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STRUCTURAL PATTERNS, GROWTH PROCESSES, CARBON STOCKS IN AN ITALIAN NETWORK OF OLD-GROWTH BEECH FORESTS

In the framework of the Project of National Interest (PRIN) "Climate change and forests - Dendroecological and ecophysiological responses, productivity and carbon balance on the Italian network of old-growth beech forests", 9 old beech (Fagus sylvatica L.) forests were sampled in the eastern Alps and in the central Apennines to assess: i) the degree of 'old-growthness' on a structural and dendroecological basis; ii) the carbon (C) stocks in the different ecosystem compartments; iii) some ecophysiological traits using stable isotopes.

Live and dead tree structure, soil features and C stocks were examined in some among the oldest and less disturbed beech forests in Italy. Furthermore, leaves, litter and wood cores were sampled for concurrent dendroecological, nutrient and stable isotope analysis, to study age structure, disturbance history and medium- and long-term response to climate and ecophysiological traits.

In all sampled stands, values of basal area, volume and large trees density reached or exceeded control values reported for European and North American old-growth forests, while total amount of deadwood was generally low, except in two sites. Diameter distribution showed a remarkable differentiation from bimodal to 'rotated-sigmoid' curve. In accordance with structural analyses, disturbance chronologies revealed the importance of frequent low-to-moderate events in generating a fine-scale structure, confirming previous

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results on beech forests. Different degrees of 'old-growthness' were identified for Alpine and Apennine beech stands through the 'structural-based approach', which proved to be a valid tool for old-growth forests detection. Preliminary results on carbon stocks on a subset of sites indicate that these old-growth forests are relevant carbon reservoirs, with 192-268 MgC ha⁻¹ of total biomass (67-73% aboveground; 27-33% belowground, 4 stands) and 7-21 MgC ha⁻¹ of deadwood. In these stands, also forest floor (excluding deadwood) and soils are stores of relevant amount of carbon (5-9 MgC ha⁻¹ litter layer, 4 stands; 168-420 MgC ha⁻¹ mineral soil, 3 stands). Finally, carbon isotope discrimination (Δ) analysis, a proxy of wateruse efficiency, was applied on tree-ring cores from a subset of sites. The perspectives of this technique for a retrospective ecophysiological interpretation of climate-change impact on old-growth forests were described.

Preliminary results obtained within this PRIN project indicate that the reported ecological indicators can be used to produce a description of forest structures and processes driving stand dynamics ('structural-based approach'). Furthermore, the use of multiple sampling and research techniques and the integration of research groups with complementary expertise can foster deeper understanding of the ecology and dynamics of old-growth forests.

Key words: old-growth forests; tree-ring; dendroecology; disturbance regime; carbon stocks; stable isotopes.

Parole chiave: foresta vetusta; dendroecologia; regime di disturbo; stock di carbonio; isotopi stabili.

1. INTRODUCTION

Studies on old-growth forests are continuously increasing: the database of SCOPUS (www.scopus.com) reports 2167 articles from 1975 to may 28th 2010, of which 81 were in 2010.

An old-growth forest is a forest ecosystem whose structural features are influenced by the presence of old trees (SPIES, 2004). However, describing old-growth forests by only considering the age of the oldest trees might appear too restrictive, since this approach could be overly linked to an anthropocentric vision of nature (FRELICH and REICH, 2003). Moreover, the oldest individuals often do not reach impressive diameters: reduced growth rates and dimensions, typical of the extreme sites where old-growth forests are often found, represent important factors contributing to tree longevity in marginal sites (PEÑUELAS, 2005). At the same time, forest structure alone is not sufficient to define the naturalness degree of a given stand. In this respect, bio-ecological processes typical of natural forests (i.e. the presence of largesized senescent and dying trees and the disturbance regime which shape the canopy structure and dynamics) should be characterized (NAGEL *et al.*, 2007).

From this point of view an old-growth structure in temperate deciduous forests can be recognized when dominant old trees start experiencing mortality after natural events (e.g. windthrows, glaze storms, droughts) and new space (canopy gaps) for the establishment of new cohorts is created (DI FILIPPO *et al.*, 2005). Old-growth forests can further be distinguished as *primary* or *secondary* (FRELICH and REICH, 2003). In primary old-growth stands there isn't evidence of anthropogenic disturbances, not even in dendroecological series, while secondary old-growth forests structures and dynamics have been influenced by human activities in the more or less recent past.

Old-growth forests are important for biodiversity conservation and as carbon reservoirs. Research on their contribution to the global carbon cycle are increasing (LUYSSAERT *et al.*, 2008; WIRTH *et al.*, 2009), even though the application of techniques used in managed forests could underestimate their role as carbon sinks (CAREY *et al.*, 2001). Recent studies highlighted that, contrary to the traditional view on their carbon neutrality, old-growth forests can continue to accumulate carbon (LUYSSAERT *et al.*, 2008), even in their soils (ZHOU *et al.*, 2006).

Hence, old-growth beech forests could provide crucial informations about structural dynamics, growth and carbon stocks in conditions of reduced human impacts. In particular, within the context of climate change impact on forests, a main concern deals with changes in frequency and extent of drought, particularly in the Mediterranean area. These occurrences are likely to determine hard selective pressures on forest stands. If not equipped with enough resilience to new conditions, many forest populations could disappear, especially across the southern distribution areas. In this context, it must be considered that the number of studies dealing with old-growth stands in the Mediterranean area is still very limited (JUMP *et al.*, 2006).

Recent studies focused on the physiological character "water-use efficiency" (WUE) to describe forest species adaptiveness to drought conditions (GUEHL et al., 1995; LAUTERI et al., 2004). The physiological significance of WUE is addressed in the plant carbon - water economy, represented by the ratio between plant carbon gain and water losses. From an ecophysiological perspective, WUE is related to the balance between ecosystem net carbon fluxes and water losses through transpiration and evaporation. To our knowledge, nothing is known about WUE of old-growth beech forests. Carbon stable isotope analysis is at present the most reliable and the least intrusive methodology to study WUE in natural environments (LAUTERI et al., 1997; BRUGNOLI and FARQUHAR, 2000). The heavy stable isotope ¹³C is discriminated during the photosynthetic CO₂ assimilation (FARQUHAR et al., 1989). As a consequence, plant carbon is invariably lighter than atmospheric C: the so-called carbon isotope discrimination (Δ). Δ is regulated by many physiological, genetic, silvicultural or environmental factors also influencing WUE (FARQUHAR et al., 1982; LAUTERI et al., 2004). Carbon isotope discrimination in tree rings (MCCARROL AND LOADER, 2004)

allows for the study of fluctuations in WUE on a long time scale (from seasons to centuries).

Dendroecology is an effective tool to investigate past forest dynamics (e.g. PIOVESAN *et al.*, 2005b), and in assessing the climate variability impact on tree-growth (e.g. PIOVESAN *et al.*, 2005a; DI FILIPPO *et al.*, 2007) and stand productivity (e.g. PIOVESAN *et al.*, 2008) at different geographic locations, while the detailed assessment of carbon stocks in the different ecosystem compartments (live and dead biomass, roots, litter, soil) can elucidate carbon allocation patterns and will provide data on overall carbon cycling. Thus, in the predicted climatic change, a comprehensive approach using dendroecological, ecological and ecophysiological techniques will provide important data and information on old-growth forests functioning and, in the end, will help to choose the proper management options for nature conservation and mitigation strategies.

In Italy, old-growth beech forests are rare biocenoses present across the Apennines and the Alps. Because of their occurrence along such a wide latitudinal gradient (climate, site, nitrogen deposition), these forests constitute an interesting study case in the topic of forest acclimation and adaptation to global changes. This paper reports the preliminary results of an Italian Research Project (PRIN) about the ecology, structure, auxology, carbon stocks and ecophysiology of primary and secondary montane and high-montane old-growth beech forests of the eastern Alps and central Italy.

2. MATERIALS AND METHODS

2.1. Study sites

A total of 9 Italian beech (*Fagus sylvatica* L.) old-growth forests were sampled for this study.

In the Alpine Region we selected 5 old-growth stands situated in the Carnic Alps (Friuli-Venezia Giulia region, Udine province), where beech was the dominant species. Elevation ranges from 825 to 1450 m a.s.l. (Table 1). With reference to the study areas, Carnic Alps pertain to temperate climate, according to the KÖPPEN-TREWARTHA classification (TREWARTHA, 1968). Almost all stands were situated above small towns where topography is generally strongly sloping (in most cases 70-100%): for this reason they have been historically protected from logging to be maintained as "protection forests" ("boschi banditi"; DI FILIPPO *et al.*, 2007) and have been publicly owned since the 16th century, when they were managed by the Republic of Venice (PAIERO *et al.*, 1975).

Table 1 - Geographi	cal features	of the sampled	l forests.							
Site	Code	Latitude (°)	Longitude (°)	Elevation (m a.s.l.)	Slope (%)	Aspect	Stand area* (ba)	Inventory method	Sampled area* (ba)	Number of plots
				Alpin	ie stands					
Lateis	LAT	46.4594	12.7489	1450 (1370-1530)	75-110	S	20	Circular plots	0.72	6
Trelli	TRL	46.4997	13.0978	950 (850-1050)	60-70	SE	7	Circular plots	0.34	5
Timau	TIM	46.5817	13.0050	1160 (825-1500)	70-90	S	20	Circular plots	0.29	7
Gracco	GRA	46.5514	12.8519	825 (750-900)	70-90	S	6	Total stand inventory	6.00	I
Cleulis	CLE	46.5584	13.0006	930	20-60	NE	14	Total stand inventory	14.00	I
				-						
				Apenn	ine stands					
Val Cervara	VCH	41.8690	13.6230	1725 (1600-1850)	40-65	M	24	Circular plots+Transects	2.20	18 + 2
Coppo del Principe	COP	41.7883	13.7381	1625 (1500-1750)	20-60	NE	45	Circular plots	0.84	7
Fonte Regna	REG	42.3247	12.7194	954 (880-1010)	50-60	M	5	Transect	0.47	1
Monte Cimino	CIM	42.4181	12.2350	1000 (950-1050)	0-35	M	62	Circular plots	3.00	25

* Areas corrected for slope.

In central Italy, 4 old-growth beech forests were sampled. In the landscape of peninsular Italy, old-growth primary forest remnants can only be found at high elevation (PIOVESAN et al., 2005b), while it could result easier to find secondary old-growth forests whose protection has been linked to their aesthetic-socio-cultural value (Monte Cimino, CIM; Fonte Regna, REG). The old-growth forests of Valle Cervara (VCH) and Coppo del Principe (COP) are located inside the Abruzzo-Lazio-Molise National Park (PNALM; Abruzzi region, L'Aquila province). The climate regime can be described as Mediterranean montane, with cold snowy winters and dry summers (PIOVESAN et al., 2003); both stands are high-elevation beech forests which escaped logging because of their difficult access and probably even because of their protective function against avalanches and landslides (PIOVESAN et al., 2005b). VCH (for acronyms see Table 1) beech forest is a primary multicohort forest (FRELICH, 2002), with an uneven-aged structure that includes the oldest known Fagus tree (more that 500 years old; tree cored by L. HERMANIN) in the Northern Hemisphere (PIOVESAN et al., 2005b). COP, located close to VCH, is a forest showing several senescent and/or dying individuals. CIM and REG are secondary old-growth mountain forests situated in the Latium region (Viterbo and Rieti province, respectively). The beech stand of Monte Cimino is at the top of the homonymous mountain; it was managed for a long time as a low-density grazing forest, but during the 19th century, under the pressure of local communities, the social-aesthetic value of the site became prominent so that timber logging ceased in 1949 (LO MONACO, 1983). Since then, no other logging practices have been performed and today the forest is in the so-called demographic transition stage (FRELICH, 2002), passing from an impressive single-layer canopy to a multi-cohort structure. The forest of REG is represented by a mixed stand of Fagus sylvatica and Quercus ilex and its preservation from massive logging was linked to the presence of an historical spring providing freshwater to the contiguous town of Roccantica (PIOVESAN et al., 1996). Grazing is still present today in the lower borders of the forest.

2.2. Structural analysis and dead wood

Structural analyses were generally conducted using circular plots with a 20 m radius (all the Apennines stands and Lateis, LAT), with the exception of the steepest Alpine site (Timau, TIM), where the radius of the sampling plots was 10 to 20 m. Within each stand, circular plots were positioned with a systematic criterion in order to cover most of the old-growth forest area. The average distance among plots was 100 m. However, due to the presence of a rugged topography and/or the fragmentation of the forest area in several

nearby small stands, the distance among plots varied between 70 and 150 m. In the small stand of Fonte Regna (Table 1) a transect of $30 \ge 180$ m was made within the core area.

Structural features (stems per ha, basal area, volume, diameter distribution) about living trees of the Alpine sites Gracco (GRA) and Cleulis (CLE) were instead obtained from Forest Management Plans (FMPs); information about dead wood structure was lacking in FMPs.

In each plot, for each tree species we measured Diameter at Breast Height (DBH, measured at 1.3 m from the ground) on trees with DBH > 2.5 cm (DBH thresholds vary for FMPs data: see Table 2). Heights were measured on a representative number of trees of all diameter classes to build height-DBH curves. For each tree, linear distance from the plot center and the azimuthal angle from the North were measured (polar coordinates). Basal area and volume distributions in DBH classes were determined, with a special attention to the relative amounts of both parameters attributable to the largest DBH classes. Volume of living trees was calculated with the two-way volume table for *Fagus* in Italy (CASTELLANI *et al.*, 1984) except for CIM where the one-way volume table for *Fagus* of Calabria (I class of fertility) was used (SGADARI, 1961).

Dead wood was divided into its standing and fallen components, called respectively snags and Coarse Woody Debris (CWD: logs and branches). Only downed debris with median diameter (D_{med}) ≥ 10 cm were ascribed to CWD, while those with $D_{med} < 10$ cm were defined as Fine Woody Debris (FWD). FWD were further divided into two dimensional classes: FWD₂₋₁₀, with D_{med} in the range 2-10 cm; FWD₀₋₂, with $D_{med} < 2$ cm.

Dead wood structure was determined for each site by recording the number and diameter distribution of snags and CWD. Polar coordinates, DBH and height of snags with DBH \geq 10 cm were measured; for truncated snags volume was calculated according to the formula:

$$V = g^* h$$

where g = basal area, h = snag height. Instead, for unbroken standing dead trees we used the two-way table for *Fagus* in Italy (CASTELLANI *et al.*, 1984).

Polar coordinates and median diameter (D_{med}) were measured for all CWD $(D_{med} \ge 10 \text{ cm})$. CWD volume was calculated according to Huber's formula (FRIDMAN and WALHEIM, 2000):

$$V = s_{0.5} * l$$

where $s_{0.5}$ = median sectional area and l = length.

Each element of dead wood (snags or CWD) was referred to a decay class ranging from class I to V with decreasing integrity (RUBINO and MCCARTHY, 2003).

Table 2 DBH>1 volume) measure	– Struct 7.5 cm; ratio; A{ d ring).	tural feat Stature=: ge _{max} =nui	tures of t mean hei mber of r	the study ght of th ings cout	√ sites (t _{DB} ne 5 tallest nted on th¢	_H =diameter s. trees; G=bas e oldest tree; <i>f</i>	ampling thre al area per h Àge5=mean ag	shold; N= lectare; V= ge of the 5 (number of woody volu oldest trees	stems per hectar ume per hectare; I (yrs); Period=cale	e; N _{17,5} =nur DV/TV=De ndar years c	nber of st ad to Tota orrespondi	ems per l volume ing to the	hectare with (live + dead first and last
Site			Live tree.	's structur	<i>∂</i> ,		Deau	d trees struc	ture			Age struc	ture	
	${t_{DBH} \over (cm)}$	$\binom{N}{(n \ ba^{-1})}$	$\binom{N_{17.5}}{(n \ ba^{-1})}$	Stature (m)	$G_{m^2 \ ba^{-1}})$	$V (m^3 ba^1)$	Dead volume	$\begin{array}{c} Snags\\ (m^3 \ ba^{-1}) \end{array}$	$CWD (m^3 ba^1)$	DV/TV(%) $(m^3 ba^{-1})$	Cores	$\mathop{Age}_{(\rm yrs)}$	$\mathop{Age}_{(yrs)}$	Period
							Alpi	ne stands						
LAT	2.5	878	505	30	40	533	18	ŗ	13	3.3	28	380	371	1625-2004
TRL	2.5	741	244	21	29	319	16	6	7	6.9	22	323	316	1686-2008
MIT	7.5	568	356	33	40	647	I	21	I	I	27	348	280	1655-2002
GRA	17.5	336	336	21	34	302	I	I	I	I	20	291	276	1685-2002
CLE	2.5	430	I	24	35	394	I	I	I	I	23	260	209	1744-2003
							Арет,	nine stands						
VCH	2.5	1590	239	28	41	497	65	24	41	11.6	32	503	425	1500-2002
COP	2.5	296	188	32	44	715	91	37	54	11.3	30	376	340	1633-2008
REG	2.5	317	446	32	42	540	42	24	18	7.3	20	179	166	1830-2008
CIM	2.5	197	132	46	48	707	19	5	14	2.6	33	205	162	1804-2008





 FWD_{2-10} was sampled using the line-intercept method (VAN WAGNER, 1968). At each site, three to six 50 m-long lines were sampled. According to this method, the volume per unit area (m³ m⁻²) is:

$$V = 9.869^* \Delta (d^2 / 8L)$$

where *d* is the piece diameter (m), and *L* is the line length (m). For the FWD category, each element was referred to a decay class ranging from I to III with decreasing integrity (D'ANDREA, 2008):

– Class I, 100% wood basal density (for beech, 0.600 Mg m⁻³);

– Class II, 66% wood basal density (0.396 Mg m⁻³);

– Class III, 33% wood basal density (0.198 Mg m⁻³).

 $FWD_{0.2}$ was estimated at each site by collecting all pieces in fixed area plots (1 m²). At each site, three random sub-plots in each 20 m radius circular area were sampled.

When presenting each stand structural feature, Alpine and Apennines sampled stands were ranked according to the Age₅ parameter (see *Dendroecological analysis* for explanation).

2.3. Carbon stocks in ecosystem compartments

2.3.1. Biomass

Aboveground biomass of stems and major branches was estimated by converting the volume values given by two-way tables for beech (CASTELLANI *et al.*, 1984) or one way table in the case of Cimino (SGADARI, 1961) using the wood basal density measured at a montane forest in Central Italy (Collelongo; SCARASCIA MUGNOZZA *et al.*, 2000).

At selected sites, leaf biomass was assessed using litter traps. Ten to twenty 0.5 m² circular litter traps were installed at CIM and REG. Litter was collected three times at the end of the growing season. Litter was brought back to the laboratory dried, weighed and three samples per site were analysed for Carbon and Nitrogen content (see under Chemical analysis).

For belowground biomass, the detailed allometric equations of Collelongo montane beech forest were used to estimate stump, coarse root (diameter > 2.5 cm), medium root (from 1.5 to 2.5 cm of diameter) and small root (from 0.5 to 1.5 cm of diameter) biomass (MASCI, 2002).

Conversion of biomass to carbon was made by using carbon content measured on the relevant component or literature data (BAUER *et al.*, 2000).

2.3.2. Litter

Accumulation of litter on the ground was determined during summer on randomly distributed positions. Samples of forest floor were obtained by pressing a 0.09 m^2 quadrat sampling frame into the forest floor and collecting all organic material of horizons L and F above the mineral soil. The L horizon is composed of fresh or slightly discoloured material, with no or weak breaking up. The F horizon is composed of medium to strongly fragmented material with many mycelia and thin roots. The number of samples was 18 for CIM, 12 for REG, 12 for COP, 8 for LAT, 8 for TIM.

2.3.3. Soil

At each site, after a preliminary series of coring to unravel diagnostic soil horizons, a representative soil profile was opened and described according to SCHOENEBERGER *et al.* (2002). Sampling was performed according to horizons using minipits and corer. Approximately 10 samples per horizon were collected. Horizon bulk densities were determined in the field, using cylinders of known volume. Three replicates per horizon were performed.

Samples were brought to the laboratory, oven-dried at 60° C to constant weight and sieved (2 mm) to separate the coarse fraction (> 2 mm) from fine earth (< 2 mm). Soil texture was estimated using the pipette method on three replicates per soil horizon, while pH was determined on all samples in water.

Soil carbon stock (SOC, kg C m⁻²) was calculated using the following equation (BOONE *et al.* (1999):

$$SOC = a * b * c * d$$

where a = C concentration in the sample (kg C kg_{soil}⁻¹), b = bulk density (kg_{soil} m⁻³), c = soil depth (m), d = 1 - coarse fraction

2.3.4. Chemical analysis

All chemical analyses were performed with an elemental analyser Carlo Erba NA1500 (Milano, Italy) on dried and milled samples. In the case of soils, samples were homogenised to less then 0.5 mm. Carbonates were removed by using a 10% HCl solution.

2.4. Dendroecological analysis

A minimum number of 20 cores were sampled in each site to perform dendroecological analyses. Trees were cored at breast height with a Pressler's increment borer: in TRL, COP, REG and CIM canopy trees representative of all DBH classes were cored, while in LAT, TIM, GRA, CLE and VCH, sampled in previous dendroclimatic studies, only dominant-codominant trees were available. A total of 235 cores were sampled in the period 2002-2008 (120 cores for Alpine stands; 115 for Apennines stands). Cores were mounted, sanded and polished and ring-widths measured to the nearest 0.01 mm using CCTRMD (Computer Controlled Tree Ring Measure Device; ANIOL, 1987) and the software CATRAS (ANIOL, 1983). Finally tree-ring series were dated after visual and statistical crossdating (STOKES and SMILEY, 1996).

Cores reaching the pith were used to investigate DBH-age relationships and to analyze age influence on forest structure. The age refers to 1.30 m. Maximum age (Age_{max}) was defined for each stand as the age of the oldest tree, but the parameter Age₅ (mean age of the 5 oldest trees per stand) was used to rank all stands according to their age.

The *boundary line* (BLACK and ABRAMS, 2003) for beech in the Italian peninsula was built considering 57796 pairs of ten-years averaged *Percent growth change* (%GC) and *Prior growth* (PG) values derived from 15 Italian beech stands (10 in the Eastern Alps and 5 in Central Italy) to detect moderate and major radial growth releases related to the occurrence of ecological disturbances (*data not shown*). Disturbance events, dated according to the *boundary line* release criteria (distinguished into moderate and major events), were organized into decades. Disturbance chronologies for 2 old sites in the Eastern Alps and 2 in the Apennines were developed. Finally, the growth history and release events of the 3 oldest trees in VCH and LAT (the oldest stand on the Apennines and the Alps, respectively) were presented to describe the disturbance history's influence on growth patterns on the oldest individuals.

2.5. Carbon isotope discrimination analysis

Isotope ratio measurements were performed on both leaf material and tree-rings obtained from wood cores. WUE dynamics were thus reconstructed for the last decade. Each isotopic series was related to dendrological data on an individual base. Particularly, isotopic analyses in this study were performed on wood cores from 3 Central Italy sites: Monte Cimino (CIM), Fonte Regna (REG) and Coppo del Principe (COP). Six trees for each site were considered, for a total of 18 cores. For the period 1999-2008, each growth ring was isolated, finely ground and analysed for carbon isotope composition. The individual isotopic series obtained were matched to Basal Area Increment (BAI). Given the possibility of a reciprocal memory effect between BAI and Δ of contiguous years (GAGEN et al., 2006; MCDOWELL et al., 2003), possible regressions were explored shifting the two series of one year in the two possible combinations. Litter material was also collected in COP and CIM and analysed for carbon isotope composition. Irrespective of the material, sub-samples of about 500 g were quantitatively burned in an elementar analyser (NA 1500, Carlo Erba). The produced CO₂ was admitted in a helium continuous flow to an isotope ratio mass spectrometer (CF-IRMS; ISOPRIME, GW, UK). Sample isotope ratios were measured to allow the

calculation of carbon isotope composition (δ^{13} C) referred to the IAEA standard VPDB. Carbon isotope discrimination (Δ) was then calculated using the classical formula reported by FARQUHAR *et al.* (1989; see also LAUTERI *et al.*, 2004) and taking into account δ^{13} C values of atmospheric CO₂ from international databases (NOAA, IPCC). The parameter Δ contains a fundamental comparative and normalization significance. Thus it allows ecophysiological comparisons among conditions that are different in time and space. Δ is widely used and accepted as a powerful proxy of Water Use Efficiency (WUE).

3. RESULTS

3.1. Live trees structure

Density of old-growth forests varies significantly from the Alps to the Apennines (Table 2). Alpine stands are characterized by values of stem ha⁻¹ ranging from 336 to 878, but considering only those stands in which sampling threshold is DBH>2.5 cm (LAT, TRL, CLE) this range reduces to 430-878 stem ha⁻¹. In the Apennines stand density is generally lower (296 - 317 stem ha⁻¹) but reaches its peak in VCH (1590 stem ha⁻¹) which presents the most developed forest structure. Alpine stands have in general lower stature, passing from 21 m in TRL to 33 m in TIM; on the other hand, in the Apennines, beech forests show higher stature (28-46 m), sometimes impressive like in Monte Cimino, where some individuals can even be higher than 50 m. Basal area ranges from 29 to 40 m² ha⁻¹ in the Alps, but less variation characterizes the Apennines beech forests, with values oscillating from the minimum of 41 m² ha⁻¹ of VCH to 48 m² ha⁻¹ of CIM. Mean volume of living trees ranges from 302 m³ ha⁻¹ to 647 m³ ha⁻¹ in the Alpine stands, and from 497 m³ ha⁻¹ to 715 m³ ha⁻¹ in Apennine stands.

In all sampled stands diameter distribution in 5 cm DBH classes was described, after log transformation, by a polynomial equation as suggested by GOFF and WEST (1975) for old-growth forests (Fig. 1). In each stand a fourth degree polynomial equation best fit the DBH logarithmic distribution, generating a "rotated sigmoid" ("diameter distributions characterized by steep decreases in tree density in the smallest and largest diameter classes and a plateau in the middle diameter classes that becomes more pronounced when displayed on semilogarithmic axes", from JANOWIAK et al., 2008) in the oldest stand on the Apennines (VCH) and the Alps (LAT), and a bimodal distribution in the other stands. Volume distribution among DBH classes tends to be normally distributed everywhere (except in TIM), negatively skewed in the Apennine stands, where large amount of woody volume are concentrated in the larger DBH classes (data not shown).

3.2. Dead trees structure

Presence of dead wood inside sampled old-growth forests varies greatly, depending on the single stand structural development, but also on the fact that often in the past CWD was removed from the forest to be used as fuelwood. This may explain the lower amount of deadwood observed in comparison to other old-growth forests in northern Europe and in the USA (see *Discussion*). The smallest amount of total dead trees volume are found in the Alps (TRL, 16 m³ ha⁻¹ and LAT, 18 m³ ha⁻¹; dead trees volumes for GRA and CLE were not reported in the FMPs). In central Italy total deadwood volume was lower in the secondary old-growth forests of CIM (19 m³ ha⁻¹) and REG (42 m³ ha⁻¹), but reached larger amounts in high-elevation old-growth forests of VCH and COP (65 and 91 m³ ha⁻¹, respectively). CWD represents the most important components of dead wood in most of the sampled stands (63-83% of total deadwood volume), while snag volume exceeds CWD volume only in TRL and REG.

3.3. Carbon stocks

Preliminary results on carbon stocks on a subset of sites are presented in Fig. 2 for different ecosystem compartments. The data indicate that these old-growth forests are relevant carbon reservoirs, with 192-268 MgC ha⁻¹ of total biomass (67-73% aboveground; 27-33% belowground, 4 stands) and 7-21 MgC ha⁻¹ of deadwood. In these stands, also forest floor (excluding



Figure 2 – Living biomass, deadwood, litter and soil carbon stocks for selected sites within the Italian old-growth beech forests network. Sites are ordered according to their age (Age_5).

deadwood) and soils are stores of relevant amount of carbon (5-9 MgC ha⁻¹ litter layer, 4 stands; 168-420 MgC ha⁻¹ mineral soil, 4 stands).

Among the sampled forest, CIM is the stand with the largest C stock, mainly due to the carbon stored in the soil.

Tree biomass represents from 37% (CIM) to 53% (COP) of total carbon pools, while deadwood makes up 1% (REG) to 4% (COP) of all ecosystem compartments. Soil and litter carbon stocks are very important at all sites sampled so far, with LAT and COP having 46% and 43% of total carbon stored in those compartments, while at CIM and REG soil and litter stocks represent 62% and 54% of the overall ecosystem C stocks.

Biomass of branches is reported as far as this component is included in the tables used for volume calculation. However, tables are generally derived from even-aged stands, generally younger and with different structure and development compared to old-growth stands. In this respect, within the PRIN project, an original methodological approach is being developed for the assessment of crown volume and biomass. Preliminary results at CIM and COP indicate that the crown of trees is old-growth forest has a larger volume and biomass compared to the crown of trees with similar diameter but grown in even-aged stands (*data not shown*).

3.4. Dendroecological analysis

Among the sampled stands VCH is by far the oldest, reaching a maximum age of 503 years; LAT, TIM, TRL and COP host various individuals older than 300-350 years; in GRA, CLE and CIM maximum age fall between 200 and 300 years, while at REG age doesn't exceed two centuries (179 years). The Age₅ parameter (mean age of the 5 oldest trees in each stand), allows a better distinction between old-growth forests in advanced structural development and secondary old-growth forests (CIM and REG). Most sampled stands exceed at least half of the maximum lifespan of beech (~ 500 years in VCH).

By plotting present DBH against age no significant relationships emerges between the two variables in the oldest stands (e.g. VCH, COP, LAT, TRL) (Fig. 3) and this is due to the growth history of cored trees (PIOVESAN *et al.*, 2005b). The DBH-age-growth graph of Fig. 3 shows clearly that trees from old-growth forests like VCH and COP and old stands such as LAT and TIM present a wide range of growth patterns, while other stands show a more regular evolution of growth through time that could be linked to a lower density (e.g. CIM, REG and GRA). Furthermore, in the secondary old-growth forest of CIM the dominance of the central cohort is evident, characterized by a biostatic structure (100-150



Figure 3 – Age-DBH relationships and auxological history of Alpine (A) and Apennine stands (B). For each site, the graph above represents the age-DBH relationship, while the lower one represents the cumulative Diameter Inside Bark (DIB). * = stands in which only dominant trees were cored.

years old) in a wide DBH range; at the same time in REG there is interesting evidence of different growth rates for younger dominant trees and older dominated individuals.

Disturbance chronologies built for LAT-GRA in the Alps and VCH-COP in the Apennines showed that in all cases old-growth beech forests experience a disturbance regime based on frequent low-to-moderate severity events (Fig. 4): release and recruitment events are present in almost all decades from 1700 to 2000, with most events involving no more than 10-20% of cored trees. Peaks of disturbances found in the Alps (e.g. 1850-1869 and 1940-1949) and in the Apennines (e.g. 1760-1769; 1820-1829; 1880-1889) could be probably synchronized in different stands by climatic events (see PIOVESAN *et al.*, 2005b).

Tree-ring series of the six oldest individuals in LAT and VCH confirmed that during their life these trees experienced few moderate disturbance events or gradual release events (Fig. 5). Often the first events occur in advanced age, meaning that the oldest beech trees were able to survive for several decades in the understorey.

3.5. Isotopic analysis

Mean Δ values recorded in the wood cores in the decade (1999-2008) were relatively different among the sites (Table 3). Coppo del Principe (COP) showed the highest Δ and Fonte Regna the lowest. The average value of CIM is intermediate. Δ values in leaf litter are higher than those of wood cores, in both COP and CIM. This finding is somewhat surprising, given the ¹³C depletion of lignin in wood material (WILSON and GRINSTED, 1977; LEAVITT and LONG, 1982), but was previously reported in another study (WEITNER *et al.*, 2007).

The analysis of C stable isotopes in tree rings, as expressed in Δ notation in relation to BAI across the referred decade, reveals an interesting negative relationship (Fig. 6A) in COP and REG when comparing shifted pairs of BAI (year *n*-1) versus Δ (year *n*). A non significant, but tendentially

Table 3 – Comparison of values of Δ in tree rings (average of 6 cores containing tree-rings from 1999 to 2008), litter (sampled on the ground in 2009) and BAI for the three sites analysed in Central Italy; mean values \pm standard errors. Uppercase letters indicate significance difference of the same variable among sites.

	Coppo del Principe	Monte Cimino	Fonte Regna
Δ tree rings (‰)	18.4 ± 1.3^{a}	18.3 ± 0.6^{a}	$17.8 \pm 1.1^{\mathrm{b}}$
Δ litter (‰)	20.2 ± 0.0	20.7 ± 0.2	-
BAI (cm ² yr ⁻¹)	23.08 ± 1.23^{a}	$41.73 \pm 2.44^{\rm b}$	$38.43 \pm 2.27^{\circ}$









Figure 6 – Relationship between in tree rings and BAI. Each data point it's the average of values from 6 trees for each year in the period 1999-2008. (A) Relationship between values of year "n" and BAI values of year "n-1" for the 3 Apennines sites; in the case of CIM the regression is not significant; dotted line represents the standard error of the mean. (B) Coppo del Principe. Relationship between BAI values of year "n" and values of year "n-1".

negative regression was observed for CIM. Thus, low Δ values are related to high BAI values. On the other side, it is noteworthy that the three analysed populations are quite clearly separated in the graph, on the basis of both Δ and BAI values. Such a negative regression would imply, in the classical Δ analysis, that high WUE is linked with high BAI, given the well tested negative relationship between WUE and Δ (BRUGNOLI and FARQUHAR, 2000).

It is, finally, noteworthy that a significant positive regression (Fig. 6B) was found relating BAI (year n) to Δ (year n-1) just for the old-growth population of COP.



Figure 7 – Old-growthness indicators. (Above) Large trees (DBH \ge 70 cm): density and their volume percentage on total living volume. (Below) Snags density and dead volume partition among snags and CWD.

4. DISCUSSION

In defining old-growth forests, a detailed analysis of pattern and processes is required. A combination of structural and botanical features may serve as important indicators (e.g. GOFF and WEST, 1975; TYRRELL and CROW, 1994; KEDDY and DRUMMOND, 1996; GREENBERG *et al.*, 1997; PONTAILLER *et al.*, 1997; EMBORG *et al.*, 2000; NILSSON *et al.*, 2002; DEBELJAK, 2006) as well as the dendroecological reconstruction of forest

dynamics (NAGEL et al., 2007). Forest naturalness could be expressed through a 'gradient' based on the gradual appearance of those structural features and processes typical of primitive stands that are lacking or insufficiently represented in managed forests (GILG, 2004). Therefore, the 'old-growthness' of a given stand can be defined to describe the degree of development of old-growth structural features (SPIES and FRANKLIN, 1988) and considered a synonym of 'naturalness'. Evaluating the degree of oldgrowthness even of a single stand could be extremely costly in terms of time, money and knowledge required (BAUHUS et al., 2009) because of the high number of indicators that could potentially be considered. Practically, it is preferable to follow a 'structural-based approach' for detecting old-growth attributes, since structure represents a "readily measured surrogate for functions (e.g. productivity) and for organisms (e.g. cavity-dwelling animals) that are difficult to measure directly" (FRANKLIN et al., 2002). A representative set of structural indicators has to be chosen (TYRRELL and CROW, 1994; WHITE and LLOYD, 1994; KEDDY and DRUMMOND, 1996; GREENBERG et al., 1997; BAUHUS et al., 2009) and the number of parameters to be measured needs to be adequately dimensioned. To assess old-growthness in the sampled beech stands we first considered four groups of indicators: basal area and volume of the stand; density of large living trees (DBH>70 cm) and their contribution to total basal area or volume; presence and volume of standing and downed dead trees, with special attention to large trees; diameter distribution (Tab. 2; Figs. 1 and 7).

Values of basal area exceeded in all stands the control/normal limit (>29 m² ha⁻¹) proposed by KEDDY and DRUMMOND (1996) for old-growth mesic hardwood deciduous forests. Mean volume per hectare is high in all stands with values comparable to those reported for other old-growth beech forests (see PIOVESAN *et al.*, 2005b for a review).

Another important old-growth feature is represented by the density of large trees, which account for a large portion of total volume in old-growth forests (BROWN *et al.*, 1997; GREENBERG *et al.*, 1997; NILSSON *et al.*, 2002; PIOVESAN *et al.*, 2005b). Control value is given by NILSSON *et al.* (2002), who found in *Fagus* dominated forests of Central Europe a density of living trees with DBH >70 cm of about 30 stem ha⁻¹. Apennine old-growth forests approach the control value of 25-30 stem ha⁻¹ (maximum value in the site of CIM with 54 stem ha⁻¹ with DBH > 70 cm) (Fig. 7). On the Alps very few individuals with DBH > 70 cm, except in TIM, where we found a large number (97) of stems ha⁻¹ with DBH > 70 cm (Fig. 7). Consequently only in the Apennine stands (with the above mentioned exception of TIM) large percentage of volume are concentrated in the largest diameter classes and the reference value of at least 20% of total woody volume included in classes with DBH > 70 cm suggested by BROWN *et al.* (1997) for old-growth forests

is reached. This discrepancy between Alps and Apennines could be interpreted as the consequence of both forests' history and site conditions. Highest values of density and volume of large trees (DBH > 70 cm) are found in CIM and REG, both characterized by fertile soils that experienced in the past at least one period of low-density grazed forests, in which canopy structure was mainly composed by large-sized scattered trees. Occurrence of large trees in the primary old-growth forest of VCH and in COP could be partially linked to mean age of the oldest trees (Age₅) of these stands, exceeding 340-400 years (even if there isn't a direct relationship between age and DBH). On the other hand, most of the Alpine stands grow on extremely steep slopes, often close to conditions of rocky soils, where diameter enlargement is limited by nutrient supply and rocky and steep topography.

Dead wood is a fundamental component of natural forests (see CHRISTENSEN et al., 2005) and it is among the most important indicators to distinguish *natural* from *managed* forests (DEBELJAK, 2006), as a consequence of reduced or no logging activities (EMBORG et al., 2000; VON OHEIMB et al., 2005). CWD is central for maintaining a wide range of ecosystem's functions and services (STEVENS, 1997) and its importance relies largely on relative abundance, state of decay and pieces size, which influences its use by the biota (PYLE and BROWN, 1999). In a review on dead wood amounts over 86 beech forest reserves across Central Europe, CHRISTENSEN et al. (2005) found that mean dead wood volume was 130 m³ ha⁻¹ with a high level of variation (from ~0 to 550 m³ ha⁻¹); PIOVESAN *et al.* (2005b) reported a range of 50-200 m³ ha⁻¹ for pure Fagus forests in Europe. Among sampled stands, only VCH and COP have total amounts of dead wood comparable to cited values (65 and 91 m³ ha⁻¹ respectively); lower amounts were found in REG (42 m³ ha⁻¹); the other showed values ranging around 16-19 m³ ha⁻¹. This scarcity of deadwood is related to CWD removal made in the past in several stands (e.g. CIM, LAT, TIM). Abundance of deadwood in natural forests varies greatly depending on the present structural phase (DEBELJAK, 2006). LEIBUNDGUT (1993, cited in WOLYNSKI, 2001) argues that deadwood volume should approach 10-15% of total live plus dead trees volume in adult stands, increasing up to 25-30% in the ageing phase. In any case, Alpine and Apennine beech forests reach these percentages, with the highest deadwood/total volume ratio found in VCH and COP (~11%). In most stands CWD (logs and stumps) contributes more than snags to dead volume, according to the results of previous studies reporting that snags represent about 20-40% of total dead trees volume (NILSSON et al., 2002). However, the density of large snags (DBH > 70 cm) is, especially on the old-growth beech stands of the Apennines, within the range values (NILSSON et al., 2002).

Diameter distribution is a key structural feature to describe natural forests and interpret their growth history (PONTAILLER *et al.*, 1997; EMBORG

et al., 2000; VON OHEIMB et al., 2005). Despite diameter distributions of oldgrowth stands have been traditionally described with a reverse J-shaped curve (see WESTPHAL et al., 2006 for a review), various findings suggested a 'rotated-sigmoid' curve being more reasonable for describing tree-sizes distribution in old-growth forests (GOFF and WEST 1975; ZHANG et al., 2001). By investigating diameter distributions in nine virgin beech forests of south-eastern Europe, WESTPHAL et al. (2006) showed how rotated-sigmoid curves derived from a Weibull function are able to fit the observed diameter distributions better than the negative exponential function, which usually underestimate the number of stems in the midsize diameter classes. The appearance of rotated-sigmoid curves has been linked to asymmetric U-shaped mortality trends found in mature and old-growth stands (LORIMER et al., 2001) where highest mortality rates are experienced by the smallest individuals, as a consequence of self-thinning (COOMES et al., 2003), and by large dominant trees, which are more exposed to natural disturbances (LORIMER et al., 2001). Intermediate size trees, instead, undergo lower mortality rates (GOFF and WEST, 1975). This trend in mortality rates generates a plateau in the midsized classes (see WESTPHAL et al., 2006; JANOWIAK et al., 2008). In our study sites rotated-sigmoid curves are evident only in those stands with the higher degree of naturalness (e.g. VCH and partially LAT) (Fig. 1). The other stands showed a bimodal distribution. generated by a two- or few-cohort stage, typical of the "demographic transition" stage (FRELICH, 2002), where numerous patches in the innovation-aggradation phases are present (EMBORG et al., 2000).

Disturbance regime directly influences auxology of forest stands, their structural dynamics, and deadwood volume and contribute to shaping the resulting forest mosaic. Anyway, disturbance frequency and distribution have been probably altered by human impacts, since few forests escaped logging (DI FILIPPO et al., 2005). From dendroecological analyses performed on sampled old-growth forests emerged a distinction between those stands with a higher degree of "old-growthness" (VCH-COP-LAT-TIM) and secondary old-growth forests (e.g. CIM), where a single cohort is clearly distinguishable (Fig. 3). Primary old-growth stands (e.g. VCH) have a multi-aged structure, typical of the last stages of stand development (FRELICH, 2002) and related to fine-scale disturbance regime in temperate deciduous forests (GILG, 2004). Disturbance chronologies produced for four of the oldest relatively undisturbed stands by using the boundary line release criteria (BLACK and ABRAMS, 2003) present the dominance of low-severity disturbances scattered in several decades from 1700 to 1999 (Fig. 4). These findings underline the importance of gap dynamics in maintaining beech forests, more than standreplacing events, which instead would synchronize recruitment rates and canopy accessions (PIOVESAN et al., 2005b). Beech is in fact well adapted to a

small gap disturbance regime thanks to its physiological and morphological plasticity (CANHAM, 1988; WAGNER *et al.*, 2010). Anyway the presence of synchronous peaks in disturbance events, as occurred in VCH and COP in the 1760 and 1820 does not exclude a certain role of periodic intermediate severity events (e.g. windstorms), which could contribute to the creation of multiple tree gaps (NAGEL *et al.*, 2007). Beech adaptation to gap dynamics is testified by the analysis of growth patterns of the oldest individuals (Fig. 5). Tree-ring series of old trees showed very low increments for several decades (suppression) and one or more disturbances (multi-gap episodes), marked on tree-rings as ecological releases. Besides multi-gap episodes, typical of late-successional old-growth forests (PIOVESAN *et al.*, 2005b), in some cases gradual, instead of abrupt, growth releases were observed, revealing the presence of very low-severity, progressive disturbance events releasing the crown of surviving trees.

The data on C stocks available for the project's sites sampled and analysed so far indicate that the studied forests are characterised by relevant values of biomass, deadwood and soil carbon stocks. The data presented here for total biomass (400-540 Mg ha⁻¹, excluding deadwood and soils) are at the highest portion of the range of other available data for managed beech forests in Europe (157-468 Mg ha⁻¹, 23 studies, closed stands, age 54-270) (MATTEUCCI et al., 1999). Mean tree carbon pool in much denser unmanaged stands sampled recently in Spain ranged between 280 and 320 MgC ha⁻¹ (Fig. 3 in MERINO et al., 2007) compared to our range of 192-268 MgC ha⁻¹ (that include only a portion of crown biomass). However, data on total ecosystem C stocks of the four forests sampled in our study (377-695 MgC ha⁻¹) are higher than the mean range reported for Spain (350-400 MgC ha⁻¹). Nevertheless, soil C in the sampled old-growth beech forests in Italy (168-420 MgC ha⁻¹) is higher than the mean values reported for Spanish unmanaged forests on different soils (40-125 MgC ha⁻¹) (MERINO et al., 2007) and similar in range to that reported for mature beech forests sampled in Germany along a precipitation gradient on a similar geological substrate (MEIER and LEUSCHNER, 2010). When calculated over 30 cm soil depth, the soil C data of this study are within the range reported for beech forests in Japan (LI et al., 2010). Nevertheless, data measured at CIM are among the highest reported for beech forests.

Finally, C isotope discrimination analyses on tree rings from old-growth beech forests revealed interesting retrospective informations on ecophysiological performances. Indeed, the 3 analysed populations (CIM, REG, COP) are quite clearly separated on the basis of both Δ and BAI values. From previous dendroclimatic investigations (e.g. PIOVESAN *et al.*, 2005a), COP can be ascribed to the high-elevation beechwood, where summer drought control on growth was limited and was important the April-May temperature; CIM and REG, both pertained to a transition group between mountain and low-elevation beechwoods where summer drought is the main climatic signal. Despite the difference in elevation, the high-elevation COP and the beechwood growing on fertile volcanic soils (CIM; see also WEITNER *et al.*, 2007) showed a higher Δ than the calcareous REG (living at the same elevation of CIM). The fact that Fonte Regna is characterised by relatively low Δ and high BAI, could indicate a relatively dry site (causing high WUE) with favourable temperature supporting an active growth through a well extended vegetative season. Accordingly to this double parameter interpretation (GAGEN et al., 2006), CIM seems affected by both favourable growth temperature (relatively high BAI) and water availability thanks to the fertile soils (high Δ). Finally, COP forest shouldn't have been limited by intense and long summer drought (high Δ) along the decade, but would have been constrained by sub-optimal temperatures for most of the vegetative seasons. This hypothesis is supported by the positive relation between Δ of the previous year (n-1) and BAI of the current one (n). Such a relation was found in this high-mountain population, but not for the other two transitional populations (REG and CIM). This effect would be visible in the population COP which is especially limited by the shortness of the vegetative period, but not in those (REG and CIM) characterized by a longer growing season and the likely occurrence of drought periods. Indeed, the presence of the positive correlation between BAI and previous year Δ at COP, could indicate the importance of reallocation of previous year assimilates on current tree productivity. Furthermore, all the three Apennine populations showed no significant relationships between concurrent growth (BAI) and WUE, as inferred from Δ analysis. The physiological response in terms of Δ seems to be partially and negatively controlled by the growth of the previous year. In fact, vigorous growth at year *n*-1 would increase the likelihood of drought constraints at year *n*, resulting in a decreased Δ because of crown expansion and consequent major exposure to the atmosphere. Whether or not these observations are consistent within a wider span of populations and ecological conditions, the capability to discriminate different sites on the basis of both physiological (Δ) and dendrochronological features (BAI) appears noteworthy.

5. CONCLUSIONS

Among all sampled stands only VCH can be defined as a primary oldgrowth forest because, besides the lack of evidence of sylvicultural intervention, its structural attributes satisfy the control values and dendroecological (Age_{max}, Age₅, growth patterns and disturbance chronologies) as well as historical analyses (e.g. chronosequences of aerial photos) confirmed that its dynamics were driven by natural processes for centuries. The remaining stands, which have not yet fully acquired advanced old-growth features, might be considered as secondary old-growth forests with different degree of naturalness, from the most developed (LAT, TRL) to the youngest ones (REG and CIM). Further historical investigations are needed to fully understand the degree of naturalness of COP.

Our study shows how defining old-growth forests following a "structural-based approach" does not allow foresters to unambiguously mark the border between old-growth forests and managed stands, since structural development is extremely complex (FRANKLIN *et al.*, 2002). Nonetheless the structural approach provides an overview on those parameters (e.g. basal area and volume distribution; large trees; deadwood) involved in defining the naturalness degree of a given forest (GILG, 2004) or, in other words, its 'old-growthness' (SPIES and FRANKLIN, 1988). Moreover, it is based on easy-to-measure attributes, which are of great help when dealing with economical restrictions for monitoring programs. The importance of the "structural-based approach" relies also on the fact that some of the mentioned structural indicators may act as functional indicators, indicating the occurrence of old-growth related processes (e.g. mortality of dominant senescent trees), thus providing a fundamental tool for assessing and monitoring old-growth forest ecosystems.

All the forests sampled within the project are important carbon reservoirs, both at tree and soil compartments. Carbon stocks of these forests are generally higher than other managed stands, both in Italy and Europe. A combined analysis enclosing both ecophysiological and dendrochronological data, if properly tuned with climatic series, could aid in reconstructing the past environmental scenarios of old-growth beech forests and their response to a varying climate. In this respect, the use of multiple sampling and research techniques and the integration of research groups with complementary expertise can foster deeper understanding of the ecology and dynamics of old-growth forests.

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RIASSUNTO

Analisi strutturale, processi di crescita e *stock* di carbonio in una rete italiana di faggete vetuste

Nell'ambito del progetto PRIN «Cambiamenti climatici e foreste - Risposte dendroecologiche ed ecofisiologiche, produttività e bilancio del carbonio in una rete italiana di faggete vetuste», sono state analizzate 9 faggete delle Alpi orientali e dell'Appennino centrale per determinarne: i) il grado di vetustà su base strutturale e dendroecologica; ii) gli *stock* di carbonio (C) nei diversi comparti ecosistemici; iii) una caratterizzazione ecofisiologica sulla base degli isotopi stabili. In alcune tra le più vecchie e meno disturbate faggete italiane sono state esaminate: la struttura delle piante vive e della necromassa; le caratteristiche del suolo; e gli *stock* di C. Inoltre foglie, lettiera e carote legnose sono state campionate per le analisi dendroecologiche, dei nutrienti e degli isotopi stabili, per studiare la struttura per età, sviluppare cronologie di disturbo, valutare le risposte climatiche di medio e lungo termine ed investigare alcuni aspetti ecofisiologici.

In tutti i popolamenti studiati i valori di area basimetrica, volume e il numero di grandi alberi per ettaro sono confrontabili con quelli di riferimento riportati per foreste vetuste europee e nord-americane, mentre il quantitativo di necromassa presente è generalmente basso, eccetto nei due siti più naturali. Le distribuzioni diametriche variano tra curve bimodali e la sigmoide-ruotata. Le analisi strutturali e le cronologie di disturbo rivelano l'importanza di eventi di medio-bassa severità nel generare una struttura a scala fine, confermando precedenti risultati ottenuti in foreste di faggio. Diversi gradi di 'vetustà' sono stati identificati per le faggete alpine ed appenniniche attraverso un approccio "strutturale", che si è dimostrato un valido strumento per l'individuazione delle foreste vetuste.

Risultati preliminari sugli *stock* di carbonio, ottenuti in un sottocampione di popolamenti, indicano come tali foreste vetuste siano rilevanti riserve di carbonio, con 192 - 268 MgC ha⁻¹ di biomassa (67-73% nella parte epigea; 27-33% nella parte ipogea; 4 popolamenti) e 7 - 21 MgC ha⁻¹ nella necromassa. In questi popolamenti anche lettiera (esclusa la necromassa) e, soprattutto, suolo stoccano notevoli quantitativi di carbonio (5 - 9 MgC ha⁻¹ nella lettiera, 4 popolamenti; 168 - 420 MgC ha⁻¹ nel suolo, 3 popolamenti). Infine l'analisi della discriminazione isotopica del carbonio (Δ), un indicatore dell'efficienza di uso idrico, è stata condotta su carote legnose estratte da un sottocampione di siti; sono state quindi descritte le prospettive di questa tecnica per una interpretazione ecofisiologica retrospettiva degli impatti del cambiamento climatico sulle foreste vetuste.

I risultati preliminari ottenuti nell'ambito del progetto PRIN indicano come gli indicatori ecologici utilizzati possano essere usati per produrre valide descrizioni delle strutture forestali e dei processi che guidano le dinamiche dei popolamenti ('approccio strutturale'). L'utilizzo di tecniche di campionamento multiple e l'integrazione di diversi gruppi di ricerca con competenze complementari può inoltre favorire una più profonda conoscenza dell'ecologia e delle dinamiche delle foreste vetuste.

REFERENCES

ANIOL R.W., 1983 – Tree-ring analysis using CATRAS. Dendrochronologia, 1: 45-53.

- ANIOL R.W., 1987 A new device for Computer Assisted Measurement of Tree-Ring Widths. Dendrochronologia, 5: 135-141.
- BAUER G.A., PERSSON H., PERSSON T., MUND M., HEIN M., KUMMETZ E., MATTEUCCI G., VAN OENE H., SCARASCIA MUGNOZZA G., SCHULZE E.-D., 2000 – Linking plant nutrition and ecosystem processes. In: Schulze E.-D. (Ed.) Carbon and Nitrogen Cycling in European forest Ecosystems, Ecological Studies, Springer Verlag, Heidelberg, p. 63-98.
- BAUHUS J., PUETTMANN K., MESSIER C., 2009 *Silviculture for old-growth attributes*. Forest Ecology and Management, 258: 525-537.
- BLACK B.A., ABRAMS M.D., 2003 Use of boundary-line growth patterns as a basis for dendroecological release criteria. Ecological Applications, 13: 1733-1749.
- BOONE RD, GRIGAL DF, SOLLINS P, AHRENS RJ, ARMSTRING DE, 1999 Soil sampling, preparation, archiving, and quality control. In: Robertson GP et al. (Eds.), Standard Soil methods for Long-term Ecological Research. Oxford University Press, New York, p. 3-28.
- BROWN S.L., SCHROEDER P., BIRDSEY R., 1997 Aboveground biomass distribution of US eastern hardwood forests and the use of large trees as an indicator of forest development. Forest Ecology and Management, 96: 37-47.
- BRUGNOLI E., FARQUHAR G.D., 2000 Photosynthetic fractionation of carbon isotopes. In: Photosynthesis: Physiology and Metabolism - Advances in Photosynthesis, vol. 9 (R.C. Leegood, T.D. Sharkey and S. von Caemmerer, Eds.), p. 399-434. Kluwer Academic Publishers, The Netherlands.
- CANHAM C.D., 1988 Growth and canopy architecture of shade-tolerant trees: response to canopy gaps. Ecology, 69: 786-795.
- CAREY E.V., SALA A., KEANE R., CALLAWAY R.N., 2001 Are old forests underestimated as global carbon sink? Global Change Biology, 7: 339-344.
- CASTELLANI C., SCRINZI G., TABACCHI G., TOSI V., 1984 *Inventario forestale nazionale italiano (I.F.N.I.), Tavole di cubatura a doppia entrata.* Istituto Sperimentale per l'Assestamento Forestale e per l'Alpicoltura, Trento, p. 71-75.
- CHRISTENSEN M., HAHN K., MOUNTFORD E.P., ÓDOR P., STANDOVÀR T., ROZENBERGAR D., DIACI J., WIJDEVEN S., MEYER P., WINTER S., VRSKA T., 2005 – *Dead wood in European beech* (Fagus sylvatica) *forest reserves*. Forest Ecology and Management, 210: 267-282.
- COOMES D.A., DUNCAN R.P., ALLEN R.B., TRUSCOTT J., 2003 Disturbances prevent stem size-density distributions in natural forests from following scaling relationships. Ecology Letters, 6: 980-989.
- D'ANDREA E. 2008 Stima della biomassa e della produzione primaria in una faggeta dell'Appennino Centrale, con particolare riferimento alle componenti a breve turn-over. Tesi di Laurea Magistrale in Scienze Forestali e Ambientali (Classe 74/S), Università degli Studi della Tuscia, Viterbo.
- DI FILIPPO A., PIOVESAN G., SCHIRONE B., 2005 Le foreste vetuste: criteri per

l'identificazione e la gestione. Atti del XIV Congresso Nazionale della Società Italiana di Ecologia (Siena, 4-6 ottobre 2004). In: Gaggi C., Nicolardi V. and Santoni S. (Eds.), Ecologia. Atti del XIV Congresso Nazionale della Società Italiana di Ecologia (Siena, 4-6 ottobre 2004). [online]URL: http://www.xivcongresso.societàitalianaecologia.org/articles/Di-Filippo-61.pdf

- DI FILIPPO A., BIONDI F., CUFAR K., DE LUIS M., GRABNER M., MAUGERI M., PRESUTTI SABA E., SCHIRONE B., PIOVESAN G., 2007 – *Bioclimatology of beech* (Fagus sylvatica L.) *in the Eastern Alps: spatial and altitudinal climatic signals identified through a tree-ring network*. Journal of Biogeography, 34: 1873-1892.
- DEBELJAK M., 2006 *Coarse woody debris in virgin and managed forest*. Ecological Indicators, 6: 733-742.
- EMBORG J., CHRISTENSEN M., HEILMANN-CLAUSEN J., 2000 *The structural dynamics of Suserup Skov, a near-natural temperate deciduous forest in Denmark.* Forest Ecology and Management, 126: 173-189.
- 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. Annuals Review of Plant Physiology and Plant Molecular Biology, 40: 503-537.
- FRANKLIN J.F., SPIES T.A., VAN PELT R., CAREY A.B., THORNBURGH D.A., BERG D.R., LINDENMAYER D.B., HARMON M.E., KEETON, W.S., SHAW D.C., BIBLE K., CHEN J., 2002 – Disturbances and structural development of natural forest ecosystems with silvicultural implications, using Douglas-fir forests as an example. Forest Ecology and Management, 155: 399-423.
- FRELICH L.E., 2002 Forest Dynamics and Disturbance Regimes. Cambridge University Press, Cambridge, UK.
- FRELICH L.E., REICH P.B., 2003 Perspectives on development of definitions and values related to old-growth forests. Environmental Reviews, 11: S9-S22.
- FRIDMAN J., WALHEIM M., 2000 Amount, structure, and dynamics of dead wood on managed forestland in Sweden. Forest Ecology and Management, 31: 23-26.
- GAGEN M., MCCARROLL D., EDOUARD J.L., 2006 *Combining Ring Width, Density* and Stable Carbon Isotope Proxies to Enhance the Climate Signal in Tree-Rings: An Example from the Southern French Alps. Climatic Change, 78: 363-379.
- GILG O., 2004 Forets a caractere naturel: caracteristiques, conservation et suivi. Cahiers Techniques de l'ATEN:74. ATEN, Montpellier, 96 p.
- GOFF F.G., WEST D., 1975 Canopy-understory interaction effects on forest population structure. Forest Science, 21: 98-108.
- GREENBERG C.H., MCLEOD D.E., LOFTIS D.L., 1997 An old-growth definition for western and mixed mesophytic forests. USDA Forest Service Technical Report GTR-SRS-16. Asheville, NC, US.
- GUEHL J.M., NGUYEN-QUEYRENS A., LOUSTAU D., FERHI A., 1995 Genetic and environmental determinants of water-use efficiency and carbon isotope

discrimination in forest trees. In: The EUROSILVA Contribution to Forest Tree Physiology (M. Bonnet-Masimbert & H. Sandermanns, Eds.), p.297-321. Editions Colloques de l'INRA, Paris.

- JANOWIAK M.K., NAGEL L.M., WEBSTER C.R., 2008 Spatial scale and stand structure in northern hardwood forests: implications for quantifying diameter distributions. Forest Science, 54 (5): 497-506.
- JUMP A.S., HUNT J.M., PEÑUELAS J., 2006 *Rapid climate change-related growth decline at the southern range edge of Fagus sylvatica*. Global Change Biology, 12: 2163-2174.
- KEDDY P.A., DRUMMOND C.G., 1996 Ecological properties for the evaluation, management, and restoration of temperate deciduous forest ecosystems. Ecological Applications, 6: 748-762.
- LAUTERI M., SCARTAZZA A., GUIDO M.C., BRUGNOLI E., 1997 Genetic variation in photosynthetic capacity, carbon isotope discrimination and mesophyll conductance in provenances of Castanea sativa adapted to different environments. Functional Ecology, 11: 675-683.
- LAUTERI M., PLIURA A., MONTEVERDI M.C., BRUGNOLI E., VILLANI F., ERIKSSON G., 2004 – Genetic variation in carbon isotope discrimination in six European populations of Castanea sativa Mill. originating from contrasting localities. Journal of Evolutionary Biology 17: 1286-1296.
- LEAVITT S.W., LONG A., 1982 Evidence for ¹³C and ¹²C fractionation between tree leaves and wood. Nature, 298: 742-743.
- LI P., WANG Q., TOMORIHO E., ZHAO X., KAKUBARI Y., 2010 Soil organic carbon stock is closely related to aboveground vegetation properties in cold-temperate mountainous forests. Geoderma, 154: 407-415.
- LORIMER C.G., DAHIR S.E., NORDHEIM E.V., 2001 Tree mortality rates and longevity in mature and old-growth hemlock-hardwood forests. Journal of Ecology, 89: 960-971.
- LO MONACO A., 1983 Proposte per un piano di valorizzazione naturalistica della *"Faggeta" del Monte Cimino (Viterbo).* Tesi di laurea in Scienze forestali, Facoltà di Agraria, Firenze.
- LUYSSAERT S., SCHULZE E.-D., BORNER A., KNOHL A., HESSENMOLLER D., LAW B.E., CIAIS P., GRACE J., 2008 – Old-growth forests as global carbon sinks. Nature, 455: 213-215.
- MASCI A., 2002 Biomassa, produttività primaria netta e mineralomassa in una faggeta dell'Appennino centrale. Tesi di Dottorato in Ecologia Forestale, Università degli Studi di Padova.
- MCCARROL D., LOADER N.J., 2004 *Stable isotopes in tree rings.* Quaternary Science Reviews, 23: 771-801.
- McDowell N., BROOKS J.R., FITZGERALD S.A., BOND B.J., 2003 Carbon isotope discrimination and growth response of old Pinus ponderosa trees to stand density reductions. Plant, Cell and Environment, 26: 631-644.
- MATTEUCCI G., DE ANGELIS P., DORE S., MASCI A., VALENTINI R., SCARASCIA MUGNOZZA G., 1999 – Il bilancio del carbonio delle faggete: dall'albero

all'ecosistema. In: Scarascia Mugnozza G. (Ed.) Ecologia strutturale e funzionale di faggete italiane. Edagricole, p. 133-183.

- MEIER I.C., LEUSCHNER C., 2010 Variation of soil and biomass carbon pools in beech forests across a precipitation gradient. Global Change Biology, 16: 1035-1045.
- MERINO A., REAL C., ALVAREZ-GONZALEZ J.G., RODRIGUEZ-GUITIAN M.A., 2007 Forest structure and C stocks in natural Fagus sylvatica forest in Southern Europe: The effects of past management. Forest Ecology and Management, 250: 206-214.
- NAGEL T.A., LEVANIC T., DIACI J., 2007 A dendroecological reconstruction of disturbance in an old-growth Fagus-Abies forest in Slovenia. Annals of Forest Sciences, 64: 891-897.
- NILSSON S.G., NIKLASSON M., HEDIN J., ARONSSON G., GUTOWSKI J.M., LINDER P., LJUNGBERG H., MIKUSÌNSKI G., RANIUS T., 2002 – Densities of large and dead trees in old-growth temperate and boreal forests. Forest Ecology and Management, 161: 189-204.
- PAIERO P., CANDIDI-TOMMASI R., CANIGLIA, G., 1975 Il bosco 'bandito' di Cleulis (Paluzza): fustaia di faggio derivata dall'invecchiamento naturale di un ceduo matricinati. Monti e Boschi, 4: 1-13.
- PEÑUELAS J., 2005 Plant physiology: a big issue for trees. Nature, 437: 965-966.
- PIOVESAN G., SCHIRONE B., SPADA F., HERMANIN L., 1996 Considerazioni sulla cenologia di alcuni popolamenti misti di faggio e leccio dei monti Sabini (Italia centrale). Atti del Seminario "Funzionalità dell'Ecosistema Faggeta", Firenze, 16-17 novembre 1995, a cura di Raffaello Giannini, p. 57-63. C.N.R, Accademia Italiana di Scienze Forestali, Grafica Tiburtina-Roma.
- PIOVESAN G., BERNABEI M., DI FILIPPO A., ROMAGNOLI M., SCHIRONE B., 2003 A long-term tree ring beech chronology from a high-elevation old-growth forest of Central Italy. Dendrochronologia, 21: 1-10.
- PIOVESAN G., BIONDI F., BERNABEI M., DI FILIPPO A., SCHIRONE B., 2005a Spatial and altitudinal bioclimatic zones of the Italian peninsula identified from a beech (Fagus sylvatica L.) tree-ring network. Acta Oecologica, 27: 197-210.
- PIOVESAN G., DI FILIPPO A., ALESSANDRINI A., BIONDI F., SCHIRONE B., 2005b Structure, dynamics and dendroecology of an old-growth Fagus forest in the Apennines. Journal of Vegetation Science, 16: 13-28.
- PIOVESAN G., BIONDI F., DI FILIPPO A., ALESSANDRINI A., MAUGERI M., 2008 Drought-driven growth reduction in old beech (Fagus sylvatica L.) forests of the Central Apennines, Italy. Global Change Biology, 14: 1265-1281.
- PONTAILLER J.Y., FAILLE A., LEMÉE G., 1997 Storms drive successional dynamics in natural forests: a case study in Fontainebleau forest (France). Forest Ecology and Management, 98: 1-15.
- PYLE C., BROWN M.M., 1999 Heterogeneity of wood decay classes within hardwood logs. Forest Ecology and Management, 144: 253-259.
- RUBINO D.L., MCCARTHY B.C., 2003 Evaluation of coarse woody debris and forest vegetation across topographic gradients in a southern Ohio forest. Forest Ecology and Management, 183: 221-238.

- SCARASCIA MUGNOZZA G., BAUER G., PERSSON H., MATTEUCCI G., MASCI A., 2000 Tree biomass, growth and nutrient pools. In: Schulze E.-D. (Ed.) Carbon and Nitrogen Cycling in European forest Ecosystems, Ecological Studies, 142. Springer Verlag, Heidelberg, p. 49-62.
- SCHOENEBERGER P.J., WYSOCKI D.A., BENHAM E.C., BRODERSON W.D., 2002 *Field Book for Describing and Sampling Soils*. Version 2.0. Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE.
- SGADARI V., 1961 Tavola dendrometrica ad una sola entrata del faggio cresciuto in fustaia prevalentemente disetanea della provincia di Cosenza (I classe di fertilità).
 In: Tavole stereometriche ed alsometriche costruite per i boschi italiani. Castellani C. (raccolte, coordinate e illustrate), Annali Istituto Sperimentale per l'Assestamento Forestale e l'Alpicoltura, 1 (1): 287.
- SPIES T.A., FRANKLIN J.F., 1988 Old growth and forest dynamics in the Douglas-fir region of western Oregon and Washington. Natural Areas Journal, 8 (3): 190-201.
- SPIES T.A., 2004 *Ecological concepts and diversity of old-growth forests.* Journal of Forestry, 102: 14-20.
- STEVENS V., 1997 The ecological role of coarse woody debris: an overview of the ecological importance of CWD in British Columbia forests. Research Program, British Columbia Ministry of Forests, Victoria, B.C. Work. Pap. 30/1997, 26 p.
- STOKES M.A., SMILEY T.L., 1996 *An introduction to tree-ring dating.* University of Arizona Press, Tucson.
- TYRRELL L.E., CROW T.R., 1994 Structural characteristics of old-growth hemlockhardwood forests in relation to age. Ecology, 75: 370-386.
- TREWARTHA G.T., 1968 An introduction to climate. McGraw-Hill, New York.
- VON OHEIMB G., WESTPHAL C., TEMPEL H., HÄRDTLE W., 2005 *Structural pattern* of a near natural beech forest (Fagus sylvatica) (*Serrahn, North-east Germany*). Forest Ecology and Management, 212: 253-263.
- WAGNER S., COLLET C., MADSEN P., NAKASHIZUKA T., NYLAND R.D., SAGHEB-TALEBI K., 2010 – *Beech regeneration research: From ecological to silvicultural aspects.* Forest Ecology and Management, 259: 2172-2182.
- VAN WAGNER C.E., 1968 *The line intercept method in forest fuel sampling*. Forest Science, 14: 20-26.
- WEITNER A., DUPOUEY J.L., LEFÈVRE Y., BRÉDA N., BADEAU V., FERHI A., DUQUESNAY A., THIMONIER A., 2007 – Roles of soil chemistry and water availability in site-related 13C variations in French beech forests. Tree Physiology, 27: 1043-1051.
- WESTPHAL C., TREMER N., VON OHEIMB G., HANSEN J., VON GADOW K., HÄRDTLE
 W., 2006 Is the reverse J-shaped diameter distribution universally applicable in European virgin beech forests? Forest Ecology and Management, 223: 75-83.
- WHITE D.L., LLOYD F.T., 1994 Defining old-growth: implications for management. Eight Biennial Southern Silvicultural Research Conference, Auburn, AL, November 1-3 1994.

- WILSON A.T., GRINSTED M.J., 1977 ¹²C/¹³C in cellulose and lignin as palaeothermometeres. Nature, 265: 133-135.
- WIRTH C., GLEIXNER G., HEIMANN M. (Eds.), 2009 Old-Growth Forests: Function, Fate and Value. Ecological Studies, 207: 1-512. Springer-Verlag, Berlin -Heidelberg. ISBN 978-3-540-92705-1.
- WOLYNSKI A., 2001 Significato della necromassa legnosa in bosco in un'ottica di gestione forestale sostenibile. Sherwood, 67: 5-12.
- ZHANG L., GOVE J.H., LIU C., LEAK W.B., 2001 A finite mixture of two Weibull distributions for modelling the diameter distributions of rotated-sigmoid, uneven-aged stands. Canadian Journal of Forest Research, 31: 1654-1659.
- ZHOU G., LIU S., LI Z., ZHANG D., TANG X., ZHOU C., JUNHUA YAN, MO J., 2006 *Old-Growth forests can accumulate carbon in soils*. Science, 314: 1417.