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Quantifying the effect of sampling plot size on estimation of structural indicators in old-growth forest stands

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Abstract

There is increasing awareness that structure-based indicators should be considered for assessing the biological value of late successional forests. In order to increase the unique habitat features critical for old-growth associated species, it is important to identify and rank candidate potential forest habitats on the basis of their distinctive structural features. Data on living and deadwood components for the identification of old-growth condition are usually acquired in the surveyed forest stands by two sampling strategies: i) census performed in large monitoring sites; ii) network of small sampling units, on which inventory practices are usually based. Several authors argued that choosing between these sampling strategies might have substantial effects on the values of common indicators of old-growth condition. Our study aims at i) assessing the total estimate differences among four common indicators measured in field plots with different sizes, and ii) defining the sample size for the reliable assessment of old-growth structural indicators. The study was carried out in six beech dominated forest stands on the Apennines range in Italy. In each stand, living and deadwood components were surveyed and geocoded in 1-ha wide areas. Circular plots with radius ranging from 4 m up to 20 m were then considered in order to quantify the effect of sampling plot size on the estimation of four structural indicators: 1) number of living trees; 2) number of large trees (dbh \geq 50 cm); 3) total deadwood volume; 4) number of large deadwood elements (snags,

dead standing trees; lying dead trees, lying deadwood) with dbh (or average diameter for lying deadwood) ≥ 30 cm. We found that the size of the sampling plots should be at least 500 m² in order to establish a reliable database for the assessment of the investigated indicators. The census approach should always be preferred to the sampling plot approach for old-growth forest stands smaller than 2-3 ha. The achieved results contribute to define assessment protocols for characterizing and ranking the naturalness of forest stands and for supporting the identification of potential old-growth stands based on standardized indicators.

Keywords: forest structure, deadwood, survey simulation, beech forests, Italy.

1. Introduction

Old-growth forests are present in many of the boreal and continental biogeographic regions of Europe and North America (e.g., Wirth et al., 2009). But just a few old-growth forests exist in mountainous Mediterranean contexts, such as in the Italian peninsula, where forests have been exploited since pre-Roman times (Blondel, 2006). However, since the second half of the 19th century, the socio-economic features in most of the mountainous areas of the Apennines changed significantly, resulting in extensive abandonment of forest and rural areas, with a significant reduction of human activities and wood exploitation (Saraceno, 1993). As a consequence, many forests have been withdrawn from regular silvicultural management for many years and their structures are now the result of both natural processes, i.e. competition and disturbances, and past human-induced changes (Das, 2012; Lombardi et al., 2008 and 2012; Vandekerckhove et al., 2009). These forests are outstanding hotspots of forest biodiversity at regional and continental scale and their monitoring is of critical relevance to understand the potential for forest functionality and ecosystem services provision (Schnitzler and Borlea, 1998). Indeed, forest developmental stage and degree of structural complexity are often considered the ecological basis for evaluating management efforts to maintain and enhance biological diversity (Bartha et al., 2006; Liira et al., 2007; Norden and Appelqvist, 2001), also in relation to the increasing international interest in silvicultural practices that mimic natural forest ecosystem processes (Lahde et al., 1999). In most European countries, sustainable silvicultural approaches are becoming more common (Ciancio et al., 1999; von Oheimb et al., 2005; Ciancio et al., 2006; Ciancio and Nocentini, 2011; Nocentini and Coll, 2013) and old-growth forests are often the management standard for comparison purposes (Barbati et al., 2012; Lahde et al., 1999; Peterken, 1996).

Under this view, there is increasing awareness that several indicators should be considered for evaluating the multiple values of late successional forests. The usefulness of indicators of old-

growth condition depends on various criteria. These indicators should discriminate between natural and anthropogenic features (Hardt and Swank, 1997; Angermeier, 2000), be reliable and objective (Uotila et al., 2002), and produce consistent and precise results (Smith and Theberge, 1987; Liira et al., 2007). Moreover, they should be readily calculable from available data (Bartha et al., 2006), sensitive to a wide range of effects (Angermeier and Karr, 1994), and appropriate at multiple spatial scales (McRoberts et al., 2009, 2012). Indicators based on structural attributes, firstly developed through studies performed in the Pacific Northwest (Spies and Franklin, 1991), are useful because they can both be a good proxy of ecosystem functions and a readily measured surrogate for habitats of many taxa (Franklin et al., 2002; Burrascano et al., 2011). Some of the most used indicators include the variation in tree size, as well as the presence of large living old trees and of multilayered canopy (Franklin and Van Pelt, 2004). Deadwood occurrence is also recognized to be one of the most typical components of old-growth forests (Siitonen et al., 2000; Laarmann et al., 2009).

In order to assess structural indicators of forest stands, various sampling approaches can be suitably exploited. When designing forest monitoring schemes, scientists should consider their specific objectives in order to define the degree of precision and accuracy required through a cost-benefit analysis (Corona and Marchetti, 2007; Corona et al., 2011; Travaglini et al., 2013). A trade-off always exists between the descriptive resolution (number of variables) and spatial resolution (extent of the surveyed area) (Corona, 2010). In general, field protocols are often misbalanced towards maximizing the number of descriptive variables and counterbalanced by poor spatial extent and resolution (Proulx, 2007). Therefore, when defining a sampling strategy it is of primary importance to take into account two main components: *i*) which information has to be acquired in the field; *ii*) how the information has to be acquired. Regarding the first point, no single variable has been pointed out for the assessment of all the aspects of forest naturalness and, therefore, a multi-criteria evaluation of forest structure is preferable (Branquart and Latham, 2007). For this reason, a relatively small set of appropriate indicators is requested, where an indicator is defined as feature of a biological system whose occurrence contributes to estimate the most relevant traits of forest conditions (Larsson et al., 2001).

In the assessment of old-growth forests, data related to the living and deadwood components for the estimation of naturalness/oldgrowthness are usually acquired in the field through sample areas (Corona et al., 2010, Marchetti et al., 2010). Among the various sampling schemes, the use of fixed-size plots, randomly or systematically located inside the surveyed area, is outlined as an effective way to obtain reliable estimates of ecological attributes together with an evaluation of their sampling variability (e.g., Barabesi and Fattorini, 1998; Schreuder et al., 1993; Gregoire and Valentine, 2008).

Plot size has a direct effect on the assessment of forest characteristics (e.g., Corona et al., 1998), but very few studies can be found in literature about how the size of monitoring areas affects indicators of old-growth forest conditions (e.g., Rubin et al., 2006; Alessandrini et al., 2011). McRoberts et al. (2012) showed that several indicators commonly used for assessing forest naturalness are sensitive, at a varying extent, to plot size and minimum diameter at breast height (dbh) threshold; however, this test has had some limitations, with respect to the objectives here considered, since: i) only a minor part of the dataset displayed old-growth forest features, and ii) the maximum plot radius used in the field was relatively low.

Very few studies deal with the statistical estimation of indicators for assessing old-growth forests (e.g. Kint et al., 2004). Considering this knowledge gap, we set up an experiment for a detailed investigation about the influence exerted by the size of sampling units on the assessment of old-growth forest conditions. First, we selected a set of structural and deadwood indicators. Second, we acquired the information needed to calculate the selected indicators on the basis of a census in six study sites where the entire living tree and the deadwood components were measured and geocoded in the field on a 1-ha area; the six study sites are all documented old-growth forest candidates (Lombardi et al., 2012). Finally, to determine the effect on the estimation of the total of each indicator exerted by the size of circular sampling plots, we calculate the variance of the Horvitz-Thompson estimator of the total with reference to plots with radius from 4 m up to 20 m, which represents the range of the most frequently plot size adopted by National Forest Inventories (NFIs, see Chirici et al., 2011).

Our study aimed at i) assessing the total estimation differences among four common indicators measured in field plots with different sizes; ii) defining the sample size for the reliable assessment of old-growth structural indicators. The ultimate goal was to give insights for the design of future inventory protocols distinctively targeted towards the identification and characterization of old-growth forests.

2. Materials and methods

2.1 Study sites

The study was conducted in six selected sites, located within undisturbed beech stands across the Italian Peninsula, in Central Apennines (Figure 1).

The investigated sites were identified as candidate old-growth forests on the basis of literature references and historical information on past anthropic activities (Lombardi et al., 2012). The study sites are located in remote and impervious mountain areas, all included in reserves, Natura 2000

network sites or National Parks, where natural development processes are the main drivers of stand evolution.

A summary description of each study site is provided in Table 1; “*ID Area*” column refers to the map in Figure 1, while the most abundant tree species occurring in these stands are listed in “*Main species*”.

The elevation of the study sites ranges between 700 and 1800 m a.s.l.. All the sites are currently unmanaged and their stand structure refers to high forest under various development stages. The forests were classified as European Forests Type 7.3, i.e. Apennine Corsican mountainous beech forest (Barbati et al., 2007; Barbati et al., 2014). The sites of “*Bosco Aschiero*”, “*Fonte Novello*”, “*Val Cervara*” and “*Pavari Area*” are covered by almost pure beech forest stands, whereas the “*Montedimezzo*” site is a mixed oak-beech stand, and the “*Abeti Soprani*” site is a relict silver fir-beech stand survived from the last glacial eve, whose structure and composition are similar to those common in the past throughout the Apennines.

The sites of “*Fonte Novello*” (1) and “*Bosco Aschiero*” (2) are located in the Abruzzo Region, inside the Gran Sasso and Monti della Laga National Park; the “*Fonte Novello*” site has not been harvested during the last centuries due to legal debates concerning the administrative boundaries; “*Bosco Aschiero*” has escaped from loggings activities for centuries, developing old-growth traits in its structure, due to the rocky and steep morphology. “*Val Cervara*” (3) site is included in the Abruzzo, Lazio and Molise National Park (Abruzzo Region); this forest has been unmanaged for centuries, due to difficult accessibility, since it is located on steep slopes, but also because of its protective role against avalanches. The sites of “*Abeti Soprani*” (4) and “*Montedimezzo*” (5) are located in the northern part of the Molise Region; the first site is not currently managed and has not been cut for more than three decades due to the low local interest in conifer timber; the second one is a mixed broadleaved forest part of the MaB Unesco Reserve Collemeluccio-Montedimezzo and, therefore, also unmanaged during the last decades. Finally, the “*Pavari Area*” beech forest (6), located on the Gargano promontory on the Adriatic Sea (Apulia Region), is part of an old forest research network established in 1952 and unharvested since 1954. For further details on the study sites, see Lombardi et al. (2012).

2.2 Indicators of old-growth forest conditions

The following naturalness/oldgrowthness indicators were considered: 1) growing stock volume of living trees (VLT); 2) number of large trees (NBL); 3) total volume of dead elements (VDE); 4) number of large dead elements (NBD).

The growing stock volume (VLT) is probably the most used traditional attribute for assessing a forest stand: for this reason it is always included in traditional forest surveys. Growing stock volume is also frequently used as indicator of naturalness/oldgrowthness since recent studies have shown the importance of old-growth forests in storing high quantities of biomass (Keith et al., 2009; Keeton et al., 2010). Indeed, it has been also demonstrated that old-growth forests host significantly higher amounts of living biomass than mature forests (Burrascano et al., 2008; Burrascano et al., 2013).

The number of large living trees (NBL) is considered since it may give useful insights on forest functions, providing habitats for a number of threatened or ecologically important taxa, ranging from fungi to lichens, saproxylic beetles, birds and bats (Brunialti et al. 2010; Jung et al. 2012; Persiani et al. 2010). We define a tree as “large” if it has dbh > 50 cm (Greenberg et al, 1997).

Deadwood traits are also considered as indicators of old-growth forest conditions. Particularly, the total volume of deadwood (VDE) is selected, since deadwood amount influences the biological diversity across all the trophic levels, representing a long-term source of nutrients due to its regular recruitment (Lombardi et al. 2012, 2013).

Finally, we also refer to the number of large deadwood elements (NBD), i.e. the number of deadwood elements (snags, dead standing trees; lying dead trees, lying deadwood) with dbh (or average diameter for lying deadwood) ≥ 30 cm (Hunter et al., 1990). Large deadwood represents an important sources of microhabitats usually not provided by fine deadwood, such as tree hollows, cavity strings and cracks. These features are useful for a variety of species, such as breeding birds, mammals, and invertebrates, but also lichens and bryophytes), saproxylics and small mammals (Brunialti et al. 2010).

2.3 Field survey

The four selected indicators were assessed within a 1-ha wide area selected in each study site. The location of all the living and dead elements surveyed was mapped and the size of all the living and the standing dead trees (height, height of the crown insertion and dbh), the snags and the stumps (minimum and maximum diameter, height), the dead downed trees (dbh and length) and the coarse woody debris (CWD) (minimum and maximum diameter, length) was measured.

All the living trees (individuals with a dbh ≥ 10 cm), the snags and the standing dead trees (individuals with a dbh ≥ 5 cm), the dead downed trees and the CWD (with a diameter ≥ 5 cm) and the stumps (cut-surface with diameter ≥ 5 cm) were identified and marked with numbered plastic tags. Then, the position of each element was recorded by measuring the distance and the azimuth from four reference points located inside each study site (Figure 2).

The coordinates of the reference points in UTM (Universal Transverse Mercator) system with WGS 84 datum were acquired in the field by GNSS (Global Navigation Satellite System) instruments. For each point the average of at least 1000 positions recorded every second were averaged, resulting in a submetric precision.

The volume of living trees, standing and downed dead trees was estimated by two-way volume equations (IFNC, 2005), while the volume of CWD, snags and stumps was calculated through the cone trunk formula.

2.4 Assessment of sampling precision

The topographical position of each living tree and deadwood element was known from the field survey, allowing the assessment of the variability in estimation of VLT, NBL, VDE and NBD by circular sampling plots with radius varying from 4 m up to 20 m.

In each study site with surface area A , there are N elements (living trees or dead elements) that belong to a planar point pattern, i.e. their 2-dimensional position is known. Coupled with the N elements are the values of interest y_j ($j = 1, 2, \dots, N$). If circular plots of radius r randomly placed

through the study site are used to estimate the total value $T_y = \sum_{j=1}^N y_j$ of an indicator (i.e. a variable), it is possible to associate with each element a K -circle K_j ($j = 1, 2, \dots, N$). The K -circle of an element j (K_j) can be considered as an imaginary circle with radius r and area $a = \pi \cdot r^2$, centred at the tree centre for standing trees (living or dead) or at the thicker end for elements lying on the

ground. An element is included into a sampling plot with probability $\gamma = \frac{a}{A^*}$, where A^* is the area of the site enlarged by the buffer adopted to overcome edge effects.

Considering a pair of elements, j and h , and the area of intersection of their K -circles a_{jh} , the variance of Horvitz-Thompson estimator of the total T_y on one plot is

$$\text{VAR}(\hat{T}_{HT}) = \frac{A^*}{a} \sum_{j=1}^N y_j + \frac{2A^*}{a^2} \sum_{h>j=1}^N a_{jh} y_j y_h - T_y^2 \quad [\text{eq. 1}]$$

If the value to be estimated is the number N of elements (as in the case of the indicators NBL and NBD), then $y_j = 1$ and the variance of the total becomes

$$VAR(\hat{N}_{HT}) = \frac{A^*}{a} N + \frac{2A^*}{a^2} \sum_{h>j=1}^N a_{jh} - N_y^2 \quad [\text{eq. 2}]$$

The relative standard error (RSE%) can then be calculated to highlight the effect of sample size (i.e. the number m of plots over the study site) on estimate precision

$$RSE\% = \frac{\sqrt{VAR}}{T_y} \cdot \frac{1}{\sqrt{m}} \cdot 100 \quad [\text{eq. 3}]$$

3. Results

The true values of total (T_y) of the considered indicators on the six study sites and the variability of the estimators based on one random plot are shown in Table 2. Setting a specific number of plots (m) over the study site, the calculation of RSE% is straightforward (see eq. 3).

The required sample size to achieve a given RSE% ranges widely across the considered indicators (Table 3), with those, like NBD, referred to sparse population and whose distribution has a contagion pattern (sensu Ricotta et al., 2003) rising to very high size. On the contrary, the indicator referred to more numerous populations (NBL) can be effectively sampled by a relatively small sample size. Obviously, the required number of sampling plots decreases by decreasing the allowable threshold of RSE% and by increasing the plot size.

Figure 3 highlights the relationship between plot size and estimate precision for the considered indicators: it is relevant that plots with radius equal to 10 m or 13 m provide quite comparable results. On the other hand, it is worth stressing that the estimate precision referred to indicators of number of elements (NBL and NBD) is always higher than that referred to volumes (VDE and VLT).

The growing stock volume (VLT) and the total volume of deadwood (VDE) show an almost identical trend across the six study sites (Fig. 3), even if the sites are characterized by very different amounts, with VLT ranging between 364 and 1060 m³ ha⁻¹ and VDE between 2 and 135 m³ ha⁻¹. The same trend is also observed for the number of large living trees (NBL): since the variability of this indicator within the considered sites is relatively low (between 58 and 106 trees per hectare), the accuracy of its estimation is much higher than that of VLT and VDE.

4. Discussion

The variability of estimators of VLT, VDE and NBD markedly drops when the plot radius is enlarged from 4 m to 13 m, and then to smoothly decreases up to a plot radius of 20 m. For NBL,

the estimate precision with respect to 20-m plot radius somewhat decreases due to the size of the study site, relatively small with respect to this plot size.

Three out of the four indicators present a trend that partially confirms the findings by McRoberts et al. (2012): the NBD trend, in those three sites where the number of large dead elements is relatively high (Fonte Novello, Pavari and Val Cervara, ranging between 33 and 71 elements per hectare), has the same pattern; whereas, for the three sites where NBD is low (especially in the Aschiero site, with only two elements per hectare) the precision is also very low, independently of the plot size.

The sample size (i.e. the number of sampling plots) required to get a given threshold of estimate precision is independent of the size of the study site to be inventoried (Gregoire and Valentine, 2008); whereas, it is related to the degree of variability of the indicators of interest to this aim, as well as to the sampling design. As a general remark, the results here obtained empirically evidence that a relatively low sample size is adequate for those sites characterized by the highest values of the considered indicators (high number of large trees and/or large amount of deadwood from dead elements), and *viceversa*, at least under the examined environmental conditions.

The number of sampling plots required for reliably estimating NBL is quite low, while, for NBD, this number might be very high (e.g., the Aschiero site); the number of plots required to achieve a given threshold of estimate precision is similar for VLT and VDE. When the sampled population is sparse, the precision is quite low, independently of the plot size. In conclusion, we are confident to suggest the adoption of sampling plots with a 13-m or 15-m radius (plot size approximately between 500 and 700 m²) to suitably inventory indicators of old-growth forest conditions.

Human-induced environmental effects, such as climate change, introduction of invasive species, intensive cuttings, and pollution pose serious threats to forest biodiversity (Ciancio and Nocentini 2011). Under this scenario, we deem effective that conventional inventory databases may be exploited also for the assessment of forest naturalness/oldgrowthness. NFIs routinely collect information potentially useful to such a purpose (Corona et al., 2011; Chirici et al., 2011; McRoberts et al., 2012), but it is relevant to stress that not all the NFI have plot size consistent with the results obtained in our study: only 13 NFIs out of the 33 reported by Tomppo et al. (2011) have plot size larger than 500 m².

When a monitoring system has to be established for a specific old-growth forest stand it is possible to establish a network of relatively small sampling plots or to apply a census approach, acquiring the information on the whole stand. On the basis of the present results (Table 3), it is difficult to give a general indication about the optimal choice: however, considering the comparative costs, it seems tentatively reasonable to apply a sampling plot approach when the investigated stand is at least 3-5 ha wide in the case of the assessment of NBL or at least 10-15 ha in the case of the other

indicators here considered. For example, plot selection for monitoring stand structure and deadwood components has been usually carried out by European countries following different national interests and, therefore, cannot be assumed to be representative neither at a continental nor at a national, regional or forest type related level, which can only be achieved by inventories based on probabilistic sampling schemes (Seidling et al., 2014).

5. Conclusions

We can derive three main conclusions from our study. First, the size of sampling plots should be at least 500 m² in order to provide a good inventory database for the assessment of indicators of old-growth/naturalness forest conditions: this finding may be considered a survey protocol reference at least in most of the forest types present in temperate and Mediterranean Europe, and Table 3 can be considered as a reference for sizing the number of plots. Second, naturalness/oldgrowthness indicators should be selected not only according to ecological reasons, but also taking into account the possibility of acquiring consistent data: notably, the estimation of indicators measured on sparse populations may be associated with high level of uncertainty (for instance, deadwood or large trunks in regularly managed forests). Third, when a new monitoring network is developed, specifically for old-growth forests, the choice between the adoption of a sample-based or a census-based approach has to be carefully conceived, with respect to three main variables: the forest characteristics, the variability of the indicators to be inventoried, the size of sampling plots; in general, on the basis of our experiment results, the census approach should be preferred for old-growth forest stands smaller than 3-5 ha.

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References

- Alessandrini A., Biondi F., Di Filippo A., Ziaco E. and Piovesan G., 2011. Tree size distribution at increasing spatial scales converges to the rotated sigmoid curve in two old-growth beech stands of the Italian Apennines. *Forest Ecology and Management* 262: 1950–1962.
- Angermeier P.L., 2000. The natural imperative for biological conservation. *Conservation Biology*, 14: 373–381.
- Angermeier P.L. and Karr J.R., 1994. Biological integrity versus biological diversity as policy directives. *BioScience* 44: 690–697.
- Barabesi L. and Fattorini L., 1998. The use of replicated plot, line and point sampling for estimating species abundances and ecological diversity. *Environmental and Ecological Statistics*, 5: 353–370.
- Barbati A., Corona P., Marchetti M., 2007. A forest typology for monitoring sustainable forest management: The case of European Forest Types. *Plant Biosystems* 141: 93-103.
- Barbati A., Salvati R., Ferrari B., Di Santo D., Quatrini A., Portoghesi L., Travaglini D., Iovino F., and Nocentini S., 2012. Assessing and promoting old-growthness of forest stands: Lessons from research in Italy. *Plant Biosystems*, 146: 167-174.
- Barbati A., Marchetti M., Chirici G. and Corona P., 2014. European Forest Types and Forest Europe SFM indicators: Tools for monitoring progress on forest biodiversity conservation. *Forest Ecology and Management*, 321: 145-157..
- Bartha D., Odor P., Horvath T., Timar G., Kenderes K., Standovar T., Boloni F., Szmorad F., Bodoncz L. and Aszalos R., 2006. Relationship of tree stand heterogeneity and forest naturalness. *Acta Silva Ling. Hung.*, 2: 7–22.
- Blondel J., 2006. The ‘Design’ of Mediterranean landscapes: A millennial story of humans and ecological systems during the historic period. *Human Ecology*, 34: 713-729.
- Branquart E. and Latham J., 2007. Selection criteria for protected forest areas dedicated to biodiversity conservation in Europe. In *Protected forest areas in Europe—Analysis and harmonization (PROFOR): Results, conclusions, and recommendations*. Frank G., Parviainen J., Vanderkerhove K., Latham J., Schuck A. and Little D. (eds.). Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Vienna, Austria.
- Burrascano S., Lombardi F. and Marchetti M., 2008. Old-growth forest structure and deadwood: Are they indicators of plant species composition? A case study from central Italy. *Plant Biosystems*, 142: 313 – 323.

- Burrascano S., Keeton W.S., Sabatini F.M. and Blasi C., 2013. Commonality and variability in the structural attributes of moist temperate old-growth forests: A global review. *Forest Ecology and Management*, 291: 458-479.
- Chirici G., Winter S. and McRoberts R.E. (eds.), 2011. *National Forest Inventories: contributions to forest biodiversity assessments*. Dordrecht Heidelberg London New York: Springer.
- Ciancio O., Corona P., Iovino F., Menguzzato G. and Scotti R., 1999. Forest management on a natural basis: the fundamentals and case studies. *Journal of Sustainable Forestry*, 1/2: 59-72.
- Ciancio O., Corona P., Lamonaca A., Portoghesi L. and Travaglini D., 2006. Conversion of clearcut beech coppices into high forests with continuous cover: a case study in central Italy. *Forest Ecology and Management*, 3: 235-240.
- Ciancio O. and Nocentini S., 2011. Biodiversity conservation and systemic silviculture: Concepts and applications. *Plant Biosystems*, 145: 411-418.
- Corona P., 2010. Integration of forest inventory and mapping to support forest management. *iForest*, 3: 59-64.
- Corona P., Saracino A. and Leone V., 1998. Plot size and shape for the early assessment of post-fire regeneration in Aleppo pine stands. *New Forests*, 16: 213-220.
- Corona P. and Marchetti M., 2007. Outlining multi-purpose forest inventories to assess the ecosystem approach in forestry *Plant Biosystems*, 141, 243-251.
- Corona P., Blasi C., Chirici G., Facioni L., Fattorini L. and Ferrari B., 2010. Monitoring and assessing old-growth forest stands by plot sampling. *Plant Biosystems*, 144: 171-179.
- Corona P., Chirici G., McRoberts R.E., Winter S., Barbati A., 2011. Contribution of large-scale forest inventories to biodiversity assessment and monitoring. *Forest Ecology and Management*, 262: 2061–2069.
- Crumpacker, D.W., 1998. Prospects for sustainability of biodiversity based on conservation biology and US Forest Service approaches to ecosystem management. *Landscape Urban Planning*, 40: 47–71.
- Das A., 2012. The effect of size and competition on tree growth rate in old-growth coniferous forests. *Canadian Journal of Forest Research*, 42:1983-1995.
- De Vries P.G., 1986. *Sampling Theory for Forest Inventory*. Springer-Verlag, Berlin.
- Franklin J.F. and Van Pelt R., 2004. Spatial aspects of structural complexity in old-growth forests. *Journal of Forestry*, 102: 22-28.
- Gregoire T.G. and Valentine H.T., 2008. *Sampling strategies for natural resources and the environment*. New York: Chapman & Hall.

- Hardt R.A. and Swank W.T., 1997. A comparison of structural and compositional characteristics of southern Appalachian young second-growth, maturing second-growth, and oldgrowth stands. *Natural Areas Journal*, 17: 42–52.
- Hunter M.L., 1990. *Wildlife, forests, and forestry: principles of managing forests for biological diversity*. Prentice Hall, Englewood Cliffs.
- INFC, 2005. *Inventario Nazionale delle Foreste e dei Serbatoi Forestali di Carbonio*. Ministero delle Politiche Agricole Alimentari e Forestali, Ispettorato Generale - Corpo Forestale dello Stato. CRA - Unità di ricerca per il Monitoraggio e la Pianificazione forestale. Available on-line at www.ifni.it.
- Kint V., De Wulf R. and Noël L., 2004. Evaluation of sampling methods for the estimation of structural indices in forest stands. *Ecological Modelling*, 180: 461–476.
- Laarmann D., Korjus H., Sims A., Stanturf J.A., Kiviste A. and Koster K., 2009. Analysis of forest naturalness and tree mortality patterns in Estonia. *Forest Ecology and Management*, 258: 187–195.
- Lahde E., Laiho O. and Norokorpi Y., 1999. Diversity-oriented silviculture in the boreal zone of Europe. *Forest Ecology and Management*, 118: 223–243.
- Lane A. and Tait J., 1990. *Practical conservation woodlands*. Hodder and Stoughton, London, UK. 128 p.
- Larsson T.B., Svensson L., Angelstam P., Balent G., Barbati A., Bijlsma R.-J., Boncina A., Bradshaw R., Bucking W., Ciancio O., Corona P., Diaci J., Dias S., Ellenberg H., Fernandes F.M., Fernandez-Gonzalez F., Ferris R., Frank G., Møller P.F., Giller P.S., Gustafsson L., Halbritter K., Hall S., Hansson L., Innes J., Jactel H., Keannel Doppertin M., Klein M., Marchetti M., Mohren F., Niemela P., Ohalloran J., Rametsteiner E., Rego F., Scheidegger C., Scotti R., Sjöberg K., Spanos I., Spanos K., Standovar T., Tømmerås Å., Trakolis D., Uuttera J., Walsh P.M., Vandekerckhove K., Watt, A.D. and Vendenmeersschant D., 2001. Biodiversity evaluation tools for European forests. *Ecological Bulletins No. 50*.
- Liira J., Sepp T. and Parrest O., 2007. The forest structure and ecosystem quality in conditions of anthropogenic disturbance along productivity gradient. *Forest Ecology and Management*, 250: 34–36.
- Lombardi F., Cherubini P., Lasserre B., Tognetti R. and Marchetti M., 2008. Tree rings used to assess time since death of deadwood of different decay classes in beech and silver fir forests in Central Apennines (Molise, Italy). *Canadian Journal of Forest Research*, 38: 821-833.
- Lombardi F., Cherubini P., Tognetti R., Cocozza C., Lasserre B., Marchetti M., 2013. Investigating biochemical processes to assess deadwood decay of beech and silver fir in Mediterranean mountain forests. *Annals of Forest Science*, 70: 101-111.

- Lombardi F., Chirici G., Marchetti M., Tognetti R., Lasserre B., Corona P., Barbati A., Ferrari B., Di Paolo S., Giuliarelli D., Mason F., Iovino F., Nicolaci A., Bianchi L., Maltoni A., Travaglini D., 2010. Deadwood in forest stands close to old-growthness under Mediterranean conditions in the Italian peninsula. *Italian Journal of Forest and Mountain Environments*, 65: 481-504.
- Lombardi F., Lasserre B., Chirici G., Tognetti R. and Marchetti M., 2012. Deadwood occurrence and forest structure as indicators of old-growth forest conditions in Mediterranean mountainous ecosystems. *Ecoscience*, 19: 344-355.
- Marchetti M., Tognetti R., Lombardi F., Chiavetta U., Palumbo G., Sellitto M., Colombo C., Iovieno P., Alfani A., Baldantoni D., Barbati A., Ferrari B., Bonacquisti S., Capotorti G., Copiz R. and Blasi C., 2010. Ecological portrayal of old-growth forests and persistent woodlands in the Cilento and Vallo di Diano National Park (southern Italy). *Plant Biosystems*, 144: 130-147.
- McRoberts R.E., Winter S., Chirici G. and LaPoint E., 2012. Assessing Forest Naturalness. *Forest Science*, 58: 294-309.
- McRoberts R., Tomppo E., Schadauer K., Vidal C., Stahl G., Chirici G., Lanz A., Cienciala E., Winter S. and Smith B., 2009. Harmonizing National Forest Inventories. *Journal of Forestry*, 107: 179-187.
- Nocentini S. and Coll L., 2013. Mediterranean forests: human use and complex adaptive systems. In: Messier C., Puettmann K.J., Coates K.D. (eds.), *Managing forests as complex adaptive systems*. Routledge, Oxon.
- Norden B. and Appelqvist T., 2001. Conceptual problems of ecological continuity and its bioindicators. *Biodiversity Conservation*, 10: 779–791.
- Nowak D.J., Walton J.T., Stevens J.C., Crane D.E., Hoehn R.E., 2008. Effect of plot and sample size on timing and precision of urban forest assessments. *Arboriculture & Urban Forestry* 34: 386-390.
- O'Regan W.G. and Palley M.N., 1965. A computer technique for the study of forest sampling methods. *Forest Science*, 11: 99-114.
- Peterken G.F., 1996. *Natural woodland. Ecology and conservation in temperate regions*. University Press, Cambridge, UK.
- Proulx R., 2007. Ecological complexity for unifying ecological theory across scales: A field ecologist's perspective. *Ecological Complexity*, 4: 85–92.
- Ricotta C., Corona P. and Marchetti M., 2003. Beware of contagion! *Landscape and Urban Planning*, 62: 173-177.
- Rubin, B.D., Manion, P.D. and Faber-Langendoen D., 2006. Diameter distributions and structural sustainability in forests. *Forest Ecology and Management* 222, 427–438.

- Saraceno E., 1993. Alternative readings of spatial differentiation: the rural versus the local economy approach in Italy. *European Review of Agricultural Economics*, 21: 451–474.
- Saudyte S., Karazihja S. and Belova O., 2005. An approach to assessment of naturalness for forest stands in Lithuania. *Baltic Forestry* 11: 39–45.
- Schnitzler A. and Borlea F., 1998. Lessons from natural forests as keys for sustainable management and improvement of naturalness in managed broadleaved forests. *Forest Ecology and Management*, 109: 293-303.
- Schreuder H.T., Gregoire T.G. and Wood G.B., 1993. *Sampling methods for multiresource forest inventory*. New York: Wiley.
- Seidling W., Travaglini D., Meyer P., Waldner P., Fischer R., Granke O., Chirici G. and Corona P., 2014. Dead wood and stand structure - relationships for forest plots across Europe. *iForest*, (early view): e1-e13 [online 2014-04-14].
- Siitonen P., Martikainen P., Punttila P. and Rauh J., 2000. Coarse woody debris and stand characteristics in mature and oldgrowth boreal mesic forests in southern Finland. *Forest Ecology and Management*, 128: 211-225.
- Smith P.G.R. and Theberge J.B., 1987. Evaluating natural areas using multiple criteria: Theory and practice. *Environmental Management*, 11: 447– 460.
- Uotila A., Kouki J., Kontkanen H. and Pulkkinen P., 2002. Assessing the naturalness of boreal forests in eastern Fennoscandia. *Forest Ecology and Management*, 161: 257–277.
- Travaglini D., Fattorini L., Barbati A., Bottalico F., Corona P., Ferretti M. and Chirici G., 2013. Towards a sampling strategy for the assessment of forest condition at European level: combining country estimates. *Environmental Monitoring and Assessment*, 185: 3255–3268.
- Vandekerkhove K., De Keersmaeker L., Menke N., Meyer P. and Verschelde P., 2009. When nature takes over from man: dead wood accumulation in previously managed oak and beech woodlands in North-western and Central Europe. *Forest Ecology and Management*, 258: 425-435.
- Von Oheimb G., Westphal C., Tempel H. and Hardtle W., 2005. Structural pattern of a near-natural beech forest (*Fagus sylvatica*) (Serrahn, North-east Germany). *Forest Ecology and Management*, 212: 253–263.
- Wirth C., Messier C., Bergeron Y., Frank D. and Fankhänel A., 2009. Old-growth forests definitions: a pragmatic view. In *Old-growth forests*, Ecological Studies. Wirth C. et al. (eds.). Springer-Verlag, Berlin, Heidelberg.

Figures

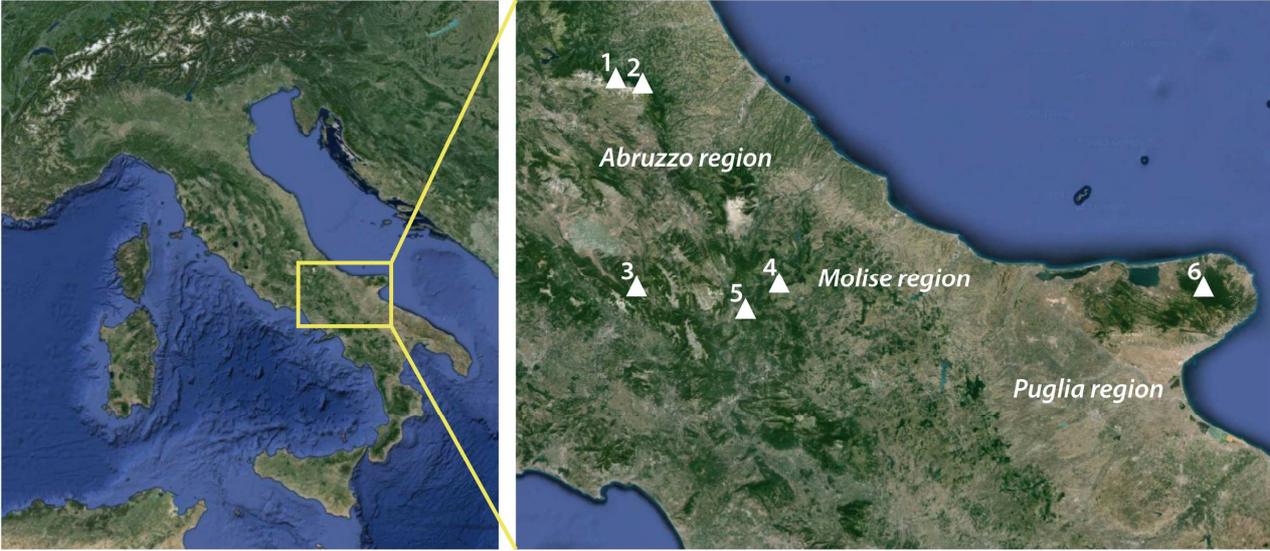


Figure 1. Location of the selected study sites. Numbers refer to the first “ID site” column of Tables 1 and 2.

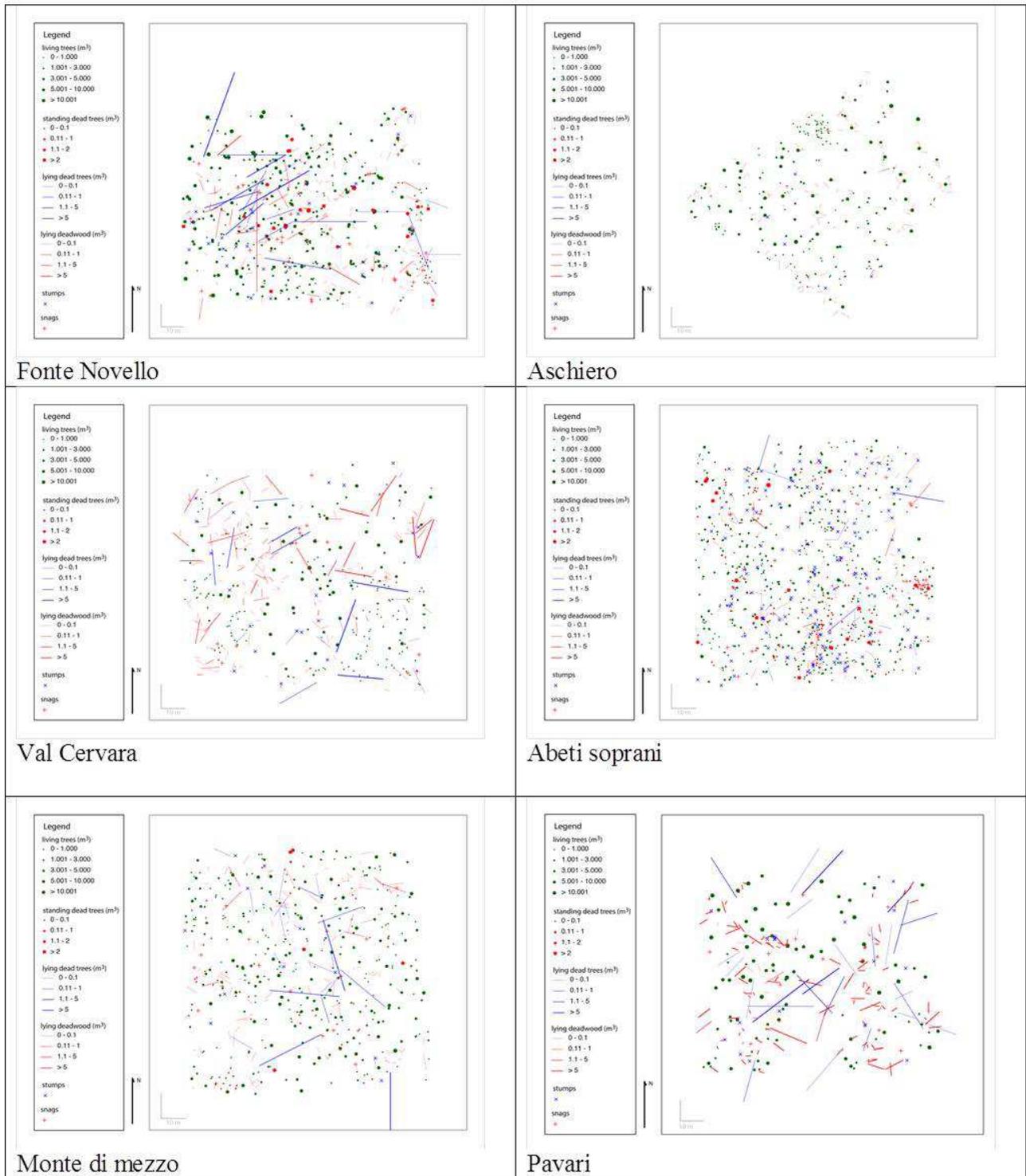


Figure 2. Maps of the living trees and deadwood elements in the old-growth forest stands censused within the selected study sites

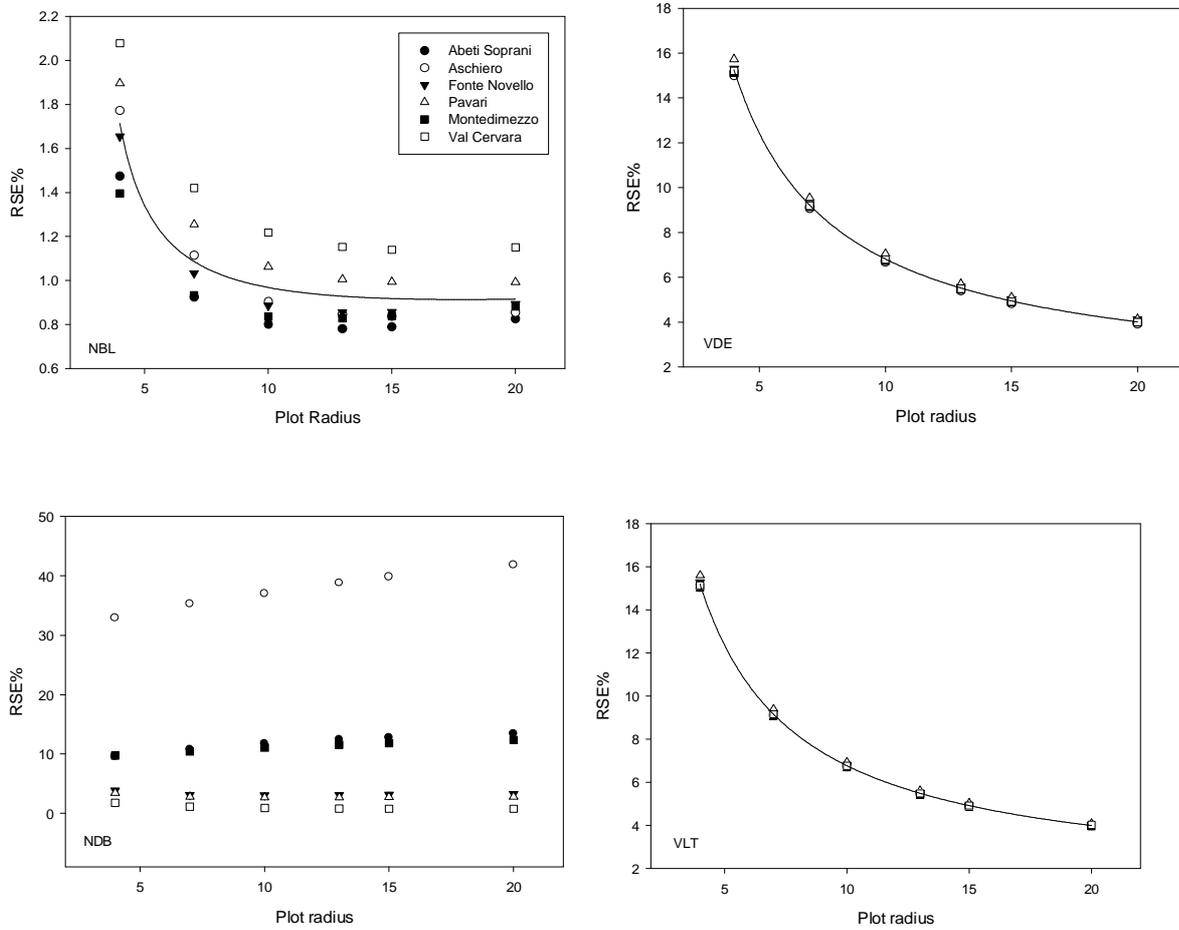


Figure 3. Relationship between plot radius and precision of the estimates for the four investigated oldgrowthness indicators.

Tables

Table 1. Main characteristics of the old-growth forest stands censused within the selected study sites. Stand mean age was determined through the mean age of trees occurring in the dominant tree layer.

ID Site	1	2	3	4	5	6	
Site	Fonte Novello	Bosco Aschiero	Val Cervera	Abeti Soprani	Monte di Mezzo	Pavari area	
Region	Abruzzo	Abruzzo	Abruzzo	Molise	Molise	Puglia	
Protected Area	Gran Sasso - Laga National Park	Gran Sasso - Laga National Park	Abruzzo, Lazio and Molise National Park	EU Natura 2000 Network site	MaB Unesco Reserve	Gargano National Park	
Coordinates	North	42° 50'	42° 50'	41° 49'	41° 86'	41° 82'	
	East	13° 50'	13° 58'	13° 43'	14° 30'	15° 99'	
Altitudinal range (m. a.s.l.)	1320 - 1360	1580 - 1640	1730 - 1830	1250 - 1450	950 - 1150	720 - 800	
Mean annual	Temperature (°C)	10.0	10.0	7.2	8.4	8.6	11.6
	Precipitation (mm)	1071	1246	1211	1124	1022	1041
Forest extension (ha)	13.4	5.21	300.0	232.0	291.0	5.3	
Main tree species	<i>F. sylvatica</i>	<i>F. sylvatica</i>	<i>F. sylvatica</i>	<i>A. alba</i>	<i>Q. cerris</i> <i>F. sylvatica</i>	<i>F. sylvatica</i>	
Additional tree species	no additional tree species	no additional tree species	no additional tree species	<i>A. Pseudoplatanus</i> <i>T. baccata</i>	<i>A. Obtusatum</i> <i>F. excelsior</i> <i>C. betulus</i>	<i>A. obtusatum</i> ,	
Stand mean age (years)	152	170	262	131	139	164	
Years since last anthropogenic disturbance	310	290	no references	31	56	59	

Table 2. Observed values of naturalness/oldgrowthness indicators on the censused forest stands and the relative standard error of the estimated values on one randomly located plot. NBL: number of biggest trees; VDE: total volume of dead elements; NDB: number of biggest dead elements; VLT: growing stock volume of living trees.

ID	Test Area	Observed values				Plot radius	RSE (%)			
		NBL	VDE	NDB	VLT		NBL	VDE	NDB	VLT
1	Abeti soprani	87	30.81	8	580.84	4	1.47	15.15	9.58	15.13
						7	0.92	9.15	10.76	9.13
						10	0.80	6.75	11.72	6.74
						13	0.78	5.45	12.38	5.46
						15	0.79	4.88	12.73	4.89
						20	0.83	3.96	13.38	3.98
2	Aschiero	68	2.19	2	414.88	4	1.77	15.01	32.87	14.97
						7	1.12	9.06	35.26	9.04
						10	0.90	6.68	36.97	6.67
						13	0.85	5.40	38.78	5.41
						15	0.84	4.83	39.82	4.85
						20	0.85	3.91	41.86	3.95
3	Fonte Novello	104	94.58	40	1060.33	4	1.65	15.31	3.86	15.29
						7	1.03	9.27	3.09	9.22
						10	0.89	6.86	3.04	6.80
						13	0.86	5.58	3.09	5.50
						15	0.86	5.02	3.13	4.93
						20	0.89	4.12	3.25	4.01
4	Pavari	71	87.73	33	698.79	4	1.90	15.73	3.43	15.60
						7	1.25	9.54	2.80	9.40
						10	1.06	7.05	2.68	6.93
						13	1.01	5.70	2.69	5.61
						15	0.99	5.10	2.71	5.03
						20	0.99	4.13	2.76	4.09
5	Monte di mezzo	106	25.06	11	689.39	4	1.40	15.13	9.74	15.05
						7	0.93	9.14	10.39	9.08
						10	0.84	6.74	11.03	6.70
						13	0.83	5.46	11.53	5.43
						15	0.84	4.89	11.79	4.86
						20	0.88	3.99	12.34	3.96
6	Val Cervara	58	135.05	71	363.97	4	2.08	15.21	1.77	15.14
						7	1.42	9.19	1.11	9.15
						10	1.22	6.78	0.88	6.76
						13	1.15	5.48	0.79	5.48
						15	1.14	4.91	0.77	4.92
						20	1.15	3.99	0.77	4.02

Table 3. Range of the numbers of plots with radius equal to 13 m to achieve a RSE% value equal to 20% under conditions similar to those of the investigated study sites (the number of plots is four times greater if a 10% RSE% is required). NBL: Number of biggest trees; VDE: total volume of dead elements; NBD: number of biggest dead elements; VLT: growing stock volume of living trees.

Plot radius	NBL		VDE		NBD		VLT	
	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
4	49	108	5629	6182	78	27015	5606	6083
7	21	50	2054	2274	31	31075	2044	2209
10	16	37	1115	1241	19	34172	1114	1201
13	15	33	728	812	16	37599	731	787
15	16	32	583	650	15	39634	588	633
20	17	33	383	427	15	43798	390	418