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**SOIL BIOLOGICAL INDICATORS AND CAESIUM-137 TO  
ESTIMATE SOIL EROSION IN AREAS WITH DIFFERENT  
FOREST SYSTEM MANAGEMENT**

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## **Abstract**

Forest management, if improperly settled, besides threatening biodiversity, could decrease and/or stop the ability of forest ecosystem to provide other services. Forests provide crucial services for human well-being and economic development. In addition to wood and fiber, they provide numerous non-wood products such as food, freshwater, and fuel, or services as climate and diseases regulation, recreation and preservation of biodiversity, driving the sustainable growth (IUCN). Nowadays, a prominent challenge is how to manage forests for timber and bioenergy production maintaining, at the same time, long-term conservation/implementation of the forest ecosystem functioning. Soil has a pivotal role in keeping high the productivity of forest ecosystem but its physical and chemical properties are highly influenced by changes in forest stand structure, density and composition of species which correlate with thinning intensity, interval, and method.

Tools are needed to verify whether the silvicultural management is feasible in practice, and how it can affect the whole ecosystem. The relevance of the subject is particularly important in regions ('marginalised contexts') where the quality of the environment is already poor. Providing high quality information on the spatial extent of land degradation under silvicultural management meet the obligations claimed by FAO. One major aspect of land degradation is the loss of top soils owing to erosional processes, which have multiple environmental and socio-economic consequence. According to FAO (2014) soil degradation is defined as the reduction in the capacity of land to provide ecosystem goods and services securing its function for all beneficiaries of the land. As such, the main objective of this study was to use a combination of different methodology, soil quality index and caesium ( $^{137}\text{Cs}$ ), to individuate the most appropriate forestry practices to manage a beech (*Fagus sylvatica* L.) forest in such a way that soil erosion is minimized. Thus, we assessed the effects of tree-oriented silviculture (innovative forest management system) on soil properties in respect to thinning (traditional forest management system) and unmanaged forest.  $^{137}\text{Cs}$  results evidenced that both thinning treatments affected soil properties. The innovative treatment showed the highest impact. The amount of small-sized particles enhanced when the intensity of thinning increased. A strong decrease in soil organic carbon (OC) was related to thinning. In the upper soil layer, OC was found positively correlated with microbial biomass (MBC), fluorescein diacetate hydrolase (FDA), water soluble phenols (WSP),



ergosterol (ERG), C/N ratio, nitrogen (N) and also with  $^{137}\text{Cs}$ . Moderate to no correlations, in the subsurface layer, highlighted the immediate impact of management techniques on the surface layer and then on the underlying ones. In the subsurface layer, OC maintained its positive correlation only with MBC, WSP and  $^{137}\text{Cs}$ .

$^{137}\text{Cs}$  was correlated in both soil layers with OC, N and WSP. The overall results suggest that WSP being always correlated to OC and  $^{137}\text{Cs}$ , may be considered as indicators of soil erosion, and can be used, even in the case of the absence of  $^{137}\text{Cs}$  in the sediment, to evidence changes in soil properties that could be the starting point of soil erosion. Regarding soil quality, OC was correlated to more than one soil parameter, suggesting that it is the data in combinations rather than a single data that reflect soil fertility loss. These results can be fruitfully used by decision makers to program and optimize the use of thinning practices for long-term forest sustainability.

## **Riassunto**

Le foreste forniscono servizi cruciali per il benessere umano e lo sviluppo economico. Oggi, una sfida importante è capire come gestire le foreste per la produzione di legname e bioenergia mantenendo, allo stesso tempo, la conservazione e il funzionamento dell'ecosistema forestale.

Per tale motivo, la comunità scientifica e gli operatori del settore hanno preso in considerazione diverse misure per mitigare gli effetti del degrado e della perdita delle foreste, tra queste, la sua gestione sostenibile. La maggior parte di questi approcci si concentra tuttavia su un singolo aspetto del problema, facendo intendere come venga a mancare il concetto di salvaguardia e conservazione del funzionamento dell'ecosistema nel suo insieme.

Il suolo ha un ruolo fondamentale nel mantenere alta la produttività dell'ecosistema forestale, e rappresenta la risorsa più scarsamente rinnovabile dell'intero comparto. I suoli forestali, oltre a costituire la base vera e propria del bosco, rappresentano una preziosa riserva idrica e nutritiva ed un importantissimo serbatoio di  $\text{CO}_2$ . Le pratiche di gestione forestale se non opportunamente indirizzate possono avere ripercussioni

negative sulle proprietà fisico chimiche del suolo e sulla biodiversità con conseguenze sull'efficienza ecologica del sistema stesso.

Fornire informazioni dettagliate sull'estensione spaziale del degrado del suolo, in regime di gestione forestale, è uno degli obblighi prefissati dalla FAO. Un aspetto importante che determina i fenomeni di degrado del suolo è la perdita di suolo superficiale dovuta ai processi erosivi, che hanno molteplici conseguenze ambientali e socio-economiche. In bosco, questi fenomeni erosivi sono influenzati in misura differente dalla continuità di copertura arborea, che è legata alle pratiche di gestione.

L'obiettivo principale di questo studio è stato quello di utilizzare una combinazione di diverse metodologie, indicatori di qualità del suolo e cesio ( $^{137}\text{Cs}$ ) per stimare il grado di erosione del suolo, al fine di individuare le pratiche forestali più appropriate per gestire boschi di faggio (*Fagus sylvatica* L.) in modo da limitarne le perdite di suolo.

Sul suolo sono stati valutati gli effetti di un sistema di gestione forestale innovativo (27% di volume totale utilizzato) di un sistema di gestione forestale tradizionale (12% totale utilizzato) rispetto alla foresta non gestita (da oltre 30 anni).

Al fine di raggiungere gli obiettivi prefissati, oltre all'erosione sono stati valutati parametri fisici, chimici e biochimici del suolo. Tra i parametri fisici sono stati determinati gli aggregati del suolo e la tessitura. Tra quelli chimici oltre al carbonio organico, sono stati determinati l'azoto, gli ioni, i fenoli, il pH e la conducibilità. Tra quelli biochimici, oltre alle attività enzimatiche (FDA, DHA, CAT), sono stati determinati, la biomassa microbica e l'ergosterolo (biomarker fungino) e gli indici di umificazione. Inoltre, sono stati monitorati i microartropodi del suolo (QBS-ar), quali marker dello stato di salute del suolo.

I risultati del  $^{137}\text{Cs}$  hanno evidenziato che entrambi i trattamenti di diradamento hanno influenzato le proprietà del suolo. Il trattamento innovativo è stato quello che ha avuto il maggiore impatto sulle proprietà del suolo innescando fenomeni erosivi. La quantità di particelle di piccole dimensioni aumentava in funzione dell'intensità del diradamento. Una forte diminuzione dell'OC del suolo era correlata al diradamento. Nello strato superficiale di suolo, il carbonio organico (OC) era positivamente correlato con la biomassa microbica (MBC), fluoresceina diacetato (FDA), fenoli (WSP), ergosterolo (ERG), rapporto C/N, azoto (N) e anche al  $^{137}\text{Cs}$ . Correlazioni quasi assenti sono state trovate nello strato sottostante (15-30 cm) tra i suddetti parametri, suggerendo che

l'impatto delle tecniche di gestione è immediato sullo strato superficiale rispetto a quello sottostante. Nello strato 15-30 cm, OC ha mantenuto la sua correlazione positiva solo con MBC, WSP e  $^{137}\text{Cs}$ .

Il  $^{137}\text{Cs}$  è risultato essere correlato in entrambi gli strati del suolo con OC, N e WSP.

I risultati complessivi suggeriscono che il WSP sempre correlato con OC e  $^{137}\text{Cs}$ , può essere considerato un indicatore dell'erosione del suolo e può essere utilizzato, anche in caso di assenza di  $^{137}\text{Cs}$  nel sedimento, per evidenziare cambiamenti nelle proprietà del suolo che potrebbero essere legate ai fenomeni erosivi. Per quanto riguarda la qualità del suolo, l'OC è stato trovato essere correlato a più di un parametro del suolo, suggerendo come siano più combinazioni anziché un singolo dato a riflettere la perdita di fertilità del suolo stesso. Questi risultati possono essere utilizzati dagli organi competenti per programmare e ottimizzare l'uso di pratiche di gestione forestale per implementare la sostenibilità delle foreste a lungo termine.

**Keywords:**  $^{137}\text{Cs}$ , biological soil properties, microarthropods, thinning intensities, soil erosion, *Fagus sylvatica*, forest management.

## 1. Introduction

Nowadays, forests play a key role in mitigating climate change, conserving biodiversity, soil and water. However, in addition to this, forests provide crucial services for human well-being and economic development. Unfortunately, forests are currently under threat by human activities and economic interests including fire, pollution, inappropriate management and erosion. In fact, forest management, if inappropriate, can be transformed into a tool capable of degrading the entire ecosystem, especially in terms of biodiversity loss and soil degradation. It is well known that incorrect management practices accelerate forest soil loss rates influencing the soil-related functions such as carbon storage, biodiversity lost and consequently soil ecosystem functioning (Van Oost et al., 2005, Ojea et al., 2012, Gamfeldt et al., 2013). Thence, forests play a key role in providing protection against runoff and soil erosion (Miura et al., 2003). Ecological factors like canopy cover, tree species, forest vertical stratification, but also different types of forest management are crucial in increasing rainfall interception and reducing the magnitude of soil loss (Elliott et al., 1998; Hartanto et al., 2003). Relationships between management and productivity are not simple but are rather extraordinarily complex, reflecting interactions among management system, soil biological activity, nutrient cycling, and climate (Muscolo et al., 2014). Natural undisturbed forests that are generally unaffected by soil erosion processes, become susceptibility to soil degradation when the area undergoes forestry activities (Swanston and Swanson, 1976; Stott et al., 2001). Thinning is a well-known silvicultural practice used for forest conservation (Fredericksen et al., 2003; Stephens et al., 2005) whose primary aim is to increase the productivity of selected trees. Thinning practices as already demonstrated by Settineri et al. (2018) may, in some cases, affect soil biological properties and their effects depend on the intensity of cutting. It is well known that the use of different types of thinning (traditional or innovative) changes the performance of soil affecting SOM dynamics Johnson (1992), Neary et al. (1999), Balboa-Murias et al. (2006) and Nilsen and Strand (2008) in different forest ecosystems. The intensity of cutting causes also significant short-term increases in sediment mobilisation and sediment yield. Increased soil loss rates have been associated with forest harvesting worldwide (see Porto et al., 2009; Altieri et al., 2018). Forest management practices could accelerate soil loss rates influencing the soil-related functions such as carbon storage, biodiversity as well as soil ecosystem functioning (Van Oost et al., 2005; Ojea et al., 2012; Gamfeldt et al., 2013).

In the disturbed mountains of Calabria, high soil loss rates (100 to 150 Mg ha<sup>-1</sup> y<sup>-1</sup>) have been observed during an experimental investigation by Sorriso-Valvo et al. (1995). For all these reasons, the attention is now focused on the use of forest management practices able to maintain/increase the productivity reducing the risk of soil degradation.

Research on the impacts of forest management activities on soil erosion and the subsequent effects on forest productivity are limited yet. Forest management if not properly settled can cause soil erosion processes, with a consequent reduction in soil productivity and environmental sustainability. In order to limit the triggering of erosive phenomena and to find useful countermeasures, there is the need to evaluate and analyze through the use of early warning indicators the effects of forest management practices in terms of soil loss.

Numerous attempts to prevent soil degradation processes have been made, mainly based on models and calculation procedures that require detailed information about the climate, topography, soil and plant characteristics (Morgan et al., 1992; Renard et al., 1994; De Roo et al., 1996). However, their utilities remain limited to the geographic areas for which calibration and validation were settled. Alternative approaches, based on the use of measurements in experimental plots or catchments (Hsieh et al., 2009; Anache et al., 2017), showed severe limitations as they were associated with point data and didn't give details on spatial distribution of erosion.

Recent work to document rates and patterns of soil redistribution by erosion processes used environmental radionuclides, particularly caesium-137 (<sup>137</sup>Cs), (Walling, 1998; Porto et al., 2001, 2003). In most environments, the <sup>137</sup>Cs fallout reaching the land surface is rapidly and strongly adsorbed by the surface soil and its subsequent redistribution within the landscape occurs in association with the erosion, transport and deposition of soil and sediment particles. Caesium-137 has a half-life of 30.2 years and the measurement of its spatial distribution in the landscape represents a validated method for estimating erosion and deposition rates, both in cultivated soils (Porto and Walling, 2012) and in forested areas (Porto et al., 2001; Di Stefano et al., 2005).

## **1.1 Forest management**

The environmental quality and the sustainability of forests are nowadays a topic of public interest and of great relevance. Often, the scientific community, and not only, links the sustainability of forests to their silvicultural management.

Forest management involves the integration of silvicultural practices and business concepts (e.g., analysing economic alternatives) in such a way as to best achieve a landowner's objectives (Bettinger et al., 2016). In addition, a recognition of the important ecological and social concerns associated with a forest may influence the choice of management practices. In a more general way, forest management can involve the collective application of silvicultural practices so that an entire forest remains healthy and vigorous by imposing treatments on the various stands (Heiligmann, 2002). The range of forest management activities can include those focused on the economics of forest businesses, or on the ecology of the ecosystem (Bettinger et al., 2016). Activities can include tree planting, herbaceous weed control, fertilization, pre-commercial thinning, commercial thinning, final harvests, harvests for habitat improvement, preservation, road construction, road obliteration, and prescribed fire, among others (Bettinger et al., 2016).

Forest management can modify the original composition of a forest by removing and/or replacing tree species, by altering the structure of the age class, by exporting the biomass and by modifying the proportion of dead wood (Paillet et al., 2010). Additionally, forest practices can be also associated with adverse environmental effects such as increased runoff and erosion, loss of soil fertility and biodiversity reduction (Cossalter and Pye-Smith, 2003).

Therefore, starting from the idea that any intervention in the forest can certainly affect ecosystem balance (negatively or positively), appropriate forest management techniques have to be selected to preserve sustainably forest functioning. Thinning is a practice in forest ecosystem management (Zak et al., 2003), with a significant influence on tree growth, species, structure composition (Kang et al., 2014; Dieler et al., 2017) and habitat conditions (Zhang et al., 2001).

As consequence, thinning affects also soil properties as result of changes in key microclimatic conditions, such as light penetration, air movement and temperature (Ma

et al., 2010; Wubet et al., 2012) as well as in microbial communities (Hu and Zhu, 1999) biomass, root density, nutrient balance and organic matter turnover (Chantigny, 2003). It has also been shown that thinning influences the understory organisms (Bender et al., 1997; Kerr, 1999; Atauri et al., 2004) which in turn affect the functioning of soil ecosystem. Zhao et al. (2014) demonstrated that the decrease in density of forest canopy generated by thinning, increased light intensity and temperature on the ground, which accelerated the decomposition rate of litter, increasing humus content and permeability of soils. Zhang et al. (2001), found 2 years after thinning and in different forest soils, an increase in microbial biomass, enzymatic activities, total porosity and nutrients. Subsequently, Chi et al. (2006) reported that faint intensities of thinning improved the physical-chemical properties of soils, while medium or intense thinning did not influence soil physical-chemical conditions compared to unmanaged forest. Contrasting results were found for enzyme activities. Garcia et al. (1997) with a 7 years thinning program, showed that the activities of dehydrogenase, catalase, urease and phosphatase in soil were significantly reduced, while protease activity did not change. Conversely, Muscolo et al. (2007), Yu et al. (2008), Xu et al. (2008) and Yang et al. (2017) showed that soil enzyme activities were positively affected by thinning. Zhao et al. (2014) confirmed the previous findings showing under moderate and intense thinning an increase in catalase and urease activities. Muscolo et al. (2015) evidenced, in a beech forest stand under different thinning intensities, that FDA (fluorescein diacetate hydrolase) was the enzyme whose activity increased strongly.

In larch-fir forest stands, with 40% thinning intensity, soil nutrients did not change significantly, indicating that the long-term effects of thinning were not significant (Wang et al., 2009). The contrasting results, reported in literature, suggested that the effects of thinning on soil properties depended on the method, time, intensity, types of forest and other minor aspects (Li et al., 2003).

Bacteria and fungi, that play an important role in the ecological processes in forest ecosystems (Levy-Booth et al., 2010), are highly sensitivity to shifts in vegetation (Maassen, 2006; Lauber et al., 2008), as well as ground dwelling arthropods and prey for vertebrates (Jokimäki et al., 1998). Besides being major engineers and potential regulators of ecosystem condition (Schowalter, 2000), soil microarthropods have been shown to respond sensitively to forest management (Buddle et al., 2006; Venier et al., 2017). Yi and Moldenke (2005) reported that thinning intensity was correlated with

high abundance and diversity of epigeic macro arthropods. This increase was correlated with a decrease in litter moisture during the dry-season. Further studies of Yi and Moldenke (2005) showed that ground-dwelling arthropod diversity were higher in heavy and medium thinning with gap treatments than control and slight thinning treatments. Strong direct effects on the population and number of microarthropods were also due to the reduction in tree density, which caused changes in the microclimate such as the availability of light, water and nutrients (Schowalter et al., 1986; Amman et al., 1988; McMillin and Wagner, 1993). In turn, changes in the amount of arthropods significantly affected forest productivity and nutrient cycling (Schowalter et al., 1986).

In conclusion, as forest management practices influence contemporarily multiple factors of soil ecosystem, if not properly settled, can cause soil quality loss and erosive phenomena.

## **1.2 Soil quality: an overview**

A more modern view of forestry must lead to study the interactions between management and the effects it can cause on the surrounded environment, for the correct preservation of the natural resources in the long term. The quality of the environment and forest should be assessed starting from the quality of all their key components, such as soil, air, water and also from the products and services that come from the forest. Forestry community up to now considered soils as simply “part of the forest”, as to a separate resource in its own right, and have not generally recalled the concept of soil as a key component of sustainable forestry (Burger and Kelting, 1998).

Soil has always provided the foundation for trees and entire forests helping to regulate important ecosystem processes, supplying nutrients, moisture for growth, storing elements for recycling back to trees. Soils are habitats for animals and microorganisms which digesting organic matter and mixing it with mineral soil, contribute to soil structure, porosity, and nutrient availability. Soils have the unique ability to sequester and store large amounts of carbon (C). They are estimated to contain about two to three times the amount of C stored in the atmosphere and vegetation combined. Soil characteristics depend on forest vegetation, climate, parent material, and organisms.



Soil quality, key factor for the growth of plants, is defined as the capacity of a soil to function within an ecosystem, to sustain biological productivity, to maintain environmental quality, and to promote plant and animal health (Doran et al., 1996b). The concept of quality is functional; includes variables that serve to evaluate the condition of the soil, or soil quality indicators (SQI). The SQI are measurement tools that provide information about the properties, processes and characteristics of the soil (Bremer and Ellert, 2004). These SQI are measurable attributes that reveal the response of the productivity or functionality of the soil to the environment, and indicate whether the quality of the soil improves, remains constant or decreases over time (Ghaemi et al., 2014). They give information on the effect of change in the use of the soil and the impact of forest practices on its degradation or functioning (Astier et al., 2002).

For this reason, soil quality cannot be separated from the sustainable management of the whole forest ecosystem, in which soil plays a key role. Physical, chemical and biological characteristics of soil are three important aspects of its quality that differ as time of response to external changes (Syers et al., 1995; Gil and Gil, 2011). Since soils often react slowly to changes in land use and management, it can be more difficult to detect changes in soil quality before non-reversible damage has occurred than for the quality of water and air (Nortcliff, 2002). Therefore, an important component of soil quality concepts is the identification of a set of sensitive indicators or attributes which reflect the capacity of a soil to fulfil its functions.

Schjønning et al. (2004) argues that it is difficult to find a proper threshold of an indicator due to the vast number of soils and ecosystems addressed. He describes that an indicator's threshold links to resilience or a boundary between sustainable and unsustainable values.

According to Hopkins and Gregorich (2013), the main challenge in developing indicator sets for SQ assessment is the identification of indicators that are meaningful, readily measurable, cost-effective and which can be compared with data in existing databases. Furthermore, SQI identify both the condition of the soil resource and the economic and environmental sustainability of land management practices (Doran, 2002). The main challenge is to identify the soil indicators that respond rapidly to soil management and show whether these practices have a positive or negative feedback (Papp, 2016).

### **1.3 Soil quality indicators**

The first soil study approaches tended to evaluate soil quality in a reductionist manner, which consisted of measuring an independent set of soil properties, sometimes physical, sometimes chemical or biological (Kibblewhite et al., 2008). To provide a complete picture of soil quality, the approach today is to identify and evaluate the set of chemical, physical and biological indicators (Frankenberger and Dick, 1983; Nannipieri et al., 1990; Dick, 1994; Gelsomino et al., 2006).

A total of 22 measurements were proposed to detect soil quality, which were grouped into i) sensitive properties such as pH, total organic carbon, bulk density, penetration resistance and earthworm counts which should be measured annually to every few years, ii) moderately sensitive properties such as cation exchange capacity and water retention which should be measured every ten years, and iii) non-sensitive properties such as particle-size distribution which should be obtained only once to establish baseline data (iSQAPER, 2016). Schipper and Sparling (2000) tested a set of 16 soil quality indicators on a set of samples from 29 sites in New Zealand and used principal component analysis (PCA) to identify the indicators with the greatest influence on the separation of samples from different land-uses (arable, grassland, native forest and plantation). A subset of six of these indicators that covered soil physical, chemical and biological properties gave a similar separation of the samples as the complete set of soil quality indicators. Numerous authors warn against using only a very limited set of indicators, they suggest instead to point out strong correlations among indicators in order to avoid unnecessary measurements, and to assess the variability of indicators measured on separate samples per site, in order to identify highly variable indicators, that are substantially scarcely sensitive to changes. Among soil physical indicators the most used are bulk density, water content, aggregate size distribution and aggregate stability. Soil organic carbon, pH, available nutrients, cation exchange capacity, and electrical conductivity, are the chemical indicators more often proposed. Soil respiration, microbial biomass and microarthropods are the biological indicators more frequently used.

**Table 1** Summary of soil health indicators used to assess soil function (Kinyangi, 2007).

| <b>Indicator</b>   | <b>Soil function</b>   |
|--|--|
| Soil organic matter (SOM)  | Soil structure, stability, nutrient retention; soil erosion (Carter, 2002)   |
| Physical: soil aggregate stability, infiltration and bulk density          | Retention and mobility of water and nutrients; habitat for macro and micro fauna (Bengtsson, 1998; Swift et al., 2004)   |
| Chemical: pH, extractable soil nutrients, N-P-K and base cations Ca Mg & K | Soil biological and chemical activity thresholds; plant available nutrients and potential for N and P as well as loss of Ca, g & K (Doran and Jones, 1996a; Drinkwater et al., 1996) |
| Biological: microbial biomass C and N; potentially mineralizable N         | Microbial catalytic potential and repository for C and N; soil productivity and N supplying potential (Cadisch and Giller, 1997; Doran and Jones, 1996b)                             |

Despite considerable effort, several authors claim that the interpretation of soil quality indicators, i.e. the establishment of target or workable ranges, will always remain contentious, which is partly due to a lack of data and in part due to the curvilinear pattern which many indicators follow (Merrington, 2006). Thus, comparative approaches may be the most intuitive and flexible way for the interpretation of data referred to soil quality.

#### **1.4 <sup>137</sup>Cs for soil erosion assessment**

Soil erosion, is regarded as one of the major and most widespread forms of land degradation, and as such, poses severe limitations to sustainable land use. Soil erosion occurs when the ground is left exposed to rain or wind energy. Raindrops hit exposed soil with great energy and easily dislodge the soil particles from the surface. In this way, raindrops remove a thin film of soil from the land surface and create what is termed sheet erosion. This erosion is the dominant form of soil degradation (Troeh et al., 1991; Oldeman, 1997). The impact of soil erosion is intensified on sloping land, where often more than half of the surface soil is carried away as the water splashes downhill into valleys and waterways. Wind energy also has great power to dislodge surface soil particles, and transport them great distances (Pimentel, 2006).

As reported by Elliot et al. (1998), erosion is also influenced by other factors such as soil structure, vegetation cover, topography, natural disturbances (fire, exceptional meteoric events, etc.) and anthropic disturbances (construction of roads, buildings, harvesting operation, forest management (Borrelli et al., 2017)). Among anthropic disturbances, incorrect forest management or deforestation can be considered important causes of rapid degradation when the soil is steep slopy or easily erodible (Pimentel, 2006).

Moving from the causes to effects, it becomes clear how soil erosion reduces the productivity of terrestrial ecosystems, increasing water runoff thereby decreasing the water infiltration and the water-storage capacity of soils (Troeh et al., 1991; Pimentel et al., 1995; Jones et al., 1997). During the erosion process also organic matter and essential plant nutrients are removed from the soil and the soil depth is reduced (Pimentel, 2006). These changes not only inhibit vegetative growth, but also reduce the presence of valuable biota and the overall biodiversity in the soil (Troeh et al., 1991; Pimentel et al., 1995). Even if it is almost impossible to separate the specific impacts of one factor from another, the loss of soil organic matter has been always correlated to other factors such as an increase in water runoff, which reduces water-storage capacity, and diminishes nutrient levels, biota biomass and biodiversity of soil ecosystems (Lal and Stewart, 1990; Jones et al., 1997). In natural stable forest ecosystems, where soil is protected by vegetation, erosion rate is relatively low, ranging from only 0.02 to 1.2 Mg ha<sup>-1</sup> (Wagenbrenner et al., 2006). In Italy, Borrelli and Schütt (2014) measured an average soil loss rate of 49Mg ha<sup>-1</sup> y<sup>-1</sup> following a tree harvesting event in the Central Apennines. However, in the disturbed mountainous areas of Calabria in Southern Apennines, high soil loss rates ranging from 100 to 150 Mg ha<sup>-1</sup> y<sup>-1</sup> were observed (Sorriso-Valvo et al., 1995).

Among the numerous studies and methodologies to quantify soil loss, erosion plots evidenced many limitations in terms of cost, representativeness reliability of the resulting data (cf. Loughran, 1989; Evans, 1995). This method is also unable to provide the detailed spatially-distributed data required to verify the new generation of distributed erosion and sediment yield models (cf. Morgan et al., 1998, De Roo et al., 1989, Nearing et al., 1989) and to interface with current developments in the application of GIS and geostatistics (e.g. Ferro et al., 1994; Desmet and Govers, 1995; Mitas and Mitasova, 1998; Molnar and Julien, 1998). The soil erosion rates can be estimated using

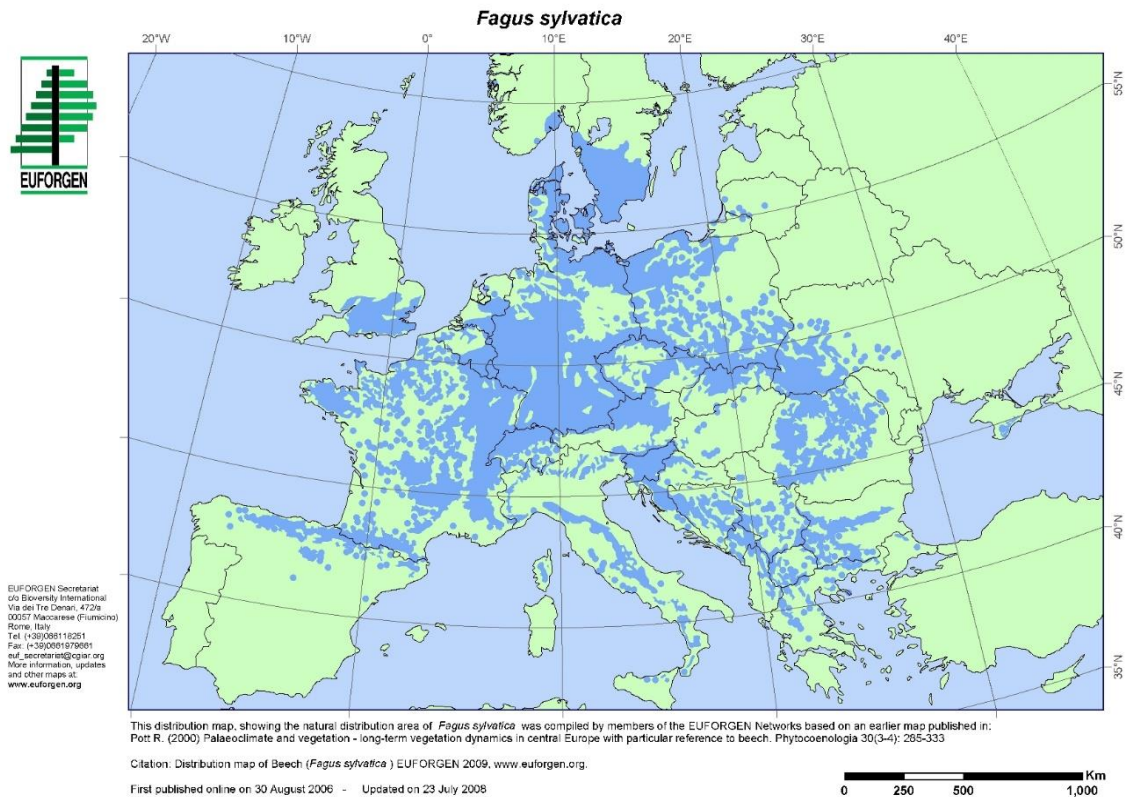
$^{137}\text{Cs}$ , a human-induced radionuclide of caesium released into the atmosphere during nuclear weapon tests more than half a century ago. (Ritchie and McHenry, 1990; Walling and Quine, 1993; Walling, 1998; Porto et al., 2001, 2009; Porto and Walling, 2012a, b).

$^{137}\text{Cs}$  technique is now widely used and offers great potential to provide retrospective information on the medium-term medium loss of soil based on a single sampling without the need to disturb the system by installing measurement equipment. The  $^{137}\text{Cs}$  technique makes use of the global fallout of bomb-derived radio caesium which occurred during a period extending from the mid-1950s to the late 1970s. In most environments, the  $^{137}\text{Cs}$  fallout reaching the land surface was rapidly adsorbed by the surface soil and its subsequent redistribution within the landscape will have occurred in association with the erosion, transport, and deposition of soil and sediment particles. Porto et al. (2001; 2014) demonstrated the feasibility of the  $^{137}\text{Cs}$  approach for forest environments, by comparing estimates of soil erosion provided by the latter with long-term measurements of sediment yield available in small afforested catchments. Correlations between the content of  $^{137}\text{Cs}$  and organic carbon in Japanese forests have been demonstrated by Takenaka et al. (1998), while Altieri et al. (2018) highlighted the relationship between  $^{137}\text{Cs}$  and the canopy cover in the Calabrian forests, demonstrating how erosion increased in absence of vegetation cover. Although  $^{137}\text{Cs}$  is a very used and efficient technique, it still requires very expensive equipment, therefore it is important to identify less demanding indicators from an economic point of view, which at least in principle give an idea of the rates of erosion in the forest.

### **1.5 *Fagus sylvatica* forest information: ecology and distribution**

The European beech (*Fagus sylvatica* L.) is a large deciduous tree that commonly reaches 30-40 m and is capable of attaining heights up to 50 m in some locations (Packham et al., 2012). Beech is widespread across Europe: it can be found from Sicily (Southern Italy) to Bergen in southern Norway (Fang et al., 2006; Hultén and Fries, 1986) (Fig.1). Longitudinally its range is from the Cantabrian Mountains in the west to the Carpathians and Balkan Mountains in the east, although there are some areas in Europe where it is not found as a native tree, such as the Po valley and the Hungarian plain (Fig. 1).

As the climate becomes more continental in the eastern parts of Europe it is replaced by oriental beech (*Fagus orientalis* Lipsky) (Houston Durrant et al., 2016). At the southern part of its range (Spain, Sicily) it is only normally present at altitudes of more than 1.000 m, and can even be found at elevations of up to 2000 m (Packham et al., 2012; Horgan et al., 2003).



**Fig.1** Distribution map of Beech (*Fagus sylvatica*) EUFORGEN (2009).

Beech is one of the most shade-tolerant broadleaved tree (Praciak et al., 2013), its natural regeneration is possible in silvicultural systems with continuous crown coverage as the seedlings are able to survive and grow below the canopy of established trees. It is not particularly soil sensitive (Walthert et al., 2013) and grows on a wide variety of soils with a pH range from 3.5 to 8.5, although it cannot tolerate the most acid conditions. Beech furthers soil conservation due to its production of a large quantity of litter (around 900 g/m<sup>2</sup> per year). In Italy, this species characterises the landscape of many mountain areas, from the Alps down to the southern regions of Campania, Basilicata, Calabria and Sicily in the Mediterranean area (Fig. 2).



**Fig. 2** Distribution of beech forests in Italy. Data from CORINE Land Cover 2000 4<sup>th</sup> Level. (by Nocentini, 2009).

On the Apennines, the optimal altimetry range of the beech forest is from 900-1000 m a.s.l. up to the limit of forest vegetation 1800-1900 m (Scoppola, 1999). However, in environments affected by drafts of humid air, beech goes up to 200-300 m a.s.l. in area that is generally proper to holm oak and to other species of the basal plain (Hofmann, 1991; Fenaroli, 1967; Montelucci, 1956; Gualdi, 1974; Pignatti, 1994). The structure of beech forest was influenced by different type of management (la Marca, 2012). According to the National Forest Inventory (INFC, 2005), the total area covered by beech in Italy is 1.042.129 hectares (about half of it is still governed by a coppice; Table

2), which corresponds to 9.4% of the country's total forest area (according to the FRA2000 definition).

In the Alps and in Central and Northern Apennine, exploitation of beech forests started in the middle ages and became very intensive in the second half of the eighteenth century when demographic pressure increased rapidly (Crivellari, 1955; Hofmann, 1991). During this long period, beech forests were extensively clear cut leaving only some seed trees, generally 30 per hectare (Gabbrielli, 1991; Rovelli, 2000). Stands were thus transformed into coppices, which were repeatedly utilized until the second half of the twentieth century for firewood and charcoal for mountain populations. Starting from the fifties, following the widespread use of other low cost energy sources and the depopulation of mountain areas, many beech coppices have been progressively abandoned.

In many areas of Southern Italy, extensive felling in beech forests started in 1826, when the Kingdom of the two Sicilies passed the "Bourbon Law", which dictated that all public owned forests be managed according to "regular felling", i.e. clear cut leaving 58 seed trees per hectare (Hofmann, 1956; Bianucci, 1982). This type of treatment caused the degradation of many beech forests on the warmer, southern slopes and where soil conditions were more difficult; in addition, notwithstanding the law, repeated grazing and fire contributed to the definitive transformation of many beech forests into degraded pastures (Hofmann, 1956; Susmel, 1957).

In Calabria, beech forests have been subject to intense exploitation. Today, from a structural point of view, there are substantially typologies monoplane deriving from the starting up of the high trunk of coppices or from the treatment in successive cuts and, more frequently, multiplane, inhomogeneous structures, where trees of various ages and sizes alternate on small spaces, holes with natural renovation of beech or beech and fir due to "selection cut" (Mercurio, 2012).

Studies conducted in various privately owned beech forests in Calabria (Iovino & Menguzzato, 2004; Ciancio et al., 2008) described selection cut treatments and the resulting stand structures. The traditional system "selection cut" eliminates the biggest trees at repeated short intervals (8-10 years). This type of felling creates small gaps - 40 to 100 m<sup>2</sup> in size- where beech regeneration quickly sets in (Ciancio et al., 2008). For beech high forests, the management approach which has been described for private



properties in Southern Italy, can provide an interesting example for sustainable use of these forest formations.

Forest policies have been increasingly directed to favour beech coppice conversion to high forests, which are considered more productive and ecologically more functional. Beech high forests have a very interesting management history, which is a very good example of the separation between classical forest management, i.e., systems defined by “scientific forestry”, and real life forest management, i.e., how forests have been, and mostly still are, actually managed (Nocentini, 2009).

**Table 2** Management types for beech forests in Italy. Data from INFC 2005.

| Region         | Coppice |      | Coppice stands in conversion to high forest |      | High forests |      | Not classified |      | Total   |
|----------------|---------|------|---|------|--------------|------|----------------|------|---------|
|                | (ha)    | %    | (ha)  | %    | (ha)         | %    | (ha)           | %    | (ha)    |
| Piedmont       | 72020   | 62.5 | 2424  | 2.1  | 29898        | 25.8 | 11158          | 9.7  | 115500  |
| Valle d'Aosta  | 0       | 0.0  | 0   | 0.0  | 771          | 66.7 | 385            | 33.3 | 1156    |
| Lombardy       | 43199   | 65.8 | 882   | 1.3  | 13224        | 20.1 | 8376           | 12.8 | 65681   |
| Alto Adige     | 1512    | 40.0 | 0   | 0.0  | 1890         | 50.0 | 378            | 10.0 | 3780    |
| Trentino       | 27027   | 43.4 | 7568  | 12.2 | 17069        | 27.4 | 10582          | 17.0 | 62246   |
| Veneto         | 39965   | 64.2 | 3735  | 4.9  | 16773        | 22.0 | 6723           | 10.0 | 67196   |
| Friuli V.G.    | 10033   | 11.3 | 11891                                       | 13.4 | 34931        | 39.3 | 31958          | 36.0 | 88813   |
| Liguria        | 24913   | 67.3 | 733   | 2.0  | 10625        | 28.7 | 733            | 2.0  | 37004   |
| Emilia Romagna | 78059   | 77.6 | 11034                                       | 10.9 | 5517         | 5.4  | 6252           | 6.2  | 100862  |
| Tuscany        | 37215   | 55.1 | 11201                                       | 14.4 | 11201        | 14.4 | 12644          | 17.5 | 72261   |
| Umbria         | 10322   | 68.3 | 369   | 2.4  | 2949         | 19.5 | 1475           | 9.8  | 15115   |
| Marche         | 11520   | 65.3 | 0   | 0.0  | 2230         | 12.2 | 4087           | 22.9 | 17837   |
| Lazio          | 33161   | 46.2 | 4053  | 5.7  | 28233        | 39.4 | 6264           | 8.7  | 71711   |
| Abruzzo        | 50703   | 41.8 | 18822                                       | 15.3 | 41293        | 33.5 | 11584          | 9.5  | 122402  |
| Molise         | 3904    | 26.3 | 0   | 0.0  | 9760         | 65.8 | 1171           | 7.9  | 14835   |
| Campania       | 4380    | 7.9  | 2210  | 4.0  | 41609        | 75.4 | 6997           | 12.7 | 55196   |
| Apulia         | 388     | 8.3  | 0   | 0.0  | 4273         | 91.7 | 0              | 0.0  | 4661    |
| Basilicata     | 2983    | 11.3 | 373   | 1.4  | 18271        | 69.1 | 4822           | 18.2 | 26449   |
| Calabria       | 14925   | 19.3 | 2985  | 3.9  | 57088        | 73.9 | 2239           | 2.9  | 77237   |
| Sicily         | 10993   | 72.5 | 0   | 0.0  | 2274         | 15.0 | 1895           | 12.5 | 15162   |
| Sardinia       | 0       | 0.0  | 0   | 0.0  | 0            | 0.0  | 0              | 0.0  | 0       |
| Italy          | 477225  | 46.1 | 78280                                       | 7.6  | 349879       | 33.8 | 129723         | 12.5 | 1035107 |

## 1.6 Objectives

Nowadays there is an urgent need to find forest sustainable management practices to maximize their positive impacts on forest sector development, minimizing their negative effects on biodiversity, soil ecosystem functioning and climate change. For the above statements, the aim of this work was to individuate early indicators of soil erosion to be applied for identifying the most appropriate forestry practices to manage a beech (*Fagus sylvatica*) forest in a sustainable way. Thus, we assessed the effect of innovative thinning (on average, ca. 27% of total volume resected), traditional thinning (on average, ca. 12% of total volume resected) on soil properties in respect to unmanaged forest. The work has been performed over two years to have a great representation of data, on a comparative basis.

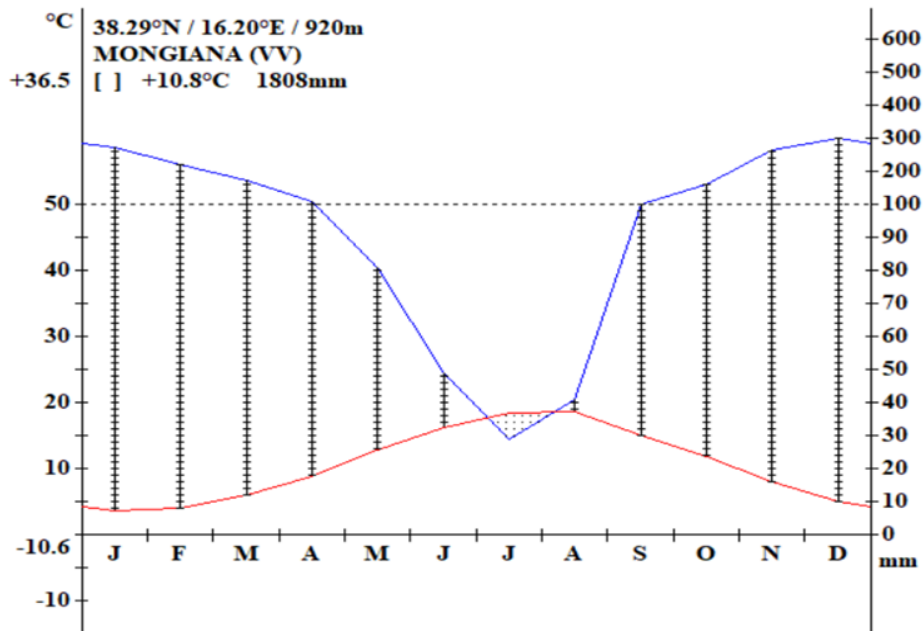
Soil erosion rate was detected by using the  $^{137}\text{Cs}$  technique in order to establish the effect of thinning on soil loss. This analysis required detailed samplings to identify undisturbed areas to deduce the reference value for  $^{137}\text{Cs}$ , and the collection of soil samples within the areas subjected to different treatments. Additional soil samples have been collected and analysed for soil physical, chemical and biochemical properties. The aim was to find a correlation between soil properties and  $^{137}\text{Cs}$  to identify specific indicators of soil erosion rate.

## 2. Materials and methods

### 2.1 Study area description and experimental design

The study area is located in the Marchesale Biogenetic Reserve (Natura 2000 site) within the highest slope of the Calabrian "Serre" mountains, in Mongiana (VV) (38° 30' N, 16° 14' E). The reserve, managed by the National Forest Service of Italy, covers 1234 hectares and consists mainly of high forest dominated by *Fagus sylvatica* L.

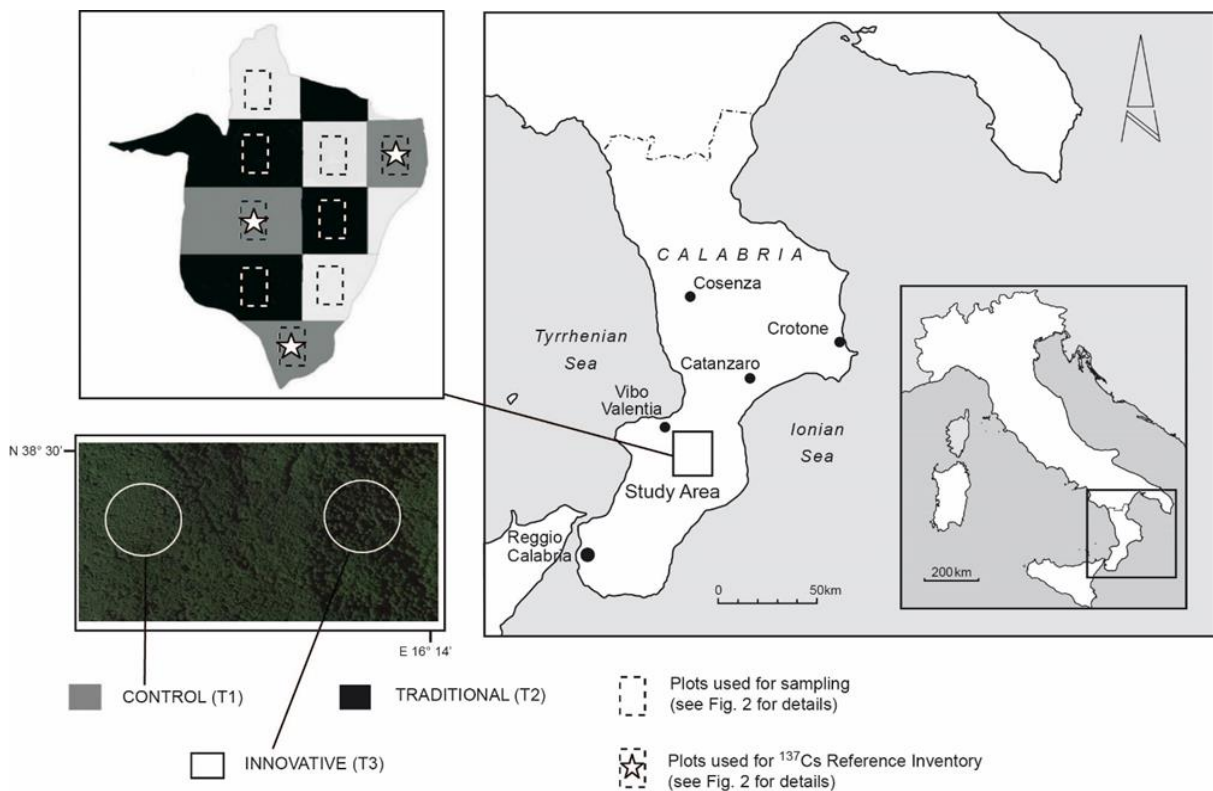
Being located in the upper part of the mountain system facing the Tyrrhenian Sea and exposed to North-West, its microclimate is influenced by the interception of fog, wet winds and precipitation (Becagli et al., 2013). The mean values of annual rainfall and temperature, based on the data obtained at Mongiana (VV) meteorological station (920 m a.s.l.) and available for the period 1928-2018, are respectively of 1808 mm e 10.8 °C (Fig. 3). The warmest month is August (18.6 °C), the coldest one is January (3.8 °C). The climate is typically Mediterranean (Csb, sensu Koppen, 1936).



**Fig. 3** Bagnouls-Gaussen diagram showing (50 years) the mean air temperatures (in red) and the monthly mean precipitations (in blue). The data refers to the meteorological station of Mongiana (VV) (Arpacal - multirischi).

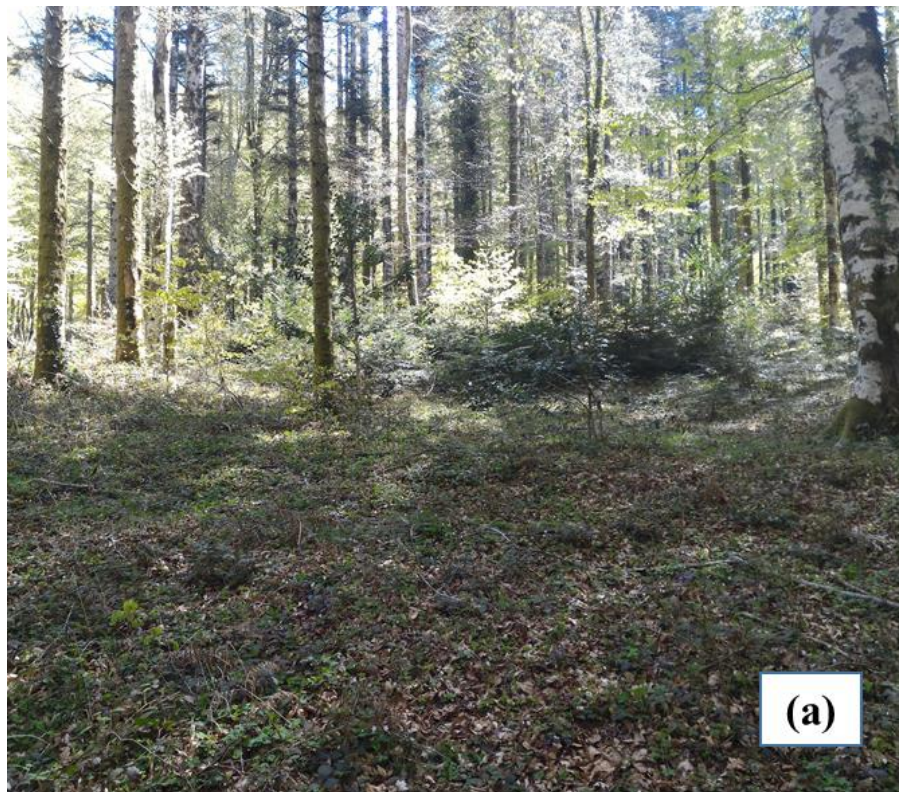
This area is geologically characterized by Paleozoic granitoid gneiss deeply fractured and with deep, versus shaped slopes (Conforti et al., 2015). According to USDA soil

classification (Soil Survey Staff, 2010), the dominant soils belong to Entisol and Inceptisol orders, with soil depths ranging between 0.2 and 1 m. Soil profiles include mainly A-Cr/R horizons and/or A-Bw-Cr horizons (Conforti et al., 2018). Generally, topsoil (A horizon) has high amounts of organic matter due to accumulation of much litter, which is promoted by humid and cool climatic conditions (Conforti et al., 2016). In the past, this area was object of the European study project ManFor C.BD., which had as objective to verify the effectiveness of different forest management practices in achieving multiple objectives (timber production, environmental protection and biodiversity conservation, etc.). For this work, we identified an experimental area of ca. 30 ha covered by a 75-year-old high beech forest (1100 m a.s.l.). In this area, three different sub-areas of about 3 ha each have been identified and subjected to different silvicultural thinning (3 treatments x 3 plots for each treatment x 2 different analysis; Fig. 4).



**Fig. 4** Study area: representation of the different treatments (T1 control, T2 traditional thinning, T3 innovative thinning) and plots established for sampling.

An unmanaged area for over 30 years (T1) was used as control; a traditional treatment (T2) and an innovative treatment (T3) have been carried out in 2012-2013. Traditional treatment (Fig. 5b; Fig. 6) was a thinning from below with a moderate intensity which removed all the dominated trees and the worst dominant trees (on average, ca. 12% of total volume resected). The innovative treatment (Fig. 5c; Fig. 6) was oriented to retain the 50 best trees per hectare and improve the structural biodiversity, collecting 5 or 6 trees closer to them, regardless of their social position (on average, ca. 27% of the total volume removed).

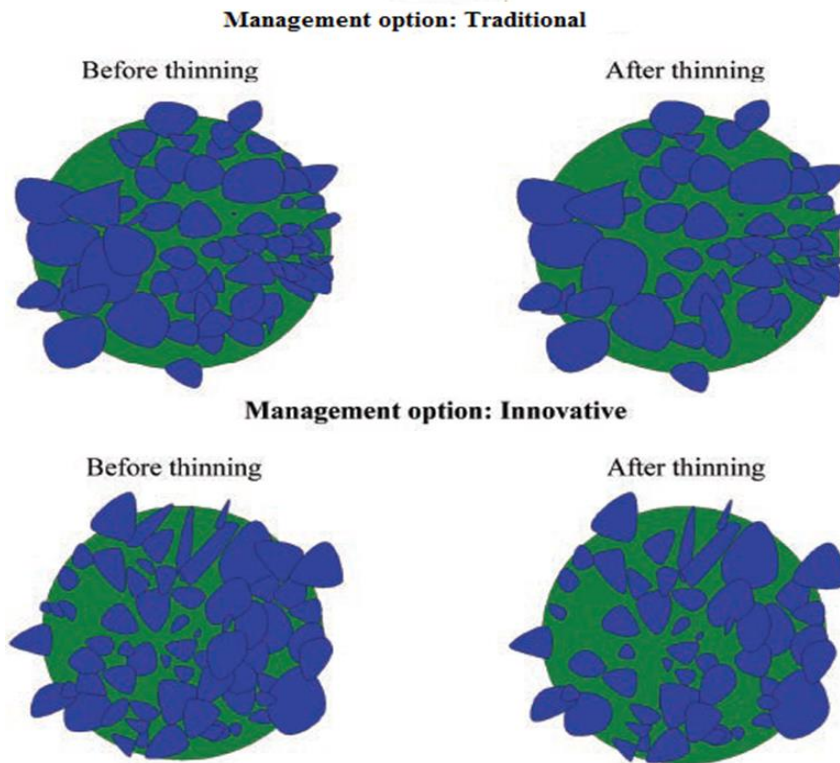






**Fig. 5** Forest conditions at the time of the first soil sampling (2017) in: **(a)** control (T1); **(b)** traditional treatment (T2); **(c)** innovative treatment (T3).

Before the silvicultural interventions the dendrometric parameters didn't show significant differences among the plots (Picchio et al., 2016) (Table 3).



**Fig. 6** Situation before and after thinning in: (T1) traditional and (T2) innovative area (ManFor C.BD).



**Table 3** Main dendrometric characteristics ( $\pm$  standard deviation), before and after thinning. T1 (control), T2 (traditional), T3 (innovative) treatments. (ManFor C.BD.; Coletta et al., 2017).

| Treatment |              | Volume<br>(m <sup>3</sup> ha <sup>-1</sup> ) | Basal area<br>(m <sup>2</sup> ha <sup>-1</sup> ) | No. tree<br>(N ha <sup>-1</sup> ) |
|-----------|--------------|--|--|-----------------------------------|
| <b>T1</b> | -            | 432.9 $\pm$ 174.1                            | 38.5 $\pm$ 11.4                                  | 334 $\pm$ 201.2                   |
| <b>T2</b> | Before       | 345.7 $\pm$ 19.8                             | 34 $\pm$ 1.2                                     | 557 $\pm$ 138.7                   |
|           | <b>After</b> | <b>301.6 <math>\pm</math> 15.6</b>           | <b>29.7 <math>\pm</math> 1</b>                   | <b>501 <math>\pm</math> 111.5</b> |
| <b>T3</b> | Before       | 338.1 $\pm$ 27.7                             | 33.9 $\pm$ 1.9                                   | 631 $\pm$ 104.4                   |
|           | <b>After</b> | <b>246.1 <math>\pm</math> 21.3</b>           | <b>26.6 <math>\pm</math> 1.6</b>                 | <b>533 <math>\pm</math> 94.3</b>  |

The managed area are fully comparable in terms of slope, orientation and soil types. The unmanaged area (T1) was used as reference location for <sup>137</sup>Cs analysis. The reference value obtained in this area was used to convert the <sup>137</sup>Cs inventories obtained in the managed area into estimates of soil erosion. More specifically, the Diffusion and Migration Model (DMM) was applied for this conversion analysis (see Porto et al., 2003). This version of the DMM is based on a simulation of <sup>137</sup>Cs activity along a soil profile following the atmospheric fallout and its temporal redistribution. Based on this assumption, the DMM attempts to reproduce the activity of <sup>137</sup>Cs with the following equation:

$$C(x,t,t') = e^{-\lambda(t-t')} \int_0^{\infty} \frac{I(t')}{H} e^{-\frac{y}{H}} \left\{ e^{\frac{V(x-y)}{2D} - \frac{V^2(t-t')}{4D}} \left[ e^{-\frac{(x+y)^2}{4D(t-t')}} + e^{-\frac{(x-y)^2}{4D(t-t')}} \right] \times \right. \\ \left. \times \frac{1}{\sqrt{4\pi D(t-t')}} - \frac{V}{2D} e^{\frac{Vx}{D}} \operatorname{erfc} \left[ \frac{x+y+V(t-t')}{\sqrt{4D(t-t')}} \right] \right\} dy \quad (1)$$

where:

$C(x,t,t')$  (Bq kg<sup>-1</sup>) indicates the <sup>137</sup>Cs activity at the mass depth  $x$  and time  $t'$ ;

$I(t')$  expressed in (Bq m<sup>-2</sup> yr<sup>-1</sup>) indicates the <sup>137</sup>Cs amount received by the soil surface at time  $t'$  with fallout;

H (kg m<sup>-2</sup>) is a basic parameter that accounts for the initial relaxation mass depth;

D (kg<sup>2</sup> m<sup>-4</sup> yr<sup>-1</sup>) is a model parameter that simulates the diffusion process;

V (kg m<sup>-2</sup> yr<sup>-1</sup>) is a model parameter that accounts for migration;

λ (= 0.023 yr<sup>-1</sup>) is the constant of radioactive decay for <sup>137</sup>Cs;

x (kg m<sup>-2</sup>) indicates the cumulative mass depth;

t (yr) is the time elapsed since the commencement of fallout in 1954;

erfc(u) is the error-function complement defined as (Crank, 1975):

$$\text{erfc}(u) = \frac{2}{\sqrt{\pi}} \int_u^{\infty} e^{-y^2} dy \quad (2)$$

Integrating Eq. (1) over time t' and assuming a continuous input I (t'), the <sup>137</sup>Cs concentration C (x,t) (Bq kg<sup>-1</sup>) in the soil profile is given by the following equation:

$$C(x,t) = \int_0^t C(x,t,t') dt \quad (3)$$

Assuming a constant soil lowering E (kg m<sup>-2</sup>), if the transport is purely diffusional, eq. (1) can be rewritten as:

$$C_e(x,t,t') = e^{-\lambda(t-t')} \int_0^{\infty} \frac{I(t')}{H} e^{-\frac{y}{H}} \left\{ \left[ e^{-\frac{[(x+E)+y]^2}{4D(t-t')}} + e^{-\frac{[(x+E)-y]^2}{4D(t-t')}} \right] \frac{1}{\sqrt{4\pi D(t-t')}} \right\} dy \quad (4)$$

where, the value of  $C_e(x,t,t')$  (Bq kg<sup>-1</sup>) indicates the concentration of <sup>137</sup>Cs in the presence of erosion, for any cumulative mass depth x and time t'.

The attempt of Eq. (1) to simulate the diffusion and migration of <sup>137</sup>Cs along the soil column was demonstrated in works carried out in southern Italy by Porto et al. (2004, 2016).

In other words, integration of C(x,t) over mass depth x gives the total <sup>137</sup>Cs inventory A<sub>u</sub> (Bq m<sup>-2</sup>) for an erosion site at time t:

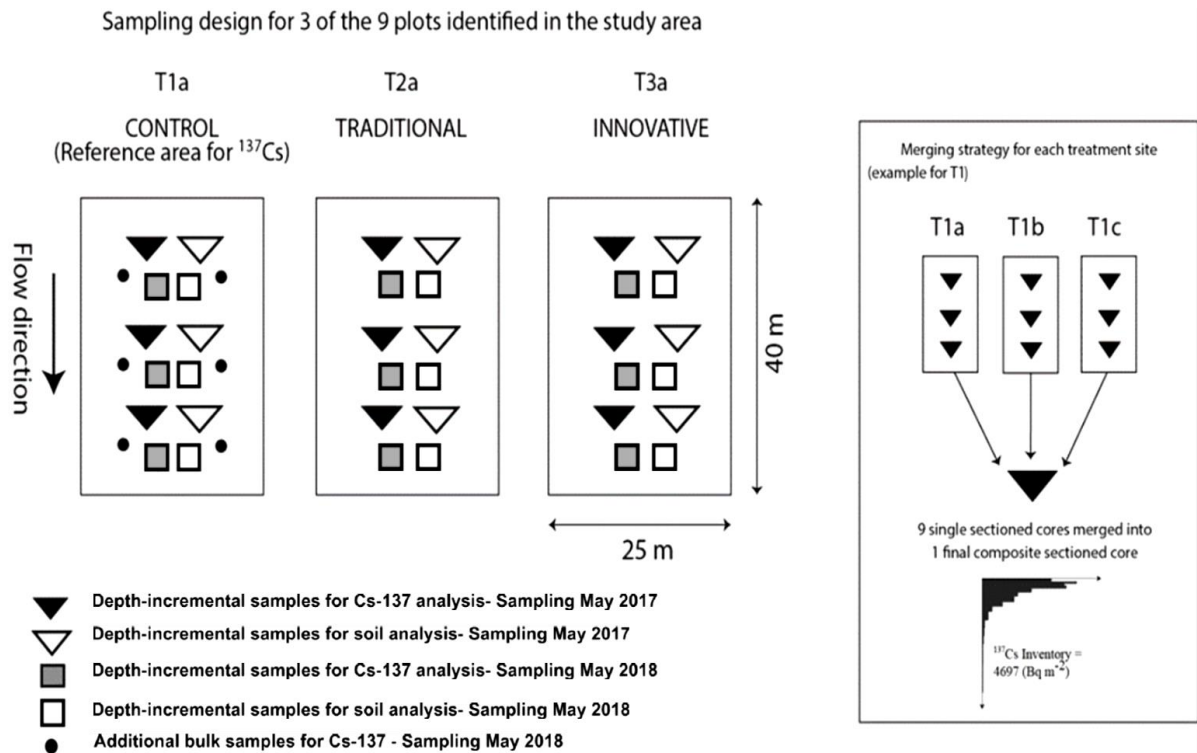
$$A_u(t) = \int_0^{\infty} C_e(x,t) dx \quad (5)$$

Eqs. (4) and (5) can then be solved simultaneously for E (kg m<sup>-2</sup>), with A<sub>u</sub> (Bq m<sup>-2</sup>) representing the measured inventory corresponding to an eroding point. Erosion rates R

( $\text{kg m}^{-2} \text{ yr}^{-1}$ ) may then be estimated by dividing the quantity E by the time  $t-t_0$  (yr) since the commencement of  $^{137}\text{Cs}$  fallout.

## **2.2 Soil sampling and preparation**

For each of the three study area (T1, T2 and T3), 3 representative plots (1000  $\text{m}^2$  in size) were established for soil sampling (see Fig. 4). In these 9 plots, five separate soil sampling were undertaken in two different years (2017-2018) following the scheme illustrated in Fig. 7. The first and the second soil sampling were carried out in May 2017 and consisted of collecting soil cores for  $^{137}\text{Cs}$  analysis separate from soil collected for chemical-physical and biological analysis. The third and fourth soil sampling, were carried out in May 2018, following the same scheme of the previous year. The cores were collected in areas with similar slope avoiding sampling points close to the tree trunks in order to minimize the effect of stemflow on the final  $^{137}\text{Cs}$  inventories. In total, in both years, 12 composite soil cores were obtained from these sampling campaigns (6 for radiometric measurements and 6 for chemical-physical and biological analysis), using a 10-cm-diameter steel core tube inserted up to a depth of ca. 35 cm. Each composite core used for  $^{137}\text{Cs}$  analysis was obtained from 9 single sectioned cores for each year (3 for each plot of the same treatment) merged layer by layer (see merging strategy explained in Fig. 7).



**Fig. 7** Sampling strategy adopted in the plots established within the three treatment areas.

More specifically, for each study area, the 9 single cores were sectioned into depth increments of 2-3 cm to a depth of 30 cm, and the deepest 5 cm were merged into one single sub-sample. This sampling strategy was necessary to obtain the distribution of  $^{137}\text{Cs}$  activity along the soil profile and to account for microscale variability. Each profile was used to fit the theoretical conversion model (DMM) able to calculate soil erosion rates.

Each composite soil core used for the chemical-physical and biological analyses consisted of 9 single soil cores (for each year), divided into 2 layers (0-15 and 15-30 cm). The corresponding layers were merged in order to obtain 3 final representative cores (one for each study area).

The fifth samplings, undertaken ever in May 2018, was necessary to further account for microscale variability associated with the  $^{137}\text{Cs}$  inventories at the reference area. These samplings provided collection of bulk cores from the three plots (T1<sub>a,b,c</sub>) within the control area (T1). In order to check the  $^{137}\text{Cs}$  reference value obtained during both the samplings, 18 additional bulk cores (six for each control plot) were collected for

comparison (see Fig. 7 for details). For the determination of microbial biomass, the samples were stored in the refrigerator at 4 ° C for up to 24 hours before being analysed. All the others samples, were air dried and sieved to separate the < 2 mm fraction. Before the radiometric assay, all the samples were oven dried at 105 °C for 48 h, disaggregated, dry sieved to separate the < 2 mm fraction, and packed in plastic pots or Petri dishes for subsequently determination of its <sup>137</sup>Cs activity by gamma spectroscopy. For QBS-ar (microarthropods) one soil sample were taken from each of 9 plots differently managed (in both years), including the control area. The samples were taken at a depth of 10 cm. Immediately upon returning from the field, the soil samples were transferred to Berlese-Tullgren funnels lined with 4 mm wire mesh, until complete extraction.

### **2.3 Radiometric method for measuring <sup>137</sup>Cs activity in soil**

The measurements of the <sup>137</sup>Cs activity and its subsequently vertical distribution in the soil profile were obtained by gamma spectrometry at the Department of Agraria of the University Mediterranea of Reggio Calabria. Two Canberra p-type HPGe detectors, model GX4020, were used for the analyses. Each detector, coupled to a Desktop Spectrum Analyzer DSA-1000 Canberra multichannel analyser, is characterised by a relative efficiency of 45.6% with a resolution of 1.1 keV at 122 keV and 2.0 keV at 1.33 MeV. The spectral analysis was provided by the Canberra Genie 2000 software package and the efficiency calibration was obtained by the Canberra's LabSOCS (Laboratory SOurceless Calibration Software) code. A certified multigamma source with a wide energy range (42.8–1274.5 keV) together with several standard materials of different geometry were used for energy calibration. Count times in the detectors were approximately 40,000 s, and the <sup>137</sup>Cs activities were obtained from the counts at 662 keV.

## 2.4 Soil physical and chemical property determination

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 (65°C for 72 hours).

Samples of soils were analysed 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 consisted 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<sub>2</sub>O<sub>2</sub>, 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 generally 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<sup>+</sup> ions in and out of the electrode, i.e., in the bathing solution. pH in H<sub>2</sub>O was 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<sub>2</sub>O deionized to determine the active acidity of soil. 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<sub>2</sub>O 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 colourless. 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.

The organic carbon (OC) was detected by treating the soil with potassium dichromate and sulfuric acid, while the difference in FeSO<sub>4</sub> added with respect to a blank titration determined its quantity (Springer and Klee, 1954).

The percentage carbon is given by the formula:

$$CO \% = M (V1 - V2 W) * 0.3 * CF \quad (6)$$

where M is the molarity of the FeSO<sub>4</sub> solution (from blank titration), V1 is the volume (mL) of FeSO<sub>4</sub> required in blank titration, V2 is the volume (mL) of FeSO<sub>4</sub> 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%).

Total water-soluble phenols (WSP) were measured by using the Folin–Ciocalteu 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 ( $\mu\text{g TAE g}^{-1}$  D.W.). 10 g dry-soil were weighed, into a conic flask and, were mixed with 50 mL  $\text{H}_2\text{O}$  deionized, the suspensions were shaken for 24h, and then centrifuged at 5000 rpm for 10 min. 1000  $\mu\text{l}$  of Folin-Ciocalteu reagent and 2.5 mL of sodium hydroxide (0.33 N) were added to the different extracts (500  $\mu\text{l}$ ). The mixtures were incubated for 15 min at room temperature. The specific absorbance was immediately measured at 760 nm.

For the measurement of dissolved organic carbon (DOC), 1 gr of air-dried and 2 mm sieved soil was taken. An extraction was carried out in 10 ml of ultrapure water (1:10), after 24 hours of rest and 2 hours of shake, centrifugation was carried out at 3000 rpm for 15 minutes. The supernatant filtered with 0.45  $\mu\text{m}$  cellulose acetate membrane filters, and the absorbance of the samples at 254 nm was measured with a Plate reader (Perkin Elmer Enspire) according to the method described in Brandstetter et al. (1996).

Soil samples were extracted with bidistilled  $\text{H}_2\text{O}$ , respectively, at a 1:10 ratio, for 24 hours at room temperature. The extract was subjected to centrifugation and the supernatant, after filtration on 0.22  $\mu\text{m}$  cellulose filters, was analysed for the qualitative and quantitative determination of ions (Wang et al., 2013). The analysis was conducted using a Dionex ICS-1100 ion chromatograph. The data are reported in mg/g of dry sample.

#### **2.4.1 Soil aggregate fraction analysis**

Soil aggregate stability (SAS) by wet sieving was determined according to ON L 1072 (2004). With this method, soil aggregates with a diameter of 2000–1000  $\mu\text{m}$  are dipped on a sieve of 250  $\mu\text{m}$ . The mass of soil (EW) used in the experiment is 4 g. The mass of stabile aggregates after dipping (mK) in 80 ml of distilled water and the mass of sand after chemical dispersion (25 ml of sodium pyrophosphate) of the remaining aggregates (mA) is determined. These parameters are used to calculate the percentage of stabile aggregates (% SAS):



$$\%SAS = \frac{m_K - m_A}{EW - m_A} \times 100 \quad (7)$$

Through the USAS method, measurable ultrasonic energy was applied to the soil-water suspension, allowing a quantitative measurement of soil aggregate stability (Mentler, 2001). The most important parameter to describe the particle dispersion during sonification and the degree of aggregate breakdown, is the specific ultrasonic energy absorbed by a soil–water mixture. Soil aggregate distribution (USAS) was carried out by a new, probe-type dispersion equipment (cite). A titanium alloy probe is inserted into the soil–water mixture and vibrates at approximately 20 kHz. The ultrasonic titanium probe has cylindrical shape and a circular cross section (diameter 30 mm). The same ultrasonic probe was used in all experiments and the insertion depth was kept constant at 10 mm.

Dispersion experiments were performed with 4 g soil in 200 ml pure degassed water. The solution was stirred with a magnetic stirring device (2 Hz, cylindrical shape with length 25 mm and thickness 8 mm). Stirring starts 10 s prior to the ultrasonic vibration and was continued during the ultrasonic experiments to obtain a homogeneous distribution of soil in the solution. All soils were tested at constant vibration amplitude of the ultrasonic probe of 2.5  $\mu\text{m}$  for 30 seconds. As reported in literature (Mayer et al., 2002; Mentler et al., 2004), the vibration amplitude was determined using electromagnetic induction coil and strain gauges. Immediately after the ultrasonic treatments, mass fractions were determined by wet sieving. The aggregates were analysed with standard sieves and classified in different aggregate fractions: macro-aggregates (1000–630  $\mu\text{m}$ ), medium aggregates (630–250  $\mu\text{m}$ ), and small aggregates (250–63  $\mu\text{m}$ ). Determination of mass fractions (accuracy 0.001 g) was performed by weighing after drying at 105°C for 24 h. Each aggregate fraction was used to determination of OC and N, using an elementary analyzer via dry combustion technique and gas chromatographic analyses (ThermoFisher *Flashsmart*, ON L 1080-99 (1999)).

## 2.5 Soil biological and 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<sub>2</sub>SO<sub>4</sub> 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<sup>-1</sup>. 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 hydrolase (FDA) 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<sup>-1</sup> 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).

Dehydrogenase (DHA) 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 (CAT) activity was measured by the method of Beck (1971). 10 mL of phosphate buffer (pH, 7) and 5 mL of a 3% H<sub>2</sub>O<sub>2</sub> substrate solution were added to 5 g of soil. The volume (mL) of O<sub>2</sub> 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<sub>3</sub>. Results were expressed as ml O<sub>2</sub> g<sup>-1</sup> dry soil.

The fungal cell membrane component ergosterol (Erg) was extracted following the method described by Gong et al. (2001) with some modifications, 10 g of methanol (Me-OH) was added to 1 g of soil. The suspension was homogenized with mechanical agitation for 15 min, and centrifuged at 3500 rpm for 15 min. The supernatant was filtered using a syringe membrane filter (4 mm, 0.45 µm polytetrafluoroethylene (PTFE) and then kept in the dark until analysis with an Agilent Technologies Infinity 1290 high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, California, USA).

Humification index (HI), degree of humification (DH%) and humification rate (HR%) have been detected, extracting humic components with a solution 0.1 M of sodium pyrophosphate and sodium hydroxide (Official Methods of Soil Chemical Analysis, 1994). TEC was fractioned into humified (humic acid, HA + fulvic acid, FA) and non-humified (NH) fractions (Ciavatta et al., 1990). Humification parameters have been calculated as shown in Eqs. 8-10 following the method proposed by Ciavatta et al. (1990) and Sidari et al. (2005).

$$HI = NH / (HA + FA) \quad (8)$$

$$HR\% = 100(HA + FA) / TOC \quad (9)$$

$$DH\% = 100(HA + FA) / TEC \quad (10)$$

Humification index (HI) represents the ratio between not humified and humified extracted carbon, while HR is the percent of humification rate and DH is the percent of humified carbon in the extract.

Microarthropods, included mites and collembola, were analyzed with Berlese-Tullgren selector (Parisi et al., 2005). Three soil samples were taken from each plot differently managed, including the control area. 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.

All the arthropods were identified at different taxonomical levels through, the determination of biological forms and calculation of QBS index, using a microscope PCE-MM200. Accordingly, the biological form (morpho-type) that is most adapted to soil was identified. Each morpho-type correspond an eco-morphological index (EMI); as a 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 was obtained from the sum of EMI index of all the collected groups (Table 4). If in a group biological forms with different EMI values are present, the higher value (more adapted to the soil form) was selected to represent the group in the QBS calculation (Parisi et al., 2005). EMI is a simplified index (Table 4), that use the microarthropod morphology assessment for generating soil biological quality index (QBS index). This analysis allows assessing the degradation level of soils (Parisi et al., 2005).

**Table 4** Eco-morphologic indices (EMI) of edaphic microarthropods groups. Qbs index is obtained from the sum of the highest values of EMI of all the collected groups.

| <b>Group</b>  | <b>EMI Score</b> |
|---|------------------|
| <i>Protura; Diplura; Acari; Palpigradi;<br/>Pseudoscorpiones; Pauropoda; Symphyla</i>                                   | 20               |
| <i>Chilopoda; Diplopoda</i>   | 10 - 20          |
| <i>Collembola; Orthoptera; Coleoptera</i>   | 1 - 20           |
| <i>Zygentomata; Microcoryphia; Embioptera;<br/>Diptera (larvae); Isopoda; Other<br/>holometabolous insects (larvae)</i> | 10               |
| <i>Blattaria</i>  | 5                |
| <i>Hymenoptera; Araneae</i>   | 1 - 5            |
| <i>Dermaptera; Psocoptera; Thysanoptera; Other<br/>holometabolous insects (adults)</i>                                  | 1                |

## 2.6 Statistical analysis

To explore relationships among soil parameters at two soil depths, and three different silvicultural treatments, datasets were analyzed using Principal Component Analysis (PCA), Multivariate Analysis of Variance (MANOVA) and T test for paired values. The results are summarized in an ordination diagram. PCA was carried out using the soil parameters in plots under different silvicultural treatments using the software PAST (Hammer et al., 2001). Because the data are expressed in different units, the results are standardized with the following formula:

$$z = \frac{(x_i - \bar{x})}{SD} \quad (11)$$

where  $x_i$  is the individual value of each parameter,  $\bar{x}$  is the mean and SD the standard deviation.

A MANOVA analysis was carried out for evaluating the effects of thinning, soil depth and their interaction on the set of soil parameters and  $^{137}\text{Cs}$ . Finally, since there are only two soil depths (0-15 cm and 15-30 cm), a T test was used for paired values to evaluate significant differences. This last analysis allowed us to verify if thinning affected soil properties according to depth, and if the impacts were similar. Pearson's correlations for both soil layers and all soil parameters, were carried out using PAST software (Hammer et al., 2001).

For each parameter analysed by ANOVA and t-test, the data matrix (sample size) from the average of the values per sub-plot, and from the average per plot, values for each experimental condition were obtained. Anova, Manova models and t-test were carried out using SPSS software (IBM Corp., 2012).

### 3. Results and discussion

#### 3.1 Year 2017

##### 3.1.1 $^{137}\text{Cs}$ distribution and soil erosion estimation

Figure 8a,b,c shows the distribution of  $^{137}\text{Cs}$  in areas under different forest managements. T1 was assumed as control area and  $^{137}\text{Cs}$  inventory obtained in this area (4697 Bq m<sup>-2</sup>; Fig. 8a) was assumed to be the  $^{137}\text{Cs}$  reference value to be compared with the values obtained in the managed area T2 and T3.

The depth scale in figure 8a,b,c has been plotted in terms of cumulative mass, rather than depth, in order to avoid the need to take account of down core variations in soil bulk density. The radionuclide distribution reported in figure 8a, together with the fitting provided by the DMM (in order to convert the loss of  $^{137}\text{Cs}$  into values of soil loss), is typical of undisturbed areas in Southern Italy (see Porto et al., 2003). It shows about 90% of the total inventory occurring in the top 15 cm and a sharp exponential decline in activity below this depth. The  $^{137}\text{Cs}$  depth distributions in T2 and T3 (Fig. 8b,c), indicate different inventory values, showing a similar shape than uncultivated soils.

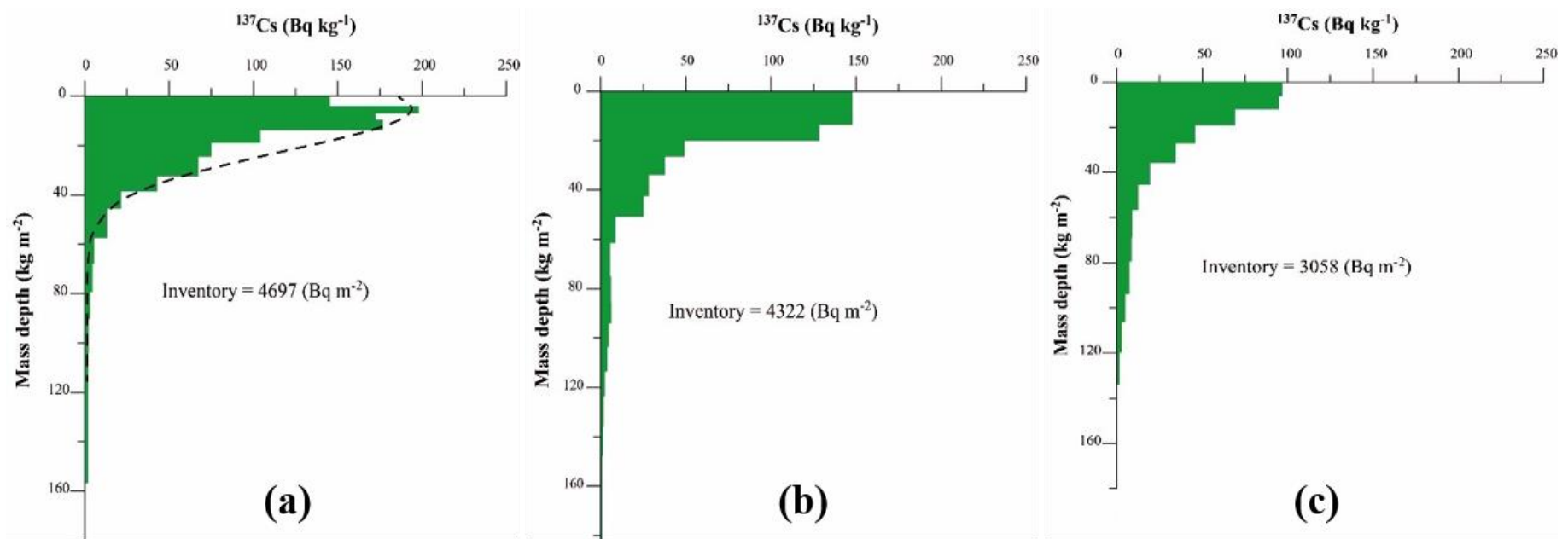
In figure 8 (b, c) is shown the distribution of  $^{137}\text{Cs}$  along soil profiles in T2 and T3 areas, that documented inventory values of 4322 Bq m<sup>-2</sup> and 3058 Bq m<sup>-2</sup> respectively.

The values of soil loss detected in T2 (1.4 t ha<sup>-1</sup> yr<sup>-1</sup>) and T3 (6.3 t ha<sup>-1</sup> yr<sup>-1</sup>) indicated that, although with different intensities, (moderate in T2 and high in T3) erosive phenomena occurred in these areas with respect to control. In short these findings demonstrated that increasing thinning intensity soil erosion increased supporting strongly the hypothesis that forests play a key role in regulating the runoff and soil erosion, due to the protection offered by the canopy cover and to their capability of improving the absorption capacity of soils as already demonstrated by Verheijen et al. (2009), reporting an upper limit of tolerable soil erosion of ca. 1.4 t ha<sup>-1</sup> year<sup>-1</sup>.

In the first 10 centimetres of soil, a decrease in  $^{137}\text{Cs}$  in both managed areas (ca. 150 Bq kg<sup>-1</sup> for T2 and 100 Bq kg<sup>-1</sup> for T3) (Fig. 8b,c), compared to reference area (ca. 200 Bq kg<sup>-1</sup>) was observed (Fig. 8a). These results highlighted that the upper part of the soil profile in T2 and T3, even if with different intensity, was removed by erosion.

Combining the profiles (Fig. 8) with the measured values of the bulk density, it was possible to calculate  $^{137}\text{Cs}$  inventory ( $\text{Bq m}^{-2}$ ) and mass activity ( $\text{Bq kg}^{-1}$ ) for a fixed depth. This calculation was made for the two soil depths (0-15 cm and 15-30 cm) in order to make possible the comparison with the soil chemical and biological properties detected in the same corresponding layers.





**Fig. 8 (2017)** -  $^{137}\text{Cs}$  profiles in the control area T1 (a); area under traditional thinning T2 (b); area under innovative thinning T3 (c).

### 3.1.2 Physical, chemical and biological soil properties

All the soil analysed belong to the sandy-loam textural class, with a silt content of about 25%, clay 14 % and sand 61 %. Our results showed that soil texture didn't change over treatments and years of sampling (data not show).

In both layers of soil (Table 5) the pH varied between the different areas, it was sub-acid and ranged from 4.8 to 5.8, increasing with forest thinning intensity, in agreement with results reported by Chen et al. (2014), Cheng et al. (2017) and Settineri et al. (2018). Electrical conductivity was 147-150  $\mu\text{S}$  and did not show significant differences among the treatments (Table 5). The phenols differed between the treatments, and decreased increasing the thinning intensity in both layers, this parameter seemed closely related to radionuclide values (Table 5). As reported by Vesterdal et al. (1995) thinning influenced nutrient dynamics, including OC, DOC and N. The loss of these nutrients in the upper layer, in fact, was proportional to thinning intensity (Table 5) and probably due to triggering erosion process.

Soil erosion, involved preferentially the removal of organic carbon (OC) because it was concentrated on soil surface and had lower density than the mineral fraction. The OC transported by water runoff is generally redistributed over the landscape and deposited in depressional sites where it is buried along with the sediments (Lal, 2005).

Data of humification parameters (DH, HR, HI), suggested that, in all the examined areas, humification prevailed over mineralization process. This was also confirmed by C/N ratios that was higher than 12, in both layers. Previous study of Sidari et al. (2005) on humic components, carried out in different ecosystems including beech forest (without particular management) in Sila mountain, showed similar results. Our results are in accord also with studies conducted in Czech young spruce and beech stands in different types of habitats by Menšík et al. (2016), showing that thinning did not negatively affect humification process (Table 6).

Despite the numerous investigations on soil microbes in managed forests, there is an uncertainty regarding the responses of microbial biomass and enzymes to thinning because they can also be affected by many other biotic and abiotic factors that occur in situ (Kim et al., 2019).

Previous studies reported either increase (Dannenmann et al., 2006; Ma et al., 2018; Pang et al., 2016; Settineri et al., 2018; Shen et al., 2018), decrease (Boerner et al., 2006; Geng et al., 2012; Schilling et al., 1999) or no changes in microbial biomass and enzyme activities as a result of thinning (Giai and Boerner, 2007; Maassen and Wirth, 2004; Overby and Hart, 2016; Tan et al., 2008).

Concerning microbial biomass, Grayston and Rennenberg (2006) stated that in cold and wet sites, forest thinning affected negatively microbial biomass. Our data, confirmed these data showing a decrease in MBC in T2 and T3 areas in respect to control and the decrease was proportional to thinning intensity (Table 6)

Enzymes were more affected by thinning in the surface rather than in the underlying layer. In 0-15 cm (Table 6), FDA was higher in T1 and T2 than in T3; DHA, on the contrary, was greater in T3 than in T1 and T2; the catalase activity (CAT) was instead the greatest in T2. Soil enzyme activities are important indicators of soil fertility. Their changes can reflect the evolution trend of 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 soil physico-chemical conditions (Wick et al., 1998; Aon and Colaneri, 2001; Baum et al., 2003).

Ergosterol is widely used to estimate fungal biomass (Seitz et al., 1979). Many studies found a significant correlation between the ergosterol content and fungal dry mass in soil and aquatic systems (Newell 1994), rice (Saxena et al., 2001). This sterol component is specific to the fungal kingdom (Weete and Gandhi, 1996) and a component of the membranes of mycelia, spores and vegetative cells (Newell, 1992). Djajakirana et al. (1996) found an average ergosterol-to-biomass C ratio of 0.72% in 0–10 cm depth in 30 German deciduous forest soils. Our results evidenced a good amount of ergosterol in the T1 and T2 areas (Table 6), indicating a good presence of fungi. In T3 area a large decrease, especially in the upper layer, was instead observed. This decrease was certainly due to degradation phenomena which occurred after the innovative thinning. In the underlying layer the variation of ergosterol among the treatments were less prominent.

The amount of  $^{137}\text{Cs}$ , decreased in both areas compared to control, especially in T3. In the deeper soil layer, no significant differences in soil parameters due to erosion process were evident in T2 and T3 compared to control (Table 5). In short, we highlighted the

gradual decrease of  $^{137}\text{Cs}$ , carbon, MBC and WSP, in respect to the increasing cutting intensity and how these parameters were correlated each other's (Fig. 9; 10).

**Table 5 (2017)** - Soil parameters detected in the two soil layers (0-15; 15-30 cm) under different forest managements (T1- Control; T2- Traditional thinning; T3- Innovative thinning): water content (WC, %); pH ( $\text{H}_2\text{O}$ ); electrical conductivity (EC,  $\mu\text{S cm}^{-1}$ ); soil aggregate stability by wet sieving (SAS, %);  $^{137}\text{Cs}$  ( $\text{Bq m}^2$ ,  $\text{Bq kg}^{-1}$ ); water soluble phenols (WSP,  $\mu\text{g TAE g}^{-1}$  dry soil); organic carbon (OC, %); dissolved organic carbon ( $\text{ml DOC L}^{-1}$ ); total nitrogen (N, %); C/N ratio. Numbers denote the standard error of the mean:  $n=3$ . Means in the same row followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

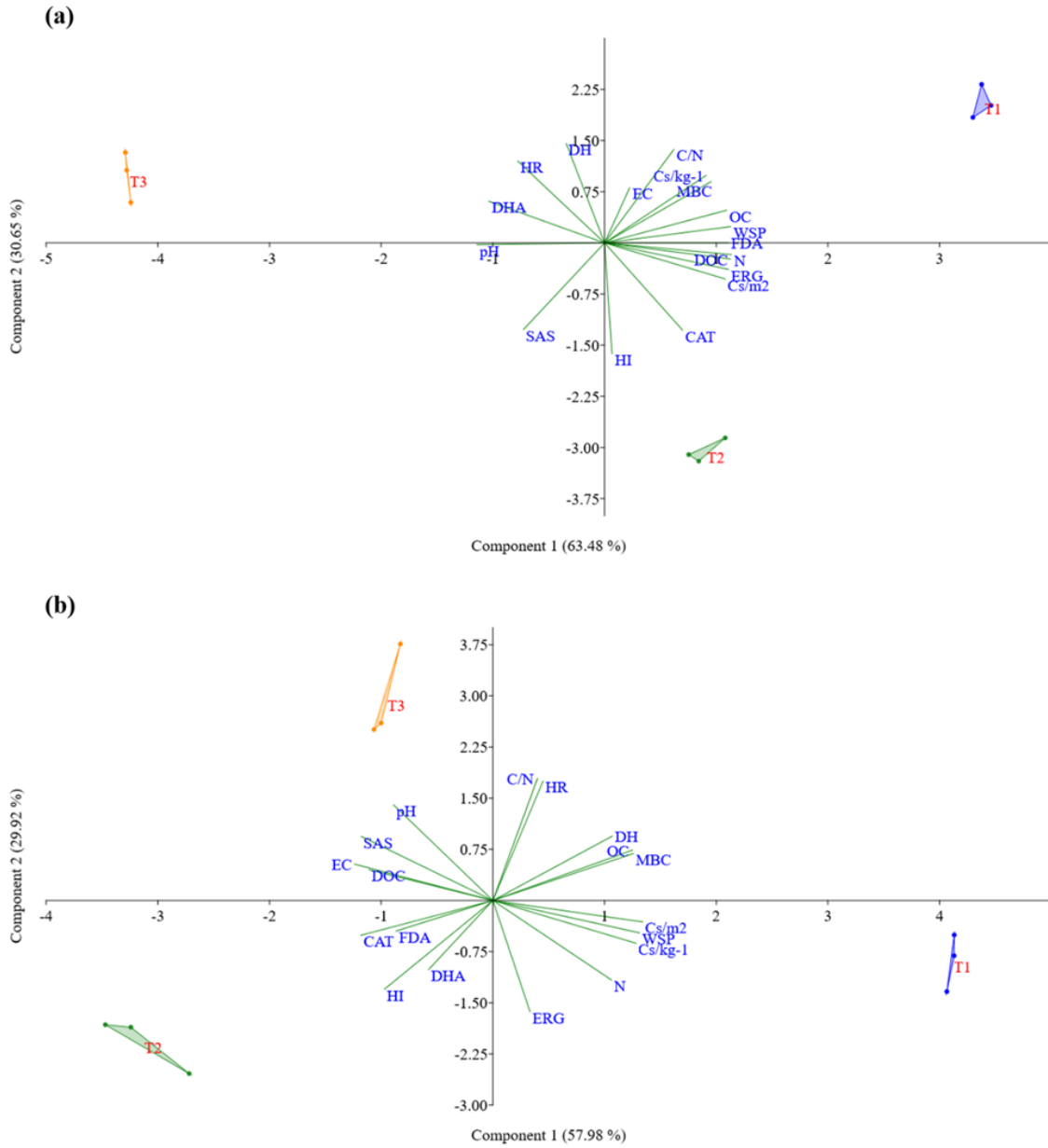
| Soil parameters           | First year (2017) 0-15 cm |                           |                           | 15 - 30 cm                |                           |                           |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                           | T1                        | T2                        | T3                        | T1                        | T2                        | T3                        |
| <b>WC</b>                 | 31 <sup>ab</sup> ± 5      | 24 <sup>b</sup> ± 3       | 43 <sup>a</sup> ± 10      | 25 <sup>ab</sup> ± 4      | 20 <sup>b</sup> ± 3       | 34 <sup>a</sup> ± 7       |
| <b>pH</b>                 | 4.83 <sup>c</sup> ± 0.01  | 5.18 <sup>b</sup> ± 0.01  | 5.84 <sup>a</sup> ± 0.01  | 5.31 <sup>b</sup> ± 0.01  | 5.54 <sup>ab</sup> ± 0.01 | 5.81 <sup>a</sup> ± 0.01  |
| <b>EC</b>                 | 150.5 <sup>a</sup> ± 0.8  | 147.6 <sup>a</sup> ± 2.0  | 148.8 <sup>a</sup> ± 4.3  | 101.4 <sup>a</sup> ± 0.6  | 106.0 <sup>a</sup> ± 1.1  | 106.4 <sup>a</sup> ± 0.9  |
| <b>SAS</b>                | 90.3 <sup>c</sup> ± 0.2   | 96.2 <sup>a</sup> ± 0.3   | 95.2 <sup>b</sup> ± 0.4   | 90.3 <sup>c</sup> ± 0.2   | 94.2 <sup>b</sup> ± 0.1   | 95.4 <sup>a</sup> ± 0.1   |
| <b>Cs m<sup>2</sup></b>   | 3994 <sup>a</sup> ± 4     | 4001 <sup>a</sup> ± 4     | 2686 <sup>b</sup> ± 2     | 703 <sup>a</sup> ± 1      | 321 <sup>c</sup> ± 1      | 373 <sup>b</sup> ± 1      |
| <b>Cs kg<sup>-1</sup></b> | 122.8 <sup>a</sup> ± 0.8  | 53.8 <sup>b</sup> ± 0.4   | 47.7 <sup>c</sup> ± 0.4   | 6.7 <sup>a</sup> ± 0.01   | 3.4 <sup>b</sup> ± 0.1    | 3.2 <sup>c</sup> ± 0.1    |
| <b>WSP</b>                | 231.7 <sup>a</sup> ± 8.8  | 160.8 <sup>b</sup> ± 4.4  | 75.8 <sup>c</sup> ± 6.2   | 445.8 <sup>a</sup> ± 13.5 | 165.2 <sup>c</sup> ± 7.1  | 176.2 <sup>b</sup> ± 8.7  |
| <b>OC</b>                 | 12.23 <sup>a</sup> ± 0.02 | 8.08 <sup>b</sup> ± 0.03  | 5.14 <sup>c</sup> ± 0.11  | 6.86 <sup>a</sup> ± 0.01  | 5.12 <sup>c</sup> ± 0.05  | 6.27 <sup>b</sup> ± 0.01  |
| <b>DOC</b>                | 16.94 <sup>a</sup> ± 0.29 | 15.84 <sup>b</sup> ± 0.25 | 11.11 <sup>c</sup> ± 0.02 | 10.86 <sup>b</sup> ± 0.22 | 11.82 <sup>a</sup> ± 0.55 | 11.73 <sup>a</sup> ± 0.35 |
| <b>N</b>                  | 0.63 <sup>a</sup> ± 0.01  | 0.56 <sup>b</sup> ± 0.01  | 0.33 <sup>c</sup> ± 0.01  | 0.42 <sup>a</sup> ± 0.01  | 0.37 <sup>b</sup> ± 0.01  | 0.34 <sup>c</sup> ± 0.01  |
| <b>C/N</b>                | 19.32 <sup>a</sup> ± 0.17 | 14.37 <sup>c</sup> ± 0.18 | 15.75 <sup>b</sup> ± 0.07 | 16.25 <sup>b</sup> ± 0.05 | 13.97 <sup>c</sup> ± 0.07 | 18.53 <sup>a</sup> ± 0.18 |

**Table 6 (2017)** - Soil parameters detected in the two soil layers (0-15; 15-30 cm) under different forest managements (T1- Control; T2- Traditional thinning; T3- Innovative thinning): humification degree (DH, %); humification rates (HR, %); humification index (HI); microbial biomass (MBC,  $\mu\text{g C g}^{-1}$  f.s.); ergosterol fungal biomarker (Erg,  $\mu\text{g g soil}^{-1}$ ); fluorescein released (FDA,  $\mu\text{g g}^{-1}$  dry soil); dehydrogenase (DHA,  $\mu\text{g INTF g}^{-1}$  dry soil  $\text{h}^{-1}$ ); catalase activity (CAT,  $\text{O}_2\%/3\text{min/g dry soil}^{-1}$ ). Numbers denote the standard error of the mean: n=3. Means in the same row followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

| Soil parameters | First year (2017) 0-15 cm |                           |                           | 15 - 30 cm                |                           |                           |
|-----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                 | T1                        | T2                        | T3                        | T1                        | T2                        | T3                        |
| <b>DH</b>       | 88.3 <sup>a</sup> ± 0.7   | 85.6 <sup>b</sup> ± 0.2   | 88.8 <sup>a</sup> ± 0.9   | 87.3 <sup>a</sup> ± 0.7   | 84.6 <sup>b</sup> ± 0.2   | 86.7 <sup>a</sup> ± 0.9   |
| <b>HR</b>       | 62.6 <sup>b</sup> ± 0.51  | 59.08 <sup>c</sup> ± 0.01 | 66.48 <sup>a</sup> ± 0.01 | 61.88 <sup>b</sup> ± 0.51 | 58.38 <sup>c</sup> ± 0.01 | 64.93 <sup>a</sup> ± 0.01 |
| <b>HI</b>       | 0.31 <sup>c</sup> ± 0.01  | 0.49 <sup>a</sup> ± 0.01  | 0.34 <sup>b</sup> ± 0.01  | 0.38 <sup>b</sup> ± 0.01  | 0.50 <sup>a</sup> ± 0.01  | 0.37 <sup>b</sup> ± 0.01  |
| <b>MBC</b>      | 1615 <sup>a</sup> ± 3     | 1126 <sup>b</sup> ± 7     | 1039 <sup>c</sup> ± 4     | 773 <sup>a</sup> ± 9      | 616 <sup>c</sup> ± 2      | 716 <sup>b</sup> ± 5      |
| <b>Erg</b>      | 11.7 <sup>a</sup> ± 0.3   | 11.0 <sup>a</sup> ± 0.5   | 3.1 <sup>b</sup> ± 0.1    | 1.2 <sup>a</sup> ± 0.2    | 1.2 <sup>ab</sup> ± 0.1   | 0.8 <sup>b</sup> ± 0.1    |
| <b>FDA</b>      | 72.0 <sup>a</sup> ± 3.2   | 60.7 <sup>a</sup> ± 7.1   | 37.1 <sup>b</sup> ± 3.9   | 25.3 <sup>a</sup> ± 4.1   | 31.0 <sup>a</sup> ± 2.6   | 27.9 <sup>a</sup> ± 2.14  |
| <b>DHA</b>      | 0.72 <sup>b</sup> ± 0.30  | 0.57 <sup>b</sup> ± 0.13  | 2.78 <sup>a</sup> ± 0.33  | 1.51 <sup>a</sup> ± 0.14  | 1.75 <sup>a</sup> ± 0.12  | 1.51 <sup>a</sup> ± 0.29  |
| <b>CAT</b>      | 2.9 <sup>b</sup> ± 0.3    | 4.4 <sup>a</sup> ± 0.3    | 1.7 <sup>c</sup> ± 0.1    | 0.8 <sup>b</sup> ± 0.1    | 1.6 <sup>a</sup> ± 0.3    | 1.1 <sup>c</sup> ± 0.1    |

PCA diagram for 0-15 cm and 15-30 cm soil depths is shown in figure 9. At both depths, the first two components (Eigenvalues >1) have been extracted. The variance was higher at 0-15 cm (94 %), than at 15-30 cm (88 %) (Table 7). At both depths, the component 1 explained  $\approx 60\%$ , while the component 2 explained  $\approx 30\%$  of the variability in all parameters (Fig. 9, Table 7).

From the PCA scatter plot, it was possible to observe how pH, DHA, soil aggregate stability and HR were positively influenced by the innovative thinning in both layers. Conversely, Erg, WSP,  $^{137}\text{Cs}$ , MBC, OC, N and HI were positioned in the opposite quadrants to innovative thinning (Fig. 9a,b). The displacement of T2 area in the left quadrant of the underlying layer (Fig. 9b) with respect to the surface layer, showed how the differences between the soil parameters in the areas subjected to forest management were reduced.



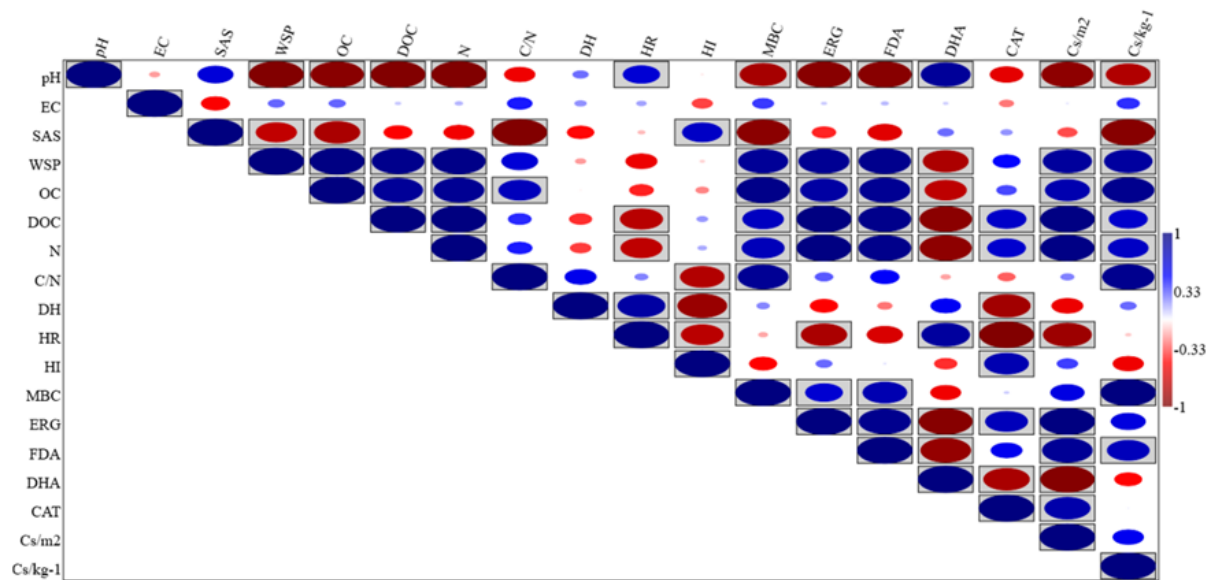
**Fig. 9 (2017)** - Principal component analysis diagram (PCA) in areas with different forest management: control area (T1), traditional thinning (T2), and innovative thinning (T3), at 0-15 cm (a) and 15-30 cm (b) soil depths.

**Table 7 (2017)** – Loading values (PC1 and PC2) of principal component analysis diagram (PCA) referred to 0-15 cm and 15-30 cm soil depths.

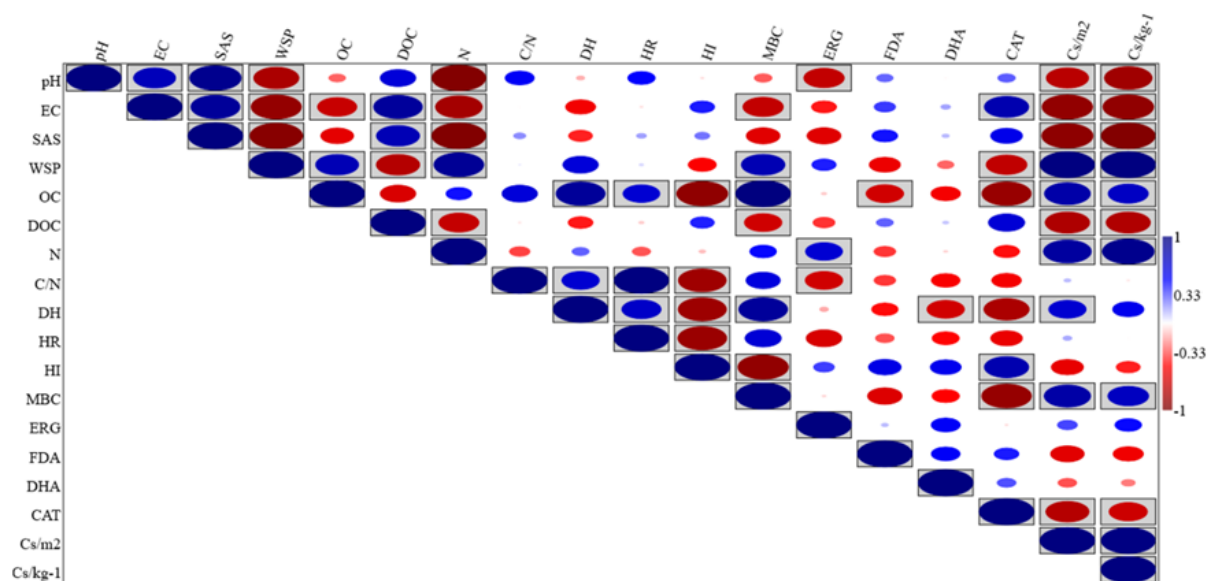
| Soil Parameters           | 0-15 cm |        | 15-30 cm |        |
|---------------------------|---------|--------|----------|--------|
|                           | PC1     | PC2    | PC1      | PC2    |
| <b>pH</b>                 | -0.999  | -0.016 | -0.651   | 0.738  |
| <b>EC</b>                 | 0.196   | 0.491  | -0.908   | 0.281  |
| <b>SAS</b>                | -0.634  | -0.771 | -0.863   | 0.494  |
| <b>WSP</b>                | 0.986   | 0.145  | 0.962    | -0.249 |
| <b>OC</b>                 | 0.955   | 0.291  | 0.916    | 0.389  |
| <b>DOC</b>                | 0.986   | -0.144 | -0.811   | 0.237  |
| <b>N</b>                  | 0.993   | -0.104 | 0.778    | -0.613 |
| <b>C/N</b>                | 0.542   | 0.832  | 0.293    | 0.943  |
| <b>DH</b>                 | -0.300  | 0.885  | 0.783    | 0.497  |
| <b>HR</b>                 | -0.680  | 0.729  | 0.329    | 0.921  |
| <b>HI</b>                 | 0.058   | -0.987 | -0.711   | -0.685 |
| <b>MBC</b>                | 0.834   | 0.546  | 0.922    | 0.362  |
| <b>ERG</b>                | 0.969   | -0.236 | 0.244    | -0.858 |
| <b>FDA</b>                | 0.969   | 0.001  | -0.635   | -0.236 |
| <b>DHA</b>                | -0.906  | 0.370  | -0.422   | -0.534 |
| <b>CAT</b>                | 0.610   | -0.777 | -0.867   | -0.269 |
| <b>Cs/m<sup>2</sup></b>   | 0.946   | -0.322 | 0.983    | -0.165 |
| <b>Cs/kg<sup>-1</sup></b> | 0.796   | 0.601  | 0.939    | -0.330 |
| Eigenvalues               | 11.427  | 5.518  | 10.468   | 5.385  |
| Total variance (%)        | 63.482  | 30.654 | 57.927   | 29.918 |
| Cumulative variance (%)   | 63.482  | 94.136 | 57.927   | 87.845 |

Pearson's correlation denoted that in the upper layer (Fig. 10a) OC was positively and significantly correlated with DOC ( $r = 0.90$ ), MBC ( $r = 0.96$ ), FDA ( $r = 0.92$ ), WSP ( $r = 0.98$ ), ERG ( $r = 0.85$ ), C/N ( $r = 0.76$ ), N ( $r = 0.92$ ) and  $^{137}\text{Cs}$  (the latter expressed in  $\text{Bq kg}^{-1}$  ( $r = 0.94$ ) and  $\text{Bq m}^2$  ( $r = 0.81$ )), while negatively and significantly correlated with pH ( $r = -0.96$ ) and DHA ( $r = -0.76$ ). In the underlying layer MBC ( $r = 0.99$ ), WSP ( $r = 0.78$ ), DH ( $r = 0.90$ ), HR ( $r = 0.67$ ) and  $^{137}\text{Cs}$  ( $\text{Bq kg}^{-1}$  and  $\text{Bq m}^2$ ) ( $r = 0.73$ ;  $0.84$ ) were positively correlated with OC. Conversely, HI ( $r = -0.93$ ), FDA ( $r = -0.68$ ), EC ( $r = -0.71$ ) and CAT ( $r = -0.90$ ) were negatively correlated with OC (Fig. 10b). In both layers,  $^{137}\text{Cs}$  were positively correlated with WSP ( $r > 0.87$ ), OC ( $r > 0.73$ ) and N ( $r > 0.73$ ), and negatively correlated with pH ( $r < -0.77$ ) (Fig. 10a,b). On the contrary, in the underlying layer  $^{137}\text{Cs}$  ( $\text{Bq m}^2$  and  $\text{Bq kg}^{-1}$ ) was negatively correlated with pH ( $r = -0.77$ ;  $-0.87$ ), EC ( $r = -0.92$ ;  $-0.93$ ), DOC ( $r = -0.81$ ;  $-0.81$ ) and catalase activity ( $r = -0.79$ ;  $-0.70$ ) (Fig. 10b).





(a)

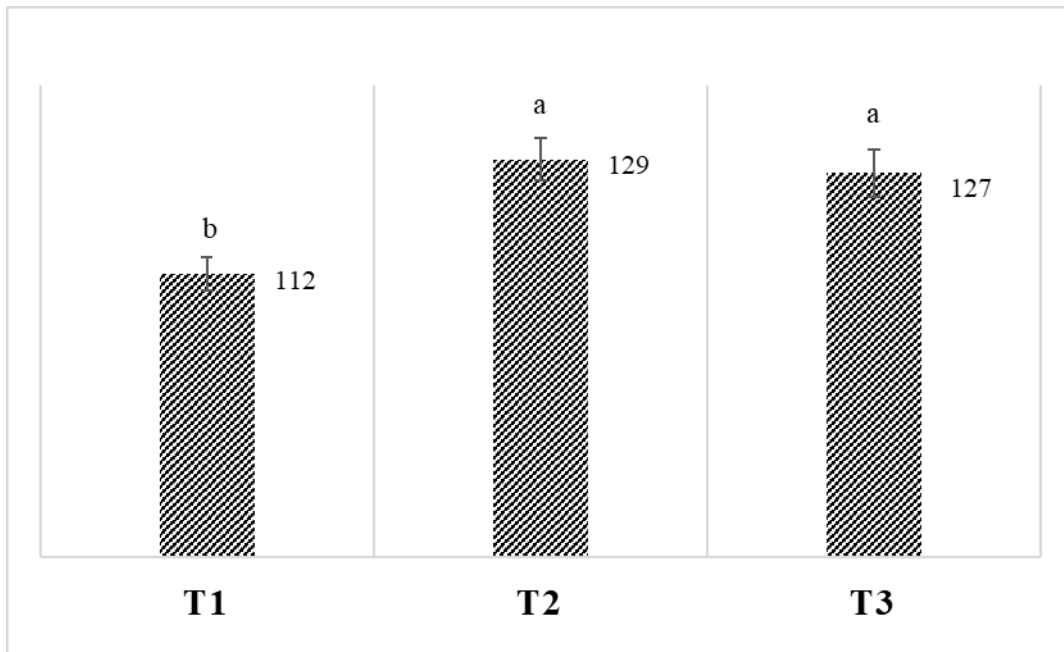


(b)

**Fig. 10 (2017)** - Pearson's correlations ( $r$ ) between the soil parameters at 0–15 cm (a) and 15–30 cm (b) depths. The boxed dots show the significant correlations between values, the magnitude shows the level (small boxed dots  $p < 0.05$ , large boxed dots  $p < 0.01$ ). The red dots the negative ones, the blue ones the positive ones (see the bars on the right of the figure).

QBS-ar value was significantly higher in T2 and T3 areas than in unmanaged area (Fig. 11) ( $p < 0.05$ ). These findings can be related to the habitat complexity, due to the presence of an undergrowth herbaceous vegetation that was missing in T1. The composition of the microarthropod community reflects changes in micro environmental conditions. Many works reported changes in population of soil microarthropods between the seasons evidencing higher populations during rainy season and a sharp decline in microarthropod community during summer months (Reddy and Venkataiah, 1990; Borah and Kakati, 2014).

Regarding the microarthropod groups a different distribution in soil differently managed was found (Table 8). Among the 16 species found (Table 8), in T1 only 9 species has been observed. Chilopoda, thysanoptera, protura, diplura, lepidoptera, formicidae and isopoda were totally absent (Table 8). In T2 only 14 species were present. The species that missed in soil under traditional thinning were blattaria and araneae. In T3 only 13 species were present, blattaria, protura and formicidae were missed. The greatest biodiversity was found in T2 in respect to the other treatments.



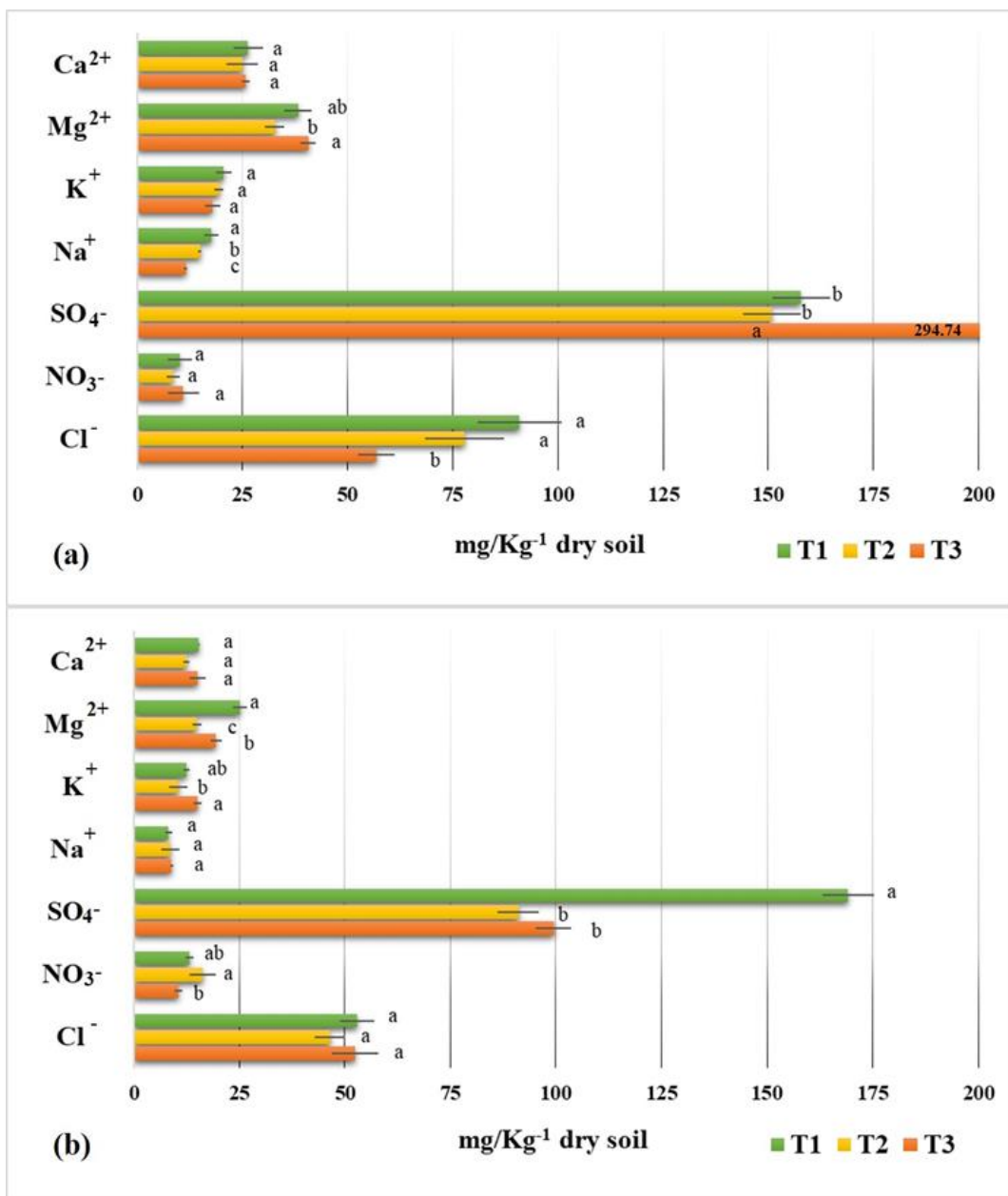
**Fig. 11 (2017)** - QBS-ar index for microarthropods, collected at 0–10 cm depths in soils under different managements: control (T1), traditional thinning (T2), innovative thinning (T3). Numbers denote the means: n=3. Bars and columns followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

**Table 8 (2017)** - Microarthropod groups found in soils under different managements: control (T1), traditional thinning (T2), innovative thinning (T3). QBs index is obtained from the sum of the highest values of EMI of all the collected groups.

| <b>Groups</b>               | <b>T1</b> | <b>T2</b> | <b>T3</b> |
|-----------------------------|-----------|-----------|-----------|
| <i>Acari</i>                | *         | *         | *         |
| <i>Collembola</i>           | *         | *         | *         |
| <i>Orthoptera</i>           | *         | *         | *         |
| <i>Hemiptera</i>            | *         | *         | *         |
| <i>Diptera (larvae)</i>     | *         | *         | *         |
| <i>Coleoptera</i>           | *         | *         | *         |
| <i>Chilopoda</i>            |           | *         | *         |
| <i>Blattaria</i>            | *         |           |           |
| <i>Symphyla</i>             | *         | *         | *         |
| <i>Thysanoptera</i>         |           | *         | *         |
| <i>Protura</i>              |           | *         |           |
| <i>Diplura</i>              |           | *         | *         |
| <i>Lepidoptera (larvae)</i> |           | *         | *         |
| <i>Araneae</i>              | *         |           | *         |
| <i>Formicidae</i>           |           | *         |           |
| <i>Isopoda</i>              |           | *         | *         |

### 3.1.2.1 Water soluble soil ions

As reported by Baeumler and Zech (1998), mean concentrations of alkali and alkaline earth cations decreased with increasing depth (Fig. 12). Our results showed a slight decrease of  $\text{Na}^+$  in the surface layer as thinning increased, a positive influence of innovative thinning (T3) on  $\text{SO}_4^-$  and  $\text{Mg}^{2+}$ , while no changes in  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentrations induced by thinning (Fig. 12a). The concentration of  $\text{NH}_4^+$  in the soil solution was below the detection limit. Deposited  $\text{NH}_4^+$  could be taken up by roots or oxidized to  $\text{NO}_3^-$  by microorganisms (Stams et al., 1991; Knoepp et al., 1993). Chloride concentration ( $\text{Cl}^-$ ) decreased in T3 area (0-15 cm), although showing a general decrease with respect to the surface of the soil, the 15-30 cm layer showed no significant differences between the different thinning (Fig. 12a,b). Sulphate ( $\text{SO}_4^-$ ) was abundant in the area with high thinning intensity, confirming previous data of Baeumler and Zech (1998). In the underlying layer, sulphate decreased in T2 and T3 areas, while it remained almost unchanged in T1, resulting greater than areas subjected to thinning (Fig. 12b). Besides leaching, the concentrations of  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Na}^+$  decreased with increasing depth (Fig. 12), presumably because the mineralization process is more intense in the surface layers with a consequent accumulation of water soluble ions from decomposing litter, while the root distribution is major in deep soil layers where a greater ion uptake occur.



**Fig. 12 (2017)** – Concentrations of water soluble ions (mg/Kg<sup>-1</sup> dry soil) in soil collected at 0–15 cm (a) and 15–30 cm (b) depths under different managements: control (T1), traditional thinning (T2), innovative thinning (T3). Bars denote the standard error of the mean: n=3. Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

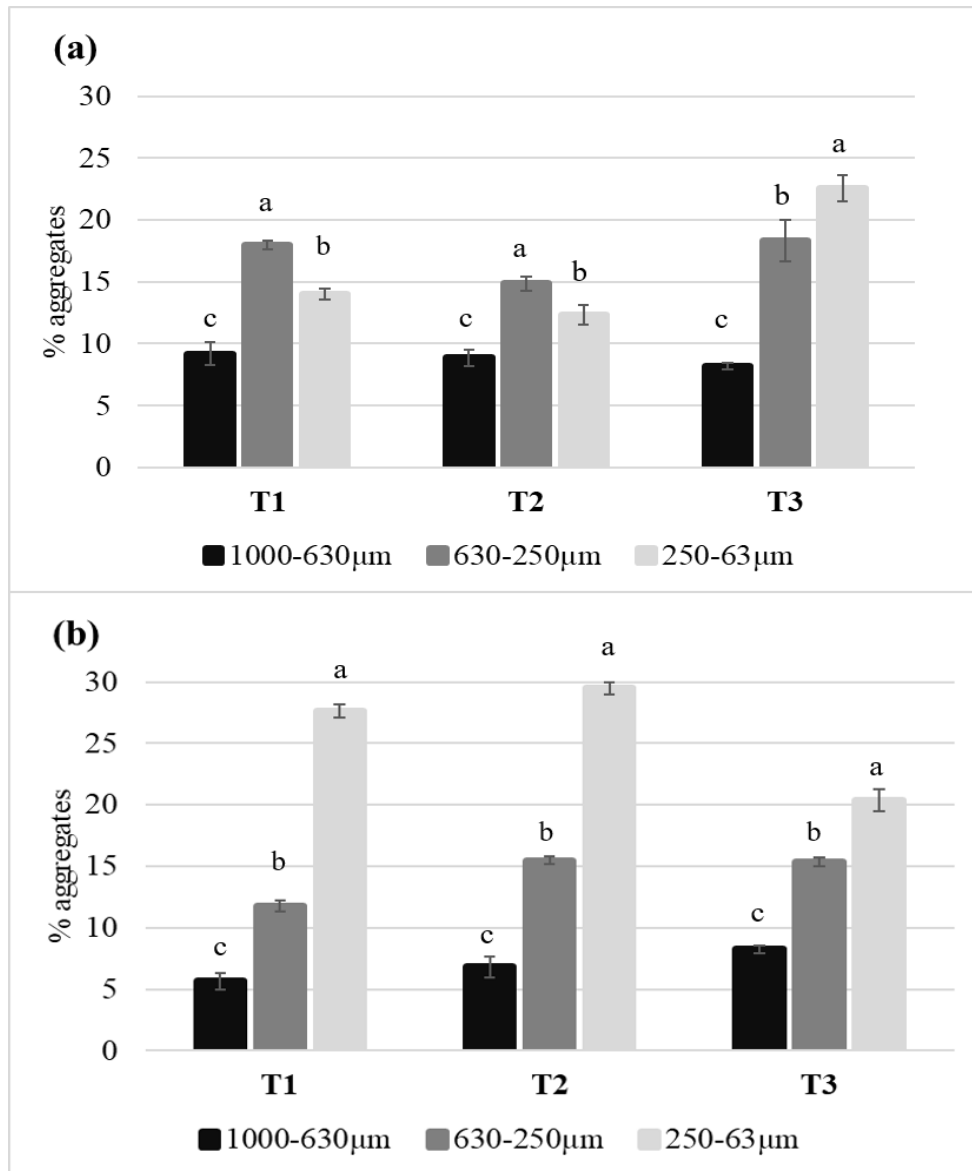
### 3.1.2.2 Soil aggregate stability

The distribution of aggregate fractions in soil under different forest management showed significant differences among the treatments at a depth of 0-15 cm (Fig. 13a). T1 and T2 contained a greater percentage of medium size aggregate fractions (250-630  $\mu\text{m}$ ) followed by small and finally large aggregates, while T3 showed a greater amount of small aggregates followed by medium and large ones. In the underlying layer (Fig. 13b), T3 maintained the same distribution of aggregate sizes along the profile, while in T1 and T2 the proportion between soil aggregates changed with a prevalence of small aggregates in respect to medium size aggregates. The distribution of OC and nitrogen in the aggregates changed not only in respect to the treatments but also with soil depth (Fig. 14a). Organic carbon and nitrogen were higher in the aggregates of the unmanaged forest (T1), at both depths, compared to the two thinning treatments (T2 and T3). Additionally, a larger content of OC and N was observed in small, medium and large aggregates in the upper layer of T1 and T2 (Fig. 14a,b). Conversely, OC and N concentrations were more abundant in the medium and small aggregates in the subsurface layer of the innovative treatment (T3) (Fig. 14a,b). In T1 the value of C/N ratio was higher in the large and medium aggregates of both soil layers, and it was significantly lower in the small aggregate fraction at both depths (Fig. 15). In T2 no significant differences among the aggregates at both depth was observed. The highest C/N ratio was observed in the large and medium aggregates in T3. Comparing the three treatments the highest values of C/N ratio was found in T3, at both depths (Fig. 15).

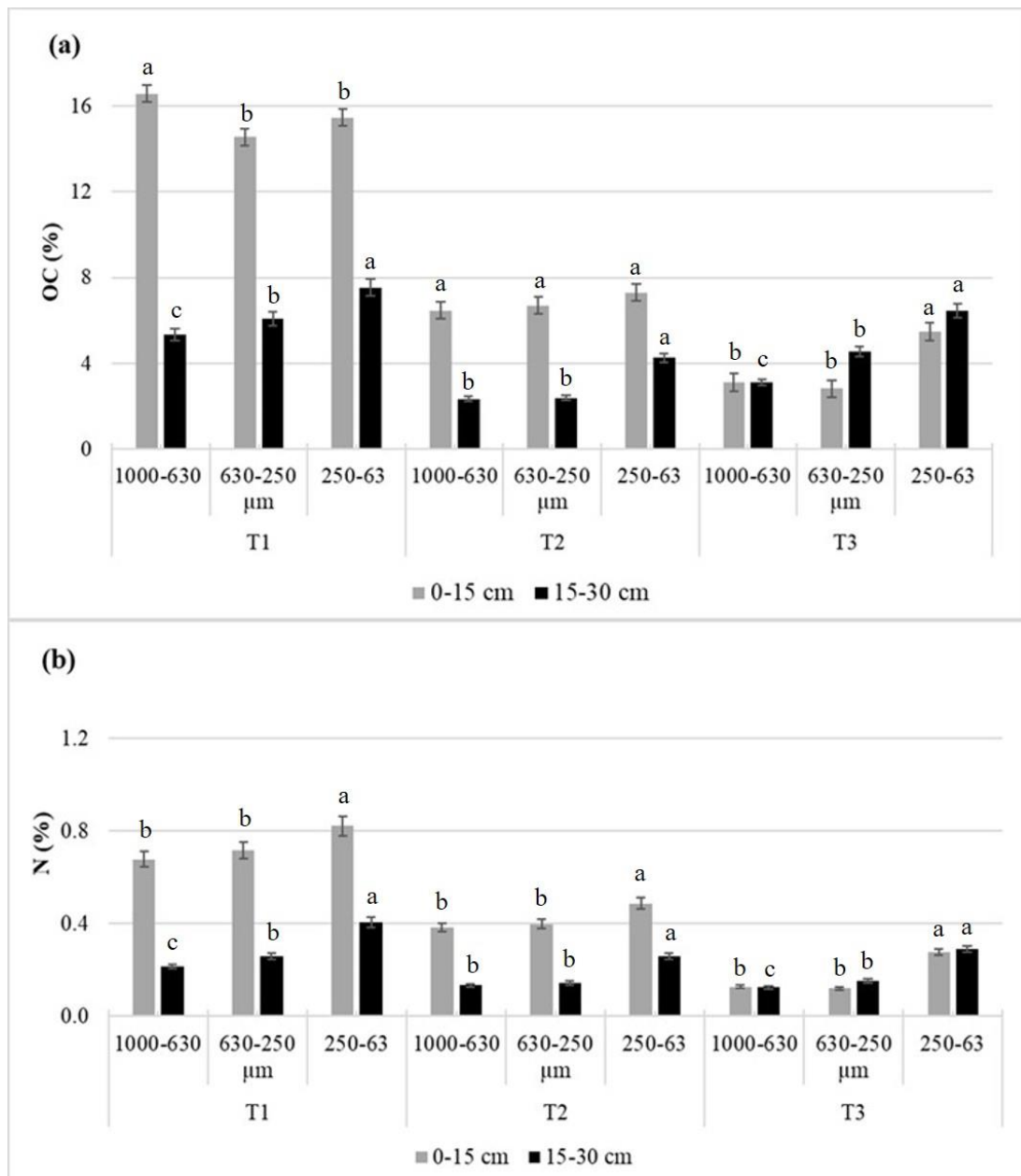
Aggregate stability is a fundamental soil property related to soil productivity, resistance to erosion and degradation (Raine and So, 1997; Six et al., 2000). Aggregate stability is a highly complex parameter influencing a wide range of soil properties, including carbon stabilization, soil porosity, water infiltration, aeration, compactability, water retention, hydraulic conductivity, resistance to erosion by water and overland flow. Maintaining a high stability of soil aggregates is essential for preserving soil productivity, minimizing soil erosion and degradation and consequently environmental pollution. Arshad and Cohen (1992) described aggregate stability as one of the soil physical property that can serve as indicator of soil quality. Hortensius and Welling (1996) included this parameter in the international standardization of soil quality measurements. The degree of association of OC with particle sizes is a qualitative

indicator of the impact of soil management. The OC associated with the medium fraction is commonly less decomposed material and has a higher C/N ratio than that associated with the fine fraction. OC adsorbed to clay is mostly of humic nature with a low C/N ratio (Christensen, 2001). Our data showed that T2 had the lowest C/N ratio in the fine fraction, suggesting that the OC is more stable and is altered more by physical and chemical process than by management practice as already demonstrated by Khanna et al. (2001). Conversely, C/N ratio, higher in T3 in the large fraction, represents a labile pool of C and reflects rapid changes in SOM quality due to changes in management.

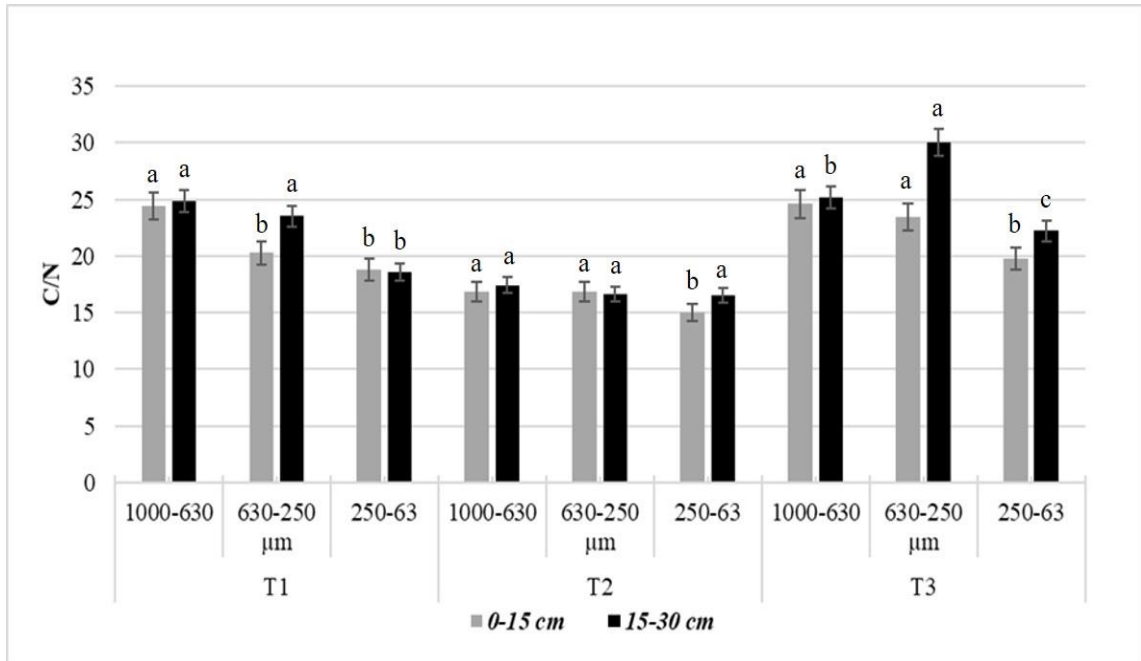




**Fig. 13 (2017)** - Distribution of soil aggregate fractions obtained with ultrasonic method (USAS) under different forest managements: control (T1), traditional thinning (T2), innovative thinning (T3); at depths of 0–15 cm (a) and 15–30 cm (b). Bars denote the standard error of the mean: n=3. Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).



**Fig. 14 (2017)** - Distribution of organic carbon (a) and total nitrogen (b) in the different aggregate fractions, at 0-15 and 15-30 cm depth, under different forests managements: control (T1), traditional thinning (T2) and innovative thinning (T3). Bars represent standard errors (n=3). Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).



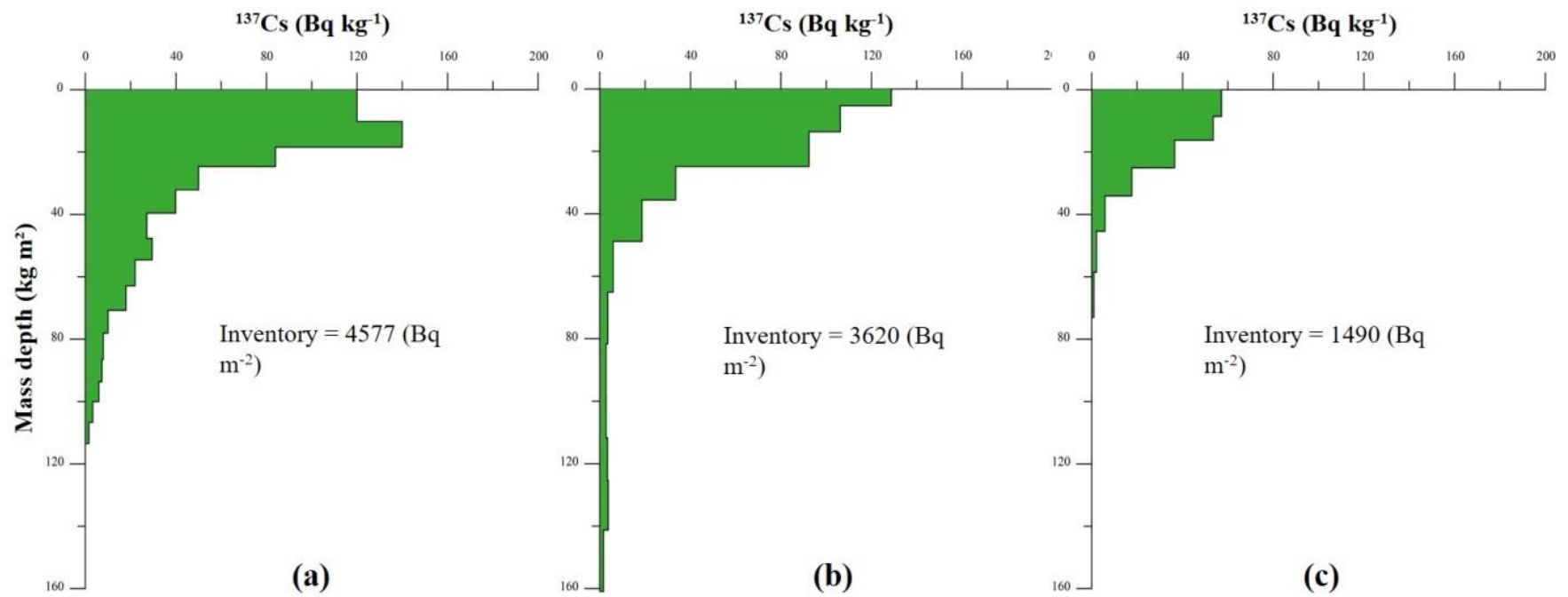
**Fig. 15 (2017)** – C/N ratio distribution in the different aggregate fractions, at 0-15 and 15-30 cm depth, under different forest managements: control (T1), traditional thinning (T2) and innovative thinning (T3). Bars represent standard errors (n=3). Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

## 3.2 Year 2018

### 3.2.1 $^{137}\text{Cs}$ distribution and soil erosion estimation

The  $^{137}\text{Cs}$  profile in T1 (Fig. 16a,b,c) shows a reference inventory value of  $4577 \text{ Bq m}^{-2}$ . T2 and T3 areas showed inventory values of  $3620 \text{ Bq m}^{-2}$  and  $1490 \text{ Bq m}^{-2}$  respectively (Fig. 16b,c).

The values of soil loss obtained by using eq. (3) for the areas T2 and T3 were equal to  $3.7 \text{ (t ha}^{-1} \text{ yr}^{-1})$  and  $13.4 \text{ (t ha}^{-1} \text{ yr}^{-1})$ , respectively, showed a higher impact of the innovative treatment if compared with the traditional one. Soil surface showed  $^{137}\text{Cs}$  activities (ca.  $120 \text{ Bq kg}^{-1}$  and  $50 \text{ Bq kg}^{-1}$  for T2 and T3, respectively) lower than control area (ca.  $150 \text{ Bq kg}^{-1}$ ) (Fig. 16a). This finding confirmed the data of the previous year, highlighting that the upper part of the soil profile was removed by erosion process with less intensity in T2 respect to T3.



**Fig. 16 (2018)** -  $^{137}\text{Cs}$  profiles in the control area T1 (a); area under traditional thinning T2 (b); area under innovative thinning T3 (c).

### 3.2.2 Physical, chemical and biological soil properties

In both soil layers, pH varied significantly among the different managed areas (Table 9), showing sub-acid reaction. The values ranged from 5.2 to 6 and showed an increase when the thinning intensity increased, the trend was the same in both soil layers. Electrical conductivity was higher in the surface layer (176  $\mu\text{S}$ ) than underlying layer (at about 111  $\mu\text{S}$ ), no significant differences among the different treatments in both layers were observed (Table 9).

WSP, as in the first year, decreased with increasing thinning intensity and showed significant differences among the silvicultural treatments in both layers (Table 9). OC, DOC and N decreased especially in the upper layer with the increase in thinning intensity (Table 9). These results reflected the trend of the previous year confirming that the worsening in soil chemical property values was probably due to the erosive phenomena that occurred.

The amount of  $^{137}\text{Cs}$  ( $\text{m}^2 \text{kg}^{-1}$ ), decreased in T2 and T3 compared to control. In addition to the strong decrease in the values of  $^{137}\text{Cs}$  ( $\text{Bq kg}^{-1}, \text{m}^{-2}$ ) in T2 with respect to the control, in T3 no trace of radionuclide was detected in the underlying layer (Table 9).

Humification parameters (DH, HR, HI) evidenced, in all the experimental areas, the prevalence of the humification over the mineralization process, as also confirmed by the C/N ratios that was much higher than 12, in both layers (Tables 9 and 10).

As already observed in the previous year, microbial biomass decreased, in both layers, in T2 and much more in T3 in respect to control (Table 10).

Enzymatic activities had different trend among the treatments. In 0-15 cm (Table 10), FDA was higher in T1 and T2 than in T3; DHA was greater in T3 than the in the other two areas; CAT was the greatest in T2. Ergosterol was greater in T2 than in control and T3, in both soil layers (Table 10).

In short, we highlighted that  $^{137}\text{Cs}$ , OC, MBC and WSP were correlated each other's and decreased gradually when the intensity of thinning increased (Tables 9 and 10).

**Table 9 (2018)** - Soil parameters detected in the two soil layers (0-15; 15-30 cm) under different forest managements (T1- Control; T2- Traditional thinning; T3- Innovative thinning): water content (WC, %); pH (H<sub>2</sub>O); electrical conductivity (EC,  $\mu\text{S cm}^{-1}$ ); soil aggregate stability by wet sieving (SAS, %); <sup>137</sup>Cs (Bq m<sup>2</sup>, Bq kg<sup>-1</sup>); water soluble phenols (WSP,  $\mu\text{g TAE g}^{-1}$  dry soil); organic carbon (OC, %); dissolved organic carbon (ml DOC L<sup>-1</sup>); total nitrogen (N, %); C/N ratio. Numbers denote the standard error of the mean: n=3. Means in the same row followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

| Soil parameters           | Second year (2018) 0-15 cm |                           |                           | 15 - 30 cm                |                           |                           |
|---------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                           | T1                         | T2                        | T3                        | T1                        | T2                        | T3                        |
| <b>WC</b>                 | 41 <sup>a</sup> ± 2        | 20.7 <sup>b</sup> ± 3     | 18.5 <sup>b</sup> ± 2     | 40.6 <sup>a</sup> ± 4     | 16.8 <sup>b</sup> ± 2     | 12 <sup>b</sup> ± 3       |
| <b>pH</b>                 | 5.36 <sup>c</sup> ± 0.01   | 5.50 <sup>b</sup> ± 0.01  | 5.98 <sup>a</sup> ± 0.10  | 5.25 <sup>c</sup> ± 0.01  | 5.54 <sup>b</sup> ± 0.01  | 5.75 <sup>a</sup> ± 0.01  |
| <b>EC</b>                 | 175.8 <sup>a</sup> ± 2.4   | 172.7 <sup>a</sup> ± 1.5  | 172.9 <sup>a</sup> ± 3.1  | 111.5 <sup>a</sup> ± 1.4  | 112.6 <sup>a</sup> ± 2.8  | 113.5 <sup>a</sup> ± 0.7  |
| <b>SAS</b>                | 92.7 <sup>b</sup> ± 0.2    | 93.2 <sup>b</sup> ± 0.4   | 97.7 <sup>a</sup> ± 0.3   | 90.2 <sup>c</sup> ± 1.1   | 97.6 <sup>b</sup> ± 0.2   | 99.5 <sup>a</sup> ± 0.1   |
| <b>Cs m<sup>2</sup></b>   | 4078 <sup>a</sup> ± 4      | 3393 <sup>b</sup> ± 3     | 1490 <sup>c</sup> ± 2     | 495 <sup>a</sup> ± 1      | 199 <sup>b</sup> ± 1      | -                         |
| <b>Cs kg<sup>-1</sup></b> | 112.6 <sup>a</sup> ± 0.4   | 72.6 <sup>b</sup> ± 0.7   | 34.2 <sup>c</sup> ± 0.2   | 5.5 <sup>a</sup> ± 0.1    | 1.3 <sup>b</sup> ± 0.1    | -                         |
| <b>WSP</b>                | 259.2 <sup>a</sup> ± 6.3   | 155.1 <sup>b</sup> ± 6.6  | 67.5 <sup>c</sup> ± 5.0   | 313.3 <sup>a</sup> ± 6.3  | 163.3 <sup>b</sup> ± 10.1 | 155.8 <sup>b</sup> ± 15.8 |
| <b>OC</b>                 | 11.54 <sup>a</sup> ± 0.37  | 6.48 <sup>b</sup> ± 0.17  | 3.06 <sup>c</sup> ± 0.06  | 5.37 <sup>a</sup> ± 0.02  | 4.17 <sup>c</sup> ± 0.02  | 4.88 <sup>b</sup> ± 0.03  |
| <b>DOC</b>                | 14.15 <sup>a</sup> ± 0.14  | 14.51 <sup>a</sup> ± 0.42 | 12.94 <sup>b</sup> ± 0.17 | 11.07 <sup>b</sup> ± 0.41 | 12.42 <sup>a</sup> ± 0.58 | 10.33 <sup>b</sup> ± 0.43 |
| <b>N</b>                  | 0.57 <sup>a</sup> ± 0.01   | 0.43 <sup>b</sup> ± 0.01  | 0.23 <sup>c</sup> ± 0.01  | 0.34 <sup>a</sup> ± 0.01  | 0.26 <sup>c</sup> ± 0.01  | 0.31 <sup>b</sup> ± 0.01  |
| <b>C/N</b>                | 20.37 <sup>a</sup> ± 0.60  | 15.10 <sup>b</sup> ± 0.41 | 13.15 <sup>c</sup> ± 0.27 | 15.67 <sup>a</sup> ± 0.11 | 15.79 <sup>a</sup> ± 0.12 | 15.89 <sup>a</sup> ± 0.09 |

**Table 10 (2018)** - Soil parameters detected in the two soil layers (0-15; 15-30 cm) under different forest managements (T1- Control; T2- Traditional thinning; T3- Innovative thinning): humification degree (DH, %); humification rates (HR, %); humification index (HI); microbial biomass (MBC,  $\mu\text{g C g}^{-1}$  f.s.); ergosterol fungal biomarker (Erg,  $\mu\text{g g soil}^{-1}$ ); fluorescein released (FDA,  $\mu\text{g g}^{-1}$  dry soil); dehydrogenase (DHA,  $\mu\text{g INTF g}^{-1}$  dry soil  $\text{h}^{-1}$ ); catalase activity (CAT,  $\text{O}_2\%/3\text{min/g dry soil}^{-1}$ ). Numbers denote the standard error of the mean: n=3. Means in the same row followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

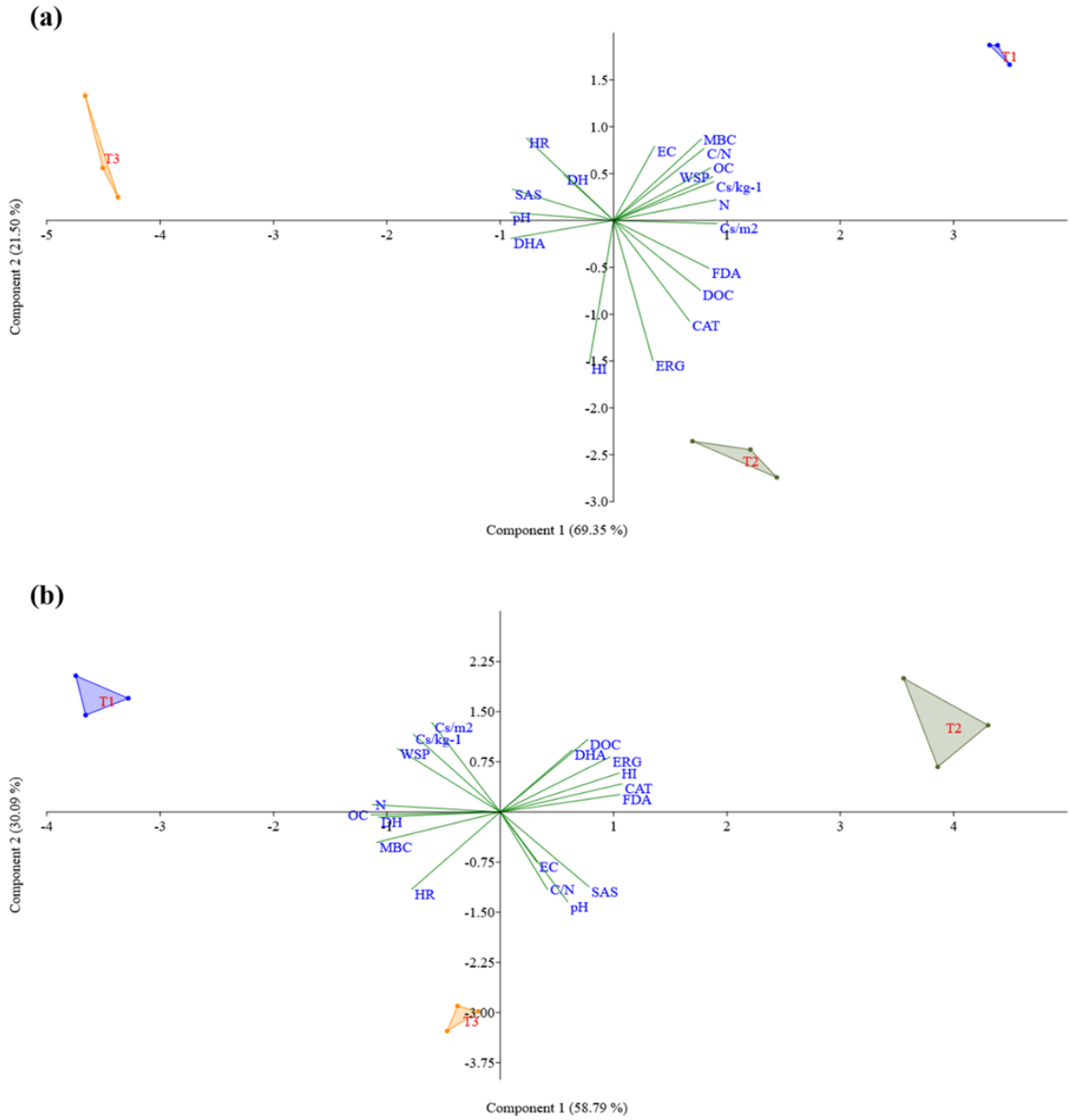
| Soil parameter | Second year (2018) 0-15 cm |                           |                           | 15 - 30 cm                |                           |                           |
|----------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                | T1                         | T2                        | T3                        | T1                        | T2                        | T3                        |
| <b>DH</b>      | 88.7 <sup>a</sup> ± 0.6    | 88.5 <sup>a</sup> ± 0.5   | 89.7 <sup>a</sup> ± 1.9   | 87.1 <sup>a</sup> ± 0.7   | 83.4 <sup>b</sup> ± 0.2   | 85.6 <sup>a</sup> ± 0.9   |
| <b>HR</b>      | 62.83 <sup>b</sup> ± 0.41  | 61.13 <sup>c</sup> ± 0.40 | 67.19 <sup>a</sup> ± 0.72 | 61.69 <sup>b</sup> ± 0.50 | 57.60 <sup>c</sup> ± 0.01 | 64.08 <sup>a</sup> ± 0.01 |
| <b>HI</b>      | 0.33 <sup>c</sup> ± 0.01   | 0.53 <sup>a</sup> ± 0.01  | 0.42 <sup>b</sup> ± 0.03  | 0.44 <sup>b</sup> ± 0.02  | 0.58 <sup>a</sup> ± 0.01  | 0.45 <sup>b</sup> ± 0.01  |
| <b>MBC</b>     | 1529 <sup>a</sup> ± 7      | 910 <sup>b</sup> ± 6      | 742 <sup>c</sup> ± 3      | 612 <sup>a</sup> ± 3      | 487 <sup>c</sup> ± 6      | 593 <sup>b</sup> ± 6      |
| <b>Erg</b>     | 6.1 <sup>b</sup> ± 0.5     | 10.3 <sup>a</sup> ± 0.5   | 5.3 <sup>b</sup> ± 0.5    | 0.96 <sup>b</sup> ± 0.02  | 2.1 <sup>a</sup> ± 0.1    | 0.1 <sup>c</sup> ± 0.02   |
| <b>FDA</b>     | 55.2 <sup>a</sup> ± 5.1    | 56.9 <sup>a</sup> ± 5.0   | 27.4 <sup>b</sup> ± 0.3   | 22.0 <sup>b</sup> ± 3.8   | 37.4 <sup>a</sup> ± 2.9   | 25.6 <sup>b</sup> ± 1.7   |
| <b>DHA</b>     | 1.28 <sup>c</sup> ± 0.02   | 1.93 <sup>b</sup> ± 0.03  | 2.96 <sup>a</sup> ± 0.04  | 1.62 <sup>a</sup> ± 0.02  | 1.87 <sup>a</sup> ± 0.28  | 1.51 <sup>a</sup> ± 0.01  |
| <b>CAT</b>     | 2.4 <sup>b</sup> ± 0.1     | 3.1 <sup>a</sup> ± 0.1    | 1.6 <sup>c</sup> ± 0.1    | 1.0 <sup>b</sup> ± 0.1    | 1.7 <sup>a</sup> ± 0.1    | 1.2 <sup>b</sup> ± 0.2    |



PCA diagram for 0-15 cm and 15-30 cm soil depths, is shown in figure 17. The variance of the component extracted (Eigenvalues >1) was high in both layers, 0-15 cm (91 %) and 15-30 cm (89 %) (Table 11). At both depths, the component 1 explained about 65%, while the component 2 explained about 25% of the variability in all parameters (Fig. 17, Table 11). As in the previous year T3 was correlated with pH, soil aggregate stability and HR in both layers. Instead, T1 was mainly correlated with WSP, <sup>137</sup>Cs, MBC and N.

In the surface layer, DHA was correlated with innovative thinning while FDA and CAT with traditional thinning (Fig. 17). The displacement of T2 and T3 in the opposite quadrants of the underlying layer (Fig. 17b), in respect to the superficial layer, showed how the differences between the soil parameters in the areas subjected to forest management were reduced.

Ergosterol appeared strictly correlated with T2 and located in the same quadrant (Fig. 17ab). In brief, from the PCA scatter plot, it was possible to observe how WSP were strictly and positively correlated, in both layers, to <sup>137</sup>Cs even more than carbon, that was also strongly correlated to both of them (Fig. 17a,b).

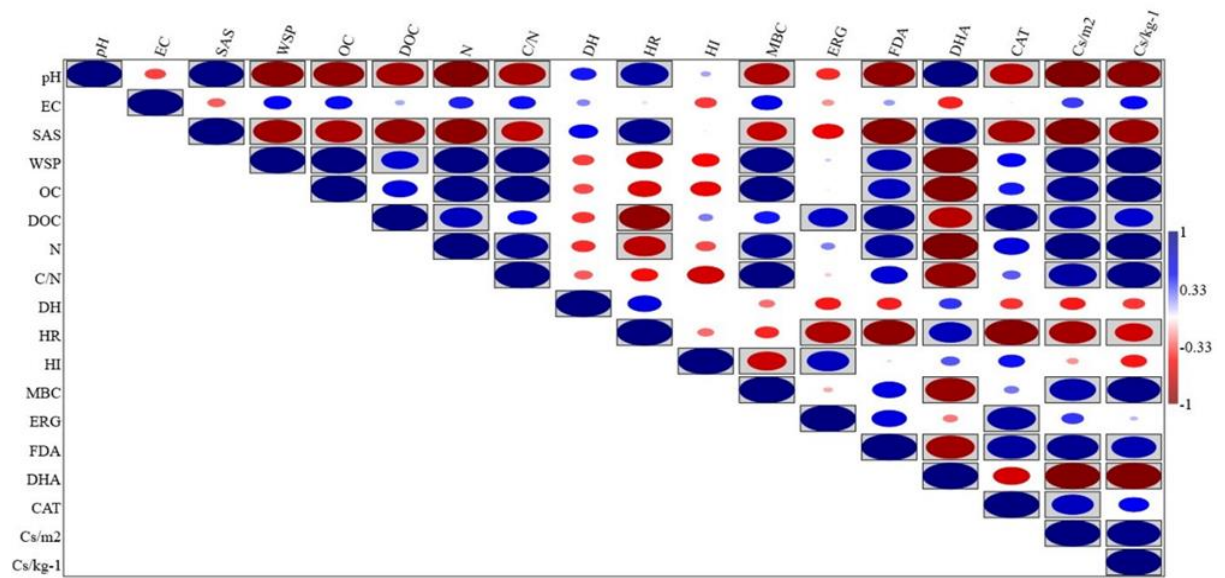


**Fig. 17 (2018)** - Principal component analysis diagram (PCA) in areas with different forest managements: control area (T1), traditional thinning (T2), and innovative thinning (T3), at 0-15 cm **(a)** and 15-30 cm **(b)** soil depths.

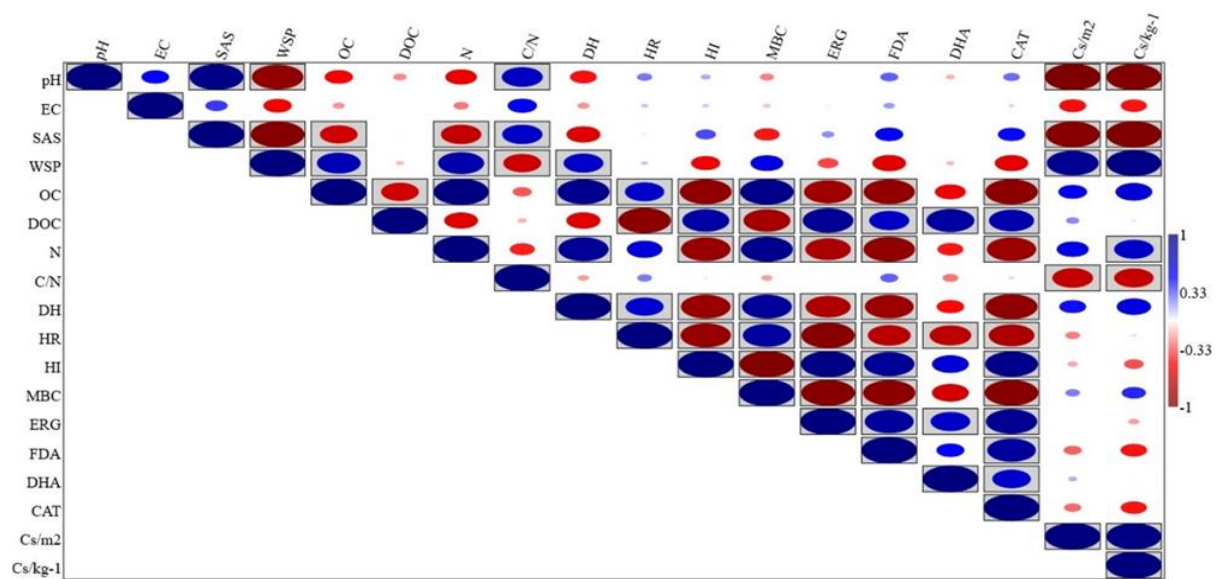
**Table 11 (2018)** – Loading values (PC1 and PC2) of principal component analysis diagram (PCA) referred to 0-15 cm and 15-30 cm soil depths.

| Soil Parameters         | 0-15 cm |        | 15-30 cm |        |
|-------------------------|---------|--------|----------|--------|
|                         | PC1     | PC2    | PC1      | PC2    |
| pH                      | -0.996  | 0.052  | 0.521    | -0.840 |
| EC                      | 0.395   | 0.482  | 0.281    | -0.466 |
| SAS                     | -0.976  | 0.203  | 0.685    | -0.699 |
| WSP                     | 0.957   | 0.284  | -0.794   | 0.593  |
| OC                      | 0.937   | 0.342  | -0.995   | -0.026 |
| DOC                     | 0.837   | -0.454 | 0.675    | 0.678  |
| N                       | 0.989   | 0.134  | -0.985   | 0.069  |
| C/N                     | 0.873   | 0.468  | 0.365    | -0.725 |
| DH                      | -0.476  | 0.296  | -0.939   | -0.047 |
| HR                      | -0.839  | 0.535  | -0.682   | -0.722 |
| HI                      | -0.235  | -0.932 | 0.916    | 0.363  |
| MBC                     | 0.846   | 0.529  | -0.954   | -0.284 |
| ERG                     | 0.379   | -0.911 | 0.846    | 0.514  |
| FDA                     | 0.920   | -0.310 | 0.922    | 0.163  |
| DHA                     | -0.990  | -0.116 | 0.554    | 0.579  |
| CAT                     | 0.732   | -0.654 | 0.940    | 0.264  |
| Cs/m <sup>2</sup>       | 0.998   | -0.020 | -0.527   | 0.835  |
| Cs/kg <sup>-1</sup>     | 0.967   | 0.251  | -0.670   | 0.727  |
| Eigenvalues             | 12.482  | 3.869  | 10.582   | 5.416  |
| Total variance (%)      | 69.346  | 21.496 | 58.786   | 30.088 |
| Cumulative variance (%) | 69.346  | 90.842 | 58.786   | 88.874 |

Pearson's correlation evidenced that, in the upper layer, (Fig. 18a) OC was positively and significantly correlated with N ( $r = 0.97$ ), C/N ( $r = 0.99$ ), MBC ( $r = 0.98$ ), FDA ( $r = 0.76$ ) and <sup>137</sup>Cs (the latter expressed in Bq kg<sup>-1</sup> ( $r = 0.99$ ) and Bq m<sup>2</sup> ( $r = 0.93$ )). In the underlying layer MBC ( $r = 0.96$ ), DH ( $r = 0.93$ ), HR ( $r = 0.70$ ) and N ( $r = 0.99$ ) were positively correlated with OC. Conversely, HI ( $r = -0.93$ ), FDA ( $r = -0.92$ ), DOC ( $r = -0.67$ ), ERG ( $r = -0.86$ ) and CAT ( $r = -0.94$ ) were negatively correlated with organic carbon (Fig. 18b). In both layers, <sup>137</sup>Cs (Bq kg<sup>-1</sup>; Bq m<sup>2</sup>) were positively correlated with WSP ( $r > 0.93$ ), while was positively correlated with OC ( $r = 0.99$ ;  $0.93$ ) and DOC ( $r = 0.69$ ;  $0.84$ ) only in the upper layer. The radionuclide was negatively correlated with pH ( $r < -0.95$ ) and SAS ( $r < -0.89$ ), in both layers (Fig. 18a,b).



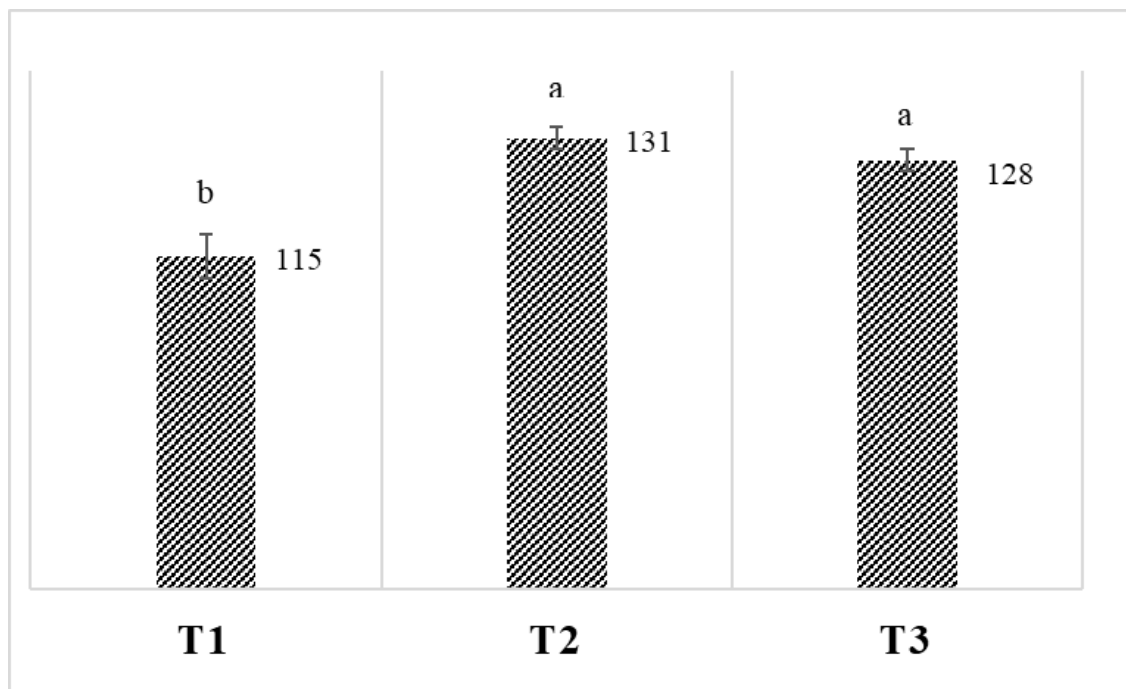
(a)



(b)

**Fig. 18 (2018)** - Pearson's correlations ( $r$ ) between the soil parameters at 0–15 cm (a) and 15–30 cm (b) depths. The boxed dots show the significant correlations between values, the magnitude shows the level (small boxed dots  $p < 0.05$ , large boxed dots  $p < 0.01$ ). The red dots the negative ones, the blue ones the positive ones (see the bars on the right of the figure).

As in the previous year, the value of QBS-ar was significantly higher in T2 and in T3 than in control area (Fig. 19). Regarding the micro-arthropod groups a different distribution in soil differently managed was found. Among the 16 species found (Table 12), in T1 only 9 species has been observed. Chilopoda, thysanoptera, protura, diplura, lepidoptera, araneae, formicidae and isopoda were totally absent (Table 12). In T2 only 14 species were present. The species that missed in soil under traditional thinning were blattaria and diplura. In T3 13 species were found, blattaria, protura and formicidae were missed (Table 12). The greatest biodiversity was found in T2 in respect to the other treatments. The difference in biodiversity can be ascribed to the different canopy cover which in turn determine different amount of litter, and changes in the microclimate in terms of humidity and temperature.



**Fig. 19 (2018)** - QBS-ar index for microarthropods, collected at 0–10 cm depths in soils under different managements: control (T1), traditional thinning (T2), innovative thinning (T3). Numbers denote the means: n=3. Bars and columns followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

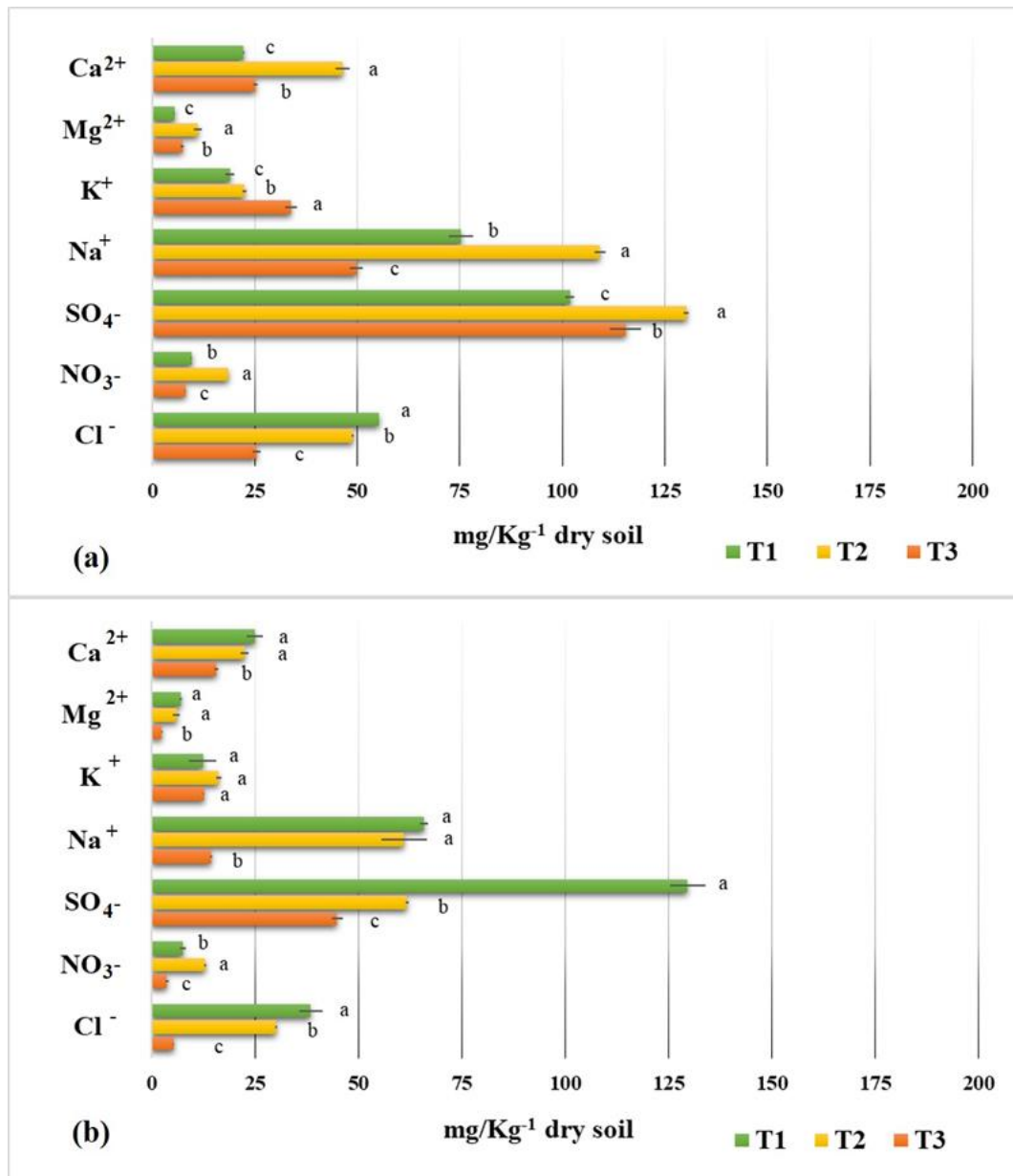
**Table 12 (2018)** - Microarthropod groups found in soils under different managements: control (T1), traditional thinning (T2), innovative thinning (T3). QBs index is obtained from the sum of the highest values of EMI of all the collected groups.

| <b>Groups</b>               | <b>T1</b> | <b>T2</b> | <b>T3</b> |
|-----------------------------|-----------|-----------|-----------|
| <i>Acari</i>                | *         | *         | *         |
| <i>Collembola</i>           | *         | *         | *         |
| <i>Orthoptera</i>           | *         | *         | *         |
| <i>Hemiptera</i>            | *         | *         | *         |
| <i>Diptera (larvae)</i>     | *         | *         | *         |
| <i>Coleoptera</i>           | *         | *         | *         |
| <i>Chilopoda</i>            |           | *         | *         |
| <i>Blattaria</i>            | *         |           |           |
| <i>Symphyla</i>             | *         | *         | *         |
| <i>Thysanoptera</i>         |           | *         | *         |
| <i>Protura</i>              |           | *         |           |
| <i>Diplura</i>              |           |           | *         |
| <i>Lepidoptera (larvae)</i> |           | *         | *         |
| <i>Araneae</i>              | *         | *         | *         |
| <i>Formicidae</i>           |           | *         |           |
| <i>Isopoda</i>              |           | *         | *         |

### 3.2.2.1 Water soluble soil ions

In the surface layer, the highest values of  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  were recorded in T2 (Fig. 20a). The only one to be positively influenced by the innovative thinning (T3) was  $\text{K}^+$  (Fig. 20a), while chloride ( $\text{Cl}^-$ ),  $\text{NO}_3^-$  and  $\text{Na}^+$  concentrations decreased in this area (0-15 cm). In the underlying soil layer, although showing a general decrease in values, water soluble ions showed roughly the same trend among the different thinning (Fig. 20b). In the upper layer, sulphate ( $\text{SO}_4^{2-}$ ) were very abundant in all the areas, especially in those managed (Fig. 20a). In the underlying layer, the

values decreased in T2 and in T3 areas, while they remained almost unchanged in T1 (Fig. 20b). Besides leaching, the concentrations of  $K^+$ ,  $Mg^{2+}$ ,  $Ca^{2+}$  and  $Na^+$  decreased with increasing soil depth presumably for ion uptake by roots.



**Fig. 20 (2018)** - Concentrations of water soluble ions (mg/Kg<sup>1</sup> dry soil) in soil collected at 0–15 cm (a) and 15–30 cm (b) depths under different managements: control (T1), traditional thinning (T2), innovative thinning (T3). Bars denote the standard error of the mean: n=3. Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

### 3.2.2.2 Soil aggregate stability

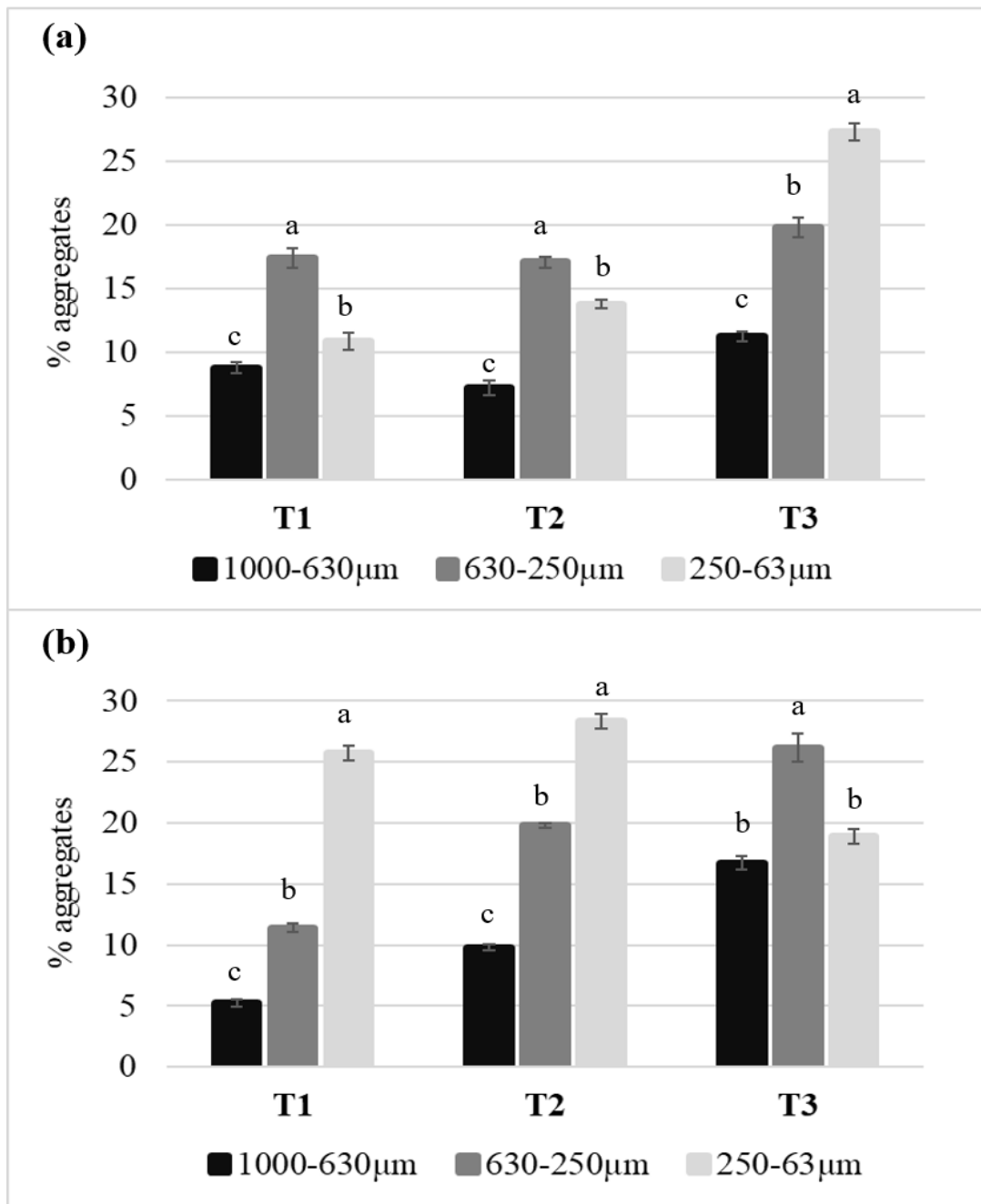
The distribution of aggregate fractions in soils under thinning showed significant differences among the treatments at both depths (Fig. 21a,b). The small aggregates (250-63  $\mu\text{m}$ ) were more abundant than medium (630-250  $\mu\text{m}$ ) and large ones (1000-630  $\mu\text{m}$ ) in the surface layer of T3 and in the underlying layer of T1 and T2. This was not recorded in underlying layer of T3.

Compared to T1 (Fig. 21a), the areas under management showed a greater percentage of medium (630-250  $\mu\text{m}$ ) and small aggregates (250-63  $\mu\text{m}$ ). In 15-30 cm layer, all the areas maintained the same size distribution of aggregates, except for T3 where a larger percentage of medium aggregates was recorded (Fig. 21b).

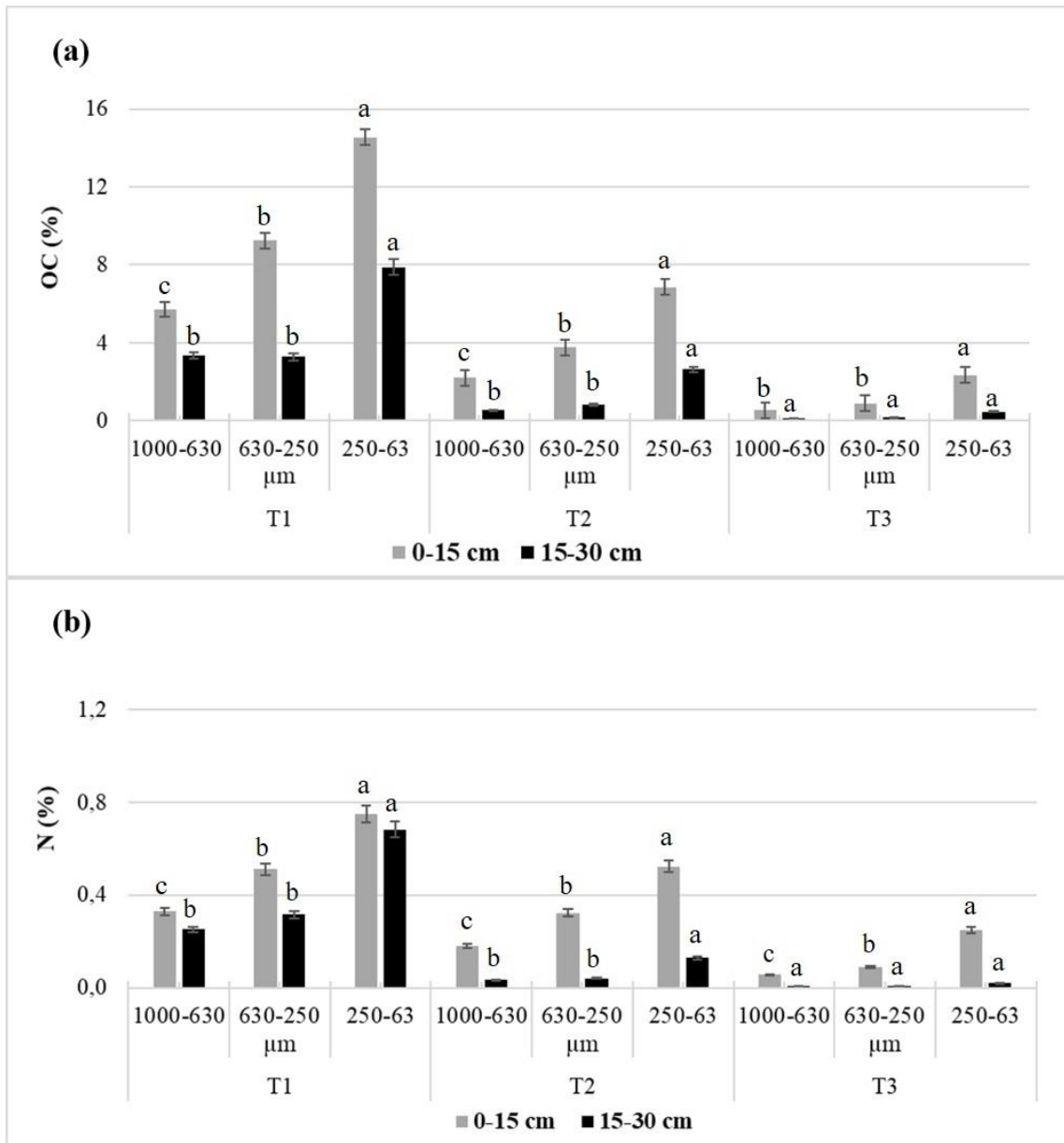
The distribution of OC and nitrogen in the aggregates changed not only in respect to the treatments but also with soil depth (Fig. 22). Organic carbon and nitrogen were higher in the aggregates of the unmanaged forest (T1) at both depths compared to the two thinning treatments (T2 and T3). Additionally, an increase in OC and N content was observed in small and medium aggregates respectively, especially in T1 and T2. In T3, despite the similar distribution trend, a strong decrease in OC and N in both layers in respect to T2 and T3 was detected (Fig. 22ab). In short, the decrease in OC and N values suggest that a gradual erosion phenomena occur (see Fig. 16), causing a loss of these nutrients.

In all areas the values of C/N ratio were generally higher in the small and medium aggregates, in the surface layer of the soil, and were significantly lower in the large aggregate fraction (Fig. 23). However, in the surface layer no significant differences were observed in C/N distribution trend among the areas. The highest C/N ratio, observed in the underlying layer of the managed areas, especially in T3, suggests that the mineralization processes prevails in these areas. Comparing the three treatments, the highest values of C/N ratio were found in T1 for the surface layer and in T3 for underlying layer (Fig. 23).

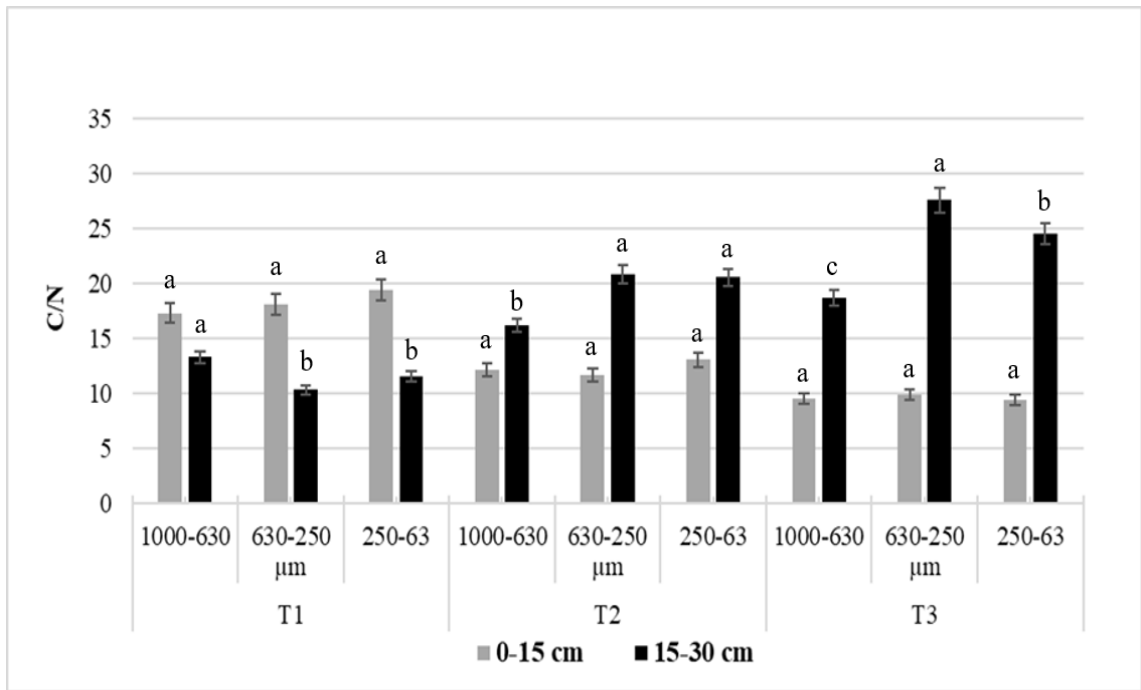




**Fig. 21 (2018)** - Distribution of soil aggregate fractions obtained with ultrasonic method (USAS) under different forest managements: control (T1), traditional thinning (T2), innovative thinning (T3); at depths of 0–15 cm (a) and 15–30 cm (b). Bars denote the standard error of the mean: n=3. Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).



**Fig. 22 (2018)** - Distribution of organic carbon (a) and total nitrogen (b) in different aggregate fractions and depth (0-15; 15-30 cm) under different forests management: control (T1), traditional thinning (T2) and innovative thinning (T3). Bars represent standard errors (n=3). Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).



**Fig. 23 (2018)** – C/N ratio distribution in different aggregate fractions and depth (0-15; 15-30 cm) under different forest managements: control (T1), traditional thinning (T2) and innovative thinning (T3). Bars represent standard errors (n=3). Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

#### 4. Conclusions and future perspectives

In short, this study showed that, at short time-scale,  $^{137}\text{Cs}$  radionuclide is a valuable tracer and a reliable indicator to estimate soil erosion.

The relationship between  $^{137}\text{Cs}$  and other soil parameters emerged from this study, highlighted as soil biological parameters are a promising additional and/or alternative diagnostic tool to quantitatively assess the soil loss in forests. In other words, the possibility to predict short-term variations in soil processes through the use of indicators represents a great advantage in the context of sustainable land management, and climate changes. Using biological indicators together with  $^{137}\text{Cs}$  can be crucial in determining the sustainability of forest management activities and can give clear and detailed information on triggering of soil loss also in terms of fertility loss. Among the soil properties to be considered, the dosage of WSP proved to be a cheap method that can be easily used to evidence changes in soil physical-chemical characteristics even in case of absence of  $^{137}\text{Cs}$  in the sediment.

Our results also evidenced that organic matter, total nitrogen, C/N ratio and water content cannot be considered, alone or in combination, indices to evaluate changes in managed soils. Rather, can be the data crossing of microbiota and ions with organic matter fractions (stable and labile) that can give important and accurate information on how management can affects the soil biological properties that are strictly correlated to soil quality.

It is by using in combination more biological indicators that we can predict the dynamic of soil processes and the impact of management practices on soil quality, allowing to highlight the sustainability of forest management activities. In other words, the possibility to predict short-term variations in soil processes through the use of indicators represents a great advantage in the context of silvicultural practices. Among the soil biological properties DOC, FDA, CAT, fungi and pedofauna have been identified as indicators of soil fertility and quality to evidence, precocely changes in forest managed areas. Conversely, Caesium, WSP, OC, correlated each other's, can be used as indicators of soil erosion process. Even if the results obtained demonstrated clearly the viability of the approach further researches, in different geomorphic and forest contexts, are required to confirm these findings and to estimate more precisely the soil erosion rates provided by  $^{137}\text{Cs}$  measurements especially in relation to the sampling strategies.

## 5. Acknowledgements

First of all I have to admit that I never thought I'd be here writing this page in a doctoral thesis. If it is true that the best of a man comes out in moments of difficulty, well, I am the example. The strong disappointment after having almost reached my sporting dream turned into courage and strength that allowed me to reach this goal. Step by step, first the bachelor degree and after that the master degree, achievements, which in the past were unimaginable for me... and now I'm almost a real doctor. Thanks to perseverance, commitment and the right people around you, this is the proof that everything is possible in life. But now it's time to say thanks to the people who made this possible.

At the beginning, I want to say thank you to my supervisor Prof. Adele Muscolo for guiding me in an exemplary way during all the research work, in the difficult and in the beautiful moments. Her immense knowledge, motivation and patience have given me more power and spirit to excel in the research. Without her, this doctoral research would not have been that easy and that successful. She is my mentor and the best advisor for my doctorate study I could ever ask for.

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Make your life a dream, and a dream a reality.  
(Antoine de Saint-Exupéry)

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## APPENDIX 1

### Scientific Publication

Romeo F, Porto P, Keiblinger K, Mentler A, Muscolo A (2019)  
**Soil biological indicators and caesium-137 to estimate soil erosion in areas with different forest system management.**  
*European Journal of Forest Research*, 1-15.



## Soil biological indicators and caesium-137 to estimate soil erosion in areas with different forest system management

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### Abstract

In this study, the effects of innovative and traditional thinning on soil properties with respect to unmanaged forest were assessed with the aim to individuate early warning indicators of soil erosion for identifying the most appropriate forestry practices to sustainably manage an Italian beech (*Fagus sylvatica*) forest. Soil organic carbon (OC), microbial biomass C (MBC), ergosterol (ERG), humification rate, water-soluble phenols (WSP), fluorescein diacetate (FDA) hydrolysis, dehydrogenase (DHA) and catalase activities (CAT), ultrasonic aggregate stability and <sup>137</sup>Cs were detected to assess soil health and erosion magnitude. The aim was to correlate <sup>137</sup>Cs, as a basic indicator of soil erosion rate, with soil aggregate stability and biological activity parameters. <sup>137</sup>Cs results evidenced that both thinning treatments affected soil properties. The innovative treatment showed the highest impact. The amount of small-sized particles enhanced when the intensity of thinning increased. A strong decrease in soil OC was related to thinning. In the upper soil layer, OC was found positively correlated with MBC, FDA, WSP, ERG, C/N, N and also with <sup>137</sup>Cs. Moderate to no correlations, in the subsurface layer, highlighted the immediate impact of management techniques on the surface layer and then on the underlying ones. In the subsurface layer, OC maintained its positive correlation only with MBC, WSP and <sup>137</sup>Cs. <sup>137</sup>Cs was correlated in both soil layers with OC, N and WSP. The overall results suggest that the latter parameters may be considered as indicators of soil erosion. More specifically, WSP can be used, even in the case of the absence of <sup>137</sup>Cs in the sediment, to evidence changes in soil properties that could be the starting point of soil fertility loss.

**Keywords** Aggregate stability · Biological indicators · Caesium-137 · Erosion · *Fagus sylvatica* · Forest management

### Introduction

Natural forests are generally unaffected by soil erosion processes. Despite these significant findings for undisturbed forests, it is prominent to know that the low susceptibility of forest to erosion and the small amount of

sediment loss from forest soils dramatically change when the area undergoes forestry activities (Swanston and Swanston 1976; Stott et al. 2001). Thinning is a well-known silvicultural practice used for forest conservation (Frederickson and Putz 2003; Stephens and Moghaddas 2005). The primary aim of forest thinning is to increase the growth of selected trees, but as already demonstrated by Settineri et al. (2018), thinning also may affect soil biological properties and its effects depend on the intensity of cutting. It is well known that the use of different types of thinning (traditional or innovative) changes the performance of soil affecting SOM dynamics as already reported by Johnson (1992), Neary et al. (1999), Balboa-Murias et al. (2006) and Nilsen and Strand (2008) who evidenced that silvicultural management practices affected C trend in different forest ecosystems. The intensity of cutting causes also significant short-term increases in sediment mobilisation and sediment yield. Increased soil loss rates have been associated with forest harvesting worldwide (see Porto

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et al. 2009; Altieri et al. 2018). Management practices could accelerate soil loss rates in forests influencing the soil-related functions such as carbon storage, biodiversity and soil ecosystem functioning (Van Oost et al. 2005; Ojea et al. 2012; Gamfeldt et al. 2013). In the disturbed mountains of Calabria, high soil loss rates (100–150 Mg ha<sup>-1</sup> year<sup>-1</sup>) have been observed during an experimental investigation by Sorriso-Valvo et al. (1995). For all these reasons, the attention is now focused on the effects of forest management practices on soil quality closely related to site productivity. Relationships between management and productivity are not simple but are rather extraordinarily complex, reflecting interactions among management system, soil biological activity, nutrient cycling and climate (Muscolo et al. 2014). Therefore, the effect of a given forest management is highly dependent on site-specific soil properties and microclimate and may also be influenced by year-to-year variation in climate.

Research on the impacts of forest management activities on soil erosion and the subsequent effects on forest productivity is limited yet. Forest management if not properly settled can cause soil erosion processes, with a consequent reduction in soil productivity and environmental sustainability. In order to limit the triggering of erosive phenomena and to find useful countermeasures, there is a need to evaluate through the use of early warning indicators if forest management practices are the cause of starting soil degradation.

Numerous attempts to prevent this phenomenon have been made, mainly based on models and calculation procedures that require more or less detailed information about the climate, topography and characteristics of the soil and of the plant (Renard and Freimund 1994; Morgan et al. 1992; De Roo et al. 1996). However, their utilities remain limited to the geographic areas for which calibration and validation are possible. Alternative approaches, based on the use of measurements in experimental plots or catchments (Hsieh et al. 2009; Anache et al. 2017), have severe limitations as they are associated with point data and do not give details on spatial distribution of erosion.

Recent work in exploring and exploiting the potential for using environmental radionuclides, and more particularly caesium-137 (<sup>137</sup>Cs), to document rates and patterns of soil redistribution by erosion processes (Walling 1998; Porto et al. 2001, 2003) can, however, now be seen as offering important new opportunities in this sector. In most environments, the <sup>137</sup>Cs fallout reaching the land surface was rapidly and strongly adsorbed by the surface soil and its subsequent redistribution within the landscape will have occurred in association with the erosion, transport and deposition of soil and sediment particles. Caesium-137 has a half-life of 30.2 years and measurement of the present spatial distribution of <sup>137</sup>Cs inventories in the landscape provides the basis for estimating erosion and deposition rates. The use of this

method was validated to estimate erosion rate both in cultivated soils (Porto and Walling 2012) and in afforested areas (Porto et al. 2001; Di Stefano et al. 2005).

Nowadays, there is an urgent need at finding forest sustainable management practices to maximize their positive impacts on forest sector development, minimizing their negative effects on biodiversity, soil ecosystem functioning and climate change. For the above statements, the aim of this work was to individuate early indicators of soil erosion to be applied for identifying the most appropriate forestry practices to manage a beech (*Fagus sylvatica*) forest in a sustainable way. Thus, we assessed the effect of tree-oriented silviculture (innovative forest management system) on soil properties with respect to thinning (traditional forest management system) and unmanaged forest. The work has been performed in different phases: a first, preliminary, phase aimed at evaluating soil erosion rates using the <sup>137</sup>Cs technique in order to establish the effect of thinning on soil loss. This analysis required detailed sampling campaigns to identify undisturbed areas to deduce the reference value for <sup>137</sup>Cs and collection of several soil samples within the areas subjected to different treatments. The second phase required collection of additional soil samples to be analysed for different soil properties that include soil organic matter (SOM), microbial biomass C (MBC), water-soluble phenols (WSP), fluorescein diacetate (FDA) hydrolysis, dehydrogenase, catalase activities and aggregates stability (USAS). The aim was to find a correlation between soil quality index and <sup>137</sup>Cs to identify early warning indicators of soil erosion rate.

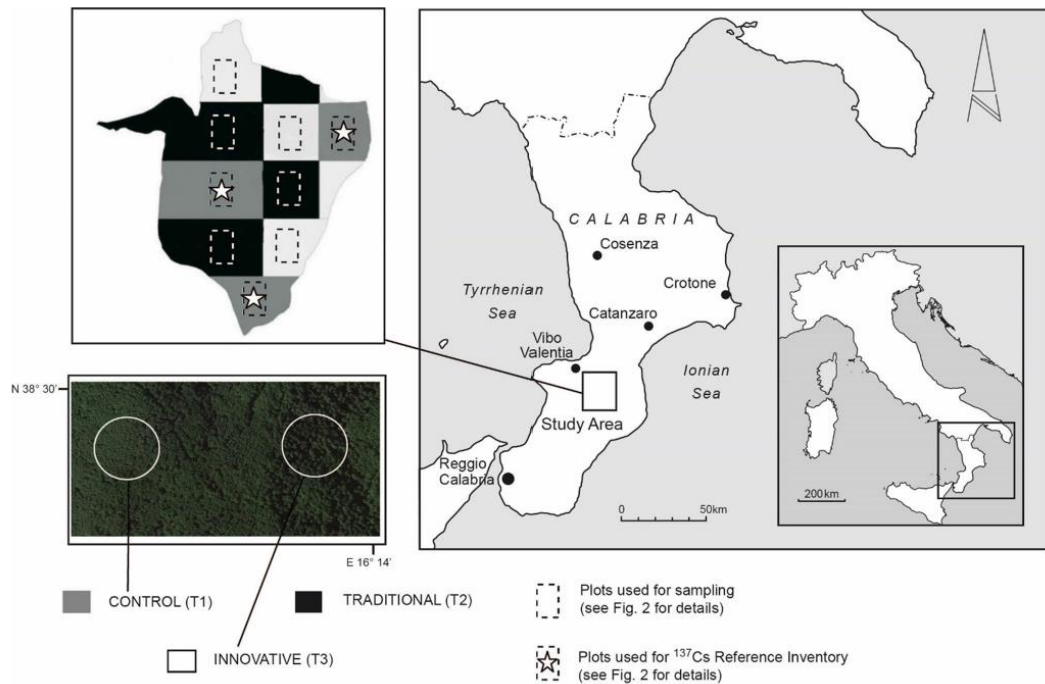
## Materials and methods

### Site description and experimental design

The study area (Fig. 1) is located in the Marchesale Biogenetic Reserve (Natura 2000 site) within the highest slope of the Calabrian “Serre” mountains, in Mongiana (VV) (38°30'N, 16°14'E). The reserve, managed by the National Forest Service of Italy, covers 1234 hectares and consists mainly of high forest dominated by *Fagus sylvatica* L.

Being located in the upper part of the mountain system facing the Tyrrhenian Sea and exposed to north-west, its microclimate is influenced by the interception of fog, wet winds and precipitation (Becagli et al. 2013). The mean values of annual rainfall and temperature, based on the data obtained at Mongiana (VV) meteorological station (920 m a.s.l.) and available for the period 1928–2018, are, respectively, of 1801.5 mm e 11.4 °C. The warmest month is August (18.6 °C), and the coldest one is January (3.8 °C). The climate is typically Mediterranean (Csb, sensu Köppen 1936).





**Fig. 1** Study area, representation of different treatments (T1—control, T2—traditional thinning and T3—innovative thinning) and location of the plots established for sampling

This area is geologically characterized by Palaeozoic granitoid deeply fractured and with deep, versus shaped slopes (Conforti et al. 2015). According to USDA soil classification, the soils are Inceptisols and Entisols (Soil Survey Staff 2010).

For this work, we identified an experimental area of ca. 30 ha covered by a 75-year-old high beech forest (1100 m a.s.l.). In this area, three different subareas of about 3 ha each have been identified and subjected to a different silvicultural thinning (3 treatments  $\times$  2 different analysis  $\times$  3 replicates of analysis, Fig. 1). An unmanaged area for over 30 years (T1) was used as control; a traditional treatment (T2) and an innovative treatment (T3) have been carried out in 2012–2013. Traditional treatment was a thinning from below with a moderate intensity which removed all the dominated trees and the worst dominant trees (on average, ca. 12% of total volume resected). The innovative treatment was oriented to retain the 50 best trees per hectare and improve the structural biodiversity, collecting 5 or 6 trees closer to them, regardless of their social position (on average, ca. 27% of the total volume removed). No significant differences were found between the dendrometric

parameters in these areas before the silvicultural interventions (Picchio et al. 2016).

The three treatment sites are fully comparable in terms of slope, orientation and soil types. The unmanaged site (T1) was also used as reference location for  $^{137}\text{Cs}$  analysis. The reference value obtained in this area was used to convert the  $^{137}\text{Cs}$  inventories obtained in the other treatment sites into estimates of soil erosion. More specifically, the diffusion and migration model (DMM) was applied for this conversion analysis (see Porto et al. 2003). This version of the DMM is based on a simulation of the  $^{137}\text{Cs}$  activity along a soil profile following the atmospheric fallout and its temporal redistribution. Based on this assumption, the DMM attempts to reproduce the activity of  $^{137}\text{Cs}$  with the following equation:

$$C(x, t, t') = e^{-\lambda(t-t')} \int_0^\infty \frac{I(t')}{H} e^{-\frac{y}{h}} \left\{ e^{-\frac{V(x-y)}{2D}} e^{-\frac{V^2(t-t')}{4D}} \left[ e^{-\frac{(x+y)^2}{4D(t-t')}} + e^{-\frac{(x-y)^2}{4D(t-t')}} \right] \right. \\ \left. \times \frac{1}{\sqrt{4\pi D(t-t')}} - \frac{V}{2D} e^{\frac{yV}{D}} \operatorname{erfc} \left[ \frac{x+y+V(t-t')}{\sqrt{4D(t-t')}} \right] \right\} dy \quad (1)$$

where  $C(x, t, t')$  ( $\text{Bq kg}^{-1}$ ) indicates the  $^{137}\text{Cs}$  activity at the mass depth  $x$  and time  $t'$ ;  $I(t')$  expressed in ( $\text{Bq m}^{-2} \text{ year}^{-1}$ ) indicates the  $^{137}\text{Cs}$  amount received by the soil surface at time  $t'$  with fallout;  $H$  ( $\text{kg m}^{-2}$ ) is a basic parameter that accounts for the initial relaxation mass depth;  $D$  ( $\text{kg}^2 \text{ m}^{-4} \text{ year}^{-1}$ ) is a model parameter that simulates the diffusion process;  $V$  ( $\text{kg m}^{-2} \text{ year}^{-1}$ ) is a model parameter that accounts for migration;  $\lambda$  ( $=0.023 \text{ year}^{-1}$ ) is the constant of radioactive decay for  $^{137}\text{Cs}$ ;  $x$  ( $\text{kg m}^{-2}$ ) indicates the cumulative mass depth;  $t$  (year) is the time elapsed since the commencement of fallout in 1954; and  $\text{erfc}(u)$  is the error function complement defined as (Crank 1975):

$$\text{erfc}(u) = \frac{2}{\sqrt{\pi}} \int_u^\infty e^{-y^2} dy \quad (2)$$

Integrating Eq. (1) over time  $t'$  and assuming a continuous input  $I(t')$ , the  $^{137}\text{Cs}$  concentration  $C(x, t)$  ( $\text{Bq kg}^{-1}$ ) in the soil profile is given by the following equation:

$$C(x, t) = \int_0^t C(x, t, t') dt \quad (3)$$

The attempt of Eq. (1) to simulate the diffusion and migration of  $^{137}\text{Cs}$  along the soil column was demonstrated in several works carried out in southern Italy (see Porto et al. 2004, 2016).

### Soil sampling and preparation

For each of the 3 treatment sites (T1, T2 and T3), 3 representative plots (1000  $\text{m}^2$  in size) were established for sampling (see Fig. 1). In these 9 plots, three separate soil sampling campaigns were undertaken following the scheme illustrated in Fig. 2. The first and the second campaigns were carried out in May 2017 and consisted of collecting soil cores for  $^{137}\text{Cs}$  analysis and for chemical–physical and biological analysis, respectively. The cores were collected in areas with similar slope avoiding sampling points close to the tree trunks in order to minimize the effect of stemflow on the final  $^{137}\text{Cs}$  inventories. In total, 6 final composite soil cores were obtained from these sampling campaigns (3 for radiometric measurements and 3 for chemical–physical and biological analysis), using a 10-cm-diameter steel core tube inserted up to a depth of ca. 35 cm. Each composite core used for  $^{137}\text{Cs}$  analysis was obtained from 9 single sectioned cores (3 for each plot of the same treatment) merged layer by layer (see merging strategy explained in Fig. 2). More specifically, for each treatment site, the 9 single cores were sectioned into depth increments of 2–3 cm to a depth of 30 cm, and the deepest 5 cm were merged into one single subsample. This sampling strategy was necessary to obtain the distribution of  $^{137}\text{Cs}$  activity along the soil profile and to account for microscale variability. Each profile was used to fit the theoretical conversion model (DMM) able to calculate soil erosion rates.

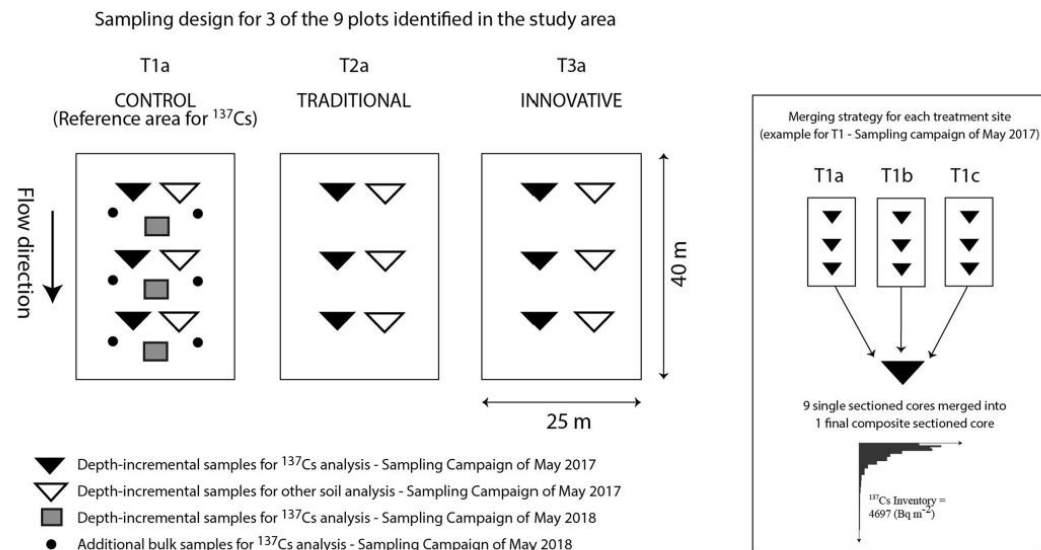


Fig. 2 The sampling strategy adopted in the plots established within the three treatment sites

Each composite soil core used for the chemical–physical and biological analyses consisted of 9 single soil cores, divided into 2 layers (0–15 and 15–30 cm). The corresponding layers were merged in order to obtain 3 final representative cores (one for each treatment site).

The third sampling campaign, undertaken in May 2018, was necessary to further account for microscale variability associated with the  $^{137}\text{Cs}$  inventories at the reference site. This sampling campaign provided collection of additional sectioned and bulk cores from the three plots ( $T_{1,a,b,c}$ ) within the control area (treatment site T1). More specifically, the sampling strategy adopted in May 2017 was repeated in May 2018 in order to increase the number of samples available for  $^{137}\text{Cs}$  analysis. This campaign allowed to obtain 9 additional sectioned cores that were merged, again, into one composite core (see merging strategy explained in Fig. 2). Also, in order to check the  $^{137}\text{Cs}$  reference value obtained during both the sampling campaigns, 18 additional bulk cores (six for each plot) were collected for comparison (see Fig. 2 for details).

All the samples were air-dried and sieved to separate the <2 mm fraction. Before the radiometric assay, all the samples were oven-dried at 105 °C for 48 h, disaggregated, dry-sieved to separate the <2 mm fraction and packed in plastic pots or Petri dishes for subsequently determination of its  $^{137}\text{Cs}$  activity by gamma spectroscopy.

#### Radiometric analyses for $^{137}\text{Cs}$ content

The measurements of the  $^{137}\text{Cs}$  activity and its subsequently vertical distribution in the soil profile were obtained by gamma spectrometry at the Department of Agraria of the Mediterranean University of Reggio Calabria. Two Canberra p-type HPGe detectors, model GX4020, were used for the analyses. Each detector, coupled to a Desktop Spectrum Analyzer DSA-1000 Canberra multichannel analyser, is characterised by a relative efficiency of 45.6% with a resolution of 1.1 keV at 122 keV and 2.0 keV at 1.33 MeV. The spectral analysis was provided by the Canberra Genie 2000 software package, and the efficiency calibration was obtained by the Canberra's LabSOCS (Laboratory SOURCEless Calibration Software) code. A certified multigamma source with a wide energy range (42.8–1274.5 keV) together with several standard materials of different geometries was used for energy calibration. Count times in the detectors were approximately 40,000 s, and the  $^{137}\text{Cs}$  activities were obtained from the counts at 662 keV.

#### Soil aggregate fraction analysis

Through the USAS method, measurable ultrasonic energy was applied to the soil–water suspension, allowing a quantitative measurement of soil aggregate stability (Mentler

2001). The most important parameter to describe the particle dispersion during sonification and the degree of aggregate breakdown is the specific ultrasonic energy absorbed by a soil–water mixture. Soil aggregate distribution (USAS) was carried out by a new, probe-type dispersion equipment (Mentler et al. 2004). A titanium alloy probe is inserted into the soil–water mixture and vibrates at approximately 20 kHz. The ultrasonic titanium probe has cylindrical shape and a circular cross section (diameter 30 mm). The same ultrasonic probe was used in all experiments, and the insertion depth was kept constant at 10 mm.

Dispersion experiments were performed with 4 g soil in 200 ml pure degassed water. The solution was stirred with a magnetic stirring device (2 Hz, cylindrical shape with length 25 mm and thickness 8 mm). Stirring starts 10 s prior to the ultrasonic vibration and was continued during the ultrasonic experiments to obtain a homogeneous distribution of soil in the solution. All soils were tested at constant vibration amplitude of the ultrasonic probe of 2.5  $\mu\text{m}$  for 30 s. As reported in the literature (Mayer et al. 2002; Mentler et al. 2004), the vibration amplitude was determined using electromagnetic induction coil and strain gauges. Immediately after the ultrasonic treatments, mass fractions were determined by wet sieving. The aggregates were analysed with standard sieves and classified in different aggregate fractions: macro-aggregates (1000–630  $\mu\text{m}$ ), medium aggregates (630–250  $\mu\text{m}$ ) and small aggregates (250–63  $\mu\text{m}$ ). Determination of mass fractions (accuracy 0.001 g) was performed by weighing after drying at 105 °C for 24 h. Each aggregate fraction was used to determination of OC and N, using an elementary analyser via dry combustion technique and gas chromatographic analyses (ThermoFisher *FlashSmart*, ON L 1080-99 1999).

#### Soil analysis

Texture was carried out by the hydrometer method using sodium hexametaphosphate as a dispersant (Bouyoucos 1962). Gravimetric water content (WC) was determined with a difference between fresh and dry weight after oven drying at 65 °C for 72 h. The pH was measured in distilled water (soil/solution ratio 1:2.5) with a glass electrode. Electric conductivity (EC) was determined in distilled water by using 1:5 residue/water suspension, mechanically shaken at 15 rpm for 1 h to dissolve soluble salts and then detected by Hanna instrument conductivity meter. Total water-soluble phenols (WSP) were measured by using the Folin–Ciocalteu reagent, following the Box method (1983). Tannic acid was used as a standard, and the concentration of WSP was expressed as tannic acid equivalents. Bulk soil organic carbon (OC) was determined by dichromate oxidation (Walkley and Black 1934), and it was converted to organic matter by multiplying the percentage of carbon by 1.72, while



total nitrogen (TN) was measured by the Kjeldahl method (1883). Fluorescein diacetate (FDA), hydrolysis reaction, was determined according to the methods of Adam and Duncan (2001). Dehydrogenase (DH) activity was determined following the method of von Mersi and Schinner (1991). Regarding the catalase activity (CAT), the disappearance of  $\text{H}_2\text{O}_2$  at 240 nm was determined according to Beaumont et al. (1990) by using extinction coefficient ( $\epsilon$ ) =  $0.036 \text{ mM}^{-1} \text{ cm}^{-1}$ . Humification index (HI), degree of humification (DH%) and humification rate (HR%) have been detected. In short, humic components were extracted with a solution 0.1 M of sodium pyrophosphate and sodium hydroxide (Official Methods of Soil Chemical Analysis 1994). The total extract (TEC) has been split into the humified component [humic acids (HA) and acids fulvic (HF)] and in the non-humified (NH) and with an oxidimetric method the organic carbon content of these purified fractions it has been evaluated using 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  (Nelson and Sommers 1982). Humification parameters have been calculated following the method proposed by Sidari et al. (2005).

The microbial biomass C (MBC) was determined by using the chloroform fumigation–extraction procedure (Vance et al. 1987) with fresh soil (equivalent to 20 g D.W.). OC of both fumigated and unfumigated samples was analysed by using Walkley and Black (1934) procedure. The estimation of the MBC was made on the basis of the differences between the fumigated and unfumigated soil, with a conversion coefficient (Vance et al. 1987). The fungal cell membrane component ergosterol (Erg) was extracted following the method described by Gong et al. (2001) with some modifications, and 10 g of methanol (Me-OH) was added to 1 g of soil. The suspension was homogenized with mechanical agitation for 15 min and centrifuged at  $10,518 \times g$  for 15 min. The supernatant was filtered using a syringe membrane filter (4 mm,  $0.45 \mu\text{m}$  polytetrafluoroethylene (PTFE) and then kept in the dark until analysis with an Agilent Technologies Infinity 1290 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, California, USA). Dissolved OC (DOC) was analysed with a visible spectrophotometer (Agilent-8453) at 254 nm according to the method described in Brandstetter et al. (1996).

### Statistical analyses

To explore relationships among soil parameters at two soil depths and three different silvicultural treatments, datasets were analysed using principal component analysis (PCA), multivariate analysis of variance (MANOVA) and *t* test for paired values. The results are summarized in an ordination diagram. PCA was carried out using the soil parameters in plots under different silvicultural treatments using the software PAST (Hammer et al. 2001). Because the data are

expressed in different units, the results are standardized with the following formula:

$$z = \frac{(x_i - \bar{x})}{\text{SD}} \quad (4)$$

where  $x_i$  is the individual value of each parameter,  $\bar{x}$  is the mean and SD the standard deviation.

A MANOVA analysis was carried out for evaluating the effects of thinning, soil depth and their interaction on the set of soil parameters and  $^{137}\text{Cs}$ . Finally, since there are only two soil depths (0–15 cm and 15–30 cm), a *t* test was used for paired values to evaluate significant differences. This last analysis allowed us to verify whether thinning affected soil properties according to depth, and whether the impacts were similar. Pearson's correlations for both soil layers and all soil parameters were carried out using PAST software (Hammer et al. 2001).

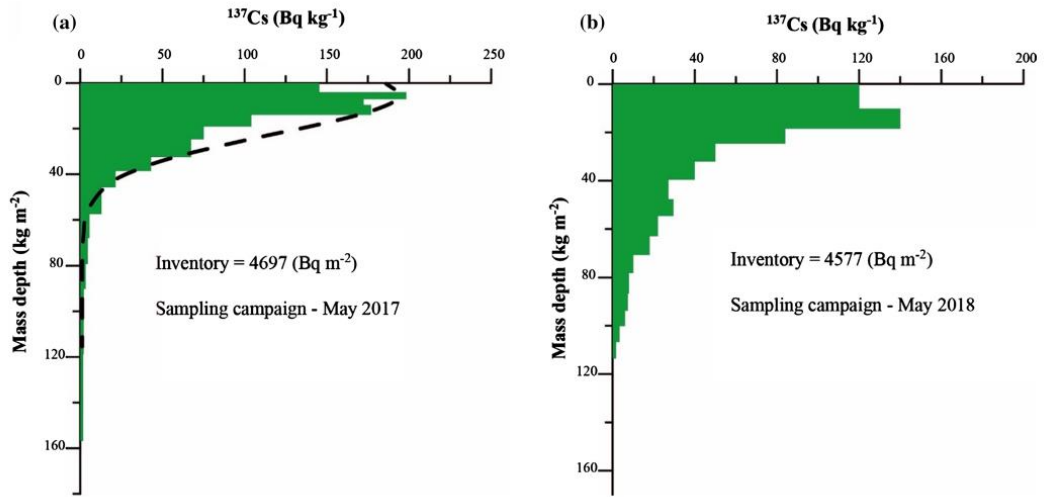
For each parameter analysed by ANOVA and *t* test, the data matrix (sample size) from the average of the values per subplot, and from the average per plot, values for each experimental condition were obtained. ANOVA, MANOVA models and *t* test were carried out using SPSS software (IBM Corp. 2012).

## Results

### The conversion of $^{137}\text{Cs}$ measurements into estimates of soil erosion

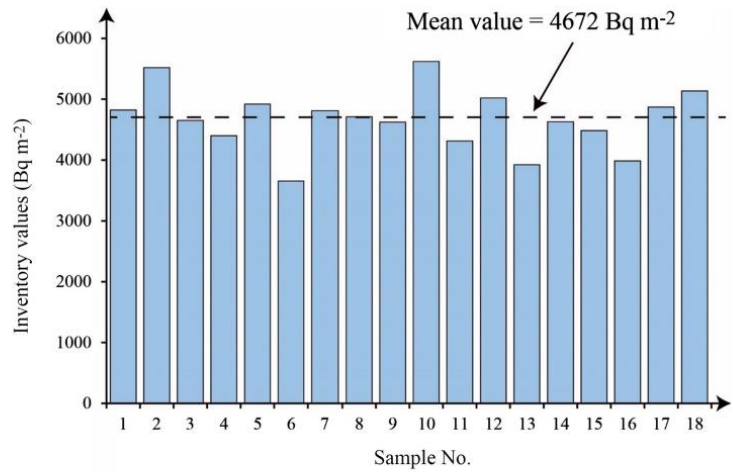
As explained above, the treatment site T1 (control area) was used as a reference location and the  $^{137}\text{Cs}$  inventory obtained in this area was assumed to be the  $^{137}\text{Cs}$  reference value to be compared with the values obtained in the other sites. The two  $^{137}\text{Cs}$  profiles related to the site T1, obtained from the sampling campaigns in 2017 and 2018, respectively, are provided in Fig. 3a, b. The depth scale in Fig. 3a, b is plotted in terms of cumulative mass ( $\text{kg m}^{-2}$ ), rather than depth (m), in order to avoid the need to take account of downcore variations in soil bulk density.

The profile obtained in May 2017 shows a reference inventory of  $4697 \text{ Bq m}^{-2}$  (Fig. 3a), while that obtained during the sampling campaign undertaken in May 2018 shows a reference value of  $4577 \text{ Bq m}^{-2}$  (Fig. 3b). If we correct the latter for decay to the year of the first sampling campaign (2017), we obtain a value of  $4683 \text{ Bq m}^{-2}$  that is very close to that in Fig. 3a and confirms the reliability of the sampling strategy adopted for the reference value. In Fig. 4, the inventory values of the 18 bulk samples collected in May 2018 (corrected for decay to 2017) are also reported for comparison. These inventory values range from 3654 to  $5621 \text{ Bq m}^{-2}$  with a mean value of  $4672 \text{ Bq m}^{-2}$  that, again,



**Fig. 3** The  $^{137}\text{Cs}$  profiles corresponding to the reference area (T1) obtained from the sampling campaigns undertaken in May 2017 (a) and in May 2018 (b)

**Fig. 4** The  $^{137}\text{Cs}$  inventories corresponding to the 18 bulk cores collected in the reference area in May 2018



is very close to the reference value related to Fig. 3a and confirms the reliability of the latter.

Even if Fig. 4 documents a logical microscale variability of the inventory values in the reference site ( $\text{SD} = 509 \text{ Bq m}^{-2}$ ), the variation coefficient (CV) related to these values is ca. 11% and falls within the usual values found in similar investigations (see Sutherland 1996). Based on the value of CV and according to Sutherland (1996), the minimum number of samples is necessary to get

a robust reference value, with an allowable error of 10% at 90% confidence would be  $N=5$ . This result confirms that the sampling strategy adopted in this study, based on 9 single sectioned cores, gives an accurate estimate of the  $^{137}\text{Cs}$  reference value. However, even if a total of 20 samples (18 bulked cores and 2 composite sectioned cores) are available for the reference area, the total  $^{137}\text{Cs}$  inventory obtained for the profile collected in May 2017 ( $4697 \text{ Bq m}^{-2}$ ) has been taken as the reference value for the study area, in view of the

greater surface area associated with the composite sample and of the sampling time (2017) that matches the other soil analysis (chemical–physical and biological).

The profile in Fig. 3a was then fitted with the theoretical DMM in order to convert the loss of  $^{137}\text{Cs}$  into values of soil loss. The example reported in Fig. 3a shows the ability of Eq. (1) to simulate the  $^{137}\text{Cs}$  distribution for the reference site T1 and confirms the basic assumptions of the model.

The profiles corresponding to the composite sectioned cores collected for sites T2 and T3 and depicted in Fig. 5a, b documented inventory values of  $4322 \text{ Bq m}^{-2}$  and  $3058 \text{ Bq m}^{-2}$  respectively.

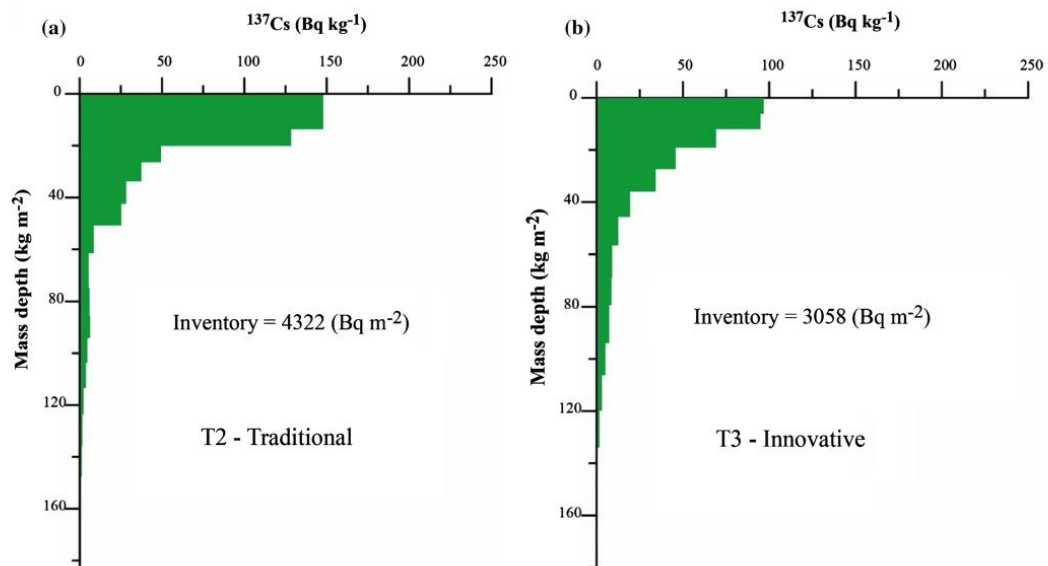
The values of soil loss obtained using Eq. (3) for the sites T2 and T3 were equal to  $1.4 \text{ (t ha}^{-1} \text{ year}^{-1}\text{)}$  and  $6.3 \text{ (t ha}^{-1} \text{ year}^{-1}\text{)}$ , respectively, indicating a higher impact of the innovative treatment if compared with the traditional one. A visual inspection of the profiles shown in Fig. 5a, b suggests that the soil surface shows  $^{137}\text{Cs}$  activities (ca.  $150 \text{ Bq kg}^{-1}$  and  $100 \text{ Bq kg}^{-1}$  for T2 and T3, respectively) lower than that documented in Fig. 3a (ca.  $200 \text{ Bq kg}^{-1}$ ) related to the reference area. This circumstance confirms that the upper part of the soil profile related to T2 and T3 was removed by erosion.

Combining the profiles shown in Fig. 5a, b with the measured values of the bulk density, it is possible to calculate  $^{137}\text{Cs}$  inventory ( $\text{Bq m}^{-2}$ ) and mass activity ( $\text{Bq kg}^{-1}$ ) for a fixed depth. This calculation was made for the two soil depths (0–15 cm and 15–30 cm) in order to make possible

the comparison with the other soil properties obtained for the corresponding layers.

### Soil properties

The particle size analysis showed that all the soils collected in the study area belong to the sandy loam textural class (Table 1), with 13.8% of clay, 25.2% of silt and 61% of sand (data not shown). In the upper layer of soil (0–15 cm) the pH, although presenting variations between the different areas was sub-acid and ranged from 4.8 to 5.8, the electrical conductivity was  $147\text{--}150 \mu\text{S}$  and did not show significant differences among the treatments. Figure 6 shows the PCA diagram for 0–15 cm and 15–30 cm soil depths. At both depths, the first two components (eigenvalues > 1) have been extracted. The variance was higher at 0–15 cm (93.5%), than at 15–30 cm (87%). At both depths, the component 1 explained about 60%, while the component 2 explained about 30% of the variability in all parameters (Fig. 6). Soil under traditional thinning, with respect to control and innovative thinning, showed a greater CAT activity and also a higher humification index, in both layers (Fig. 6, Table 1). On the contrary, soil under innovative thinning, in both layers, (located in the quadrant with both components positive) showed a greater water retention capacity, a slightly higher pH and higher values of humification rate and humification degree (Fig. 6, Table 1), while a decrease in total phenol,



**Fig. 5** The  $^{137}\text{Cs}$  profiles corresponding to the area T2 treated using a traditional thinning (a) and the area T3 treated with the innovative thinning (b)



**Table 1** Mean values and standard deviations of soil parameters referred to two layers of soil (0–15; 15–30 cm): texture; water content (WC, %); pH (H<sub>2</sub>O); electrical conductivity (EC,  $\mu\text{S cm}^{-1}$ ); <sup>137</sup>Cs (Bq m<sup>2</sup>, Bq kg<sup>-1</sup>); water-soluble phenols (WSP,  $\mu\text{g TAE g}^{-1}$  dry soil); organic carbon (OC, %); dissolved organic carbon (ml DOC L<sup>-1</sup>); total nitrogen (N, %); C/N ratio; humification degree (DH, %); humification rates (HR, %); humification index (HI); microbial biomass (MBC,  $\mu\text{g C g}^{-1}$  f.s.); ergosterol fungal biomarker (ERG,  $\mu\text{g g soil}^{-1}$ ); fluorescein released (FDA,  $\mu\text{g g}^{-1}$  dry soil); dehydrogenase (DHA,  $\mu\text{g INTF g}^{-1}$  dry soil h<sup>-1</sup>); catalase activity (CAT, O<sub>2</sub>/3 min/g dry soil<sup>-1</sup>)

| Soil parameters           | 0–15 cm                    |                           |                           | 15–30 cm                   |                           |                            |
|---------------------------|----------------------------|---------------------------|---------------------------|----------------------------|---------------------------|----------------------------|
|                           | T1                         | T2                        | T3                        | T1                         | T2                        | T3                         |
| Texture                   | Sandy loam                 | Sandy loam                | Sandy loam                | Sandy loam                 | Sandy loam                | Sandy loam                 |
| WC                        | 30.6                       | 23.5                      | 43.6                      | 25                         | 20.3                      | 34.2                       |
| pH                        | 4.83 <sup>c</sup> ± 0.01   | 5.18 <sup>b</sup> ± 0.01  | 5.84 <sup>a</sup> ± 0.01  | 5.31 <sup>c</sup> ± 0.01   | 5.54 <sup>b</sup> ± 0.01  | 5.81 <sup>a</sup> ± 0.01   |
| EC                        | 150.5 <sup>a</sup> ± 0.81  | 147.6 <sup>a</sup> ± 1.98 | 148.8 <sup>a</sup> ± 4.27 | 101.4 <sup>b</sup> ± 0.55  | 106 <sup>a</sup> ± 1.46   | 106.4 <sup>a</sup> ± 0.92  |
| Cs (Bq m <sup>2</sup> )   | 3994 <sup>a</sup> ± 4.10   | 4001 <sup>a</sup> ± 3.82  | 2686 <sup>b</sup> ± 1.81  | 703 <sup>a</sup> ± 1.21    | 321 <sup>c</sup> ± 0.81   | 373 <sup>b</sup> ± 0.94    |
| Cs (Bq kg <sup>-1</sup> ) | 122.78 <sup>a</sup> ± 0.82 | 53.76 <sup>b</sup> ± 0.43 | 47.66 <sup>c</sup> ± 0.36 | 6.68 <sup>a</sup> ± 0.01   | 3.34 <sup>b</sup> ± 0.01  | 3.15 <sup>c</sup> ± 0.01   |
| WSP                       | 231.7 <sup>a</sup> ± 8.78  | 160.8 <sup>b</sup> ± 4.41 | 75.8 <sup>c</sup> ± 6.21  | 445.8 <sup>a</sup> ± 13.53 | 165.2 <sup>b</sup> ± 7.01 | 176.2 <sup>b</sup> ± 8.66  |
| OC                        | 12.23 <sup>a</sup> ± 0.02  | 8.08 <sup>b</sup> ± 0.03  | 5.14 <sup>c</sup> ± 0.11  | 6.86 <sup>a</sup> ± 0.01   | 5.12 <sup>c</sup> ± 0.05  | 6.27 <sup>b</sup> ± 0.01   |
| DOC                       | 16.94 <sup>a</sup> ± 0.29  | 15.84 <sup>b</sup> ± 0.25 | 11.11 <sup>c</sup> ± 0.02 | 10.86 <sup>b</sup> ± 0.22  | 11.82 <sup>a</sup> ± 0.55 | 11.73 <sup>ab</sup> ± 0.35 |
| N                         | 0.63 <sup>a</sup> ± 0.01   | 0.56 <sup>b</sup> ± 0.01  | 0.33 <sup>c</sup> ± 0.01  | 0.42 <sup>a</sup> ± 0.01   | 0.37 <sup>b</sup> ± 0.01  | 0.34 <sup>c</sup> ± 0.01   |
| C/N                       | 19.32 <sup>a</sup> ± 0.17  | 14.37 <sup>c</sup> ± 0.18 | 15.75 <sup>b</sup> ± 0.07 | 16.25 <sup>b</sup> ± 0.05  | 13.97 <sup>c</sup> ± 0.07 | 18.53 <sup>a</sup> ± 0.18  |
| DH                        | 88.33 <sup>a</sup> ± 0.72  | 85.59 <sup>b</sup> ± 0.25 | 88.79 <sup>a</sup> ± 0.95 | 87.32 <sup>a</sup> ± 0.71  | 84.57 <sup>b</sup> ± 0.24 | 86.72 <sup>a</sup> ± 0.93  |
| HR                        | 62.6 <sup>b</sup> ± 0.51   | 59.08 <sup>c</sup> ± 0.01 | 66.48 <sup>a</sup> ± 0.01 | 61.88 <sup>b</sup> ± 0.51  | 58.38 <sup>c</sup> ± 0.01 | 64.93 <sup>a</sup> ± 0.01  |
| HI                        | 0.31 <sup>c</sup> ± 0.01   | 0.49 <sup>a</sup> ± 0.01  | 0.34 <sup>b</sup> ± 0.01  | 0.38 <sup>b</sup> ± 0.01   | 0.50 <sup>a</sup> ± 0.01  | 0.37 <sup>b</sup> ± 0.01   |
| MBC                       | 1615 <sup>a</sup> ± 2.75   | 1126 <sup>b</sup> ± 7.34  | 1039 <sup>c</sup> ± 4.01  | 773 <sup>a</sup> ± 8.87    | 616 <sup>c</sup> ± 1.57   | 716 <sup>b</sup> ± 5.51    |
| ERG                       | 11.74 <sup>a</sup> ± 0.27  | 10.96 <sup>a</sup> ± 0.46 | 3.12 <sup>b</sup> ± 0.12  | 1.20 <sup>a</sup> ± 0.18   | 1.17 <sup>ab</sup> ± 0.13 | 0.85 <sup>b</sup> ± 0.06   |
| FDA                       | 71.99 <sup>a</sup> ± 3.21  | 60.68 <sup>a</sup> ± 7.06 | 37.07 <sup>b</sup> ± 3.87 | 25.32 <sup>a</sup> ± 4.14  | 31.03 <sup>a</sup> ± 2.60 | 27.93 <sup>a</sup> ± 2.14  |
| DHA                       | 0.72 <sup>b</sup> ± 0.30   | 0.57 <sup>b</sup> ± 0.13  | 2.78 <sup>a</sup> ± 0.33  | 1.51 <sup>a</sup> ± 0.14   | 1.75 <sup>a</sup> ± 0.12  | 1.51 <sup>a</sup> ± 0.29   |
| CAT                       | 2.95 <sup>b</sup> ± 0.28   | 4.44 <sup>a</sup> ± 0.29  | 1.69 <sup>c</sup> ± 0.09  | 0.84 <sup>b</sup> ± 0.10   | 1.61 <sup>a</sup> ± 0.28  | 1.15 <sup>b</sup> ± 0.10   |

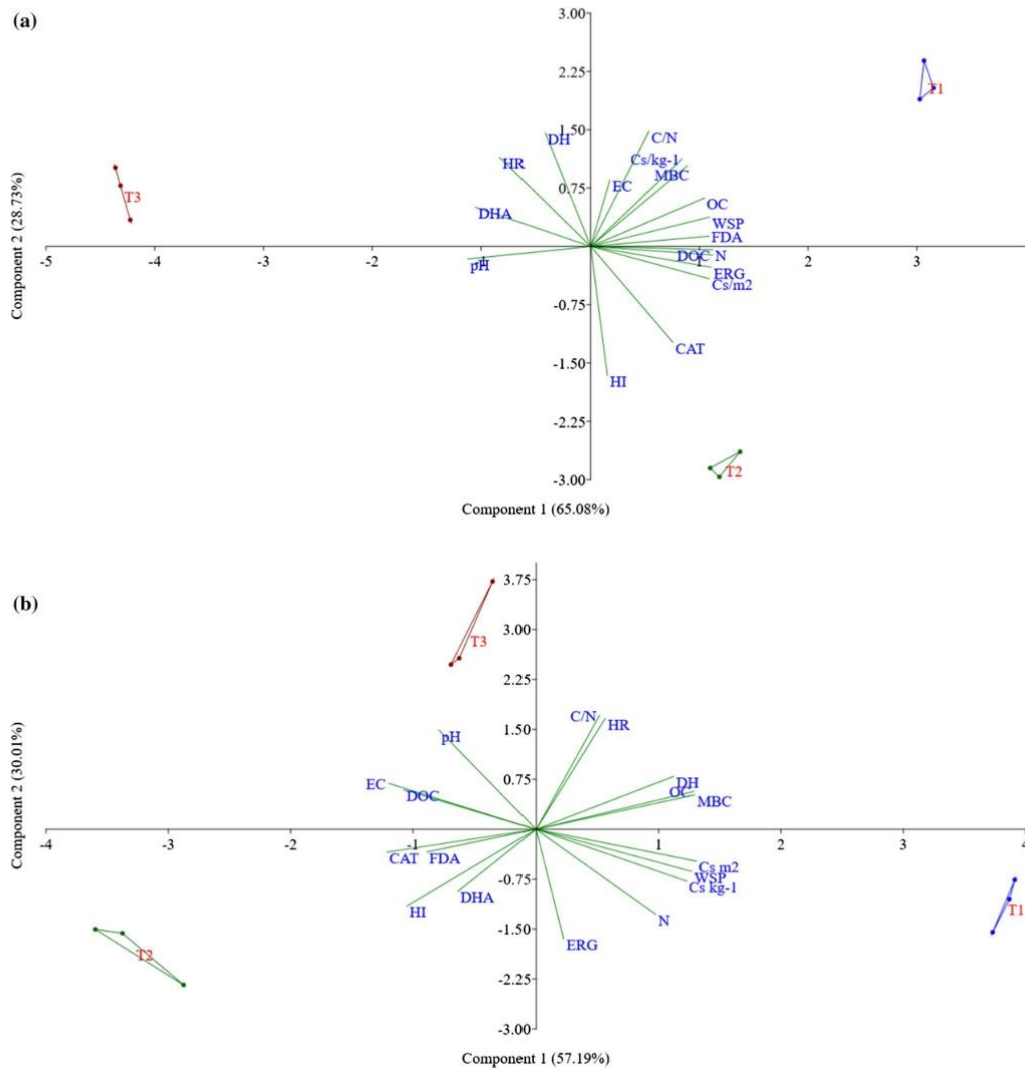
For each parameter the sample size was  $n=3$  for treatment (T1—control; T2—traditional thinning; and T3—innovative thinning). Means in the same column followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test)

<sup>137</sup>Cs, carbon and nitrogen contents was found, especially in the upper horizon (Fig. 6a, Table 1). ERG and MBC, reflecting soil decomposition activity, showed the lowest values in the upper layer under innovative thinning (located in opposite quadrants in Fig. 6a). In the underlying layer (15–30 cm), while the soil physicochemical properties such as WC, EC and pH maintained the same trend of upper layer among the treatments, soil biochemical properties changed. In particular, we observed an increase in soil organic carbon and dissolved organic carbon in T3 with respect to their values in the upper layer, and the values were similar to those detected in T1 and T2 (Fig. 6b). C/N ratio and HR and DH values were the highest in T3 (Table 1). Ergosterol showed the lowest value in T3 (always located in opposite quadrants, Fig. 6), while MBC that showed the lowest value in the upper layer of T3 was higher than that related to T2 in 15–30 cm depth. FDA decreased and DHA increased in T1 and T2, while decreased in T3 (Table 1). The displacement of the T2 area in the left quadrant of the underlying layer (Fig. 6b) with respect to the superficial layer showed how the differences between the soil parameters in the areas subjected to forest management were reduced. The amount of

<sup>137</sup>Cs decreased in both areas compared to control, especially in the T3 located in the opposite quadrant (Fig. 6). In the deeper soil layer, no significant differences in soil parameters due to erosion process were evident in T2 and T3 compared to control. Pearson's correlation denoted that in the upper layer (Fig. 7a) OC was positively and significantly correlated with DOC, MBC, FDA, WSP, ERG, C/N, N and <sup>137</sup>Cs (the latter expressed in Bq kg<sup>-1</sup> and Bq m<sup>2</sup>), while negatively and significantly correlated with pH and DHA. In the underlying layer, MBC, WSP, DH, HR and <sup>137</sup>Cs (Bq kg<sup>-1</sup> and Bq m<sup>2</sup>) were positively correlated with OC. Conversely, HI, FDA, EC and CAT were negatively correlated with OC (Fig. 7b). In both horizons, <sup>137</sup>Cs was positively correlated with WSP, OC and N and negatively correlated with pH (Fig. 7a). On the contrary, in the underlying layer <sup>137</sup>Cs was negatively correlated with pH, EC, DOC and catalase activity (Fig. 7b).

### Soil aggregates

The distribution of soil aggregate fractions (obtained by ultrasonic method) under different forest management showed significant differences among the treatments at a depth of

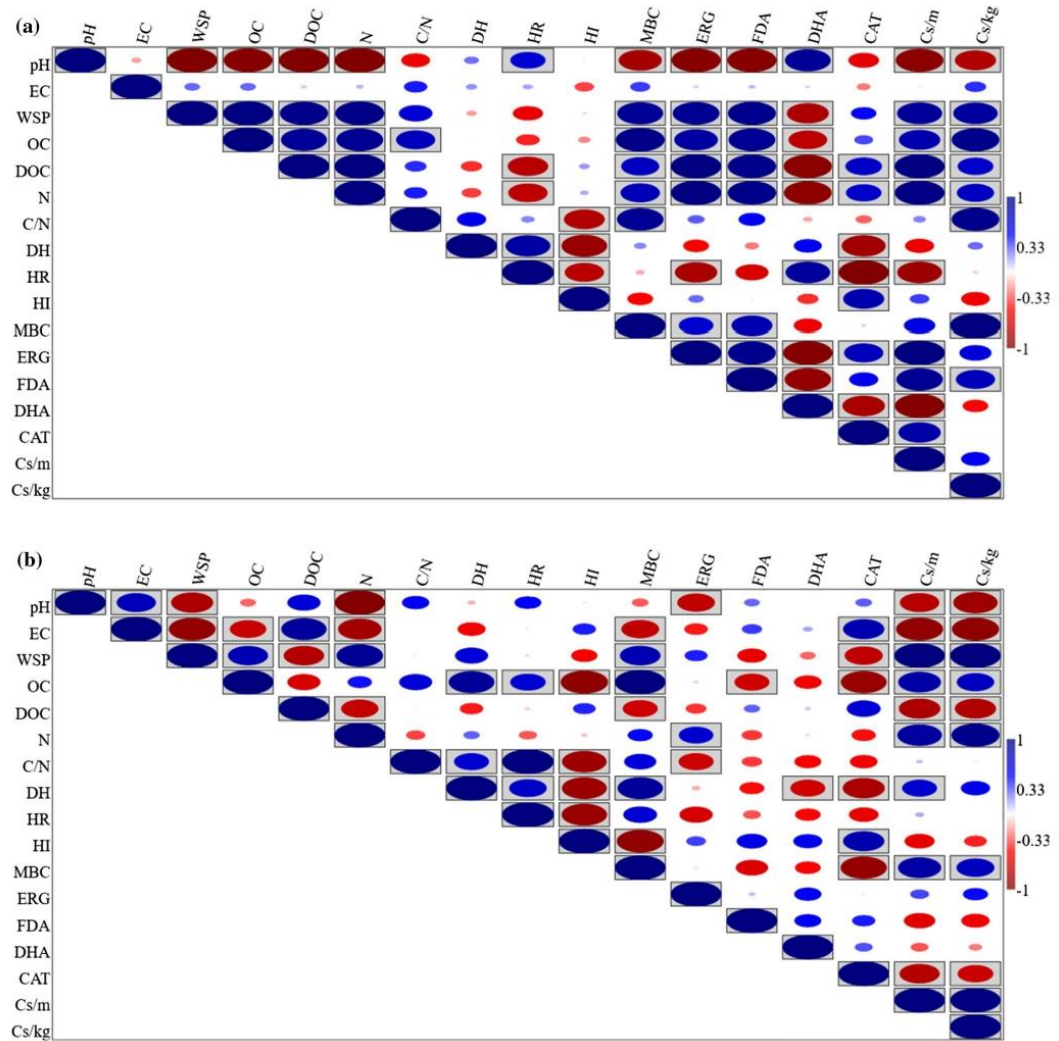


**Fig. 6** Principal component analysis diagram (PCA) in area with different forest management: reference areas (T1), traditional thinning (T2) and innovative thinning (T3), at 0–15 cm (a) and 15–30 cm (b) soil depths

0–15 cm (Fig. 8a). T1 and T2 contained a greater percentage of medium size aggregate (250–630  $\mu\text{m}$ ) followed by small and finally large aggregates, while T3 showed a greater amount of small aggregates followed by medium and large ones. In the subsoil layer (Fig. 8b), T3 maintained the same distribution of aggregate sizes, while in T1 and T2 the proportion of aggregates changed, with both treatments showing a larger percentage of small aggregates rather than medium-sized. The

distribution of OC and nitrogen in the aggregates changed not only with respect to the treatments but also with soil depth (Fig. 9). Organic carbon and nitrogen were higher in the aggregates of the unmanaged treatment (T1) at both depths compared to the two thinning treatments (T2 and T3). Also, a larger content of OC and N was observed in small, medium and large aggregates in the upper layer of T1 and T2. Conversely, OC and N concentrations were more abundant in the



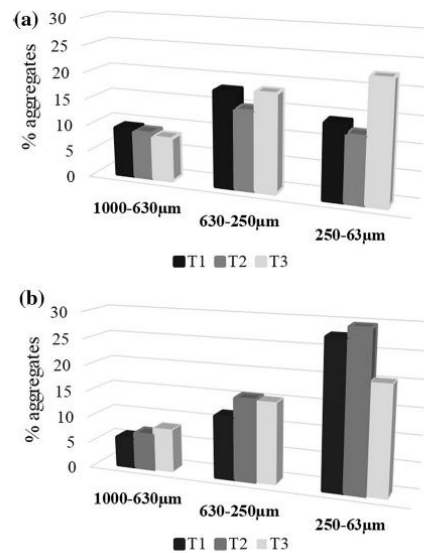


**Fig. 7** Pearson's correlations ( $r$ ) between the soil parameters at 0–15 cm (**a**) and 15–30 cm (**b**) depths. The boxed dots show the significant correlations between values, and the magnitude shows the

level (small boxed dots  $p < 0.05$  and large boxed dots  $p < 0.01$ ). The red dots the negative ones, and the blue ones the positive ones (see the bars on the right of the figure). (Color figure online)

medium and small aggregates in the subsurface layer of the innovative treatment (T3). In T1 the value of C/N ratio was higher in the large and medium aggregates of both soil layers, and it was significantly lower in the small aggregate fraction at both depths (data not shown). In T2 no significant difference among the aggregates at both depths was observed. The highest C/N ratio was observed in the large and medium aggregates

in T3. Comparing the three treatments, the highest values of C/N ratio were found in T3, at both depths (data not shown).



**Fig. 8** Distribution of soil aggregate fractions by ultrasonic method (USAS) under different forest management: unmanaged (T1), traditional thinning (T2), innovative thinning (T3); at depths of 0–15 cm (a) and 15–30 cm (b)

## Discussion

The results presented in Figs. 3 and 5 emphasize that there is clear evidence of active erosion in the soil surface when forests are affected by thinning. The estimate of erosion rates provided by the  $^{137}\text{Cs}$  technique suggests that care must be taken when cutting operations in forest areas are planned. There are, however, important differences between the estimates of soil erosion in relation to the type of treatment. These differences are emphasized by the lower content (and activity) of  $^{137}\text{Cs}$  in the first 0–15 cm of soil with respect to an undisturbed area, and this confirms the reliability of this radionuclide to be considered as an effective tracer to estimate soil erosion even in forested areas. The decrease in  $^{137}\text{Cs}$  inventory in the areas affected by thinning seems to be proportional to the intensity of cutting (see Table 1), and this result gives even more strength to the use of this tracer for similar investigations.

The results presented in Table 1 highlighted also that OC, MBC and WSP decreased gradually when the intensity of cutting increased, and this suggests that these parameters are correlated with  $^{137}\text{Cs}$  and can be considered as possible indicators of soil erosion. Correlation between OC and  $^{137}\text{Cs}$  is well documented in the literature (see, among the others, Li et al. 2004; Teramage et al. 2013; Zhang et al. 2006). However, from the PCA scatter plot illustrated in Figs. 6 and

7, it is possible to observe that WSP was strictly and positively correlated, in both horizons (0–15 and 15–30 cm), to  $^{137}\text{Cs}$  even more than OC, that was also strongly correlated with both of them.

More specifically, results of the present study support a decline in OC and a decrease in the size of aggregates mainly in the innovative thinning, suggesting that the stability of aggregates and the content of the organic component were gradually reduced, as well the amount of ergosterol. Besides being the biomarker for soil fungi, ergosterol is also an important indicator for soil erosion and aggregate stability because, as fungal hyphae, it can contribute to aggregate formation in soils, and may protect soil from erosion. The decrease in OC, aggregate size and ERG resulted in large and medium aggregates being broken into small ones thereby affecting a set of other parameters important for soil health and productivity.

In the upper layer of soil (0–15 cm), other than with  $^{137}\text{Cs}$ , OC was found positively correlated (Pearson's coefficient) with labile organic matter components in terms of MBC, FDA, WSP ERG, C/N, N. These values tended to balance in the lower horizon (15–30 cm), highlighting that management techniques first influenced the surface horizon and subsequently the deeper layers. This finding is supported by the value of OC in the subsurface layer (15–30 cm) that maintains its positive correlation only with MBC, WSP and  $^{137}\text{Cs}$ .

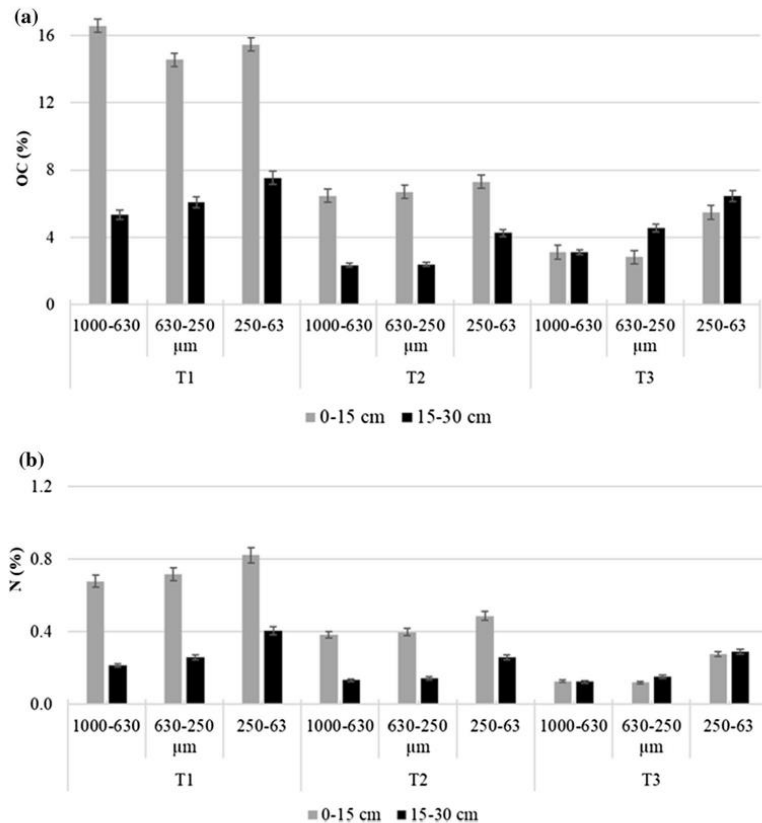
On the other hand, no correlation was found between OC and FDA, ERG, C/N and N, but a significant correlation is documented with DH and HR, parameters that reflect the humification of organic matter.

In short, we can reassume that OC and WSP were the only biological soil parameters that followed the  $^{137}\text{Cs}$  trend in both layers, and thus, they could be considered as indicators of soil loss. However, it is very difficult to assess the soil quality, by measuring only changes in SOM in a short-term (Liang et al. 1997). Estimating WSP, the labile part of SOM, may be more indicative of alteration in place in a short time because WSP is considered an index strictly related to change in situ, induced mainly by the interaction of soil horizon and climate, independently of the type of vegetation cover (Muscolo et al. 2015).

## Conclusions

The results obtained in this study provide a useful tool to evaluate the effect of thinning on soil loss in forested areas. The preliminary analysis, based on the use of the  $^{137}\text{Cs}$  technique, confirmed that this radionuclide is a valuable tracer to estimate soil erosion even in forest contexts. In this respect, the analysis demonstrated that, when traditional monitoring practices cannot be used to evaluate the impacts of forestry

**Fig. 9** Distribution of organic carbon (a) and total nitrogen (b) in different aggregate fractions and depths (0–15; 15–30 cm) under different forest management: unmanaged (T1), traditional thinning (T2) and innovative thinning (T3). Bars represent standard errors



practices on soil at short timescale,  $^{137}\text{Cs}$  proved to be a reliable indicator if a proper sampling strategy is applied.

The established relationship between  $^{137}\text{Cs}$  and other soil parameters, measured in soils affected by different treatments, provides a promising alternative diagnostic tool to quantitatively assess the soil loss in forests. In other words, the possibility to predict short-term variations in soil processes through the use of indicators represents a great advantage in the context of sustainable land management and climate changes. Using biological indicators together with  $^{137}\text{Cs}$  can be crucial in determining the sustainability of forest management activities and will give clear and detailed information on triggering of soil loss. Among the soil properties to be considered, the dosage of WSP proved to be a cheap method that can be easily used to monitor changes in soil characteristics even in case of absence of  $^{137}\text{Cs}$  in soil.

Further work is clearly required to confirm the findings of this investigation in different geomorphic contexts and for different forest species. Additional measurements are also necessary to establish the effectiveness of the  $^{137}\text{Cs}$

technique in different forest ecosystems. In this respect, the need to quantify more precisely the uncertainties associated with the estimates of soil erosion rates provided by  $^{137}\text{Cs}$  measurements has to be considered especially in relation to the sampling strategies adopted in this study. However, the results obtained in this study can be considered as a useful demonstration of the viability of the approach.

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## APPENDIX 2

### Scientific Publication (under review)

Romeo F, Settineri G, Sidari M, Mallamaci C, Muscolo A (2019)

**Responses of soil quality indicators to innovative and traditional thinning in a beech (*Fagus sylvatica* L.) forest**

*Forest Ecology and Management*

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### Abstract

Soil has a pivotal role in keeping high the productivity of forest ecosystem but its physical and chemical properties are highly influenced by changes in forest species composition and forest management practices. Thinning is the most effective silvicultural practice used in Europe to increase the ecological and economic value of forest stands. In this study, biological indicators were used to assess the effects of innovative (T3) and traditional (T2) thinning on soil properties with respect to unmanaged forest (T1), because forest practices are among the main causes of soil fertility and biodiversity loss. The aim was to identify the most appropriate forestry practices to sustainably manage beech (*Fagus sylvatica* L.) forest. Results showed that T2 had the highest dissolved organic carbon (DOC) and the lowest water content (WC), organic carbon (OC) and nitrogen (N) amount. The humification index was the highest in T2 as well as fungi, ergosterol fluorescein diacetate hydrolase (FDA) and catalase. The highest values of NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>-</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> were found in T2. QBS-ar was significantly higher in T2 and T3 than in T1. In short, our results evidenced that organic matter, total nitrogen, C/N ratio and water content cannot be considered alone or in combination indices of quality to evaluate the effect of thinning on soils. Rather, is the data crossing of microbiota and ions with organic matter fractions (stable and labile) that can give important and accurate information on how thinning can affect soil biological properties that are strictly correlated to soil fertility and quality.

|   |   |
|---|---|
| <b>Keywords</b>                           | Biodiversity, QBS-ar, thinning intensities, soil biological indicators          |
| <b>Corresponding Author</b>               | A. Muscolo  |
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## Highlights

- Biological indicators were used to assess the effects of thinning on soil properties.
- Thinning decreased organic C and water content in a *Fagus sylvatica* dominated-soil.
- QBS-ar was significantly higher in the thinned soils than in the control area
- High thinning intensity reduced the activity of soil enzymes and MBC
- The highest values of ions were found in the traditional thinning





Improved

Organic carbon, nitrogen, water content, phenols, C/N ratio, MBC



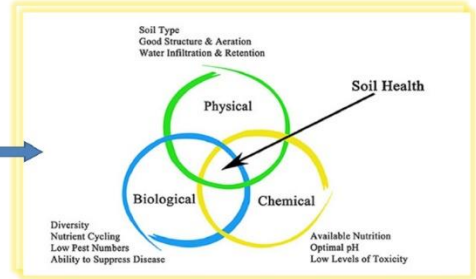
Increased

DOC, nutrients FBC, enzymes, HI, QBS-ar



decreased

Organic carbon, nitrogen, water content, phenols, C/N ratio, MBC, DOC, nutrients FBC, enzymes, HI



1 **Responses of soil quality indicators to innovative and traditional thinning in a**  
2 **beech (*Fagus sylvatica* L.) forest**

3

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4

5 **Abstract**

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20 Rather, is the data crossing of microbiota and ions with organic matter fractions (stable and labile)

1

21 that can give important and accurate information on how thinning can affect soil biological  
22 properties that are strictly correlated to soil fertility and quality.

23

24 *Keywords:* Biodiversity, QBS-ar, thinning intensities, soil biological indicators

25

26

## 27 **1. Introduction**

28 Forests provide crucial services for human well-being and economic development. In addition to  
29 wood and fiber, they provide numerous non-wood products such as food, freshwater, and fuel, or  
30 services as climate and diseases regulation, recreation and preservation of biodiversity, driving the  
31 sustainable growth (IUCN). Now a days, a prominent challenge is how to manage forests for timber  
32 and bioenergy production maintaining, at the same time, long-term conservation/implementation of  
33 the forest ecosystem functioning. Soil has a pivotal role in keeping high the productivity of forest  
34 ecosystem but its physical and chemical properties are highly influenced by changes in forest stand  
35 structure, density and composition of species which correlates with thinning intensity, interval, and  
36 method. The reduction in tree density due to thinning is reported to alter microclimate (Masyagina  
37 et al., 2006; Trentini et al., 2017), increasing soil temperature and decreasing soil humidity (Ma et  
38 al., 2010) but also to affect soil properties, increasing compaction (Elliot et al., 1998; Picchio et al.,  
39 2012; Marchi et al., 2014), soil bulk density and decreasing water holding capacity and nutrients.  
40 Thinning can also affect organic matter (Smolander et al., 2013) which govern most of the soil  
41 properties, and hence soil quality and health. Soil organic matter (SOM) changes in a short time are  
42 difficult to measure directly because they don't vary only on the basis of flux by which carbon enter  
43 or leaves soil ecosystem but also for the flux of carbon that occurs in the soil ecosystem (Chapin III  
44 et al., 2002), total SOM by itself is thus not able to highlight changes in soil ecosystem functioning.  
45 Organic matter contains numerous fractions (phenolics, microorganisms, enzymes) each with a

2

46 different residence time and different functional roles in soil (Zagal et al., 2009) which singularly  
47 could give a measure of subtle, or early changes in soil quality. Organic matter fractions can be  
48 considered as fine indicators of soil quality that influence soil function in specific ways and that are  
49 much more sensitive to changes in soil management practices (Muscolo et al., 2014). Generally,  
50 changes in SOM fractions can sensitively respond to changes in plant vegetation, climate and land  
51 use in agroforestry ecosystems (Schmidt et al., 2011; Wang and Wang, 2011; Wagg et al., 2014).  
52 Muscolo et al. (2014) identified organic matter fractions such as microbial biomass carbon (MBC),  
53 water soluble phenols (WSP), and fluorescein diacetate hydrolase (FDA), as effective tools in the  
54 evaluation of soil quality changes, in the short term, in a coniferous stand of southern Italy.  
55 Additionally, Muscolo et al. (2015) showed in *Pinus laricio* Poiret ssp. *calabrica*, *Abies alba* Mill.  
56 and *Fagus sylvatica* stands in Calabrian Apennines, southern Italy, that these three indicators  
57 reflected soil quality change due to different factors. MBC primarily showed variation related to  
58 vegetation, FDA evidenced changes caused by climatic factors, and WSP was influenced by  
59 changes related to soil depth. Thinning might have a great impact on the different fractions of SOM  
60 in the soil. Recently, there were a number of studies on the effects of thinning on soil respiration  
61 and SOM. Settineri et al. (2018), studying the effects of different thinning intensities on soil carbon  
62 stock showed that high intensity thinning was the best silvicultural practice-approach method to  
63 manage *Pinus laricio* forest for increasing soil carbon storage. Kim et al. (2019) highlighted the  
64 interaction between soil properties and microbes evidencing that inconsistent thinning effects on  
65 soil properties changed microbial biomass and enzymes in thinned oak and larch forest ecosystems.  
66 Zhang et al. (2018) analyzing 53 published studies on forest thinning, evidenced that thinning did  
67 not significantly change total carbon, and MBC, but enhanced soil temperature, soil total nitrogen  
68 and decreased the soil C/N ratio. Since there is still little information on the effect of thinning on  
69 organic matter fractions, further studies on the variations in soil organic matter fractions and soil  
70 biodiversity are thus crucial for evaluating the stability and the sustainability of forest production  
71 following a thinning treatment. In Italy, thinning of beech forests represent the most used

3

72 silvicultural treatment to enhance the economic value of these stands maintaining biodiversity  
73 (Lombardi et al., 2018). Therefore, this research aims to understand how thinning affects organic  
74 matter and each of its component pools in beech forest ecosystem. The objectives of this study were  
75 to: (1) compare the effects of two different thinning practices (traditional and innovative) to  
76 unmanaged beech (*Fagus sylvatica*) forest; (2) determine the variation of soil organic matter  
77 fractions under different thinning practices, to identify which type of forest management practice  
78 was more sustainable for maintaining/improving soil quality in Calabrian beech forests.

79

## 80 **2. Material and methods**

### 81 *2.1. Study area*

82 The study area is located in the Marchesale Biogenetic Reserve (Natura 2000 site) within the  
83 highest slope of the Calabrian "Serre" mountains, in Mongiana (VV) (38° 30' N, 16° 14' E). The  
84 entire reserve, that covers 1234 hectares, is managed by the National Forest Service of Italy and  
85 consists mainly of 75-year-old high beech forest *Fagus sylvatica*.

86 Being located in the upper part of the mountain system facing the Tyrrhenian Sea (1100 m a.s.l.)  
87 and exposed to North-West, its microclimate is influenced by the interception of fog, wet winds and  
88 precipitation (Becagli et al., 2013). The mean values of annual rainfall and temperature, based on  
89 the data obtained at Mongiana (VV) meteorological station (920 m a.s.l.) and available for the  
90 period 1928-2018, are respectively of 1801.5 mm e 11.4 °C. The warmest month is August (18.6  
91 °C), the coldest one is January (3.8 °C). The climate is typically Mediterranean (Csb, sensu  
92 Koppen, 1936).

93 This area is geologically characterized by Paleozoic granitoid deeply fractured and with deep,  
94 versus shaped slopes (Conforti et al., 2015). According to USDA (soil classification), the  
95 predominant soils are Inceptisols and Entisols (Soil Survey Staff, 2010).

96 Between 2012 and 2013, an experimental forest management was carried out on a surface of 30  
97 hectares of the entire reserve, chosen subsequently as the object of our study. In this area three  
98 different sub-areas of about 3 ha each have been identified for each different silvicultural thinning  
99 (3 treatments x 3 replicates). Specifically, an unmanaged area for over 30 years (T1) was used as  
100 control; a traditional treatment (T2) and an innovative treatment (T3) have been identified.  
101 Traditional treatment was a thinning from below with a moderate intensity which removed all the  
102 dominated trees and the worst dominant trees (on average, ca. 12% of total volume resected). The  
103 innovative treatment was oriented to retain the 50 best trees per hectare and improve the structural  
104 biodiversity, collecting 5 or 6 trees closer to them, regardless of their social position (on average,  
105 ca. 27% of the total volume removed). No significant differences were found between the  
106 dendrometric parameters in these areas before the silvicultural interventions (Picchio et al., 2016).  
107 The three treatment sites are fully comparable in terms of slope, orientation and soil types.

108

#### 109 2.2. *Sampling procedure*

110 For each of the three treatment sites (T1, T2 and T3), 3 representative plots (1000 m<sup>2</sup> in size) were  
111 established for sampling (3 treatments x 3 plots = 9 plots). The soil sampling campaign was carried  
112 out in May 2018, and consisted of collecting soil cores for chemical-physical and biological  
113 analysis. The cores were collected in areas with similar slope using a 10-cm-diameter steel core  
114 tube inserted up to a depth of ca. 35 cm.

115 Each soil core (9 in total) used for the chemical-physical and biological analyses, was divided into 2  
116 layers (0-15 and 15-30 cm). The corresponding layers were merged in order to obtain 3 final  
117 representative cores (one for each treatment site) and all analysis made tripled. Separate samples  
118 (10 cm depth), following the same scheme, were performed to establish the micro-arthropods QBS-  
119 ar index. All the samples were air dried and sieved to separate the < 2 mm fraction, except for  
120 microbial biomass, QBS-ar (fresh soil) and water content (oven drying).

121



148 1 g of soil. The suspension was homogenized with mechanical agitation for 15 min, and centrifuged  
149 at 3500 rpm for 15 min. The supernatant was filtered using a syringe membrane filter (4 mm, 0.45  
150  $\mu\text{m}$  polytetrafluoroethylene (PTFE) and then kept in the dark until analysis with an Agilent  
151 Technologies Infinity 1290 high performance liquid chromatography (HPLC) system (Agilent  
152 Technologies, Santa Clara, California, USA). Soil samples were extracted with bidistilled water  
153 (ratio soil/water 1:10) (Wang et al., 2013) for 24 h at 25 °C to detect ion concentration by using a  
154 chromatography systems (Dionex ICS-1100).

155

#### 156 2.4. Fungal and Microbial biomass C

157 MBC was determined in fresh soil samples by the chloroform fumigation-extraction method (Vance  
158 et al., 1987). The filtered soil extracts of both fumigated and non-fumigated samples were analyzed  
159 for soluble organic C using the method of Walkley and Black (1934). The estimation of the MBC  
160 was made on the basis of the differences between the fumigated and non-fumigated soil, and an  
161 extraction efficiency coefficient of 0.38 was used to convert soluble C in biomass (Vance et al.,  
162 1987). As reported by Montgomery et al. (2000), measurement of ergosterol concentration in the  
163 soil is useful for estimating the soil fungal biomass carbon (FBC) content. Following the method  
164 proposed by Montgomery et al. (2000) it was possible to determine the content of living FBC  
165 starting from measurements of ergosterol content in the soil. Several mycelial carpets have been  
166 studied in different types of soil, and ergosterol transformed by the following formula into FB  
167 (fungal biomass):

$$168 \quad FB (\mu\text{g g}^{-1} \text{ soil}) = \text{Ergosterol} (\mu\text{g g}^{-1} \text{ soil}) \times f \times Rf \quad (4)$$

169 where  $f = 250$  ( $1/4 \times 1000$ , mg biomass  $\mu\text{g}^{-1}$  ergosterol), and  $Rf = 1.61$  (correction factor for  
170 average percent recovery,  $1/0.62$ ).

171 FB can also be expressed in terms of the C content of the mycelial mats (Montgomery et al., 2000):

$$172 \quad FB - C = FB \times C \quad (5)$$

173 where FB-C = fungal biomass-carbon, and  $C = 0.43$ , average C content detected in fungal species.

7



174

175       2.5.       *Enzymatic assay*

176   Fluorescein dyacetate hydrolase (FDA) hydrolysis reaction was determined according to the  
177   methods of Adam and Duncan (2001). Briefly to 2 g of soil (fresh weight, sieved <2mm) was added  
178   15 mL of 60 mM potassium phosphate pH 7.6 and 0.2 mL 1000 mg FDA mL<sup>-1</sup> and then placed in  
179   an orbital incubator at 30,8°C for 20 min. Once removed from the incubator 15 mL of chloroform/  
180   methanol (2:1, v/v) was added to terminate the reaction. After this, the content of the flask was  
181   centrifuged at 2000 rpm for 3 min. The supernatant was filtered and measured at 490 nm on a  
182   spectrophotometer (Shimadzu UV-vis 2100, Japan).

183   Dehydrogenase (DH) activity was determined by the method of von Mersi and Schinner (1991). In  
184   brief, to a sample of fresh soil equivalent to 1 g of oven dried (105 °C) soil were added 1.5 ml of 1  
185   M Tris-HCl buffer of pH 7.5 followed by 2 ml of 0.5% INT solution (Sigma product No I 8377),  
186   and the suspension was kept at 40 °C for 1 h. Then 10 ml of extractant (methanol) were added and  
187   the samples were mixed and then leaved in the dark for 10 min. Finally, the solutions were filtered  
188   (Whatman's n° 40 paper), and the absorbance of the filtrate was determined at 490 nm.

189   Urease (URE) was determined using the method of Kandeler and Gerber (1988). Soil (5 g fresh  
190   weight) was mixed with 2.5 ml of urea (80mM) and 20 ml 0.1 M borate buffer pH (10.0), allowed  
191   to react for 2 h in an orbital shaker at 37 °C. After incubation, pipette 2.5 ml of urea to the control,  
192   add 30 ml of KCl (2M) to both sample and control, and shake for 30 min. Filter the contents of the  
193   flasks through folded filters. Aliquots of 1 ml of the filtered solution were mixed with 9ml of  
194   distilled water, 5 ml of sodium/salicylate solution and 2 ml of dichloroisocyanuric acid (Na<sup>+</sup> salt).  
195   The colour intensity of the solution was measured at 690 nm. Ammonium concentrations were  
196   determined by using a calibration curve of ammonium chloride standard solution.

197   Catalase (CAT) activity was measured by the method of Beck (1971). Results were expressed as  
198   O<sub>2</sub>% g<sup>-1</sup> soil 3min<sup>-1</sup>.

199

8

200        2.6.        *Microarthropods*

201    Micro-arthropods, included mites and collembola, were analyzed with Berlese-Tullgren selector  
202    (Parisi et al., 2005). One soil samples were taken from each site differently managed, including the  
203    control site. Each plot was sampled at soil depths of 0-10 cm. Immediately upon returning from the  
204    field, the soil samples were transferred to Berlese-Tullgren funnels lined with 4 mm wire mesh.  
205    Arthropods were extracted for 7 days and collected in a beaker filled with preservative liquid (2  
206    parts 75% ethanol and 1 part glycerol) beneath the funnel.

207    All the arthropods were identified at different taxonomical levels through, the determination of  
208    biological forms and calculation of QBS index, using a microscope PCE-MM200. Accordingly, the  
209    biological form (morpho-type) that is most adapted to soil was identified. Each morpho-type  
210    correspond an ecomorphological index (EMI); as a rule, eu-edaphic (i.e. deep soil living) forms  
211    correspond to an EMI=20, hemi-edaphic (i.e. intermediate) forms have an index rating  
212    proportionate to their degree of specialization, while epi-edaphic (surface living) have an EMI = 1.  
213    The QBS index value was obtained from the sum of EMI index of all the collected groups (*Acari*,  
214    *Collembola*, *Areneae*, *Diptera*, *Hemiptera*, *Coleoptera*, *Hymenoptera*, *Protura*, *Diplura*,  
215    *Diplopoda*). If in a group biological forms with different EMI values are present, the higher value  
216    (more adapted to the soil form) was selected to represent the group in the QBS calculation (Parisi et  
217    al., 2005). EMI is a simplified index, that use the microartopod morphology assessment for  
218    generating Soil Biological Quality index (QBS index). This analysis allows assessing the  
219    degradation level of soils (Parisi et al., 2005).

220

221        2.7.        *Statistical analyses*

222    To test the relationships among soil parameters at two soil depths and for three different  
223    silvicultural treatments, datasets were analyzed using Principal Component Analysis (PCA),  
224    Multivariate Analysis of Variance (MANOVA) and T test for paired values. The results are  
225    summarized in an ordination diagram. PCA was carried out using the soil parameters in plots under

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226 different silvicultural treatments using the software PAST (Hammer et al., 2001). Because the data  
227 are expressed in different units, the results are standardized with the following formula:

$$228 \quad z = \frac{(x_i - \bar{x})}{SD} \quad (6)$$

229 where  $x_i$  is the individual value of each parameter,  $\bar{x}$  is the mean and SD the standard deviation.

230 A MANOVA analysis was carried out for evaluating the effects of thinning, soil depth and their  
231 interaction on the set of soil parameters. Finally, since there are only two soil depths (0-15 cm and  
232 15-30 cm), a T test was used for paired values to evaluate significant differences. This last analysis  
233 allowed us to verify if thinning affected soil properties according to depth, and if the impacts were  
234 similar. Pearson's correlations for both soil layers and all soil parameters, were carried out using  
235 PAST software (Hammer et al., 2001).

236 One way ANOVA and t-test was carried out to determine statistical difference for QBS-ar among  
237 the treatments. Anova, Manova models and t-test were carried out using SPSS software (IBM  
238 Corp., 2012).

239

### 240 **3. Results**

#### 241 *3.1. Soil chemical and biochemical features*

242 All the soils analyzed belong to the sandy-loam textural class, with 25% silt, 13% clay and 62%  
243 sand (data not shown). Soil texture did not change over treatments (data not shown). Water content  
244 (WC) decreased in both layers (Table 1) following tree thinning intensity. In the first layer of soil  
245 (0-15 cm) WSP, OC, N, C/N decreased in T2 and much more in T3 in comparison to T1. DOC  
246 decreased only in the innovative thinning. In the underlying layer a similar trend was observed for  
247 WC (Table 1). T3 and T2 showed the lowest WSP. T2 showed the highest DOC amount and the  
248 lowest OC and N amount, while C/N ratio did not show significant change among the treatments  
249 and control (Table 1). The humification degree did not change in the 0-15 cm between the  
250 treatments and control (Table 2). Humification rate was the highest in T3 while the humification

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251 index was the highest in T2 (Table 2). MBC decreased in T2 (-40%) and T3 (-51%) in respect to  
252 control. Ergosterol and FBC were the highest in T2 and they were almost double than T3.  
253 Regarding the enzymatic analysis, FDA in T3 was half than T1 and T2. DHA was instead greater in  
254 T3 than T1 and T2. CAT was the highest in T2 followed by T1 and T3. URE was the lowest in T3  
255 and the highest in T1. In the 15-30 cm layer DH, HI and HR had a trend similar to those observed in  
256 0-15 cm layer. MBC was always higher in T1 than T2 and T3. FBC and Erg were also the highest  
257 in T2. FDA was always the highest in T2. FDA was less than half in T1 in respect to the same  
258 treatment in the upper layer. DHA did not show significant variation among the treatments and  
259 control. Catalase and urease maintained the same trend in the upper and lower layers, and it was  
260 higher in T2 than T3 (Table 2).

261 For PCA analysis the first two components (Eigenvalues >1) have been extracted. The variance was  
262 higher at 0-15 cm (94.6 %), than at 15-30 cm (91 %). At both depths, the component 1 explained  
263 about 55 %, while the component 2 explained about 35 % of the variability in all parameters (Fig.  
264 1). PCA diagram for 0-15 cm showed that T3 influenced only DHA, DH and HR while T1 and T2  
265 were similar in fitting with all the other parameters. At 15-30 cm T3 influenced only HR, T2  
266 influenced mainly the enzyme activities, Erg, FBC and DOC. All the other soil parameters were  
267 equally distributed in T2 and T1 (Fig. 1). Soil under traditional thinning, in respect to control and  
268 innovative thinning, showed a greater CAT, Erg, FBC and HI in both layers (Table 3). Still in the  
269 T2 area, FDA activity was the highest in both soil layers (Table 2). These assumptions are clearly  
270 evident in Fig. 1, where the PCA showed these values significantly correlated to T2 area and placed  
271 in the same quadrants. On the contrary, soil under innovative thinning, in both layers, (located in the  
272 quadrant with both negative components) showed only a greater humification rate. T3 positively  
273 influenced DHA in the upper layer and DH, MBC and OC in the lower layer (Fig. 1a,b). Lowest  
274 values of WSP, OC, DOC, N, C/N, Erg, MBC, FDA, CAT and URE, were detected in the upper  
275 layer under innovative thinning (located in opposite quadrants in Fig. 1a, Table 1,2). In the 15-30  
276 cm DOC, N, DH, HR, HI, CAT and URE maintained the same trend of the upper layer for all the

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277 treatments (Table 1,2). OC, C/N ratio and WSP increased in T3 in respect to its own values detected  
278 in the upper layer. A strong decrease in MBC, Erg and FBC content was instead observed (Table 2).  
279 In the underlying layer, FDA, URE and CAT decreased and DHA increased in T1 and T2, while all  
280 these parameters decreased in T3 in respect to 0-15 cm layer (Table 2). OC in both layer and in all  
281 treatments resulted positively correlated to WC, WSP, N, and MBC (Table 4).  
282 FDA, C/N and URE were positively correlated to OC only in the first layer (Table 4). FBC was not  
283 correlated to OC, but showed significant and positive correlation in both layer with DOC, HI, ERG,  
284 CAT and  $\text{NO}_3^-$  (table 4).

285

### 286 3.2. *Soil ions*

287 In the surface layer, the highest values of  $\text{NO}_3^-$ ,  $\text{SO}_4^-$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  were measured in the T2  
288 area (Fig. 2a). The only one to be positively influenced by the innovative thinning was  $\text{K}^+$  (Fig. 1a-  
289 b), while chloride ( $\text{Cl}^-$ ),  $\text{NO}_3^-$  and  $\text{Na}^+$  decreased in T3 (0-15 cm) (Fig. 2a). In the 15-30 soil layer,  
290 ions showed a general decrease in values,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  were higher in T1 and T2 in respect to  
291 T3 (Fig. 2b).  $\text{K}^+$  did not show significant differences between treatments and control,  $\text{SO}_4^-$  and  $\text{Cl}^-$   
292 were higher in T1 than T2 and T3,  $\text{NO}_3^-$  was the highest in T2 (Fig. 2b). The concentration of  $\text{NH}_4^+$   
293 in soil was below the detection limit.

294 PCA diagram for 0-15 cm showed that T3 influenced only  $\text{K}^+$ , while T1 and T2 similarly fitted with  
295 all the other parameters. At 15-30 cm T2 influenced mainly  $\text{K}^+$  and  $\text{NO}_3^-$  (Fig. 1b). It was also  
296 evident from the PCA analysis how the innovative area was the only one with the lowest ion related  
297 parameters (Fig. 1a-b). In fact, except for  $\text{K}^+$  in the surface layer (Fig. 2a), all the parameters were  
298 completely translated in the quadrants opposite to T3 (always with negative components).

299

### 300 3.3. *Microarthropods features*

301 QBS-ar was significantly higher in the T2 and T3 than in the control area (Fig. 3). This is certainly  
302 due to microclimatic properties, solar irradiation and different presence of herbaceous vegetation

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303 between the examined areas. Regarding the micro-arthropod groups a different distribution in soil  
304 differently managed was found. Among the 16 species found (Table 3), in T1 9 species has been  
305 observed. Chilopoda, thysanoptera, protura, diplura, lepidoptera, araneae, formicidae and isopoda  
306 were totally absent (Table 3). In T2 14 species were present. The species that missed in soil under  
307 traditional thinning were blattaria and diplura. In T3 13 species were present, blattaria, protura and  
308 formicidae were missed. The greatest biodiversity was found in T2 in respect to all the other  
309 treatments.

310

#### 311 **4. Discussion**

312 Soil quality reflects the capacity of a soil to promote biological productivity, plant and animal  
313 health, and to maintain environmental quality (Doran and Parkin, 1994). In agro and forestry-  
314 ecosystems the assessment of soil quality through biological indicators allows early evidence of  
315 changes in soil physical and/or biological characteristics determined also due to forest management.  
316 Roscoe and Buurman (2003) quantified the effects of forest management on soil estimating total  
317 organic carbon, but as subsequently demonstrated forest management affect not only the organic  
318 carbon storage (Lal, 2006), but also nutrient concentrations (Ashagrie et al., 2007), soil enzyme  
319 activities (Kim et al., 2019), soil biodiversity (Lukac, 2017) and water retention (Resck et al., 2008).  
320 Thus testing only the changes in SOM would be reductive, to assess instead SOM fractions (labile  
321 and stable), and correlating them each others can give important information to evaluate the impact  
322 that forest management can have in a short term on soil fertility. Our results demonstrated a  
323 decrease in OC, C/N and labile fraction of organic matter in terms of MBC and WSP mainly in the  
324 innovative thinning than traditional one in respect to unthinned forest. These results highlighted that  
325 under thinning, the relative rate of decomposition increased mainly in T3, as demonstrated by the  
326 greatest decrease in C/N ratio. The humification index, the ratio between not humified and humified  
327 extracted carbon, and the percent of humification rate detected because they are considered key

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328 indicators of the humification status of organic matter in soil (Gigliotti et al., 1999), evidenced that  
329 T2 had a good balance between humification and mineralization process in respect to T1 and T3  
330 where a shift to humification and mineralization process respectively were observed. HI was at  
331 about 0.5 in T2, lower values indicate more humified extracts (Sidari et al., 2005). HR parameter  
332 that is proportional to the state of humification of the soil organic matter, was similar in T2 and T1  
333 as well as DH that is generally 100% when the extracted organic carbon is completely humified.  
334 Pedofauna and microflora detected because soil ecosystems with large biodiversity and microbial  
335 biomass improve soil carbon cycle more than soil ecosystem with reduced amount of microbiota (de  
336 Graaff et al., 2015). Hooper et al. (2005) highlighted that taxonomic and functional compositions of  
337 soil communities are effective drivers of SOM processes. Results evidenced that the abundance,  
338 species richness of micro-arthropods, as well as fungi and bacteria colonies and soil properties  
339 changed with the treatments. Under T2 we found the greatest biodiversity and the highest amount of  
340 micro-arthropods. Because of their high abundance, specie richness, habitat fidelity (Andersen and  
341 Majer, 2004), and high sensitivity to external perturbations, micro-arthropods are considered  
342 important bio-indicators of environmental quality and can be used for monitoring short-term  
343 changes in soil ecosystem. The diversity in number of species, found among the treatments, can  
344 explain in part the differences in soil quality. The faeces of arthropods are the basis for the  
345 formation of soil aggregates and humus, which physically stabilize the soil and increase its capacity  
346 to store nutrients improving its quality and represent also a substrate for microbial decomposition  
347 and to fostering the growth and dispersal of microbial populations (Culliney, 2013). Under T2, the  
348 soils had the highest amount of fungi as well as ergosterol, but a low amount of MBC. Generally  
349 high fungi amount correspond to higher ability of such soils to improve nutrient cycling (Hodge et  
350 al., 2001; Smith & Smith, 2011). A shift toward a fungal dominance in the microbial community is  
351 thought to enhance soil aggregation and soil nutrient interception and availability to plants. (Augè,  
352 2004; Six et al., 2006). Ergosterol an important indicator of fungal growth on organic compounds  
353 and mineralization activity was higher in T2 than T1 and T3 confirming the greatest presence of

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354 fungi amount and activity in traditionally managed forest stand. For instance, fungi are thought to  
355 express a broader suite of enzymes capable of transforming and stabilizing inputs; and fungal  
356 biomass has greater C/N ratio which results in increased carbon use efficiency (Strickland and  
357 Rousk, 2010; Waring et al., 2013). Intensively managed soils often exhibit lower fungal/microbial  
358 biomass ratio. Our data apparently in contrast with data on OC, MBC and WSP that could suggest  
359 in thinned forest a greater carbon loss with a decrease in fertility, evidenced a higher  
360 fungi/microbial biomass ratio in managed areas and in particular in T2 than T1 and T3 as well as a  
361 higher activity of FDA and catalase, enzymes fungal-produced, as demonstrated by correlation  
362 analysis. Catalase activity is usually higher in high quality soils and its activity may decrease or  
363 cease when soil pH, nutrients, or temperature extremes occur (Xun et al., 2015). Catalase is also an  
364 important cellular antioxidant enzyme that defends soil against oxidative stress. The highest  
365 catalase activity in T2 suggests that the traditional thinning positively affected soil quality. In T2,  
366 the greatest amount of FDA, enzyme markers of hydrolytic soil activity, reliable estimator of fungal  
367 biomass was found, suggesting that in this site a great decomposition transformation, and  
368 mineralization of organic matter with a major nutrient release occurred. This data were also  
369 confirmed by the increase in nutrients and DOC and by the contemporary decrease in SOM and  
370 were in agreement with the findings of Bardgett et al. (2003), Lee and Jose (2003), Allison (2006),  
371 De Deyn et al. (2009) and Billings et al. (2010), showing that microflora components have a key  
372 role in soil nutrient and organic matter cycle. In short from this data crossing resulted that T2  
373 improved soil quality even if the organic matter amount decreased resulting a sustainable forest  
374 management practice.

375

## 376 **5. Conclusion**

377 In short, our results evidenced that organic matter, total nitrogen, C/N ratio and water content  
378 cannot be considered alone or in combination indices of quality to evaluate the effect of thinning on

15



379 soils. Rather, is the data crossing of microbiota and ions with organic matter fractions (stable and  
380 labile) that can give important and accurate information on how thinning can affect soil biological  
381 properties that are strictly correlated to soil fertility and quality. It is by using specific and pertinent  
382 biological indicators in combination, that we can predict the dynamic behaviour of soil processes  
383 and the impact of management practices on soil quality allowing to determining the sustainability of  
384 forest management activities. In other words, the possibility to predict short-term variations in soil  
385 processes through the use of indicators represents a great advantage in the context of sustainable  
386 land management. Using biological indicators forest management activities will give clear and  
387 detailed information on triggering of soil fertility loss. Among the soil properties DOC, FDA, CAT,  
388 fungi and pedofauna have been identified as effective tools to evaluate performance and quality of  
389 managed soil in a short time.

390

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394

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397 analyzed the data, performed statistical analyses. MS critically reviewed and edited the manuscript,  
398 GS performed the laboratory experiments, CM conducted fieldwork.

399

#### 400 **Conflict of interest statement**

401 None declared.

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574



575 **Figure Captions**

576 **Fig. 1** Principal component analysis diagram (PCA) in area with different forest management:  
577 reference areas (T1), traditional thinning (T2), and innovative thinning (T3), at 0-15 cm (a) and 15-  
578 30 cm (b) soil depths.

579 **Fig. 2** Distribution of mean values and standard deviations (bars) of soil ions (mg/Kg-1 dry soil),  
580 collected at 0–15 cm (a) and 15–30 cm (b) depths; referred to different management: control (T1),  
581 traditional thinning (T2), innovative thinning (T3). For each parameter the sample size was  $n = 3$   
582 for treatment. Bars followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test)

583 **Fig. 3** Distribution of mean values and standard deviations (bars) of QBS-ar index for micro-  
584 arthropods, collected at 0–10 cm depths; referred to different management: control (T1), traditional  
585 thinning (T2), innovative thinning (T3). For each parameter the sample size was  $n = 3$  for treatment.  
586 Bars and columns followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test)

587 **Fig. 4** Pearson's correlations ( $r$ ) between the soil parameters at 0–15 cm (a) and 15–30 cm (b)  
588 depths. The boxed dots show the significant correlations between values, the magnitude shows the  
589 level (small boxed dots  $p < 0.05$ , large boxed dots  $p < 0.01$ ). The red dots the negative ones, the blue  
590 ones the positive ones (see the bars on the right of the figure)

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593

594 **Table 1** Mean values and standard deviations of soil parameters referred to two layers of soil (0-15;  
595 15-30 cm): water content (WC, %); water soluble phenols (WSP,  $\mu\text{g TAE g}^{-1}$  dry soil); organic  
596 carbon (OC, %); dissolved organic carbon (ml DOC L<sup>-1</sup>); total nitrogen (N, %); C/N ratio. For each  
597 parameter the sample size was n = 3 for treatment (T1- Control; T2- Traditional thinning; T3-  
598 Innovative thinning). Means in the same column followed by the same letter are not statistically  
599 different at  $p \leq 0.05$  (Tukey test)

600

|            | 0-15 cm                  |                          |                          | 15 - 30 cm               |                          |                          |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|            | T1                       | T2                       | T3                       | T1                       | T2                       | T3                       |
| <b>WC</b>  | 41 <sup>a</sup> ± 2      | 20.7 <sup>b</sup> ± 3    | 18.5 <sup>b</sup> ± 2    | 40.6 <sup>a</sup> ± 4    | 16.8 <sup>b</sup> ± 2    | 12 <sup>b</sup> ± 3      |
| <b>WSP</b> | 259 <sup>a</sup> ± 6     | 155 <sup>b</sup> ± 7     | 67 <sup>c</sup> ± 5      | 313 <sup>a</sup> ± 6     | 163 <sup>b</sup> ± 10    | 156 <sup>b</sup> ± 16    |
| <b>OC</b>  | 11.5 <sup>a</sup> ± 0.4  | 6.5 <sup>b</sup> ± 0.2   | 3.1 <sup>c</sup> ± 0.1   | 5.4 <sup>a</sup> ± 0.02  | 4.2 <sup>c</sup> ± 0.02  | 4.9 <sup>b</sup> ± 0.03  |
| <b>DOC</b> | 14.1 <sup>a</sup> ± 0.1  | 14.5 <sup>a</sup> ± 0.4  | 12.9 <sup>b</sup> ± 0.2  | 11.1 <sup>b</sup> ± 0.4  | 12.4 <sup>a</sup> ± 0.6  | 10.3 <sup>b</sup> ± 0.4  |
| <b>N</b>   | 0.57 <sup>a</sup> ± 0.01 | 0.43 <sup>b</sup> ± 0.01 | 0.23 <sup>c</sup> ± 0.01 | 0.34 <sup>a</sup> ± 0.01 | 0.26 <sup>c</sup> ± 0.01 | 0.31 <sup>b</sup> ± 0.01 |
| <b>C/N</b> | 20.4 <sup>a</sup> ± 0.6  | 15.1 <sup>b</sup> ± 0.4  | 13.1 <sup>c</sup> ± 0.3  | 15.7 <sup>a</sup> ± 0.1  | 15.8 <sup>a</sup> ± 0.1  | 15.9 <sup>a</sup> ± 0.1  |

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613 **Table 2** Mean values and standard deviations of soil parameters referred to two layers of soil (0-15;  
614 15-30 cm): humification degree (DH, %); humification rates (HR, %); humification index (HI);  
615 microbial biomass (MBC,  $\mu\text{g C g}^{-1}$  f.s.); Fungal biomass-carbon (FBC,  $\mu\text{g g}^{-1}$ ); ergosterol fungal  
616 biomarker (ERG,  $\mu\text{g g soil}^{-1}$ ); fluorescein released (FDA,  $\mu\text{g g}^{-1}$  dry soil); dehydrogenase (DHA,  $\mu\text{g}$   
617 INTF  $\text{g}^{-1}$  dry soil  $\text{h}^{-1}$ ); catalase activity (CAT,  $\text{O}_2\%/3\text{min/g dry soil}^{-1}$ ); urease activity (URE,  $\text{mg}$   
618  $\text{NH}_4^+\text{-N g}^{-1}$  dry soil  $2\text{h}^{-1}$ ). For each parameter the sample size was  $n = 3$  for treatment (T1- Control;  
619 T2- Traditional thinning; T3- Innovative thinning). Means in the same column followed by the  
620 same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

|            | 0-15 cm                  |                          |                          | 15 - 30 cm               |                          |                          |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|            | T1                       | T2                       | T3                       | T1                       | T2                       | T3                       |
| <b>DH</b>  | 88.7 <sup>a</sup> ± 0.6  | 88.5 <sup>a</sup> ± 0.5  | 89.7 <sup>a</sup> ± 1.9  | 87.1 <sup>a</sup> ± 0.7  | 83.4 <sup>b</sup> ± 0.2  | 85.6 <sup>a</sup> ± 0.9  |
| <b>HR</b>  | 62.8 <sup>b</sup> ± 0.4  | 61.1 <sup>c</sup> ± 0.4  | 67.2 <sup>a</sup> ± 0.7  | 61.7 <sup>b</sup> ± 0.5  | 57.6 <sup>c</sup> ± 0.1  | 64.1 <sup>a</sup> ± 0.1  |
| <b>HI</b>  | 0.33 <sup>c</sup> ± 0.01 | 0.53 <sup>a</sup> ± 0.01 | 0.42 <sup>b</sup> ± 0.03 | 0.44 <sup>b</sup> ± 0.02 | 0.58 <sup>a</sup> ± 0.01 | 0.45 <sup>b</sup> ± 0.01 |
| <b>MBC</b> | 1529 <sup>a</sup> ± 7    | 910 <sup>b</sup> ± 6     | 742 <sup>c</sup> ± 3     | 612 <sup>a</sup> ± 3     | 487 <sup>c</sup> ± 6     | 593 <sup>b</sup> ± 6     |
| <b>FBC</b> | 1051 <sup>b</sup> ± 95   | 1778 <sup>a</sup> ± 87   | 911 <sup>b</sup> ± 94    | 167 <sup>b</sup> ± 4     | 373 <sup>a</sup> ± 9     | 140 <sup>b</sup> ± 17    |
| <b>Erg</b> | 6.1 <sup>b</sup> ± 0.5   | 10.3 <sup>a</sup> ± 0.5  | 5.3 <sup>b</sup> ± 0.5   | 0.96 <sup>b</sup> ± 0.02 | 2.15 <sup>a</sup> ± 0.05 | 0.08 <sup>c</sup> ± 0.02 |
| <b>FDA</b> | 55.2 <sup>a</sup> ± 5.1  | 56.9 <sup>a</sup> ± 4.9  | 27.4 <sup>b</sup> ± 0.3  | 21.9 <sup>b</sup> ± 3.8  | 37.4 <sup>a</sup> ± 2.9  | 25.6 <sup>b</sup> ± 1.7  |
| <b>DHA</b> | 1.28 <sup>c</sup> ± 0.02 | 1.93 <sup>b</sup> ± 0.03 | 2.96 <sup>a</sup> ± 0.04 | 1.62 <sup>a</sup> ± 0.02 | 1.87 <sup>a</sup> ± 0.28 | 1.51 <sup>b</sup> ± 0.01 |
| <b>CAT</b> | 2.41 <sup>b</sup> ± 0.05 | 3.08 <sup>a</sup> ± 0.09 | 1.56 <sup>c</sup> ± 0.08 | 1.01 <sup>b</sup> ± 0.11 | 1.75 <sup>a</sup> ± 0.06 | 1.16 <sup>b</sup> ± 0.19 |
| <b>URE</b> | 87.5 <sup>a</sup> ± 1.1  | 76.2 <sup>b</sup> ± 2.2  | 68.1 <sup>c</sup> ± 2.7  | 39.8 <sup>a</sup> ± 1.7  | 36.5 <sup>a</sup> ± 1.8  | 28.3 <sup>b</sup> ± 1.1  |

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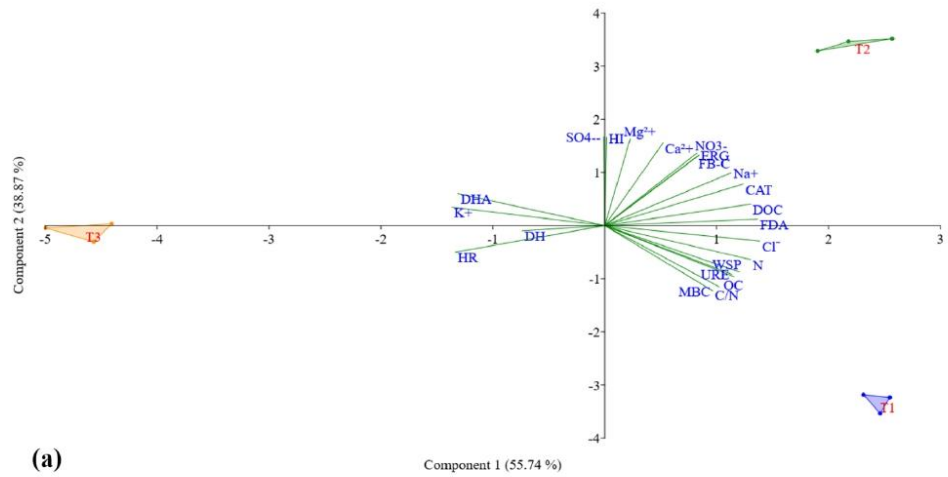
623 **Table 3** Micro-arthropod groups found in soils under different management: control (T1),  
 624 traditional thinning (T2), innovative thinning (T3). QBs index is obtained from the sum of the  
 625 highest values of EMI of all the collected groups.

| <b>Species</b>              | <b>T1</b> | <b>T2</b> | <b>T3</b> |
|-----------------------------|-----------|-----------|-----------|
| <i>Acari</i>                | *         | *         | *         |
| <i>Collembola</i>           | *         | *         | *         |
| <i>Orthoptera</i>           | *         | *         | *         |
| <i>Hemiptera</i>            | *         | *         | *         |
| <i>Diptera (larvae)</i>     | *         | *         | *         |
| <i>Coleoptera</i>           | *         | *         | *         |
| <i>Chilopoda</i>            |           | *         | *         |
| <i>Blattaria</i>            | *         |           |           |
| <i>Symphyla</i>             | *         | *         | *         |
| <i>Thysanoptera</i>         |           | *         | *         |
| <i>Protura</i>              |           | *         |           |
| <i>Diplura</i>              |           |           | *         |
| <i>Lepidoptera (larvae)</i> |           | *         | *         |
| <i>Araneae</i>              | *         | *         | *         |
| <i>Formicidae</i>           |           | *         |           |
| <i>Isopoda</i>              |           | *         | *         |

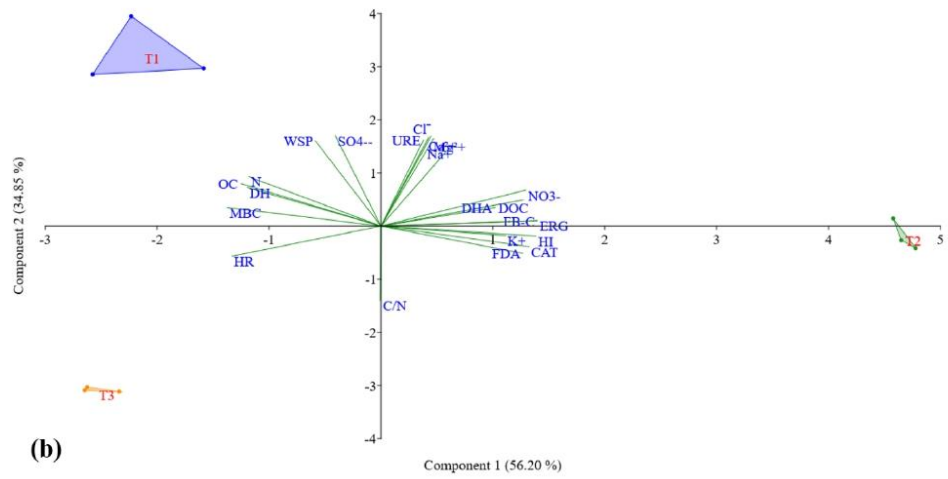
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(a)



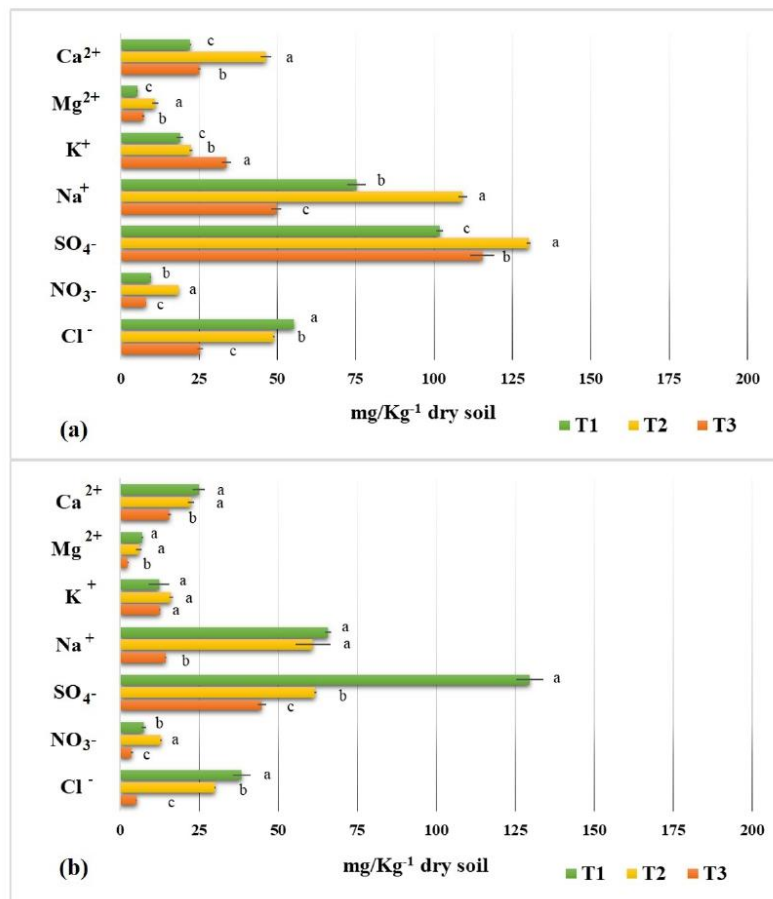
(b)

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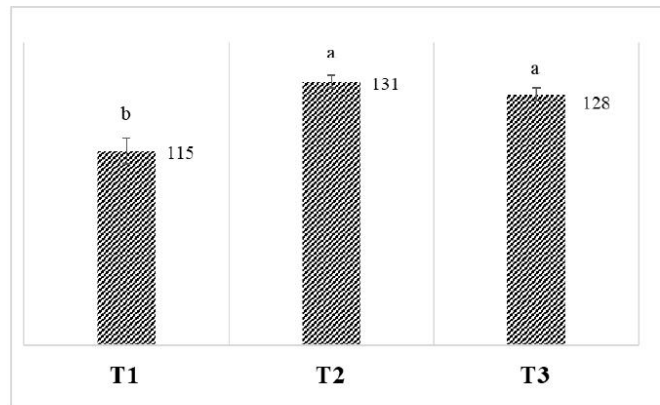
631 **Fig. 1**

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 635 **Fig. 2**  
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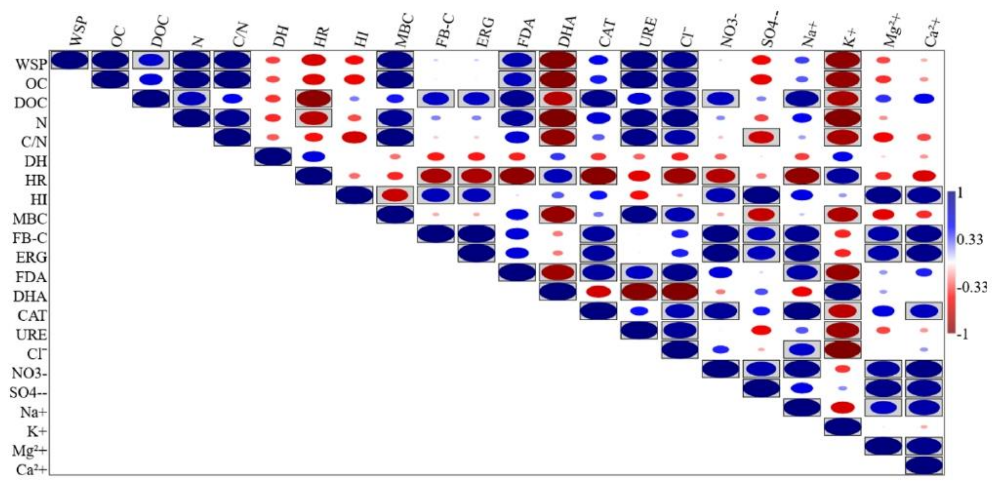
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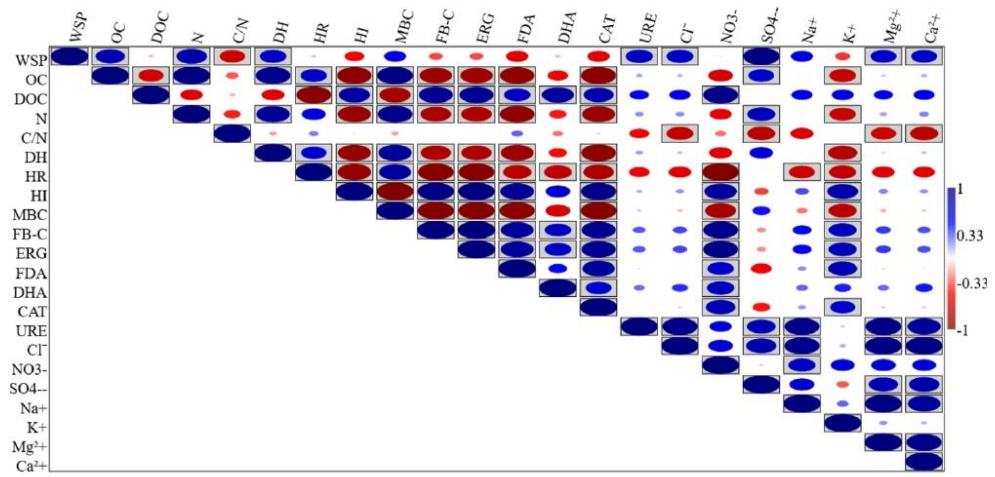
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640 **Fig. 3**



(a)



(b)

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643 **Fig. 4**



## APPENDIX 3

### Scientific Publication (under review)

Settineri G, Romeo F, Mallamaci C, Muscolo A (2019)

**Effects of different thinning intensity on soil biodiversity in a  
*Pinus laricio* forest of Calabria Apennine South Italy**

*Forestry: An International Journal of Forest Research*

*Submission number: Forestry-2019-229*



**Effects of different thinning intensity on soil biodiversity in a *Pinus laricio* forest of Calabria Apennine South Italy**

|                               |   |
|-------------------------------|---|
| Journal:                      | <i>Forestry: An International Journal of Forest Research</i>  |
| Manuscript ID:                | Forestry-2019-229   |
| Manuscript Type:              | Original Article  |
| Date Submitted by the Author: | 30-Oct-2019   |
| Complete List of Authors:     | Settineri, Giovanna; Universita degli Studi Mediterranea di Reggio Calabria Dipartimento di Agraria<br>Romeo, Federico; Universita degli Studi Mediterranea di Reggio Calabria Dipartimento di Agraria,<br>Mallamaci, Carmelo; Universita degli Studi Mediterranea di Reggio Calabria Dipartimento di Agraria<br>Muscolo, Adele; Mediterranea University, GESAF |
| Keywords:                     | Arthropod, biodiversity index, bulk density, thinning intensities   |
|                               |   |

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2 **Effects of different thinning intensity on soil biodiversity in a *Pinus laricio* forest of Calabria Apennine**  
3  
4 **South Italy**

5 **Giovanna Settineri <sup>a</sup>, Federico Romeo <sup>a</sup>, Carmelo Mallamaci <sup>a</sup>, Muscolo Adele<sup>a\*</sup>**

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8  
9 **Abstract**

10 Soil properties, along with soil biodiversity, are greatly affected by forest management practices. Thinning is  
11 the most effective silvicultural treatment used in Europe to increase the ecological value of coniferous  
12 stands. Uncorrected forest practices are among the main causes of soil degradation and biodiversity loss.  
13 Considering the importance of *Pinus laricio* forests in Calabria and their economic and natural scenery role,  
14 it is important to appropriately manage this forest to avoid the triggering of degradation phenomena with  
15 heavy consequences on the environment and local economy. In this study moderate thinning (MT), intense  
16 thinning (HT) and clearcutting (CC) were used to manage *Pinus laricio* stands. The effects of the different  
17 thinning intensities were evaluated, in two contrasting seasons (summer and winter), on the abundance,  
18 species richness, diversity of arthropods, fungi and bacteria colonies as well as on selected soil properties  
19 (organic matter, humication index, bulk density, pH), related to soil habitability. The aim of this study was to  
20 identify for each degree of thinning, the intensity less invasive and eco-sustainable. The outcomes of this  
21 study will help to formulate forestry policy recommendations to benefit biodiversity.  
22 Results evidenced that the abundance, species richness and diversity of arthropods, as well as fungi and  
23 bacteria colonies and soil properties changed with the treatments and seasons. Under HT we found the  
24 greatest biodiversity and the highest amount of arthropods, fungi and bacteria in both seasons as well as the  
25 greatest organic carbon content, humication index and the lowest bulk density value as consequence of the

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3 26 greatest occurrence of herbaceous species that was affected by decrease in canopy closure, in particular by  
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5 27 low tree density. The greatest richness of understory in HT positively affected soil biodiversity. This study  
6  
7 28 gives insights on ecological relationships between understory composition related to tree species abundance  
8  
9 29 and soil community.

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13 31 *Keywords:* Arthropod, biodiversity index, bulk density, thinning intensities  
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## 1. Introduction

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24 37 Among the silvicultural techniques used to preserve forests, thinning is a practice defined as selective  
25  
26 38 removing of a small number of trees (Bianchi *et al.*, 2010; Cantiani and Chiavetta, 2015), generally required  
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28 39 to maintain healthy and vigorous forests, to drive the abundance and composition of undergrowth vegetation  
29  
30 40 and to increase the economic value of forests. Sometime, when improperly used, silvicultural treatments can  
31  
32 41 cause disturbance to the forest equilibrium, affecting species succession, understory vegetation distribution  
33  
34 42 and soil ecosystem functioning, with negative impact on tree growth (Santa-Regina and Tarazona, 2001;  
35  
36 43 Thom *et al.*, 2017), forest community structure, species composition, habitat conditions and soil fertility  
37  
38 44 (Zhang *et al.*, 2001). Thinning, creating gaps, changes the entity of light penetration, air movement and  
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40 45 temperature which in turn affect (Ma *et al.*, 2010; Wubet *et al.*, 2012; Muscolo *et al.*, 2015; Muscolo *et al.*,  
41  
42 46 2017) the amounts of litter and organic matter, soil nutrient cycles, soil microorganisms (Hu and Zhu, 1999)  
43  
44 47 and arthropod communities (Lassau *et al.*, 2005), factors, that all together regulate soil fertility. Soil  
45  
46 48 microorganisms, have an essential role in soil organic matter decomposition (Burton *et al.*, 2010) and in  
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48 49 biogeochemical cycling of nutrients and for their high sensitivity to external perturbation are considered  
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50 50 early warning indicators of changes in soil properties (Muscolo *et al.*, 2015). Arthropods with their 1.2  
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52 51 million species are litter transformers and pulverizes (Schowalter, 2000), contributing to improve soil  
53  
54 52 physical and chemical properties (Schowalter and Ganio 1998; Parisi *et al.*, 2005; Chakravarthy *et al.*, 2016).  
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56 53 Because of their high abundance, specie richness, habitat fidelity (Andersen, 2004), and high sensitivity to  
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3 54 external perturbations, arthropods are considered important bio-indicators of environmental quality and can  
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5 55 be used for monitoring short-term changes in soil ecosystem. For the above considerations, changes in forest  
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7 56 vegetation related to thinning are expected to affect soil quality through changes in microbial and arthropod  
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9 57 communities. Previous works evidenced that soil microorganisms were affected by gap creation in forest  
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11 58 (Muscolo *et al.*, 2007; Muscolo *et al.*, 2014; Chen *et al.*, 2015; Lewandowski, 2015), highlighting that small  
12  
13 59 gaps (185 meter square) created the best environmental conditions for microorganism development. Kwon *et*  
14  
15 60 *al.* (2010) showed that there was no differences in the total arthropod amount between thinned and un-  
16  
17 61 thinned areas because the differences were annulled by the increase or decrease in taxa abundance. Richards  
18  
19 62 and Windsorf (2007) showed that arthropod biodiversity was positively related to humidity, while arthropod  
20  
21 63 abundance was negatively correlated with light intensity. At present, researches on relationships between  
22  
23 64 arthropod abundance and biodiversity, plant distribution and soil abiotic factors are still scarce, even if these  
24  
25 65 information are essential for understanding soil processes linked to site productivity. *Pinus laricio* is an  
26  
27 66 endemic species in Calabria, South Italy with great value for the local economy and deserve an appropriate  
28  
29 67 management to avoid triggering of forest degradation with soil fertility loss in area already subjected to  
30  
31 68 climatic changes. For the above consideration, forty years old *Pinus laricio* stand have been managed with  
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33 69 thinning of different intensities (Moderate Thinning, MT and Intense Thinning, HT) and Clear Cut, CC to  
34  
35 70 identify the better silvicultural practice to preserve the multi-functionality of this forest respecting at the  
36  
37 71 same time the quality of soil. Our starting hypothesis was that different thinning intensities would differently  
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39 72 affected soil biodiversity and fertility. Our specific aims were to (1) assess changes caused by different  
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41 73 thinning intensities on soil properties, microorganism and arthropod community composition and diversity;  
42  
43 74 (2) explore the relationships among soil properties, microorganisms and arthropod communities in  
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45 75 differently thinned plantations.  
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## 51 77 **2. Material and methods**

### 52 78 53 79 *2.1 Study area*

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3 81 The study was conducted in Aspromonte Mountain (Zervò, Calabria) (38°14'37" N; 16°01'11" E), 1100 m  
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5 82 above sea level in a *Pinus laricio* forest of 60 years-old. The study area was approximately of 45 ha. Four  
6  
7 83 study plots: no thinning stand (15 ha) named control (CTR: with 1935 tree ha<sup>-1</sup>); moderate thinning (10 ha)  
8  
9 84 (MT: 25% basal area (BA) removed, 1354 tree ha<sup>-1</sup>); intense thinning (10 ha) (HT: 45% BA removed, 780  
10  
11 85 tree ha<sup>-1</sup>); and clear cut (10 ha) (CC: 100% thinning, 0 tree ha<sup>-1</sup>) were established, in the study area, in a  
12  
13 86 randomized design with five replicates for each of the four treatments (Figure 1). Each block in the CTR was  
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15 87 3 ha, while in the MT, HT and CC was 2 ha. Thinnings were designed to reduce stand density, removing all  
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17 88 of the trees present in the stand. The study areas is characterized by homogeneous features regarding the  
18  
19 89 slope, exposure, elevation and climate conditions. The area's climate is typically Mediterranean, with mean  
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21 90 annual precipitation is 1838 mm, with the total precipitation occurring from October to June. Mean annual  
22  
23 91 temperature was about 10 °C, and the lowest and highest monthly mean temperatures are 3°C in January and  
24  
25 92 17°C in July, respectively (climate data from Santa Cristina d'Aspromonte Meteorological Station). The  
26  
27 93 areas appertain to the Castanetum zone according to Pavari's phytoclimatic classification (Pavari, 1959). The  
28  
29 94 soil, with a xeric soil regime moisture, generated from schist and biotitic gneisses, and were classified as  
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31 95 Humic Cambisols, according to the IUSS WRB (2014).  
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### 37 97 *2.2 Measurement of microclimatic variables*

38  
39 98 The microclimate in the gaps was assessed by measuring air temperature, soil temperature and moisture, and  
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41 99 photosynthetically active radiation (PAR, measured at 400–700 nm). PAR was detected on clear days, at  
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43 100 12.00 h in each plot for all treatments. PAR was measured by using a Ceptometer (AccuPAR, Degagon  
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45 101 Devices Inc., Pullman, WA, USA), at 1.00 m above the ground, with the instrument held horizontally  
46  
47 102 (Gendron *et al.*, 1998). Corresponding PAR values were used to calculate PAR transmittance using the  
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49 103 following formula: PAR transmittance = (PAR subplot/PAR full open) x 100. Soil temperature was  
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51 104 measured using a soil thermometer (Elite). In addition, litter thickness was measured using a millimeter  
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53 105 scale.  
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### 56 106 57 58 107 *2.3 Experimental design*

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3 109 Surface soil samples at 0-10 cm depth were collected in each stands (HT, MT and CC) and control stand  
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5 110 (CTR), for two consecutive years and, in two different seasons (Spring, June 2014-2015 and Autumn,  
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7 111 November, 2014-2015). Soil samples were randomly taken using a soil borer from 3 points within 20x20m  
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9 112 quadrat (5) of each block, after removing the litter layers. A total of 120 samples were collected. The  
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11 113 samples were brought to the laboratory on the same day and soil water content (WC), soil fauna, fungi and  
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13 114 bacteria were detected in fresh soil within 24 h of sample collection. A part of the soil samples were on air-  
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15 115 dried, sieved and passed through a 2 mm diameter mesh, and visible roots were removed.  
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#### 20 117 *2.4 Soil chemical and physical analysis*

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24 119 Water content (WC) in soil was determined by oven-drying method, based on removing soil moisture by  
25  
26 120 oven-drying at 105 °C until the weight remains constant. The moisture content (%) was calculated from the  
27  
28 121 sample weight before and after drying. pH determination was based on soil:water suspensions at a ratio of  
29  
30 122 1:2.5 (w/v) using 10 gr of soil and 25 mL water. Immediately after the addition of the water the suspensions  
31  
32 123 were thoroughly mixed on an orbital shaker for 2 h, pH readings were taken after sedimentation. Organic  
33  
34 124 carbon (OC) was determined by dichromate oxidation method, according to Springer and Klee (1954).  
35  
36 125 Organic carbon was quantified by titration with iron-sulfate (FeSO<sub>4</sub>, 0.2 N).  
37  
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39 126 The determination of humic acid, fulvic acid (HC, FC) and humification rate (HR) was performed,  
40  
41 127 respectively, according to Ciavatta *et al.* (1990) and Ciavatta and Govi (1993). Bulk density (BD) was  
42  
43 128 measured by taking samples of soil using corer with a 250 cm<sup>3</sup> volume. The samples were weighted and  
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45 129 dried (105°C) until they reached a constant mass, the total dry mass was divided by the sampled volume to  
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47 130 obtain the BD value.  
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#### 52 132 *2.5 Soil microbial analysis*

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56 134 Bacteria, fungi and actinomycetes were extracted by adding 95 mL of 0.1% (w/v) solution of sodium  
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58 135 pyrophosphate to 10 grams of each soil sample. 95 mL of 0.1% (w/v) solution of sodium pyrophosphate.  
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60 136 This solution was decimally diluted (10<sup>-1</sup> to 10<sup>-7</sup>) and aliquots were plated on specific agarized culture media

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3 137 (Elliot and Jardin, 1999). Bacteria and fungi colony forming units (CFU) were counted, according to Picci  
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5 138 and Nannipieri (2003) and Eaton *et al.* (2005).  
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## 9 140 *2.6 Soil fauna determination*

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14 142 Berlese (1905) procedure was performed for Arthropod extraction: soil samples were placed on the sieve  
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16 143 mesh at the top of each funnel selector for 7 days and collected in a beaker with preservative liquid with 70%  
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18 144 ethanol and glycerol 2:1 v/v) under the funnel. Since the arthropods are macroscopic organisms, not easily  
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20 145 visible, extracted specimens, were observed with a stereomicroscope, to identify, classify and count them.  
21  
22 146 Larvae and imago belonging to the same taxonomic group were grouped together. Were used identification  
23  
24 147 keys, to classify arthropods (Angelini *et al.*, 2002; Ruiz and Lavelle, 2008; Duyar, 2014).  
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## 28 149 *2.7 Data analysis*

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33 151 All sets of experiments were repeated five times. All datasets were tested for normality using Shapiro–Wilk  
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35 152 and Jarque–Bera tests. Soil micro-arthropod biodiversity was calculated by using Shannon and Pielou’s  
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37 153 evenness indices (Shannon and Weaver, 1949; Heip *et al.*, 1998) and species richness was calculated by  
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39 154 counting the number of species present in each soil sample as reported in Whittaker (Whittaker, 1972). All  
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41 155 statistical analyses were performed using Systat v. 8.0 software package (SPSS Inc., Evanston, IL, USA).  
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43 156 Tukey’s test (Sokal and Rohlf, 1981) was used to compare treatment means and to determine which means  
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45 157 differed significantly at  $p \leq 0.05$ . Analysis of variance (one-way ANOVA) was utilized to test the differences  
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47 158 among the treatments, while two-way ANOVA to detect the relationships between treatments and seasons.  
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49 159 Significant differences and effects were determined as  $p \leq 0.05$ .  
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## 53 161 **3. Results and discussion**

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### 57 163 *3.1 Microclimate variables*

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2  
3 165 Microclimate parameters were significantly different between the silvicultural treatments (Table 1). PAR,  
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5 166 was representative of light levels at solar noon and it was highest in summer than winter. PAR transmittance  
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7 167 significantly increased with increasing tree cutting. Air and soil temperatures were greater in summer than  
8  
9 168 winter. Soil temperature was highest in CC in summer followed by HT, MT and CTR. In winter the highest  
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11 169 soil temperature was detected in HT. Litter thickness was always greater in HT in both seasons than the  
12  
13 170 others treatments (Table 1), suggesting that microclimate changed in respect to the canopy closure and  
14  
15 171 influenced the understory vegetation as amount and typology (Table 1). In fact in HT we found the greatest  
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17 172 understory biodiversity expressed by *Brachypodium sylvaticum* (Huds.) *Erica arborea* L., *Dactylis*  
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19 173 *glomerata* L. subsp. *Glomerata*, *Oxalis acetosella* L., *Pteridium aquilinum* L., *Kuhn* subsp. *Aquilinum*, and  
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21 174 *Rubus hirtus*. In CC and MT we found *Erica arborea* L., *Genista sagittalis* L., *Rubus hirtus* and *Fragaria*  
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23 175 *vesca* L. subsp. *Vesca*. Under CTR no understory vegetation was found.  
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### 31 178 3.2 Soil chemical and physical features

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35 180 Soil texture was sandy-loam with 10% silt, 8 % clay and 82% sand (data not shown). Water content was  
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37 181 significantly higher in winter than summer in CC, CTR, MT and HT. CC was the treatment with the highest  
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39 182 WC value in winter and the lowest in summer (Table 2). This was due to the total cut of trees that caused an  
40  
41 183 excessive infiltration of rainwater in winter and an excessive water evaporation in summer time. Conversely,  
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43 184 canopy shade reduced the amount of total soil water evaporation as already reported by Stormont *et al.*  
44  
45 185 (2009). pH was moderately acidic and didn't differ significantly among the treatments and seasons (Table 2)  
46  
47 186 confirming the typical acidic nature of the soil under conifers (Balduccio *et al.*, 1992). OC content was the  
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49 187 highest in summer in all the treatments. The greatest amount of OC was detected in the HT with values of  
50  
51 188 12.81% in summer and 9.40% in winter, respectively (Table 2), evidencing also as seasonality influence the  
52  
53 189 OC accumulation (Table 2). Regarding humic and fulvic acids (HC, FC) the highest values were registered  
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55 190 in summer for all the treatments. In the managed soils and in CTR the HC was significantly higher than FC  
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57 191 indicating that in all the area prevailed the humification process in respect to the mineralization process. HT  
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59 192 showed the greatest HC values in both seasons. No significant differences were observed between the two

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3 193 seasons for the humification rate (Table 2); the greatest values were detected in HT in both seasons. The  
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5 194 components of soil organic matter are sensitive to climatic changes and to site-specific factors such as stand  
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7 195 productivity, vegetation management and land use history. Our results evidenced as treatments more than  
8  
9 196 seasons and their interaction influenced the humification process (Table 2). In HT we found the greatest  
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11 197 carbon storage and the best humification process in both seasons as shown by the highest values of  
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13 198 humification rate and HC (Schindlbacher *et al.*, 2010; Muscolo *et al.*, 2010; Settineri *et al.*, 2018). The  
14  
15 199 carbon storage increase in HT were due to the low tree density which determining a different regime of light,  
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17 200 temperature and humidity at soil level, increased the growth and biodiversity of understory herbaceous  
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19 201 vegetation producing more easily degradable litter with a consequent increase in OC. Bulk density was  
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21 202 significantly different among the treatments, both in summer and in winter, with the lowest values (< 1 g cm<sup>-3</sup>)  
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23 203 in HT (Figure 2). The values in MT, CC and CTR were always > 1 g cm<sup>-3</sup>. Bulk density was more  
24  
25 204 influenced by treatments than season and their interaction (Table 5). It is well known that bulk density is  
26  
27 205 directly correlated to soil compaction and consolidation and it is highly and inversely correlated to the soil  
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29 206 organic carbon content (Chaudhari *et al.*, 2013). Our data in agreement with findings of other authors  
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31 207 (Chaudhari *et al.*, 2013; Ahad, 2015 *et al.*, 2015) evidenced the strongest negative correlation between bulk  
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33 208 density and organic matter in HT, with the lowest BD, and the highest OC in both seasons. Additionally, we  
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35 209 found also a significant negative correlation between species richness, fungi and bacteria colonies and soil  
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37 210 bulk density. In both seasons, populations of soil aerobic bacteria, fungi and arthropods decreased when bulk  
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39 211 density increased.  
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### 44 213 *3.3 Soil microbiological features*

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46  
47 215 Soil bulk density and organic matter in turn influenced the amount of fungi and bacteria. Fungi and bacteria  
48  
49 216 colonies, in each stand, were mainly present in summer (Figure 3) the season with the highest light and  
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51 217 temperature, at ground level. The highest number of colonies was detected in HT, where the lowest BD and  
52  
53 218 the highest OC, and HC values were detected. The less microbial amount in the other treatments were due to  
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55 219 the negative impact of soil compaction on soil water/air ratio that consequently affected the aerobic soil  
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57 220 bacteria and fungi as already reported by Li *et al.* (2001).  
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3 221 Although biotic factors in terms of resource quality and availability have been always considered as the main  
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5 222 drivers of fungal and bacterial fluctuation in soil (Pollierer and Scheu, 2017), we put in light as seasonality  
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7 223 (Figure 2) and abiotic factors, that differed in the study area under different management intensity, were  
8  
9 224 important in steering the variations in soil communities. The greater intensity of light, temperature and  
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11 225 humidity, parameters closely related to each other's and observed in HT compared to the other treatments,  
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13 226 were able to increase the understory vegetation diversity which in turn promoted the proliferation of soil  
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15 227 fungi and bacteria colonies as already reported by Swenson *et al.* (2012).  
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20 229 *3.4 Soil biodiversity*

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24 231 Significant differences were observed in arthropod community and number among the treatments in both  
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26 232 seasons. The highest number of individuals, species richness and biodiversity both in winter and in summer  
27  
28 233 were recorded in HT (Tables 3, 4), while the lowest ones in CC (Figure 4, 5, and 6). In all treatments the  
29  
30 234 individuals, as well the richness of species were more abundant in winter than in summer. These differences  
31  
32 235 were due to the variations in temperature and rainfall between summer and winter in a strongly  
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34 236 Mediterranean-climate which caused asynchrony between resource availability and plant growth (Legakis,  
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36 237 1994; Lionello *et al.*, 2006). The lower biodiversity indices found in summer in the sites differently  
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38 238 managed, and in the unmanaged forest were due to the limited amount of rain and to the consequent scarce  
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40 239 litter humidity that strongly influenced the invertebrate diversity as already reported by other authors  
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42 240 (Mueller *et al.*, 2016; Santonja *et al.*, 2017). Shannon diversity and evenness showed significant differences  
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44 241 among treatments but did not show any difference between seasons. This trend in species richness, can be  
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46 242 simply due to an increase in resources correlated with the increasing thinning intensity. The greater amount  
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48 243 of undergrowth vegetation and the consequent mixed litter at different decomposition stages present in HT,  
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50 244 strongly influenced the amount and the diversity of arthropod community as already reported by Santonja *et*  
51  
52 245 *al.* (2017) and Jiménez-Chacón (2018). These results evidenced as thinning intensities affecting pedoclimatic  
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54 246 conditions, drove understory richness and in turn modified the relationships between arthropod richness,  
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56 247 evenness, and proportional diversity with consequent effects on soil microorganism amount. Seasonal  
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58 248 changes didn't affect the thinning trend, specifically, in summer season we found in HT a greater amount of



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3 249 micro-arthropods (collembola, acarina, dipteri, hemynoptera and pseudoscorpionida) in respect to all the  
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5 250 other treatments (Table 3). All these micro-arthropods as already reported by Rusek (1998) and Tsurho and  
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7 251 Ao (2014), play an important role in maintaining the sustainability of an ecosystem through the  
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9 252 decomposition and mineralization of leaf litter. Additionally, all these micro-arthropods are considered  
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11 253 important bio-indicators of soil ecosystem functioning because they include numerous groups that respond  
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13 254 quickly to external disturbance varying their distribution and amount in space and time. In winter, in HT we  
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15 255 found the greatest abundance of the above mentioned micro-arthropods (Table 4), and surprisingly we  
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17 256 observed also the appearance of other groups that were completely absent in the other treatments (Chilopoda,  
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19 257 Psocoptera, Symphyla and Thysanoptera) and in HT in summer season (Tables 3, 4). These findings  
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21 258 highlighted an increase in species richness in soil under high thinning intensity, suggesting that HT created  
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23 259 independently by seasons the better habitat allowing the developing of trophic interactions that played a key  
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25 260 role in ecological soil processes as already demonstrated by Wardle (2006) and Dyer *et al.* (2010). Analysis  
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27 261 of variance of the effects of treatments, seasons and their interactions on arthropod taxa confirmed the above  
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29 262 statement, evidencing that the majority of taxa were mainly influenced by treatments than seasons and their  
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31 263 interaction (Table 5) except for acarina, diptera, hymenoptera and protura which were mainly influenced by  
32  
33 264 seasonality (Table 5). The highest amount of micro-arthropods in HT in both seasons with the consequent  
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35 265 greater ingestion and excretion of dead plant material could have increased the interfacial contact facilitating  
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37 266 the colonization of plant residues by fungi and bacteria justifying their increase in number. In short our  
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39 267 results evidenced a strict correlation among soil community, seasonality, stand density, understory  
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41 268 abundance and litter diversity.  
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#### 46 270 **4. Conclusions**

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48 272 Despite the complex nature of soil community interactions, we found that the soil community responses to  
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50 273 forest management in coniferous forest depended on the provided higher resources (light, below-ground  
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52 274 resources) and better habitat conditions for shade-intolerant species that thinning treatments established  
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54 275 along with resident vegetation. The highest thinning intensity (which removed a greater amount of basal  
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56 276 area) favoured the highest understory species richness with positive effects on soil organic matter,  
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3 277 humification process as well as on soil community in terms of arthropod amount and biodiversity and soil  
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5 278 microorganisms. The relationship found between thinning intensity and soil quality allowed to individuate  
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7 279 the high intensity thinning as a sustainable forest management practice not only from a forestry point of view  
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9 280 but mainly for its eco-compatibility with soil ecosystem.

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21  
22 286 manuscript. GS and FR analyzed the data, performed statistical analyses and critically reviewed and edited  
23  
24 287 the manuscript. SG and FR performed the laboratory experiments, collected the data, CM conducted  
25  
26 288 fieldwork

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29 289 **Conflict of interest statement**

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32 290 None declared.

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432 **Figure captions**

433 **Figure 1** Map of the study site including the experimental design.

434 **Figure 2** Bulk density (BD, g cm<sup>-3</sup>) in soil under *Pinus laricio* plantation differently managed: intensive  
435 thinning (HT), moderate thinning (MT), clear cut, (CC) and control (CTR), two way ANOVA shows the  
436 effects of treatments, seasons and their interactions. Lowercase letter and capital letter, show significant  
437 differences among the management intensities (Tukey's test,  $p$ -level  $\leq 0.05$ ), in summer and winter  
438 respectively.

439 **Figure 3** Fungi (CFU 10<sup>4</sup> g<sup>-1</sup> dry soil) and bacteria (CFU 10<sup>5</sup> g<sup>-1</sup> dry soil) in soil under *Pinus laricio*  
440 plantation differently managed: intensive thinning (HT), moderate thinning (MT), clear cut, (CC) and control  
441 (CTR), two way ANOVA shows the effects of treatments, seasons and their interactions. Lowercase letter  
442 and capital letter, show significant differences among the management intensities (Tukey's test,  $p$ -level  $\leq$   
443 0.05), in summer and winter respectively.

444 **Figure 4** Species richness in soil under *Pinus laricio* plantation differently managed: intensive thinning  
445 (HT), moderate thinning (MT), clear cut, (CC) and control (CTR), two way ANOVA shows the effects of  
446 treatments, seasons and their interactions. Lowercase letter and capital letter, show significant differences  
447 among the management intensities (Tukey's test,  $p$ -level  $\leq 0.05$ ), in summer and winter respectively.

448 **Figure 5** Shannon-Wiener index of diversity in soil under *Pinus laricio* plantation differently managed:  
449 intensive thinning (HT), moderate thinning (MT), clear cut, (CC) and control (CTR), two way ANOVA  
450 shows the effects of treatments, seasons and their interactions. Lowercase letter and capital letter, show  
451 significant differences among the management intensities (Tukey's test,  $p$ -level  $\leq 0.05$ ), in summer and  
452 winter respectively.

453 **Figure 6** Species Evenness in soil under *Pinus laricio* plantation differently managed: intensive thinning  
454 (HT), moderate thinning (MT), clear cut, (CC) and control (CTR), two way ANOVA shows the effects of  
455 treatments, seasons and their interactions. Lowercase letter and capital letter, show significant differences  
456 among the management intensities (Tukey's test,  $p$ -level  $\leq 0.05$ ), in summer and winter respectively.

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2 458 **Table 1.** Micro-environmental variables in high intensity thinning (HT), medium intensity thinning (MT), clear cut (CC) and control (CTR) in summer and  
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| Seasons       | Parameters                              | HT                                | MT                               | CC                                | CTR                              |
|---------------|---|-----------------------------------|----------------------------------|-----------------------------------|----------------------------------|
| <b>Summer</b> | PAR transmittance (%)                   | 12.60 <sup>a</sup> ( $\pm 2.72$ ) | 2.3 <sup>b</sup> ( $\pm 0.50$ )  | 16.45 <sup>a</sup> ( $\pm 3.43$ ) | 1.42 <sup>b</sup> ( $\pm 0.82$ ) |
|               | Air temperature ( $^{\circ}\text{C}$ )  | 12.48 <sup>b</sup> ( $\pm 0.74$ ) | 9.72 <sup>c</sup> ( $\pm 0.50$ ) | 16.74 <sup>a</sup> ( $\pm 0.61$ ) | 7.82 <sup>d</sup> ( $\pm 0.73$ ) |
|               | Soil temperature ( $^{\circ}\text{C}$ ) | 11.52 <sup>b</sup> ( $\pm 0.72$ ) | 7.20 <sup>c</sup> ( $\pm 0.94$ ) | 15.78 <sup>a</sup> ( $\pm 0.53$ ) | 5.89 <sup>d</sup> ( $\pm 0.75$ ) |
|               | Litter thickness (cm)                   | 3.5 <sup>a</sup> ( $\pm 0.11$ )   | 1.11 <sup>b</sup> ( $\pm 0.45$ ) | 0.2 <sup>c</sup> ( $\pm 0.05$ )   | 1.5 <sup>b</sup> ( $\pm 0.25$ )  |
| <b>Winter</b> | PAR transmittance (%)                   | 9.52 <sup>a</sup> ( $\pm 1.25$ )  | 1.21 <sup>b</sup> ( $\pm 0.92$ ) | 11.27 <sup>a</sup> ( $\pm 1.48$ ) | 0.81 <sup>b</sup> ( $\pm 0.32$ ) |
|               | Air temperature ( $^{\circ}\text{C}$ )  | 7.58 <sup>a</sup> ( $\pm 1.42$ )  | 5.12 <sup>b</sup> ( $\pm 0.53$ ) | 5.24 <sup>b</sup> ( $\pm 0.92$ )  | 4.64 <sup>b</sup> ( $\pm 0.71$ ) |
|               | Soil temperature ( $^{\circ}\text{C}$ ) | 6.32 <sup>a</sup> ( $\pm 1.01$ )  | 4.21 <sup>b</sup> ( $\pm 0.94$ ) | 3.54 <sup>b</sup> ( $\pm 0.63$ )  | 3.87 <sup>b</sup> ( $\pm 1.02$ ) |
|               | Litter thickness (cm)                   | 3.21 <sup>a</sup> ( $\pm 0.62$ )  | 1.05 <sup>b</sup> ( $\pm 0.26$ ) | 0.2 <sup>c</sup> ( $\pm 0.07$ )   | 1.27 <sup>b</sup> ( $\pm 0.98$ ) |

28 468 Different letters in the same row indicate, within each season, significant differences (Tukey's test,  $p \leq 0.05$ ).

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2 469 **Table 2.** Water content (WC), pH, Organic Carbon (OC), humic acid (HC), fulvic acid (FC) and humification rate (HR) in soil under *Pinus laricio* plantation  
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4 470 differently managed: intensive thinning (HT), moderate thinning (MT), clear cut, (CC) and control (CTR) in summer and winter season. Analysis of variance  
5 471 show the effects of treatments, seasons and their interactions.  
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| Seasons | Management  | WC (%)                | pH                      | OC (%)                   | HC (%)                  | FC (%)                  | HR(%)                    |
|---------|-------------|-----------------------|-------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| Summer  | MT          | 48 <sup>a</sup> ±1.80 | 4.96 <sup>a</sup> ±0.49 | 10.52 <sup>b</sup> ±0.43 | 3.87 <sup>b</sup> ±0.26 | 2.80 <sup>b</sup> ±0.16 | 63.40 <sup>b</sup> ±5.15 |
|         | HT          | 51 <sup>a</sup> ±1.40 | 5.39 <sup>a</sup> ±0.39 | 12.81 <sup>a</sup> ±0.61 | 5.56 <sup>a</sup> ±0.24 | 4.28 <sup>a</sup> ±0.32 | 76.81 <sup>a</sup> ±4.88 |
|         | CC          | 38 <sup>b</sup> ±1.25 | 5.10 <sup>a</sup> ±0.36 | 7.59 <sup>a</sup> ±0.58  | 3.11 <sup>c</sup> ±0.13 | 1.82 <sup>a</sup> ±0.28 | 64.95 <sup>b</sup> ±5.70 |
|         | CTR         | 48 <sup>a</sup> ±1.95 | 5.20 <sup>a</sup> ±0.50 | 9.71 <sup>b</sup> ±0.23  | 3.55 <sup>b</sup> ±0.12 | 2.60 <sup>b</sup> ±0.10 | 63.33 <sup>b</sup> ±5.36 |
| Winter  | MT          | 60 <sup>b</sup> ±1.80 | 5.59 <sup>a</sup> ±0.35 | 8.31 <sup>b</sup> ±0.26  | 3.17 <sup>b</sup> ±0.10 | 1.85 <sup>b</sup> ±0.42 | 60.40 <sup>b</sup> ±4.72 |
|         | HT          | 65 <sup>b</sup> ±1.20 | 5.64 <sup>a</sup> ±0.31 | 9.40 <sup>a</sup> ±0.43  | 3.98 <sup>a</sup> ±0.16 | 3.14 <sup>a</sup> ±0.12 | 75.74 <sup>a</sup> ±5.31 |
|         | CC          | 82 <sup>a</sup> ±1.00 | 5.37 <sup>a</sup> ±0.29 | 8.08 <sup>b</sup> ±0.22  | 2.91 <sup>b</sup> ±0.33 | 1.62 <sup>b</sup> ±0.13 | 56.06 <sup>b</sup> ±5.23 |
|         | CTR         | 61 <sup>b</sup> ±2.02 | 5.53 <sup>a</sup> ±0.30 | 7.80 <sup>b</sup> ±0.20  | 3.04 <sup>b</sup> ±0.17 | 1.53 <sup>b</sup> ±0.14 | 58.58 <sup>b</sup> ±5.11 |
| F-ratio | Treatments  | 11.478*               | 0.625                   | 70.611*                  | 86.976*                 | 82.725*                 | 12.843*                  |
|         | Seasons     | 609.151*              | 5.565*                  | 114.852*                 | 80.687*                 | 76.661*                 | 4.360                    |
|         | Interaction | 87.075*               | 0.328                   | 24.674*                  | 12.653*                 | 5.112*                  | 0.617                    |

28 472 Different letters in the same column indicate, within each season, significant differences (Tukey's test,  $p \leq 0.05$ ).

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475 **Table 3.** Total number of individuals (Ab) and percentage abundance (% Ab) of taxa captured in summer in  
476 in soil under *Pinus laricio* plantation differently managed: intensive thinning (HT), moderate thinning (MT),  
477 clear cut, (CC) and control (CTR).

| Taxa              | Summer                  |        |                         |        |                        |        |                         |        |
|-------------------|-------------------------|--------|-------------------------|--------|------------------------|--------|-------------------------|--------|
|                   | MT                      |        | HT                      |        | CC                     |        | CTR                     |        |
|                   | Ab                      | Ab (%) | Ab                      | Ab (%) | Ab                     | Ab (%) | Ab                      | Ab (%) |
| Acarina           | 10667 <sup>c</sup> ±558 | 52.52  | 15833 <sup>a</sup> ±721 | 44.49  | 9417 <sup>d</sup> ±65  | 71.56  | 13417 <sup>b</sup> ±172 | 62.46  |
| Araneidae         | 333 <sup>b</sup> ±18    | 1.64   | 833 <sup>a</sup> ±79    | 2.34   | 0                      | 0.00   | 250 <sup>b</sup> ±9     | 1.16   |
| Coleoptera        | 250 <sup>b</sup> ±32    | 1.23   | 833 <sup>a</sup> ±29    | 2.34   | 250 <sup>b</sup> ±11   | 1.90   | 50 <sup>c</sup> ±86     | 0.22   |
| Collembola        | 7833 <sup>b</sup> ±388  | 38.57  | 10583 <sup>a</sup> ±399 | 29.75  | 2500 <sup>d</sup> ±84  | 18.99  | 5167 <sup>c</sup> ±184  | 24.04  |
| Diplopoda         | 250 <sup>b</sup> ±16    | 1.23   | 500 <sup>a</sup> ±37    | 1.40   | 30 <sup>d</sup> ±51    | 0.22   | 116 <sup>cd</sup> ±87   | 0.53   |
| Diplura           | 116 <sup>b</sup> ±101   | 0.55   | 667 <sup>a</sup> ±90    | 1.87   | 0                      | 0.00   | 115 <sup>b</sup> ±99    | 0.52   |
| Diptera           | 171 <sup>d</sup> ±118   | 0.81   | 2083 <sup>a</sup> ±111  | 5.85   | 333 <sup>c</sup> ±98   | 2.53   | 1167 <sup>b</sup> ±105  | 5.43   |
| Hemiptera         | 51 <sup>c</sup> ±38     | 0.23   | 583 <sup>a</sup> ±121   | 1.63   | 167 <sup>bc</sup> ±119 | 1.25   | 117 <sup>c</sup> ±82    | 0.53   |
| Hymenoptera       | 250 <sup>d</sup> ±84    | 1.23   | 1000 <sup>a</sup> ±148  | 2.81   | 417 <sup>c</sup> ±87   | 3.17   | 667 <sup>b</sup> ±124   | 3.10   |
| Isopoda           | 79 <sup>a</sup> ±35     | 0.36   | 53 <sup>a</sup> ±19     | 0.15   | 0                      | 0.00   | 51 <sup>a</sup> ±20     | 0.23   |
| Protura           | 333 <sup>b</sup> ±102   | 1.64   | 750 <sup>a</sup> ±145   | 2.11   | 53 <sup>c</sup> ±26    | 0.39   | 52 <sup>c</sup> ±33     | 0.23   |
| Pseudoscorpionida | 0                       | 0.00   | 1833 <sup>a</sup> ±124  | 5.16   | 0                      | 0.00   | 333 <sup>b</sup> ±97    | 1.55   |
| Symphyla          | 0                       | 0.00   | 30±17                   | 0.09   | 0                      | 0.00   | 0                       | 0.00   |
| Total             | 20333                   | 100.00 | 35581                   | 100.00 | 13167                  | 100.00 | 21502                   | 100.00 |

478 Different letters in the same column indicate, within each season, significant differences (Tukey's test,  $p \leq$   
479 0.05).

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480 **Table 4.** Total number of individuals (Ab) and percentage abundance (% Ab) of taxa captured in winter in in  
481 soil under *Pinus laricio* plantation differently managed: intensive thinning (HT), moderate thinning (MT),

| Taxa              | Winter                  |        |                          |        |                         |        |                         |        |
|-------------------|-------------------------|--------|--------------------------|--------|-------------------------|--------|-------------------------|--------|
|                   | MT                      |        | HT                       |        | CC                      |        | CTR                     |        |
|                   | Ab                      | Ab (%) | Ab                       | Ab (%) | Ab                      | Ab (%) | Ab                      | Ab (%) |
| Acarina           | 25417 <sup>a</sup> ±259 | 59.76  | 19250 <sup>b</sup> ±272  | 36.62  | 24333 <sup>a</sup> ±294 | 70.79  | 25083 <sup>a</sup> ±248 | 63.98  |
| Araneidae         | 667 <sup>b</sup> ±142   | 1.57   | 2083 <sup>a</sup> ±214   | 3.96   | 208 <sup>c</sup> ±124   | 0.60   | 83 <sup>c</sup> ±43     | 0.21   |
| Chilopoda         | 0                       | 0.00   | 82±32                    | 0.15   | 0                       | 0.00   | 0                       | 0.00   |
| Coleoptera        | 1167 <sup>b</sup> ±174  | 2.59   | 2000 <sup>a</sup> ±207   | 3.80   | 28 <sup>d</sup> ±12     | 0.08   | 750 <sup>c</sup> ±142   | 1.91   |
| Collembola        | 9333 <sup>b</sup> ±1142 | 22.07  | 16167 <sup>a</sup> ±1537 | 30.75  | 4667 <sup>d</sup> ±942  | 13.58  | 6250 <sup>c</sup> ±1021 | 15.94  |
| Diplopoda         | 833 <sup>b</sup> ±314   | 1.97   | 168 <sup>c</sup> ±48     | 0.32   | 1333 <sup>a</sup> ±523  | 3.88   | 167 <sup>c</sup> ±51    | 0.43   |
| Diplura           | 1417 <sup>a</sup> ±231  | 3.35   | 1500 <sup>a</sup> ±227   | 2.85   | 28 <sup>c</sup> ±10     | 0.08   | 167 <sup>b</sup> ±46    | 0.43   |
| Diptera           | 2417 <sup>b</sup> ±745  | 5.54   | 4583 <sup>a</sup> ±974   | 8.72   | 1833 <sup>c</sup> ±428  | 5.33   | 2417 <sup>b</sup> ±681  | 6.16   |
| Hemiptera         | 28 <sup>b</sup> ±7      | 0.07   | 417 <sup>a</sup> ±74     | 0.79   | 0                       | 0.00   | 0                       | 0.00   |
| Hymenoptera       | 1083 <sup>c</sup> ±179  | 2.54   | 2167 <sup>a</sup> ±211   | 4.12   | 674 <sup>d</sup> ±107   | 1.97   | 1417 <sup>b</sup> ±183  | 3.61   |
| Isopoda           | 167 <sup>b</sup> ±62    | 0.39   | 417 <sup>a</sup> ±183    | 0.79   | 119 <sup>c</sup> ±54    | 0.35   | 124 <sup>c</sup> ±53    | 0.32   |
| Protura           | 28 <sup>d</sup> ±12     | 0.07   | 1667 <sup>b</sup> ±61    | 3.17   | 1159 <sup>c</sup> ±324  | 3.35   | 1917 <sup>a</sup> ±421  | 4.89   |
| Pseudoscorpionida | 30 <sup>c</sup> ±17     | 0.08   | 1583 <sup>a</sup> ±374   | 3.01   | 0                       | 0.00   | 833 <sup>b</sup> ±211   | 2.12   |
| Psocoptera        | 0                       | 0.00   | 115±55                   | 0.22   | 0                       | 0.00   | 0                       | 0.00   |
| Symphyla          | 0                       | 0.00   | 152±42                   | 0.09   | 0                       | 0.00   | 0                       | 0.00   |
| Thysanoptera      | 0                       | 0.00   | 333±89                   | 0.63   | 0                       | 0.00   | 0                       | 0.00   |
| Total             | 42586                   | 100.00 | 52583                    | 100.00 | 34381                   | 100.00 | 39208                   | 100.00 |

482 clear cut, (CC) and control (CTR).

483 Different letters in the same column indicate, within each season, significant differences (Tukey's test,  $p \leq$   
484 0.05).



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485 **Table 5.** Analysis of variance of the effects of treatments, seasons and their interactions on arthropod taxa.

| Taxa              | <i>F</i> -ratio |          |             |
|-------------------|-----------------|----------|-------------|
|                   | Treatments      | Seasons  | Interaction |
| Acarina           | 2.967           | 871.066* | 57.942*     |
| Araneidae         | 418.725*        | 193.862* | 83.547*     |
| Chilopoda         | 0.921           | 0.882    | 0.921       |
| Coleoptera        | 1.613           | 1.677    | 0.988       |
| Collembola        | 3.345           | 0.029    | 1.696       |
| Diplopoda         | 3.638*          | 4.735*   | 3.139       |
| Diplura           | 8.040*          | 6.664*   | 6.868*      |
| Diptera           | 2.167           | 17.107*  | 2.057       |
| Hemiptera         | 6.525*          | 2.472    | 3.589*      |
| Hymenoptera       | 1.203           | 5.387*   | 2.599       |
| Isopoda           | 5.792*          | 8.116*   | 5.611*      |
| Protura           | 5.135*          | 25.827*  | 11.417*     |
| Pseudoscorpionida | 5519.388*       | 61.935*  | 587.760*    |
| Psocoptera        | 5.270*          | 5.049*   | 5.270*      |
| Raphidioptera     | 0.921           | 0.882    | 0.921       |
| Symphyla          | 1.729           | 0.109    | 0.113       |
| Thysanoptera      | 531.951*        | 509.600* | 531.951*    |

\* $p < 0.05$

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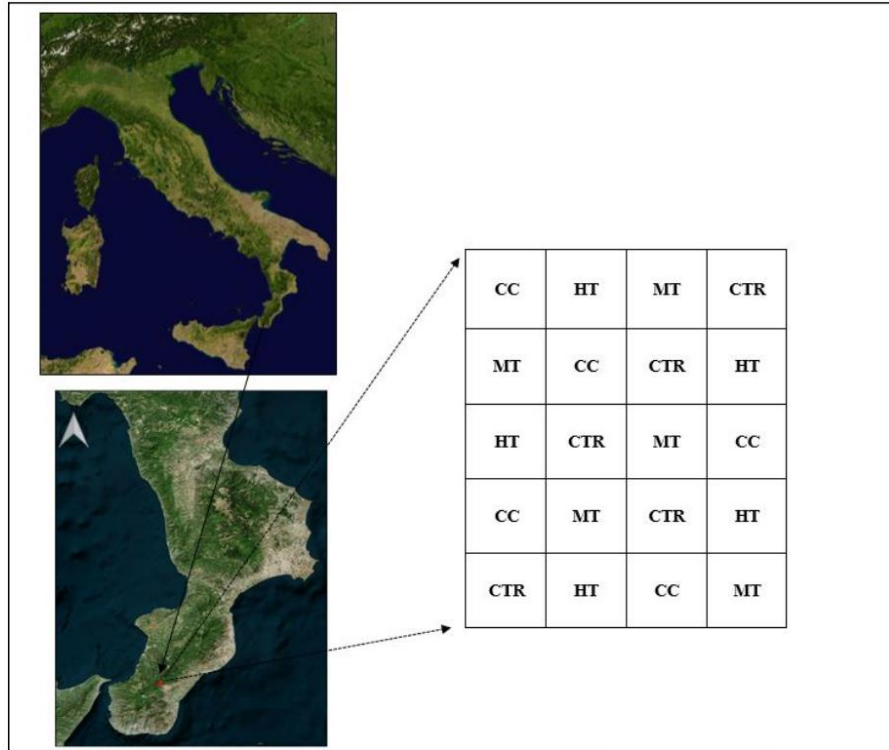
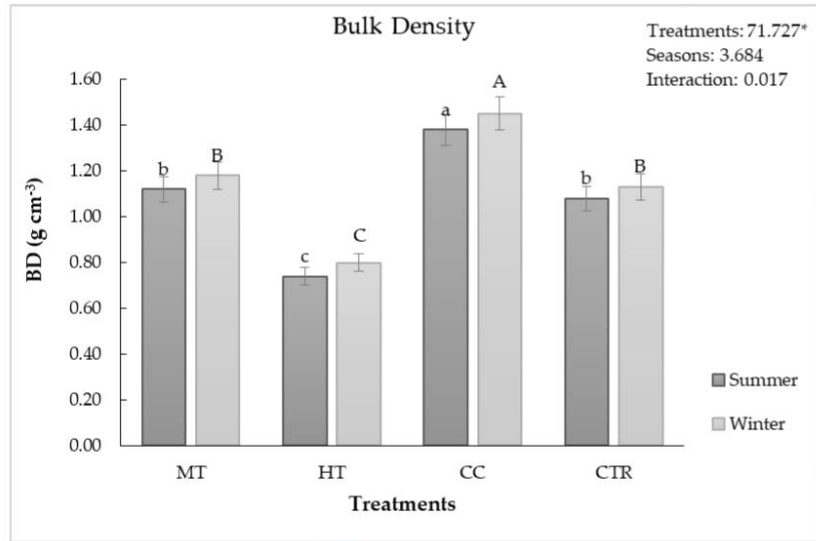


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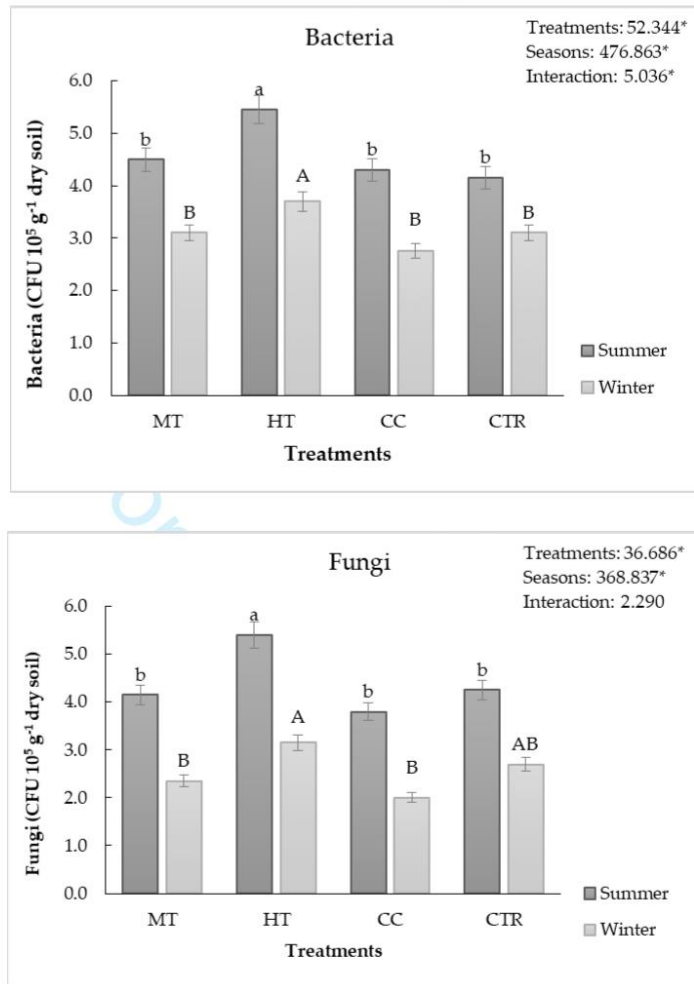
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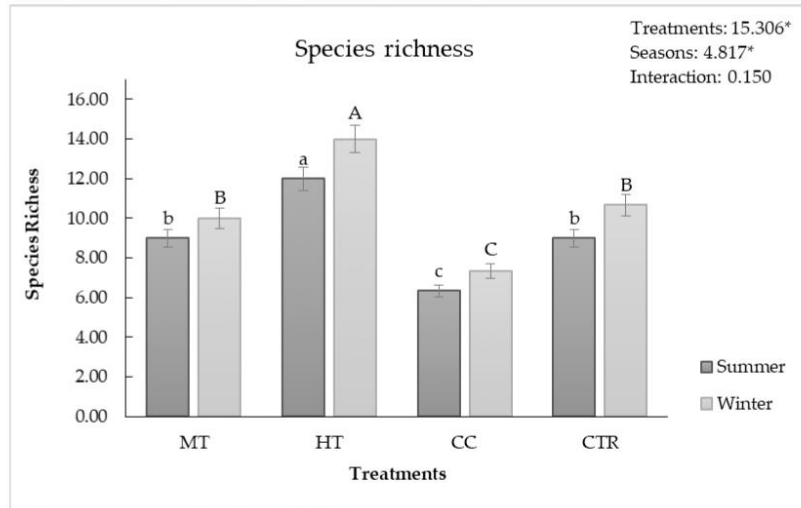
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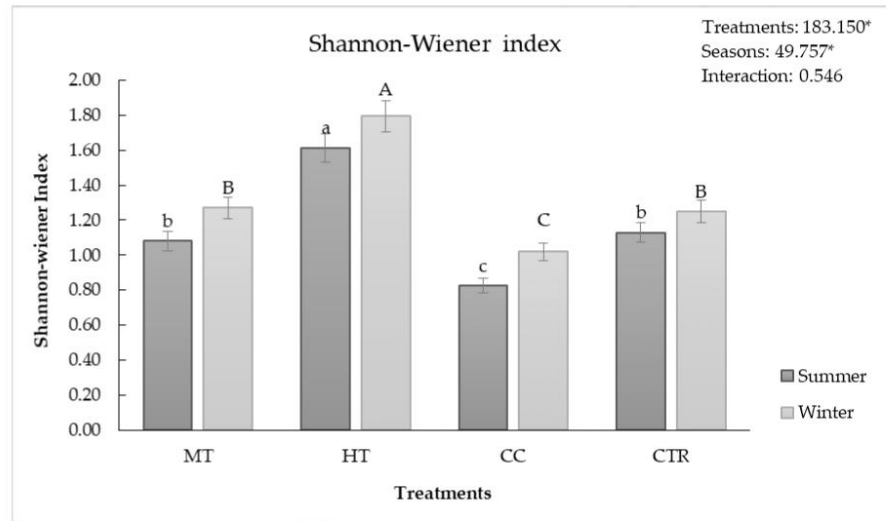


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511  
512 **Figure 4.**

Review Only

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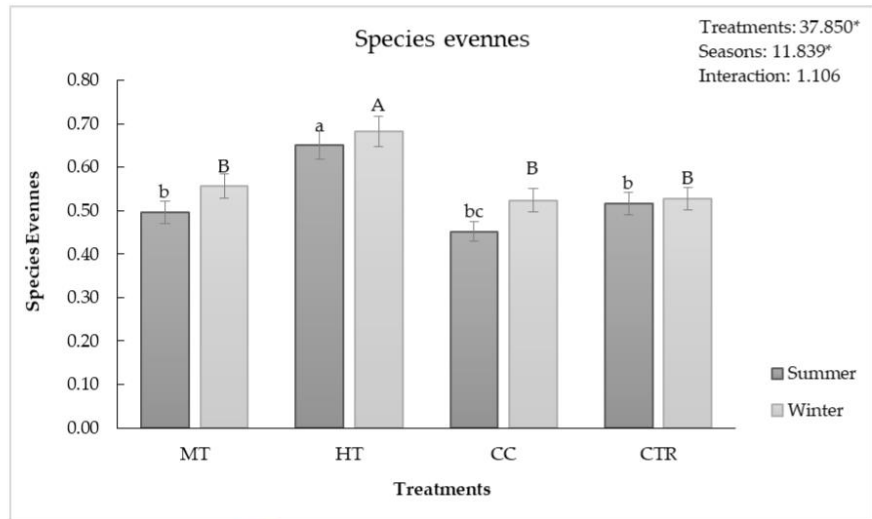


\* $p < 0.05$

Figure 5.

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Figure 6.

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**APPENDIX 4**  
**Posters presentations**





## **Soil Biological Indicators and Caesium-137 to Estimate Soil Erosion in Areas with Different Forest System Management**

Federico Romeo, Paolo Porto, Carmelo Mallamaci, and Adele Muscolo

Dipartimento di Agraria, Università Mediterranea, Feo di Vito, 89124 Reggio Calabria Italy (amuscolo@unirc.it)

In natural forests, surface runoff and soil erosion are generally low because of the surface litter cover, but can be triggered by natural disturbances or anthropic interventions. Many researches have been carried out on the impact of natural disturbance on soil ecosystem service, but researches on the impacts of forest management activities on soil erosion and the subsequent effects on forest productivity are limited yet. Relationships between management and productivity are not simple but are rather extraordinarily complex, reflecting interactions among management system, soil biological activity, nutrient cycling, and climate. Therefore, the effect of a given forest management is highly dependent on site-specific soil properties and microclimate and may also be influenced by year-to-year variation in climate. Forest management if not properly settled can cause soil erosion process, reducing consequently soil productivity and environmental sustainability. In order to reduce these phenomena and to find useful countermeasures, we need to evaluate the intensity of the erosion process under forest management practices, and the reasons that cause it. Numerous attempts to prevent this phenomenon have been made, mainly based on models and calculation procedures however, their utilities remain limited to the geographic areas for which calibration and validation are possible. The aim of this work was to estimate if innovative thinning (preselect 50 trees/ha and removal of direct competitors) can induce soil erosion in beech forest in respect to traditional thinning (cutting of 45% total trees/ha) or control (no thinning). The soil erosion rate has been estimated by using the technique of the radionuclide cesium-137 ( $^{137}\text{Cs}$ ) jointly with soil biological indicators (soil organic matter, microbial biomass C, water soluble phenols, fluorescein diacetate hydrolase, and dehydrogenase activities) of soil ecosystem functioning. The aim was to individuate early warning indicators of soil erosion process and to find a connection between biological indices and the estimates of soil erosion rate provided by the radionuclide measurements. The experiments was carried out on a comparative basis in a beech forest, located in Calabria Apennine, Southern Italy and included in the Marchesale Biogenetic Reserve (VV), Natura 2000 site. Our results based on the cesium-137 technique showed that in both selvicultural treatments soil erosion rates were very low in respect to the undisturbed area. The innovative thinning increases soil water holding capacity, pH, cations and anions content in respect to the no thinning (unmanaged forest, control) and traditional thinning. EMI index was highest in both managed sites suggesting that the treatments increased soil biodiversity. Dehydrogenase activity was highest in soil under innovative thinning, conversely FDA activity was highest under traditional thinning. In short our data indicated that both selvicultural practices have not triggered soil erosion processes, even if they affected soil ecosystem functioning differently. In the traditional thinning prevailed the mineralization process and a hydrolytic soil activity instead, in the innovative thinning prevailed the oxidoreductasic activity and a balance between mineralization and humification process. Overall results suggest that cesium-137, DHA and FDA were the parameters that can be used to estimate early on the onset of erosive phenomena.

# Book of Abstracts



## ECOLOGY – MEETING THE SCIENTIFIC CHALLENGES OF A COMPLEX WORLD

48<sup>th</sup> Annual Meeting of the Ecological Society  
of Germany, Austria and Switzerland

Universität für Bodenkultur Wien, 10 – 14 September 2018



# POSTER PRESENTATIONS

## SESSION 13-P1

### **Thinning practice influences soil microbial biomass, enzyme activities and organic matter dynamics in *Fagus sylvatica* plantation**

Federico Romeo<sup>1</sup>, Katharina M. Keiblinger<sup>2</sup>, Axel Mentler<sup>2</sup>, Carmelo Mallamaci<sup>1</sup>, Adele Muscolo<sup>1</sup>

<sup>1</sup>*Università Mediterranea, Reggio Calabria, IT, federico.romeo@unirc.it*

<sup>2</sup>*University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, AT*

For centuries forests have been managed to maximize timber production for economic profit neglecting other forest ecosystem service. Nowadays, forests are recognized as key elements to combating climate change, and reducing risk of natural disasters. Thus it is imperative to schedule appropriate management practices to reach sustainable development goals. Incorrect forest management can cause negative effects on the stability of ecosystems in each of its forms, inducing soil erosion and loss of biodiversity and consequently forest productivity. Numerous studies have been carried out to understand the relationships between forest management, soil erosion and productivity, but few studies focused on the effects of forest managements on soil biological quality. The aim of this work was to assess if and how two different forest management practices, influence soil biological quality in terms of organic matter dynamic and microbial biomass activity, with the specific objective to identify a forest management practice to improve productivity and at the same time preserve forest soil quality. The effects of innovative thinning (preselected 50 trees/ha and removal of direct competitors) and traditional thinning (cutting of 12 % total trees/ha) have been evaluated and compared to unmanaged area (no thinning). Microbial biomass carbon, enzymatic activities, organic matter content, humification rates, ergosterol, dissolved organic carbon and microfauna community (EMI index) have been assessed. The results obtained showed highest dehydrogenase activity in soil under innovative thinning where a balance between mineralization and humification process was also observed. Conversely, fluorescein diacetate and catalase activities, were highest under traditional thinning, where the mineralization process prevailed. Both managed sites had the highest EMI index, suggesting that thinning increased soil biodiversity. Ergosterol, a fungal biomarker, was low in the innovative thinning area supporting lower hydrolytic soil activity. Largest amount of humified OM under innovative thinning, support this management strategy as a sustainable way to improve fertility and physicochemical soil stability.





XXXVI CONVEGNO NAZIONALE DELLA SOCIETÀ ITALIANA DI CHIMICA AGRARIA



# SICA 2018

IL RUOLO DELLA CHIMICA AGRARIA  
PER LA GESTIONE SOSTENIBILE  
DELLE RISORSE AGRARIE E FORESTALI

24-26 SETTEMBRE 2018  
DIPARTIMENTO DI AGRARIA - UNIVERSITÀ MEDITERRANEA  
REGGIO CALABRIA

**S9 Effetti di differenti tipi di gestione forestale sulle proprietà chimico-fisiche del suolo e sull'erosione stimata con la tecnica del cesio-137, in boschi di faggio**

**F. Romeo\***, G. Settineri, C. Mallamaci & A. Muscolo

*Dipartimento di Agraria, Università Mediterranea, Reggio Calabria, Italia*

*\*E-mail: [federico.romeo@unirc.it](mailto:federico.romeo@unirc.it)*

Nelle foreste naturali, il deflusso superficiale e l'erosione del suolo sono generalmente fenomeni poco rilevanti grazie alla presenza della lettiera ed alla copertura vegetale che svolgono un'azione protettiva. I fenomeni erosivi vengono generalmente innescati o accelerati da cambiamenti climatici e/o attività antropica tra cui una non corretta gestione dei boschi. Per contrastare e limitare i fenomeni erosivi è necessario quindi individuare pratiche di gestione forestale sostenibili in grado di ridurre la perdita di biodiversità e di garantire i servizi eco-sistemici per costruire una nuova e buona economia attraverso la valorizzazione e la gestione della risorsa bosco. Per fare ciò è necessario monitorare durante le pratiche di gestione forestale il sistema suolo per valutare l'intensità del processo di erosione e individuarne le cause. Lo scopo di questo lavoro è stato quello di mettere a confronto gli effetti di differenti tipi di gestione forestale sul suolo, al fine di individuarne la pratica più sostenibile. Sono stati comparati gli effetti di una gestione forestale di tipo innovativo (preselezione di 50 alberi/ha e la rimozione di concorrenti diretti, 27% totale di alberi sottoposti al taglio) con un diradamento tradizionale (taglio del 12% totale alberi/ha), e un bosco non gestito (senza diradamento da oltre 30 anni). L'obiettivo specifico è stato quello di individuare i principali cambiamenti nelle caratteristiche fisico-chimiche del suolo, cercando di trovare una correlazione con le stime dei tassi di erosione del suolo. Gli esperimenti sono stati condotti in una faggeta, situata nell'Appennino calabrese, nel Sud Italia e inclusa nella Riserva Biogenetica Marchesale (VV), nonché sito Natura 2000. Sui suoli prelevati a due differenti profondità (0-15 cm e 15-30 cm), sono stati determinati: tessitura, umidità, pH (H<sub>2</sub>O), conducibilità elettrica, fenoli totali, carbonio organico (%), azoto (%) e rapporto C/N. I tassi di erosione del suolo sono stati stimati utilizzando la tecnica del radionuclide Cesio-137 (137-Cs). I risultati hanno evidenziato che il suolo sottoposto ad una gestione innovativa presentava una maggiore capacità di ritenzione idrica, un maggiore rapporto C/N, ed un pH più elevato rispetto agli altri due siti studiati. Una diminuzione del contenuto di fenoli totali, carbonio e azoto è invece stata riscontrata in entrambi gli strati di suolo prelevato nell'area sottoposta a gestione innovativa. Un aumento proporzionale di perdita di suolo, seppur limitata, è stato osservato all'aumentare dell'intensità di taglio. In breve, i nostri dati indicano che anche se le pratiche di gestione forestale influenzano diversamente le proprietà chimiche del suolo favorendo il processo di umificazione nel taglio innovativo e di mineralizzazione in quello tradizionale entrambe le pratiche selvicolturali non sembrano al momento aver innescato processi di erosione significativi rispetto all'area controllo.



**S11**      **Effetti di diverse intensità di diradamento sulla biodiversità del suolo in un popolamento di *Pino laricio* nell'Appennino Meridionale Calabrese**

**G. Settineri\*, F. Romeo & A. Muscolo**

*Dipartimento di Agraria Università Mediterranea Reggio Calabria, Italia*

*\*E-mail: [giovanna.settineri@unirc.it](mailto:giovanna.settineri@unirc.it)*

Il monitoraggio dei cambiamenti che avvengono nei boschi, in risposta alle pratiche selvicolturali, risulta necessario per poter progettare una gestione forestale sostenibile. Un obiettivo importante della moderna selvicoltura è la conservazione delle risorse ambientali e naturali a lungo termine in modo da mantenere la multifunzionalità delle foreste. Gli ecosistemi forestali sono tra i più ricchi di biodiversità e gli artropodi, in molti ecosistemi sono il phylum più numeroso. Gli artropodi del suolo hanno un ruolo fondamentale nell'ecosistema forestale in quanto frammentano la sostanza organica facilitandone la decomposizione e l'umificazione inoltre, per la loro sensibilità ai cambiamenti ambientali sono considerati importanti bioindicatori della salute dell'ecosistema. Le pratiche forestali non corrette sono tra le cause principali del degrado del suolo e della perdita di biodiversità, con ricadute negative sull'intero funzionamento dell'ecosistema forestale. Considerando l'importanza delle foreste di pino laricio (*Pinus nigra* J.F. Arnold subsp. *laricio* Palib. ex Maire) in Calabria e il loro ruolo economico ed ecologico, una gestione sostenibile è importante per evitare ripercussioni negative sia sull'ambiente che sull'economia locale. Il diradamento è il trattamento selvicolturale più utilizzato in Europa per migliorare il valore ecologico dei boschi di conifere. Il presente studio ha valutato gli effetti di diverse intensità di diradamento sulla biodiversità del suolo, allo scopo di identificare la pratica più adatta alla gestione dei boschi di pino laricio. Gli effetti delle diverse intensità di diradamento (MI, moderata intensità, 30% ed AI, alta intensità, 60%) e del taglio raso rispetto un bosco non trattato (CTRL, controllo) sono stati valutati monitorando alcuni parametri chimico-fisici (pH, umidità, carbonio organico, tasso di umificazione, acidi umici e fulvici) e l'abbondanza, la ricchezza in specie, l'indice di Shannon-Wiener e l'indice di equitabilità (Evenness) degli artropodi. I siti di studio, omogenei dal punto di vista climatico, altitudinale e topografico, erano situati nell'Appennino calabrese (sud Italia) in una pineta di *Pinus nigra* J.F. Arnold subsp. *laricio* Palib. ex Maire di 40 anni. Il diradamento è stato effettuato nel 2011, ed i risultati dopo 4 anni di monitoraggio hanno evidenziato che sia i dati relativi ai parametri chimico-fisici che quelli riguardanti l'abbondanza, la ricchezza delle specie e la diversità degli artropodi sono stati modificati dai trattamenti. Nel diradamento AI abbiamo trovato la maggiore biodiversità e la più alta quantità di carbonio organico, dovute a una minore densità di piante, che conseguentemente determinavano diversi regimi di luce, temperatura e umidità a livello del suolo e, quindi la creazione di un microclima favorevole allo sviluppo del sottobosco con produzione di lettiera facilmente degradabile, e conseguente aumento di biodiversità.

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**Table 4** Eco-morphologic indices (EMI) of edaphic microarthropods groups. Qbs index is obtained from the sum of the highest values of EMI of all the collected groups.

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**Table 6 (2017)** - Soil parameters detected in the two soil layers (0-15; 15-30 cm) under different forest managements (T1- Control; T2- Traditional thinning; T3- Innovative thinning): humification degree (DH, %); humification rates (HR, %); humification index (HI); microbial biomass (MBC,  $\mu\text{g C g}^{-1}$  f.s.); ergosterol fungal biomarker (Erg,  $\mu\text{g g soil}^{-1}$ ); fluorescein released (FDA,  $\mu\text{g g}^{-1}$  dry soil); dehydrogenase (DHA,  $\mu\text{g INTF g}^{-1}$  dry soil h<sup>-1</sup>); catalase activity (CAT, O<sub>2</sub>%/3min/g dry soil<sup>-1</sup>). Numbers denote the standard error of the mean: n=3. Means in the same row followed by the same letter are not statistically different at  $p \leq 0.05$  (Tukey test).

**Table 7 (2017)** – Loading values (PC1 and PC2) of principal component analysis diagram (PCA) referred to 0-15 cm and 15-30 cm soil depths.

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