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Effects of different forest management practices on soil chemical and biological properties, and on the accretion of plants in coniferous stands located in Aspromonte (Southern Italy)

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SHORT ABSTRACT

This PhD thesis aimed to assess the effects of thinning of different intensities on soil quality, plant accretion, and wood quality in a 60-year-old *Pinus nigra* Arn. *laricio* Poiret var. *calabrica* forests, located in the Calabrian Apennines South Italy. Thinning of different intensities, carried on in 2011, was applied for evaluating changes in chemical, physical and biological soil properties and in dendrometric parameters of plants, with the specific objective to identify the silvicultural practice to be adopted for a sustainable forest management of Calabrian pine. The results showed that high intensity thinning, improved soil fertility, and had the best effects on plant accretion and wood quality. The results of this thesis provide functional information to design a silvicultural system useful to manage Coniferous Mediterranean forests, minimizing the environmental and visual impact.

Key words: Forest management, Plant accretion, Soil quality, Thinning intensities, Wood density

ABSTRACT

The main aim of this thesis was to assess the effects of different forest management practices on soil quality and plant growth in *Pinus nigra* Arn. spp. *laricio* Poiret var. *calabrica* Delamare forests, located in the Calabrian Apennines, South Italy. The effects of clearcutting and thinning at different intensities (i.e. 60 and 30% plant removed) were evaluated in respect to the unmanaged forest, monitoring changes in chemical, physical and biological soil parameters and in dendrometric parameters of plants. The specific aim was to identify the silvicultural practice to be adopted for a sustainable management of Calabrian pine forests. In this context, the effects of forest management on soil physical characteristics, amount of nutrients, organic matter trend, enzyme activities, pedofauna, soil microbial biomass and microorganisms, in terms of fungi, bacteria and actinomycetes colonies, were monitored for three years, in two contrasting seasons (i.e. summer and winter) to evaluate also if the effects of management on soil were affected by climatic conditions (i.e. temperature and humidity). The modifications of soil characteristics, in short term, were evaluated through the analysis of early warning indicators of changes in soil ecosystem functioning. Additionally, we evaluated the effects of different silvicultural practices on pine accretion, wood density and H/D ratio as parameter of tree stability. The soils in the study area, developed from high rank metamorphic rocks such as schist and biotitic gneisses, were classified as Humic Cambisols, with a xeric soil regime moisture. The soils belonged to the sandy-loam textural class. The pH was acid. The different types of management caused significant changes in the content of organic matter, nitrogen, enzymes, MBC, mites and collembola.

In clear cutting all biological parameter values were lower respect the other treatments, evidencing that tree removal reduced the water holding capacity leading to the leaching of soil nutrients and which in turn affected soil microbial biomass and activity.

Under 60% thinning the soils had the highest amount of organic matter, FDA, protease, catalase, MBC, and mites and collembola. These findings were due to a lower density of plants compared to natural forest and 30% thinning, that determined different regimes of light, temperature and humidity at soil level, increasing herbaceous vegetation undergrowth with the production of litter easily degradable. All this leaded to an increase in soil organic matter which in turn promoted soil biodiversity, a major soil activity and a better soil ecosystem functioning. 60% thinning had the best effects on soil quality. The results related to dendro-auxometric parameters evidenced that pinus accretion (diameter and height) and wood density changed with the treatments. H/D ratio in 60% thinning was lower than in 30 % thinning and natural forest suggesting a positive effect of 60% thinning on the mechanical stability of the trees.

The results of this thesis provide functional information to design a silvicultural system useful to manage Coniferous Mediterranean forests minimizing the environmental and visual impact.

1. INTRODUCTION

Forests provide a wide variety of goods, services and processes needed by society including wood and fiber, non-timber products, clean water and air, wildlife and fish habitat, recreation, esthetics and preservation of biodiversity. The economic and social well-being of modern society depends, to a great extent, on the presence of abundant and productive forests. In the years to the present, there have been huge changes in the distribution and nature of forests, caused by natural phenomena (disease and pests, storms, fire, drought and other climatic stresses) or human intervention, or by the interaction of human impacts and natural causes (European Environment Agency http://www.eea.europa.eu/publications/92-826-5409-5/page034new.html). Appropriate measures should be taken to protect forests in order to maintain their full multiple values. The incorrect silvicultural practices are the main causes of soil quality loss, timber productivity decrease, and poor quality material production. The sustainable management of natural resources is therefore a guiding principle of the proactive approach for maintaining forest productivity and combating land degradation.

1.1. *Pinus nigra* distribution and use

Pinus nigra J.F. Arnold, known as European black pine or black pine, is a fast-growing conifer with a wide but fragmented distribution across Europe and Asia Minor (Figure 1), predominantly in mountain areas. It has also become naturalised in some areas in North America. It is subdivided into several distinct subspecies and its taxonomic status is still a subject of debate among specialists (Enescu *et al*., 2016).

In Central Europe, Balkans, and Mediterranean countries *Pinus nigra* Arn. ssp. *laricio* is commonly planted for protection purposes (Bussotti, 2002). In Italy, this practice was established at the national level in the 20th century in accordance to a policy developed to protect land from erosion (Piermattei *et al*., 2012). *Pinus nigra* was used in central and south Italy to reforest mountainous areas depleted by the intensive use of natural resources, especially during the I and II World Wars. Toward the end of the 1970s, the mandatory requirement to reforest these areas, using coniferous species, was discontinued. In Italy, black pine occurs mostly in pure stands of even-aged forests (50 years old on average) and covers an area of 236.467 hectares (Gasparini and Tabacchi, 2011).

In Calabria it grows on Sila and Aspromonte mountains, covering approximately 114.000 ha, of which more than 50% are pure stands of both natural and artificial origin. The latter originated from extensive reforestation projects carried out between 1950 and 1970 on the basis of specific State laws

(Iovino and Menguzzato, 2002a; 2002b; Nicolaci *et al*., 2014), generally the *Pinus nigra* ssp. *laricio* plantations vary between 1250 and 2200 trees per hectares (Callegari *et al.*, 1994).

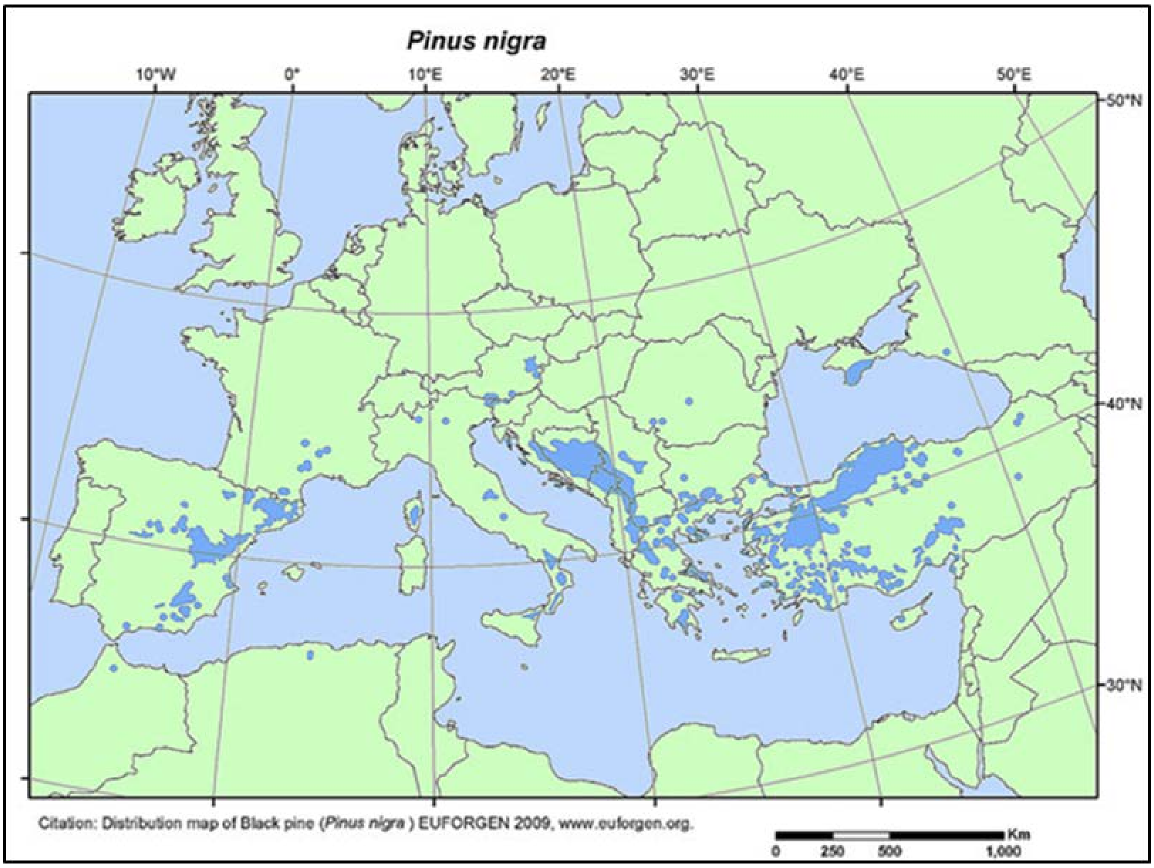

Figure 1. Map showing the wide but scattered distribution of *Pinus nigra* in the mountain area of Europe and Asia minor (blue areas).

Pinus nigra was widely used across Italy because of its typical characteristics of a pioneer species: low mortality, rapid juvenile growth, low incidence of health issues, and the ability to improve both chemical and physical characteristics of the soil. Among the sub-species of *Pinus nigra* Arn., the European black pine (*Pinus nigra* Arn. spp. *nigra*) was used more commonly on calcareous soils while *Pinus nigra* Arn. spp. *laricio* was more commonly used on sandstone soils. The main aims of establishing pine forests in Italy were to protect the soil from erosion and to facilitate the natural succession toward mixed forests with a strong component of deciduous species (Cantiani *et al.*, 2010). Together with soil conservation and watershed protection, *Pinus nigra* Arn. spp. *laricio* has an important role in the local forest economy.

1.2. Main habitat features, ecology and variability of *Pinus nigra* in south Italy

Pinus nigra forests, comprising a variety of subspecies, generally occupy medium and high mountain zones, often on substrates rich in magnesium (dolomites, in the case of the Western subspecies, and dolomites and serpentines in the case of the Eastern subspecies) (Regato *et al*., 1991). Some sub-types are present on deep soils, but in most cases the soil is superficial and not mature (Zaghi, 2008). The climate in the distribution area is sub-Mediterranean-continental. Winters are cold and summers are drought, compensated at times by frequent storms. Mature *Pinus nigra* forests can develop a high and closed arboreal canopy, with several classes of ages and individuals far above 30 m high, and shady under growth. *Pinus nigra* can occur with other coniferous species, mainly *Juniperus* L sp. in areas where pine forest is less thick and there is only a thin intermediate layer with non-evergreen tree and shrub species. *Pinus nigra* is a long-lived frugal species able to adapt ecologically and physiologically to very different environmental conditions; it colonizes various ecological niches and plays a leading role in different stages within forest succession. *Pinus nigra* forests occurs in eastern Italian, Austrian and Slovenian Alps and Apennines (EC, 2007). *Pinus nigra* is adapted to many soil types and topographic conditions. It is a long-lived species, with medium to fast growth. Pines mature at about 80 years old, commonly developing a flat, round, or spreading crown (Van Haverbeke, 1990). *Pinus nigra* seed viability is very short on the ground. In addition, seed predators further decrease its low survival capacity. In winter, at the beginning of dispersal period, rodents are the main seed predators, while in spring the ants are the major ones (Zaghi, 2008). This high predation rate makes the probability of *P. nigra* seeds germinating in autumn almost zero (Zaghi, 2008). Characteristic species that coexist with *Pinus nigra* in Alpino-Apennine forests are: *Fagus sylvatica*, *Fraxinus ornus*, *Ostrya carpinifolia, Picea abies, Pinus sylvestris, Quercus pubescens, Sorbus aria* (Ellmauer, 2005; Zaghi, 2008).

1.3. *Pinus laricio* forests in Calabria

Pinus laricio ssp. *calabrica* forests occur in southern Italy on the Sila and Aspromonte massif (Fig. 2), and Etna mountain (EC, 2007). They are found mainly on siliceous and poorly developed soils, with sparse rock outcrops, between 1.200 and 1.800 m a.s.l. (Minelli, 2007). Pinewoods are characterised by a well-developed tree layer, often containing old specimens (up to 50 cm and 90 cm in diameter in Sila). In contrast, the understory has very few species. As pedogenesis proceeds, mature-site native forest species (beech, downy oak) colonize microenvironments with a more developed soil profile (EEA, 2006).

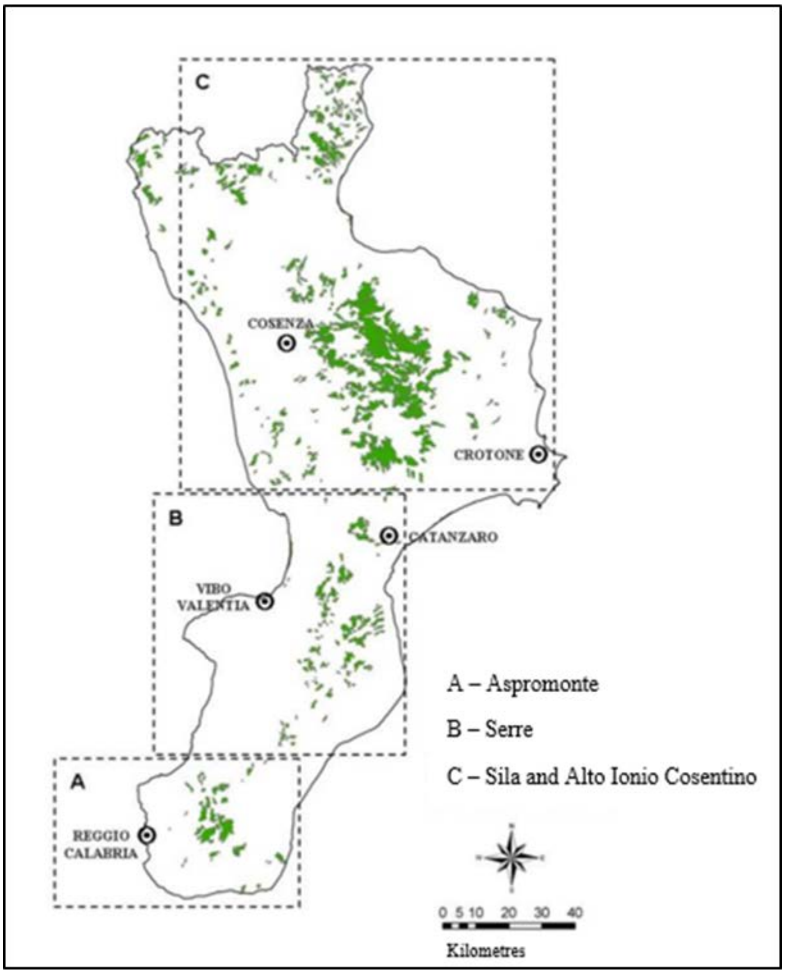

Figure 2. Green colour shows the distribution of pine forests in three calabrian areas: Aspromonte (A), Serre (B) and Sila (C) mountains. Source: CORINE Land Cover (CLC).

A recent study, based on molecular analyses, demonstrated that *Pinus laricio* ssp. *calabrica* derives from the Spanish black pine and not from the Austrian pine as believed until now (Zanghi, 2008). *Pinus laricio* plays an important role in soil development on substrates that are difficult for plants to colonize, as in the case of dolomites. Under the environmental conditions in which these forests grow, soil processes are very slow. The presence of good soil conditions is crucial to ensuring sound forest regeneration and development. Some forestry treatments, such as clear cutting in steep slopes, can result in the loss or degradation of forest land, leading to regressive sequential stages and significantly reducing the pines “potential” for growth and their regeneration capacity (Zaghi, 2008).

1.4. Management of Mediterranean forest pine

The management of the Mediterranean forests should aim at the improvement of the ecosystem functions through the conservation of biological and functional diversity (De Dios, 2006). The high variability of the (sub)-Mediterranean pine forests with the endemic *Pinus nigra* and the diverse ecological conditions in which it occurs determine differences in productivity and regeneration capacity that should be taken into account together with their sustainable management. Also species richness is an important parameter to be considered (Alejano and Martínez, 2003).

The concept of sustainability, although cumbersome to actually define, has been accepted globally by forest resource managers and policy makers as an appropriate medium for the fight against the fast eroding resource base vis-a-vis its multi-facet functions. Use of forest resources and assessment of progress towards its sustainability, over the years by conventional forest management practices, has not yielded successful results. Outcome of credible reports such as the forest resources assessment of the Food and Agriculture Organization (FAO, 2005), International Tropical Timber Organization's report (ITTO, 2005) on tropical forest management and the Millennium Ecosystem Assessment (MEA, 2005) all indicate that global forest cover continue to dwindle on a daily bases at a rate of 25.000 hectares with the magnitude weighing more on tropical forests. Sustainable forest management (SFM) is recognized by the United Nations Conference on Environment and Development (UNCED) as the most important contribution the forestry sector can inject into sustainable development objectives of any country (UNCED, 1992). The role of SFM as a key element in sustainable development equations has already been identified and enshrined by UNCED under its forest policy provisions.

The study agrees with the underlying principles of the new forest management paradigm which sees the wisdom in the collective use of criteria and indicators as a tool in tracking performance progress (FAO, 2001) towards a nationwide sustainable forest management program. It also subscribes to the numerous proliferations of conventions and treaties on biodiversity, conservation and environmental care with the understanding that arriving at appropriate criteria and indicators will present a formidable and an efficient system scientifically based, to give a picture of the forest (Cook *et al*., 1999). Soil and vegetation have a reciprocal relationship. Fertility and quality of soils encourage plant growth by providing nutrients, acting as water holding tank, and serving as substrate to which plants anchor their roots. In return, forestry vegetation covers prevent soil degradation and desertification by stabilizing soil, maintaining water and nutrient cycling, and reducing water and wind erosion. Although there is an integrated culture that recognizes the importance of soil in the forest sector, there is an urgent need to monitor its "status" so that forestry practices adopted may be changed, as soon as negative and irreversible changes begin to occur. Unsustainable forest management is able to modify the physical (Wang *et al*., 2006) and biological (Kennedy and Papendick, 1995) soil properties, altering the nutrient cycles in soil (Kong *et al*., 2006), and decreasing the amount of organic matter, causing in turn an increase in erosion process (Brandt and Thornes, 1996; Drake and Vafeidis, 2004). In addition, incorrect forestry practices can cause loss of biodiversity resulting in a simplification of the soil system which in turn reduce the growth and the stability of the plants. The health status of a soil is evaluated through the analysis of its biological and biochemical properties, as these properties respond quickly to environmental changes and to pressure of external agents (Scotti *et al*., 2013; Muscolo *et al*., 2014). One of the current challenges of forest management is thus to develop and improve forest management systems in order to maintain or enhance soil quality. The impacts of management on soil quality and subsequent tree growth can be positive, neutral or negative; the direction and magnitude of the impact depends on the specific management practice soil physical, chemical and biological properties. In order to understand and predict the impacts of management, the factors limiting productivity on each specific site must be understood.

Management to change an ecosystem process in support of one regulating ecosystem service can either provide co-benefits to other services or require tradeoffs (Robinson *et al*., 2013; Dominati *et al*., 2010). Forest management acts through coherent sets of silvicultural operations at the stand level to achieve specific objectives. This implies purposeful manipulation of one or more key variables, e.g., tree species composition, stand density and age structure, and stand edges, or other site attributes that result in a changed ecosystem (Duncker *et al*., 2012). A use which respects the natural cycle of renewal resources (ecological sustainability) is the essential prerogative of sustainable management of natural resources and at the same time ensuring fairness, making it possible to use, existing and future resources, by multiple beneficiaries (economic and social sustainability). Accordingly, various models of forest management systems have evolved over time. They range from “exploitive” to “back-to-nature”, with all intended to satisfy the diverse human demands on the forest (Seymour and Hunter, 1999; Woods, 2001; Gamborg and Larsen, 2003). On recent decades, increasing emphasis has been placed on designing silvicultural treatments that mimic natural disturbance to restore forests in a natural way, preserving biodiversity and ecosystem function while optimizing harvest and yield (Lindenmayer and Franklin, 2002; Abrari Vajari *et al*., 2012).

1.5. Soil quality

An important aim of modern silviculture is the preservation of the environmental and natural resources in the long-term. The quality of the environment can be assessed by the quality of its main tasks, such as air, water site and soil.

Recently, the need for assessing soil properties has expanded because of growing public interest in determining consequences of management practices on soil quality relative to the sustainability of forest ecosystem functions in addition to plant productivity. The forestry community frequently emphasize soils as simply “part of the forest”, as opposed to a separate resource in its own right, and have not generally invoked the concept of soil quality as a component of sustainable forestry (Burger and Kelting, 1998). However, the concept of site quality that includes climate, geologic, and topographic factors as well as soil, is well understood and widely used by foresters. Site quality is usually indexed with the height of the tree canopy at a given age (Carmean, 1975). By definition, soil quality, as part of site quality, is expressed often in terms of tree growth or biomass production, which was only a part of several important functions of soil (i.e. regulating water quality and quantity, carbon sequestration, remediation of human and animal wastes, regulating energy flow). Furthermore, the contribution of soil to site quality is confounded by the interactions of other site factors, tree breeding, and silvicultural practices that affect soil functioning ecosystem. The concept of soil quality (SQ) includes assessment of soil properties and processes as they relate to ability of soil to function effectively as a component of a healthy ecosystem. Soil quality, like site quality or forest productivity, is a value-based concept related to the objectives of ecosystem management, and hence will be management and ecosystem-dependent. Foresters, by comparison, have traditionally measured soil productivity using tree growth or wood yield (Ford, 1983). On the other hand, agronomists and farmers most often define soil quality as the suitability of a soil to function for different uses (Warkentin, 1995; Schoenholtz *et al.*, 2000), which illustrates a broader concept, and the fact that agriculture has traditionally been more soil-interactive than silviculture. In short, soil quality includes a measure of a soil’s ability to produce plant biomass, maintain animal health and production, recycle nutrients, store carbon, partition rainfall, buffer anthropogenic acidity, remediate added animal and human wastes, and regulate energy transformations. Evaluating and measuring the quality of the soil resource was prompted by this increasing awareness that soil serves multiple functions in maintaining worldwide environmental quality (Doran and Parkin, 1994). Evaluation would be based upon soil function and soil indicators that measure function. Soil function would be defined in terms of physical, chemical, and biological properties and processes and measured against some definable standard to determine whether a soil is being improved or degraded (Karlen *et al.*, 1997). Soils are being degraded worldwide through processes of erosion, anaerobiosis, salinization, compaction and hard-setting, organic matter depletion, and nutrient imbalance and uncorrected forest practices. Measuring soil quality serves to evaluate in short term the soil's capacity to produce safe and nutritious food, enhance human and animal health, and overcome degradative processes (Papendick and Parr, 1992). Therefore, it is imperative to develop sensitive and precise methods to assess soil's conditions, how soil responds to management, and its resilience to stresses imposed by cultural and management practices. Because of, soil is the foundation of the forest system, forest management must be based on a holistic understanding of forest soil ecosystem functioning, to avoid silvicultural practices that affect forest soil quality. Measuring and monitoring parts of the forest that change in response to silviculture is necessary for the process of adaptive forest management for a sustainable forestry. The rationale for managing the forest soil resource, especially in plantation systems, is the same as that used for managing the soil resource in agroecosystems. Forest management, on a sustainable basis, has to consistently include maintenance or enhancement of forest soil quality as a criterion of sustainability (Schoenholtz *et al*., 2000).

Soil quality may deteriorate quickly due to bad land management, stabilize with time under proper management, vary slightly because of the weather and growing conditions, and improve in the long time for the supply of organic matter. The assessment of soil quality has many implications, such as the attitude of soil to a specific use, and the assessment of the human activities and management practices carried out on it. To ensure a correct approach, it is firstly necessary to make the distinction between intrinsic and dynamic quality (Karlen *et al*., 2001). The first depends on the intrinsic characteristics of a soil, defined as those that are not modifiable in the short term. The dynamic quality is rather determined by all properties that change in the short term. For example, when a study aims to assess the impact on soil quality of specific techniques the intrinsic properties of soil must be considered. For deeper understanding of the soil quality it is not enough to assess only their chemical characteristics (texture, cation exchange capacity and pH), which are mostly determined by the characteristics of the parent rock and the pedogenic processes that formed that land. It is more appropriate, instead, to focus the attention on the organic components of soil, assessing the amount (total content of a soil) and quality (properties and relative importance of different fractions) or the properties of its vital components (meso and microorganisms). Two concepts are useful to assess changes in soil quality: the resistance to degradation, and the resilience, that is the ability of a soil to recover its functional and structural integrity after an external disturbance (Griffith *et al*., 2001). Soil quality can be assessed by estimating changes in its characteristics as defined by indicators capable of being synthesized in harmonious, complex and interrelated phenomena.

1.5.1 Soil quality indicators

Soil quality and soil health are very often used interchangeably (Doran and Safley, 1997). "Soil health" was define as the continued capacity of soil to function as a vital living system, within natural or managed ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health (Doran *et al*., 1996; Doran and Zeiss, 2000; Karlen *et al*., 2001). Soil quality is the end product of soil degradative or conserving processes and is a combination of the physical, chemical and biological properties that are essential for plant growth, regulation and partitioning of surface to ground water, and buffering, detoxifying and scrubbing of hazardous chemicals. Soil quality is rather dynamic and can affect the sustainability and productivity of land use proposing as an integrative indicator of environmental quality (National Research Council, 1993; Monreal *et al*., 1998), food security (Lal, 1999) and economic viability (Hillel, 1991). Soil quality indicators should well correlate with ecosystem functions such as C and N cycling (Visser and Parkinson, 1992), should integrate soil physical, chemical, and biological properties and processes, and serve as basic inputs, needed for the estimation of soil properties or functions which are more difficult to measure directly. Additionally soil quality indicators should be measurable by as many users as possible and not limited to a select group of research scientists; should be applicable to field conditions, and they should describe the major ecological processes in soil ensuring that measurements reflect conditions as they exist in the field under a given management system. Soil quality indicators have to be sensitive to variations in management and climate; and be components of existing soil data bases where possible (Doran and Parkin, 1994). Quantifying these variables through long-term monitoring may lead to an understanding about the effects of land management practices and natural or human-caused disturbances on the soil component of ecosystems (Knoepp *et al*., 2000). It is often difficult to separate soil functions into chemical, physical, and biological processes because of the dynamic, interactive nature of these processes (Schoenholtz *et al*., 2000). Because of these interactions, soil indices are extremely variable. Many soil chemical properties directly influence microbiological processes. These processes, together with soil physical-chemical processes, determine the capacity of soils to hold and supply nutrients cycles (including carbon), and the movement and availability of water. Therefore, soil chemical indicators are used mostly in the context of nutrient relations and may also be referred to as "indices of nutrient supply" (Powers *et al*., 1998). By contrast, biological and biochemical properties, including soil respiration, microbial biomass and the activities of soil enzymes, are most useful for detecting the deterioration of soil quality (Visser and Parkinson, 1992) because of their importance in cycling of organic matter and regulating active nutrient pools in soils (Caravaca and Roldán, 2003). The identification of biological indicators of soil quality is important because soil quality is strongly influenced by microorganism mediated processes. nutrient capacity, aggregate stability, whereby the key is to identifying those components that rapidly respond to changes in soil quality (Doran and Parkin, 1994). Nevertheless, there is the problem of knowing which indicator responds to a specific soil treatment or contaminant. Therefore, the use of multiple biological and biochemical properties is often suggested (Ros *et al*., 2006). General biochemical properties such as microbial biomass carbon (Brookes, 1995), as well as specific biochemical properties such as hydrolytic soil enzymes related to C, N and P cycles (Nannipieri *et al*., 1990) are suggested. One of the limitation in using biological assays for soil quality indication is the lack of standard methodologies. Considerable variation exists among assay procedures used by various researchers, making actual activity comparison between sites difficult. It was thus emphasized that if bioassays have to be used as soil quality indicators, soil sample pretreatment, assay procedures and units of measurement have to be standardized (Dick, 1994).

1.5.2 Soil organic matter

Organic matter (OM) is one of the main factors of soil fertility; it has significant influence on all aspects of life in soil, on its evolution and on the organisms living there. It has a crucial role in soil structure due to its contributes to the aggregation of mineral particles, promoting a correct balance among the components of soil, air, water and solid phase, which is essential for the containment measures for erosion, compaction and crusting. OM is the source of energy for all soil microorganisms. This is very important for their role in the demolition and transformation of organic materials and, indirectly, in the cycle of nutrients, useful for the growth of plants. OM explicates a defensive action against plants for several reasons: the balanced intake of nutrients that strengthen the plant; the diversification and multiplication of soil microorganisms that depend on the quality and quantity of OM (Vizioli, 1997). The contribution of OM to plant nutrition is direct, as a sink of nutrients, and indirectly, by multiple actions, such as availability and solubility of elements and functions of plant root absorption. As carbon is the most important element for living organisms, OM is the main source of CO_2 in nature. OM is, then, an abundant reserve of all the nutrients that may be fully assimilated by plants. As carbon stored in soils accounts for over two -thirds of the C in forest ecosystems (Dixon *et al*., 1994) and is more stable than that stored in living plant biomass (Vesterdal *et al*., 2002; Chen *et al*., 2005; Laganière *et al*., 2010), a better knowledge of dynamics of soil organic carbon (SOC) stock following forest management is needed. The microbial activity is strongly influenced by the presence of OM in soils. The concomitant presence of microorganisms and OM explicates a positive action against the solubilization of some poorly soluble, mineral elements. An usual case in many soils of central Italy, characterized by alkaline pH and presence of calcium, is the lack of availability of important elements such as phosphorus and potassium. The reaction between carbon dioxide, produced by respiration of microorganisms that attack the OM, with soil water and limestone shifts the balance toward calcium carbonate, which is made less stable. A greater amount of OM in soil increases the microbial activity, and then the production of carbon dioxide thus shifting to right the reaction and favouring the solubility of some nutrients. By contrast, a smaller amount of OM leads to less microbial activity and more likely to the insolubility of some elements. Because of OM important functions, it is clear that its decrease can have a significant impact on the fertility and quality of soil (Costantini, 1995). Therefore, it is essential to maintain an adequate level of OM through a sustainable soil management. For these reasons, the understanding of the mechanisms involved in the interaction between OM and the other chemical and biological soil properties in the environment is of great importance.

1.5.3 Soil enzymes

Nutrient cycling in soils involves biochemical, chemical and physico-chemical reactions, with biochemical processes being mediated by microorganisms, plant roots, and soil organism. It is well known that all biochemical reactions are catalysed by enzymes, which are proteins that act as catalysts without undergoing permanent alteration and causing chemical reactions to proceed at faster rates. In addition, they are specific for the types of chemical reactions in which they participate (Tabatabai, 1994). Burns (1982) classified soil enzymes according to their location in soil. Three main enzyme categories (termed biotic enzymes) are associated with viable proliferating cells. They are located: intracellularly in cell cytoplasm, in the periplasmic space, and at the outer cell surfaces. Enzymes in the soil solution are generally short-lived because they are readily inactivated by physical adsorption, denaturation or degradation (Burns, 1986). The remaining categories are broadly characterized as abiontic (Skujinš, 1976). Abiontic enzymes are those exclusive of live cells that include enzymes excreted by living cells during cell growth and division from extant or lysed cells but whose original functional location was on or within the cell. Additionally, abiontic enzymes can exist as stabilized enzymes in two locations: adsorbed to internal or external clay surfaces, and complexed with humic colloids through adsorption, entrapment or copolymerization during humic matter genesis (Boyd and Mortland, 1990). Enzymes associated with humic substances and, to a lesser extent, with clay particulates are protected against thermal denaturation, proteolysis, dehydration or decomposition. They are part of a persistent extracellular enzyme pool that is independent of the existing microbiota (Burns, 1982; Sarkar and Burns, 1984; Miller and Dick, 1995). The humic-enzyme fractions retain the original properties of the enzymes (Busto and Perez-Mateos, 1995) as stable enzyme-organic matter complexes and they were found to allow diffusion of substrates to the active enzyme site (Burns, 1982). Therefore, soil can be considered as a sink and source of indigenous and persistent enzymatic capacity, which is independent of current or recent microbial and plant activity (Galstian, 1974; Burns, 1986; Lähdesmäki and Piispanen, 1992; Busto and Perez-Mateos, 1995). Moreover, the enzymatic activity of a soil is conditioned by land use history, since enzymes are produced by living organisms which contribute to the biological soil formation. The activity and stability of enzymes in soil is regulated by pH (Frankenberger and Johanson, 1983; Trasar-Cepeda and Gil-Sotres, 1987; Dick *et al*., 1988), microbial biomass (Häussling and Marschner, 1989; Saffigna *et al*., 1989; Carter, 1991; Srivastava and Singh, 1991), vegetation (Juma and Tabatabai, 1978; Harrison, 1983; Perucci *et al*., 1984; Helal and Sauerbeck, 1987; Tarafdar and Jungk, 1987), soil and crop management practices (Perucci and Scarponi, 1985; Beck, 1990; Martens *et al*., 1992; Kandeler and Eder, 1993), soil OM (Juma and Tabatabai, 1978; Chhonkar and Tarafdar, 1984; Sparling *et al*., 1986), and soil moisture content (Harrison, 1983; West *et al*., 1988a,b). Enzyme activities are important indicators of soil biological activity because they are involved in the dynamics of soil nutrient cycling and energy transfer. Indeed, they reflect the intensity and direction of biochemical processes in the soil matrix. Hence, their activity indicates the biological capacity of a soil to carry out the biochemical processes which are important to maintaining the soil fertility (Galstian, 1974; Dkhar and Mishra, 1983; Burns, 1986; Garcia *et al*., 1994), as soil fertility depends not only on the nutrient status and availability but also on the turnover of N, P and other nutrients (Lopez-Hernandez *et al*., 1989). Actually, enzymatic processes are closely associated with soil fertility as they mediate the conversion of unavailable forms of nutrients to forms that are readily assimilable by plants and microbial biomass (Sarathchandra *et al*., 1984; Dick *et al*., 1988; Sarkar *et al*., 1989; Dick, 1992; Martens *et al*., 1992; Sinsabaugh, 1994). Soil enzymes also participate in the decomposition and synthesis of organic substances and are important for the formation of recalcitrant organic molecules (Galstian, 1974; Martens *et al*., 1992). As enzymes do not react readily to environmental changes respect to soil microbial biomass, their activity is a more stable indicator of biological processes (Galstian, 1974). In soil, the most common enzymes belong essentially to four classes: oxidoreductases, lyases, hydrolases and transferases. Among them, hydrolases and oxidoreductases are the most common in soil.

2. STATE OF THE ART

2.1 History of forest management

The forest management has evolved over time according to the interests and needs of human population with respect to the use of natural resources.

During the whole of the Middle Ages, forests were very much exploited, as a wood reserve, since wood was used as firewood or to build different items, such as barrels, tools, hurdles for enclosures, fencing, boats and ships. Forests were also seen as potential arable land, after being cleared of trees and shrubs (Paletto *et al*., 2008). Across the centuries these unplanned and more-and- more intensive activities seriously endangered the survival of forests and forced local communities to elaborate specific laws to limit pasture rights, tree-cutting, and land clearance (Naso, 1988).

In the Modern age (the late 15th century and 16th, 17th centuries) the multi-products forest (silvopastoral economics) came to be replaced with the traditional agricultural forest. Medieval forest management, based on selective cutting and uneven-aged stands, (Agnoletti, 2006) was replaced by tillage and the intensification of clearcutting (Thomas, 1983).

During the Modern age, particularly in the plain areas of Europe, it is assisted to a progressive increase of the agriculture to damage of the other land uses. Specifically, in those years we assist to the increasing elimination of uncultivated areas and to the reduction of the extent of forests (deforestation) (Le Master and Schmithüsen, 2006) and swamps. These events, that were important in the development of agricultural economies, created limitation of silvopastoral economics typical of the collective communities of the Middle ages (limitation in the rights of hunting and product collection), and increase of hydrogeological instability (the protection function of forest) in mountain areas, and flooding in the plain zones (e.g.: flooding in Italy of the Tevere river in the years 1495 and 1530) (Paletto, 2008).

In conclusion man bends nature to his own wishes, and consequently, the management of forests has been decided only on the basis on the human needs (Thomas, 1983). The end of Modern Age coincides with the advent of the Industrial Revolution, during this period new social needs and demands arose and, as consequence, the management of natural resources changed radically. The industrial revolution modified deeply the economy of European society, production increased considerably and the technological efficiency improved significantly. These factors caused an increase in the demand not only of raw materials (wood, cotton, iron, coal) but also of roundwood for construction, fuelwood for domestic use, pole wood, charcoal and tannin (Paletto, 2008).

In this context the work of Georg Ludwig Hartig and Heinrich von Cotta developed the concept of “modern” silviculture. The old mixed forests were replaced with monospecific forests managed with new techniques (fertilisation, selected exotic species, artificial regeneration), and harvested by clear cutting (Vos, 1996). In the Mediterranean regions coppice using short rotations of 3 to 10 years replaced the high forest (Agnoletti, 2002). The economic approach to forestry management was imperative, the need to achieve maximum forest revenue has concurred to the affirmation of timber market, industrial silviculture and different forestry branches (wood technology, forest economics, forest mensuration and assessment (Agnoletti, 2006). After the industrial revolution the social perception of the forest resource changed. In the new cultural context two main principles in forest management emerged: multifunctionality and sustainability. The concept of forest multifunctionality was created in Germany in 1953 with the elaboration of the "Theory of Forestry Function" published in the "Forstwirtschaftspolitik" by professor Viktor Dieterich of the University of Munich. In this theory, the concept of multiple-use, widespread in North America (Canada and United States), was developed and widened through a less anthropocentric vision where the functions had an intrinsic importance (vitality and health of ecosystem).

The modern formulation of the concept of sustainability was elaborated in the report of the World Commission on Environment and Development (WCED, 1987), better known as the Brundtland Report (1987). In this report sustainability was defined as: "Meeting the needs of the present generation without compromising the ability of future generations to meet their needs". This definition represents the conceptual basis of a new forest management paradigm that changed radically the traditional forest management in Sustainable Forest Management (SFM). Sustainable forest management (SFM) is currently widely accepted as the overriding objective for forest policy and practice. SFM is a concept developed over time, for the increasing needs to find new strategies and methods for a rational management of forest resources (Paletto, 2008).

The first international definition of SFM appeared in the early 1990s in a number of important documents: Agenda 21, the Rio de Janeiro Declaration on Environment and Development and the Statement of Principles for the Sustainable Management of Forests. This was adopted by many Governments at the United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro (Brazil, 3-14 June 1992). The term, sustainable forest management asserts that: "Forest resources and forest lands should be sustainably managed to meet the social, economic, ecological, cultural and spiritual human needs of present and future generations. These needs are for forest products and services, such as wood and wood products, water, soil, food, fodder, medicine, fuel, shelter, employment, recreation, habitats for wildlife, landscape diversity, carbon sinks and reservoirs, and for other forest products". In Europe the first definition of SFM was worked out during the second Ministerial Conference on the Protection of Forests in Europe (MCPFE) in Helsinki (1993): "sustainable management means the stewardship and use of forests and forest lands in a way, and at a rate, that maintains their biodiversity, productivity, regeneration capacity, vitality and their potential to fulfill, now and in the future, relevant ecological, economic and social functions, at local, national, and global levels, and that does not cause damage to other ecosystems" (Resolution H1 point D, MCPFE 2000). The final evolution of forest management doctrine is Ecosystem Management (EM). EM "integrates scientific knowledge of ecological relationships within a complex sociopolitical and values framework toward the general goal of protecting native ecosystem integrity over the long- term" (Grumbine, 1994; Paletto, 2008).

The best management had to maintain a good level of health and vitality of the forest ecosystem (Wolynski, 1998). In order to achieve these objectives, managers must avoid traumatic actions against the forest ecosystem (for examples: clear cutting on wide surface, artificial regeneration with exotic species, etc.). The four major criteria for the sustainability of a forest ecosystem are: maintenance of biological diversity, viability of ecological processes, productivity of soil, water and air and the ability to provide for sustained human use (Georgia Forestry Commission, 2000). Contemporary sustainable forest management aims to ensure that goods and services derived from forests meet present-day needs while at the same time securing the forests' continued viability and contributions to long-term development. As such, forest management itself requires prudent management in order to conserve essential ecosystem services such as soil fertility, water quality, as well as, carbon sequestration, maintenance of biodiversity, and recreational values. One of the most important questions for the future is how to manage the forest for timber production while conserving or improving the other important ecosystem services. (Paletto, 2008; Duncker *et al*,. 2012).

2.2 *Pinus nigra* forest management

Most forests of *Pinus nigra* occur in mountainous areas between 1000 and 1500 m a.s.l. As a result, its populations are fragmented and exhibit high morphological, physiological and ecological variability. Some *Pinus nigra* forests are or were managed for timber production, which usually results in quite homogeneous stands in terms of age and size of the trees. Some of these exploited forests are very far from the typical structure of a mature forest. Considering that *Pinus nigra* is a very long-lived species which can attain over thousand years, with individuals of up to 2 m diameter and over 30 m height, current stands are usually well below their natural longevity, being managed with a rotation age that does not exceed 150 years, where no large trees can be normally found. It is recommended to find a balance between production objectives and conservation needs and try to reach in some areas a similar status to that found in non-intervened forests, whose dynamics depends mainly on natural perturbations. Proper management of (sub)-Mediterranean *Pinus nigra* with endemic black pines should try to maintain irregular structures with trees of different ages (MATT, 2004), including very old specimens, in order to secure the kind of broad genetic variability that allows the forest to survive the various natural disturbances and environmental stresses of the continental sub-Mediterranean climate. Good coverage of associated species should also be encouraged. Suitable management should also promote a closed structure with a well developed canopy, which offer suitable microclimatic conditions (shade and humidity) for regeneration. It is also recommended to maintain a number of old trees as well as an appropriate amount of dead wood, which play an important role in the conservation of biodiversity. Some particular recommendations that may be applied under certain conditions are presented below. In general regeneration of forests composed by long-lived species can be reached with clear cuttings, which mimic the natural disturbances to the ecosystem (e.g. fires) (De Dios, 2006; Zaghi, 2008). In the state forests of Cyprus, for instance, the Ministry of Agriculture, Natural Resources and Environment encourages the natural regeneration of uneven-aged stands by the repeated removal at relatively short intervals (felling cycle of 10-15 years) of the oldest and largest *P. nigra* subsp. *pallasiana* trees, either as single, scattered individuals or in small groups, with the aim of preserving the naturalness of the forest. Rules for marking the plants of *P. nigra* to be removed have been created. When no natural regeneration occurs in the stand within an acceptable time frame or when regeneration is unsatisfactory, creating poor and under-stocked stands, planting and direct seeding are carried out to hasten natural regeneration (Forest Department, 2006). In Corsica, to favour regeneration by seeds for *Pinus nigra* subsp. *laricio* var. *Corsicana*, when forest productivity is higher than 5-6 m^3/ha per year and the forest has accesses (tracks or cable), cuttings can be applied on trees 150 - 200 years old and rather large (70 cm on average in the best stations, 55 cm in the worst). When productivity is low, cuttings with an interval of 30-40 years can be made to encourage natural regeneration of *P. nigra*. This technique is the best option to manage small homogeneous areas (Bensettiti *et al*., 2001).

According to the results of the LIFE Nature project 03NAT/E/000059 “Habitats management in the Nord Este region, Murcia (Spain)”, clearing of competitive species (mainly *Pinus halepensis*) and pruning of trees can favour the regeneration of the *P. nigra* subsp. *salzmannii* forest. When natural regeneration continues to be poor, *P. nigra* can be planted after clearing of suitable areas, preparing holes and installing protective devices. Planting of black pines can be carried out with a density of 900 -1.200 plants/ha. Accompanying species should also be planted in order to increase biodiversity (Murcia Region, 2007). In Italy, the National Forestry Corp manages a seed bank of Italian autochthonous trees, among which the different subspecies of *P. nigra* are included. The seeds, of known origin, are used for the production of saplings utilized for planting programs (Zaghi, 2008.), promoting an irregular structure. In public properties of southern Italy, strip and patch clear cuttings were applied in the past in order to obtain stands with an even-aged structure over vast areas. In private properties, instead, *Pinus nigra* ssp. *laricio* stands have a complex structure that is the result of a particular form of selection cutting based on traditional local knowledge. Cuts of marked trees are repeated approximately every 20 years on the same section of the forest. Results show that stand structure consists of very small clusters (60-100 m^2) of trees in four distinct age classes which are the result of the natural regeneration occurring in the gaps opened by the periodic removal of the biggest trees in the stand. The volume of harvested trees is slightly less than the volume increment for the average cutting cycle (Ciancio *et al*., 2006). (Sub)-Mediterranean pine forests with endemic *Pinus nigra* in good conditions have around 10-20% coverage of associated species. To conserve some of these species, e.g. fir trees, which live less than *Pinus nigra*, the following forestry technique has proven reliable: each turn of cutting pine should be matched by two turns of the other species until they are deemed to be regenerating (Bensettiti *et al*., 2001).

The most common silvicultural treatments in Europe currently aim at maximizing the stand stability, facilitating the transition from pure stands to mixed stands with a stronger presence of native tree species (Mosandl and Kessner, 1999; Heitz and Hasenauer, 2000; Kenk and Guehne, 2001; Malcolm *et al.*, 2001; Kint *et al*., 2006). Black pine was usually planted in pure stands at a density of 2500 trees per hectare with a rotation period of approximately 90 years depending on the site characteristics. During the rotation period multiple thinning usually should be applied to facilitate the transition toward a mixed forest. The average stand age of Italian pine forest is 50 years; consequently, there is still a need for additional research to understand the most effective treatments required to convert these pure pine forests.

On privately owned forests and public land, the most common treatment is to clear cut in small patches to facilitate the natural regeneration of native species (e.g., *Acer pseudoplatanus* L., *Acer opalus* Mill., *Quercus cerris* L., *Fraxinus ornus* L., etc.).

Thinning is the most effective silvicultural treatment to enhance the ecological value of these stands. Pure pine stands reach canopy closure at an early age; therefore, it is important to conduct pre-commercial thinning when the plants have 30 years old, followed by additional thinning every 15 years (Cameron, 2002; Brüchert and Gardiner, 2006). However, multiple thinning are really expensive and the necessary treatments are not applied regularly. In these forests, the most common treatment is the thinning from below, which means removing from below the layer of the main canopy, only trees with phytosanitary issues or diseased stems. Cantiani *et al*., (2010) indicated that *Pinus nigra* can still benefit from late thinning (at the age of 45), thus quickly reaching crown closure when treatments are carried out on the dominant layer. This operation can cause an initial disruption in the crown coverage. Consequently, it is important to evaluate the vigor of the crown and the social status of each tree before proceeding with a silvicultural treatment similar to thinning from above. The most frequently used indicators are: (a) the height/DBH ratio (slenderness coefficient or taper); (b) the relative depth of the crown; (c) the crown projection; and (d) the crown eccentricity (Cantiani and Chiavetta, 2015).

La Fauci *et al*., (2006) recommend the presence of old trees, selective cuttings and the prolongation of the utilization cycles to favor the creation of multi-stratified structures and to increase the number of old and large trees. The Giganti della Sila and the Gallopane biogenetic state reserves (Italy) have the specific objective of conserving *Pinus nigra* forests and veteran trees of *Pinus laricio*. The reserves include uneven-aged high forests with individuals up to 200-250 years old and 50 veteran trees (six of which left on the ground as dead wood). The latter are over 350 years old, have diameters (at 1.30 m height) between 71 and 187 cm, height between 35 and 43 m, with a volume of wood of 30-62 m^3. The forests are left to natural evolution as the local conditions favour the permanence of *P. laricio*, which ages and regenerates abundantly without human interventions (Zaghi, 2008.). The importance of maintaining a certain volume of dead wood to ensure conservation of forest biodiversity and functionality has already been recognized at international level and in particular in the European Union forest policy. Up to a third of European forest species depend on the presence of dead wood (Dudley and Vallauri, 2004). According to Ammer (1991) and Vallauri *et al*. (2003), the amount of dead wood in pine forests in Germany and France ranged from 5-10 to 15 m^3/ha. It includes both standing dead trees and coarse woody debris. It is usually sufficient to leave decaying trees to natural evolution, but in certain cases it might be necessary to increase the volume of dead and decaying wood. The techniques that can be used differ greatly one from another (for an overview, consult Cavalli and Donini, 2003).

2.3 Effect of forest management on soil

Forest management can change the original composition of a forest through the removal and/or replacement of tree species, and by altering the age class structure, exporting biomass and modifying the proportion of dead wood (Paillet *et al*., 2010). Different forest management techniques can be used to conserve and use forests in a sustainable way. Thinning is a crucial practice in the forest ecosystem management (Couteaux *et al*,. 1995; Zak *et al*,. 2003), with significant influence on tree growth (Santa-Regina and Tarazona, 1995; 2001), forest community structure, species composition, habitat conditions and forest soil (Zhang *et al*., 2001) affecting its productivity and fertility status. It has also been shown that thinning influences the understory organisms (Bender *et al*., 1997; Tsui *et al*., 1998; Kerr, 1999) affecting soil ecosystem functioning (Bengtsson *et al*., 2000). The decreased forest canopy density generated by thinning, leads to improved lighting conditions and light intensity in forested stands, speeding directly up litter decomposition, resulting in increased soil humus content, which promoted the surface soil formation crumb structure and the soil's air permeability (Zhao *et al*., 2014). The properties of the soil affected by forest thinning are the result of changes in key microclimatic conditions such as light penetration, air movement and temperature (Ma *et al*., 2010; Wubet *et al*., 2012), microbial communities (Hu and Zhu, 1999) and biomass, root density, nutrient budgets and organic matter turnover (Ilyas, 2014). Zhang *et al*. (2001) demonstrated that soil microbial quantity, enzyme activity, total porosity, available nutrients and soil fertility were improved while soil bulk density was reduced, after 2 year thinning in *Cunninghamia Lanceolata*, *Pinus massoniana*, *Fokienia hodginsii*, *Cryptomeria fortunei* and *Schima superba.* Weak intensity thinning improved soil physiochemical properties. Medium or intensive thinning did not affect physicochemical soil condition in respect to the control stand (Chi *et al*., 2006). Soil enzyme activities including catalase, urease, alkaline phosphatase, invertase and polyphenol oxidase were instead improved (Guo *et al*., 2007). The 18 year thinning program in pine forests indicated that with enhanced thinning intensity, the forest soil total porosity, capillary moisture holding capacity, soil nutrient level and enzyme activities were significantly enhanced, demonstrating that forest thinning can improve soil quality (Yu *et al*., 2008). Thinning significantly improved soil alkaline phosphatase activity and other soil properties in *Platycladus orientalis* forests, especially in intensively thinned forests (Xu *et al*., 2008). In contrast, some researches showed that thinning did not significantly improve soil quality, even damaging soil structure. For instance, after 5 years of thinning in a pine forest of northeast Germany, soil microbial activity was not significantly improved and soil enzyme activities including xylanase, phenol oxidase and peroxidase in both the organic layer and mineral layer were not significantly different from the control stand (Maassen *et al*., 2006). Forest soil nutrients in Larch-fir forest stands did not significantly change under <40% thinning intensity, indicating that long-term effects of thinning were not significant (Wang *et al*., 2009). With a 7 years thinning program, the activities of dehydrogenase, catalase, urease and phosphatase in soil were significantly reduced but protease activity did not change (Garcia *et al*., 1997). In *Pinus massoniana* forests, thinning reduced soil structure stability, soil total porosity, soil moisture storage capacity and holding capacity, but increased soil volume quality (Jing and Xiao, 1998).

The contrasting results reported in literature suggest that the effect of thinning on soil properties depend on the method, time, intensity of thinning, forest types and other aspects (Li *et al*., 2003). Zhao *et al*. (2014) studied soil physiochemical properties of *Pinus tabulaeformis* plantations under, intensive (50%), medium (45%), and weak (33%) thinning intensity and the results showed that: organic matter content rose the highest was after 5 years in weak thinning. Furthermore, soil urease and catalase contents were increased by medium thinning and intensive thinning. Following 5 years of different intensity thinning on *P. tabulaeform* forested stands intensive thinning (50%) led to the greatest improvement of the forests soil physiochemical properties.

Soils are also habitats for a wide range of organisms, involved in soil ecosystem functioning (Deacon, 2009), that can shaped by a number of biotic and abiotic factors (Goldmann *et al*., 2015). Bacteria, fungi, and other soil microorganisms play important roles in the ecological processes and nutrient dynamics of forest ecosystems (Delvasto *et al*., 2006; Levy-Booth *et al*., 2010). Fungi account for the majority of the microbial biomass in forest soil and convert recalcitrant organic material into forms useable by other organisms (Dighton, 2003).

Because of their close associations with plants, fungi show particularly high sensitivity to shifts in vegetation (Lauber *et al*., 2008). Trees, as the main plants in forest ecosystems, affect soil fungi passively by shading the ground and by regulating soil temperature and moisture as well as by influencing understory vegetation (Gömöryová *et al*., 2013).

Most studies that examined the influence of tree thinning on these organisms have focused on the impact of this major disturbance on soil bacteria or ectomycorrhizal fungi (Shaw, 2003), and concluded that the effects of thinning on soil microorganisms appear to be complex. Thinning has been shown to significantly change soil microbial communities in a pine forest (Maassen, 2006), Jarrah (*Eucalyptus marginata*) forest (Cookson, 2008), and *Chamaecyparis formosensis* forest (Lin *et al*., 2011). In a study conducted by Lin *et al*. (2016) the thinned plots had significantly higher fungal counts than those of the controls.

Forest management practices can also affect ground dwelling arthropods and can affect the availability of prey for vertebrates (Jokimäki *et al*., 1998). Besides being major engineers and potential regulators of ecosystem conditions (Schowalter, 2000), soil micro-arthropods have been shown to respond sensitively to land management (Schowalter *et al*., 1986; Stork *et al*., 1997; Schowalter and Ganio, 1998; Parisi, 2005). Yi and Moldenke (2005) reported that thinning intensity was correlated with higher abundance and diversity of epigeic macro-arthropods collected by pitfall-trapping. This increase was correlated with a decrease in litter moisture during the dry-season. Studies of Yi and Moldenke (2005) showed that abundance and diversity of ground-dwelling arthropods were higher in heavy thinning and light thinning with gap treatments than control and light thinning treatments.

Reduction of tree density had also strong direct effects on micro arthropod populations and number, due to changes in microclimate such as availability of light, water and nutrients (Lorio, 1980; Schowalter *et al*., 1986; Walker *et al*., 1986; Amman *et al*., 1988; Tappeiner and Alaback, 1990;

McMillin and Wagner, 1993). In turn, changes in the amount of arthropods significantly affected forest productivity and nutrient cycling (Schowalter *et al*., 1986).

2.4 Effect of forest management on plant

Forest management acts through coherent sets of silvicultural operations at the stand level to achieve specific objectives. Therefore, shifts in forest system management practices can change tree species composition and richness, stand density and age structure, either slowly over a number of decades, or perhaps suddenly because of clear-felling or burning of old growth stands (Chazdon, 2008; Torras and Saura, 2008; Duncker *et al*., 2012). Management practices also increase the quality and quantity of merchantable timber and reduced damage from disease and fire (Zeide, 2001; Franklin *et al*., 2002; Frey *et al*., 2003; Skov *et al*., 2004; Gilmore and Palik, 2006; Ostaff *et al*., 2006). With thinning, poorly performing trees are selectively removed, leaving a vigorous stand, increasing the growth of standing trees, developing structural characteristics needed for wildlife habitats, and generally producing healthy and vigorous forests with less risk of insect infestation, destructive fire, and wind damage (Bradford and Palik, 2009, Demers *et al*., 2013; Harrington, 2001). In short, thinning is a tool for stand density control aimed to improve the growth of the remaining shafts (Grant *et al*., 2007) on a sustainable basis (e.g. Houtzagers, 2002; Tullus, 2002; Zeide, 2004), decreasing also tree mortality rate (Brissette *et al.*, 1999). The greatest difference between unmanaged and managed stands is the lower density of trees and the minor volume of large snags and logs in managed plantations (Spies and Cline, 1988; Spies *et al*., 1988; Spies, 1991; Spies and Franklin, 1991; Hunter, 1993).

Studies on coniferous tree species have shown that thinning of forest stands can result in increased tree growth, by apportioning the available soil water among fewer trees (Black *et al*., 1980; Morikawa *et al*., 1986; Aussenac and Granier, 1988). The growth of thinned stands of several species has been related to reduced soil water stress (Zahner, 1968), as a consequence of a reduction of both canopy interception and stand transpiration. Juodvalkis *et al*. (2005) in a study conducted in six tree species (ash, aspen, birch, oak, pine and spruce) showed the same trend in stands of all six investigated tree species, and faster expansion of crowns was recorded in stands thinned to a larger degree. To date, a number of studies had been carried out on crown dynamics of pine forest following thinning. The results demonstrated that in thinned stands residual trees aimed to occupy newly available space and enhanced their crown increment. This has been also observed in stands of pine (Toma, 1940; Georgievsky, 1957; Baldwin *et al*., 2000; Lockow, 2003).

Numerous reports (Hamilton, 1981; Lockow, 2003; Oyen, 2003; Canellas *et al*., 2004; Makinen and Isomaki, 2004a; 2004b; Varmola and Salminen, 2004; Sorg *et al*., 2016; Jackson *et al*., 2016) presented data on the effects of thinning on the diameter accretion of residual trees of many species from wide geographical ranges. Data on pine, spruce and oak in temperate stands of Europe clearly indicated that thinning promoted diameter breast height increment, especially in younger stands, and that increase in diameter breast height increment was positively correlated with thinning degree. A controversial but widespread timber harvesting technique (Bliss, 2000; Hansis, 1995) used in most fast-growing commercial forests, particularly conifer plantations, is the clearcutting (Kerr, 1999; Spellerberg and Sawyer, 1996). Concerns regarding this technique are related to its potentially detrimental effects on soil erosion (Iroumé *et al*., 2006), nutrient cycling (Prescott, 1997), wildlife habitat (Mannan and Meslow, 1984), alien invasions (Selmants and Knight, 2003), and aesthetics (Levine and Langenau, 1979), among others. Several authors (Sterba, 1988; Merganicová *et al*., 2005; Helmisaari *et al*., 2011) proved the negative effects of intensified forest management on productivity.

2.5 Objectives

Aim of this study was to evaluate the effects of three different silvicultural practices in a forest pine of Southern Italy to understand which management practice preserves soil fertility improving at the same time the growth and the quality of plants.

In this context, the effects of forest management on soil physical characteristics, amount of nutrients, organic matter trend, enzyme activities, pedofauna, soil microbial biomass C and microorganisms in terms of fungi, bacteria and actinomycetes colonies were monitored over three years, in two contrasting seasons (winter and summer) to evaluate if the effects of management on soil were also affected by climatic conditions (temperature and humidity).

The modifications in soil characteristics, in short term, were evaluated through the analysis of early warning indicators of changes in soil ecosystem functioning (soil microbial biomass carbon, fluorescein diacetate and dehydrogenase activity). Additionally, the effects of different silvicultural practices on pine accretion, was assessed by monitoring the slenderness coefficient (H/D ratio) as parameter of tree stability and wood density as parameter of timber quality.

3. MATERIAL AND METHODS

3.1 Study area

The study area was located in *Pinus laricio* stands on Aspromonte Mountain (Zervò, Calabria) (38° 24' 24" N; 16° 01' 82" E), at an elevation of 1100 m above sea level; and covers a surface of 45 hectares. The last forest utilization was carried out in 2011, by using a “selective” thinning system based on the number of plants removed: low thinning intensity (30% plants removed), high thinning intensity (60% plants removed), and clear cutting (no plants) (Figures 3, 4, 5, 6).

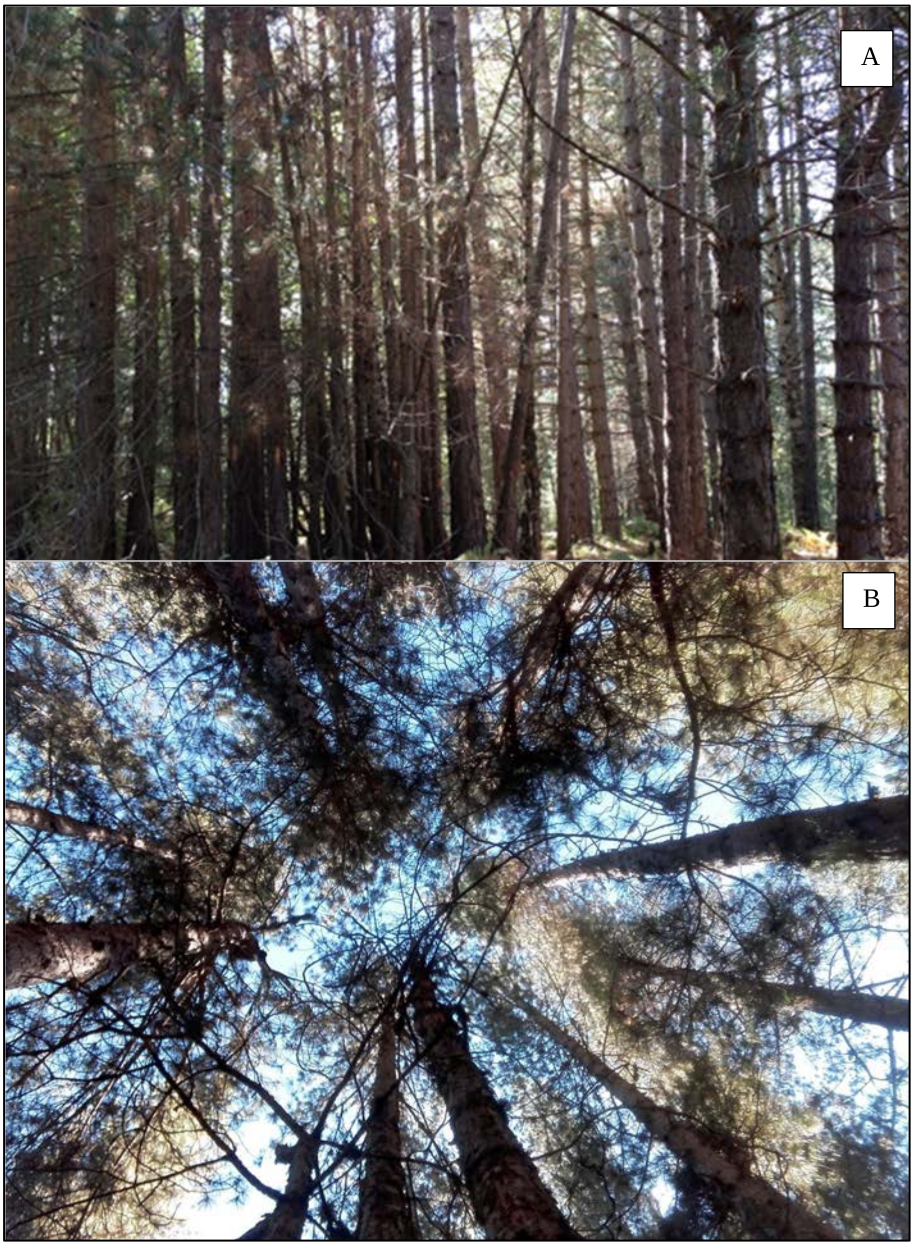

Figure 3. Absence of thinning shows the high density of trees (A) of *Pinus nigra* spp. *laricio* var. *calabrica* and relative coverage (B) in Aspromonte mountains (Zervò, RC, Italy).

Figure 4. Density of trees (A) and relative coverage (B) after 30% thinning in *Pinus nigra* spp. *laricio* var. *calabrica* forest, Aspromonte mountains (Zervò, RC, Italy).

Italy).

Figure 5. Density of trees (A) and relative coverage (B) after 60 % thinning in *Pinus nigra* spp. *laricio* var. *calabrica* forest, Aspromonte mountains (Zervò, RC, Italy).

Figure 6. Clear cut in *Pinus nigra* spp. *laricio* var. *calabrica* forest, Aspromonte mountains (Zervò, RC, Italy).

A typically Mediterranean climate prevailed in the study area, with an annual temperature about of 10 °C, an average temperature of the coldest month of 2-5 °C, and an average temperature of the warmest month of 17 °C. The annual rainfall is 1838 mm, with minimum precipitation in summer (Figure 7). Rainfalls are unevenly distributed over the year. According to Pavari's phytoclimatic classification (Pavari, 1959) the stands belong to the *Castanetum* zone.

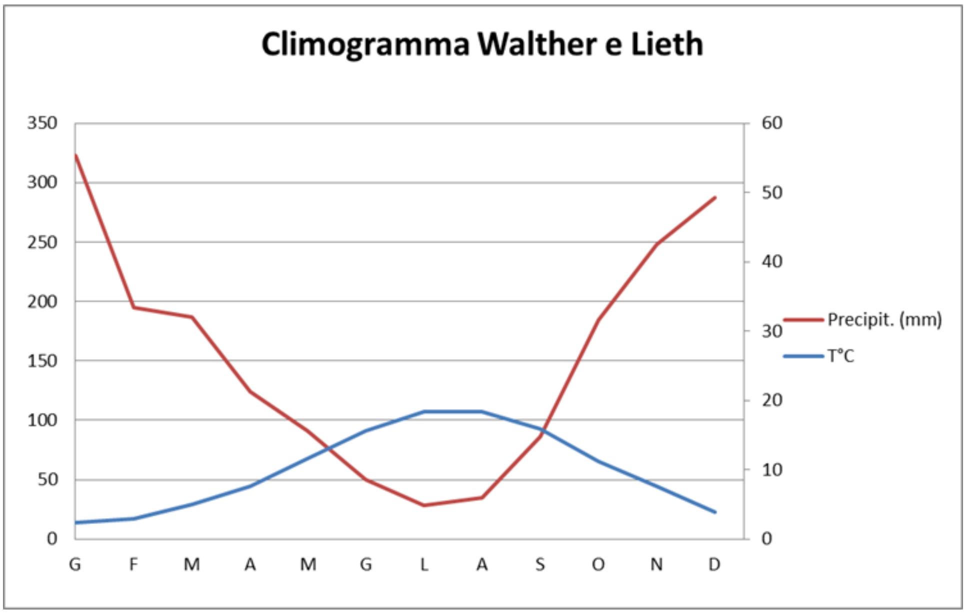

Figure 7. Ombrothermic diagram showing monthly mean air temperatures (in blue) and monthly mean precipitations (in red). Source: Meteorological station of S. Cristina d'Aspromonte (RC), Italy.

The soils developed from high rank metamorphic rocks, such as schist and biotitic gneisses, are Humic Cambisols (Sidari *et al*., 2008), with a xeric soil regime moisture.

The study area belonging to the Aspromonte National Park, consists mainly of *Pinus laricio*, *Pseudotsuga menziesii*, *Larix kaempferi*, *Pinus strobus*, and F*agus sylvatica* vegetation and in minor part of former farmland and pastures. The forest is managed by AFoR (Azienda Forestale Regionale) and before reforestation was cultivated with wheat and potatoes. The reforestation was carried out in the years 1960-70.

The forest was classified as I class for roughness, while the slope was included between I and II class, in agreement with terrain classification of UK Forestry Commission (1995) (Figure 8).

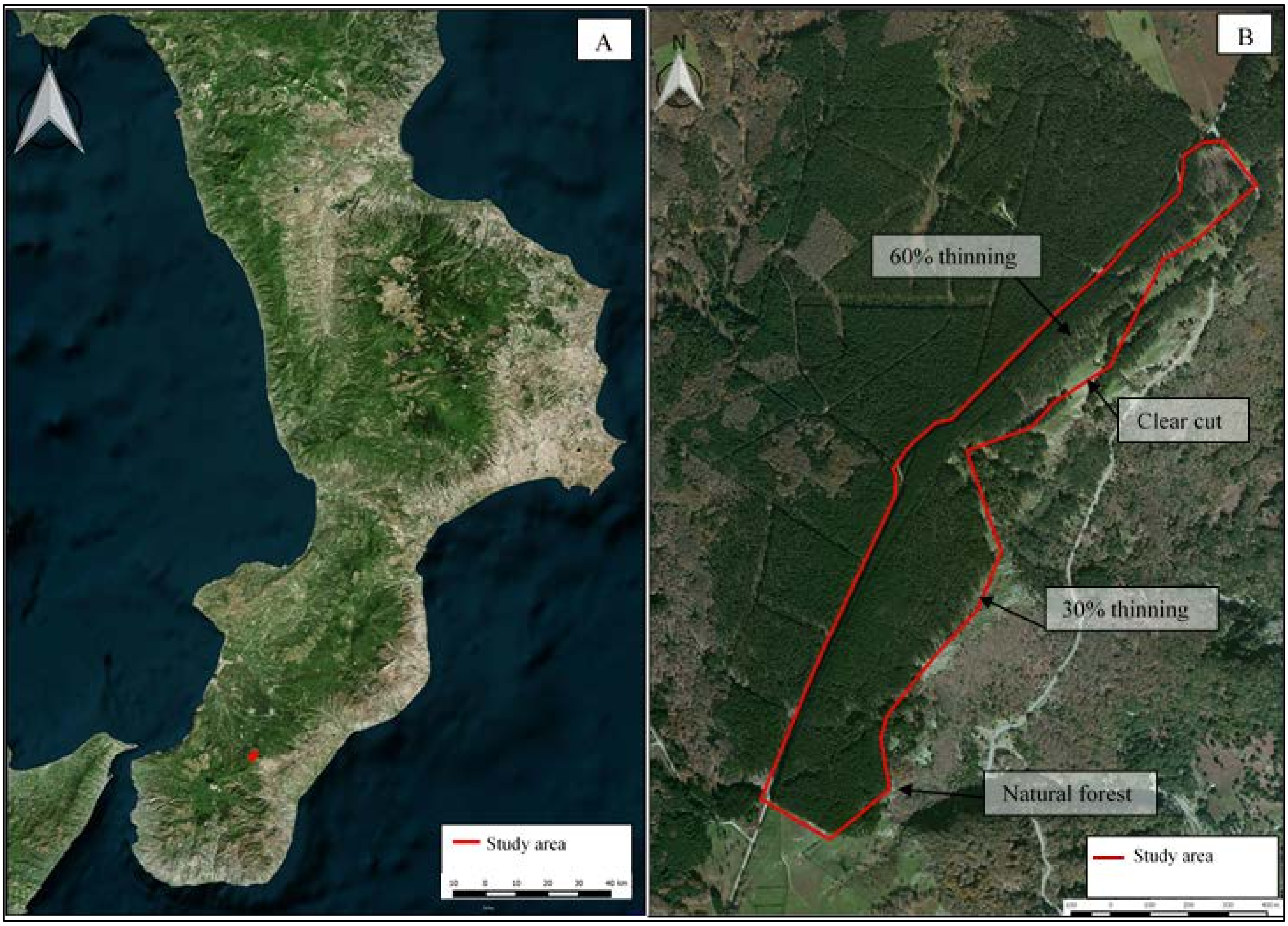

Figure 8. A) Study area located in *Pinus laricio* stands Aspromante Mountain (Zervò, Calabria, 38° 24' 24" N and 16° 01' 82" E) at an elevation of 1100 m a.s.l. B) Ortophoto showing location of the study sites: natural forest (control), selective thinning (30%, 60%) and clear cut.

3.2 Soil analysis

Soils were sampled in summer (June 2014, 2015, 2016) and in winter (December 2014, 2015) in undisturbed stand (natural forest) and in managed stands.
In detail, one circular plot of about 500 m^2 (representative area) in each study site was selected. Soil samples (0-10 cm) were taken from each site after removing the litter layers. In each site, five samples of soil were collected at a distance of about 10 m from each other. The samples were brought to the laboratory on the same day and kept in the refrigerator at 4 °C for up to 24 h until processing. Prior to the soil analysis, except for soil water content (SWC), soil fauna and microbial biomass, all the soil samples were air-dried, sieved (<2mm), and visible roots were removed.

3.2.1 Soil chemical and physical analysis

The Water Content (WC) was determined in according to the "Official Methods of Chemical Soil Analysis" (DM, 13/09/1999). The method covers the determination of the moisture content of a soil as a percentage of its oven-dried weight, up to a constant weight.
Samples of soils were analyzed to determine the size distribution of the mineral particles (texture). The texture was measured using the method of Bouyoucos (1962). This method is based on Stoke's law, governing the rate of sedimentation of particles suspended in water. The Bouyoucos method consistes of the destruction of the SOM with hydrogen peroxide and its further dispersion with sodium hexametaphosphate (Calgon). The SOM was destroyed in a 50 g soil sample by applying successive aliquots (approximately three times) of 40 mL of hydrogen peroxide (H_2O_2, 130 volumes) until the effervescence of the reaction was minimal. Dispersion was obtained by shaking 50 g of dry soil sample with 100 mL of 25% sodium hexametaphosphate (technical Calgon) in a reciprocating shaker. The mixture was then placed in a Bouyoucos' blender cup and stirred for two minutes with an electrical mixer. The contents of each cup were transferred to a 1 L sedimentation cylinder, and the cylinder was filled with deionized water to the 1000 mL mark. The mixture was then homogenized using manual agitation. Immediately after the cylinder was placed on the table and the hydrometer was gently inserted into the suspension.The solids in the suspension were measured with a hydrometer following 4 minutes of decantation with a second lecture taken after 24 hours. The measurement was made when the suspension was between 20 and 22 °C and then corrected due to the temperature. The first reading was for estimating the silt content whereas the second one at 24 hours was to estimate the clay. A soil texture triangle was used to determine the texture category. Results are reported as percentages of the mineral fraction, % sand, % silt, and % clay.

The acidity, neutrality or alkalinity of a soil are measured in terms of hydrogen ion activity of the soil water system. This method is essentially based on the measurement of potential, developed across an indicator or the glass electrode on account of the difference activity of H+ ions in and out of the electrode, i.e., in the bathing solution. pH in H_2O and KCl were measured according to the "Official Methods of Chemical Soil Analysis" (DM, 13/09/1999). 10 g air-dried soils were weighed, into a glass beaker and were mixed with 25 mL H_2O deionized to determine the active acidity of soil. 10 g of air-dried soils were weighed, into a glass beaker and were mixed with 25 mL of KCl (1:2.5) to determine the potential acidity. The suspensions were shaken for 2 hours, and after decantation, the electrode of pH-meter was immersed into the samples. The pH range normally found in soils varies from 3 to 9.

Electrical conductivity (EC) was detected according the method described by Blakemore et al (1987): 10 g of dry soil were weighed, into a glass beaker and mixed with 50 mL H_2O deionized, the suspension was shaken for 30 minutes and after decantation the conductivity was measured by using a conductimeter.

Total nitrogen (N) was detected by Kjeldahl method (Kjeldahl, 1883). The method consists of heating soil with sulphuric acid, which decomposes the organic substances by oxidation to liberate the reduced nitrogen as ammonium sulphate. In this step potassium sulphate is added to increase the boiling point of the medium (from 337 °C to 373 °C). Chemical decomposition of the sample is complete when the initially very dark-coloured medium has become clear and colorless. The solution is then distilled with a small quantity of sodium hydroxide, which converts the ammonium salt to ammonia. The amount of ammonia present, and thus the amount of nitrogen present in the sample, is determined by back titration. The end of the condenser is dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid is then titrated with a sodium carbonate solution by way of a methyl orange pH indicator.

- Mineralization: Sample + $H_2SO_4 \rightarrow (NH_4)_2SO_4(aq) + CO_2(g) + SO_2(g) + H_2O(g)$
- Liberation of ammonia: $(NH_4)_2SO_4(aq) + 2NaOH \rightarrow Na_2SO_4(aq) + 2H_2O(l) + 2NH_3(g)$
- Capture of ammonia: $B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$
- Back-titration: $B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_3(aq) + NaB(OH)_4(aq) + CO_2(g) + H_2O$

Organic carbon (OC) was determined by dichromate oxidation method, this procedure was developed in 1934 (Walkley and Black, 1934) based on the Schollenberger method, and further refined by Walkley (1947). Concentrated H_2SO_4 was added to a mixture of soil and aqueous $K_2Cr_2O_7$. The heat of dilution raises the temperature sufficiently to induce a substantial, but not complete, oxidation by the acidified dichromate. Residual dichromate was back titrated using ferrous sulphate (Figure 9).

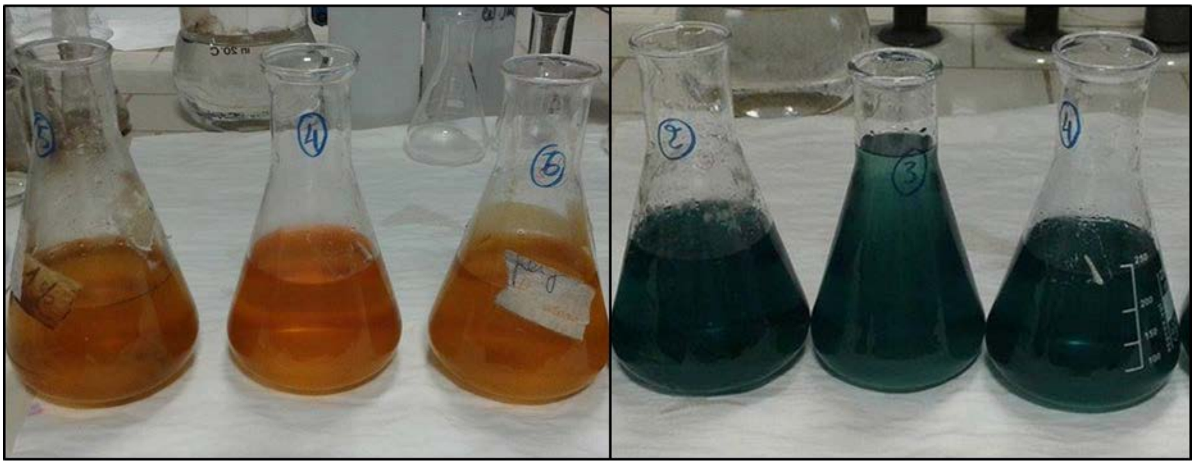

Figure 9. Organic carbon, detected according to the Walkley and Black procedure.

The difference in added $FeSO_4$ compared with a blank titration determines the amount of easy oxidizable organic carbon.
The percentage carbon is given by the formula:

$$C\ ORG.\% = M(\frac{V1-V2}{W})\text{ x 0.3 x CF}$$

where M is the molarity of the $FeSO_4$ solution (from blank titration), V_1 is the volume (mL) of $FeSO_4$ required in blank titration, V_2 is the volume (mL) of $FeSO_4$ required in actual titration, W is the weight (g) of the oven-dried soil sample and CF is the correction factor. The CF is a compensation for the incomplete oxidation and is the inverse of the recovery. This CF was set by Walkley and Black (1934) to 1.72 (recovery of 76%).
The cation-exchange capacity (CEC) is a measure of the amount of ions that can be adsorbed, in an exchangeable way, on the negative charge sites of the soil colloids (Bache, 1976). 2.0 g of soil into 30 ml of 1 M $BaCl_2$ solution, were shaked for 1 h at room temperature. Then the soil solution was centrifuged at 5000 g for 10 min. The supernatant was then transferred to a 100 mL volumetric flask. This procedure was repeated three times, collecting all the supernatants in the same 100 mL volumetric tanks. After the third decantation, 10 mL of 5 mM $MgSO_4$ was added and shaked gently for one hour. At the end, the conductivity of 1.5 mM $MgSO_4$ solution (it should be ~300 µS) was determined and the tube was weighed Calculations for CEC:

a. **Total solution (mL) [assumes 1 mL weighs 1 g] =**

final tube weight (g) - tube tare weight (g) - 2 g [weight of soil used]

b. **Mg in solution, not on CEC (meq) =**

total solution (mL) x 0.003 (meq/mL) [1.5 mM $MgSO_4$ has 0.003 meq/mL]

c. Total Mg added (meq) =

0.1 meq [meq in 10 mL of 5 mM $MgSO_4$] + meq added in 0.1 M $MgSO_4$

[mL of 0.1 M $MgSO_4$ x 0.2 meq/mL (0.1 M $MgSO_4$ has 0.2 meq/mL)]

d. CEC (meq/100g) = (c - b) x 50

[Total Mg added - Mg in final solution; 50 is to convert 2 g of soil to 100 g of soil]

Total water-soluble phenols (WSP) were measured by using the Folin–Ciocalteau reagent, following the Box method (1983). Tannic acid was used as standard and the concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (µg TAE g^{-1} D.W.). 10 g dry-soil were weighed, into a conic flask and, were mixed with 50 mL H_2O deionized, the suspensions were shaken for 24h, and then centrifuged at 5000 rpm for 10 min. 1000 µl of Folin-Ciolcateu reagent and 2.5 mL of sodium hydroxide (0.33 N) were added to the different extracts (500 µl). The mixtures were incubated for 15 min at room temperature. The specific absorbance was immediately measured at 760 nm.

3.2.2 Soil biochemical analysis

Soil microbial biomass carbon (MBC) was detected following the method of Vance *et al.* (1987): 5 g of each soil were fumigated with ethanol-free chloroform for 24 h at 25 °C in a sealed desiccator in the dark. 5 g of non-fumigated soil for each sample, were stored at 8 °C during this period. The next day, after removing the beaker with chloroform, the desiccator was evacuated six times to remove the chloroform from the soils. Both fumigated and non-fumigated samples were extracted with freshly prepared 0.5 M K_2SO_4 at 1:4 ratio and filtered. Dissolved organic carbon in all filtrates was determined after dichromate digestion by titrating with acidified ferrous ammonium sulphate. Biomass C is the difference between C extracted from the fumigated and non-fumigated treatments, both expressed as µg C g^{-1}. Finally the result was divided for 0.45 to convert the chloroform-labile C pool to the microbial biomass C (Beck *et al*. 1997).

Fluorescein diacetate (FDA) hydrolysis activity was determined according to the methods of Adam and Duncan (2001). To 2 g of soil (fresh weight, sieved <2 mm) 15 mL of 60 mM potassium phosphate pH 7.6 and 0.2 mL 1000 mg FDA mL^{-1} were added. The flask was then placed in an orbital incubator at 30 °C for 20 min. Once removed from the incubator, 15 mL of chloroform/methanol (2:1

v/v) was added to terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3 min. The supernatant was filtered and the filtrates measured at 490 nm on a spectrophotometer (Shimadzu UV–vis 2100, Japan). Hydrolysing coefficient (Hc): mmol of fluorescein diacetate hydrolysed/mmol of total fluorescein diacetate before hydrolysis (Perucci, 1992).

Dehydrogenase (DH) activity was determined by the method of von Mersi and Schinner (1991). Dehydrogenase assays based on the reduction of 2, 3, 5- triphenyltetrazolium chloride (TTC) to the creaming red-colored formazan (TPF), have been used to determine microbial activity in soil. In each tube was added 1 ml of TTC, except for the control, which received water instead. After the addition, of water, substrate and TTC were simultaneously mixed through the soil with a sterile glass rod; then rubber stoppers were inserted, and the tubes were incubated at 37°C at 24h. Upon completion of incubation, the tubes were extracted immediately. Each soil sample was transferred with methanol to a funnel containing Whatman no. 5 filter paper. The red methanolic solutions of the formazan were read at 485 nm against the extract from the non-TTC soil blank by using a spectrophotometer (Shimadzu UV–Vis 2100, Japan).

Catalase activity (CAT) was measured by the method of Beck (1971). 10 mL of phosphate buffer (pH, 7) and 5 mL of a 3% H_2O_2 substrate solution were added to 5 g of soil. The volume (mL) of O_2 released within 3 minutes at 20°C was determined. The controls were tested in the same way, but with the addition of 2 mL of 6.5% (w/v) NaN_3. Results were expressed as ml O_2 g^{-1} dry soil.

Protease (PROT) was measured as reported in Ladd and Butler (1972). Moist soil (1 g) was shaken, with 2-5 mL sodium caseinate in 0.1M Tris buffer, pH 8 for 120 min at 50°C. The tubes were then quickly cooled and enzyme activity stopped by adding 1 mL 15% trichloroacetic acid (TCA). After centrifugation of the reaction tubes, 2 mL samples of the supernatants were rapidly mixed with 3 mL alkaline reagent, and 1 mL of three-fold diluted Folin reagent (Lowry *et al.*, 1951). After 10 min, absorbances at 700 nm were determined and related to those of similarly-treated tyrosine standards.

3.2.3 Soil microbial analysis

Soil bacteria, fungi and actinomycetes were detected according the following method. Extraction procedure: ten grams of each soil sample were added to 90 mL of 0.1% (w/v) sterile solution of sodium pyrophosphate and 1% glycerol, pH 7.0 (Elliot and Des Jardin, 1999). After homogenization for 15 min at 1000 rpm, this solution was decimally diluted (10^{-1} to 10^{-7}) in a 0.85% NaCl sterile solution and aliquots of the resulting solutions plated on appropriate culture media.

For total bacteria count, Tryptone Soya Broth (Oxoid Ltd, UK) diluted (3 g L^{-1}), containing cycloeximide (100 µg mL^{-1}, agent that inhibit fungal growth) and solidified with agar (15 g L^{-1}), adjusted to pH 7 and sterilized at 121°C for 15 min was used. After incubation at 28°C for 7 days the colony forming units (CFU) were counted.

For fungal estimation, DifcoTM Malt Extract Agar containing chloramphenicol (100 µg mL^{-1} to inhibit bacteria growth) was used (Picci and Nannipieri, 2003). The incubation was performed at 28°C for 7 days (Figure 10).

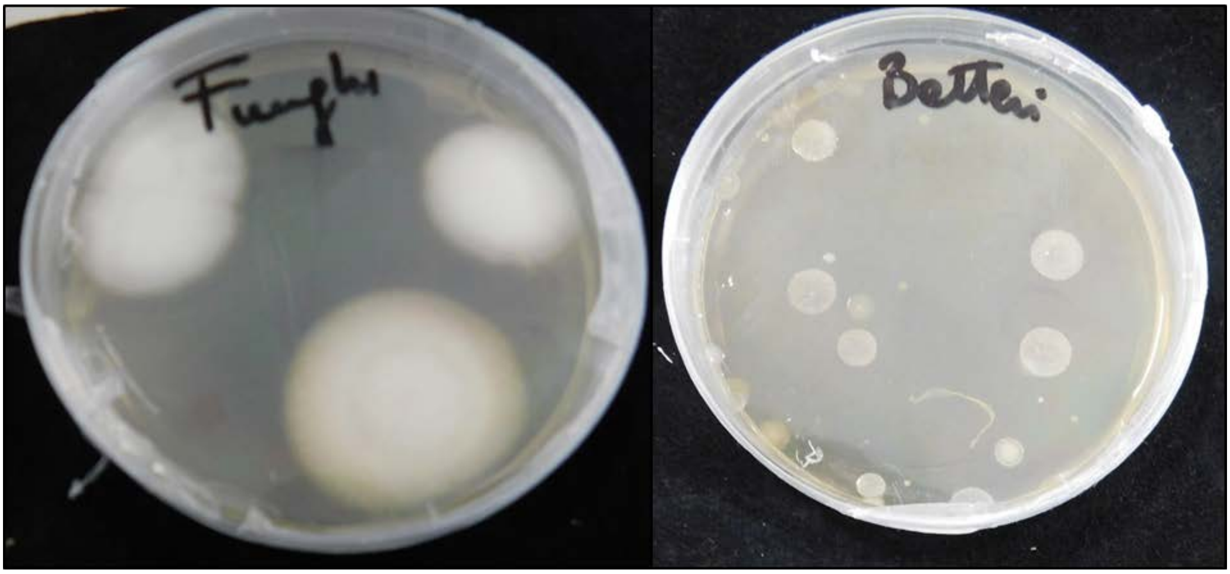

Figure 10. Example of fungi and bacteria colonies on agar plate, incubated for 7 days in growth chamber at 28°C.

For actinomycetes detections sodium caseinate (2 g L^{-1}), asparagine (0.1 g L^{-1}), sodium propionate (4 g L^{-1}), dipotassium phosphate (0.5 g L^{-1}), magnesium sulphate (0.1 g L^{-1}), ferrous sulphate (0.001 g L^{-1}), agar (15 g L^{-1}), containing 50 µg mL^{-1}, chloramphenicol (to inhibit bacteria growth), and cycloeximide (100 µg mL^{-1}, agent that inhibit fungal growth) was used (Lechevalier, 1975; Eaton *et al*., 2005). Sterilize by autoclaving at 121°C for 15 minutes. The incubation was performed at 28°C for 14 days.

3.2.4 Soil fauna determination

Micro-arthropods, included mites and collembola, were analyzed with Berlese-Tullgren selector (Parisi *et al*., 2005). 5 soil samples were taken from each site differently managed, including the control site. Each plot was sampled at soil depths of 0-10 cm. Immediately upon returning from the field, the soil samples were transferred to Berlese-Tullgren funnels lined with 4 mm wire mesh. Arthropods were extracted for 7 days and collected in a beaker filled with preservative liquid (2 parts 75% ethanol and 1 part glycerol) beneath the funnel. Extracted specimens were observed under a stereomicroscope at low magnification (usually 20-40× is sufficient) in the same preservative liquid. For mites and collembola a quantitative evaluation test was performed. All the arthropods were identified at different taxonomical levels through, the determination of biological forms and calculation of QBS index. Accordingly, the biological form (morpho-type) that is most adapted to soil was identified. Each morpho-type correspond an ecomorphological index (EMI); as a general rule, eu-edaphic (i.e. deep soil-living) forms correspond to an EMI = 20, hemi-edaphic (i.e. intermediate) forms have an index rating proportionate to their degree of specialization, while epi-edaphic (surface-living) have an EMI = 1. The QBS index value is obtained from the sum of EMI index of all the collected groups (Acari, Collembola, Areneae, Diptera, Hemiptera, Coleoptera, Hymenoptera, Protura, Diplura, Diplopoda) (Figures 11, 12). If in a group biological forms with different EMI values are present, the higher value (more adapted to the soil form) is selected to represent the group in the QBS calculation (Parisi *et al*., 2005). EMI is a semplified index, that use the microartopod morphology assessment for generating Soil Biological Quality index (QBS index). This analysis allows assessing the degradation level of soils (Parisi *et al*., 2005).

Eco-morphologic indices (EMIs) of edaphic microarthropod groups[a]

Group	EMI score
Protura	20
Diplura	20
Collembola	1–20
Microcoryphia	10
Zygentomata	10
Dermaptera	1
Orthoptera	1–20
Embioptera	10
Blattaria	5
Psocoptera	1
Hemiptera	1–10
Thysanoptera	1
Coleoptera	1–20
Hymenoptera	1–5
Diptera (larvae)	10
Other holometabolous insects (larvae)	10
Other holometabolous insects (adults)	1
Acari	20
Araneae	1–5
Opiliones	10
Palpigradi	20
Pseudoscorpiones	20
Isopoda	10
Chilopoda	10–20
Diplopoda	10–20
Pauropoda	20
Symphyla	20

Figure 11. Eco-morphologic indices (EMI). The value of QBS index is obtained from the sum of the highest values of EMI of all the collected groups (Acari, Collembola, Areneae, Diptera, Hemiptera, Coleoptera, Hymenoptera, Protura, Diplura, Diplopoda).

A simplified scheme to calculate collembolan's EMI

Character	EMI score
(1) Clearly epigeous forms: middle to large size, complex pigmentation present, long, well-developed appendages, well developed visual apparatus (eye spot and eyes)	1
(2) Epigeous forms not related with grass, shrubs or trees well-developed appendages (possible), well-developed setae or protective cover of scales, well-developed visual apparatus	2
(3) Small size—though not necessarily—forms, usually limited to litter, with modest pigmentation, average length of appendages, developed visual apparatus	4
(4) Hemi-edaphic forms with visual apparatus still developed, not elongated appendages, cuticle with pigmentation	6
(5) Hemi-edaphic forms with reduced number of ommatidia, scarcely developed appendages, often short or absent furca, pigmentation present	8
(6) Eu-edaphic forms with no pigmentation, reduction or absence of ommatidia, furca present—but reduced	10
(7) Clearly eu-edaphic forms: no pigmentation, absent furca, short appendages, presence of typical structures such as pseudo-oculi, developed postantennal organs (character not necessarily present), apomorphic sensorial structures	20

Orthoptera	In general	EMI 1
	Except for Grillidae family	Whose EMI is 20
Hemiptera	In general, mostly epigeous (above-ground) or root-feeding forms	EMI 1
	Cicada larvae	EMI 10
Coleoptera	Clearly epigeous forms	EMI 1
	Main adaptations to underground life that can be detected by direct examination of specimens are:	
	(a) dimensions smaller than 2 mm points 4; (b) thin integument, often testaceous (tan-brown) color points 5; (c) hind wings highly reduced or absent points 5; (d) microphtalmy or anophtalmy points 5;	
	For these forms, the EMI value is equal to the sum of points relative to the detected characters—e.g. if only (a) and (b) are present, then EMI score is = 1 + 4 + 5 = 10.	
Hymenoptera	In general	EMI 1
	Formicidae	EMI 5
Araneae	Small forms, scarcely pigmented	EMI 5
	Forms >5 mm	EMI 1
Diplopoda	Forms >5 mm	EMI 5
	Forms <5 mm	EMI 20
Chilopoda	Forms >5 mm, well-developed legs	EMI 10
	Other forms	EMI 20

Figure 12. Detailled scheme of Eco-morphologic indices (EMI).

3.3 Plant analysis

We analyzed the plants in correspondence of the analyzed soil samples in all silvicultural treatment, except for the clear cutting in which the plants were absents.

In detail, in these circular plots of about 500 m^2, the dendrometric parameters (diameter at breast height and height) were measured and wood samples were collected. This analysis was carried out at the end of the experimental trial (June 2016).

3.3.1 Dendrometric analysis

The dendrometric analyses were carried out with standard methods. The diameter at breast height (DBH) was measured by normal caliper, while the total height of all the trees was measured with Vertex ultrasonic measuring device. By using these dendrometric parameters it was possible to determine the H/D ratio (height/diameter at breast height) indicating population mechanical stability.

3.3.2 Wood quality analysis

The wood samples for densitometric analyses were obtained nondestructively. Increment wood core was taken by a Pressler borer at the steam breast height (Figure 13). The wood samples were stored in a conditioning chamber (12 h, 20°C, 50% RH). The wood density, was measured with a densitometer that uses X-ray to make a totally noninvasive analysis.

Figure 13. Wood core sections taken at breast height with a Pressler borer in *Pinus nigr*a spp. *laricio* var. *calabrica* forest, Aspromonte mountains (Zervò, RC, Italy), five years after treatments (June 2016).

Wood X-ray densitometry is a radiation detection method in which X-ray attenuation is converted to wood density measurement. This densitometer was projected and assembled at Italian National Research Council – Trees and Timber Institute, in S. Michele all'Adige (TN) (Figure 14).

Figure 14. X-ray densitometer, for wood density analyses, located at Italian National Research Council – Trees and Timber Institute, in S. Michele all'Adige (TN, Italy).

The scanning was done using following settings: voltage of 30 kV, current value of 25 mA. Through a software LabVIEW v. 6i of National Instruments, was obtained a radiographic image (Figure 15). Subsequently, through a series of formulas it is possible to determine directly the density of the wood.

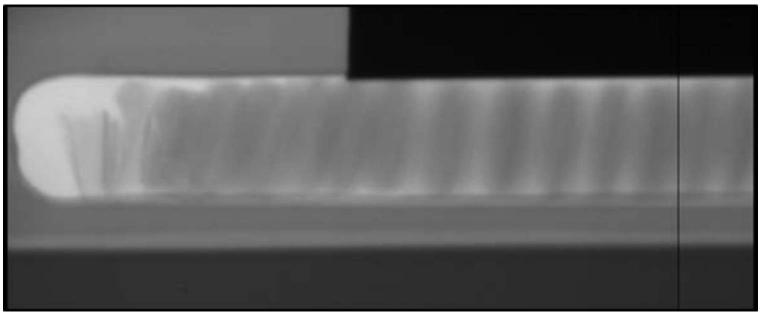
Figure 15. Radiographic image of wood core. Analyses was performed at Italian National Research Council – Trees and Timber Institute, in S. Michele all'Adige (TN).

The radiological analysis of the wood was carried out by using the mitigation power of material, that absorbs a quantity of X-rays as a function of the thickness and density. The X-rays have an effect on wood at the atomic level. All the process of X-rays absorption is adjusted by the attenuation law exponential:

$$I/I0 = \exp\left[-(\mu/r)\, x\right] \quad \text{(eq 1)}$$

Where:

• I/I_0 is the ratio between current intensity read by the photodiode with the specimen and vacuum,

• (μ/r) is the attenuation coefficient for the material analysis, expressed in cm^2/g,

• r is the density of the sample (in the case of the wood is the density) [g/cm^3]

• μ is the linear attenuation coefficient [cm^{-1}]

• x is the specimen thickness [cm]

Equation 1 is easily found that:

$$\mu/r = x^{-1} \ln (I/I0) \tag{eq 2}$$

Assuming that the wood is homogenous, and doing the due proportions it is possible to calculate the coefficient of total absorption of the samples as sum of the different absorption coefficients of the single atoms, calculated proportionally to the weight of sample and expressed in:

$$(\mu/r)_{Wood} = \Sigma_i w_i (\mu/r)_i \tag{eq 3}$$

Where:

• $(\mu/r)_{Wood}$ is the coefficient of total wood consumption

• w_i is the part of the element weight of the total

• $(\mu/r)_i$ is the absorption coefficient of the element i.

The value of the coefficient μ/r expressed in cm^2/g is multiplied for the wood thickness and then used in the first equation to calculate the density of materials (Negri *et al.*, 2004; Fellin, 2004).

3.4 Statistical analysis

Data analysis are the mean of five replicates and were performed by using SPSS software. All datasets were tested for normality using Shapiro-Wilk and Jarque-Bera tests. Tukey's test determined differences among treatments. One-way analysis of variance (one-way ANOVA) was used to test the differences in soil and plant quality under different thinning. Two-way ANOVA was used to test relationships between treatments and seasons. Significant differences and effects were determined for $p \leq 0.05$.

4. RESULTS AND DISCUSSION

4.1 Soil chemical and physical features

All the soils analyzed belong to the sandy-loam textural class, with silt varying from 10 to 16 %, clay ranged from 4 to 8 % and sand varying from 78 to 82 %. Our results showed that soil texture didn't change over seasons and over treatments (data not show).

WC differently and significantly changed both with treatment and season (p <0.05) (Tables 1, 2). The detected differences among the treatments, were caused by the lower density of the plants (clear cutting> 60% thinning> 30 % thinning), that determined different regimes of light, temperature and humidity at soil level. The differences observed between summer and winter were due to the different regimes of temperatures and rainfalls, typical of the seasonal variation of Mediterranean climate (Figure 7).

EC showed significant differences (p <0.05) among treatments for both summer and winter season (Tables 2, 4, 6). Analysis of variance showed that seasonal variations affected EC and WC more than treatments.

Generally, pH was acid in all sites and ranged from 4.99 to 5.29 (summer 2014), 4.93 to 5.49 (summer 2015) and 5.00 to 5.76 (summer 2016). In winter 2014 and 2015 varied from 5.33 to 5.68 and 5.4 to 5.62, respectively, highlighting the typical acidic nature of the soil under conifers (Badalucco *et al*., 1992). No significant differences among treatments were observed (p=0.6). On the contrary, season influenced pH values that were significantly higher (p <0.05) in winter than in summer (Tables 2, 4, 6).

WSP differed significantly between seasons (p < 0.05) but not in respect to the treatments (p=0.8); WSP content was higher in winter than in summer (Tables 1, 3).

The observed increase in phenol concentration, in winter, was due to the high leaching rates of phenols from freshly fallen litter during the first stage of decomposition that enriched the underlying soil layers as already reported by Kuiters and Denneman (1987).

60% thinning increased the amount of soil organic matter compared to control (natural forest) and to the other treatments (Tables 1, 3, 5). Same results were obtained for nitrogen and C:N ratio. These data suggested that the lower density of plants in the managed sites, determining a different regime of light, temperature and humidity at soil level, increased the undergrowth herbaceous vegetation that produced litter easily degradable. All this, leaded to an increase in organic matter and nitrogen in soil, which in turn promoted soil biodiversity, an increased soil activity and a better soil ecosystem functioning. Significant differences p <0.05 (Tables 2, 4) in organic matter, nitrogen content and C/N ratio were observed between winter and summer seasons, with highest values in summer. These findings are the result of the different rainfall and temperature regime between the two different seasons.

CEC changed significantly not only between the different treatments $p < 0.05$ (Table 4), but also between the different seasons (Table 2), an increase in CEC in the soil samples collected in summer (dry season), was observed compared to soils sampled in winter (wet season). In the dry season the observed increase in organic matter justified the increase in CEC. In short, our results demonstrated that in summer the amount of OM, N, C/N ratio and CEC were greater than in winter. The analysis of variance confirmed that the changes in soil properties between summer and winter (Table 4) were highly significant per $p < 0.05$.

The data obtained are in agreement with findings of other authors recognizing that the climate (particularly temperature and precipitation) is one of the most important factors regulating SOM (Sims and Nielsen, 1986; Homann *et al*., 1995; Alvarez and Lavado, 1998). In effect, seasonal variations were able to affect vegetation cover, quantity and quality of soil organic matters, SOM mineralization rate and litter decomposition (Quideau *et al*., 2001; Heviaa *et al*., 2003). Results of Azlan *et al*. (2012) showed that TOC and SOM concentrations were significantly different ($p < 0.05$) over seasons, with the greater amounts during dry season. Buschiazzo *et al*., (2004) demonstrated also that tree coverage affecting soil temperature and moisture, created microclimatic condition which in turn affected SOM pools.

4.2 Soil biochemical features

Microbial Biomass Carbon also changed significantly under the different forest managements ($p < 0.05$) (Tables 8 and 10). 60% thinning was the silvicultural practice that mainly increased MBC amount. Soil MBC peaked in summer when both soil temperature and soil water content were balanced.

FDA changed significantly in respect to the different forest management practice per $p < 0.05$ (Tables 8, 10, 12). 60% thinning increased soil FDA activity in respect to natural forest and other treatments. Significant differences were observed between summer and winter ($p < 0.05$) (Tables 8, 10), with the maximum activity in summer. These results are fully in agreement with other studies showing that FDA was the soil biological parameter mostly affected by environmental factors (Sicardi *et al*., 2004; Pesaro *et al*., 2004; Son *et al*., 2006; Muscolo *et al*., 2014).

Forest management, influenced significantly also the activity of dehydrogenase in soil ($p < 0.05$) (Tables 8 and 10). The highest DHA activity was detected in soil under 60% thinning. The activity of DHA in soil during the summer season was almost double than in winter, in all the plots analyzed.

Protease activity changed significantly in function of the different treatments (p <0.05). Protease in forest soil, in summer, was higher than in winter in all plots (p <0.05) (Tables 8 and 10).

Catalase varied significantly in respect to the different forest management practices per p <0.05 (Tables 8, 10, 12). 60% thinning showed the highest value of catalase activity in soil. Significant differences were observed between summer and winter (p <0.05) (Tables 8, 10), with the maximum activity in summer.

Soil enzyme activities are important indicators of soil fertility. Their changes, to a certain extent, can reflect the evolution trend of forest soil quality and the effects of forest management on soil ecosystem functioning. Soil enzyme activities are 'sensors' of soil degradation since they integrate information about microbial status, and also, from soil physico-chemical conditions (Wick *et al*., 1998; Aon and Colaneri, 2001; Baum *et al*., 2003). They are used as sensors in studies on the influence of soil treatments on soil fertility (Chen *et al*., 2003). They may correlate well with nutrient availability (Asmar *et al*., 1994). Regarding the role of the enzymes tested in soil, it is well known that protease plays a significant role in N mineralization (Ladd and Jackson, 1982), an important process regulating the amount of plant available N and plant growth. This enzyme is generally associated with inorganic and organic colloids (Burns, 1982; Nannipieri *et al*., 1996). The amount of this extra cellular enzyme activity may be indicative not only of the biological capacity of soil for the enzymatic conversion of the substrate, which is independent of the extent of microbial activity, but might also have an important role in the ecology of microorganisms in the ecosystem (Burns, 1982). The dehydrogenase enzyme activity is commonly used as indicator of biological activity in soils (Burns, 1978). This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. DHA enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions (Kandeler, 1996; Glinski and Stepniewski, 1985). Based on these assertions, studies on the activities of soil dehydrogenase enzyme are very important for giving indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility as well as soil health. A study of Brzezinska *et al.* (1998) suggested that soil water content and temperature influenced dehydrogenase activity indirectly by affecting the soil redox status. Redox transformations are closely connected with respiration activity of soil microorganisms, and are considered a possible measure of microbial oxidative activity (Tabatabai, 1982; Trevors, 1984, Garcia and Hernandez, 1997), for these reasons dehydrogenase enzyme is considered on indirect measure of any disruption caused by management practices to the soil (Reddy and Faza, 1989; Wilke, 1991; Frank and Malkomes, 1993).

Catalase, as dehydrogenase, is widely distributed in soil and it is very sensitive to environmental changes. It is used as enzyme marker to analyze harmful effects of H_2O_2 to plant growth (Dai and Wang, 1994). Its activity is related to soil respiration intensity and can also reflect microbial activity intensity (Dai and Bai, 1995). In short the results coming from the comparison of the enzyme activity of soils under management with soils under unmanaged forest, show that: in soil under intensive thinning (60%), the activity of all the enzymes analyzed, increased. We observed the greatest increase in the enzyme activities during summer time probably caused by on increase in soil temperature which in turn stimulated soil MBC. High levels of biological activity in soil are strictly dependent on SOM availability. Our data confirm this statements and are in agreement with the results of Azlan *et al*. 2012 showing that, soils with low level of SOM have a reduced biological activity. The obtained results on enzyme activities are in line with the data related to physical and chemical soil analysis and confirm that 60% thinning has the best effects on soil ecosystem functioning.

Table 1. Summer and Winter 2014 - Chemical and physical analysis of soil under *Pinus laricio* plantation differently managed. Water content (WC%); pH (H_2O); pH (KCl); electrical conductivity (EC μs cm^{-1}); organic matter (OM%); total phenols (WSP μg TAE g^{-1} dry soil); total nitrogen (N%); cation exchange capacity (CEC, cmol(+)/ Kg).

Seasons	**Management**	**WC**	**pH (H_2O)**	**pH (KCl)**	**EC**	**WSP**	**OM**	**N**	**C/N**	**CEC**
Summer	**Thinning 30%**	49^{b}	4.99^{a}	4.15^{b}	33.10^{b}	200^{a}	18.35^{b}	0.55^{c}	19.00^{a}	34.30^{b}
	Thinning 60%	67^{a}	5.29^{a}	4.41^{a}	50.90^{a}	200^{a}	24.21^{a}	0.94^{a}	15.00^{c}	39.20^{a}
	Clear cut	38^{c}	5.13^{a}	4.41^{a}	50.10^{a}	195^{a}	16.86^{b}	0.72^{b}	14.00^{d}	33.50^{b}
	Natural forest	27^{d}	5.25^{a}	4.24^{ab}	48.50^{a}	200^{a}	7.68^{c}	0.27^{d}	16.00^{b}	18.50^{c}
Winter	**Thinning 30%**	48^{c}	5.56^{ab}	4.55^{a}	42.60^{c}	233^{a}	14.54^{a}	0.69^{b}	12.00^{a}	31.40^{a}
	Thinning 60%	64^{b}	5.68^{a}	4.67^{a}	74.10^{a}	221^{a}	14.49^{a}	0.84^{a}	10.00^{c}	31.30^{a}
	Clear cut	81^{a}	5.33^{b}	4.64^{a}	76.20^{a}	228^{a}	13.48^{b}	0.83^{a}	9.00^{d}	30.00^{a}
	Natural forest	48^{c}	5.56^{ab}	4.58^{a}	60.90^{b}	229^{a}	12.32^{c}	0.67^{b}	11.00^{b}	28.00^{b}

*Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$).

Table 2. Analysis of variance of the effects of treatments, seasons and their interactions on chemical and physical soil parameters (Summer and Winter 2014). Water content (WC); pH (H_2O); pH (KCl); electrical conductivity (EC); organic matter (OM); total phenols (WSP); total nitrogen (N); cation exchange capacity (CEC).

		WC	**pH (H_2O)**	**pH (KCl)**	**EC**	**WSP**	**OM**	**N**	**C/N**	**CEC**
R^2		0.937	0.206	0.129	0.969	0.391	0.923	0.836	0.892	0.853
	Treatments	87.381**	0.745	0.405	219.838**	0.228	90.167**	45.373**	24.071**	50.529**
***F*-ratio**	**Seasons**	128.571**	7.168**	4.494**	502.219**	24.304**	55.566**	24.999**	249.635**	2.665**
	Interaction	66.667**	0.329	0.073	25.903**	0.236	51.332**	14.217**	2.767**	25.842**

**p<0.05

Table 3. Summer and Winter 2015 - Chemical and physical analysis of soil under *Pinus laricio* plantation differently managed. Water content (WC%); pH (H_2O); pH (KCl); electrical conductivity (EC µs cm^{-1}); organic matter (OM%); total phenols (WSP µg TAE g^{-1} dry soil); total nitrogen (N%); cation exchange capacity (CEC, cmol(+)/ Kg).

Seasons	Management	WC	pH (H_2O)	pH (KCl)	EC	WSP	OM	N	C/N	CEC
Summer	Thinning 30%	47^{b}	4.93^{b}	4.24^{b}	55.50^{b}	217^{a}	17.85^{b}	0.65^{b}	16.00^{a}	35.30^{ab}
	Thinning 60%	35^{c}	5.49^{a}	4.52^{a}	36.70^{c}	211^{a}	19.85^{a}	0.75^{a}	15.00^{b}	37.00^{a}
	Clear cut	57^{a}	5.07^{b}	4.42^{ab}	60.91^{a}	208^{a}	16.54^{c}	0.71^{a}	14.00^{c}	34.70^{b}
	Natural Forest	48^{b}	5.15^{b}	4.36^{ab}	62.15^{a}	216^{a}	18.43^{b}	0.72^{a}	15.00^{b}	36.00^{ab}
Winter	Thinning 30%	72^{b}	5.62^{a}	4.75^{a}	68.13^{a}	237^{a}	14.05^{b}	0.61^{c}	13.38^{a}	31.20^{b}
	Thinning 60%	65^{c}	5.59^{a}	4.63^{a}	62.27^{b}	227^{a}	17.85^{a}	0.88^{a}	11.78^{b}	33.45^{a}
	Clear cut	83^{a}	5.40^{a}	4.56^{a}	52.36^{c}	232^{a}	14.30^{b}	0.78^{b}	10.57^{c}	31.40^{b}
	Natural Forest	74^{b}	5.49^{a}	4.60^{a}	55.12^{c}	230^{a}	14.50^{b}	0.75^{b}	11.42^{b}	34.50^{a}

*Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$).

Table 4. Analysis of variance of the effects of treatments, seasons and their interactions on chemical and physical soil parameters (Summer and Winter 2015). Water content (WC); pH (H_2O); pH (KCl); electrical conductivity (EC); organic matter (OM); total phenols (WSP); total nitrogen (N); cation exchange capacity (CEC).

		WC	**pH (H_2O)**	**pH (KCl)**	**EC**	**WSP**	**OM**	**N**	**C/N**	**CEC**
R^2		0.955	0.167	0.190	0.841	0.106	0.523	0.424	0.667	0.188
***F*-ratio**	**Treatments**	61.314**	0.639	0.077	18.296**	0.148	6.062**	7.227**	6.160**	0.914
	Seasons	654.229**	4.593**	8.173**	21.418**	4.131**	23.686**	2.792	61.373**	5.950**
	Interaction	1.124	0.511	0.334	45.093**	0.056	0.679	1.652	0.139	0.194

**$p<0.05$

Table 5. Summer 2016 – Chemical and physical analysis of soil under *Pinus laricio* plantation differently managed. Water content (WC%); pH (H_2O); pH (KCl); electrical conductivity (EC µs cm^{-1}); organic matter (OM%); total phenols (WSP µg TAE g^{-1} dry soil); total nitrogen (N%); cation exchange capacity (CEC, cmol(+)/ Kg).

Management	**WC**	**pH (H_2O)**	**pH (KCl)**	**EC**	**WSP**	**OM**	**N**	**C/N**	**CEC**
Thinning 30%	55^{b}	5.02^{b}	4.40^{a}	48.50^{b}	210^{b}	18.20^{b}	0.73^{b}	14.64^{b}	34.50^{b}
Thinning 60%	39^{c}	5.56^{a}	4.42^{a}	51.50^{a}	207^{b}	21.05^{a}	0.95^{a}	13.26^{c}	37.20^{a}
Clear cut	60^{a}	5.76^{a}	4.42^{a}	50.30^{a}	230^{a}	17.00^{c}	0.68^{c}	15.00^{b}	33.70^{b}
Natural Forest	52^{b}	5.00^{b}	4.40^{a}	$48,10^{b}$	215^{b}	18.50^{b}	0.67^{c}	16.02^{a}	34.80^{b}

*Different letters in the same column indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 6. Analysis of variance of the effects of treatments, on chemical and physical soil parameters (Summer 2016). Water content (WC); pH (H_2O); pH (KCl); electrical conductivity (EC); organic matter (OM); total phenols (WSP); total nitrogen (N); cation exchange capacity (CEC).

		WC	pH (H_2O)	pH (KCl)	EC	WSP	OM	N	C/N	CEC
R^2		0.955	0.167	0.190	0.841	0.106	0.523	0.424	0.667	0.188
***F*-ratio**	**Treatments**	12.072**	4.579**	0.020	14.035**	1.010	6.700**	7.001**	3.873**	0.610

**$p<0.05$

Table 7. Summer and Winter 2014 - Biological analysis of soil under *Pinus laricio* plantation differently managed. Fluorescein diacetate (FDA, µg fluorescein released g^{-1} d.s.); protease (PROT, µg tyrosine g^{-1}d.s./2h); catalase (CAT, $\%O_2$/3min/g d.s.); dehydrogenase (DHA, µg TTF g^{-1} h^{-1} and microbial biomass C (MBC, µg C g^{-1}d.s.).

Seasons	Management	FDA	PROT	CAT	DHA	MBC
	Thinning 30%	58.52^{b}	80.35^{b}	1.69^{b}	7.36^{b}	7574^{b}
Summer	**Thinning 60%**	71.92^{a}	90.90^{a}	1.88^{a}	11.15^{a}	7957^{a}
	Clear Cut	51.18^{c}	76.47^{b}	1.13^{c}	6.23^{c}	6810^{c}
	Natural forest	45.86^{d}	68.22^{c}	0.74^{d}	5.89^{c}	6378^{d}
	Thinning 30%	53.25^{b}	59.86^{b}	1.32^{b}	3.37^{b}	6800^{b}
Winter	**Thinning 60%**	61.80^{a}	63.01^{a}	1.41^{a}	4.40^{a}	7571^{a}
	Clear Cut	41.10^{c}	56.91^{bc}	1.03^{c}	2.24^{c}	6752^{b}
	Natural forest	42.85^{c}	55.79^{c}	0.94^{d}	1.53^{d}	6027^{c}

*Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$).

Table 8. Analysis of variance of the effects of treatments, seasons and their interactions on biological soil parameters (Summer and Winter 2014). Fluorescein diacetate (FDA); protease (PROT); catalase (CAT); dehydrogenase (DHA); microbial biomass C (MBC).

		FDA	**PROT**	**CAT**	**DHA**	**MBC**
R^2		0.892	0.950	0.763	0.874	0.653
	Treatments	97.761**	19.957**	34.420**	74.902**	21.497**
***F* ratio**	**Seasons**	23.271**	203.537**	8.479**	504.224**	7.616**
	Interaction	4.899**	11.125**	5.592**	5.040**	1.069

***p <0.05*

Table 9. Summer and Winter 2015 - Biological analysis of soil under *Pinus laricio* plantation differently managed. Fluorescein diacetate (FDA, μg fluorescein released g^{-1} d.s.); Protease (PROT, μg tyrosine g^{-1}d.s./2h); catalase (CAT, $\%O_2$/3min/g d.s.); dehydrogenase (DHA, μg TTF g^{-1} h^{-1} and microbial biomass C (MBC, μg C g^{-1}d.s.).

Seasons	Management	FDA	PROT	CAT	DHA	MBC
Summer	**Thinning 30%**	53.85^{b}	77.99^{b}	1.98^{b}	6.07^{b}	6899^{c}
	Thinning 60%	58.87^{a}	85.89^{a}	2.64^{a}	9.45^{a}	7785^{a}
	Clear cut	48.81^{c}	74.56^{c}	0.94^{c}	5.09^{c}	6290^{d}
	Natural Forest	56.35^{b}	83.27^{a}	2.16^{b}	6.86^{b}	7308^{b}
Winter	**Thinning 30%**	45.23^{b}	62.46^{b}	1.32^{b}	5.03^{b}	6512^{b}
	Thinning 60%	50.73^{a}	69.95^{a}	1.88^{a}	5.95^{a}	7255^{a}
	Clear cut	40.94^{c}	60.35^{bc}	0.90^{c}	3.71^{c}	6058^{c}
	Natural Forest	45.85^{b}	64.14^{b}	1.37^{b}	4.46^{b}	6672^{b}

*Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$).

Table 10. Analysis of variance of the effects of treatments, seasons and their interactions on biological soil parameters (Summer and Winter 2015). Fluorescein diacetate (FDA); protease (PROT); catalase (CAT); dehydrogenase (DHA); microbial biomass C (MBC).

		FDA	**PROT**	**CAT**	**DHA**	**MBC**
R^2		0.668	0.924	0.829	0.883	0.707
***F* ratio**	**Treatments**	10.718**	83.610**	45.134**	17.523**	26.033**
	Seasons	47.087**	192.305**	44.981**	244.253**	16.435**
	Interaction	0.454	14.564**	4.489**	1.804	0.636

**p <0.05

Table 11. Summer 2016 - Biological analysis of soil under *Pinus laricio* plantation differently managed. FDA (Fluorescein diacetate, µg fluorescein released g^{-1} d.s.); protease (PROT, µg tyrosine g^{-1}d.s./2h); catalase (CAT, $\%O_2$/3min/g d.s.); dehydrogenase (DHA, µg TTF g^{-1} h^{-1} and microbial biomass C (MBC, µg C g^{-1}d.s.).

Management	**FDA**	**DHA**	**PROT**	**CAT**	**MBC**
Thinning 30%	55.65^{b}	5.87^{b}	68.7^{b}	1.51^{b}	6724^{b}
Thinning 60%	61.60^{a}	7.48^{a}	76.5^{a}	1.87^{a}	7484^{a}
Clear cut	50.55^{c}	5.08^{c}	62.1^{c}	1.14^{c}	6258^{c}
Natural Forest	55.56^{b}	5.60^{b}	68.5^{b}	1.45^{b}	6789^{b}

*Different letters in the same column indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 12. Analysis of variance of the effects of treatments on biological soil parameters (Summer 2016). Fluorescein diacetate (FDA); protease (PROT); catalase (CAT); dehydrogenase (DHA); microbial biomass C (MBC).

		FDA	**PROT**	**CAT**	**DHA**	**MBC**
$\mathbf{R^2}$		0.590	0.845	0.376	0.364	0.881
***F* ratio**	**Treatments**	9.603^{**}	36.422^{**}	4.021^{**}	3.814^{**}	49.537^{**}

$^{**}p < 0.05$

4.3 Soil microbial features

Soil microbes, play diverse and often critical roles in ecosystem services. The vast metabolic diversity of soil microbes means their activities drive or contribute to the cycling of all major elements (e.g. C, N, P), and this cycling affects the structure and the functions of soil ecosystems as well as the ability of soils to provide services to people. The soils of all the sites analyzed had higher population of bacteria than fungi. No actinomycetes were counted.

The development of microorganisms is conditioned by the soil pH. Generally, the soil pH vary between 4.5 and 8.5. Most microorganisms prefer soils with neutral pH (between 6 and 7), as under these conditions, the capacity for using nutrients is maximal. For instance, actinomycetes develop in a strict interval of pH close to the neutrality (Lim, 1998). This is the reason why in all the sites analyzed, no actinomycete colonies were found. Highly significant microbial population were observed in summer, and 60% thinning was the most effective treatment that significantly increased the number of colonies of fungi and bacteria (Tables 13, 15, 17). Analysis of variance showed that seasonal variations affected fungal and bacterial populations more than treatments. These changes can be ascribed to the increase in soil temperature which in turn stimulated the reproduction of soil microorganisms. The majority of soil microorganisms prefer temperature ranging from 20 to 45 degree for their growth rates (Castro *et al*., 2010; Gray *et al*., 2011; Lennon *et al*., 2012; Briones *et al*., 2014; Delgado-Baquerizo *et al*., 2014; Whitaker *et al*., 2014). Thinning of over story trees can influence both understory plants and soil microbial communities affecting the microclimate (Jones *et al*., 2003; Owen *et al*., 2009; Pickles *et al*., 2010) the chemical properties of soils (DeBano *et al*., 1998; Waldrop *et al*., 2003; Prusky and Yakoby, 2003; Garbeva *et al*., 2004; Grayston and Renneberg, 2006; Lauber *et al*., 2008; De Angelis *et al*., 2013; Zheng *et al*., 2015) and the plant communities, on which fungi depend, which provides them with resources and acts as hosts for some fungal groups (Ferrer and Gilbert, 2003; McGuire *et al*., 2011; Wardle *et al*., 2011; Mueller *et al*., 2014; Urbanová *et al*., 2015). The quantity and nutritional quality of litter produced by the plant community may favor specific soil fungi (Gholz *et al*., 2000), thereby structuring fungal community composition (Prescott and Grayston, 2013). Among soil properties, soil carbon affects soil fungal decomposers (De Graaff *et al*., 2010; Chapin *et al*., 2011) and arbuscular mycorrhizal (AM) fungi by enhancing their mineral nutrient uptake (Leigh *et al*., 2009) and by influencing plant growth (Zink and Allen, 1998). Because of their close associations with plants, fungi showed particularly high sensitivity to shifts in vegetation (Lauber *et al*., 2008). Trees, as the main plants in forest ecosystems, affect soil fungi passively by shading the ground and by regulating soil temperature and moisture as well as by influencing understory vegetation (Gömöryová *et al*., 2013). Soil fungal communities are also shaped by the local plant community. Our results evidenced that management is an important global driver of change in below ground organisms. 60% thinning was associated with changes in soil fungal communities and these effects can persist long after management as demonstrated by data showing that the number of fungi and bacteria colonies was much more in 60% thinning in respect to natural forest and other treatments. This means that the seasonal climatic variation, influenced the number of colonies with a larger quantity of microorganisms in summer and less in winter. These data demonstrate that the fungi and bacteria reached a condition of balance, over time, both with the climate and with the treatments. Although abiotic factors have been considered as the main driver of variation in soil of fungal and bacteria community, we put in light the importance of biotic factors that differed between the study area with differing management. Biotic factors shaped the composition of soil communities more strongly in areas with 60% thinning, suggesting that the biotic heterogeneity drives the number of fungal and bacteria colonies more strongly than other factors. The greater number of colonies observed in 60% thinning compared to the other treatments, was associated with the undercanopy vegetation diversity as already showed by (Swenson *et al*., 2012), edaphic factors become very important in areas subject to forest management in regulating soil ecosystem.

Table 13. Summer and Winter 2014. Colony forming unit (CFU x 10^4 g^{-1} dry soil) for fungi, (CFU x 10^5 g^{-1} dry soil) for bacteria in soil under *Pinus laricio* plantation differently managed.

Seasons	**Management**	**Fungi**	**Bacteria**
	Thinning 30%	3.5^{b}	4.0^{bc}
Summer	**Thinning 60%**	4.9^{a}	4.8^{a}
	Clear cut	2.7^{d}	3.8^{c}
	Natural forest	3.3c	3.0^{d}
	Thinning 30%	2.6^{a}	3.4^{b}
Winter	**Thinning 60%**	2.7^{a}	3.6^{a}
	Clear cut	2.8^{c}	3.0^{c}
	Natural forest	3.0^{b}	3.1^{c}

*Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$).

Table 14. Analysis of variance of the effects of treatments, seasons and their interactions on fungi and bacteria colonies in soil under *Pinus laricio* (Summer and Winter 2014).

		Fungi	**Bacteria**
R^2		0.985	0.978
	Treatments	263.438**	326.250**
***F* ratio**	**Seasons**	1020.938**	540.000**
	Interaction	408.437**	97.500**

***p<0.05*

Table 15. Summer and Winter 2015 Colony forming unit (CFU x 10^4 g^{-1} dry soil) for fungi, (CFU x 10^5 g^{-1} dry soil) for bacteria in soil under *Pinus laricio* plantation differently managed.

Seasons	**Management**	**Fungi**	**Bacteria**
	Thinning 30%	4.8^c	5.0^c
Summer	**Thinning 60%**	5.9^a	6.1^a
	Clear cut	4.9^c	4.8^c
	Natural forest	5.2^b	5.3^b
	Thinning 30%	2.1^c	2.8^c
Winter	**Thinning 60%**	3.6^a	3.8^a
	Clear cut	1.2^d	2.5^d
	Natural forest	2.4^b	3.1^b

*Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$).

Table 16. Analysis of variance of the effects of treatments, seasons and their interactions on fungi and bacteria colonies in soil under *Pinus laricio* (Summer and Winter 2015).

		Fungi	**Bacteria**
R^2		0.997	0.996
	Treatments	790.937**	534.659**
***F* ratio**	**Seasons**	12398.437**	8345.568**
	Interaction	130.937**	2.841**

**p<0.05

Table 17. Summer 2016. Colony forming unit (CFU x 10^4 g^{-1} dry soil) for fungi, (CFU x 10^5 g^{-1} dry soil) for bacteria in soil under *Pinus laricio* plantation differently managed.

Management	Fungi	Bacteria
Thinning 30%	5.1^c	5.3^b
Thinning 60%	6.2^a	6.5^a
Clear cutt	4.9^c	5.0^c
Natural forest	5.5^b	5.4^b

*Different letters in the same column indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 18. Analysis of variance of the effects of treatments on fungi and bacteria colonies in soil under *Pinus laricio* (Summer 2016).

		Fungi	**Bacteria**
$\mathbf{R^2}$		0.960	0.980
***F* ratio**	**Treatments**	161.364^{**}	322.500^{**}

$^{**}P$ <0.05

4.4 Soil microarthropods

Soil fauna is essential for ecosystem dynamics as it is involved in biogeochemical processes, promotes nutrient availability, and affects the animal communities associated with plants. Soil fauna has been found to accelerate litter decomposition in some ecosystems, with calls for the need to include them in global models of C and N cycling. Among soil microarthropods, collembola and mites play a primary role in organic matter breakdown and its incorporation to the soil system (Hooper et *al*., 2000). The ingestion and subsequent excretion of dead plant material accelerate plant material decomposition, thus facilitating the colonization of plant material by fungi and other microorganisms by increasing interfacial contact (Van Vliet and Hendrix, 2007; Siddiky et *al*., 2012). They also redistribute spores and bacteria across the soil layers (Palacios-Vargas et *al*., 2000). The composition and abundance of the soil microarthropod assemblage are determined by several factors, including interactions with other soil inhabitants (nematodes, enchytraeids, fungi, and bacteria), and soil organic matter content (Eaton *et al*. 2004), temperature, moisture, pH, (De la Peña, 2009), and compaction (Cutz-Pool *et al*., 2006). Thus, changes in these conditions might affect the abundance and distribution of soil microarthropods (Filser *et al*., 2002). Understanding the relationship between the diversity of organisms above and below the ground is necessary to infer the processes determining the structure and functioning of communities, both locally and regionally (Hooper *et al*., 2000; Soler *et al*., 2005; Moreau *et al*., 2006; González and Muller, 2010; Kostenko *et al*., 2012). This knowledge can also provide useful information for better understanding the dynamics between-species, which can support the formulation of successful ecosystem conservation and restoration programs. Authors as Razo-González *et al*. (2014) have found that the vegetation cover not only affected soil characteristics at a given site (open vs. closed), but also influenced the diversity and composition of soil microarthropod community, evidencing that a more dense vegetation cover at closed sites created a more stable environment, with higher soil moisture and less temperature variations. Van Straalen (1998) and Cutz-Pool *et al*. (2006) reported a higher diversity for the arthropod community inhabiting the soil of closed sites. However, no significant difference was observed in the taxonomic diversity of the microarthropod community of the open versus closed site, even if the mean value of the Shannon–Wiener index was slightly higher in the closed site. The composition of the microarthropod community reflects changes in micro environmental conditions. Our results showed that QBS-ar value was significantly higher in 60% thinning than in other sites (Table 19) ($p < 0.05$). This findings can be related to the habitat complexity, due to the presence of an undergrowth herbaceous vegetation which is missing in the others treatments. QBS-ar value significantly changed both summer and winter. The highest values of QBS-ar observed in winter in all the experimental plots (Table 19), were due to the presence of larvae, which were lacking in the summer season. Many works reported a high population of soil microarthropods during rainy season and a sharp decline during summer months (Reddy and Venkataiah, 1990; Borah and Kakati, 2014). Analysis of variance showed that treatments affected QBS-ar more than seasons; the interactions of the two factors, treatment and season, had the lowest effect with respect to the factors individually analyzed (Table 20). 60% thinning had the highest amount both of mites and collembola. Mites were more in number than collembola (Table 21). Mites and collembola numbers were high in winter than in summer. Analysis of variance show than seasonal variation affected mites more than treatments, while treatments influenced collembola more than seasons (Table 22).The soil microflora and fauna complement each other in communition of litter, mineralization of essential plant nutrients and conservation of these nutrients within the soil system (Marshall, 2000). Free living soil mites and collembola are abundant soil organisms that are sensitive to soil perturbations in forest practices and their number and diversity often get reduced affecting their ecosystem services (Minor and Cianciolo, 2007). Mites and collembola are thus the expression of soil quality; our results are in agreement with the findings of Parisi *et al*. (2005) suggesting that the higher is the soil quality, greater will be the number of microarthropods well adapted to soil habitats. Our results also showed that the abundance of the microarthropods and the QBS-ar index changed in respect to soil moisture and temperature. Since QBS-ar is based on qualitative data of soil fauna, in a pine forest this index seems to be a good soil quality indicator. In forest ecosystem, QBS-ar could be used also as an efficient index for evaluating the impacts of forest management on soil. At the same time, QBS-ar can be a valuable tool in ecosystem restoration programs to monitor the development of soil functions and biodiversity and to prevent the negative effects of soil management practices.

Table 19. Biological quality index expressed as QBS-ar of soils under *Pinus laricio* plantation differently managed and over seasons.

	Year 2014		Year 2015		Year 2016
Management	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**
Thinning 30%	125^{b*}	136^{b}	131^{b}	141^{b}	134^{b}
Thinning 60%	149^{a}	161^{a}	151^{a}	166^{a}	156^{a}
Clear cut	99^{c}	113^{c}	102^{c}	118^{c}	105^{c}
Natural forest	129^{b}	138^{b}	132^{b}	140^{b}	133^{b}

*Different letters in the same column indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 20. Analysis of variance of the effects of treatments, seasons and their interactions on biological quality index expressed as QBS-ar.

		Summer and Winter 2014	Summer and Winter 2015	Summer 2016
R^2		0.998	0.998	0.998
***F* ratio**	**Treatments**	6036.250**	5895.938**	3275.00**
	Seasons	1983.750**	2250.938**	-------
	Interactions	16.250**	55.938**	--------

**p<0.05

Table 21. Effects of different silvicultural treatments, over seasons, on mites and collembola amount in *Pinus nigra* spp. *laricio*.

Management	Year 2014				Year 2015				Year 2016	
	Summer		Winter		Summer		Winter		Summer	
	Mites	**Collembola**	**Mites**	**Collembola**	**Mites**	**Collembola**	**Mites**	**Collembola**	**Mites**	**Collembola**
Thinning 30%	63^{b}	45^{b}	144^{c}	54^{b}	65^{c}	49^{b}	164^{b}	62^{a}	78^{b}	47^{c}
Thinning 60%	83^{a}	48^{a}	358^{a}	57^{a}	113^{a}	52^{a}	282^{a}	56^{b}	130^{a}	55^{a}
Clear cut	55^{c}	12^{d}	126^{d}	26^{c}	48^{d}	22^{d}	132^{c}	30^{d}	66^{c}	51^{b}
Natural forest	60^{b}	30^{c}	210^{b}	25^{c}	71^{b}	32^{c}	289^{a}	42^{c}	81^{b}	31^{d}

*Different letters in the same column indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 22. Analysis of variance of the effects of treatments, seasons and their interactions on mites and collembolan amount.

		Summer and Winter 2014		Summer and Winter 2015		Summer 2016	
		Mites	**Collembola**	**Mites**	**Collembola**	**Mites**	**Collembola**
R^2		1.00	0.997	1.00	0.996	0.999	0.992
***F* ratio**	**Treatments**	51300.938**	3692.500**	39667.500**	3010.938**	5986.875**	830.00**
	Seasons	312120.938**	735.00**	304593.750**	1148.438**	-------	-------
	Interactions	33118.438**	257.500**	14646.250**	53.437**	--------	--------

$^{**}p<0.05$

4.5 Dendrometric parameters and wood quality

The dendrometric parameters (diameter and height) changed with the treatments. H/D ratio in 60% thinning was significantly lower than in 30 % thinning and natural forest. H/D ratio is considered an index of tree stability, and of resistance to wind throw (Navratil, 1996). Our results suggested a positive effect of 60% thinning on the mechanical stability of the trees (Table 23). A low H/D ratio usually indicates a longer crown, lower centre of gravity, and a better developed root system. Therefore, trees with higher H/D ratio are much more susceptible to wind damage (Eguakun and Oyebade, 2015). Pardé and Bouchon (1988) consider stable stands with H/D ratio <80 and unstable with H/D ratio >100. Minimal damage occurred in stands for which the value of this coefficient was below 80. For South African radiata pine plantations, Gadow and Bredenkamp (1992) and Hinze and Wessels (2002) reported that an individual H/D ratio greater than 85 implies high tree instability due to wind or snow. Castedo-Dorado *et al*. (2000) found that snow and wind damage to radiata pine plantations in Galicia mainly occurred in stands with H/D ratio greater than 88, especially in mountainous areas. Since H/D is primarily a function of spacing, it enables improvement of stand stability through density management (e.g. Wonn and O'Hara, 2001; Cameron, 2002). In according with our results when intertree spacing increases, H/D decreases; the additional growing space provided by wider initial spacing or thinning allows residual trees to maintain rapid diameter growth, thereby reducing their susceptibility to wind or snow damage. Information on the H/D for tree species is therefore essential when wind or snow damage must be addressed in thinning schedules (Castedo-Dorado *et al*., 2009).

In addition, Crechi *et al*. (2005) showed an effect of thinning on DBH, evidencing that intense thinning improved product quality by increasing DBH mean. Our results were in agreement with these findings showing significant differences (p <0.05) in DBH between the different treatments, and evidencing that 60% thinning was the management practice that mostly and significantly increased DBH (Table 23). The greater value of the wood density was observed in 60% thinning compared to 30% thinning and natural forest (Table 23).

Wood density considered an indicator of wood quality (Barnett and Jeronimidis, 2003), is a key functional trait of woody plant species, with important effects on ecosystem processes including carbon storage in biomass and necromass (Chave *et al.*, 2009). Basic density is closely related to end-use quality parameters such as pulp yield and structural timber strength (Harvald and Olesen, 1987). Schniewind (1989) showed that much of the variation in wood strength, both between and within species, can be attributed to differences in wood density, that is recognized as a general indicator of cell size and a good predictor of strength, stiffness, ease of drying, machining, hardness and various paper making properties (Cown, 1992). Previously, Brazier and Howell (1979) expressed the opinion that density is one of the most important properties influencing the use of timber. Researches has successively shown that higher density species tend to have stronger timber than lower density species (Addis *et al.*, 1995; Walker and Butterfield, 1996).

In short we can suggest that thinning from below approach should create open large enough to allow sufficient light penetration to the understory, to favour a balance between above and below ground processes, and a better ecosystem functioning. Thus selective thinning should be encouraged for a proper and sustainable forest management that also increase forest productivity and quality.

Table 23. Number of plants (NP), dendrometric parameters (diameter at breast height (DBH, cm), height (H, m), ratio H/D (height/diameter at breast height)), and wood density (WD, g/cm^3) of *Pinus laricio* trees differently managed.

Management	**NP**	**DBH**	**H**	**H/D**	**WD**
Thinning 30%	1354^b	26.9^b	20.9^a	77.6^a	0.53^b
Thinning 60%	780^c	30.3^a	20.8^a	68.6^b	0.58^a
Natural forest	1935^a	23.6^c	18.4^b	77.9^a	0.52^b

*Different letters in the same column indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 24. Analysis of variance of treatments number of plants (NP), dendrometric parameters (diameter at breast height (DBH, cm), height (H, m), ratio H/D (height/diameter at breast height)), and wood density (WD, g/cm^3) of *Pinus laricio* trees differently managed.

		NP	DBH	H	H/D	WD
R^2		1.00	0.900	0.511	0.961	0.538
***F* ratio**	**Treatments**	19270.691**	67.500**	7.850**	186.577**	8.750**

***p<0.05*

5. CONCLUSION AND FUTURE PERSPECTIVES

Close to nature forest management appears to be a flexible toolbox, in which thinning is a highly useful tool to secure the sustainable development of forests. These tools mimic the openings of various sizes that follow moderate disturbance events. In particular, this study provide the necessary information to design a sustainable forest management system, putting in evidence the importance of thinning size in maintaining or improving soil and plant quality. Our results showed that in general *Pinus laricio* forest stand the ranking of soil quality among the treatments, was 60% thinning> natural forest > 30% thinning and clear cut. The same ranking was observed for plant quality parameters as suggested by the results related to dendrometric parameters evidencing that pinus accretion (diameter and height), wood density and mechanical stability of the trees were increased by 60% thinning. From an ecological point of view the range of different thinning sizes can create diverse habitats with serious impact on microclimate, fauna and microflora, with consequence on soil fertility and quality. Therefore, a 60% thinning system is particularly suited to the restoration of forest stand also inside protected areas as Aspromonte because is able to improve plant stability, wood density and merchantable timber quality, respecting the soil ecosystem functioning.

Further investigation will be focused on the effects of different thinning intensities on forest carbon sequestration. Changes in soil dynamics drive CO_2, CH_4, and N_2O emissions, thus the challange is to find the best thinning option to minimize the impact on green hause gas (GHG) emissions and at the same time to improve forest productivity and quality.

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