

**Vezzola** L.C., Michelozzi M., Calamai L., Gonthier P., Giordano L., Cherubini P., Pelfini M. Volatile Terpenes and tree-ring analyses indicate fungal infection in asymptomatic adult Norway spruce trees in the Alps.

## 10. ACKNOWLEDGEMENTS

When I began my studies at the University of Milan, in 2007, I remember thinking "Let's see if I can pass the first exam, if not, I will just give up and do something else in life". Well, at that time I never, never, never thought I could get here. I never thought I could actually deliver a Ph.D. thesis in Environmental Sciences. So let me say that I am very proud of me, today. But if I am here, it's because many people allowed me to get here, and this is the right moment to thank them.

First of all, I would like to express my sincere gratitude to my supervisor, Prof. Manuela Pelfini, for the encouragement, the kindness, the understanding and the patience she always demonstrated to me. Thank you for the scientific talks and for always caring about me and my academic success.
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