Geogr. Fis. Dinam. Quat.
37 (2014), 101-111, 6 figg.

DOI 10.4461/GFDQ.2014.37.09

GIOVANNI LEONELLI (*), MANUELA PELFINI (*), SARA PANSERI (**),
GIOVANNA BATTIPAGLIA (***), LAURA VEZZOLA (*) & ANNA GIORGI (**)

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)

ABSTRACT: LEONELLI G., PELFINI M., PANSERI S., BATTIPAGLIA G., VEZZOLA L. & GIORGI A., *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).* (IT ISSN 0391-9838, 2014).

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 were 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 (β-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.

KEY WORDS: Tree-ring stable isotopes, Needle VOCs, Debris covered Miage Glacier, European Alps.

RIASSUNTO: LEONELLI G., PELFINI M., PANSERI S., BATTIPAGLIA G., VEZZOLA L. & GIORGI A., *Isotopi stabili negli anelli di accrescimento arborei, anomalie di crescita e composti organici volatili nelle foglie quali indicatori di stress ambientali al Ghiacciaio del Miage (Monte Bianco).* (IT ISSN 0391-9838, 2014).

Nel presente lavoro vengono riportati i primi risultati ottenuti mediante un approccio *multi-proxy* finalizzato alla valutazione dell'influenza del clima e della tipologia di substrato sulle caratteristiche degli anelli di accrescimento di piante arboree epiglaciali e periglaciali s.s. e sui composti organici volatili (VOC) emessi dalle foglie. A tal fine sono stati analizzati gli isotopi stabili e gli andamenti di crescita degli anelli di accrescimento e i VOC emessi dagli aghi degli alberi, selezionando cinque esemplari appartenenti alla specie *Larix decidua* Mill. di altezza e caratteristiche simili, per due siti campione caratterizzati da condizioni stazionali simili ma da caratteristiche contrastanti relativamente al substrato e alla morfologia del terreno: il sito sul ghiacciaio nero del Miage (*Glacier*) e il sito su morena laterale (*Control*). Nel periodo 2003-2012, gli anelli di accrescimento al sito *Glacier* hanno mostrato di essere più arricchiti in valori medi di ^{13}C ($p<0.05$) rispetto al sito *Control*, verosimilmente a causa di una minore conduttanza stomatica indotta dalla scarsa ritenzione idrica del suolo, delle elevate escursioni termiche e dell'elevata esposizione alla radiazione solare diretta. Anche i valori medi di ^{18}O sono risultati più elevati ($p<0.001$) al sito *Glacier*, in seguito all'assimilazione di acque poco profonde da parte di un sistema radicale superficiale, in contrasto con quanto si verifica al sito *Control*, caratterizzato da un suolo più sviluppato e da un substrato più stabile. L'analisi degli andamenti di crescita annuale degli esemplari campionati ha fornito una caratterizzazione temporale degli stress climatici e geomorfologici negli alberi al sito Glacier dove sono stati osservati tassi più elevati di brusche variazioni di crescita (AGCs), ma dove non sono state riscontrate differenze nella percentuale di legno tardivo. I composti organici volatili (VOC) hanno mostrato differenze significative per alcuni composti di mono- di- e sesquiterpeni. Quelli con elevate concentrazioni (β-mircene ed estragolo) hanno eviden-

---

(*) *Dipartimento di Scienza della Terra, Università degli Studi di Milano, via Mangiagalli 34 - I-20133 Milano, Italy.*

(**) *Centro Interdipartimentale di Studi Applicati per la Gestione Sostenibile e la Difesa della Montagna - Ge.s.di.mont., Università degli Studi di Milano, via Morino 8 - I-25048 Edolo (BS), Italy.*

(***) *Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche, Seconda Università di Napoli, via Vivaldi, 43 - 81100 Caserta, Italy.*

This study was supported by the Club Alpino Italiano - (Project "Ricostruzione dei fenomeni dinamici legati ai ghiacciai alpini per un turismo consapevole e partecipativo in alta montagna") and by the PRIN 2010-11 (2010AYKTAB_006) "Dinamica dei sistemi morfoclimatici in risposta ai cambiamenti globali e rischi geomorfologici indotti" local coordinator C. Smiraglia, national coordinator C. Baroni.

ziato anche le differenze maggiori con concentrazioni più elevate al sito *Glacier*. Gli isotopi stabili e le AGCs negli anelli di accrescimento, così come i VOC negli aghi degli alberi possono essere utilizzati, rispettivamente, come indicatori di stress nel medio e breve termine, in quanto forniscono importanti informazioni per la valutazione degli impatti del cambiamento climatico e del controllo geomorfologico negli ambienti glaciali alpini.

TERMINI CHIAVE: Dendro-isotopi, composti organici volatili, Ghiacciaio del Miage, Alpi europee.

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 & 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 & 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 & 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 & 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 & 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 & 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 & *alii*, 2013; 2014). In the glacial environment of the debris-covered Miage Glacier, Leonelli & *alii* (accepted) 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 & Tumlinson, 1999; Guerrieri & 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 & 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 & Staudt, 1999): their concentration in the air affects the tropospheric chemistry, the production of air pollutants, aerosols and greenhouse gases (Kesselmeier & 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 & 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 & Pelfini, 2013) as also documented by aerial photos (Giardino & *alii*, 2001) and glaciological investigation (Smiraglia & *alii*, 2000). Moreover Leonelli & 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 (45° 47'04.08'' N; 6° 53' 35.94'' E) and five larches of the same height in a Control site (45° 47' 10.13'' N; 6° 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 Mi-

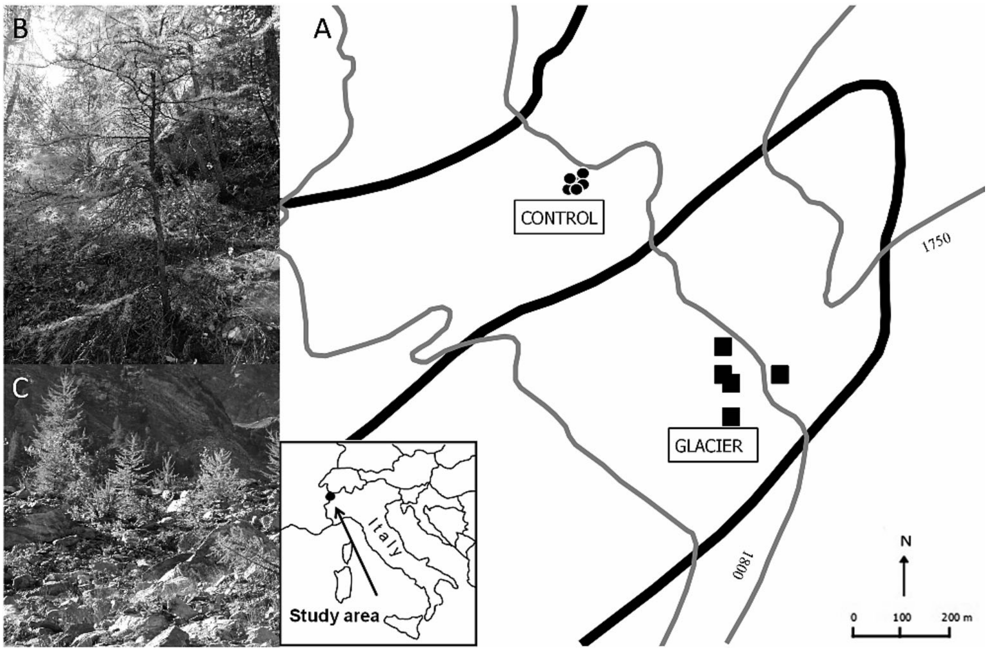

FIG. 1 - Sketch map of the study area (A). The points correspond to sampled trees at the Control site on the moraine (photo in B), the squares correspond to the sampled trees at the Glacier site (C).

age 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.

Tree-ring methods

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 & 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°C in a solution of 5% NaOH), for removing the lignin (3 baths of 8 hours at 60°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 & 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 °C 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

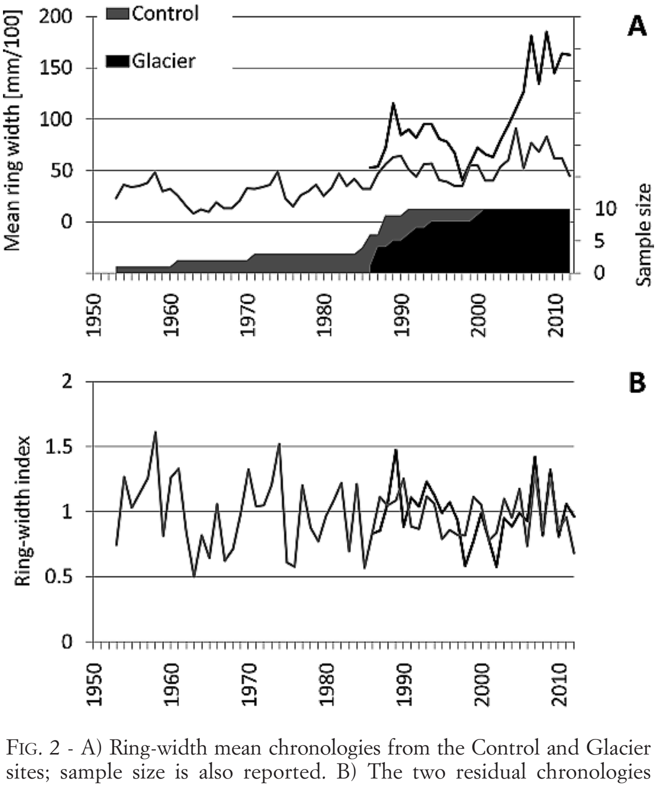

FIG. 2 - A) Ring-width mean chronologies from the Control and Glacier sites; sample size is also reported. B) The two residual chronologies derived from tree-ring measurements.

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.

Tree-ring stable isotopes

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 ($p<0.01$).

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).

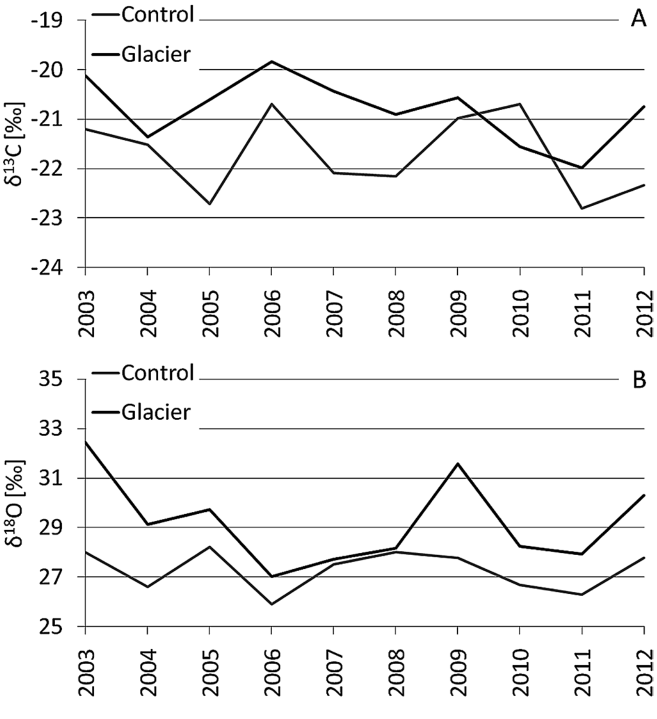

FIG. 3 - The stable isotope ratio series constructed at the Control and Glacier sites: tree-ring $\delta^{13}C$ (A) and $\delta^{18}O$ (B) values are reported for the period 2003-2012.

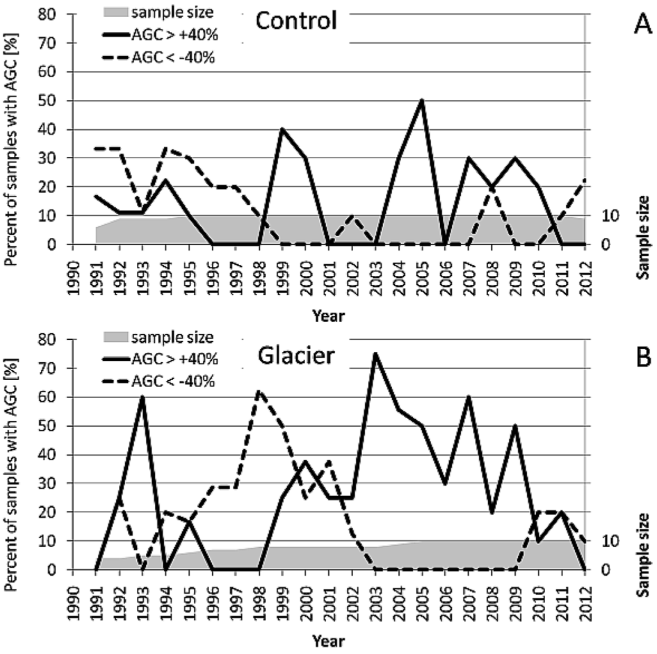

FIG. 4 - Percent of samples presenting abrupt growth changes (AGCs) >+40% and <–40% over the common period 1991-2012 where at least 4 samples per site are present in the Control site (A) and on the Glacier site (B); sample size is also reported.

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).

VOCs in needles

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 dif-

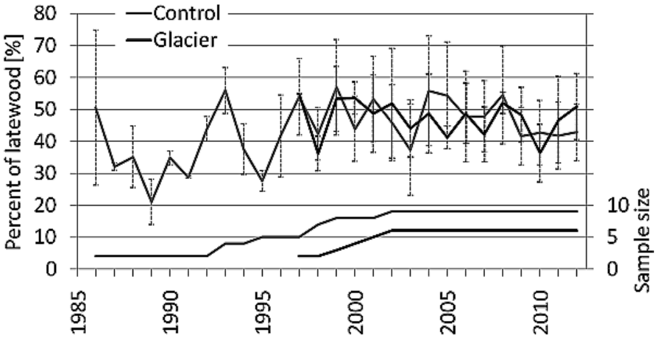

FIG. 5 - Percent of latewood calculated with respect to the total ring width both for Control and Glacier sites; error bars indicate ±1 standard deviation. Sample size for the measured cores (see methods) is also reported.

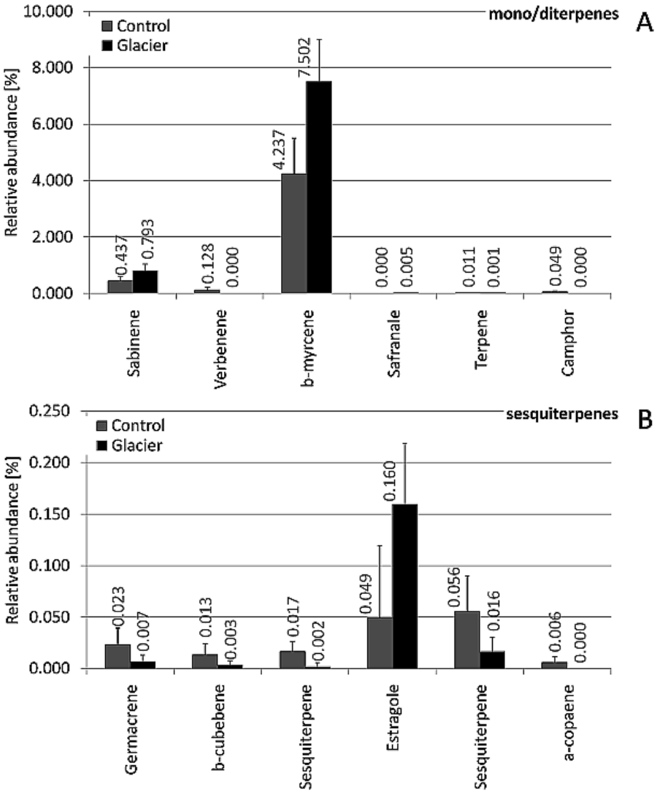

FIG. 6 - Concentration of the mono- and diterpenes (A) and of the sesquiterpenes (B) in the larch needles showing significant differences in mean values between Control and Glacier sites. Error bars indicate +1 standard deviation. The mean values of concentrations are also reported as relative abundance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

ferences between sites, the largest differences in concentrations at the two sites were observed for those terpenes showing the highest concentrations: namely, the β-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).

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 & 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 δ^{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 δ^{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 δ^{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 & Long, 1979; Schleser, 1992; Buchmann & 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 δD, 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 & 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 & Stasova, 1993; Lebourgeois, 2000). However, a different percentage of latewood at the Glacier site may be also induced by a differential responses 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 β-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 10°C 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 10°C (reaching a mean value of 30°C) 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 & 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.

REFERENCES

Antonova G.F. & Stasova V.V. (1993) - *Effects of environmental factors on wood formation in Scots pine stems*. Trees, 7, 214-219.

Arthur C.L. & Pawliszyn J. (1990) - *Solid-phase microextraction with thermal desorption using silica optical fibers.* Analytical Chemistry, 62, 2145-2148.

Auer I. & alii (2007) - *HISTALP - historical instrumental climatological surface time series of the Greater Alpine Region.* International Journal of Climatology, 27, 17-46.

Barbour M.M. (2007) - *Stable oxygen isotope composition of plant tissue: a review*. Functional Plant Biology, 34, 83-94.

Battipaglia G., Jäggi M., Saurer M., Siegwolf R.T.W. & Cotrufo M.F. (2008) - *Climatic sensitivity of* $\delta^{18}O$ *in the wood and cellulose of tree rings: results from a mixed stand of Acer pseudoplatanus L. and Fagus sylvatica L.* Palaeogeography, Palaeoclimatology, Palaeoecology, 261, 193-202.

Battipaglia G., Saurer M., Cherubini P., Siegwolf R.T.W. & Cotrufo M.F. (2009) - *Tree rings indicate different drought resistance of a native (Abies alba Mill.) and a nonnative (Picea abies (L.) Karst.) species co-occurring at a dry site in Southern Italy*. Forest Ecology and Management, 257, 820-828.

Battipaglia G., De Micco V., Brand W.A., Linke P., Aronne G., Saurer M. & Cherubini P. (2010) - *Variations of vessel diameter and* $\delta^{13}C$ *in false rings of Arbutus unedo L. reflect different environmental conditions*. New Phytologist, 188, 1099-1112.

Battipaglia G., Saurer M., Cherubini P., Calfapietra C., McCarthy H.R., Norby R.J. & Cotrufo, M.F. (2013) - *Elevated* CO_2 *increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites*. New Phytologist, 197, 544-554.

Battipaglia G., De Micco V., Brand W.A., Saurer M., Aronne G., Linke P. & Cherubini P. (2014) - *Drought impact on water use efficiency and intra-annual density fluctuations in Erica arborea on Elba (Italy)*. Plant, Cell & Environment, 37, 382-391.

Beniston M., Stephenson D.B., Christensen O.B., Ferro C.A.T., Frei C., Goyette S., Halsnaes K., Holt T., Jylhä K., Koffi B., Palutikof J., Schöll R., Semmler T. & Woth K. (2007) - *Future extreme events in European climate: an exploration of regional climate model projections*. Climatic Change, 81, 71-95.

Bidart-Bouzat M.G. & Imeh-Nathaniel A. (2008) - *Global change effects on plant chemical defenses against insect herbivores*. Journal of Integrative Plant Biology, 50, 1339-1354.

Bond B.J. (2000) - *Age-related changes in photosynthesis of woody plants*. Trends in Plant Science, 5, 349-353.

Braithwaite R.J., Raper S.C.B. & Candela R. (2013) - *Recent changes (1991-2010) in glacier mass balance and air temperature in the European Alps.* Annals of Glaciology, 54(63), 139-146.

Branda E., Turchetti B., Diolaiuti G., Pecci M., Smiraglia C. & Buzzini P. (2010) - *Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy)*. Fems Microbiology Ecology, 72, 354-369.

Brown C.L. (1970) - *Physiology of wood formation in conifers*. Wood Science, 3, 8-22.

Brunetti M., Maugeri M., Monti F. & Nanni T. (2004) - *Changes in daily precipitation frequency and distribution in Italy over the last 120 years*. Journal of Geophysical Research: Atmospheres, 109, D05102, doi:10.1029/2003JD004296.

Buchmann N. & Ehleringer J.R. (1998) - CO_2 *concentration profiles, and carbon and oxygen isotopes in C3 and C4 crop canopies.* Agricultural and Forest Meteorology, 89, 45-58.

Büntgen U., Frankm D.C., Nievergelt D. & Esper J. (2006) - *Summer temperature variations in the European Alps, A.D. 755-2004*. Journal of Climate, 19, 5606-5623.

Büntgen U., Tegel W., Nicolussi K., McCormick M., Frank D., Trouet V., Kaplan J.O., Herzig F., Heussner K.-U., Wanner H., Luterbacher J. & Esper J. (2011) - *2500 years of European climate variability and human susceptibility*. Science, 331, 578-583, doi:10.1126/science.1197175.

Caccianiga M., Andreis C., Diolaiuti G., Mihalcea C. & Smiraglia C. (2010) - *Alpine debris-covered glaciers as a habitat for plant life*. The Holocene, 21, 1011-1020.

Cannone N., Diolaiuti G., Guglielmin M. & Smiraglia C. (2008) - *Accelerating climate change impacts on Alpine glacier forefield ecosystems in the European Alps*. Ecological Application, 18, 637-648.

Christensen O.B. & Christensen J.H. (2004) - *Intensification of extreme European summer precipitation in a warmer climate*. Global and Planetary Change, 44, 107-117.

Cook E.R. & Briffa K.R. (1990) - *Data analysis*. In: Cook E.R. & Kairiukstis L.A. (Eds), «Methods of dendrochronology. Applications in the environmental sciences» Kluwer, Boston, 97-162.

Coppola A., Leonelli G., Salvatore M.C., Pelfini M. & Baroni C. (2013) - *Tree-ring-based summer mean temperature variations in the Adamello-Presanella Group (Italian Central Alps), 1610-2008 AD*. Climate of the Past, 9, 211-221.

Dawson T.E., Mambelli S., Plamboeck A.H., Templer P.H. & Tu K.P. (2002) - *Stable isotopes in plant ecology*. Annual Review of Ecology and Systematics, 33, 507-559.

Deline P. (2005) - *Change in surface debris cover on Mont Blanc massif glaciers after the "Little Ice Age" termination*. The Holocene, 15(2), 302-309.

Dorado Linan I., Gutierrez E., Heinrich I., Andreu-Hayles L., Muntàn E., Campelo F. & Helle G. (2012) - *Age effects and climate response in trees: a multi-proxy tree-ring test in old-growth life stages*. European Journal of Forest Research, 131(4), 933-944.

Duhl T.R., Helmig D. & Guenther A., 2008: *Sesquiterpene emissions from vegetation: a review*. Biogeosciences, 5, 761-777.

Duhl T.R., Gochis D., Guenther A., Ferrenberg S. & Pendall E. (2013) - *Emissions of BVOC from lodgepole pine in response to mountain pine beetle attack in high and low mortality forest stands*. Biogeosciences, 10, 483-499.

Esper J., Frank D.C., Büntgen U., Verstege A., Hantemirov R.M. & Kirdyanov A.V. (2010) - *Trends and uncertainties in Siberian indicators of 20th century warming*. Global Change Biology, 16, 386-398.

Farquhar G.D., O'Leary M.H. & Berry J.A. (1982) - *On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves*. Australian Journal of Plant Physiology, 9, 121-137

Farquhar G.D., Ehleringer J.R. & Hubick K.T. (1989) - *Carbon isotope discrimination and photosynthesis*. Annual Review of Plant Physiology and Plant Molocular Biology, 40, 503-537.

Farquhar G.D., Cernusak L.A. & Barnes B. (2007) - *Heavy water fractionation during transpiration*. Plant Physiology, 143, 11-18.

Ferrio J.P., Mateo M.A., Bort J., Abdalla O., Voltas J. & Araus J.L. (2007) - *Relationships of grain* $\delta^{13}C$ *and* $\delta^{18}O$ *with wheat phenology and yield under water-limited conditions*. Annals of Applied Biology, 150, 207-215.

Francey R.J., Allison C.E., Etheridge D.M., Trudinger C.M., Enting I.G., Leuenberger M., Langenfelds R.L., Michel E. & Steele L.P. (1999) - *A 1000-year high precision record of* $\delta^{13}C$ *in atmospheric* CO_2. Tellus 51B: 170-193.

Fritts H.C. (1976) - «*Tree rings and climate*». Academic Press, New York, 567 pp.

Gagen M., Mccarroll D., Loader N.J., Robertson I., Jalkanen R. & Anchukaitis K.J. (2007) - *Exorcising the "segment length curse": summer temperature reconstruction since AD 1640 using non-detrended stable carbon isotope ratios from pine trees in northern Finland*. The Holocene, 17, 435-446.

Gairola S., Shariff N.M., Bhatt A. & Kala C.P. (2010) - *Influence of climate change on production of secondary chemicals in high altitude medicinal plants: Issues needs immediate attention.* Journal of Medicinal Plants Research, 4(18), 1825-1829.

Giardino M., Mortara G. & Bonetto F. (2001) - *Proposta per la realizzazione di un catalogo aerofotografico dei ghiacciai italiani.* Geografia Fisica Dinamica Quaternaria Supplementi, 5, 89-98.

Giorgi A., Panseri S., Nanayakkara N.N.M.C. & Chiesa L.M. (2012a) - *HS-SPMEGC/MS analysis of the volatile compounds of Achillea collina: evaluation of the emissions fingerprint induced by Myzus persicae infestation*. Journal of Plant Biology, 55, 251-260.

Giorgi A., Panseri S., Mattara M.S., Andreis C. & Chiesa L.M. (2012b) - *Secondary metabolites and antioxidant capacities of Waldheimia Glabra (Decne.) Regel from Nepal.* Journal of the Science of Food and Agriculture, DOI:10.1002/jsfa.5839.

Gobbi M., Isaia M. & De Bernardi F. (2011) - *Arthropod colonisation of a debris-covered glacier*. The Holocene, 21, 343-349.

Grissino-Mayer H.D. (2001) - *Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA.* Tree-Ring Research, 57, 205-221.

Guerrieri E. & Digilio M.C. (2008) - *Aphid-plant interactions: a review*. Journal of Plant Interaction, 3(4), 223-232.

Hengxiao G., McMillin J.D., Wagner M.R., Zhou J., Zhou Z. & Xu X. (1999) - *Altitudinal variation in foliar chemistry and anatomy of Yunnan pine, Pinus yunnanensis, and pine sawfly (Hym., Diprionidae) performance*. Journal of Applied Entomology, 123, 465-471.

Holmes R.L. (1983) - *Computer-assisted quality control in tree-ring dating and measurement*. Tree-Ring Bulletin, 43(1), 69-78.

Janson R.W. (1993) - *Monoterpene emissions from Scots pine and Norwegian spruce*. Journal of Geophysical Research-Atmospheres, 98, 2839-2850.

Jolly W.M., Dobbertin M., Zimmerman N.E. & Reichstein M. (2005) - *Divergent vegetation growth responses to the 2003 heat wave in the Swiss Alps*. Geophysical Research Letters, 32: L18409. doi:10.1029/2005GL023252.

Kesselmeier J. & Staudt M. (1999) - *Biogenic volatile compounds (VOC): an overview on emission, physiology and ecology*. Journal of Atmospheric Chemistry, 33, 23-88.

Larson P.R. (1960) - *A physiological consideration of the springwood summerwood transition in red pine*. Forest Science, 6, 110-122.

Leavitt S.W., Treydte K. & Yu L. (2010) - *Environment in time and space: opportunities for tree-ring isotpope networks*. In: West J.B., Bowen G.J., Dawson T.E. & Tu K.P. (Eds), «Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping». Springer, Dordrecht, The Netherlands.

Lebourgeois F. (2000) - *Climatic signals in earlywood, latewood and total ring width of Corsican pine from western France*. Annals of Forest Science, 57, 155-164.

Leonelli G. & Pelfini M. (2008) - *Influence of climate and climate anomalies on Norway spruce tree-ring growth at different altitude and on glacier responses: examples from the Central Italian Alps*. Geografiska Annaler, Series A, 90, 75-86.

Leonelli G. & Pelfini M. (2013) - *Past surface instability of Miage debris-covered glacier tongue (Mont Blanc Massif, Italy): a decadal-scale tree-ring-based reconstruction.* Boreas, 42, 613-622.

Leonelli G., Pelfini M., Battipaglia G., Saurer M., Siegwolf R.T.W. & 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.

Lerman J.C. & Long A. (1979) - *Carbon-13 in tree rings: local or canopy effect?* In Jacoby G. (Ed), «Proceedings of the international meeting on stable isotopes in tree ring research», New York, pp 22-32.

Li Q., Nakatsuka T., Kawamura K., Liu Y. & Song H. (2011) - *Hydroclimate variability in the North China Plain and its link with El Niño-Southern Oscillation since 1784 A.D.: Insights from tree-ring cellulose* $\delta^{18}O$. Journal of Geophysical Research, Atmospheres 116(D22), D22106, DOI: 10.1029/2011JD015987.

Loader N.J., Robertson I., Barker A.C., Switsur V.R. & Waterhouse J.S. (1997) - *An improved technique for the batch processing of small whole wood samples to alpha-cellulose*. Chemical Geology, 136, 313-317.

Loader N.J., McCarroll D., Gagen M., Robertson I. & Jalkanen R. (2007) - *Extracting climatic information from stable isotopes in tree rings*. In: Dawson T.E. & Siegwolf R.T.W. (Eds), «Stable isotopes as indicators of Ecological Change». Elsevier Inc., New York, pp. 27-48.

Loader N.J, Young G.H.F., McCarroll D. & Wilson R.J.S. (2013) - *Quantifying uncertainty in isotope dendroclimatology.* The Holocene, 23(9), 1221-1226.

Loreto F. & Schnitzler J.P. (2010) - *Abiotic stresses and induced BVOCs*. Trends in Plant Science, 15, 154-166.

Maffei M.E. (2010) - *Sites of synthesis, biochemistry and functional role of plant volatiles*. South African Journal of Botany, 76, 612-631.

Mattson L.E. & Gardner J.S. (1989) - *Energy exchange and ablation rates on the debris-covered Rakhiot Glacier, Pakistan*. Zeitschrift für Gletscherkunde und Glazialgeologie, 25, 17-32.

Mc Carrol D. & Loader N.J. (2004) - *Stable isotopes in tree rings*. Quaternary Science Reviews, 23, 771-801.

Mello M.O. & Silva-Filho M.C. (2002) - *Plant-insect interactions: an evolutionary arms race between two distinct defense mechanisms*. Brazilian Journal of Plant Physiology, 14, 71-81.

Mihalcea C., Brock B., Diolaiuti G., D'Agata C., Citterio M., Kirkbride M., Smiraglia C. & Cutler M. (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.

Moreno-Gutiérrez C., Battipaglia G., Cherubini P., Saurer M., Nicolás E., Contreras S. & Querejeta J.I. (2012) - *Stand structure modulates the long-term vulnerability of Pinus halepensis to climatic drought in a semiarid Mediterranean ecosystem*. Plant, Cell and Environment, 35, 1026-1039.

Nakatsuka T., Ohnishi K. & Hara T. (2008) - *Reconstruction of summer temperature variation by hydrogen and oxygen isotopic ratios of larch tree-ring cellulose in Kamchatka Peninsula*, Chikyu Mon, 30, 207-215.

Ogaya R. & Peñuelas J. (2003) - *Comparative field study of Quercus ilex and Phillyrea latifolia: photosynthetic response to experimental drought conditions*. Environmental and Experimental Botany, 50, 137-148.

Oliveira A.P., Silva L.R., Andrade P.B., Valentão P., Silva B.M., Pereira J.A. & de Pinho P.G. (2010) - *Determination of low molecular weight volatiles in Ficus carica using HS-SPME and GC/FID*. Food Chemistry, 121(4), 1289-1295.

Paré P.W. & Tumlinson J.H. (1999) - *Plant volatiles as a defense against insects herbivores*. Plant Physiology, 121, 325-332.

Pelfini M., Santilli M., Leonelli G. & Bozzoni M. (2007) - *Investigating surface movements of debris-covered Miage glacier,Western Italian Alps, using dendroglaciological analysis*. Journal of Glaciology, 53(180), 141-152.

Pelfini M. & Santilli M. (2008) - *Frequency of debris flows and their relation with precipitation: A case study in the Central Alps, Italy*. Geomorphology, 101, 721-730.

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: The case study of the Miage Glacier, Italian Alps*. The Holocene, 22(8), 847-856.

Raffali-Delerce G., Masson-Delmotte V., Dupoey J.L., Stievenard M., Breda N. & Moisselin J.M. (2004) - *Reconstruction of summer droughts using tree-ring cellulose isotopes: a calibration study with living oaks from Britanny (western France).* Tellus B, 56, 160-174.

Räisänen T., Ryyppö A. & Kellomäki S. (2009) - *Monoterpene emission of a boreal Scots pine (Pinus sylvestris L.) forest.* Agricultural and Forest Meteorology, 149, 808-819.

Richter M., Fichter T. & Grüninger F. (2004) - *Pflanzen Auf Schuttbedeckten Gletschern - Wandernde Kuriositäten*. Geoöko, 25, 225-256.

Rinn F. (2005) - *TSAPWin - Time Series Analysis and Presentation for Dendrochronology and Related Applications, Version 0.53,* «User Reference», Heidelberg. 91 pp.

Ripullone F., Guerrieri M., Saurer M., Siegwolf R., Jäggi M., Guarini R. & Magnani F. (2009) - *Testing a dual isotope model to track carbon and water gas exchanges in a Mediterranean forest*. iForest - Biogeosciences and Forestry, 2, 59-66.

Roden J.S. & Farquhar G.D. (2012) - *A controlled test of the dual-isotope approach for the interpretation of stable carbon and oxygen isotope ratio variation in tree rings*. Tree Physiology, 32(4), 490-503.

Saurer M., Siegwolf R.T.W. & Schweingruber F.H. (2004) - *Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years*. Global Change Biology, 10, 2109-2120.

Scheidegger Y., Saurer M., Bahn M. & Siegwolf R. (2000) - *Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: A conceptual model*. Oecologia, 125, 350-357.

Schleser G.H. (1992) - *δ^{13}C pattern in a forest tree as an indicator of carbon transfer in trees*. Ecology, 73, 1922-1925.

Schweingruber F.H., Wehrli U., Aellen-Rumo K. & Aellen M. (1991) - *Weiserjahre als Zeiger extremer Standortenseinflusse.* Schweizerische Zeitschrift für Forstwesen*,* 142, 33-52.

Smiraglia C., Diolaiuti G., Casati D. & Kirkbride M.P. (2000) - *Recent areal and altimetric variations of Miage Glacier (Monte Bianco massif, Italian Alps).* In: Nakawo M., Raymond C. & Fountain A. (Eds), «Debris-Covered Glaciers». International Association of Hydrological Sciences (IAHS). Publication 264, 227-233.

Staudt M. & Seufert G. (1995) - *Light-dependent emission of monoterpenes by Holm Oak (Quercus ilex L.).* Naturwissenschaften*,* 82, 89-92.

Stoffel M. & Bollschweiler M. (2009) - *What tree rings can tell about earth-surface processes: teaching the principles of dendrogeomorphology*. Geography Compass, 3/3, 1013-1037.

Strunk H. (1997) - *Dating of geomorphical processes using dendrogeomorphical methods*. Catena*,* 31, 137-151.

Tiiva P., Rinnan R., Faubert P., Räsänen J., Holopainen T., Holopainen J.K. & Kirö E. (2007) - *Isoprene emission from a subarctic peatland under enhanced UV-B radiation*. New Phytologist, 176, 346-355.

Treydte K., Schleser G.H., Helle G., Frank D.C., Winiger M., Haug G.H. & Esper J. (2006) - *The twentieth century was the wettest period innorthern Pakistan over the past millennium*. Nature, 440, 1179-1182.

Turtola S., Rousi M., Pusenius J., Yamaji K., Heiska S., Tirkkonen V., Meier B. & Riitta J.T. (2006) - *Genotypic variation in drought response of willows grown under ambient and enhanced UV-B radiation*. Environmental and Experimental Botany, 6(1), 80-86.

Wallis C.M., Huber D.P.W. & Lewis K.J. (2011) - *Ecosystem, location, and climate effects on foliar secondary metabolites of Lodgepole pine populations from central British Columbia.* Journal of Chemical Ecology, 37, 607-621.

Yoder B.J., Ryan M.G., Waring R.H., Schoettle A.W. & Kaufmann M.R. (1994) - *Evidence of reduced photosynthetic rates in old trees*. Forest Science, 40, 513-527.

Zhang C., Qi M., Shao Q., Zhou S. & Fu R. (2007) - *Analysis of the volatile compounds in Ligusticum chuanxiong Hort. using HS-SPME-GC-MS.* Journal of Pharmaceutical and Biomedical Analysis, 44(2), 464-470.

Zhang Y. & Li H. (2010) - *Innovation search of new ventures in a technology cluster: the role of ties with service intermediaries.* Strategic Management Journal, 31, 88-109.

(Ms. received 31 October 2013; accepted 15 June 2014)