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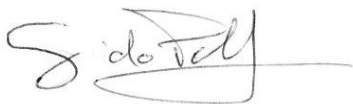
**Scienze Tecnologie e Biotecnologie per la Sostenibilità – Curriculum Ecologia Forestale e
Tecnologie Ambientali – XXIX Ciclo**

**SOIL ORGANIC CARBON, SOIL MICROBIAL COMMUNITIES AND PLANT BIOMASS CHANGES
ALONG WOODY ENCROACHMENTS OVER ABANDONED PASTURES IN ITALY**

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1. LIST OF ABBREVIATIONS

ABG = aboveground biomass

AI = aridity index

ARPA = Agenzia regionale per l'ambiente

B = biomass

BD = bulk density

BGB = belowground biomass

C = carbon

C/N = carbon to nitrogen ratio

CEC= cation exchange capacity

CLC = CORINE land cover

C_{mic} = microbial biomass carbon

C_{mic}/N_{mic} = microbial biomass carbon to microbial biomass nitrogen

C_{org} = organic carbon

CWD = coarse woody debris

DBH = diameter at breast height

DCH = diameter at the collar height

DEM = digital elevation model

DIBAF = Dipartimento per la Innovazione dei sistemi Biologici, Agroalimentari e Forestali

E = East

EDTA = ethylenediaminetetraacetic acid

EEA = European environmental agency

ESD = equivalent soil depth

ESM = equivalent soil mass

F = forest

FAO = The Food and Agriculture Organisation

FWC = fine woody debris

GHG = greenhouse gas

GIS = geographic information system

GPS = global positioning system

H = height

H' = Shannon's diversity index

H_m = mean height

IGM = Istituto Geografico Militare

INFC = inventario nazionale delle foreste e dei serbatoi forestali di carbonio

IPCC = Intergovernmental Panel on Climate Change

IUTI = inventario dell'uso delle terre d'Italia

L = length

LUC = land-use change

MAP = mean annual precipitation

MAPF = Ministero delle Politiche Agricole e Forestali

MAT = mean annual temperature

Maxis = major axis

MUF = methylumbelliferone

N = nitrogen

N = North

N_{mic} = microbial biomass nitrogen

O = origin, central point

P = pasture

R/S = root to shoot ratio

S = South

SA = sampling area

SDS = summer dry stress

SEIc = Synthetic Enzymatic Index for the carbon cycle enzymes

SOC = soil organic carbon

SPA = special protection area

SUE = substrate use efficiency

T1 = encroached stage at time 1

T2 = encroached stage at time 2

T3 = encroached stage at time 3

T4 = encroached stage at time 4

UNFCCC = United Nation Framework Convention on Climate Change

UTM = Universal Transverse Mercator

V = volume

W = West

WCS = winter cold stress

WGS = world geographic system

WHC = water holding capacity

WRB = World Reference Base

2. SHORT ABSTRACT

The abandonment of mountain pastoral practices after the World War II has led to a widespread woody encroachment process in Europe. It implicates an increase in living biomass and dead organic matter C pools, but contradictory results on soil organic C (SOC) stock have been reported.

The aim of this study was to investigate the effect of this process – in different climatic conditions along the Italian peninsula – firstly on IPCC terrestrial ecosystem C pools and secondly on the soil microbial biomass and enzyme activity. A chronosequence approach was adopted to identify, in each site, several stages. Seven chronosequences were selected along Italy also filling the knowledge lack about this process in the Central and Southern mainland territory.

Considering the whole soil profile, SOC stocks changed differently in the Alps and the Apennines. In the Alps an initial increase by 40% in the SOC stock was followed by a decrease to approximately initial pasture values. In the Apennines, an initially similar increase (31%) continued to reach 104% in the forest. N stocks showed similar trends. The subsoil (below 30cm) was found to be a substantial pool, often containing C stock comparable to the topsoil (0-30cm). SOC stock changes among the sites selected in mainland Italy appeared statistically correlated not with mean annual precipitations, as suggested in the literature, but rather with temperatures and winter cold stress. All the other C pools increased along the process in each site. A peak in soil enzyme activity and plant functional-type diversity was measured in the intermediate stage, but with no simultaneous peak in microbial biomass per unit of C, suggestive of microbial population stress.

Even if it was possible that the SOC stock changes between pastures and forest stages were not detectable, the results showed that the woody encroachment in the Italian mainland territory always leads to a substantial increase in the ecosystem C stock (+147%, on average).

3. KEYWORDS

Woody encroachment; pasture abandonment; SOC stock; subsoil; climatic gradient; ecosystem C pools; soil enzyme activity

4. EXTENDED ABSTRACT

4.1. Introduction

The role of land-use change (LUC) in the global carbon (C) budget is of great importance and it has effects, at the ecosystem level, both on C stored in the vegetation and in the soil (Foley *et al.*, 2005). Among the LUC typologies, woody plant encroachment into pastures and grasslands has been representing a significant phenomenon, particularly in the mountain areas after the Second World War (Alberti *et al.*, 2008). In effect, Fuchs *et al.* (2013) estimated that – between 1950 to 2010 – 7.64% of the Southern Europe land was subject to this process.

As a result of woody encroachment, it is widely accepted that C stored in the aboveground biomass (AGB) increases along a secondary succession process from grassland to forest (Thuille and Schulze, 2006; Alberti *et al.*, 2008; Risch *et al.*, 2008; Guidi *et al.*, 2014a). However, the trend is not equally clear when the soil organic carbon (SOC) pool is considered (Guo and Gifford, 2002; Poeplau and Don, 2013) and, according to Jackson *et al.* (2002), it is possible for the SOC stock decrease to even counterbalance the C biomass storing effect. In addition, despite the numerous papers published on this LUC – see e.g. Guo and Gifford (2002), Jackson *et al.* (2002), Lal (2005), Alberti *et al.* (2008, 2011), Guidi *et al.* (2014a) –, an evaluation of the IPCC terrestrial C pools changes occurring during woody encroachment on abandoned pastures and grasslands is still lacking, particularly for the Central and Southern part of the mainland Italian territory.

To understand the SOC stocks changes, it is fundamental to identify and control the balance between C inputs and outputs from the soil and all the factors that can control C stabilisation and destabilisation (Sollins *et al.*, 1996). Considering the effect on SOC stock changes along the woody encroachment process, Lal (2005) and Paul *et al.* (2002) jointly stress the relevance of: climate variables, in particular the yearly precipitation (Jackson *et al.*, 2002; Alberti *et al.*, 2011); orography and substrate morphology; soil chemistry, properties and clay content; natural and anthropogenic disturbances; previous land use, its management and its age; species of the new forest formation; time from the abandonment.

Therefore, the aim of this study was to investigate the role of woody encroachment on C sequestration over abandoned pastures and grasslands in Alpine and Apennine ecosystems. This work is particularly focussed on SOC because of the greater soil C store capacity, which was estimated to be about three times the amount of C stored in vegetation and twice the amount in the atmosphere (Scharlemann *et al.*, 2014).

4.2. Materials and Methods

The study of the effect of woody encroachment on pastures and grasslands process on the IPCC's terrestrial C pools was performed on seven selected sites located along a latitudinal gradient in Italy.

Following Walker *et al.* (2010), a chronosequence approach was applied to study the temporal dynamics on plant communities and soil developments. The chronosequence concept adopted for this study follows the space-for-time substitutions purposed by Austin (1981). At each site, a chronosequence was identified collecting a series of local airborne photographs. Images were uploaded on a GIS and used to identify definitive chronostages (five or six) as homogeneous as possible. Finally, chronostages were ordered on the base of abandonment period and labelled *P*, *T1*, *T2*, *T3*, *T4* and *F* (*P* being a current pasture/grassland, *Tx* transitional phases where the index increases with time since

abandonment and *F* forest older than 60 years). The selected sites are located in the following municipality: Castello Tesino, Mel, Chianocco in the Alps ridge, and Firenzuola, Farindola, Vastogirardi, Pignola in the Apennines.

Soil sampling for organic C and nitrogen (N) analysis were collected following the European Commission sampling protocols to certify SOC changes (Stolbovoy *et al.*, 2007) with some modifications. First, the depth intervals – which were originally 0-10, 10-20 and 20-30 cm depth – were reset to 0-5, 5-15 and 15-30 cm, to achieve a more refined analysis of the topsoil; second, the analysis was extended to the subsoil, using the 30-50 and 50-70 cm depth intervals (or stopping at the R horizon). In each chronostage three composite samples for C and N analysis and three bulk density (BD) samples were collected for each depth intervals and analysed. A comparison of mean SOC and N stocks values between the different stages of each chronosequence was tested with a repeated ANOVA measurement and Tukey's post-hoc test. Effects of climate variables on SOC stock changes were tested with linear regression models.

A deeper analysis of *P*, *T2* (which generally is a scrubland) and *F* chronostages was conducted, in which, in addition to the SOC, living biomass and dead organic matter C pools were considered. AGB and dead organic matter estimation followed a protocol inspired by the third phase INFC protocol of Ministero delle Politiche Agricole e Forestali (MPAF, 2006), Alberti *et al.* (2008) and Bovio *et al.* (2014). Shrubs AGB was measured following specific allometric equations (when available in the consulted literature) or weighing the dry AGB of the collected plants (for those species without a species-specific allometric equation). A value for the belowground biomass (BGB) was estimated adopting vegetation-specific root to shoot ratio (R/S) according to Mokany *et al.* (2006), an approach adopted also in the IPCC Guideline for National Greenhouse Gas Inventories (2006). Concerning dead organic matter, litter and fine woody debris (FWD) samples were directly collected in the field, dried out in the oven at 70 °C, and then weighed. For the coarse woody debris (CWD), because no samples were collected to estimate the wood density data, it was only possible to estimate the volume according to the methods of Harmon and Sexton (1996). Then, the measurements were converted into mass by means of a wood density estimation measured by species-specific fresh wood density (Global Wood Density Database, 2015) and the decrease decay wood density class values described by Alberti *et al.* (2008). Then, the living biomass and dead organic matter pools were converted in C estimates adopting the 0.475 conversion factor proposed by Magnussen and Reed (2004). Given that the living biomass and dead organic matter were estimated from direct measurements only in three stages (*P*, *T2* and *F*), the values in the remaining stages were estimated by linear interpolation. Finally, the five C pools (above- and belowground living biomass, dead wood, litter and soil) were added together in order to estimate the total ecosystem C stock for each stage in each chronosequence.

A plant functional-type diversity index was also estimated on the basis of shrubs and trees Shannon diversity indices (Shannon and Weaver, 1948) and the grasses aboveground biomass. Each parameter was normalised on the maximum values of each chronosequence and then they were summed together, grouping separately conifers and broadleaves (see Figs. 4.1A and 4.1B).

The three stages (*P*, *T2* and *F*) selected for the living biomass and dead organic matter estimation, were also considered for a deeper soil investigation, which was based on the microbial biomass (*C_{mic}*) and enzyme activities linked to the C and N cycles (β -cellobiohydrolase, α -glucosidase, β -glucosidase, xylosidase, β -N-acetyl-glucosaminidase). The measurements were performed only at two soil depth layers: 0-5 and 5-15 cm. The sum of the C-cycle enzyme activities was considered as a Synthetic Enzymatic Index for carbon (SEIc), as Dumontet *et al.* (2001) suggested for all the enzyme measurements. Finally, according to Trasar-Cepeda *et al.* (2008), the SEIc and the chitinase (the only considered enzyme linked to the N cycle) were both normalised dividing by the SOC, in order to estimate each enzyme-

specific activity. Preliminary results suggested that the major part of the microbial biomass and its activity was confined to the upper layer. Therefore, the above- and belowground interaction was explored only in the 0-5 cm depth interval.

4.3. Results

In all the chronosequences, it was possible to highlight some variations (which are usually statistically significant) along the woody encroachment process both in SOC and N stock in the two part of the soil profile (topsoil and subsoil) (an example is shown Fig. 1A and B). C and N concentrations progressively decreased along the soil profile in each chronostage (data not shown).

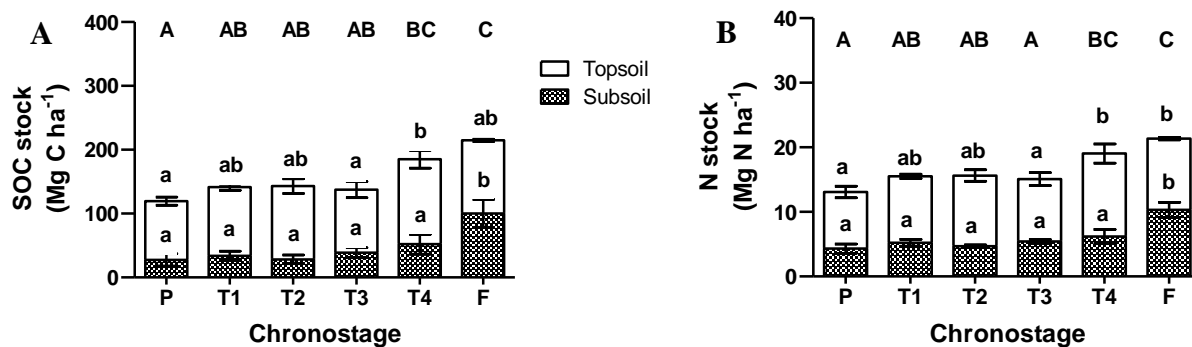


Figure 1 Topsoil (empty bars) and subsoil (dark bars) SOC stocks (graph A) and N stocks (graph B) changes along the woody encroachment process in Firenzuola study area. Statistically significant differences among the stages are labelled with low case letter (considering topsoil and subsoil separately) or with capital letters (whole soil profile).

SOC stock changes along the woody encroachment are summarised in Fig. 2A and 2B for Alps and Apennine study areas, respectively. In the Alps study areas, in the first 22 years of the process the SOC stock increases by almost 41% (range 34 – 49%) and, then, it decreases reaching the forest stages with a value of +7% (range +0.5 – +15%) more than the previous pasture. In the Apennine study areas, after a similar increase (mean 31%, range 20 – 42%) in the first part of the process, the SOC stock continues to increase until the forest stage (a total increment by 104% with respect to the initial pasture, range 80 – 147%). Similar trends were observed also for the nitrogen stocks (data not shown). Vastogirardi displayed an uncommon trend when compared to the other study areas, in particular in terms of its marked variability in the aspect (i.e. the slope orientation) of the chronostages, which spanned the full range of directions from N to S. This is the only study area displaying this level of heterogeneity between stages, which could have important effects on the organic matter input in the soil (Lonzano-García *et al.*, 2016) and which, therefore, could weaken the validity the chronostages approach. For this reason, this study area was excluded from the following analyses and results.

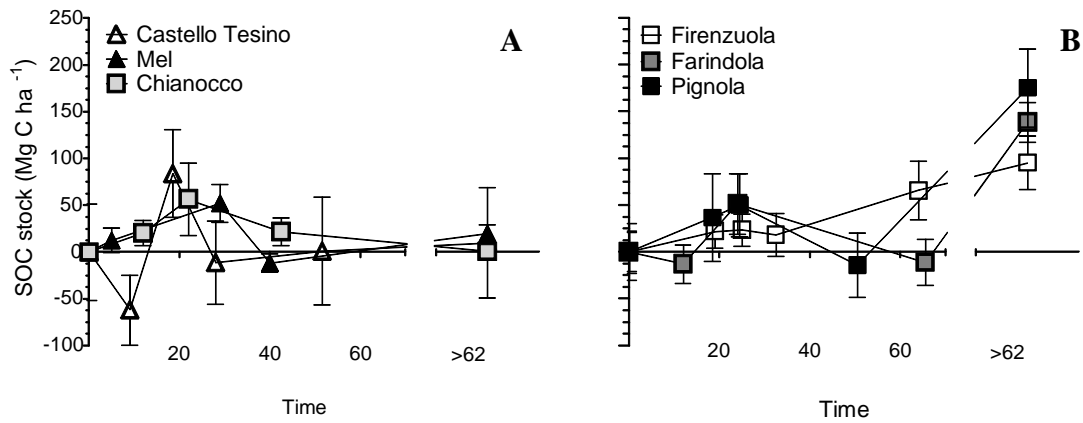


Figure 2 SOC stock variations, reported as difference between the values in each chronostage and the values in the pasture stage of each chronosequence. The horizontal axis measures the chronostages' "age", i.e. the time since the woody encroachment process started, measured in years before present (Time). Graph A and B correspond to Alps and Apennine study areas, respectively. Triangle symbols refer to chronosequences, which are characterised by a conifer-dominated forest stand, while square symbols refer to broadleaves-dominated chronosequences

No significant relations were observed between SOC stock proportional change (i.e. the forest to previous pasture ratio) and mean annual precipitation (MAP). Similarly, no statistically significant results were obtained using the most common Aridity Indices (as the Lang pluviometer, the Gaussen aridity index, De Martonne pluviometer, De Martonne-Gottmann aridity indices). Differently, statistically significant correlation was observed when SOC stock proportional changes were plotted against mean annual temperature (MAT) and Mitrakos winter cold stress index (WCS), as shown in Fig 3A and 4B, respectively.

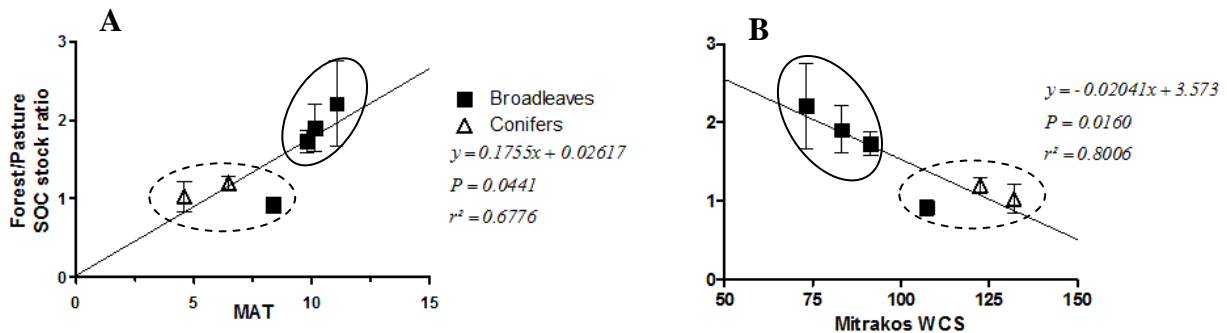


Figure 3: Forest to Pasture SOC stock ratios per each site as a function of MAT (A) and WCS index (B). Filled squares refer to the broadleaves stands while empty triangles refer to conifers. Linear regressions (thin lines) were added to the graph. Sites in Alps (dotted line) and Apennines (continuous line) were grouped separately.

Fig. 3 clearly shows the presence of the two groups of study areas previously described: the Alps, with a SOC stock ratio close to one (i.e. a SOC stock that does not change substantially between pasture and forest stages) and the Apennines, with a ratio markedly higher than one. Similar results were obtained when the both the variations (measured as the differences of the values between forest and previous pasture stages) in the SOC stock and in the litter C stock pool are plotted against MAT and Mitrakos WCS (data not shown).

Living biomass and dead organic matter C stocks increased progressively along the secondary successions. At the beginning of the process, the amount of C stored in both these pools was negligible, especially if compared to the total ecosystem C stock. On the opposite, at the forest stages, the C stored in the living biomass played a marked role, ranging between the 40 and the 55% of the total ecosystem carbon stock. In all the study areas, independently of the group they are included in, the woody encroachment process led to an increase in the ecosystem C stock (data not shown) of, on average, 105% (range: 86-144%) in the Alps and 220% (range: 180-280%) in the Apennines. In the Alpine study areas, the living biomass and dead organic matter pools of the oldest stages were fundamental in the increment of the ecosystem C stocks. Given the living biomass and dead organic matter pools in the initial stages of the process were negligible, the SOC stock played a much larger role early on than in the older stages.

No significant correlations were observed when each biochemical index (C_{mic} , C_{mic}/SOC , enzyme activities and enzyme-specific activities) was plotted against each soil chemical index separately. However, maximum values for enzyme-specific activities have been generally observed in the intermediate stages, which were characterised by the maximum plant functional type diversity index (Fig. 4).

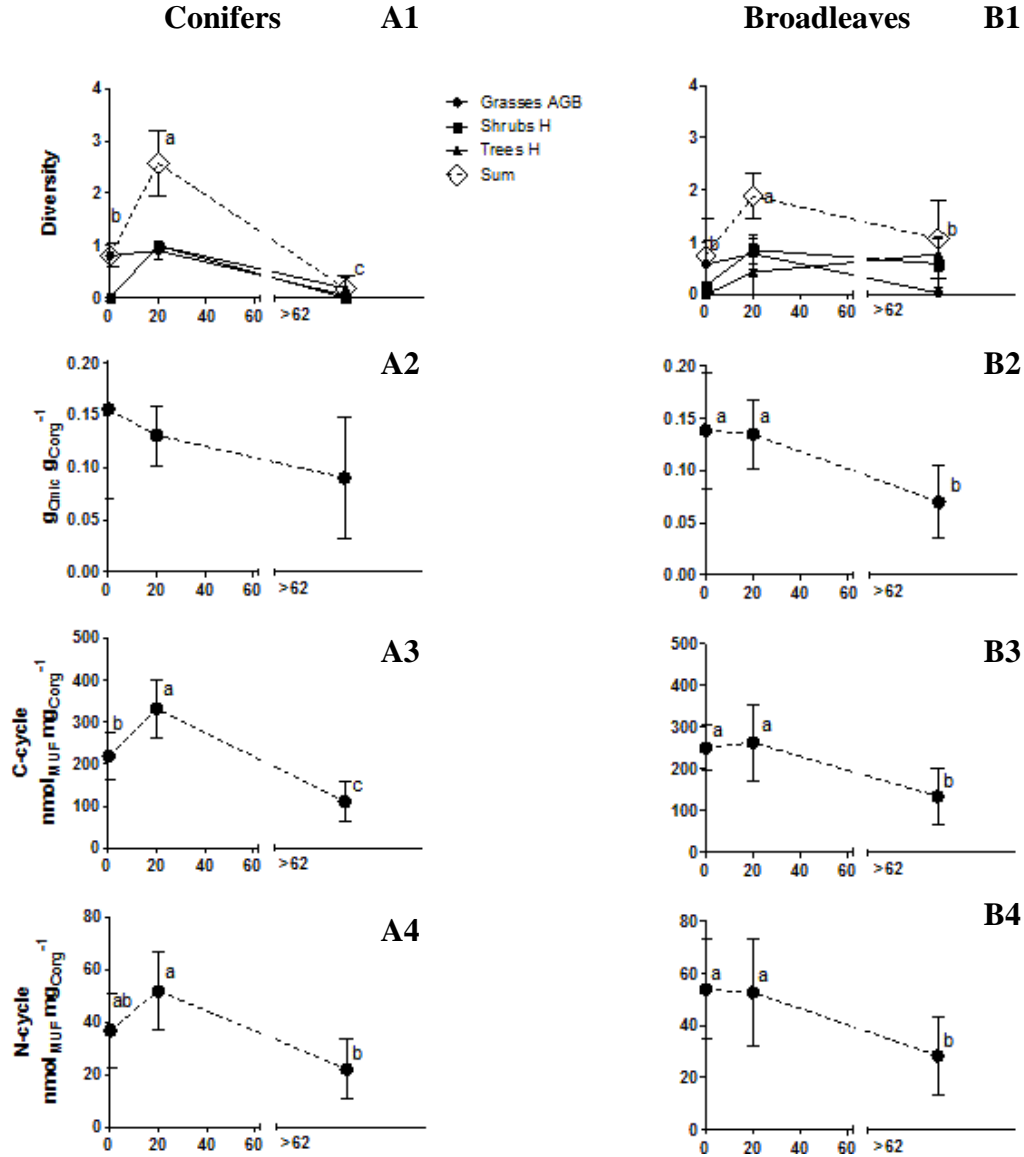


Figure 4: Aboveground and belowground comparison between conifers (A column) and broadleaves (B column) sites. In all the graphs, the horizontal axis is the time, in years before present, and refers to the age of each stage (Pasture, Intermediate and Forest). Graphs A1 and B1 presents the shrubs Shannon diversity index, the trees Shannon diversity index and the grasses aboveground biomass, as well as the aboveground functional type diversity index obtained from their sum. All the other graphs (except for A5 and B5) take into account, separately, the main biochemical indices only in the 0-5 cm soil depth interval. In particular, graphs A2 and B2 show the change in microbial carbon per unit of soil organic carbon; A3 and B3 show the change in the SEIc; and A4 and B4 show the variation in Chitinase activity per unit of soil organic. Significant differences are marked with lowercase labels next to the points.

4.4. Discussion

According to the scientific literature (Jobbágy and Jackson, 2000; Zhao *et al.*, 2016), the C concentration decreases along the soil profile.

Although, in general, it was not considered in similar studies, Fig. 2 shows that the subsoil represented a substantial C reservoir, which in some cases could contain an organic C and N stocks comparable to that of the topsoil. Therefore, it

is strongly recommended to include the deeper part of the soil profile in the terrestrial ecosystem C budget estimation, and particularly when investigating the SOC changes as consequence of a LUC.

Results in Fig. 3 shows that secondary successions over abandoned pastures / grasslands in Italian mountain areas led to SOC stock trends that vary among sites. According to the scientific literature, the woody encroachment process can allocate (sink effect) or can release (source effect) organic C differently on the basis of different environmental conditions. Jackson *et al.*, (2002), Guo and Gifford (2002), Alberti *et al.* (2011) remark upon the climate influence on the encroached sites, particularly stressing on the mean annual precipitation role on soil C dynamics. Therefore, the clearly different trend in SOC stock changes in two study area groups found in this work (corresponding to Alps and Apennine) should not be considered as a result of the mountain chains orogenetic processes themselves, but rather a consequence of the different climatic conditions between the two mountain ranges. Differently from what expected, the SOC stock proportional change was found to be statistically significantly correlated not with the mean annual precipitation (MAP), as suggested in the literature, but both with mean annual temperature (MAT) and Mitrakos winter colder stress index (WCS) (both Spearman's correlation $P < 0.05$). It is possible that the MAP spectrum of values considered in this study is too narrow to observe a significant trend. Indeed, the site distribution – limited to the mountain areas – and the difficulties related to chronosequence identification and chronostage dimensions has lead to range of MAP values consistently reduced compared to the whole spectrum observed in Italy. In addition, the small number of sites unavoidably limits on the robustness of the regression and correlation analyses. Nevertheless, it is clear that temperatures had a stronger effect than precipitations on variations in C stock. Indeed, both the indices against which significant variations were found (MAT and Mitrakos WCS) were affected by temperature values only, while all the other aridity indices investigated include at least one parameter linked to precipitations.

The sum of the C stock stored in the living biomass (aboveground and belowground), in the dead organic matter (litter and dead wood) and in the soil was considered as the ecosystem C stock (IPCC, 2003). As observed also by Thuille and Schulze (2006), Alberti *et al.* (2008), Risch *et al.* (2008) Guidi *et al.* (2014a), the relative contribution of the biomass increases along the secondary succession in all the study areas considered. More specifically, the living biomass acted as a significant C sink mostly in the last part of the process, while in the initial stages its contribution was limited, so that the ecosystem C stock dynamics were driven by the much more variable SOC stock (Fig. 2). The same observation could be made concerning the dead organic matter. Similar results were observed by Thuille *et al.* (2000), Risch *et al.* (2008), Alberti *et al.* (2008) and Hiltbrunner *et al.* (2013) considering only the aboveground biomass. Unlike the results of Thuille and Schulze (2006), the results of the present study showed a marked contribution of the SOC pool in all the stages in each study areas.

Considering the soil biochemical analyses, an overall reduction in both microbial biomass and soil enzyme activities between pasture and forest was observed in almost all the study areas (Fig. 4). However, some differences could be pointed out concerning the transient dynamics of microbial biomass per unit of carbon and enzyme-specific activities, with the first showing a progressive decrease along the process (graphs A2 and B2), while the latter showing an transient increase in the intermediate stage (graphs A3, B3, A4 and B4), especially in the conifer-dominated study areas (graphs A3 and A4), where it was strongly statistically significant. It was observed that this positive peak in the enzyme-specific activity is simultaneous with an increase of the plant functional-type diversity (graphs A1 and B1) and that the variables were positively and statistically significant correlated in the conifer-dominated study areas (data not shown). The peak in enzyme-specific activity was in line with what observed for grasses ecosystem by Stephan *et al.* (2000), Zak *et al.*

(2003) and Steinauer *et al.* (2015). However, in contrast with those studies, the microbial biomass per unit of SOC showed a progressive decrease. According to Trasar-Cepeda *et al.* (2008), a peak in enzyme-specific activity with no peak in microbial biomass per unit of SOC could be indicative of a sort of stress on the soil microbial community equilibrium. Because of the limited number of studies on this topic (see Liao and Button, 2008; Cable *et al.*, 2009; Hollister *et al.*, 2010; García-Morote *et al.*, 2012), all of which focussed on different climatic conditions, it could only be hypothesised that the stress could be a reaction of a "grassland-adapted" microbial community to the scattered encroachment of the first conifer plants, which were able to produce allelopathic substances (Singh *et al.*, 1999).

4.5. Conclusions and Future Perspectives

This study showed that in the Italian peninsula the woody encroachment affects the SOC stock over time differently in different climatic conditions, with the distinction of sites based on different SOC stock trends naturally leading to two groups, corresponding to Alps and Apennines. The first group was characterised by an increase of the SOC stock in the whole profile (mean 41%, range 34 - 49%) of the first phase of the process and a subsequent decrease to the previous pasture values, while the second group was characterised by a progressive increase in the SOC stock until the forest stage (mean 104%, range 80 – 147%). Although in the literature the mean annual precipitation was considered as the best climatic predictor for the SOC stock trend, the results of this work identify both the mean annual temperature and the Mitrakos winter cold stress index as the main factors.

The main SOC pool was, generally, the topsoil, even if the SOC stock in the subsoil could reach comparable values. Moreover, although the subsoil is infrequently measured in similar studies, its changes along the secondary succession process were proportionally more pronounced than those of the topsoil and, therefore, they can be crucial to estimate statistically significant differences among stages in the whole profile. In all study areas, high levels of N stock were estimated both in the topsoil and in the subsoil, even in the forest stages. The trends observed of the N stock were similar to those estimated for the SOC stock everywhere. The results of this research confirmed that both the living biomass and dead organic C pools increased along the process in all the study areas considered. Furthermore, the living biomass C pool could reach values that are comparable or higher than those of the soil, but only in the forest stages.

Overall, summing all the pools (SOC, above- and belowground living biomass, dead wood and litter) the whole ecosystem C stock increased along the process (mean 147%, range 55 – 280%); therefore, the woody encroachment in Italy acts as a sink of C at all latitudes and in all climatic conditions examined. These results were particularly important for future refined quantifications of the ecosystem C pools linked to the LUC at the National level.

Concerning the biochemical analyses, an unexpected and general peak in the enzyme-specific activities was measured in the intermediate stage, especially in the conifer-dominated study areas. This peak could be explained by the statistically significant correlation between the enzyme-specific activities and plant functional-type diversity index, especially in the conifer-dominated study areas. However, the increase in the specific enzyme activity without significant change in the microbial biomass per unit of carbon was arguably better associated with microbial population stress. No conclusive explanation could be provided from the data collected, also because of the limited number of scientific work on this topic in temperate and semi-continental climatic conditions.

All the results (SOC and N contents, SOC and N stocks, living biomass, dead organic carbon, microbial biomass and enzymatic activities) pointed out that between the stable conditions of both pasture / grassland and forest, there is a transient phase that is as yet poorly understood.

5. INTRODUCTION

5.1. General considerations

Although not all political leaders agree, climate change is happening. Human activities are continuing to affect the Earth's atmospheric concentration of greenhouse gases (GHGs) and its land surface properties (IPCC, 2013a). A GHG is a gas present in the atmosphere and that absorbs energy and emits radiation within the thermal infrared range. The most important GHGs are water vapour, CO₂, CH₄, CO, N₂O and NO_x (IPCC, 2003). Among all the molecules that were recognised as GHGs, the most important is the CO₂ because it is the one that is mostly released by fossil fuel combustion.

CO₂ is strongly linked to the biotic and abiotic processes as the photosynthesis, respiration, decomposition and combustion (IPCC, 2006). Therefore, it is crucial to enhance our understanding of the linkage between the climate system alteration and the biogeochemical cycles, particularly in a perspective of formulation of accurate GHGs emission mitigation scenarios (Guo and Gifford, 2002; Lal, 2005; Stolbovoy *et al.*, 2007; Alberti *et al.*, 2011; IPCC, 2013b).

In addition to fossil fuel combustion, a key activity responsible for the increment of GHGs, and in particular CO₂, is land-use change (LUC) (IPCC, 2014). Indeed, the role of LUC in the global carbon (C) budget is of great importance and has effects, at the ecosystem level, affecting the C stored both in the vegetation and in the soil (Foley *et al.*, 2005). In the Earth System, the soil represents one of the largest C reservoirs, storing an amount of C of about 1500-2400 PgC (1Pg = 10¹⁵g), which is 1.8-3 times the size of atmospheric pool (828 PgC) and 2.3-5.5 times the size of the organic compound of vegetation living biomass (450-650 PgC) (IPCC, 2013b; Lal, 2004).

The soil organic carbon (SOC) concentration in the soil depends on a complex interaction of biotic and abiotic factors. According to Sollins *et al.* (1996), these could be summarised in a balance between an input flux and an output flux, under the effect of the stabilisation and destabilisation of organic matter due to physical, chemical and biochemical processes determined by the soil characteristics.

The study of biogeochemical cycles is based on estimating the amount of chemical elements in the different pools or in measuring the fluxes of such elements between pools (IPCC, 2006). Among the approaches to measure the latter, the most important at the ecosystem level is the eddy covariance technique (see, for example, Goulden *et al.*, 1996; Aubinet *et al.*, 2000; Baldocchi, 2003; Aubinet *et al.*, 2012). The eddy covariance technique focuses on measuring the exchange of GHGs between land or water areas and the atmosphere. Although it is a very robust methodology for estimation of processes occurring over short or medium time intervals, it has not been used yet for processes spanning longer time intervals, such as those involved in LUCs. Because this work focuses on LUC, the approach that was used here is based on the estimation of the terrestrial ecosystem C pools.

5.2. Terminology

As in numerous other areas of the natural sciences, the terminology used by different authors is not always consistent, with the same name sometimes used to refer to, or more likely to group together, slightly different processes. It is therefore worth reviewing briefly some of the expressions that appeared in the literature and clarify the definitions adopted in this work.

The expression *land cover* is used to describe the biophysical characteristics of the terrestrial surface, while *land use* is instead used to describe the human use and management of the soil cover (Marchetti *et al.*, 2012). Although the definitions are clear, in practice their application in the field of environmental research is often the result of a fusion of the two concepts: classifications typically start from categories of land cover (e.g. grasslands), but are then refined based on the soil use (e.g. pastures). Because for studies similar to this one the term *land-use change* is more common than *land cover change* in the literature, the former will be used throughout this work despite the fact that the identification of the sites studied here is based on biophysical characteristics of the soil surface.

A *forest* is defined by FAO (2012) as a land with tree crown cover of more than 10% and area of more than 0.5 hectares (ha), where the trees at maturity are able to reach a minimum height of 5 meters (m). The increase in forest cover on inland territories is due to three main processes defined in the Kyoto Protocol (UNFCCC, 2006):

- *Afforestation*, the direct human-induced conversion of land that has not been forested for a period of at least 50 years to;
- *Reforestation*, the direct human-induced conversion of non-forested land to forested land was forested but that has been converted to non-forested land;
- *Revegetation*, a direct human-induced activity to increase carbon stocks on sites through the establishment of vegetation that covers a minimum area of 0.05 hectares and does not meet the definitions of afforestation and reforestation.

Although not explicitly specified in the Kyoto Protocol, it is generally recognised that afforestation and reforestation are due to a more active human intervention (e.g. direct tree plantation), which exerts a pressure on the land. On the contrary, revegetation is commonly interpreted as the result of a more passive human cause, such as the release of a previously exerted pressure, which allows natural processes to take place.

A *secondary succession* is a particular type of revegetation process characterised by the re-emergence of the plant species that were present locally beforehand. The secondary succession process therefore leads to a progressive vegetation trend towards a climax stage that depend to the site environmental characteristics (Tasser and Tappeiner, 2002).

Woody encroachment or *woody vegetation invasion* is a form of land cover change that leads to the emergence of woody vegetation. This particular expression has been used extensively to describe a vegetation succession leading to the emergence of shrubs and tree species in Central and North America (see, for example, Jackson *et al.*, 2002; Cable *et al.*, 2009; Hollister *et al.*, 2010; Pino and Wilson, 2011). A woody encroachment is not necessarily a secondary succession, if the woody vegetation was not the natural vegetation present originally (e.g. an example of a woody encroachment that is not a secondary succession is the increase in altitude of the tree-line following global warming). However, the same terminology is also used in studies that describe secondary successions in forests of temperate (e.g. Alberti *et al.*, 2011) and inter-tropical regions (e.g. Chiti *et al.*, 2016). Therefore, in the present work, the expressions secondary succession and woody encroachments will be used as synonyms. It is possible that in the literature other authors have used slightly different terminologies to describe the same process, i.e. a natural development of forest following the release of anthropic pressure (e.g. the term *forest expansion*, used by Guidi *et al.*, 2014a).

All the processes described above could follow the abandonment of a land that was previously used for a broad range of different activities. It is reasonable to expect that the land use preceding the succession process can have an effect on the soil ability to absorb or release GHGs. Therefore, some studies distinguish the processes based the different previous land use (Post and Kwon, 2000; Guo and Gifford, 2002; Poeplau *et al.*, 2011). Although these reviews have highlighted differences, other studies tend to group together processes following different land uses, making it more difficult to interpret to obtained results.

According to the IPCC (2003), the terrestrial ecosystem contains five C pools:

- *Aboveground biomass*, consisting of all living biomass above the soil that takes the form of stem, stamp, branches, bark, seeds and foliage;
- *Belowground biomass*, consisting of all living biomass of live roots (though fine roots are often excluded);
- *Deadwood*, i.e. the non-living wood biomass;
- *Litter*, consisting of all non-living biomass smaller than a certain size threshold; and
- *Soil organic carbon (SOC)*, which includes the organic carbon present in mineral soil and in organic soil.

The five pools are typically grouped in three categories:

- *Living biomass* (aboveground and belowground biomass);
- *Dead organic matter* (deadwood and litter); and
- *Soil*.

As previously expressed, among all the C pools, the soil is the most considerable one (Scharlemann *et al.*, 2014). The soil is a complex, multiphase and multicomponent system with an intrinsic spatial heterogeneity. Physical, chemical and biochemical processes are able to modify its characteristics and its ability to stabilise and destabilise the organic C included in it (Sollins *et al.*, 1996; Doetterl *et al.*, 2016). Therefore, in order to correctly estimate the SOC amount it is important to understand which are the main processes that can affect its dynamics. The net concentration of SOC is the result of the two effects of C input and output flows. According to Sollins *at al.* (1996), the organic C reaches the soil trough litter deposition (above and belowground), roots exudates, dissolved organic carbon transported by means of the throughfall and sediment deposition. On the contrary, the organic C leaves the soil system by means of respiration (essentially heterotrophic), leaching, erosion and other organic gasses losses.

6. STATE-OF-THE-ART

6.1. Land-Use Change

From the beginning of its appearance, the human population has transformed between one-third and one-half of the land Earth planet (Vitousek *et al.*, 1997). A significant land transformation process – due to LUC – has occurred after the industrial revolution (Goldewijk and Battjes, 1997) and the causes are basically linked to the response of the people to economic opportunities (Lambin *et al.*, 2001). In more recent times, especially after the Second World War, the changes in land use were documented all around the world and were essentially linked to the abandonment of agricultural practices (Guidi *et al.*, 2014a).

Focusing on the European area, according to Fuchs *et al.* (2013), the 15.5% of the EU27 + Switzerland surface (approximately 700,000 km²) was subjected to a land-cover change between 1950 and 2010. Höchtl *et al.* (2005) confirmed that after the Second World War a wide part of the European rural territory was subjected to the progressive abandonment of agricultural practices. When only the Mediterranean country are considered, the value of the land-cover change increases to 19% (Fuchs *et al.*, 2013). Moreover, according to Tappeiner *et al.* (2008) and Zimmermann *et al.* (2010), the areas more subjected to this phenomenon in Europe are the mountain territories where, usually, the land abandonment leads to a new forest establishment.

A similar trend was also described for the Italian territory. Indeed, Agnoletti (2005) observed that the forest surface has constantly increased from the Second World War until the present and that the actual coverage has more or less doubled with respect to the first decade of 1900. According to Piussi (2002), Corona *et al.* (2005) Falcucci *et al.* (2007), after the '50s, the forest vegetation cover has increased, especially in the Alp and Apennine ridges. Similar phenomena were described in localised areas of the Alps by Tasser and Tappeiner (2002), Höchtl *et al.* (2005), Tasser *et al.* (2007), Tappeiner *et al.* (2008). The forest cover increment appears due to the progressive decrease of the demographic pressure in the mountain territories (Falcucci *et al.*, 2007), which has operated differently at different elevations (Busi, 1973). The consequence was a scattered – but also widespread – woody plant species invasion on the previous pastures and croplands, especially in the less accessible and less productive areas (Tappeiner *et al.*, 2008).

6.2. Establishment of woody plant species

In addition to a transformation of the species composition and modification of ecological dynamics, typical of any LUC, a process that leads to the establishment woody plant species, either artificially or naturally, exhibits also an increase in the structure and biomass of the plant community (Thuille and Schulze, 2006; Risch *et al.*, 2008; Guidi *et al.*, 2014a) and an alteration of SOC stocks and of the different SOC fractions (Alberti *et al.*, 2008, Guidi *et al.*, 2014a, 2014b). Indeed, according to the results of Post and Kwon (2000), Guo and Gifford (2002), and Poeplau and Don (2013) the woody plant species establishment over cropland tends to lead to a significant increase in the SOC stock. On opposite, according to the results of the same authors, the changes in the SOC stock from pastures to woody plant species establishment are not as predictable as those on cropland, and more recent studies show discordant results. Indeed, according to the literature, the variation in the SOC stock can be negative (Thuille *et al.*, 2000; Alberti *et al.* 2008; Guidi *et al.* 2014a; Thuille and Schulze, 2006; Pinno and Wilson, 2011; La Mantia *et al.*, 2013), positive (Brejda, 1997; Feldpausch *et al.*, 2004; Montané *et al.*, 2007; McKinley and Blair, 2008; Fonseca *et al.*, 2011; La Mantia *et al.*, 2013; Chiti *et al.*, 2016) or statistically not significant (Risch *et al.*, 2008; Hiltbrunner *et al.*, 2013; La Mantia *et al.*, 2013).

Despite the large number of studies of woody plant species establishment over cropland or pasture / grassland, only a few of them considered, in addition to the soil, the other ecosystem C pools (Alberti *et al.*, 2008; Pinno and Wilson, 2011; Thuille and Schulze, 2006; Risch *et al.*, 2008; Hiltbrunner *et al.*, 2013; Guidi *et al.*, 2014a).

Although the previous land use is recognised as a key factor in determining the changes on SOC stock over the woody plant establishment process, other factors could also play a secondary, though still important, role. One of these is whether the woody vegetation is planted (i.e. afforestation / reforestation) or it is the result of a natural process, i.e. a woody encroachment or secondary succession (hereafter used as synonyms). The main reason is that the afforestation and reforestation generally involve a site preparation, which increases the soil organic matter mineralisation (Guo and Gifford, 2002; Don *et al.*, 2009; Poeplau *et al.*, 2011; Guidi *et al.*, 2014a).

In general, the number of studies focusing on afforestation and reforestation over pasture is significantly larger than those involving secondary succession processes. This is true not only at an international level (Guo and Gifford, 2002), but also on the Italian territory, where the vast majority of the studies focus on the Alp ridge and on afforestation and reforestation (Thuille *et al.*, 2000; Thuille and Schulze, 2006; Risch *et al.*, 2008; Hiltbrunner *et al.*, 2013). As for all successions leading to the establishment of woody plant species following croplands abandonment, these studies confirm the general loss of SOC stock when pastures are followed by an afforestation or reforestation, as described in the reviews by Guo and Gifford (2002) and Poeplau *et al.* (2011). However, this is not the case in general when pastures or grassland are followed by a secondary succession, and while the only Italian studies that focus on secondary successions over pastures or grasslands on the Alps (Thuille and Schulze, 2006; Alberti *et al.*, 2008; Guidi *et al.*, 2014a) still find a loss in SOC stock, similar studies in Sicily show discordant results (La Mantia *et al.*, 2013).

As previously described, among the LUC typology, woody plant encroachment into pastures and grasslands has been representing a significant phenomenon, particularly in the Italian mountain areas after the Second World War (Alberti *et al.*, 2008). In effect, Fuchs *et al.* (2013) estimated that – between 1950 to 2010 – 7.64% of the Southern Europe land was subject to this process. According to Corona *et al.* (2012) and Marchetti *et al.* (2012), between 1990 and 2008, the extension of pastures have been reduced by an amount equal to 1.07% of Italian land area. However, although a few studies mentioned above have focussed on this process in the Alps, no published studies have been found to focus on the Apennine region (although one is in preparation: personal communication).

Considering the effect on SOC stock change along the woody species establishment, Lal (2004), Paul *et al.* (2002), Alberti *et al.* (2011) and La Mantia *et al.* (2013) jointly stress the relevance of climate variables, in particular the annual precipitation (Guo e Gifford, 2002; Jackson *et al.*, 2002; Alberti *et al.*, 2011), the orography and substrate morphology, soil chemistry properties, clay content, natural and anthropogenic disturbances, the previous land use, its management and its age; the species of the new forest formation and the time from the abandonment.

The studies that have focussed on the impact of climate on SOC changes over woody plant species establishment are either reviews (e.g. Guo and Gifford, 2002) or are based on the pair-site comparison approach, i.e. only observe the difference in SOC between two time points (the woody plant species area and the previous land use). Therefore, in order to fill in the knowledge gaps highlighted above, it was deemed important to find different study areas in the Italian Peninsula that:

- results from a woody encroachment process over pastures or grasslands,

- are subject to different climatic conditions, and
- would also take into account the different stages of the secondary succession process.

6.3. The chronosequences approach

Studying a secondary succession requires working with observations temporally far apart from each other. According to Piussi (2000), the rate of a woody encroachment is generally quite slow and could be extremely variable on the basis of several factors. Among them, the most important ones are: the degree of the environmental change that has to occur before a plant community could replace the previous one; the productivity and the efficiency of the plant species that participate in the process; the longevity of these species; and the resistance of each stage community to the invasion of other species. More generally, according to Gleason (1926) and Kimmins (1997), the rate of woody encroachment depends on the migration and environmental selection processes, which firstly affect the rate of the species arrival and, secondly, define the establishing and survival success of specific plant species (Alberti *et al.*, 2009). Therefore, two possible methodologies can be adopted to investigate the secondary successions. One is the *direct* or *diachronic method*, which is based on the delimitation of long-term study areas. These areas have to be studied in different moments over time and therefore the research project has to continue for the same duration. However, when successional processes exceed a reasonable time span for the study development, Walker *et al.* (2010) suggested adopting another solution – the *synchronic method* – which is based on the chronosequence approach (Austin, 1981; Huggett, 1998). All the studies that have focussed on monitoring the SOC stock changes at different points in time over a woody plant species establishment have used this method.

In order to consider a chronosequence as a *space-for-time* substitution it is necessary that younger stages (chronostages) have developed and are developing as the older ones had (Walker *et al.*, 2010), and that all forming-factors are constant, with the exception of time (Huggett, 1998). In addition, based on how long it is thought the mineral soil C pool takes to reach a steady-state, the IPCC (2003) suggests it should be proved that the land has been used as a pasture for at least 20 years before the start of the LUC process. Under ideal conditions, when all such assumptions can be satisfied, the chronosequence approach is the best instrument to study a long-term dynamic process, such as a secondary succession. In fact, this methodology allows excluding the effect of the climatic variability among the chronostage and the environmental heterogeneity. Therefore, the only variable remaining is the time, which drives the vegetation changes, which in turn determine the quantity and quality of organic matter input and output flows.

However, two main difficulties linked with the application of the chronosequence approach had to be faced in the present work. The first one is the identification of the areas interested by a process of woody encroachment over abandoned pastures, because they are typically small and highly fragmented. The CORINE Land Cover (CLC) inventories, with a resolution of 5 ha (EEA, 2012), are often too coarse for the detection and monitoring of the different species involved in the successional phases. On the other extreme, the *Inventario dell'Uso delle Terre d'Italia* (IUTI) inventory system provides land use observations at specific points across the Italian Peninsula, rather than information on land areas. Although on the basis of this data it is possible to evaluate an aggregate, national-level measure of land use and its change between 1990 and 2008 (Corona *et al.*, 2012; Marchetti *et al.*, 2012), the lack of information concerning the land use between points makes it impossible to use this tool to identify the land use in the different stages of a potential chronosequence. Therefore, with only these two data sources, it would be excessively difficult to identify suitable sites for the application of the chronosequence approach. Fortunately, Alberti *et al.* (2008) observed

that, on the Italian territory, the secondary successions over abandoned pastures have spread sporadically on the mountain areas. This suggests mountain regions as promising areas where to look for examples of woody encroachment over abandoned pastures.

Therefore, the solution for chronosequence identification adopted in this work was to combine interpretation of historical airborne photograph collection for a specific territory (Walker *et al.*, 2010) with literature consultation, collection of direct information by local population and on-field surveys (Guidi *et al.*, 2014a).

A second difficulty faced in this work was that near-ideal conditions for the application of the chronosequence approach are easier to find over flat regions. On the contrary, the main assumptions of the chronosequence approach were challenged by the intrinsic small-scale landscape heterogeneity that characterised the studied mountain areas. Indeed, even if chronostages are defined in a close-range area, it is possible that some small-scale variability could affect the conceptual model of Sollins *et al.* (1996), which, as described above, takes into account organic matter input-output flows and C stabilisation/destabilisation processes. For example, according to Yiemer *et al.* (2006) and Sigua and Coleman (2010), slope aspect affects microclimatic conditions – due to different radiation input – and, therefore, net primary productivity, which in turn affects the organic matter input-output flow. In addition, slope steepness can have an effect on erosion processes and, consequently, SOC removal and/or deposition (Liu *et al.*, 2006; Miller *et al.*, 2015; Doetterl *et al.*, 2016), although in some cases a positive correlation was observed between slope steepness and SOC content (Hontoria *et al.*, 1999 Wang *et al.*, 2012; Campos *et al.*, 2014), while a negative correlation was measured in others (Liu *et al.*, 2006; Singh *et al.*, 2011; Chuai *et al.*, 2012; Muñoz-Rojas *et al.*, 2012; Wang *et al.*, 2012).

6.4. Vegetation and microbiology

As previously described, the main parameter that changes along the secondary succession is the vegetation. According to Hiltbrunner *et al.* (2013), most of the studies focussing on the SOC stock changes along secondary succession or afforestation over abandoned pastures do not take into account the processes that could take place during the LUC.

A first effect of the vegetation change is an alteration of the microclimatic conditions due to the closure of the woody plant species canopy. In addition to the closure of the plant canopy, the increase in the plant biomass and plant height reduces the solar radiation that reaches the soil (Yannarell *et al.*, 2014). Therefore, the woody plant species increases the shading effect and, consequently, the soil temperature (Smith and Johnson, 2004), especially in the vegetative period.

A second effect is the variation in the pathway through which the litter reaches the soil system and a variation of its quantity and quality (De Deyn *et al.*, 2008). According to Poeplau *et al.* (2013), the woody plant species on previous grasses vegetation tend to reduce the aggregate formation and increase the particulate organic matter (POM) amount, with a consecutive reduction in the organic matter stability in the soil. According to Condon and Newman (1998), along the woody encroachment the C/N ratio (i.e. a indices of organic matter quality) tends to increase simultaneously with the amount of lignin produced by the woody species. Therefore, the soil organic matter (SOM) quality tends to decrease steadily in the transition from pasture to woody plant establishment, even if each species specific variability could also play a major role. Moreover, according to Guo and Gifford (2002) and Solly *et al.* (2013), the tree root system lives for many years and, therefore, the annual fine root turnover drastically decreases when comparing grasses species with trees, probably because of a reduction in the litter quality.

Both the alteration in the microclimatic conditions and in quality (and quantity) of the organic matter inputs has a marked effect on the soil microbial community composition and activity. Indeed, because of the former change, according to Smith and Johnson (2004) and Yannarell *et al.* (2014), the reduction of the temperature under woody species causes a significant reduction in the soil organic matter mineralisation by the heterotrophic respiration. Because of the latter change, the reduction of the aboveground and belowground litter quality (due to the increase of C/N ratio) tends to reduce the pH and the nutrient availability for microbes (Miles, 1985; Lucas-Borja *et al.*, 2010). These parameters also affect the soil macrofauna – and in particular the earthworms community – which is one of the main drivers of organic matter translocation along the soil profile. Similarly, Muys *et al.* (1992) observed that the decomposition activity related to the earthworms presence can change along the forest development on a formerly grassland. In particular they observed an increased earthworms' activity in some cases and its reduction in others, and the authors suggested that these differences are due to the changes in both pH and litter palatability. However, in a subalpine area subject to pasture abandonment, Seeber *et al.* (2005) described a decrease in both the macrofauna community abundance and its activity along the encroachment process.

Even if numerous studies of LUC focussed on microbial biomass and activity, the effect of different vegetation covers on the biochemical and microbiological properties of the soil is yet incompletely understood, especially when the changes in the vegetation cover are gradual as in a secondary succession process. This is also due to the fact that the number of studies on this topic linked to the woody encroachment over pastures and grasslands is extremely limited and that they usually refer to arid or semiarid ecosystems (see for example Liao and Button, 2008; Cable *et al.*, 2009; Hollister *et al.*, 2010; García-Morote *et al.*, 2012).

Finally, in addition to an increment of the biomass and a variation in its quality, the secondary succession leads also to a variation in plant species community composition. The ecological indices historically used to measure potential changes in the community composition are the richness – number of the species – and diversity, which in addition to the richness also considers the species evenness (Shannon and Weaver, 1949).

In addition to biomass and its quality variation, several studies have shown how plant diversity may also strongly affect soil functioning linked to microbes (see for example Stephan *et al.*, 2000; Zak *et al.*, 2003; Steinauer *et al.*, 2015). Moreover, Tillman *et al.* (1997), suggest the use of a functional diversity index that takes into account the functional traits of the organisms, highlighting the fact that it can be linked to an increasing exploration of niches and optimisation of the productivity of the ecosystem. Furthermore, Stephan *et al.* (2000) observed that the increase in the plant functional type diversity could have a positive effect on the overall microbial catabolic activity. Unfortunately, all these studies were referred to grassland environments and none of them was focused on the woody encroachment process.

7. AIMS OF THE STUDY

As it emerges from the literature, although the abandonment of agricultural practices is a widespread phenomenon, the understanding of the effect of this process is still incomplete. This is even more so in the case of natural encroachment of the woody plant species over abandoned pastures and grasslands.

Therefore, the aim of this study was to investigate the effect of woody encroachments over abandoned pastures and grasslands on the terrestrial ecosystem C pools in different stages over time. The main effort was focussed on the estimation of the organic C and N stocks in the soil system over at least 5, and in some cases 6, successional stages for each chronosequence. Indeed, the soil is characterised both by a great C storing capacity and by the fact that its C pool reacts slowly to the pressures. On the contrary, the living biomass and dead organic matter C pools can generally be lost faster than the soil C pool because of harvesting, human removal and fires. However, living biomass and dead organic matter pools were also estimated, though only at three time points along the woody encroachment process. Finally, on the same three time points, soil microbial biomass and enzyme activities were investigated in order to examine the soil system more in detail.

Seven study areas were selected along the latitudinal gradient of the Italian peninsula in order to understand better the dynamics of this process in the different parts of the national territory. Indeed, according to Alberti *et al.* (2011) an evaluation of the SOC changes occurring during a secondary forest succession is still lacking in the central and Southern part of Italian peninsula. In addition, the latitudinal gradient of the peninsula gave also the possibility to consider the effect of the different climatic parameters on the process.

This work is valuable not only scientifically but also for future political planning. Indeed, the results of this research could be used to refine the national forestry inventory and should be taken into account in the estimation of the sink/source effect of the ecosystems subjected to a LUC, as requested by the European (decision n. 529/2013/UE) and international level (Kyoto Protocol).

8. MATERIALS AND METHODS

8.1. Sites selection

The study areas were identified using the following steps:

1. Information on potential woody encroachments was collected from local research groups, forest rangers, literature – where available – and from people who live in the possible suitable territories;
2. A list of airborne photos was consulted for each possible site in order to verify the truthfulness on the information collected during Step 1. Recent images (from around 1988) were obtained from the *Geoportale Nazionale* website (MATTM, 2015) or from regional cartographic websites. Older images were consulted from the *Istituto Geografico Militare* (2014) website.
3. Among all consulted images, only a few of them, selected as best describing the secondary successions, were downloaded on a GIS software by means of a Web Map Service (WMS) connection to the *Geoportale Nazionale* website (MATTM, 2015) or from regional cartographic websites, or, if prior to 1988, were bought from the *Istituto Geografico Militare* – IGM (2014) and uploaded on the same software. The time interval between the selected images was not identical among sites, but varied based on factors such as the speed of the woody encroachment process and the availability of images from the sources used.
4. Two extremities for each series (pasture / grassland and forest) were defined comparing the airborne photograph collections and matching them with the information collected in Step 1. The pasture / grassland was considered as the reference stage for the woody encroachment process, while the forest as the stand towards which the process is expected to tend. The temporal sequences of photos of these two areas should show no variation in land cover.
5. Other intermediate stages (three or four) of the process were defined following each airborne photograph series. Each intermediate stage was taken into account only for the areas where, in the first photo or at the beginning of the secondary succession process, a pasture / grassland was present. In addition, each intermediate stage per chronosequence had to be encroached by woody plant species at different times in the past. Discussion with local people was very informative in determining the age of pasture abandonment. However, to avoid relying on anecdotal evidence, the intermediate date between the two subsequent airborne images showing interpretable differences in the canopy cover was considered as the date of secondary succession start for each intermediate stage. The interval between such two images varied among stages, so its half-length was used as a measure of uncertainty in the estimating the stages' "age".
6. A minimum area of 0.5 ha was initially considered as a threshold but, in one site (Chianocco site, described in Section 8.2.3.), it was not possible to respect this assumption. According to FAO (2012), 0.5 ha is the minimum width of woody vegetation that can be considered a forest in the future, even if, for example, one of the intermediate stages did not grow up sufficiently close to the previous forest to merge into it.
7. All selected areas had to be in a close-range (maximum a couple of km apart) to exclude significant lithological and climatic variations between them. The upload of pedological, and phytoclimatic layers (available from the *Geoportale Nazionale*) in the same GIS project of the airborne images and delimited polygonal stages was performed in order to test these conditions.

Therefore, seven sites were selected on Alps and Apennines along a latitudinal gradient in Italy (Tab. 1, Fig. 5A).

Table 1 *List of selected chronosequences with essential biophysical characteristics. Chronosequence names refer to the municipality area in which they are located. Bioclimatic regions refer to those of MATTM (2015). Mean annual precipitations (MAP) and mean annual temperatures (MAT) refer to local estimated data provided by Bologna ISAC-CNR according to the methodology described in Brunetti et al. (2012). Mean annual direct precipitation data for nearest meteorological stations were also summarised (data provided by local Regional Environmental Protection Agency – ARPA –, civil defence – Protezione Civile – and Ferretti (2014)).*

Site name	Coordinate (WGS 84) Lon / Lat decimal deg	Elevation mean m.a.s.l.	Soil type WRB (2015)	Bioclimatic region	MAP mm yr ⁻¹	MAT °C	Direct MAP mm yr ⁻¹
Castello Tesino	11.650 / 46.125	1700	Phaeozems	Temperate oceanic	1286	4.6	1185
Mel	12.071 / 45.969	1250	Cambisols	Temperate oceanic / semi- continental	1670	6.5	1428
Chianocco	7.202 / 45.177	1100	Cambisols	Temperate / semi-continental	967	8.4	806
Firenzuola	11.320 / 44.140	890	Cambisols	Temperate oceanic / semi- continental	1620	10.1	1128
Farindola	13.783 / 42.433	1160	Phaeozems	Transitional temperate oceanic / temperate semi-	1136	9.8	834
Vastogirardi	14.228 / 41.772	1090	Chernozems	Temperate oceanic/semi- continental	1039	10.0	1080
Pignola	15.819 / 40.583	1040	Phaeozems	Transitional temperate oceanic / temperate semi-	957	11.0	694

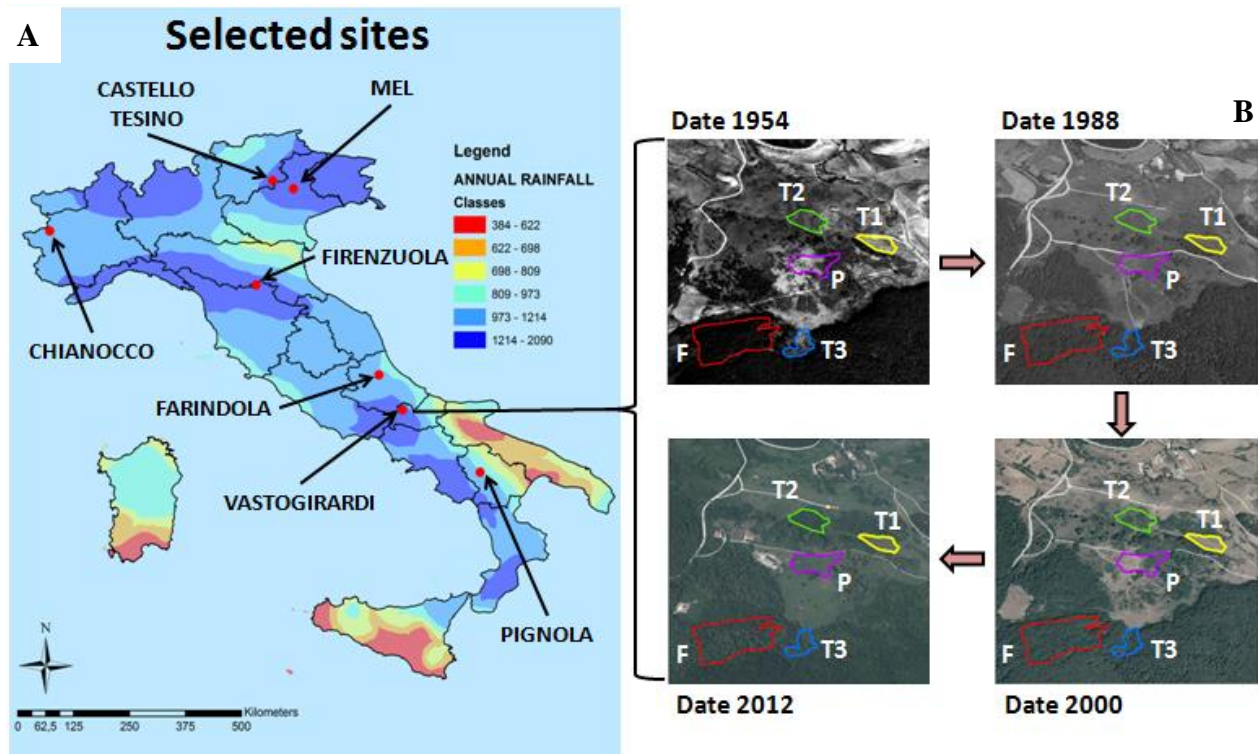


Figure 5 A) Selected sites along the Italian latitudinal gradient. Colour gradient refer to a coarse rainfall spatial distribution classification. Additional details are reported in Tab. 1.
 B) Vastogirardi chronosequence, composed by five stages: a pasture (P), a pastured abandoned 3 to 10 years before present (T1), a pasture abandoned 10 to 15 years before present (T2), a pasture/grassland abandoned 34 to 60 years before present (T3) and a forest (F).

For each selected site a local IGM 1:25000 topographic map (available from MATTM (2015)) raster file was uploaded in a GIS environment. Contour lines were drawn on a new polyline shape-file, and a local Digital Elevation Model (DEM) was built up. In each delimited chronostage, three sampling circular areas were delimited around a central point. The centre of each area coincided with the central point of each plot selected for the soil sampling design (see below) and the radius was fixed as the double the length of the side of each squared plot. On the basis of the site-specific DEM, a mean value in each circular area was estimated for exposure and steepness. Finally, a qualitative description for slope position and vegetation type was recorded for each stage.

The identification of species was performed using “Flora d’Italia” (Pignatti, 1982) and actualised using The Plant List (2013).

8.2. Sites description

8.2.1. Castello Tesino

Castello Tesino chronosequence is located in the North-East Alps ridge in the Trento province (Trentino-Alto Adige administrative region) on the Southern slope of Monte Tolva. The selected area extends over the Alpine huts of Malga Cavallara and Malga Telvagola, on the Northern side of the street that connects Val Malene to Passo Brocon, just before the cross with the road for Castello Tesino village.

The ombrothermic diagram of Bagnouls and Gaussen (1957) according to Walter (1984) is shown in Fig. 6. According to the shapefile available on the WebGIS (i.e. the GIS available online) of the MATTM website (2015), the site is in the temperate oceanic climate class, which consists of a constant abundant precipitation trend throughout the year. According to the diagram in Fig. 6, the precipitation trend is always higher than those of temperature and, therefore, no dry stress periods are present. The peak precipitation was observed in May, with high precipitation levels continuing throughout the vegetative period. Minimum monthly precipitation values of approximately 60 mm were observed in winter, and a frozen period was always observed between December and March.

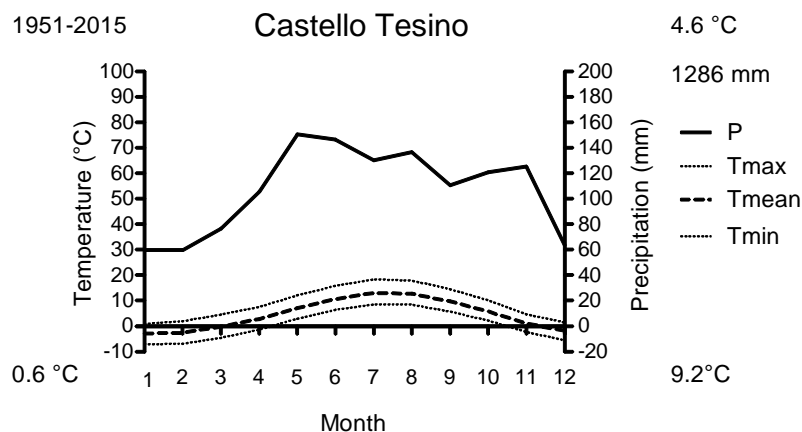


Figure 6: Ombrothermic diagram of Castello Tesino based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

The bedrock is calcareous, with a predominance of micritic limestone and clay-rich micrites. However, granitic rock agglomerates are also present, probably transported by landslides or collapses of rocky materials from the upper part of the slope. Indeed, according to the geolithologic map, the main rock compound of the Monte Tolva has a plutonic or metamorphic origin.

Generally, the soil is not well-developed because of the typical steep slope of the area. The soil depth reaches 60-70 cm but it could be greater especially where the steepness is lower and the pastures are still present. The main soil type is a Lepthic Phaeozems, which is common for grassland and forest in moderately continental climates. In addition, it is characterised by a dark and humus-rich surface and an intense leaching (IUSS Working group WRB, 2015).

The chronosequence has a mean elevation of 1700 m.a.s.l. and a predominant S-SE exposure. The steepness is extremely marked, especially in the less productive areas where the rearing activity has not been continued or was not practised at all. In this site, six stages were selected. Table 2 summarises the stages' main characteristics, while their spatial arrangement is shown in Fig. 7.

Table 2: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations ($n=3$ replicates).

Characteristic \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 3 (T3)	Encroached time 4 (T4)	Forest (F)
Age	0	9 (6)	19 (3)	28 (1)	52 (10)	> 62
Elevation	1700	1650	1900	1450	1750	1750
Aspect	S	SE	S	SE	S	SE
Steepness	26.24 (5.34)	48.85 (6.9)	62.82 (5.68)	27.9 (3.78)	64.13 (8.29)	71.01 (10.4)
Slope position	Footslope	Backslope	Backslope	Footslope	Backslope	Backslope

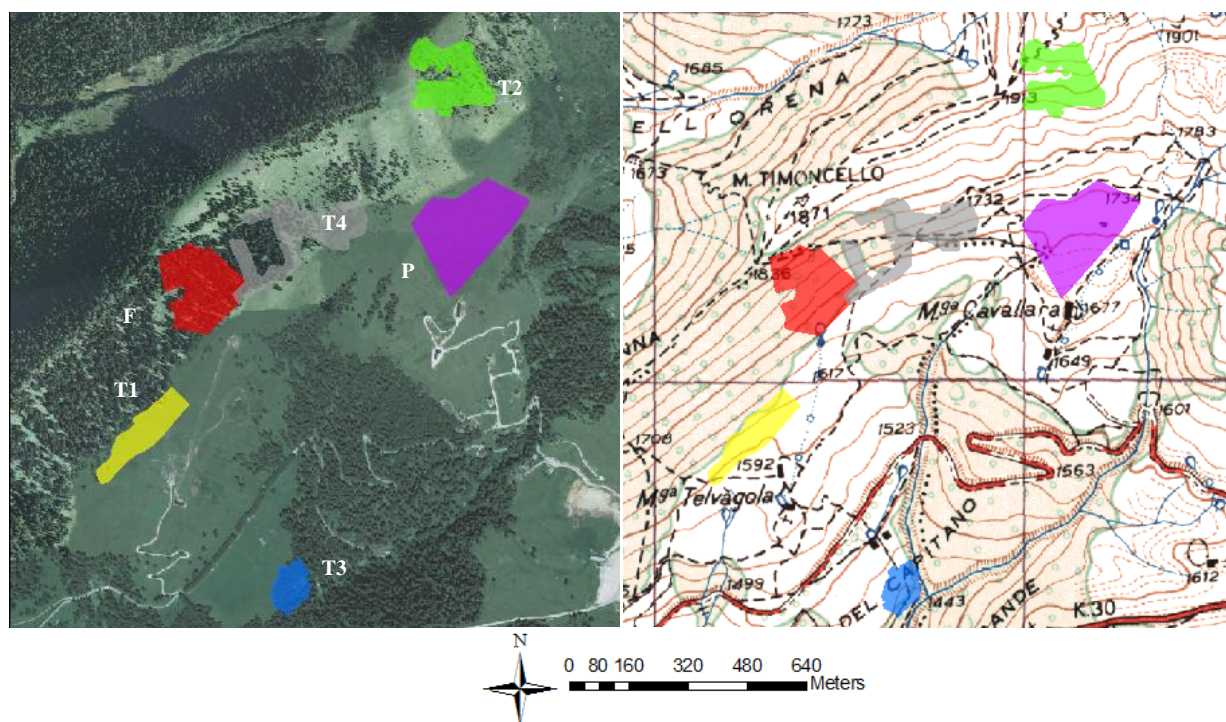


Figure 7 The Castello Tesino chronosequence (see Tab. 2), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab.2.

The pasture stage is an active cow summer pasture, generally used from May or June to September. On average, the number of livestock is one per hectare (shepherds, personal communication). The younger encroached stage is an active pasture with – more or less – the same livestock density, but with a widespread presence of young *Picea abies* (L.) H.Karst. and *Larix decidua* Mill. tree plants. The intermediate stage, encroached 19 years before present, is the most heterogeneous of the chronosequences and all the plant functional type are present: grasses, shrubs and trees. In addition to *P. abies* and *L. decidua* the other woody plant species are *Rhododendron hirsutum* L., *Calluna vulgaris* (L.) Hull, and

Juniperus communis L. The other stages, those of 28, those of 52 and more than 62 years old are forests. The first one has a young and dense monospecific *P. abies* canopy without undergrowth vegetation and was used in the past as a patch clearcut without artificial regeneration by the local population (Forestry corps, personal communication). The two older forest stages are similar, with a mix *P. abies* and *L. deciduas* tree vegetation and a few gaps caused by tree falls due to the high steepness values, winter snow pressures and small avalanches.

8.2.2. Mel

The study area is located in the North-East Alps, on the first mountain ridge of the Alps chain (Prealpi) between Belluno and Treviso province (Veneto administrative region). The whole site is included in the *Dorsale Prealpina tra Valdobbiadene e Serravalle* (IT3240024) Special Protection Area (SPA). The stock grazing activities in this area have been sustained by the population of the nearest Eastern Po plain (just South of the study area) and have reduced over time. At present, active livestock grazing is limited to the grasslands, which circumscribe the Malga Mont Alpine hut, near to the Monte Crep peak. The grazing pressure is approximately of one cow per hectare (shepherd personal communication).

According to the WebGIS (MATTM, 2015), this area is characterised by a temperate oceanic/semi-continental climate. Indeed, because of its position, in this area there is a combination of winter rigid temperatures – which are typical of the inner Alps massifs – and very high air humidity and precipitations. These are caused by both the condensation of the Po plain wet air, which is pressed to the Southern Alpine ridges, and by humid winds which blow from the Northern Adriatic Sea. Looking at the ombrothermic diagram of Fig. 8, it is noticeable that the mean monthly precipitations reach, for ten consecutive months, values higher than 100 mm. On the other side, temperatures follow the classical seasonal trend and are usually below 0 °C in December, January and February.

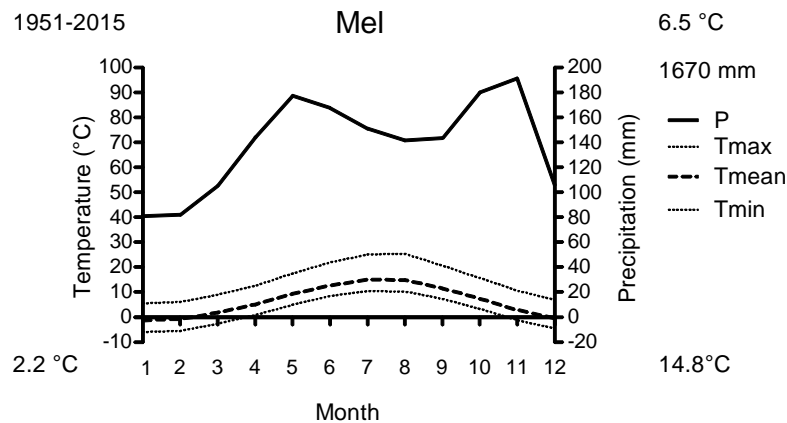


Figure 8: Ombrothermic diagram of Mel based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

A sedimentary calcaric, marly-calcaric and dolomitic lithology is the most common one in this site. The main soil formations are included in the Edoleptic Cambisols (IUSS Working group WRB, 2015). They are characterised by a

reduced subsurface soil formation and are widespread on Alpine and Apennine mountain ridges. Soils reach a maximum depth of nearly 60 cm.

The mean elevation of the chronosequence is approximately 1250 m.a.s.l. and NW is the prevalent aspect. Steepness is variable, from moderate to considerable. Five stages were delimited for the chronosequence arrangement (Tab. 3 and Fig. 9).

Table 3: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations (n=3 replicates).

Characteristic \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 4 (T4)	Forest (F)
Age	0	5	29 (2)	40 (8)	> 62
Elevation	1325	1270	1260	1250	1225
Aspect	N	N	W	N	NW
Steepness	31.6 (9.91)	29.97 (8.35)	53.07 (11.2)	38.63 (3.26)	29.21 (5.04)
Slope position	Shoulder	Shoulder	Backslope	Backslope	Backslope

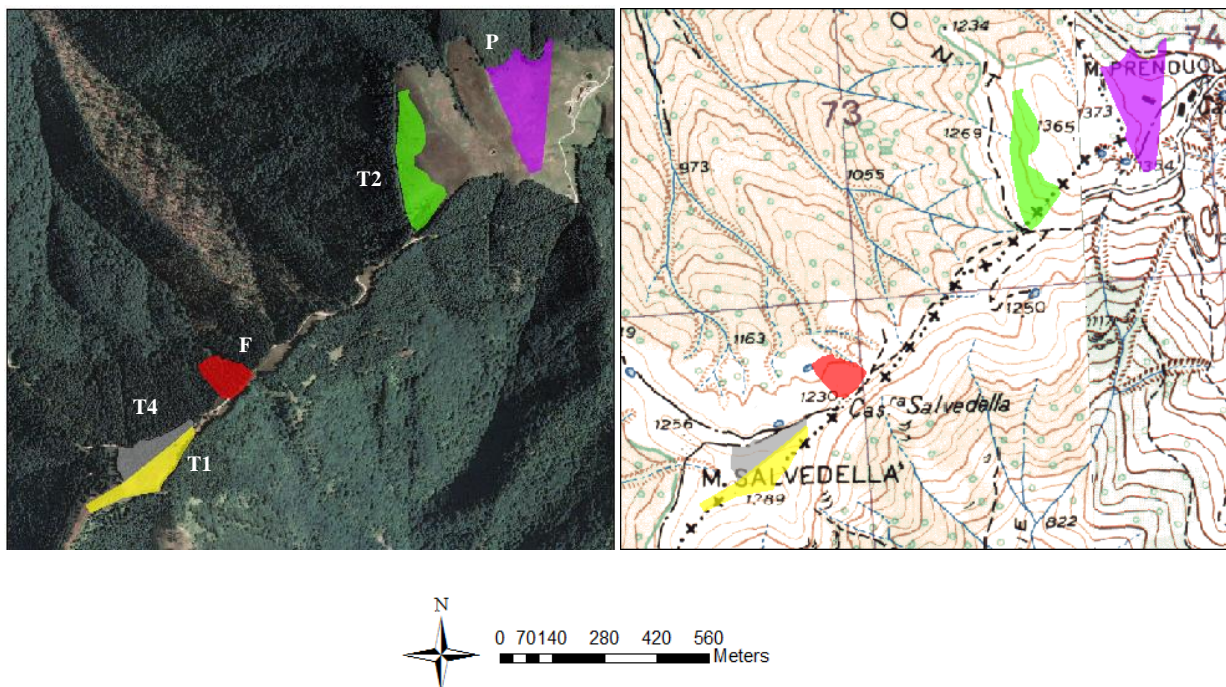


Figure 9 The Mel chronosequence (see Tab. 3), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab.3.

The pasture stage is managed and livestock grazing is restricted to the summer period (May – September). On average, the number of livestock is one per hectare (shepherd personal communication). The younger encroached stage is an abandoned pasture with annual and perennial grasses species. Grazing patches, typical of wild boar were observed during fieldwork. The intermediate stage (T2), where the encroachment started around 29 years before, is included in the Malga Mont enclosure. Even if shrubs and young trees are present, the faeces observed on the field prove a widespread wild grazing activity. Shrubs species were identified as *Rubus idaeus* L., *J. communis*, *Corylus avellana* L. and *Sorbus aria* (L.) Crantz. The only tree species is *P. abies*. The older encroached stage and the forest are the results of a monospecific anthropogenic *P. abies* reforestation. While under the tree canopy of older encroached stage there was a dense *Vaccinium myrtillus* L. population, under the forest stage no undergrowth vegetation was present. Indeed, afforested areas are generally characterised by an initial losses of SOC due to the site preparation, rather than an increase as in the case of a secondary succession. However, in the present case, only the oldest stages were subjected to a human-induced reforestation and it could be argued that, once a stable forest stage has developed, all previous SOC dynamics have reached an equilibrium condition which is unaffected by whether the reforestation was natural or human-induced. Therefore, this study area was not excluded from the current research.

8.2.3. Chianocco

Chianocco site is located in the North-West Alps ridge, in the Turin province (Piedmont administrative region). The site is nearly halfway through the Val di Susa. It is an East to West valley, which connects Turin city to the higher Alps massifs on the Italian-France border. Chianocco village is on the base of the Southern aspect slope of the valley, just below the Grand Uia peak. The chronosequence has a mean elevation of 1100 m.a.s.l.

The ombrothermic diagram on Fig. 10 shows that in this site, the precipitation trend is lower than those of Castello Tesino and Mel. In addition, two main precipitation peaks are present: one in the spring and one in the autumn. The frost period, which is not always present, is generally limited to January. No summer dry stress period is detectable, even if July is one of the driest months of the year. According to the WebGIS (MATTM, 2015) climate map, the site is characterised by a temperate semi-continental climate.

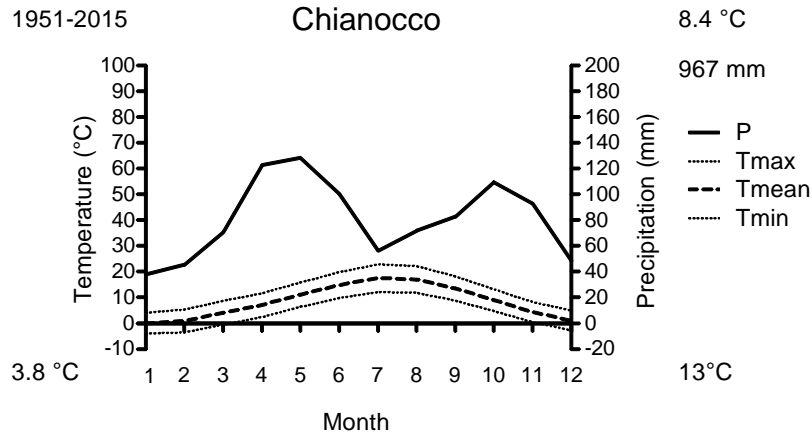


Figure 10: Ombrothermic diagram of Chianocco based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

The bedrock in the site is mainly based on shists, which derived from the limestone and marl metamorphose after the Jurassic sedimentation. Other rock materials derived from the translocation or the collapse of parent material of the upper part of the slope, where the lithology has to be included in the serpentine category.

The site is included in the *Western and central Alps on igneous and metamorphic rocks* pedological region and the main soil type is a Skeletic Cambisols (IUSS Working group WRB, 2015). The average soil depth reaches no more than 70 cm with a not well-developed horizons differentiation.

The chronosequence has a mean elevation of 1100 m.a.s.l., a mean S-W aspect and it was observed on slopes of steepness from consistent to marked. Five stages were delimited. Ech of them is briefly described in Tab. 4 and the spatial distribution of all the stages is shown in Fig. 11.

Table 4: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations (n=3 replicates).

Characteristic \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 4 (T4)	Forest (F)
Age	0	12 (3)	22 (3)	43 (17)	> 62
Elevation	1200	1200	920	1100	1100
Aspect	SW	SW	W	SW	W
Steepness	37.35 (1.63)	47.49 (3.49)	45.12 (8.36)	40.96 (3.54)	46.89 (5.6)
Slope position	Backslope	Backslope	Backslope	Backslope	Backslope

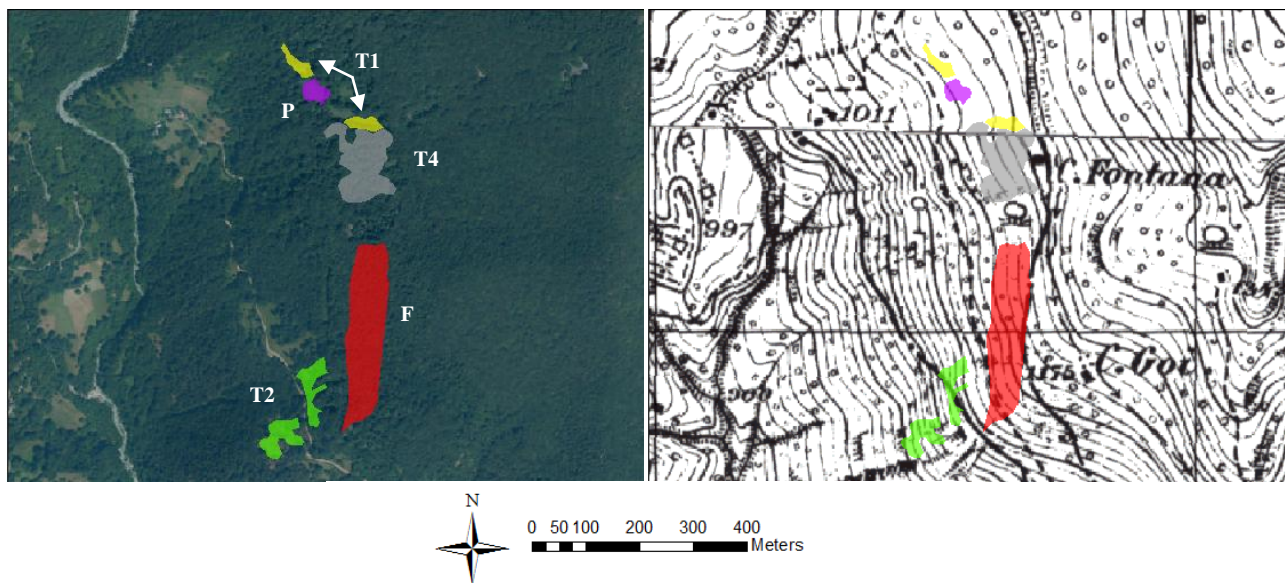


Figure 11 The Chianocco chronosequence (see Tab. 4), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab.4.

The first chronostage is grassland with no evident grazing pressure (neither from livestock nor from wildlife). The second stage is a *Rubus ulmifolius* Schott encroached area with grass and shrub patches. Grass and soil excavations prove the active presence of wild boar. The intermediate stage (T2) is an area with both young and sparse trees and shrubs. A widespread grass cover is present too. The main species are *Tilia cordata* Mill, *Fraxinus excelsior* L., *Quercus pubescens* Willd., *C. avellana* L., *Laburnum alpinum* (Mill.) Brecht. & J.Presl, *Pinus sylvestris* L., *Acer pseudoplatanus* L. and *J. communis*. The oldest stages are both young forests, of different ages. The main tree species are *Fagus sylvatica* L. and *P. sylvestris*. Small grasses patches are present in both these stages.

8.2.4. Firenzuola

The Firenzuola site is located in the northern Apennine ridge in the Florence province, near the border between Tuscany and Emilia-Romagna regions. The closest village to the site is Covigliaio. The chronosequence was arranged on the southern ridge of the Monte Beni peak. All the forests in the surrounding area were harvested before and during the Second World War. Indeed, here there was a part of the German defensive Gothic Line. Therefore, no forests older than seventy years are present. In this territory, the pasture practices have declined after the conflict because of people migrating to the main cities. This decline is still considerable because rural activities did not pass from one generation to the next.

The ombrothermic diagram in Fig. 12 shows a very high precipitation trend. A positive maximum is reached in late autumn, with values higher than 200 mm per month. Instead, the minimum occurs in summer, at the same time of temperatures maximum. Precipitation and mean temperature trend do not cross and therefore no summer dry stress affects the vegetation. The climate WebGIS (MATTM, 2015) map includes this area in the temperate oceanic/semi-continental climate class.

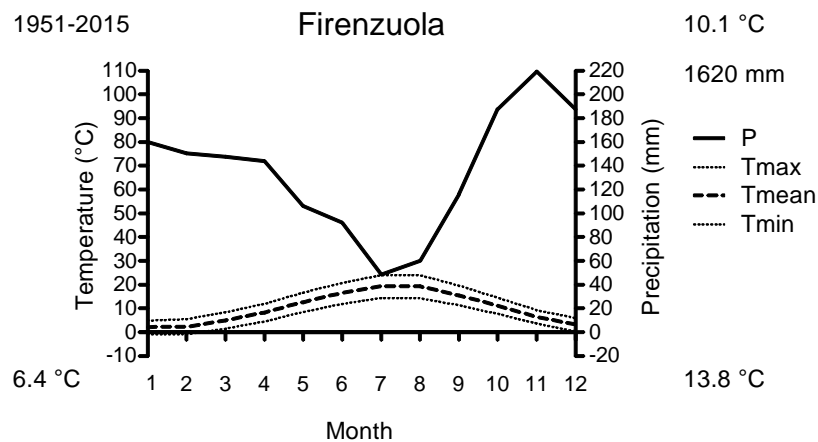


Figure 12: Ombrothermic diagram of Firenzuola based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

The site lithology is chaotic, due to the overlay of different parent material complexes, which have been regimented in different geological period. This bedrock mixing is common for all the chronostages.

Northern and central Apennine is the pedological region that includes this chronosequence. The soil is not well developed even if the slope steepness is not pronounced. The maximum soil depth is approximately 70 cm. The soils of all the stages are described as Eutric Cambisols (IUSS Working group WRB, 2015).

The chronosequence has a mean elevation of 890 m.a.s.l. and the prevalent aspect is S with some small variation among sites. Six stages were delimited, all in close proximity of each other. The main characteristics are summarised in Tab. 5 and the stages spatial distribution is shown in Fig. 13.

Table 5: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations (n=3 replicates).

Characteristics \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 3 (T3)	Encroached time 4 (T4)	Forest (F)
Age	0	19 (4)	25 (3)	33 (2)	64 (3)	> 68
Elevation	875	900	925	860	850	925
Aspect	SE	SE	SW	SE	S	SE
Steepness	15.03 (1.7)	20.23 (5.47)	16.28 (1.51)	37.34 (7.59)	17.44 (3.06)	26.07 (4.27)
Slope position	Backslope	Backslope	Backslope	Backslope	Backslope	Backslope

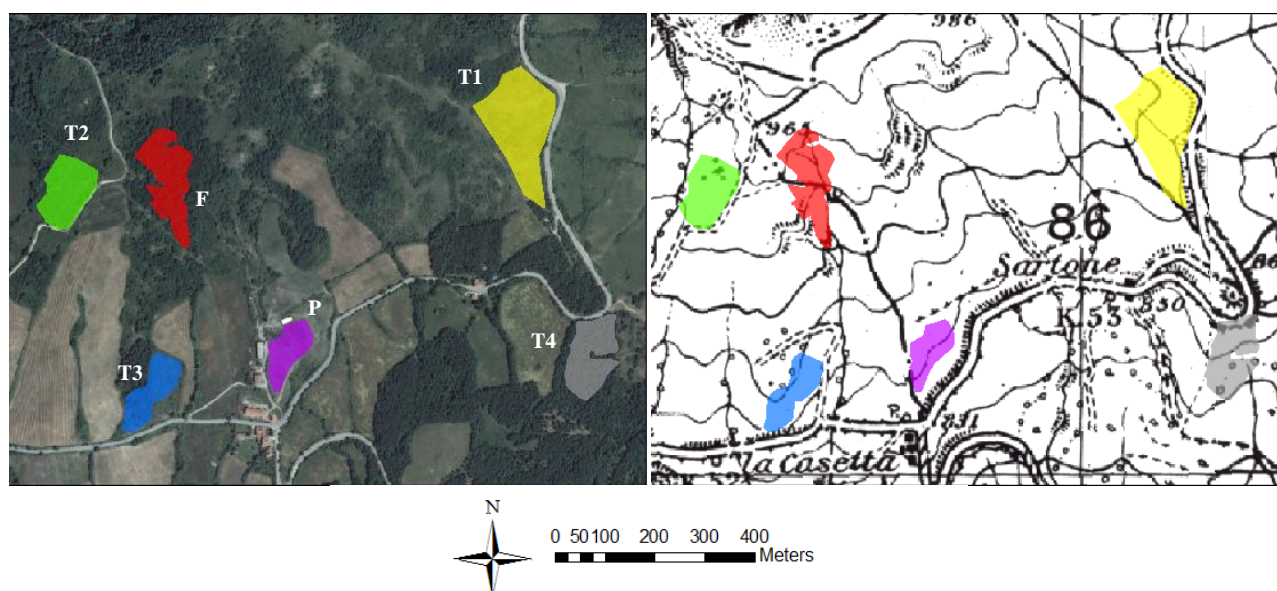


Figure 13 The Firenzuola chronosequence (see Tab. 5), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab. 5.

The pasture stage is included in a fenced area where the cows can graze during the summer period. Although used as a pasture, a few *Rosa canina* L. shrubs are present nonetheless because a regional law prevents the cut and eradication of this species. The first encroach stage is a mixture of grasses and shrubs. The main woody plant species are *J. communis* and *R. canina*. In this stage livestock and wild animals trampling paths are present and it is possible that both of them graze when passing throw this area. In the encroached stage number two, the vegetation is more heterogeneous: grasses, shrubs and young trees are present together. The woody plant species are *Prunus spinosa* L., *Crataegus monogyna* Jacq., *R. canina*, *J. communis*, *Ligustrum vulgare* L. and *Pyrus communis* L. In this stage animal trampling paths are visible too. The 33 year-old encroached stage is similar to the previous one, but the trees species are more abundant and the vegetation cover is denser than in the previous stage. In addition to the species just listed, *Quercus cerris* L. was

present too. This species tends to dominate the woody composition in the last two stages. In the 64 year-old stand it coexists with the *Q. pubescens* and an undergrowth shrubs component is also well developed. On the opposite, in the last chronostage, the tree canopy cover is denser and the shrubs are not so common any more. In addition to *Q. cerris*, the most abundant tree species are *Acer campestre* L., *P. communis*, *Ulmus minor* Mill., *Fraxinus ornus* L. and *Acer opalus* Mill. The poorly represented shrub species are *C. monogyna* and *L. vulgare*.

8.2.5. Farindola

The Farindola site is in the Central Apennine ridge in the Pescara province (Abruzzi region). The area is inside the borders of the Gran Sasso e Monti della Laga National Park and it is situated approximately three km West of Farindola village. All the chronostages are near the Rifugio Tito Acerbo hut. This area, named Rigopiano, is located beyond the Monte Sella peak, at a mean elevation of 1150 m.a.s.l.

The ombrothermic diagram of Fig. 14 shows the seasonal trend both for precipitation and temperature. Two main high rainfall peaks are present: one at the beginning of spring and one at the end of autumn. The minimum precipitation level is in the summer, even if it does not lead to a dry stress event. Mean temperatures never reach values below 0 °C, even if freezing periods could occur in January or February. Maximum temperatures occur in July and August, with mean values that nearly reach the 19 °C. The bioclimatic condition is classified as transitional between temperate oceanic and temperate semi-continental.

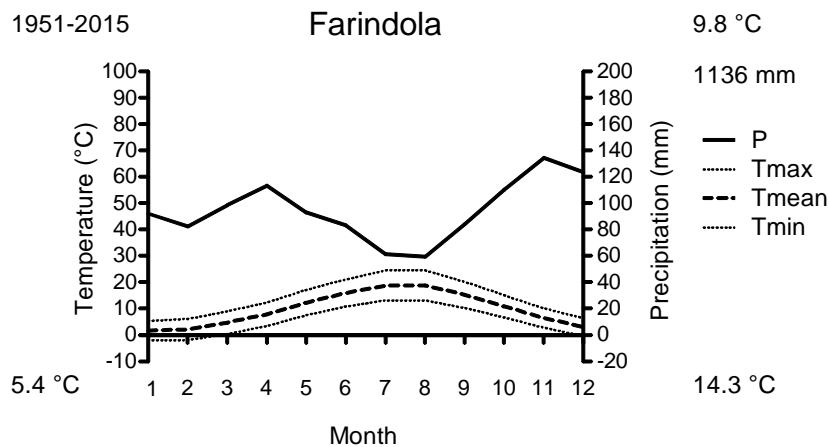


Figure 14: Ombrothermic diagram of Farindola based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

The prevalent lithotype is a mix of limestone and calcareous marls of sedimentary origin. The soil generally is not well developed because of the morphological asperity and the prevalent rockiness typical of the screes. The pedological region is *Apennine relieves on limestone and intra-mountain plains*. The soil, generally only in an incipient formation stage, was described as Calcaric Phaeozems (IUSS Working group WRB, 2015) for all the stages.

The chronosequence mean elevation is 1150 m.a.s.l. and the mean exposure is South. The steepness is generally moderate with lower values for the younger stages. Tab. 6 includes a summary of the stages and Fig. 15 shows the stages spatial distribution.

Table 6: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations (n=3 replicates).

Characteristics \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 4 (T4)	Forest (F)
Age	0	12 (3)	24 (3)	66 (4)	> 70
Elevation	1140	1160	1050	1190	1270
Aspect	SW	S	S	SW	E
Steepness	19.52 (1.53)	28.29 (8.03)	27.94 (4.6)	39.48 (7.48)	32.64 (4.56)
Slope position	Backslope	Backslope	Backslope	Backslope	Backslope

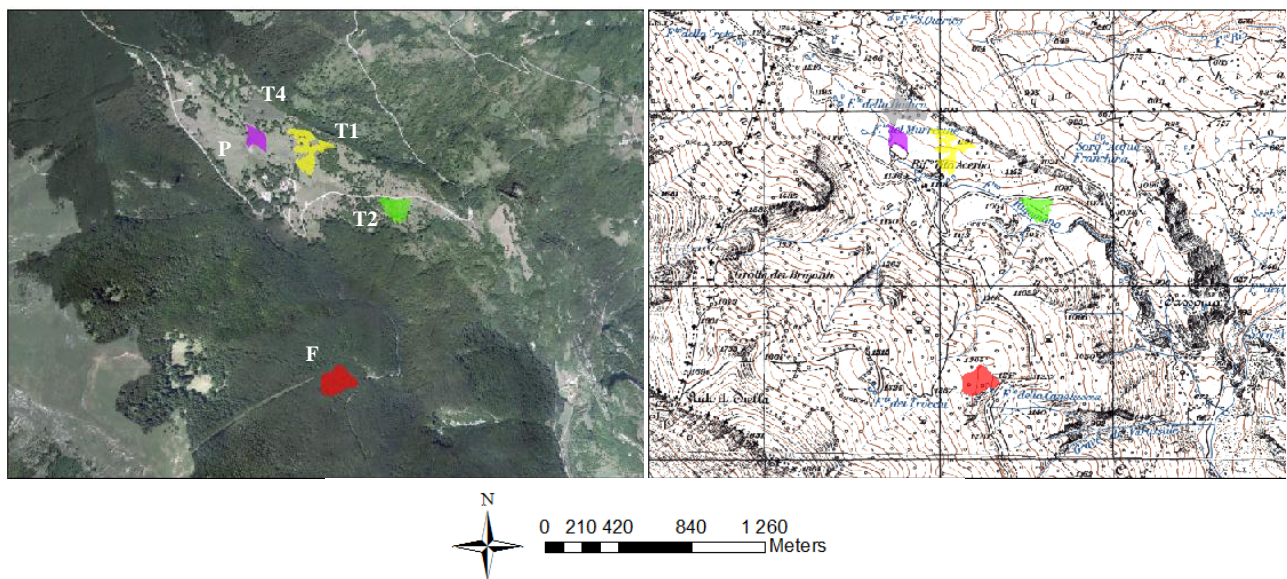


Figure 15 The *Farinfolia* chronosequence (see Tab. 6), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab. 6.

The pasture is a public area where shepherds use to lead the flocks of sheep during the summer period. It is not a fenced area and the sheep tend to pass through it during the daily movement from lower areas to higher altitudes. However, the grazing pressure is generally intense because of the flock dimension (300-400 cattle specimen). The first encroached area is grassland with a few shrubs species. Even if sheep are free to move everywhere, no intense grazing activity was detected during the fieldwork. The most common shrub species are *R. canina* and *P. spinosa*. The second encroachment stage is the most heterogeneous, both for the number of woody species and for the plant life-forms. Indeed, shrubs and

young trees stand over the grass canopy. Most common woody species are: *R. canina*, *C. monogyna*, *R. ulmifolius*, *P. spinosa*, *P. communis* and *A. campestre*. Trampling paths are present, but grazing is limited or absent. In the older encroached stage the *F. sylvatica* is the only woody species and, below the tree canopy, off road vehicles tracks cross the area. The forest stand is a mono-specific beech stand, which was not managed in the recent past.

8.2.6. Vastogirardi

Vastogirardi site is located a couple of kilometres South of the homonymous municipality in the Isernia province, Molise region. The chronosequence lies on a hilly area between a place called Sterparo and one called Valle Rubia.

The ombrothermic diagram in Fig. 16 shows precipitation and temperature seasonal trends. Concerning precipitation, a single maximum is clearly visible in November and a single minimum in August. Mean temperatures range from 5.4 °C in January to 19.2 °C in August. In this month, summer dry stresses, thought uncommon, could sometimes arise. According to the bioclimatic classification, this is a site with a temperate oceanic/semi-continental climate.

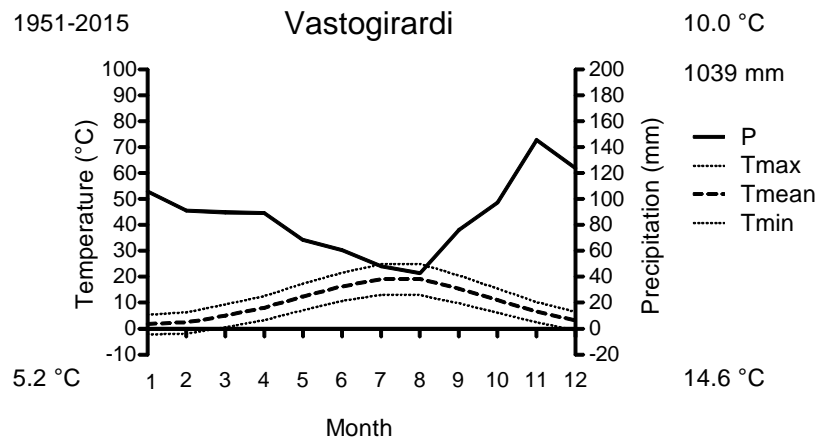


Figure 16: Ombrothermic diagram of Vastogirardi based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

A mixture of marls and calcareous marls is the main parent material. The site is included in the *hills and mountains on limestone covered by volcanic ashes of southern Italy, and included alluvial and coastal plains* pedological region. The main soil type is a Leptic Chernozems (IUSS Working group WRB, 2015), with a typical thick blackish surface layer rich in organic matter. Generally, the soil profile is not well-developed and it reaches a 50-60 cm depth.

The chronosequence has a mean elevation of 1100 m.a.s.l. The stage aspects differ and range from South to North. The site was sampled even if this extreme exposure variation does not satisfy the necessary requisite for application of the chronosequence approach. Steepness values are moderate. Tab. 7 summarises the stage characteristics and Fig.17 shows the stages spatial distribution.

Table 7: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations (n=3 replicates).

Characteristics \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 4 (T4)	Forest (F)
Age	0	6 (3)	13 (2)	48 (13)	> 62
Elevation	1100	1080	1090	1090	1120
Aspect	SEE	E	S	N	N
Steepness	8.75 (2.82)	8.45 (1.6)	14.9 (2.76)	5.77 (2.11)	13.91 (3.24)
Slope position	Shoulder	Footslope	Backslope	Footslope	Backslope

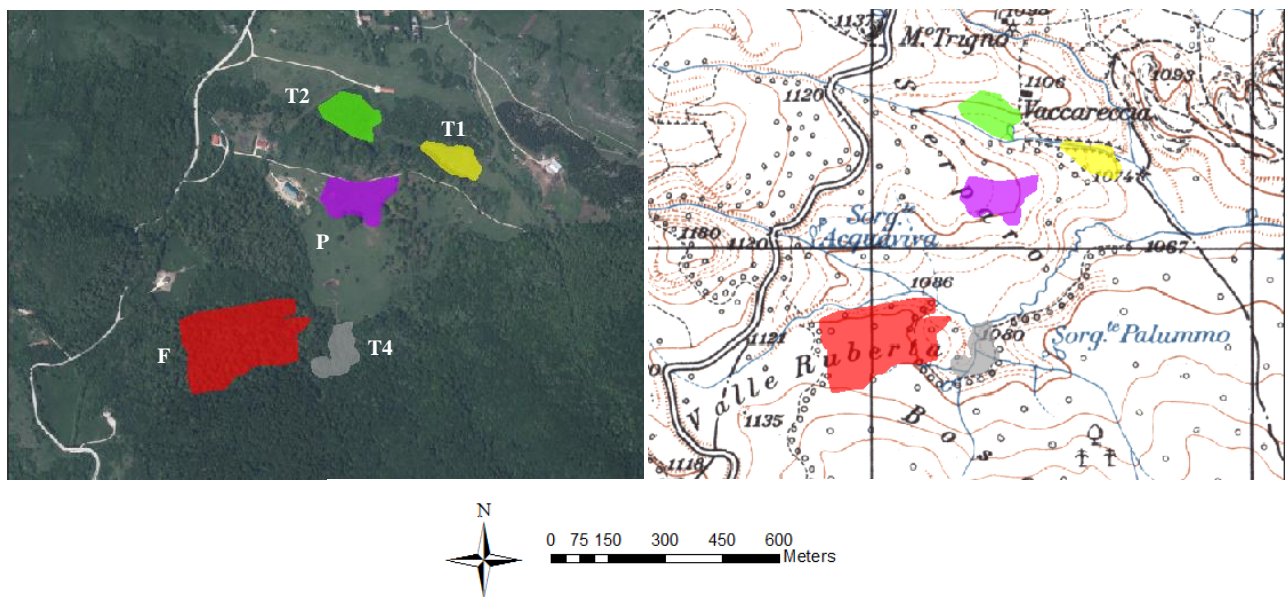


Figure 17 The Vastogirardi chronosequence (see Tab. 7), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab. 7.

The pasture stage is a fenced public area. Each 4-5 years, the municipality gives it in concession to private shepherds for domestic animal grazing. During the sampling period, the pasture pressure was of approximately one head of cattle per hectare. In addition to the typical annual and perennial grasses species, ferns plants were also present. No other information on plant species composition was collected. The first encroachment stage is grassland with only a few young *P. communis* tree species. Cows faeces confirm that a grazing pressure is present, even if it is not particularly intense. The approximately 12 year-old encroached stage is characterised by a dense shrubs and young tree species vegetation. The *P. communis* dominates this stage and the abundance of other tree and shrub species confers to this area the highest woody plant diversity. In addition to *P. communis* the other trees identified in this stage are *A. campestre* and *Q. cerris*. The most common shrub species are: *R. ulmifolius*, *P. spinosa*, *Prunus cerasifera* Ehrh., *Prunus cerasus*

L., *C. monogyna*, *L. vulgare* and *Sambucus nigra* L. The oldest stages are similar in plant species composition. *Q. cerris* and *F. sylvatica* dominate the tree cover, while *A. campestre* and *Prunus mahaleb* L. are less represented. *R. ulmifolius* and *L. vulgare* dominate the undergrowth vegetation.

8.2.7. Pignola

The Pignola site is located approximately 4 km East of the homonymous village in the Potenza province, Basilicata region. The chronosequence lied in the Rifreddo valley on an East aspect slope, near the forestry corps house.

The ombrothermic diagram in Fig. 18 shows that most of the yearly rainfall is limited to October – April period. The monthly maximum of 127 mm coincides with November and the minimum of approximately 30 mm is in July and August. In these months a summer dry stress period generally occurs. The mean temperatures range from 3.0 °C in January to 20.5 °C in August and follow the typical seasonal trend. The bioclimatic region is classified as transitional between temperate oceanic and temperate semi-continental.

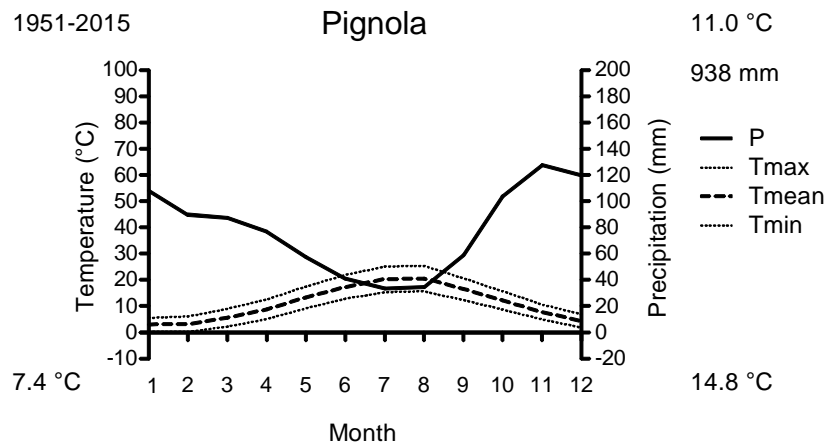


Figure 18: Ombrothermic diagram of Pignola based on the local estimated data provided by Bologna ISAC-CNR according to methodology described in Brunetti et al. (2012). The graph reports mean values over the period defined on upper left side. Solid line on the top is the mean monthly precipitation, with values on the right vertical axis. Monthly mean temperatures (maximum, mean and minimum) are shown with dotted and dashed lines and refer to the left axis values. Horizontal axis values represent the month number. The other values on the graph are: average of the yearly mean temperature and precipitation on the upper right corner, yearly maximum temperatures mean on the bottom right corner, yearly minimum temperature mean on the bottom left corner.

The site is included in the Apennine and anti-Apennines relieves on sedimentary rocks of Central and Southern Italy. The main lithologies are limestone, calcareous marls and calcareous clays. The sedimentation process – which caused bedrock formation – occurred between the Cretaceous and the upper Jurassic eras.

In all the stages, the main soil class is Pheozems (IUSS Working group WRB, 2015). However, the forest soil appeared quite different from those of the other stages because of a limited percentage of clay particles. The maximum soil depth is of approximately 70 cm.

The mean elevation of the site is 1050 m.a.s.l. and the prevalent exposure is NE. The steepness ranges from limited to moderate. The main chronostages characteristics are summarised in Tab. 8 and their spatial distribution ins shown in Fig. 19.

Table 8: List of main site characteristics for each chronostage (column). “Age” represents the time since the woody encroachment started and is measured in years before present, with values in brackets expressing estimation uncertainty (see Step 5 in Section 8.1). Elevation, aspect and steepness were measured from the DEM as described in the text. Steepness is expressed in %. Bracket values in steepness are standard deviations (n=3 replicates).

Characteristics \ Stage label	Pasture (P)	Encroached time 1 (T1)	Encroached time 2 (T2)	Encroached time 4 (T4)	Forest (F)
Age	0	18 (3)	25 (2)	51 (9)	> 60
Elevation	1000	980	1000	1030	1200
Aspect	E	N	N	N	E
Steepness	20.15 (2.85)	18.1 (4.5)	16.7 (3.82)	22.26 (4.7)	37.53 (2.64)
Slope position	Backslope	Backslope	Backslope	Backslope	Backslope

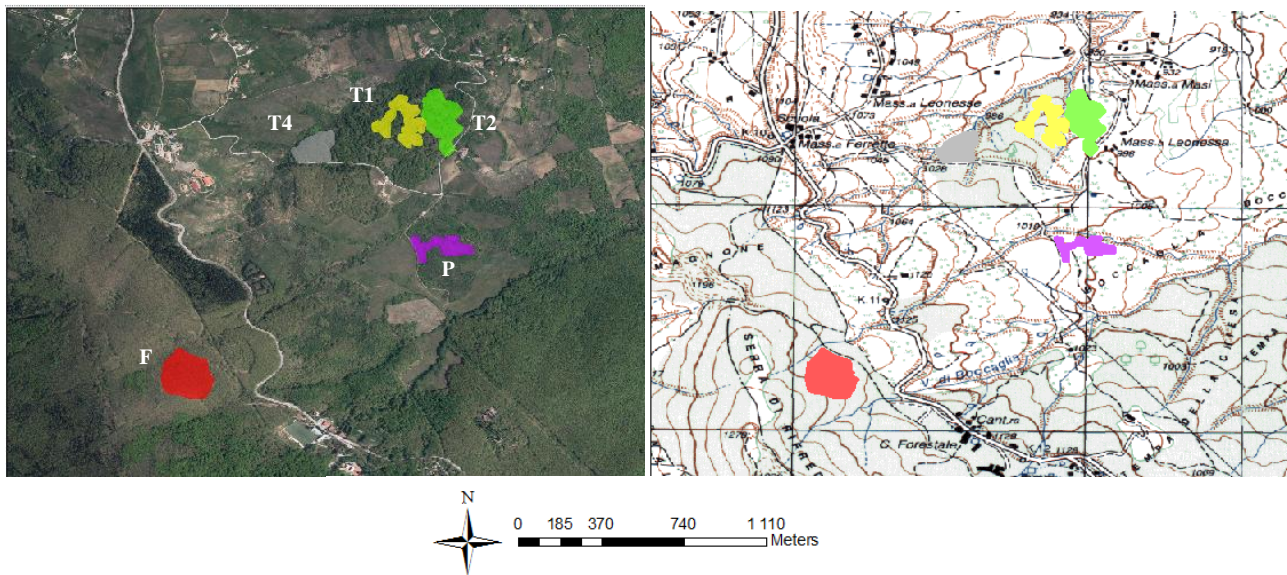


Figure 19 The Pignola chronosequence (see Tab. 8), identified by interpretation of an airborne photograph series. Chronostages are labelled as described in Tab. 8.

The pasture stage is grazed by sheep and donkeys, which are free to move in a fairly wide enclosure area. The pasture pressure is limited and the shrubs vegetation is going to colonise the area rapidly. The first encroached stage is a mixture of grass and shrub vegetation. The most common woody species are *Crategus spp.*, *P. spinosa*, *R. canina* and *R. ulmifolius*. Similar vegetation is also present in the second encroached stage. In addition to the previous species, *Spartium junceum* L., *Lonicera caprifolium* L. and *P. communis* were also identified. Both these stages are still used for sheep grazing. In the third encroached stage there is a mono-specific *Q. cerris* cover without undergrowth vegetation. During the sampling work, a cemented soil was observed in this stage, which was hard and extremely difficult to break. *Q. cerris* is the main tree species in the managed forest stand. The most common undergrowth vegetation is composed of *Ilex aquifolium* L. shrubs and a thin grasses layer.

8.3. Soil sampling design

In order to study the SOC variations along the different stages of a chronosequence, the soil sampling protocol defined by Stolbovoy *et al.* (2007) was adopted, which was specifically designed to examine the changes in SOC in the mineral soil for different land uses within the European Union. The general acceptance of this protocol at the European level allows comparing standardised results from different research activities and – as for this specific case – between different chronosequences. In addition, it has the benefit that almost all its procedures, from the sampling design to the SOC stock computation, are standardised.

According to the protocol, the sampling design was based on a randomised data collection procedure. For each chronosequence stage, the smallest N-S and E-W oriented square area was drawn around the stage polygon. The side length of this large square is hereafter referred to as the *Maxis* (Fig. 20). Given that the polygon varies from one chronostage to another, the *Maxis* also varies in length. Then, the square has to be divided in a grid of 10 x 10 square cells. Therefore, each of the 100 cells has a side length (*Gs*, in Fig. 20) that is one tenth of the *Maxis*. According to the original protocol, cells are labelled with numbers from 1 to 100, drawn uniformly at random without replacement, subject to the further constraint that no two contiguous cells can have consecutive labels (i.e. if a cell is labelled x , none of the four adjacent cells can be labelled $x+1$ or $x-1$).

For the sampling data collection, the cells that have to be considered are only those completely included in the inscribed sampling unit. According to the protocol, the number of the completely included cells, which have to be sampled, varies as a function of the sampling unit area dimension as summarised in Tab. 9.

Table 9: *The number of sampling cell varies as a function of the sampling unit area dimension according Stolbovoy et al. (2007).*

Sampling unit dimension	Number of sampling cell
< 5 ha	3
5 – 10 ha	4
10 – 25 ha	5
> 25 ha	6

Because the woody encroachment generally takes place in small and fragmented areas, a maximum sampling unit dimension of 5 ha was used in this study, leading to a total of 3 sampling cells for each chronostage.

In each selected cell – hereafter called sampling site – 25 ordered points were selected for the soil sampling collection (ISO, 2002). The position of each point is based on the geometrical subdivision of the sampling site as shown in Fig. 16.

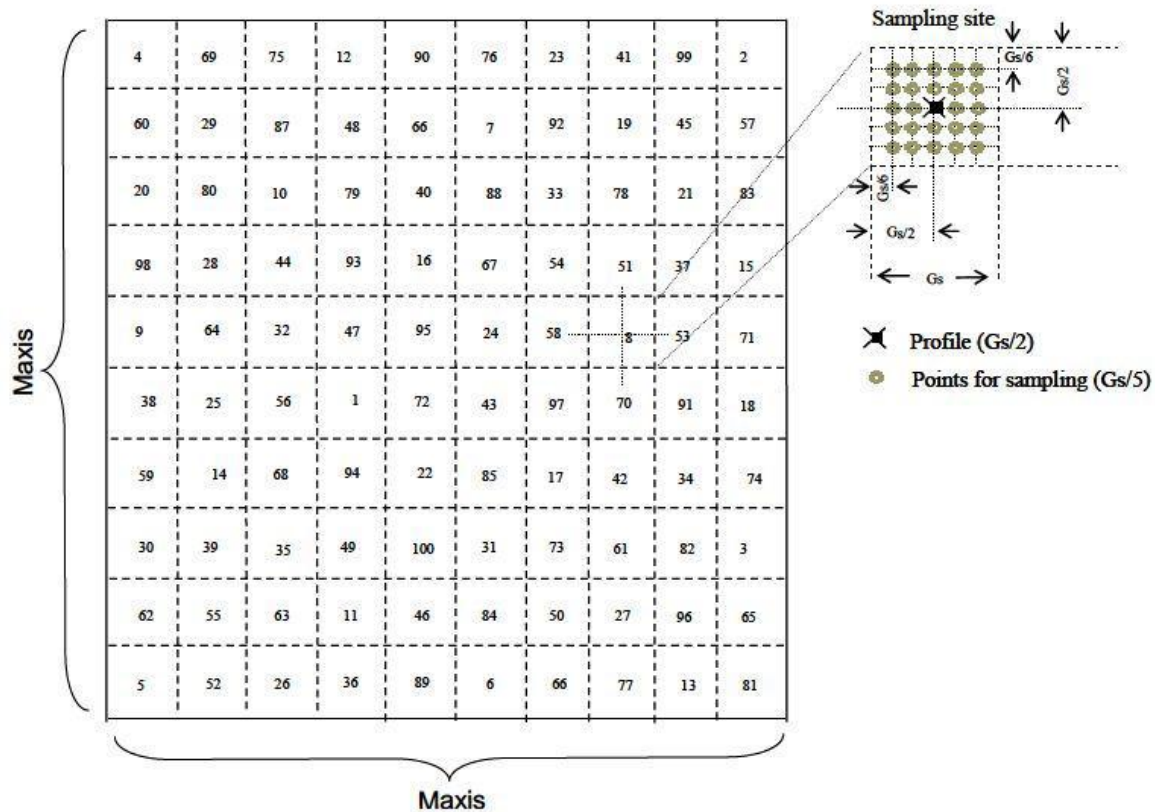


Figure 20: Soil sampling design according to Stolbovoy *et al.* (2007), from where the figure is copied.

In ArcGIS 9.3 a sampling project was generated for each chronosequence by following the protocol of Stolbovoy *et al.* (2007) through the subsequent steps:

1. The metric coordinate system UTM 32 or 33 was set up (the number depend on the geographical position of each chronosequence).
2. The *Bounding container* function was applied to create the smallest circumscribed rectangle over each chronostage.
3. For each rectangle, the shorter side was extended to the length of the longer one. The result was the smallest square area, circumscribing the chronostage.
4. This square area was subdivided in a 10 x 10 grid by means of the *Create Vector Grid (line/polygon)* tool included in the *HawthsTool* package.
5. Only the cells that were completely included in the chronostage were selected, copied and added to a new polygon layer.
6. The *Create Random Selection* tool (available from the same package) was used to randomly select three non-contiguous cells among all of them. This process gives the possibility to bypass the standard cells numeration and selection that was suggested in Stolbovoy *et al.* (2007), and still satisfy the more important non-contiguity constraint in cell numbering.
7. *Generate Polygon Centroid Points* was used to create a new layer with the coordinate of the central point of each sampling site.
8. Finally, the central points coordinates were transformed in the WGS 84 coordinate system in order to upload this data into a GPS system.

Through this procedure, it was also possible to determine the length of the Maxis, the side of the circumscribing square area (see Fig. 20) and, then, to calculate the length of each sampling site side.

8.4. Soil and litter sampling collection

Soil samples for organic C and total N estimations have been collected between July 2014 and November 2015. For each study area, a sampling campaign was organised independently.

The coordinates of the central point of each plot were reached on the field by means of the GPS system. The N-S direction was then identified by means of a compass and each sampling site, as drawn in the GIS project phase, was reconstructed on the field. The correct positioning of each sampling site was fixed by means of a compass. Due to the considerable amount of work and the short distances between the points for sampling (see Fig. 20), the position of each of them was roughly estimated knowing each sampling site dimension and position. The original form of the protocol was based on the rules list included in the IPCC guidelines (2003). Considering pastures land-use, it suggests to focus only to the upper 30 cm soil layer depth. However, for this study it was decided to make some modifications to the original protocol version. Firstly, the depth of the topsoil intervals in Stolbovoy et al. (2007) was reset in order to refine the analysis in the upper layers. The new intervals considered were 0-5, 5-15, 15-30 cm in depth. Secondly, the analysis was implemented to other subsoil layers, collecting samples at 30-50, 50-70, 70-100 cm of depth (or to R horizon).

From each of these 25 sampling point, a soil sample was collected for each soil layer by means of a hand drill. Then, a composite sample ($n = 25$) per each layer was created directly in the field mixing the 25 samples. In the central point of each sampling site, a mini pit was dug in order to describe the soil profile, based on pedogenetic horizons, according to Schoeneberger *et al.* (2002). In the central point, the BD samples – which are then used to estimate the soil density – were collected in the three upper layers using a small metal cylinder with a known volume (diameter = 5 cm, height = 5 cm; Blake, 1965). Overall, in each chronostage, six soil samples were collected for each soil depth. Three independent BD samples for each layer and three independent composite samples to be used for physical and chemical analyses.

The organic horizon, namely the litter layer was also collected in the stages where it was present. A 20 x 20 cm plot was randomly placed inside each sampling site. The whole organic layer included in the 400 cm² plot was removed in order to estimate the concentration of organic C (see sections 8.6 and 8.7).

8.5. Soil physical and chemical analyses

All samples were transported to the laboratory and then dried in an aired oven at 50 °C until a constant weight was achieved. The physical and chemical analyses performed on the soil samples are summarised in table 10.

Table 10: List of physical and chemical analyses performed on completely dry soil samples

Physical analyses	Chemical analyses
Bulk density	pH
Texture	CEC
	C _{org}
	N tot

The BD is a fundamental parameter to estimate the *SOC stock*, defined as the SOC amount per surface unit. Since the volume of the metal cylinder used to collect BD was known (98.17 cm³), the dried BD soil samples were weighed and the density was estimated as mass per unit volume. BD samples were collected only for the three upper depth intervals (0-5, 5-15 and 15-30 cm) because of the abundance of the rock fragments in the *subsoil*, i.e. the deeper part of the soil profile (generally below 30 cm of depth). Therefore, for the deeper layers, BD was estimated using the pedotransfer function proposed by Adams (1973) as:

$$BD = \frac{1}{(a + b * \%C)} \quad (1)$$

where $a = 0.686$ and $b = 0.085$ are the constant values suggested by Chiti *et al.* (2012) for the 30-100 cm soil compartment and %C is the soil C percentage as expressed by elemental analysers.

The other samples – those not specifically collected for the BD estimations – were crushed and sieved at 2 mm. Both the coarse rock fragments (> 2 mm) and the fine earth (< 2 mm) fraction were weighed in order to estimate the percentage of each component. All the physical and chemical analyses were performed on the fine earth only.

The particle size distribution was estimated only for the pasture / grassland and forest stages. For each of them, a composite sample was assembled mixing together the three soil replicates (one per sampling sites) for each depth interval considered. The adopted procedure follows protocol by the Ministero per le Politiche Agricole (1999), which is based on the sedimentation process speed of solid particles in a still liquid according the Stokes' law. The procedure is summarised as follows:

1. 10 g of dry soil were weighed;
2. 50 ml of 5% sodium hexametaphosphate – (NaPO₃)₆ – solution was added to the soil and the 250 ml volume was reached by adding deionised water;
3. The solution was shaken at approximately 150 cycles per minutes for 2 hours;
4. The whole solution was poured into an Andreasen pipette and deionised water was added to reach the pipette limit volume;
5. The Andreasen pipette was manually shaken with standardised movements in order to homogenise its content;
6. A first 10 ml solution sample was taken out 9 minutes and 36 seconds after the shaking of the Andreson pipette was stopped;
7. A second 10 ml solution was taken out after 16 hours.

Both the solutions were dried out in an oven at 105 °C and the mass of the solid particles was weighed. The percentage of the three textural classes were estimated according to the following formulae:

$$silt \% = (Pn_{9'36''} - Pn_{16h}) * V \quad (2)$$

$$clay \% = \left[Pn_{16h} - \left(2.5 * \frac{10}{V} \right) \right] * V \quad (3)$$

$$sand \% = 100\% - (silt \% + clay \%), \quad (4)$$

where $Pn_{9'36''}$ is the net dry mass of the solution collected after 9 min and 36 seconds; Pn_{16h} is the net dry mass of the solution picked out the pipette after 16 hours; V is the pipette limit volume; $2.5*10/V$ is a conversion factor which considers the (NaPO₃)₆ solution at 5% which was used as a dispersion solution.

The soil size-particles and texture classes subdivision refers to the International Union of Soil Science categorisation (IUSS Working group WRB, 2015).

The pH was measured for all soil samples. The adopted procedure followed Van Reeuwijk (2002) and was performed by means of a Mettler Toledo Easy pH Titrator System. The procedure consists of a potentiometrical pH determination in a 1:2.5 (w/v) soil-deionised water.

The cation exchange capacity (CEC) was measured for only three stages of each chronosequence: the pastures / grasslands, the intermediate stages (T2) and the forests. The CEC determination was performed for each of the soil depth intervals considered. The adopted methodology followed Gillman (1979) as summarised in the following steps:

1. 2 g of soil were weighed for each sample;
2. 25 ml of pH 8.2 barium chlorite ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) solution were added to the soil;
3. The mixture was shaken for one hour and centrifuged at 3000 rpm, then the suspension was removed;
4. Steps 2 and 3 were repeated again, this time using 30 ml of water in order to wash the soil sample;
5. 25 ml of a 5 cmol l^{-1} magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) were added to the soil sample and the mixture has was shaken for 5 minutes and then centrifuged in a plastic tube;
6. 10 ml of the suspension were collected and added to 100 ml of a pH 10 buffer solution (previously prepared with ammonium chloride and ammonium hydroxide) and a very small amount of an indicator previously prepared with 20 g of sodium chloride and 0.2 g of eriochrome black T;

The solution was titrated with a 2.5 cmol l^{-1} ethylenediaminetetraacetic acid (EDTA) solution until an azure coloration appeared.

The same titration was done on a blank control prepared with 10 ml of magnesium sulfate solution, 10 ml of the 10 pH buffer solution and a small portion of the indicator described in step 6.

The CEC was estimated according to the following formula:

$$CEC = \left[\frac{[(V_B - V_A) \cdot M \cdot 1000]}{m \cdot 1000} \cdot \frac{25}{10} \cdot \frac{(25 + B - A)}{25} \cdot 2 \right], \quad (5)$$

where CEC is expressed in $\text{cmol}(+) \cdot \text{kg}_{\text{soil}}^{-1}$; V_A is the volume (ml) of the EDTA solution which was used for the titration of the sample; V_B is the volume (ml) of the EDTA solution which was used for the blank control solution; A is the sum mass (g) of the tube used for the centrifugation and the soil sample; B is the sum mass (g) of the tube and the soil after the barium chlorite saturation, centrifugation and the washing with the water; M is the EDTA concentration in the solution (in cmol l^{-1}); m is the sample mass (g).

Estimation of C_{org} and total soil N was performed on all soil samples collected in the field. The adopted procedure follows the Method VII.1 suggested by Ministero delle Politiche Agricole e Forestali (MPAF, 2000). Two different flash combustion analysers were used because of the breakdown of the first of them. One was a Flash E/A 112 Series Thermo Electron and it was used in the Innovation in Biological system, Food and Forestry Department (DIBAF) of the University of Tuscia. The other one was a Flash 2000 combustion CHNS/O of the Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche of the Università degli Studi della Campania “Luigi Vanvitelli”. Both the analysers work in accordance with the Dumas (1831) methodology, which consists in the flash combustion of the solid particles and the successive series of oxidation and reduction reactions of the emanated gasses. Before the

analyses, both the instruments were calibrated with the same standard samples with a $\pm 0.07\%$ and $\pm 0.01\%$ certified uncertainty for C and N, respectively.

From each sample, a small soil amount was isolated and pulverised in order to obtain soil dust. Then, it was stored in a 1.5 ml eppendorf and only a portion of it (approximately 20 mg) was weighed in a silver capsule. The precision scale that was used had a precision of $\pm 1\mu\text{g}$.

Because almost all selected areas have calcareous bedrock formations, all samples were treated with hydrochloric acid (HCl) before the C_{org} and total N analyses. In particular the following procedure was adopted:

1. In each silver capsule, 40 μl of 10% HCl was added;
2. The sample was left for one night at the environmental temperature in order to give time to the solution to dissolve the inorganic carbonates and partially evaporate;
3. 40 μl of 10% HCl was added again;
4. Four hours after the second treatment, the sample was heated on a metal plate at 65 °C for three hours.

Then, all the silver capsules were closed and inserted in the instruments.

Finally, the SOC stock was calculated for each layer of the profiles according to Eq. (6) (Boone *et al.*, 1999):

$$Y = a * b * c * d \quad (6)$$

where Y is the SOC stock per unit area (kgC m^{-2}), a is the C concentration in the soil sample ($\text{kgC kg}_{\text{soil}}^{-1}$), b is the soil bulk density ($\text{kg}_{\text{soil m}^{-3}}$), c is the depth of the layer (m) and d is the percentage in mass of mineral soil without rock fragments [$1 - (\% \text{ rock volume}/100)$]. The same equation was used to calculate the N stock in the soil. Finally, stocks values were expressed in MgC ha^{-1} .

The comparison on the stock between different soil profiles could be applied according two main schools of thought: the classic approach, based on the equivalent soil depth (ESD) comparison, and the equivalent soil mass (ESM) approach proposed by Ellert and Bettany (1995). In the first case, the stocks of two or more soil profiles have to be compared considering equivalent volumes of soil, while the latter fully accounts for variations in soil mass. Ellert and Bettany (1995) highly recommend their approach for stocks comparisons among ploughed and un-ploughed soils. In their work, the authors showed that the comparison of the same soil stocks estimated just before and immediately after the tillage might be affected only by the BD changes (due to the atrophic ploughing) and not by an effective difference in the C concentration. In addition, the authors who supported this approach (see Lee *et al.*, 2009) generally compare the soil stocks estimated only in the upper part of the soil, which is the more affected by the BD variation due to a LUC.

The present case study is substantially different from those of Ellert and Bettany (1995) and Lee *et al.* (2009). Indeed, firstly, it is highly probable that the root system of the different vegetation types that characterise each chronostage does affect the soil BD. Therefore, the application of the ESM approach could mask important factors that are fundamentally due to the woody encroachment process itself. Secondly, both the topsoil and subsoil are investigated, reducing the problem of BD changes linked to the upper part of the soil. In addition, the information obtained from the ESM comparison could be completely re-derived by comparing C and N concentrations in the respective depth layer. Finally, the application of the ESM makes it generally more difficult to identify explicitly in which part of the soil the most important changes are located.

For all these reasons, in the present work the ESD method was applied, a method that fits better within the sampling design suggested by Stolbovoy *et al.* (2007) compared to the ESM one.

8.6. Living biomass estimation

Plant biomass and necromass were estimated in only in three chronostages per chronosequence: pasture / grassland (P), the intermediate stage, i.e. the second encroached stage (T2) and the forest (F). In each of them, three sampling plots were centred on the pit excavated for soil description (see soil sampling design, Section 8.3).

In the forest chronostages, the essential attributes necessary to estimate the aboveground biomass (AGB) were collected following a sampling design inspired by the third phase INFC protocol of MPAF (2006) and the sampling design proposed in Bovio *et al.* (2014). In the next paragraph the procedure that was used on the field is briefly described. Tab. 11 and Fig. 21 summarise and graphically illustrate the protocol:

- A Sampling Area with a 13 m radius length (SA 13) was delimited around the central point (O) used for the soil sampling site. In the SA 13, all alive and standing dead trees with a diameter at breast height (shoot diameter at a 1.3 m height, hereafter *DBH*) greater than 9.5 cm were considered. *DBH* and total plant height (hereafter *H*) were estimated.
- A smaller and concentric area of 4 m radius length (SA 4) was delimited inside the SA 13. In the SA 4, tree plants with a *DBH* measure greater than 2.5 but lower than 9.5 cm were measured. The *DBH* and *H* measure estimations were collected for each plant.
- Two perpendicular diameters of SA 13, those along the N-S and E-W directions, were identified. Along these diameters, the Coarse Woody Debris (CWD, woody debris with a diameter > 5 cm) data were collected. In particular, the diameter of each CWD was measured and, for each element, the relative decay class according Alberti *et al.* (2008) was recorded.
- Two circular areas of 2 m radius (SAs 2) were delimited on the E-W diameter at a 10 m distance from the central point O, one left and one right of it (Fig. 21). The shrubs included in each SA 2 were pruned at the collar height and collected.
- Four square areas of 0.5 x 0.5 m (SAs 0.5) were delimited at 10 m from point O on the diameter lines. Fine Woody Debris (FWD, woody debris with a diameter < 5 cm) and grass plants were collected and stored separately. The grasses were pruned at the collar height.
- In a 0.2 x 0.2 randomly positioned square area litter was collected as described in the *Soil and litter sampling data collection* paragraph (section 8.4).

Table 11 A brief description of the areas represented and labelled in Fig. 21. Each of them refers to the vegetation component considered for the biomass and necromass estimation in forest chronostages. The last column on the right includes all the annotations and attributes considered (DBH, H, DCH = diameter at the base of the stem). The threshold diameter dimension between FWD and CWD is 5 cm. Decay classes refer to Alberti *et al.* (2008) and are described in the text.

Graphic element	Dimensions	Vegetation component	Measures and annotation
SA 13	13 m radius circle	trees with $DBH \geq 9.5$ cm; dead standing trees, remain stumps	DBH, H and species
SA 4	4 m radius circle	trees with a $DBH > 2.5$ cm	DBH, H and species
SA 2	2 m radius circles	shrubs	Direct collection
SA 0.5	0.5 x 0.5 m plots	grasses and FWD	Direct collection
Dotted diameters	26 m lines	CWD	2 diameters and decay class
Central point O	0.2 x 0.2 m plot	litter	Direct collection

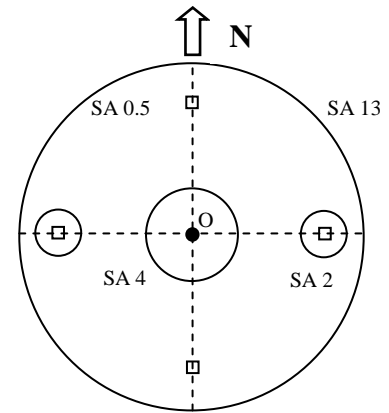


Figure 21: Sampling design adopted for biomass and necromass estimation. All the areas and relative labels are explained in Tab. 11.

The main instruments used during the field work are:

- a tree measuring caliper to measure the trees DBH ;
- a small caliper for the DBH , and small dead woody debris diameters;
- a roll-up measuring tape to delimit the sampling areas;
- a clinometer (Suunto PM-5/360 PC) to measure the angles for the plant height estimation.

The H was estimated according to trigonometric laws by means the clinometers angles and the distances between viewpoints and the plants. Tree biomass of SA 13 and SA 4 was estimated by means of species-specific allometric equations according to Tabacchi *et al.* (2011). However, the model of Tabacchi *et al.* (2011) does not include plants with $DBH < 5$ cm or $H < 5$ m. Literature on small trees and shrubs biomass is extremely limited. Therefore, for plants with a DBH and H lower than the thresholds, the model suggested by Sah *et al.* (2004) for tree-like mixed-species was adopted to obtain an indicative biomass value, even if this model was tested in a Florida ecosystem on completely different species. The adopted formula is shown in equation (9):

$$B = -0.06 + 0.227 * (DBH)^2 + 0.002 * H, \quad (9)$$

where B is the general plant biomass.

Grasses were directly collected on the field. Shrubs were collected too because, unlike for trees, species-specific allometric equations are very rare in the literature. Grass and shrub samples were transported to the DIBAF laboratory, dried completely out at 70 °C and, finally, weighed to obtain the total biomass.

Differently from the forest stages, the intermediate ones are usually characterised by heterogeneous distribution of shrubs species and young trees. Therefore, the forest protocol was slightly modified in order to reduce the possible bias caused by the shrubs spatial heterogeneous distribution. In addition, as before, the problem of the lack of shrub allometric equations in the literature needed to be dealt with. In the attempt to simplify the sampling data collection as much as possible, the following solutions were adopted:

- In the case of shrub species with objectively identifiable separate individuals (the most common situation), only one representative plant per species was taken into account. The selection of these plants was based on the mean of the most cited independent allometric parameters (diameter at collar height, total plant height, and two crown diameters, the maximum and the one orthogonal to it) of all the plants of the same species present in the chronostage. Alberti *et al.* (2008) suggested an equation to estimate the volume of shrub species:

$$V = 0.5 * \frac{\pi}{4} * DBH_m^2 * H_m * n, \quad (10)$$

where V is the volume of the whole plant, DBH_m is the average DBH of the stems for each stump, H_m is the average height and n is the number of stems. However, the study area of Alberti *et al.* (2008) essentially consists only in *C. avellana*. Therefore, the volume of *C. avellana* was estimated by means of Eq. (10). In order to obtain the biomass of *C. avellana*, its volume was multiplied by the wood density of 520 kg m⁻³ (IPPC, 2003). The biomass of *R. canina*, instead, was estimated, according to Blujdea *et al.* (2012) by means of equation:

$$\ln(ABG) = \ln(a) + b * \ln(DCH), \quad (11)$$

where $\ln(a) = -2.8316 \pm 2.2779$ and $b = 2.4364 \pm 0.5952$ and DCH = diameter at the base of the stem (i.e. the diameter at the collar height).

For the other shrub species, the vast majority of which does not reach 1.30 m of height, and for which it is therefore not possible to measure the DBH, the selected plant was cut at collar height and transported to the laboratory where it was dried and weighed. Finally, all the individuals of each species were counted in each SA 13 considered in the chronostage.

- In the case of shrubs species with not objectively identifiable separate individuals (essentially *Rubus ssp.*), the following procedure was followed: a square 0.5x0.5 m plot was placed on a region where the species of interest showed homogeneous distribution and cover, and that was visually assessed to be representative of the overall above ground phytomass cover; all above ground phytomass in the plot was collected; finally, the estimated density inside the plot was extrapolated to the entire SA 13 area.

Although the measurement might be inaccurate, the estimated shrub biomass was so low that it hardly affects the overall estimates beyond the uncertainty levels discussed below.

Pasture / grassland stages (P), are characterised by only grass vegetation and the biomass was directly collected in the SAs 0.5.

A value for the belowground biomass (BGB) was estimated by adopting vegetation-specific root to shoot ratio (R/S) according to Mokany *et al.* (2006). Even if the use of a vegetation-specific ratio, rather than species-specific ones, is quite a crude approach, it was preferred because it bypassed the usual problems related to the lack, in the literature, of species-specific allometric equations to estimate the AGB and species-specific R/S ratios for the shrub vegetation. The same approach was also adopted in the IPCC (2006) and is briefly described in Table 14.

Table 14: Root to shoot ratio adopted in this work. Data from Moganay *et al.* (2006).

Vegetation type	R/S ratio
Temperate conifers (AGB > 150 tonnes ha ⁻¹)	0.201
Temperate other broadleaf (AGB > 150 tonnes ha ⁻¹)	0.241
Temperate <i>Quercus spp.</i> (AGB >70 tonnes ha ⁻¹)	0.295
Shrublands	1.837
Cold and warm temperate grassland	4.422

8.7. Dead organic matter estimation

The sampling protocol for necromass estimation was inspired by Alberti *et al.* (2008) and is summarised in Tab. 11 and Fig. 21.

Litter and FWD were collected, transported to the laboratories, dried out in the oven at 70 °C and then weighed. For the CWD, because of the lack of wood density data for each decay class, it was possible to estimate only the volume (V of CWD in m³ ha⁻¹) for each transect according to the following formula, suggested by Harmon and Sexton (1996):

$$V_{CWD} = 9.869 * \sum \left(\frac{d^2}{8L} \right), \quad (12)$$

where d is the fragment diameter (m) and L is the sum of the lengths of both transects (26 m).

The volume of standing dead trees (V_{sdt} in m³) was estimated using the formula suggested by Alberti *et al.* (2008):

$$V_{sdt} = 0.5 * \left(\frac{\pi}{4} \right) * DBH^2 * H, \quad (13)$$

where DBH and H are diameter at breast height and total height respectively.

According to Harmon *et al.* (2000), in the case of decomposition evaluation for very large areas with unknown temporal patterns of woody detritus input, it is reasonable to assume that the decomposition rate does not vary with time. Then, it was assumed that the CWD and standing dead trees decay classes defined by Alberti *et al.* (2008) – adopted in the present work – correspond to equally-wide time intervals. Therefore, the mean CWD density summarised in Alberti *et al.* (2008) was used to obtain a linear density regression. The slope of this linear regression was considered as a constant wood-density decrease rate, even if Harmon *et al.* (2000) asserts that decomposition rates are species-dependent. Finally, the fresh woody density for the species of each CWD was obtained from the Global Wood Density Database (2015) and was set up as the intercept of the species-specific linear regression models.

Finally, the above- and belowground living biomass, CWD, FWD and litter pools were converted in C estimates, adopting the 0.475 conversion factor proposed by Magnussen and Reed (2004).

8.8. Soil biochemical analyses

Soil sampling collection for biochemical analyses was carried out, in each chronosequence, for the same three chronostages (P, T2 and F) used for the living biomass and dead organic matter estimation. The sampling plots (n=3) were the same as defined for the SOC and soil N stocks. Each sample was composed of 5 subsamples randomly selected on the field. All of them were collected only in the two upper mineral soil layers (0-5 and 5-15 cm deep). Finally, the samples were dried at air temperature and stored until the start of the analyses.

According to Vance *et al.* (1987), the microbial biomass (C_{mic}) and nitrogen (N_{mic}) were measured by the fumigation-extraction method. Two 10 g of soil portions were collected from each soil sample and, subsequently, conditioned. This process consisted in adjusting each portion to 60% of their water holding capacity (WHC) and storing them in a dark room at 25°C for 3 days. For each pair of portions, one was fumigated for 24 hours in ethanol-free chloroform, while the other was not fumigated. Then, for each portion, 80 ml of 0.5 M K_2SO_4 were added to the soil. The solution was homogenised for 30 minutes and filtered with Whatman paper (no. 42).

For each extract, the amount of organic C and N were measured by a V CSN and TNM-1 analyser (Shimadzu). The measurement obtained by the non-fumigated portions are the total extractable C and extractable N and these values were used to calculate microbial carbon (C_{mic}) and microbial nitrogen (N_{mic}) according the following formulas:

$$C_{mic} = E_C * k_{EC} \quad (14)$$

$$N_{mic} = E_N * k_{EN} \quad (15)$$

where E_C and E_N are the difference, respectively, in the C and N content between fumigated and non-fumigated extracts and $k_{EC} = 2.54$ and $k_{EN} = 2.22$ are the constant values relative to carbon and nitrogen, respectively.

Soil enzymes were measured according to Marx *et al.* (2001) and using fluorogenic methylumbelliferyl (MUF)-substrates. The activity of five enzymes were measured and grouped on the base of the principal element (C, N cycles). For the C cycle the enzymes considered were:

- β -cellobiohydrolase and α -glucosidase, which are involved in the degradation of cellulose;
- β -glucosidase, contributing to the degradation of starch;
- β -N-acetyl-glucosaminidase (chitinase), which plays a role in the degradation of the chitin;
- xylosidase, involved in the degradation of hemicelluloses.

For the N cycle, the only enzyme considered was β -N-acetyl-glucosaminidase (chitinase).

For each enzyme, the fluorogenic substrates were, respectively, 4-MUF- β -D-cellobioside, 4-MUF- β -D-glucoside, 4-MUF-N-acetyl- β -glucosaminide, 4-MUF- α -D-glucoside, 4-MUF-phosphate, 4-MUF-7- β -D-xyloside, 4-MUF-sulphate and 4-MUF-butyrate.

Before the analyses, 2 g of soil were collected for each sample and then conditioned at 60% of their WHC at 25°C in the dark room for 3 days. 50 ml of sterile water were added and shaken with the 2 g of soil by means of an Ultra Turrax at 9600 rpm for 3 min. Finally, 50 μ l of Sodium Acetate buffer 0.5 M pH 5.5 and 100 μ l of 1 mM substrate solution were added in order to obtain a final substrate concentration of 500 μ M. Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent) and readings were taken after 0, 30, 60, 120 and 180 min of incubation at 30°C (Marinari *et al.*, 2013).

The sum of the C-cycle enzyme activities was considered as a Synthetic Enzymatic Index for carbon (SEIc), as Dumontet *et al.* (2001) suggested for all the enzyme measurements.

Finally, according to Trasar-Cepeda *et al.* (2008), the SEIc and the chitinase were both normalised by dividing by C_{org} , in order to estimate each enzyme-specific activity.

8.9. Statistical analyses

8.9.1. Soil chemical characteristic: pH and C/N

Within each chronosequence, the variation over time of the pH and C/N were analysed using a repeated measurements ANOVA. The normality and equality variances tests were not performed because of the reduced number of soil replicates ($n=3$). However, it was assumed that measured values were both normally distributed and with equal variance because the ecological variables considered in this work generally respect these assumptions. Furthermore, even if this was not the case, the fact that the analysis were performed on composite soil samples would support the normal distribution and equal variance assumptions by virtue of the Central Limit Theorem. In case of statistically significant differences between stages, a post-hoc Tukey test was performed to compare each pair of stages separately.

The same analyses were also performed to identify significant differences in C_{org} and N concentrations among the stages of each chronosequence, separately for each depth interval considered, and in the SOC and N stocks, separately for the topsoil and subsoil, as well as for the whole soil profile.

Then, within each chronosequence, the differences in SOC stock between each stage and the pasture stage was calculated, and is hereafter referred to as the stage-to-pasture SOC difference. This was calculated separately for the topsoil and the subsoil, and for the whole soil profile.

The sites were then grouped on the basis of similar changes in time in the SOC stock in the entire soil profile.

An average profile of SOC stock changes over time in each group (Alps and Apennines) was then generated by taking, in each stage, the mean of the stage-to-pasture SOC stock differences over all chronosequences in each group.

As the next step, the changes in SOC from pasture to forest in each chronosequence was measured both as the ratio and as the difference of the values in the two stages, and the impact of climate on both measures was explored. More specifically, the most common climatic indices were considered (see Tab. 18 in section 9.4). For each index, either measure (ratio or difference of forest SOC and pasture SOC) for each chronosequence was plotted against the values of the climatic index in that site, and the Pearson's correlation coefficient was calculated.

The same analysis was then repeated for the litter C pool. However, in this case only the pasture-to-forest difference was used, as computing the ratio was not possible because the litter was absent in the pasture stages.

8.9.2. Living biomass and dead organic matter and Ecosystem C stocks

Living biomass and dead organic matter were estimated from direct measurements only in the stages of pasture and forest, and in one intermediate stage.

The values in the remaining stages were instead estimated by linear interpolation.

Then, the five C pools (above- and belowground living biomass, dead wood and litter and soil) were added together in order to estimate the total ecosystem C stock for each stage in each chronosequence. Within each chronosequence, the differences in ecosystem C stock between each stage and the pasture stage were calculated, and are hereafter referred to as the stage-to-pasture ecosystem C stock differences.

An average profile of ecosystem C stock changes over time in each group (Alps and Apennines) was then generated by taking, in each stage, the mean of the stage-to-pasture ecosystem C stock differences over all chronosequences in each group.

Then, the percentage of C_{org} in each pool (living biomass, dead organic matter, topsoil and subsoil) in each stage was obtained dividing the absolute value of C_{org} in each pool by the total ecosystem C stock. This normalisation process was repeated in each chronosequence.

An above-ground functional type diversity index was estimated for pasture, intermediate and forest stages according to the following steps:

1. All the plant species were subdivided in functional types (grasses, shrubs and trees) and each of them was considered separately.
2. The diversity index of shrubs and trees was based on Shannon's diversity index H' (Shannon and Waver, 1949):

$$H' = -\sum_{i=1}^R p_i * \ln(p_i), \quad (16)$$

where p_i is the proportion of individuals belonging to the i th species and R is the species richness (i.e. the total number of species). Grass species were not identified and classified. Instead, the total grass aboveground biomass was used as a proxy for their diversity.

3. A mean and a standard deviation were measured for each plant functional type in each stage on the basis of the values of the field measurement replicates ($n = 3$).
4. The plant functional type mean value in each stage was normalised dividing it by the maximum functional type mean value in the same chronosequence, obtaining values that range from 0 to 1 along the chronosequence and reach 1 in the stage where they are highest.
5. Study areas were grouped in conifers and broadleaves, on the basis of the characteristics of the vegetation of the relative forest stage.
6. In each of the two groups, an average of the normalised functional type mean values was estimated for each stage.
7. Finally, in each group, these three averages were added together, leading to an aboveground functional diversity index that ranges from 0 to 3.

An aboveground functional diversity index of 0 indicates that the stage is characterised by grasses absence and monospecific tree and/or monospecific shrub coverage. An aboveground functional type diversity index of 3 indicates that the stage is characterised by the maximum aboveground grasses biomass in the chronosequence and maximum tree diversity and shrub diversity.

8.9.3. Soil biochemical indices

Within each chronosequence, the variation over time (pasture, intermediate and forest) of the C_{mic} , SEIc and chitinase (related to N cycle) enzyme activity were analysed using a one-way ANOVA. In case of statistically significant differences between stages, a post-hoc Tukey test was performed to compare each pair of stages separately. The analyses were performed separately for each depth interval considered (0-5 and 5-15 cm).

The study areas were grouped in conifers and broadleaves, on the base of the characteristic of the vegetation of the relative forest stage. Since the maximum microbial biomass and enzyme activities values were observed in the upper

part of the soil profile, the following analyses were performed only on the 0-5 cm depth layer. Microbial biomass, SEIc and Chitinase activity were normalised per unit of carbon and the variation over time was analysed using a one-way ANOVA. Tukey test was performed when statistically significant differences were detected. The same analyses were performed on the sum of litter and SOC stock of the 0-5 cm layer in each stage.

Finally, the overall above- and below-ground interaction was explored. All the SEIc and chitinase specific enzyme activities were plotted against all the relative plant functional type index values, and the Pearson's correlation coefficient was calculated.

All statistical analyses were performed using the GraphPad Prism 5 software.

9. RESULTS

9.1. Soil physico-chemical properties

The main soil physical properties observed in this study are summarised in Tab. 15. All the sites, except for Castello Tesino and Mel, were characterised in general by a low or very low rock fragments content. A rather marked variability could be detected when comparing the different stages in each study area. On the contrary, BD values were much more consistent among stages in each study area. Generally, the subsoil compartment was characterised by a higher soil density with respect to the topsoil one. Changes in rock and BD values were not analysed because these parameter were taken into account in the SOC and total N stock estimations.

Concerning the texture, in some of the study areas, in particular Farindola, Vastogirardi and Pignola, a variation in the percentage of clay, silt and sand was observed when comparing pastures / grasslands and the relative forest stages. More specifically, the percentage of clay, silt and sand increases markedly in Vastogirardi and decreases markedly in Farindola and Pignola. No mineralogical analysis was performed on the clay particles class. However, the possible effect of clay content in the soil on SOC amount was explored for the topsoil and subsoil, as well as for the whole profile (see Fig. 22). In all three cases, a statistically significant negative correlation was observed.

The variation of pH along the woody encroachment process was marked and typically in the direction of higher acidity in the older stages. Only two study areas showed a different trend. In the first of the two, Castello Tesino, the pH oscillated throughout the entire process, but around values that were generally lower in the initial part than in the final one. In the second case, in Vastogirardi, no significant pH variability was observed.

Concerning the C to N ratio, the forest stages of all study areas showed unexpectedly low values. The C to N ratio showed a trend similar to that of pH, i.e. lower values were observed for younger stages, while higher values were measured in the older woody encroachment stages.

In all chronostages, the encroached time 2 stage was the only one other than pasture (*P*) and forest (*F*) for which the cation exchange capacity was measured, and is hereafter referred to as the second encroached stage (*T2*) or simply as the *intermediate stage*. This is also the only stage other than pasture and forest where the plant living biomass and dead organic matter were measured and the microbial analyses performed (see Sections 8.6, 8.7, 8.8).

Table 15: Main soil physico-chemical variables measured in each chronostage. Chronostages are grouped by study area, and are identified by their mean age (see Section 8.1, Step 5). Rockiness and BD are reported separately for topsoil and subsoil, and were computed as a weighted average over the depth layers in each compartment (0-5, 5-15 and 15-30 cm for the topsoil; 30-50 and 50-70 cm); soil texture, pH, Cation Exchange Capacity (CEC) and C/N were computed as a weighted average over all soil layers. Only in Vastogirardi the 50-70 cm soil depth interval was not considered because of the thinner soil profile with respect to all the other study areas. Values in brackets are standard deviations across measurement replicates in each chronostage ($n = 3$); soil texture and CEC were computed from a single composite sample, so a single measurement was available. Capital letters for pH and C/N show the grouping resulting from Tukey's test.

Study area	Stage age	Rockiness (%)		Bulk density (Mg ha ⁻¹)		Soil texture			pH	CEC (cmol kg ⁻¹)	C/N
		Topsoil	Subsoil	Topsoil	Subsoil	Clay %	Silt %	Sand %	Class		
Castello Tesino	P 0	8.35 (3.42)	9.77 (7.18)	0.79 (0.05)	1.13 (0.06)	13.59	11.72	74.70	Sandy loam	4.94 (0.10) A	11.05 (2.55) A
	T1 9	7.09 (2.22)	34.60 (16.83)	0.80 (0.08)	1.16 (0.10)					6.79 (0.13) B	10.64 (0.15) A
	T2 19	10.98 (4.17)	23.75 (15.04)	0.69 (0.08)	0.93 (0.18)					4.91 (0.17) A	15.63 (2.37) B
	T3 28	9.17 (2.76)	25.08 (7.53)	0.72 (0.08)	1.02 (0.09)					5.08 (0.04) C	12.60 (0.42) AB
	T4 52	7.94 (1.40)	39.46 (2.27)	0.79 (0.05)	1.03 (0.15)					6.41 (0.17) B	10.89 (0.40) A
	F >62	4.83 (2.29)	7.50 (3.77)	0.70 (0.09)	1.08 (0.06)	23.16	25.34	51.50	Sandy clay loam	5.67 (0.25) C	11.53 (0.59) AB
Mel	P 0	48.29 (4.34)	42.89 (5.43)	0.97 (0.06)	1.15 (0.05)	12.75	22.29	64.96	Sandy loam	4.79 (0.08) AB	12.66 (0.38) ABC
	T1 5	30.74 (9.56)	39.44 (12.98)	0.90 (0.10)	1.14 (0.06)					4.71 (0.28) AB	11.76 (0.88) A
	T2 29	30.62 (5.48)	35.67 (3.38)	0.82 (0.05)	1.02 (0.07)					5.15 (0.33) A	9.99 (0.51) B
	T4 40	49.41 (7.85)	46.26 (15.01)	0.98 (0.06)	1.14 (0.10)					4.33 (0.16) B	13.89 (0.93) C
	F >62	51.03 (6.05)	42.78 (3.40)	0.88 (0.04)	1.14 (0.06)	13.97	22.85	63.17	Sandy loam	4.10 (0.23) B	11.23 (0.29) AB
	P 0	8.87 (5.09)	2.32 (2.31)	1.12 (0.02)	1.33 (0.02)	5.97	13.45	80.58	Sandy loam	6.45 (0.22) A	9.11 (0.36) A
Chianocco	T1 12	2.93 (1.10)	5.10 (5.82)	1.11 (0.08)	1.28 (0.02)					6.34 (0.17) AB	9.83 (0.57) AB
	T2 22	8.19 (1.54)	4.07 (4.10)	1.00 (0.09)	1.19 (0.06)					6.85 (0.34) A	11.51 (1.56) B
	T4 43	9.58 (3.86)	12.71 (9.22)	1.08 (0.08)	1.31 (0.02)					6.19 (0.07) AB	9.97 (0.47) AB
	F >62	15.29 (5.79)	3.01 (3.72)	1.16 (0.05)	1.30 (0.02)	9.83	12.29	77.89	Sandy loam	5.60 (0.27) B	14.83 (0.35) C
	P 0	28.47 (8.59)	23.58 (15.30)	1.42 (0.09)	1.03 (0.04)	22.18	17.55	60.27	Sandy clay loam	7.15 (0.13) AB	8.97 (0.73) AB
	T1 19	9.31 (1.72)	3.71 (0.76)	1.46 (0.07)	1.29 (0.04)					7.37 (0.07) A	8.31 (0.35) A
Firenzuola	T2 25	10.80 (2.80)	17.35 (8.42)	1.42 (0.06)	1.27 (0.12)					7.12 (0.09) AB	8.09 (0.56) A
	T3 33	9.75 (1.83)	23.92 (6.66)	1.39 (0.11)	1.29 (0.02)					7.01 (0.09) AB	8.52 (0.99) A
	T4 64	3.53 (1.47)	7.22 (5.44)	1.38 (0.08)	1.27 (0.04)					6.83 (0.11) B	9.97 (0.78) AB
	F >68	5.76 (2.97)	2.46 (4.10)	1.13 (0.05)	1.14 (0.06)	41.82	32.13	26.05	Silty clay loam	6.72 (0.11) B	11.05 (0.97) B
	P 0	6.47 (2.76)	5.16 (2.93)	1.17 (0.02)	1.30 (0.06)	42.51	27.16	30.33	Clay	6.36 (0.24) A	8.39 (0.36)
	T1 12	1.63 (0.68)	9.57 (8.19)	1.23 (0.10)	1.24 (0.03)					7.13 (0.12) B	11.31 (2.86)
Farindola	T2 24	7.71 (1.84)	4.15 (0.83)	1.23 (0.05)	1.15 (0.03)					6.30 (0.09) A	9.90 (0.51)
	T4 66	12.08 (4.34)	9.63 (6.95)	1.21 (0.10)	1.28 (0.03)					6.73 (0.21) AB	9.52 (0.32)
	F >70	12.11 (1.64)	10.81 (1.47)	0.92 (0.07)	0.97 (0.03)	11.12	22.24	66.64	Sandy loam	6.45 (0.17) A	10.39 (0.71)
	P 0	15.56 (2.65)	14.28 (9.01)	1.01 (0.05)	0.99 (0.04)	20.29	16.91	62.80	Sandy clay loam	5.20 (0.15)	9.57 (0.25) A
	T1 6	3.22 (1.22)	0.48 (0.71)	1.13 (0.06)	1.10 (0.00)					6.17 (0.13)	9.61 (0.0) A
	T2 13	1.46 (0.37)	3.09 (4.20)	1.21 (0.09)	1.16 (0.05)					6.20 (0.14)	11.21 (0.46) B
Vastogirardi	T4 48	3.17 (1.28)	2.10 (0.81)	1.06 (0.06)	1.06 (0.06)					6.05 (0.33)	10.92 (0.82) AB
	F >62	4.42 (4.72)	5.29 (7.35)	1.15 (0.06)	1.30 (0.03)	48.12	23.29	28.59	Clay	5.50 (0.46)	10.07 (0.36) AB
	P 0	16.93 (16.29)	5.01 (4.14)	1.32 (0.03)	1.35 (0.02)	50.01	19.92	30.07	Clay	7.29 (0.15) A	8.24 (1.03) A
	T1 18	4.38 (3.41)	1.66 (0.96)	1.33 (0.04)	1.26 (0.06)					6.64 (0.19) ABC	10.44 (2.00) AB
	T2 25	5.92 (6.42)	10.54 (15.91)	1.19 (0.06)	1.23 (0.06)					7.08 (0.28) AB	10.50 (0.87) AB
	T4 51	5.28 (1.74)	8.22 (5.13)	1.41 (0.04)	1.32 (0.05)					6.12 (0.28) BC	8.79 (2.15) A
Pignola	F >60	15.84 (4.98)	5.68 (1.81)	0.94 (0.08)	1.07 (0.13)	11.04	21.45	67.52	Sandy loam	6.03 (0.08) C	12.98 (0.82) B

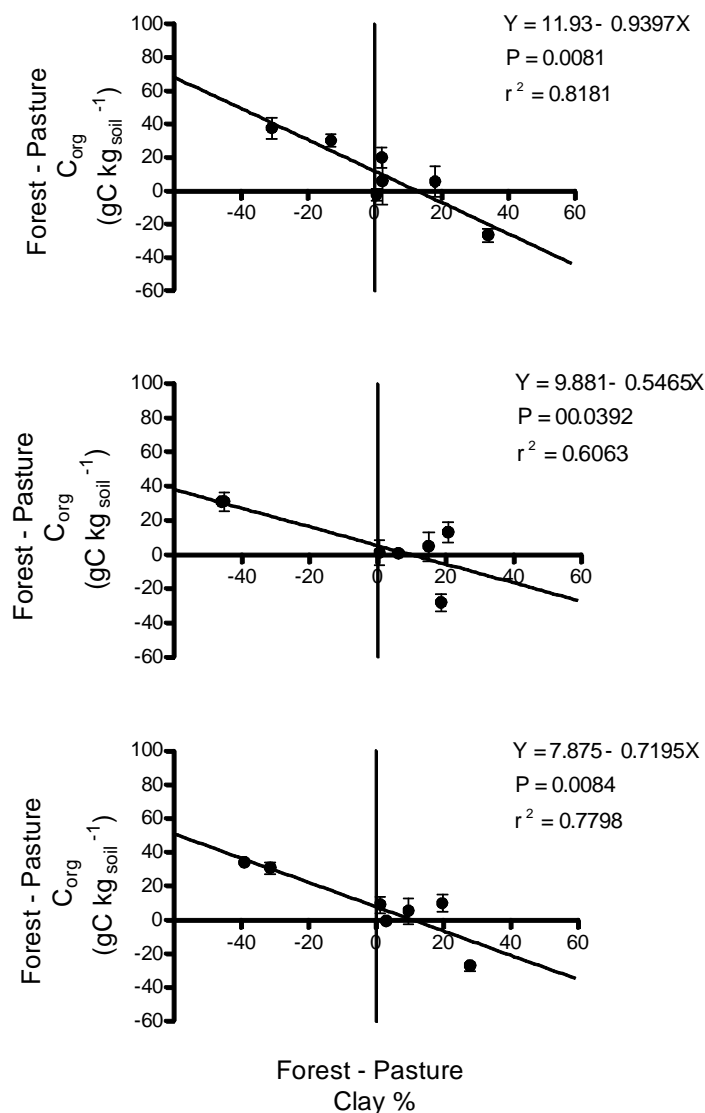


Figure 22: Linear regression analyses between mean forest to pasture SOC content differences and forest to pasture differences in the clay content percentage. Graphs 1, 2 and 3 refers to topsoil, subsoil and the whole soil profile, respectively. Each value represents the mean and the bars the sd ($n=3$).

9.2. SOC and total N concentration

The SOC variation along the soil profile of the different study areas is shown in Fig. 23. When considering all the stages together, the SOC concentration was found to decrease with increasing depth.

The differences between the stages in each study area were further investigated. In Castello Tesino (Fig. 23A), the 19-year-old intermediate stage was characterised by the highest SOC values (even if not always significantly) in all the soil depth intervals considered. In particular, the C concentration in this stage decreased from a mean of $177 \text{ gC kg}_{\text{soil}}^{-1}$ on the upper part of the mineral soil (0-5 cm of depth) to a value of $54 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the deepest interval (50-70 cm). On the contrary, the 9-year-old stage showed the lowest values at all soil depth considered. In this stage, the C concentration decreased from a mean value of $90 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the 0-5 cm interval to a mean value of $17 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the 50-70 cm interval. The stages at 9 and 19 years were statistically significantly different in each soil depth layer.

For all the other stages, intermediate values were observed. Statistically significant differences from the intermediate stage (19 years old) were measured in the 0-5 cm (for all the stages), in the 30-50 cm (for the pasture, the 28-year-old stage and the forest), and in the 50-70 cm depth layer (only for the pasture). No statistically significant differences were measured comparing the 9-year-old stage with all the others (except for the intermediate one) for each soil depth interval considered.

In the Mel study areas (Fig. 23B), no statistically significant differences between stages were observed at each depth layer. The maximum C concentration in the upper layer (at a depth of 0-5 cm) was observed in the forest stage ($132 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$) while, in contrast, the maximum values in the deepest layers (30-50 and 50-70 cm) were observed in the intermediate stage (29 years old), with 42 and $29 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$, respectively.

The mean C concentration of the Chianocco site (Fig. 23C) was generally lower than the previous study areas in all chronosequences. The values varied from a maximum of $47 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the upper soil layer in the intermediate stage (22 years old) to a minimum of $7 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the deepest layers at pasture stage. Statistical differences in the SOC concentration were observed only in the central part of the soil at a depth of 15-30 and 30-50 cm. In particular, in the 15-30 cm layer, the C concentration in the forest was significantly different from that of the 43-year-old stage, while, in the 30-50 cm layer, the pasture C concentration was lower than in the encroached stages of 12 and 22 years, and in the older successional stages (43 years and forest) it was lower than in the intermediate stage (22 years).

In the Firenzuola study areas (Fig. 23D), the C concentration ranged between a mean of $68 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the upper layer of the intermediate stage (25 years old) to a mean of $5 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the deepest layer of the 19-year-old stage. Statistically significant differences were observed only in upper three layers: at 0-5, 30-50 and 50-70 cm. In the upper one, the 25-year-old stage was statistically significantly different from the 33-year-old one, while all the other stages were not significantly different from either the 25- or the 33-year-old ones. In contrast, both in the 30-50 and 50-70 cm layers, the maximum C concentration was measured in the forest stage. In the first case, the forest C concentration was statistically significantly different from all the previous stages of the woody encroachment (pasture, 19 and 25 years old), while in the latter it was significantly different from three intermediate stages (19, 25 and 33 years old).

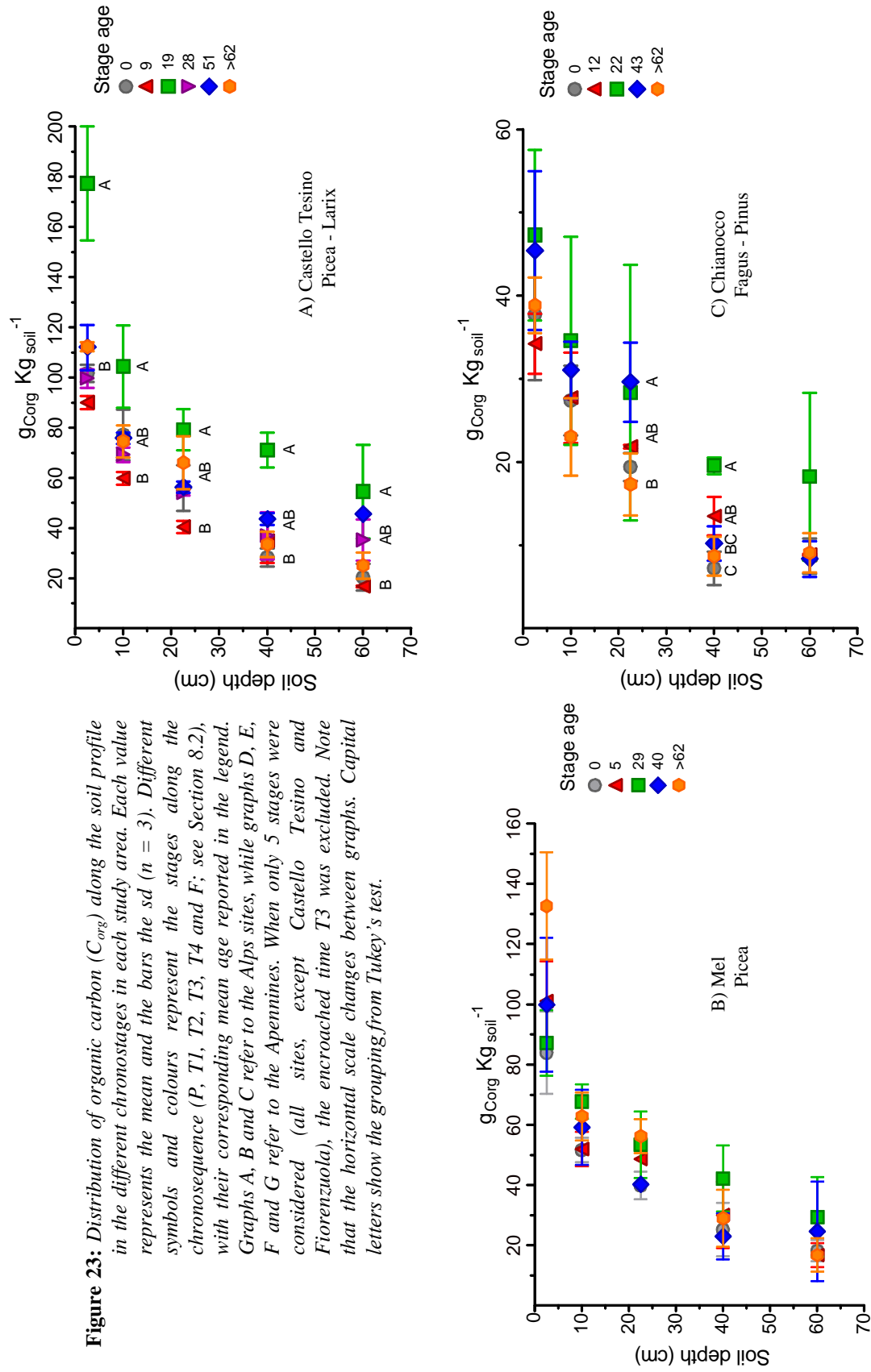
In the Farindola study area (Fig. 23E), the C concentration values ranged from a mean of $82 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ to a mean of $7 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the upper forest soil layer and deeper grassland soil layer, respectively. The C concentration in the forest soil was generally higher than in all other stages at each depth interval except for the upper one, where no statistically significant differences were observed between forest stage values and those of the pasture and the first encroached stage (12 years old). In the other layers, the difference between forest and all the other stages were more pronounced. Considering all the stages other than the forest, the C concentration varied more markedly in the upper part of the soil (0-5 cm) and in the deepest ones (30-50 and 50-70 cm) than in other layers. More in detail, in the 0-5 cm layer, in the 66-year-old stage the C concentration was significantly lower only compared to that of the 12-years-old stage. In the 30-50 cm layer, the C concentration in the 12 and 24 years-old stages was statistically significantly higher than that of the pasture and of the 66-year-old stage. In the deepest layer, the amount of carbon in the 12-year-old stage was lower only in comparison to the intermediate stage (24 years old).

Similarly to what was described for the Farindola study area, in the Pignola one (Fig. 23F), the C concentration of the forest stage was significantly different from that of all the other stages in each soil depth interval. The C concentration mean values ranged from minimum of $6 \text{ gC}_{\text{org}} \text{ kg}_{\text{soil}}^{-1}$ in the deepest layer of the pasture to a maximum of $95 \text{ gC}_{\text{org}}$

$\text{kg}_{\text{soil}}^{-1}$ measured for the forest upper soil interval. When looking at the difference among the layers other than the forest, in the two upper intervals (0-5 and 5-15 cm), the 51-year-old stage had a lower C concentration than the intermediate stage (25 years old), while in the 30-50 cm layer the pasture stage had a lower C concentration with respect to that of the 25-year-old stage. In the other soil depth layers (15-30 and 50-70 cm), no statistically significant differences were measured in the non-forest stages.

Although Vastogirardi was located North of Pignola and was introduced before Pignola in Section 8.2 and Tab. 15, it is here discussed last (and reported as last in all figures) because its results were different with respect to all other study areas. Indeed for Vastogirardi (Fig. 23G), the C concentration of the forest stage was generally the lowest in all depth intervals. In addition, the mean pasture C concentration was the highest in each depth layer. Moreover, this study area was characterised by a thinner soil profile (0-50 cm) compared to the others. More in detail, in the 0-5 cm layer, the C concentration tended to decrease along the woody encroachment process, with a maximum value in the pasture and a minimum in the 48-year-old stage. This stage was statistically significantly different from the pasture and the 6-year-old stage. The stage at 13 years and the forest had lower values only when compared to the pasture. In the layer at 5-15 cm, pasture C concentration was higher than for all the other stages, among which no statistically significant differences were observed. The high C concentration variability measured in each stage in the 15-30 cm did not permit to detect statistical differences among the stages. In the deepest layer, pasture C concentration was statistically different to the 6-, 13year-old and forest stages. In addition, in the forest stage, the C concentration was lower than in all other stages.

Figure 23: Distribution of organic carbon (C_{org}) along the soil profile in the different chronostages in each study area. Each value represents the mean and the bars the sd ($n = 3$). Different symbols and colours represent the stages along the chronosequence (P, T1, T2, T3, T4 and F; see Section 8.2), with their corresponding mean age reported in the legend. Graphs A, B and C refer to the Alps sites, while graphs D, E, F and G refer to the Apennines. When only 5 stages were considered (all sites, except Castello Tesino and Fiorenzuola), the encroached time T3 was excluded. Note that the horizontal scale changes between graphs. Capital letters show the grouping from Tukey's test.



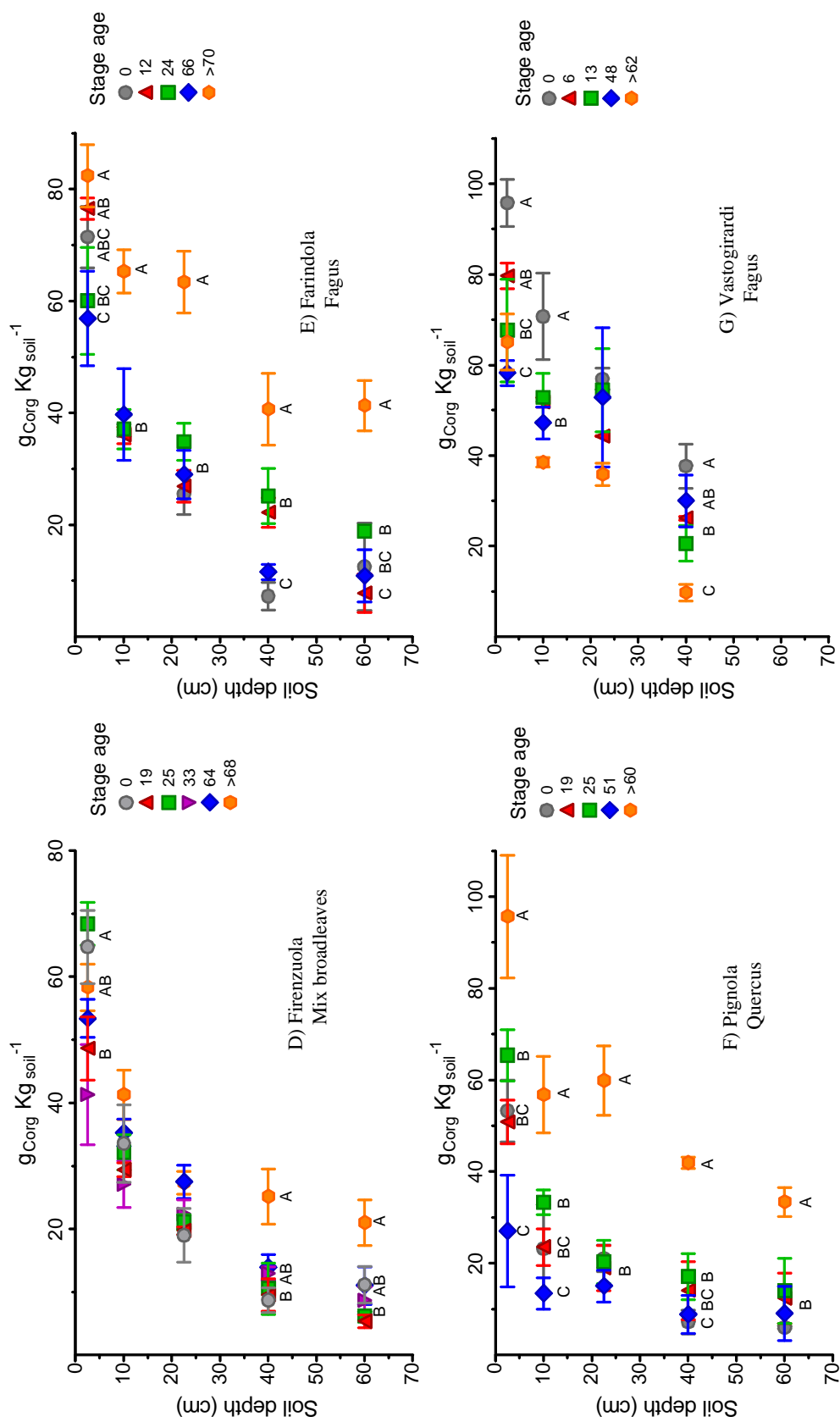


Fig. 24 shows the total N concentration along the soil profile in each study area. In each of them, the differences among stages were tested for statistical significance. As observed for the C concentration, the total N concentration decreased progressively with the soil depth.

In Castello Tesino (Fig. 24A), statistically significant differences among the stages were observed in the upper and deepest soil layers. More in detail, in the 0-5 cm layer, the highest N concentration was observed in the intermediate stage (19 years old). Its N concentration was statistically different from that of the 9-, 28-, 51-year-old and forest stages. The pasture stage N concentration was not significantly different from that of all the other stages. In the 50-70 cm layer, the maximum N concentration was measured in the 51-year-old stage. Its N concentration was different to the respective values of the pasture, 9-, 28-year-old and forest stages. The intermediate stage did not show significant differences to any of the other stages.

In Mel study area (Fig. 24B) statistically significant differences in N concentration among stages were observed only in the topsoil compartment. In particular, in the 0-5 cm layer, the N concentration in the forest stage was statistically higher than that of the 40-year-old stage. In the 5-15 cm layer, the intermediate stage of 29 years had a N concentration significantly higher than that of all the other stages. In the 15-30 cm layer, the N concentration in the intermediate stage was significantly higher than only that of the 40-year-old stage.

The Chianocco study area (Fig. 24C) was characterised by a lowest N concentration in the forest stage in each soil depth interval. More in detail, the N concentration in the forest is statistically significantly different from that of all the other stages in the topsoil. In the 30-50 cm layer, a maximum N concentration was measured in the intermediate stage (22 years old). Statistically significantly lower values of N concentration were measured in the pasture, 43-year-old and forest stages. In addition, the forest N concentration was statistically lower than that of the 12-year-old stage. In the deepest layer (50-70 cm), a statistically significant difference was observed among the intermediate stage of 22 years (where the value is maximum) and the forest stage.

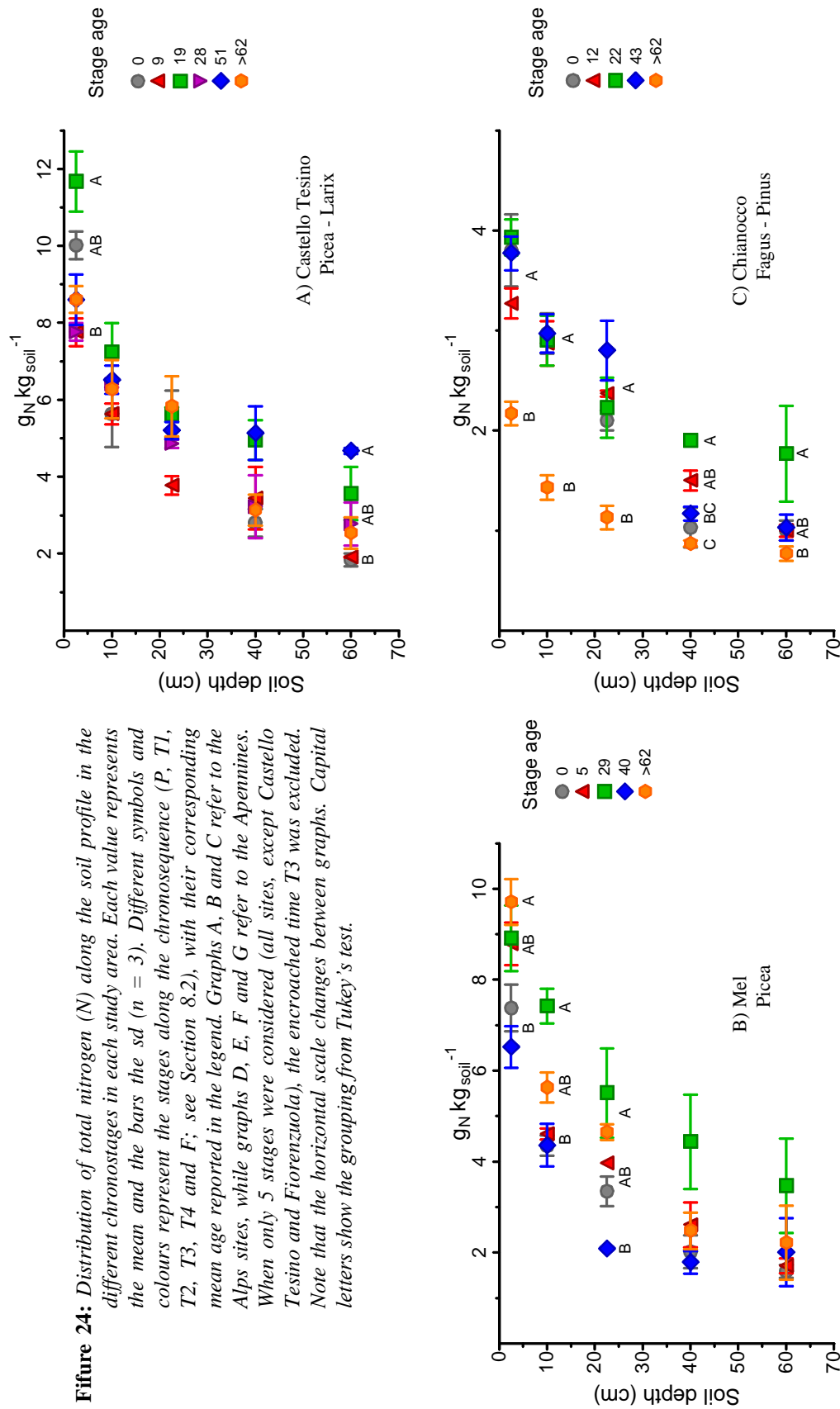
Differently from what measured in the Mel study area, in the Firenzuola one (Fig. 24D) the differences in N concentration among sites were statistically significant only in the subsoil. In addition, the maximum values were measured in the forest stage. In the 30-50 cm layer the forest N concentration was statistically significantly different from those of pasture, 19-, 25- and 64-year-old stages, while in the 50-70 cm layer significant differences were measured among the forest stage and the stages at 19 and 25 years.

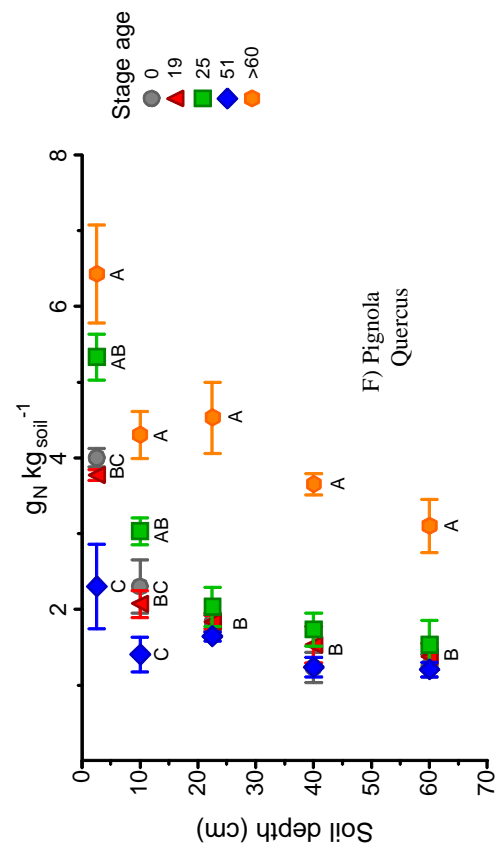
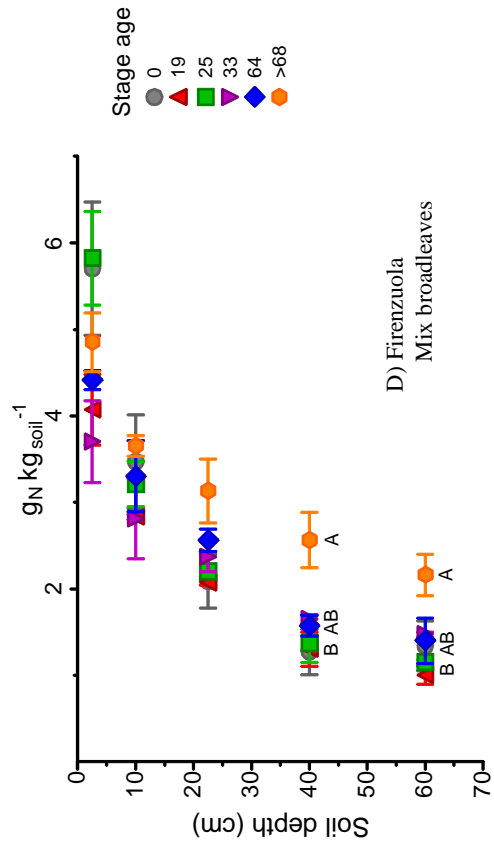
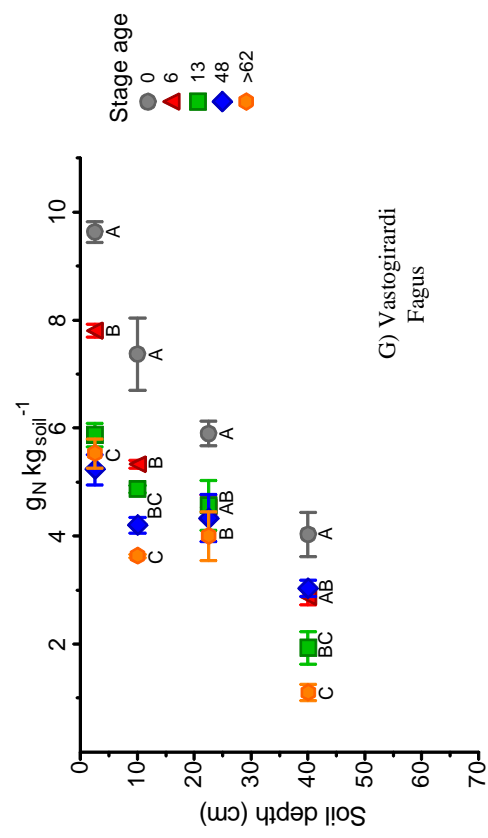
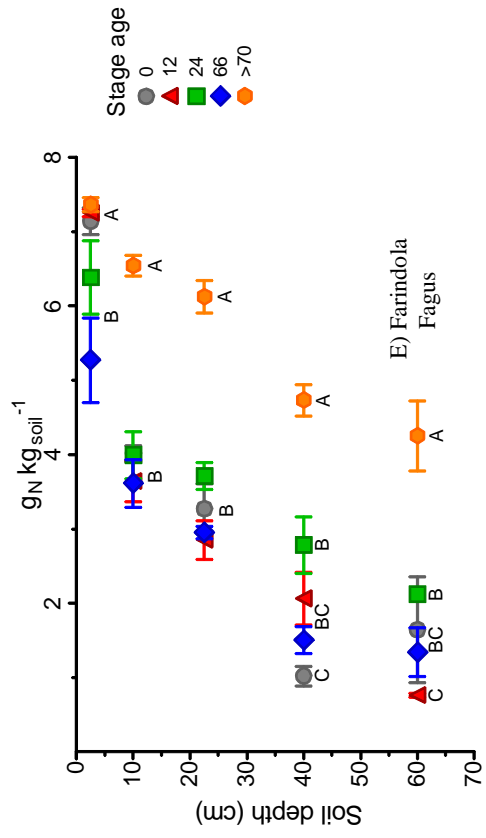
As described for Firenzuola, in the Farindola study area (Fig. 24E) the higher N concentration were measured in the forest stage in each depth interval considered. In the 0-5 cm layer, in the pasture, 12-year-old and the forest stages the N concentration was statistically significantly higher than that measured in the 66-year-old stage. In the 5-15 and 15-30 cm layers, the N concentration in the forest was statistically significantly higher than of all the other stages. In the 30-50 cm layer the maximum value measured in the forest stage was statistically different to that of all the other stages. In addition, Tukey's test showed a significant difference between the pasture and the 24-year-old intermediate stage, with the lowest value measured in the pasture. In the 50-70 cm layer, a similar pattern was measured: the forest stage was that with the highest N concentration and it was statistically significantly different from all other stages. Other differences were measured between the intermediate stage (24 years old) and that of 12 years. In this last one, the lowest value was measured.

In Pignola (Fig. 24F), the forest stage showed the highest values in all the soil depth layers and the Tukey's test showed that it was always statistically significantly different from all the other stages except for the 25-year-old intermediate ones in the 0-5 and 5-15 cm intervals. Other differences were measured among the other stages only in these two layers. In particular, in both of them, the lowest N concentration values were observed in the 51-year-old stage and the Tukey's test showed that values was statistically significantly different from that of the intermediate one (25 years old).

In the Vastogirardi study area (Fig. 24G), the repeated measurement ANOVA and Tukey's test results showed a progressive reduction in the N concentration along the woody encroachment process in each soil depth layers. Therefore, the N concentration results respected the trend described and shown for the C_{org} . The maximum N concentration values were measured in the pasture stage and the minimum ones in the forest. The differences among them were statistically significant in all the layers. More in detail, in the 0-5 cm layer, the 6-year-old stage had a N concentration statistically significantly lower than that of the pasture and, at the same time, significantly higher than that of the three older stages. In the 5-15 cm layer, again the 6-year-old stage had a statistically lower N concentration with respect to the pasture, but statistically significantly higher only to that of the forest stage. No significant differences were confirmed comparing the 25- and 51-year-old stages and both the 6-year-old and forest stages. In the 15-30 cm layer, statistically significant differences were observed only between pasture and forest. The three intermediate stages did not show different values with respect to both the pasture and the forest. In the 30-50 cm layer, the intermediate stage of 25 years and the forest, which were not significantly different in N concentration, showed significantly lower values compared to the pasture stage. Moreover, only the forest stage had a statistically significantly lower N concentration amount than the 6- and 51-year-old stages. No significant differences were measured among these stages and the 25-year-old one.

Figure 24: Distribution of total nitrogen (N) along the soil profile in the different chronostages in each study area. Each value represents the mean and the bars the sd ($n = 3$). Different symbols and colours represent the stages along the chronosequence (P, T1, T2, T3, T4 and F; see Section 8.2), with their corresponding mean age reported in the legend. Graphs A, B and C refer to the Alps sites, while graphs D, E, F and G refer to the Apennines. When only 5 stages were considered (all sites, except Castello Tesino and Fiorenzuola), the encroached time T3 was excluded. Note that the horizontal scale changes between graphs. Capital letters show the grouping from Tukey's test.





9.3. Soil organic carbon and nitrogen stocks

SOC and total N stocks were determined for each soil depth interval on the basis of the relative C concentration, layer thickness, soil bulk density and rock percentage as described in Section 8.5. The C and N stock amount in the topsoil (0-30 cm depth) and subsoil (30-70 cm depth) were estimated as the sum of the C and N stock of each interval included in these two compartments. The subsoil stocks in the Vastogirardi study area refer to the estimation performed for the 30-50 cm depth interval only, due to the presence of shallow soils in this area. Topsoil, subsoil and whole soil profile stocks were summarised in Tab. 16, Fig. 25, Tab.17, and Fig. 26 for C and N, respectively. In order to simplify the comparison between sites, the stages in all chronosequences are labelled, in increasing order, P (pasture), T1, T2 (the *intermediate* stage), T3, T4 and F (forest), with stage T3 being present only in the sites of Castello Tesino and Firenzuola (6 stages rather than 5). Given the age of such stages varies between chronosequences (e.g. the mean age of T2 is 19 years in Castello Tesino and 25 years in Firenzuola), for simplicity in the Tabs. 16 and 17 and Figs. 25 and 26, all stages with the same label are associated, rather than with their actual age, with the mean of their ages across all chronosequences.

Table 16: Changes in SOC stock along the chronosequences in each study area. Topsoil (0–30 cm depth) and subsoil (30–bedrock) are considered both separately and grouped together for the SOC stock estimation of the whole soil profile. Values are the mean and sd (in brackets) over the measure replicates ($n = 3$). Numbers with different letters are significantly different ($P < 0.05$) among stage ages.

Study area	Soil depth (cm)	Stage mean age				
		P (0 year) SOC (MgC ha ⁻¹)	T1 (14 years) SOC (MgC ha ⁻¹)	T2 (22 years) SOC (MgC ha ⁻¹)	T3 (30 years) SOC (MgC ha ⁻¹)	T4 (51 years) SOC (MgC ha ⁻¹)
Castello Tesino	0-30	148.44 (21.17)	114.56 (5.83)	172.63 (29.90)	125.06 (18.73)	131.77 (36.60)
	30-70	95.17 (18.48)	67.15 (11.09)	154.99 (49.29)	107.24 (38.46)	112.94 (14.54)
	0-70	243.61 (36.58) AB	181.71 (7.92) B	327.62 (28.72) A	232.30 (25.42) AB	244.71 (44.72) AB
Mel	0-30	75.87 (7.58)	69.72 (10.86)	81.23 (15.35)	71.42 (11.50)	97.60 (10.14)
	30-70	51.37 (6.58) BC	70.08 (20.10) AB	97.97 (16.64) A	43.95 (7.26) C	49.55 (3.89) BC
	0-70	127.24 (6.19) B	139.79 (11.61) B	179.20 (19.16) A	115.36 (18.74) B	147.16 (6.58) AB
Chianocco	0-30	74.75 (5.03)	82.90 (9.96)	90.35 (36.90)	95.62 (12.42)	65.05 (1.59)
	30-70	40.87 (3.51)	53.16 (4.33)	81.83 (17.73)	41.17 (3.01)	51.18 (4.22)
	0-70	115.62 (2.83) B	136.06 (12.99) AB	172.17 (38.95) A	136.78 (14.52) AB	116.23 (4.05) B
Firenzuola	0-30	91.96 (6.38) B	107.47 (4.80) AB	114.68 (11.55) AB	98.83 (12.71) B	133.10 (14.13) A
	30-70	27.50 (9.66) B	33.98 (7.32) B	28.32 (6.87) B	38.72 (7.93) B	52.09 (15.53) B
	0-70	119.45 (16.04) C	141.45 (7.41) BC	143.01 (5.68) BC	137.55 (16.19) BC	185.19 (26.70) AB
Farindola	0-30	116.08 (3.72) B	86.93 (16.21) B	119.11 (28.32) AB	101.39 (21.80) B	161.16 (10.11) A
	30-70	47.33 (11.14) C	63.65 (1.69) B	95.78 (7.38) B	50.80 (3.36) C	140.85 (6.35) A
	0-70	163.40 (14.77) BC	150.58 (14.56) BC	214.90 (28.57) B	152.19 (19.62) C	302.01 (15.58) A
Vastogirardi	0-30	169.10 (16.79)	167.18 (10.62)	199.02 (33.67)	157.00 (21.07)	133.07 (7.80)
	30-50	64.34 (11.54) A	57.27 (0.26) A	46.32 (8.92) A	62.11 (9.35) A	24.02 (5.44) B
	0-50	233.44 (27.34) A	224.45 (10.82) AB	245.34 (26.67) A	219.11 (27.30) AB	157.09 (13.24) B
Pignola	0-30	84.97 (15.58) B	91.84 (16.69) AB	104.49 (13.53) AB	62.95 (10.58) B	151.77 (32.78) A
	30-70	33.70 (7.26) B	63.74 (24.87) B	64.23 (13.02) B	41.84 (16.47) B	142.10 (3.62) A
	0-70	118.67 (21.34) B	155.57 (41.55) B	168.71 (25.92) B	104.79 (26.97) B	293.86 (35.87) A

The graphs in Fig. 25 were grouped in Alps (left column) and Apennine (right column), as it was done for the C and N concentration. For uniformity, all percentage values described hereafter express the SOC stock in any stage as increment with respect to the corresponding initial pasture value.

In the Castello Tesino study area (Fig. 25A), the Tukey's test did not reveal any statistically significant difference among the woody encroachment stages, either in the topsoil or in the subsoil compartment. However, it is possible to observe that SOC stock changed over time in both the compartments. In particular, in the topsoil, the SOC stock decreased by 23% between stages P and T1, and then increased reaching the maximum, in the *intermediate stage* (T2), of +16% more than the initial pasture value. After this peak, the SOC stocks values decreased again, reaching 13% less than the original pasture value in T3 and, even if a slight increase was visible, in the forest stage the SOC stock mean value was 4% less than that of the previous pasture. On the other hand, in the subsoil, the mean SOC stock initially decreased by 29% between P and T1 stages, and then it increased reaching a maximum of +63% more than in P in the intermediate stage (T2). After this peak, the SOC stocks values decreased again to +13% with respect to P in T3 and, this percentage did not consistently change over time in the last part of the process. The forest stage mean SOC stock is 16% higher than that of the previous pasture. Differently with respect to both the topsoil and subsoil in isolation, statistically significant differences were observed when the whole soil profile was considered. Even though the SOC stock in the intermediate stage (T2, 22 years old) was higher than that of all the other stages, a statistically significant difference was confirmed only between that and the 14-year-old stage (T1). The estimation of percentage changes with respect to the previous pasture highlighted that the major changes appeared in the first part of the woody encroachment process. After an initial decrease of 25% (between P and T1), a marked increment in the SOC stock was measure in T2 (reaching +34% of the initial value in P). Subsequently, the SOC stock decreased to 4% less than P and then it reached mean values similar to that of the pasture (0.5% more than P in T4). The forest stage is characterised by a +4% increase in the SOC stock with respect to P.

In the Mel study area (Fig. 25B), the SOC stock changes in the topsoil were moderate and did not show any statistically significant difference among the stages. More in detail, considering their variation in percentage with respect to pasture, the SOC stock decreased by 8% between P and T1 and, subsequently, it increased to 7% more than the value in P between T1 and T2. Another decrease was registered between T2 and T4 (to 5% less than P). Finally, a more pronounced increment was measured between T4 and the forest stage, where the mean SOC stock was 29% higher than that of the previous pasture. Differently to what described for the topsoil, Tukey's test showed statistically significant differences among the stages both in the subsoil and in the whole profile. In both the cases, the maximum value was measured in the intermediate stage (T2). More in detail, considering the subsoil, the changes in the mean SOC stock were marked in initial part of the process (reaching +36 and +91% more than the previous pasture in T1 and T2, respectively) and then decreased to a constant values in T4 and F (to 14 and 4% less than P). When considering the whole profile, a general increase was measured in the first part of the process with an increment of 10 and 40% with respect to P in T1 and T2, respectively. A marked decrease to 9% less than P was measured in T4. Finally, the forest stage was characterised by a SOC stock of 16% higher than that of the P.

The changes in the SOC stock in Chianocco chronosequence (Fig. 25C) were similar to those described for the previous study areas. In particular, when considering the whole soil profile SOC stock, the maximum value was observed in the intermediate stage. In addition, this value was statistically significantly different from the SOC stock estimated in the pasture and forest stages. No statistically significant differences were detected for either the topsoil or subsoil

separately. In the first case (topsoil), initially the mean SOC stock increased along the first three steps (with values of, respectively, +11, +21, +28% more than the previous pasture) and then drastically decreases in the last part of the process. The forest mean SOC stock is 13% lower than that of the initial stage. Similarly to what described for the topsoil, in the subsoil, the mean SOC stock initially increased along the first two steps (+30 and +100% more than the previous pasture) and then decreased to 1% more than P in T4 stage. The SOC stock in stage F is 25% higher than that of P. Finally, considering the whole soil profile, all the encroached stages had a higher SOC stock with respect to the pasture. The percentage estimates confirm the positive trend in the first part of the encroaching process (+17 and +49% more than P in T1 and T2 respectively), and then a progressive decrease (to +18% and +0.5% more than in P) in stages T4 and F.

In the Firenzuola study area (Fig. 25D), a gradual increase in the SOC stock was estimated both in the topsoil and in the subsoil. Maximum stock values were measured in T4 and F. In the topsoil, the SOC stock increased by 17% between P and T1 and then increased further by 8% of P between T1 and T2 (reaching 25% more than P). Between T2 and T3, the SOC stock decreased to 7% more than P and then increased again to 45% of P between T3 and T4. The SOC stock of the forest stage was 25% higher than that of the previous pasture. In the deeper compartment (subsoil), the SOC stock increased by 24% between P and T1 and then decreased by 19% between T1 and T2. In T3, the SOC stock was 41% higher than that of the pasture. This percentage increased in the latter stages of the process reaching values of 89 and 264% more than those of the pasture. The repeated measurement ANOVA verified that differences were statistically significant when considering the whole profile too. The following post-hoc comparison among stages proved that both the 51-year-old (T4) and the forest stages were statistically significantly different from the previous pasture. The changes in percentage with respect to pasture value highlighted a progressive increase in the SOC stock in the first part of the process (+18 and +20% in T1 and T2, respectively). Then, the SOC stock slightly decreased to +15% more than P in stage T3. Finally, a marked increment to +55% and +80% more than the initial pasture value was measured in the latter part of the secondary succession (stages T4 and F, respectively).

In the Farindola study area (Fig. 25E), the SOC stock changed significantly along the woody encroachment both in the topsoil and subsoil. Similarly to what was shown for Firenzuola, there was a general and gradual increment in the stocks along the process. However, minimum stock values (both in the topsoil and in the subsoil) were observed in the T4 stage. The SOC stocks in this stage was not statistically significantly different from the previous pasture even if a significant increase in the subsoil stock amount compared to the pasture stage was observed both in the T1 and T2 stages. The maximum SOC stock was observed in the forest stage in the topsoil (higher stock with respect to pasture, T1 and T2 stages), in the subsoil (higher than all the other stages) and in the whole profile (higher than all the other stages). Considering the percentage values in the topsoil, initially the SOC stock markedly decreased by 25% between the pasture and T1, then it increased between T1 and T2 reaching a mean values that is 3% higher than that of the previous pasture. Then, the SOC stock decreased another time (to 13% less than the previous pasture) and finally increased reaching the maximum value in the forest stage (+39% more than in P). More marked changes were estimated in the subsoil compartment. As previously described, the SOC stock increased over time until the intermediate stage with values of +34 and +102% more than those of P, in T1 and T2, respectively. Then, it substantially decreased reaching, in T4, a +7% more than in P. Finally, the SOC stock in the forest stage was 198% higher than that of the first stage of the chronosequence. When considering the whole profile, the huge percentage values described for the subsoil were reduced by the lower variability observed in the topsoil. With respect to the previous pasture, the SOC stock mean percentages were -8, +32, -7 and +84% more than in the pasture in T1, T2, T4 and F, respectively.

The same changes in the topsoil subsoil and whole profile SOC stock were measured in the Pignola study area (Fig. 25F). More in detail, in the topsoil, the SOC stock progressively increased between P and T2 (+8% and 23% more than P in T1 and in T2 respectively) and then decreased, reaching a minimum value in T3 (of 26% less than the previous pasture). The SOC stock in the forest stage was 78% higher than that of the previous pasture. In the subsoil, with the exception of the T4 stage, a substantial increment in the SOC stock was measured in T1, T2 and F stage (+89, +91 and +322% more than the previous pasture, respectively). Similarly to what was described for the Farindola study area, in Pignola the SOC stock changes in the whole profile with respect to the previous pasture are lower than that measured for the subsoil separately. Indeed, the measured percentage in the SOC stock changes from the initial pasture value were +31, +42, -12 and +148% in T1, T2, T4 and F stages, respectively.

Similarly to what was described for the C concentration, the Vastogirardi SOC stock trend (Fig. 25G) was quite different compared to all the other study areas. In particular, no variation was observed in the topsoil compartment, while a gradual reduction of the SOC stock was detected in the subsoil. Considering this compartment alone, the value of the forest stage was significantly lower than that of all the other stages. A reduction of the SOC stock was detectable also when considering the whole profile. Again, the minimum value was estimated in the forest stage and a statistically significant difference was confirmed between this stage and the pasture or the T2 ones. Considering the percentage values, in the topsoil the SOC stock did not change in the first step of the process (-1% between P and T1). Then a maximum SOC stock values was measured in the intermediate stage (+18% more than the previous pasture). Finally, the SOC stock in the topsoil tended progressively to decrease to 7 and 21% less than the initial value of the pasture in T4 and F stages respectively. Considering only the subsoil compartment, all the stages had a lower SOC stock with respect to the pasture. In particular, the percentage values of reductions from pasture are -11, -28, -3 and -63% in T1, T2, T4 and F stages, respectively. Finally, considering the whole soil profile, the main part of the secondary succession process do not lead the SOC stock to change considerably. Mean percentage values of variations from the initial pasture value were -4, +5 and -6% in T1, T2 and T3, respectively. The forest SOC stock was 33% lower than that measured in stage P.

Overall, looking at Fig. 25, if Vastogirardi is not considered, the study areas included in each group show a similar C stock changes along the woody encroachment process. Indeed, in the Alps group a variation in the C stock was detected with a maximum value in the intermediate stage (T2, around 22 years old) and a following decrease until the forest stage, which was however not statistically significantly different to the previous pasture. Considered in percentage, the mean SOC stock changes for the Alps study areas highlighted the initial increment of 0.7% and 41% with respect to P in T1 and T2 respectively. Then, the SOC stock tended to rapidly decrease to 4% less than P in the T3 stages and to stabilise around similar values with respect to that of the pastures. SOC stock percentage values of +3 and +6% more than P were measured for T4 and F stages, respectively.

On the opposite, in the Apennine group, a general progressive increase in the C stock was detected along the woody encroachment, which led to a consistent different C stock among the forest stages and the previous pastures. On average, the different successional stages were characterised by a SOC stock 14 (for T1), 31 (for T2), 15 (for T3), 12 (for T4) and 104% (for F) higher than the SOC stock of the pasture.

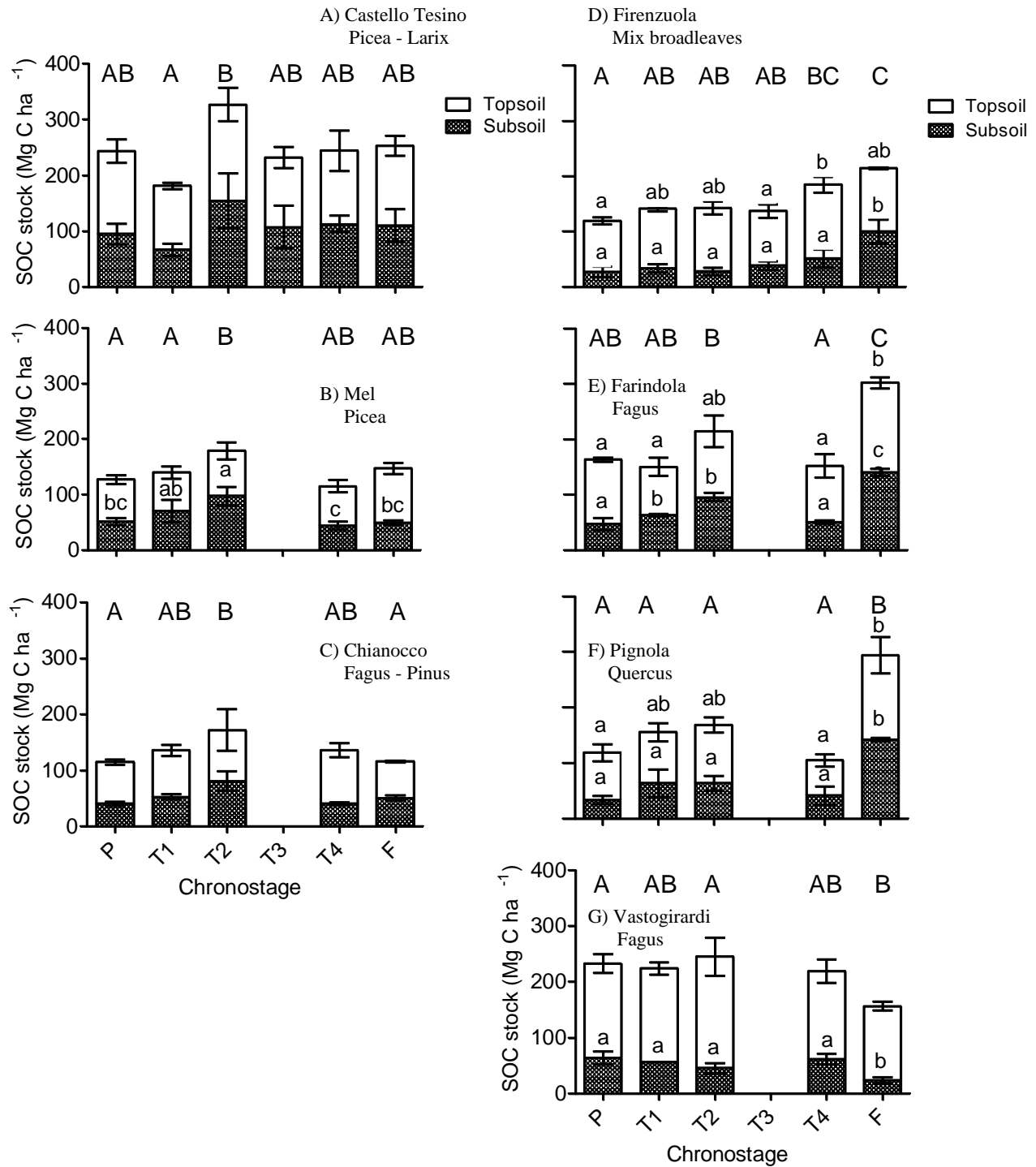


Figure 25: Changes in SOC stock along the woody encroachment process. Stages are classified by means of their average age. Statistically significant differences among the stages are labelled with case letter (considering topsoil and subsoil separately) or with capital letters (whole soil profile). Lowercase and capital letter are added only in case of significant statistical differences.

Table 17: Changes in N stock along the chronosequences in each study area. Topsoil (0-30 cm depth) and subsoil (30-bedrock) are considered both separately and grouped together for the N stock estimation of the whole soil profile. Values are the mean and sd (in brackets) over the measure replicates ($n = 3$). Numbers with different letters are significantly different ($P < 0.05$) among stage ages.

Study area	Soil depth (cm)	Pasture (0 year) (MgN ha ⁻¹)	Stage mean age					Forest (>62) (MgN ha ⁻¹)
			T1 (14 years) (MgN ha ⁻¹)	T2 (22 years) (MgN ha ⁻¹)	T3 (30 years) (MgN ha ⁻¹)	T4 (51 years) (MgN ha ⁻¹)		
Castello Tesino	0-30	13.17 (4.42)	10.56 (0.57)	12.18 (2.34)	11.05 (1.55)	13.40 (1.60)	12.20 (1.60)	
	30-70	9.37 (2.19)	7.14 (1.06)	10.58 (2.69)	8.88 (2.89)	11.57 (2.96)	10.78 (22.98)	
	0-70	22.54 (4.41)	17.70 (1.25)	22.76 (1.88)	19.93 (1.59)	24.97 (4.51)	22.98 (3.68)	
Mel	0-30	7.40 (0.69)	7.65 (0.50)	8.20 (1.60)		6.34 (0.79)	8.74 (0.82)	
	30-70	4.31 (0.29) A	6.50 (1.08) A	10.68 (2.50) B		3.61 (0.53) A	5.17 (0.64) A	
	0-70	11.71 (0.49) A	14.15 (1.57) AB	18.88 (3.12) B		9.95 (1.24) A	13.90 (0.20) A	
Chianocco	0-30	7.49 (0.43)	7.86 (0.83)	8.72 (3.32)		9.19 (1.27)	6.79 (0.14)	
	30-70	5.84 (0.78) AB	6.08 (0.62) AB	7.11 (0.77) A		5.34 (0.38) AB	4.49 (0.54) B	
	0-70	13.33 (0.91)	13.94 (1.42)	15.83 (3.82)		14.53 (1.65)	10.28 (0.52)	
Firenzuola	0-30	8.78 (0.90) A	10.29 (0.34) AB	10.96 (0.89) AB	9.66 (0.99) A	12.87 (1.48) B	11.02 (0.20) B	
	30-70	4.30 (0.71) A	5.22 (0.54) A	4.67 (0.22) A	5.42 (0.28) A	6.17 (1.10) A	10.31 (1.17) B	
	0-70	13.08 (1.27) A	15.52 (0.57) AB	15.63 (0.84) AB	15.08 (1.08) A	19.05 (2.39) BC	21.34 (1.31) C	
Farindola	0-30	13.22 (0.29) AB	13.43 (3.09) AB	14.08 (1.07) AB		9.43 (1.70) A	15.62 (1.40) B	
	30-70	6.42 (1.62) A	6.24 (2.04) A	10.68 (1.06) B		6.45 (0.49) A	14.99 (0.94) B	
	0-70	19.64 (1.43) AB	19.67 (4.78) AB	24.76 (2.09) BC		15.88 (2.16) A	30.62 (2.33) C	
Vastogirardi	0-30	16.93 (1.71)	14.54 (3.28)	19.50 (3.44)		15.48 (1.88)	12.88 (0.81)	
	30-50	6.90 (1.50) A	6.28 (0.50) A	4.35 (1.10) AB		6.29 (0.70) A	2.72 (0.7) B	
	0-50	23.83 (3.14)	20.82 (4.28)	23.85 (2.31)		21.78 (3.32)	15.60 (1.52)	
Pignola	0-30	7.84 (1.79) A	8.28 (1.31) A	9.87 (1.40) AB		6.12 (0.96) A	14.15 (3.02) B	
	30-70	6.54 (1.09) A	7.02 (1.19) A	7.20 (1.17) A		6.74 (0.88) A	12.75 (1.08) B	
	0-70	14.38 (2.66) A	15.30 (2.49) A	17.07 (2.53) A		12.86 (1.37) A	26.90 (4.01) B	

The total N stock changes along the woody encroachment process are shown in Fig. 26. In each graph, topsoil, subsoil and whole profile N stocks were considered and analysed by the repeated measurement ANOVA. In Castello Tesino (Fig. 26A), Mel, (Fig. 26B) and Chianocco (Fig. 26C) study areas the N stock in the forest stage soil is not significantly different to that of the previous pasture. However, both in Mel and Chianocco, a statistically significant increase was observed until the intermediate stage of about 22 years (T2) and then a gradual decrease until the forest stage, especially in the subsoil compartment. Considering the Alps group, in the topsoil, the N stock percentage increased by +1 and +26% with respect to the pastures, in T1 and T2 respectively. Then, a marked reduction in the N stock was measured in T3 (to 12% less than P) and, finally a stabilisation around the pastures values was measured in the final part of the process (to +2 more and 0.7% less than the initial P value in stages T4 and F, respectively). A similar trend was measured in the subsoil. The N stock increased by 7% with respect to P in T2 and then a remarkable decrease was measured in T3 (to 16% less than P). In the oldest stages the N stock is comparable to that of the pasture (to 4% less than P in F). Considering the whole profile, a consistent increment in the N stock was measured in the initial part of the succession, with a +10 and +61% more than in P in the T1 and T2 stages, respectively. Then, the N stock decreased to 10% less than P in the T3 stage and then tended to increase in the older stages, with values of 7 and 15% more than P measured in T4 and F, respectively.

The changes in the N stock in the other study areas, those of the Apennine ridge, are similar to what was described for the relative SOC stock changes. Maximum and statistically significantly different values were estimated in the forest stages in Firenzuola (Fig. 26D), Farindola (Fig. 26E) and Pignola (Fig. 26F) study areas. Considering the topsoil, the N stock increased to +9 and +22% more than the previous pasture, in T1 and T2 respectively. Then, a sharp decrease was measured in T3 and T4 (reaching +15 and +3% more than P). Finally, the forest stage had, on average, a N stock 70% higher than P. Similar results were obtained for the subsoil compartment. The N stock increased in the first part of the succession and values of +8 and +19% more than P were measured in T1 and T2. Then, the decrease led the N stock to a +10 more and 1% less than the initial value in P in T3 and T4. Finally, N stock markedly increased to 41% more than P, in the forest stage. When the whole profile is considered, all the successional stages had a higher N stock than the pasture. In particular, values of +9, +28, +26, +15 and +123% more than the pasture stage were measured in T1, T2, T3, T4 and F stages, respectively.

The opposite trend was observed for Vastogirardi (Fig. 26G). In the whole profile, the N stock initially decreased by 13% between P and T1, then it reached similar values to the previous pasture (+0%) in T2. Finally it progressively decreased to values of 9 and 35% less than in T4 and F stages, respectively.

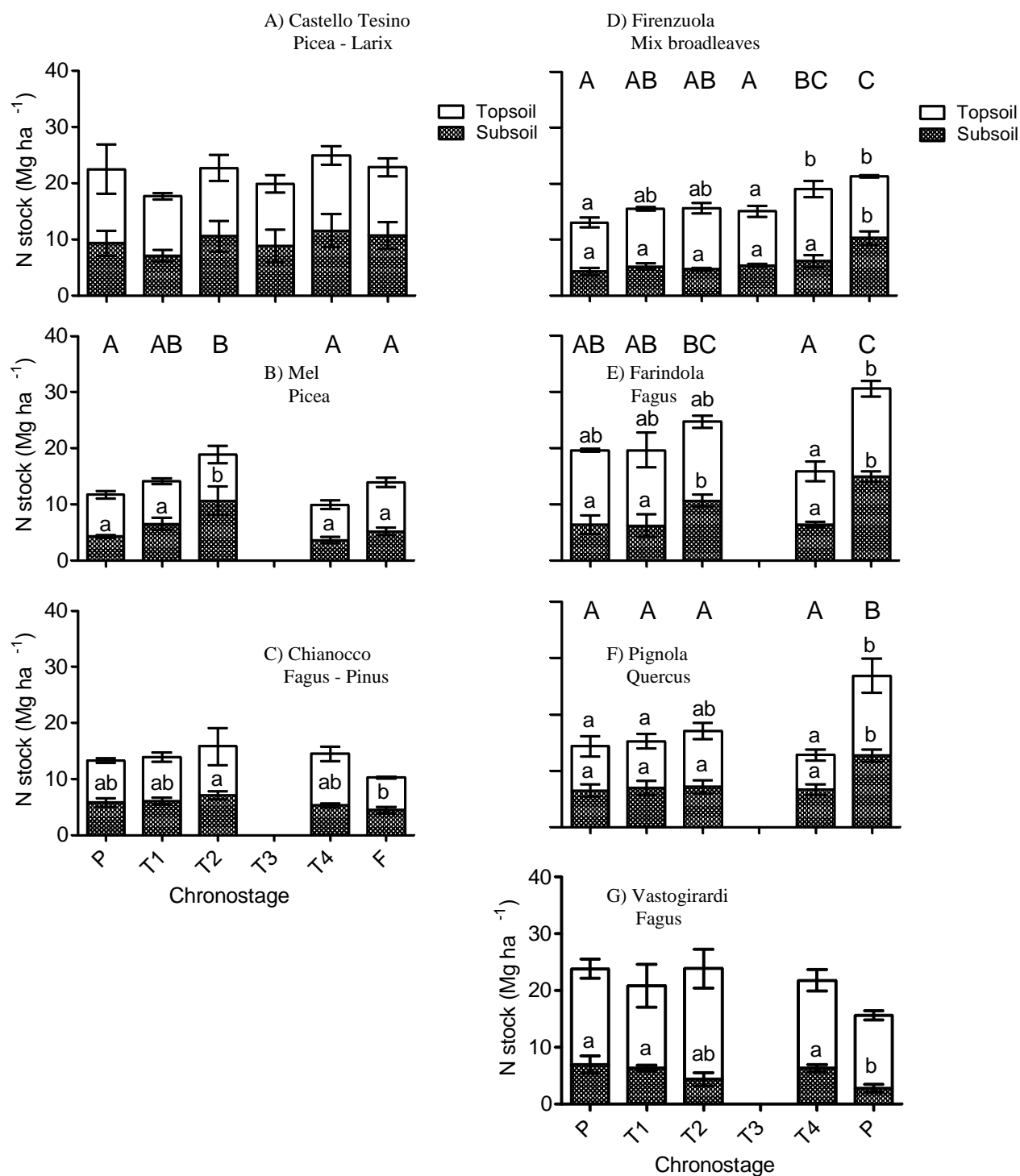


Figure 26: Changes in N stock along the woody encroachment process in each study area. Stages are classified by means of their average age. Statistically significant differences among the stages are labelled with case letter (considering topsoil and subsoil) or with capital letters (whole soil profile). Lowercase and capital letter are added only in case of significant statistical differences.

SOC and N stocks clearly revealed the presence of two main groups of sites, plus the exceptional case of Vastogirardi. The groups correspond to the Alps and the Apennines. The first group (Alps) was characterised by the fact that pastures and forests were not statistically significantly different when considering the SOC and N stocks. In addition, this group was characterised by values increasing in the intermediate part of the woody encroachment process and subsequently decreasing in the second part. On the contrary, the concentrations and stocks trend of the Apennine sites showed an initial variability that, ultimately, led to a significant increase in the forest stage. In addition, in all the Apennine study areas the forest stage was characterised by a statistically significantly higher value of all the other stages, especially in comparison to the pasture.

Vastogirardi showed an uncommon trend when compared to the other study areas, in particular in terms of its marked variability in the aspect of the chronostages, which span the full range of directions from N to S. Indeed, according to what was described in the Section 8.2.6, this is the only study area that was affected by this confounding effect on the chronostages arrangement. For this reason, this study area was excluded from the following analyses and results.

Figs. 27, 28, 29 and 30 describe the variation (difference) in SOC stock between the value in each stage and the value in the previous pasture stage of the same chronosequence. In addition, they were subdivided in two graphs, with the left one referring to the Alps study areas (graphs 1), and the right one referring to the Apennine (graphs 2).

Fig. 27 shows the SOC stock variation over the time from the start of the woody encroachment process in the topsoil (0-30 cm depth). Although Alps (graph 1) and Apennine sites (graph 2) have a very similar trend in the beginning part of the process, they reacted differently in the last part of the process. Indeed, while in the Alps sites no significant variation is shown for the forest stage, a marked increase is visible for the Apennine counterparts.

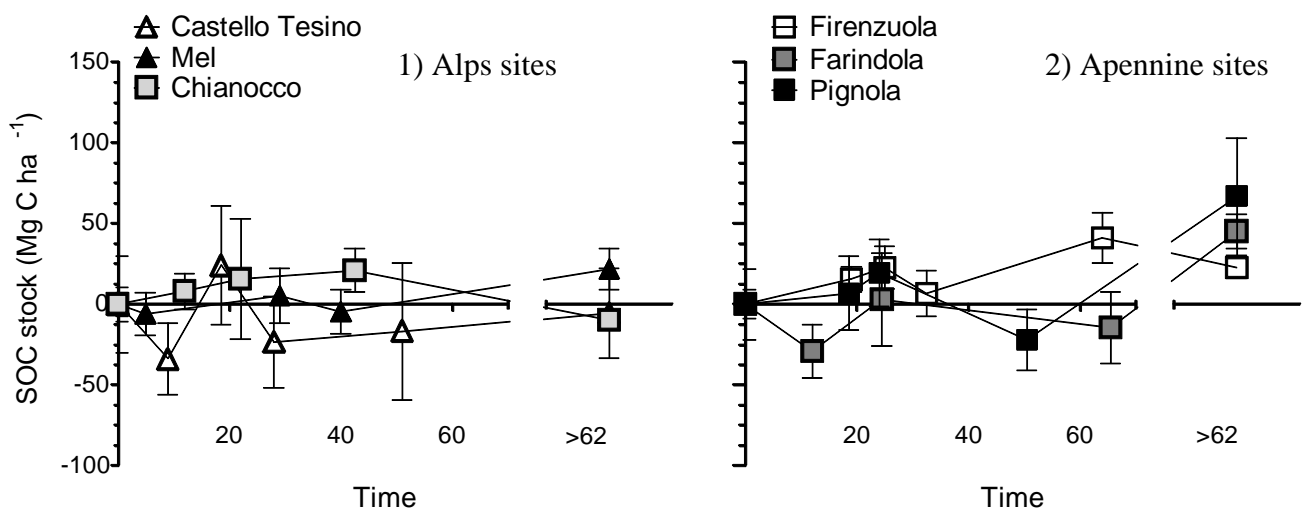


Figure 27: Variation (difference) in SOC stock from pasture/grassland values in the topsoil (0-30 cm of depth). Graph 1 refers to the sites in the Alps, while graph 2 refers to the sites in the Apennines. Triangle and square symbols refer to conifer and broadleaves stands, respectively. Each value represents the mean and the bars the sd ($n=3$).

Fig. 28 shows the SOC stock variations over the time in the subsoil. The previous grouping is still effective, with Alps study areas (graph 1) characterised by negligible values in the forest stage, while Apennine forest stages values that were markedly positive. Finally, both the groups were characterised by a notable increase in the SOC stock in the

intermediate part of the woody encroachment. However, this variation was more pronounced in the Alps study areas (graph 1) than in the Apennine ones (graph 2).

Fig. 28 not only shows that the subsoil is surprisingly dynamic, but also that – in some cases – its variability is more pronounced than that of the topsoil (Fig.27).

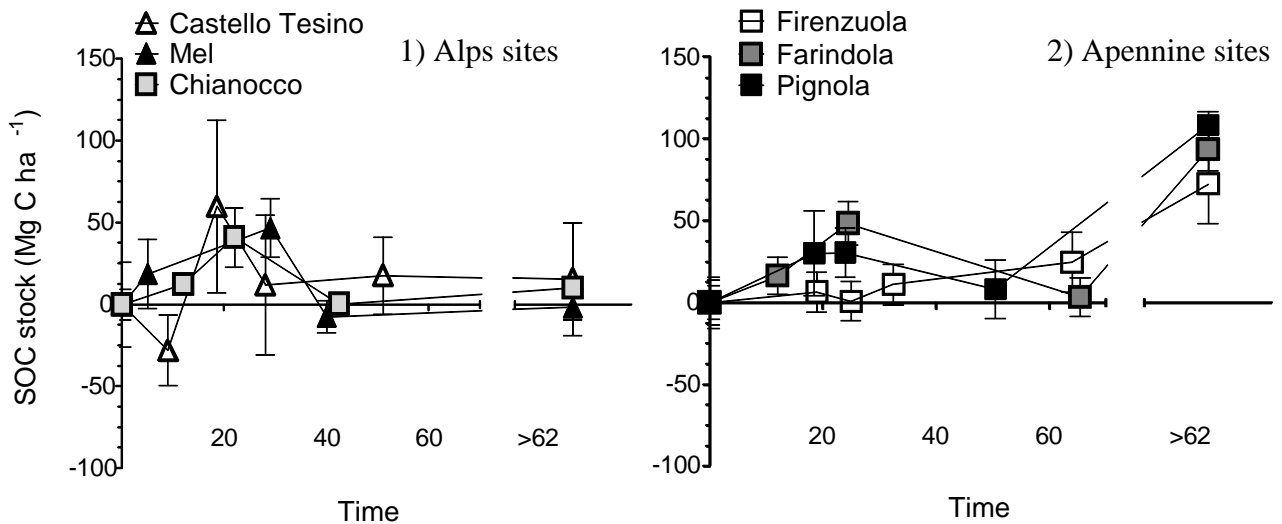


Figure 28: Variation (difference) in SOC stock from pasture/grassland values in the subsoil (30-70 cm of depth). Graph 1 refers to the sites in the Alps, while graph 2 refers to the sites in the Apennine. Triangle and square symbols refer to conifer and broadleaves stands, respectively. Each value represents the mean and the bars the sd (n=3).

Both Figs. 29 and 30 describe the soil considering the whole profile. In particular, Fig. 29 includes the variation trends of all the study areas in each group, while Fig. 30 describes only the average trend in each group. Comparing the graphs of Fig. 29 with those of Fig. 27 and 28, it is clear that the shapes of the trend variations in the whole profile are more similar to those of the subsoil component than those of the topsoil. This means that the role of the subsoil is dominant over that on the topsoil.

Fig. 30 summarised the variation in SOC stock in the two groups. It is possible to conclude that the woody encroachment in the Alps led to a consistent increase in the SOC stock until the intermediate stage (which is generally characterised by a marked plant biodiversity and plant spatial heterogeneity) and, after this stage, the SOC stock tends gradually to reduce, reaching values similar to those of the previous pastures. On the contrary, the variation in the SOC stock in the Apennine study areas (graph 2) shows a maximum in the forest stages where the stock is dramatically higher than that of the previous pastures. In addition, similarly to the Alps group (graph 1), another peak is visible (even if less pronounced than for the Alps) in the intermediate stage.

The results described in Fig. 27, 28 and 29 seem to validate a posteriori the assumption adopted concerning Mel's oldest chronostage (paragraph 8.2.2), i.e. that the SOC stock values measured in the forest stage are comparable to what expected in a secondary succession even if the observed forest was known to result from human-induced afforestation. Indeed, the SOC stock variation along Mel's chronosequence show a trend that is not significantly dissimilar to that of the other Alps chronosequences.

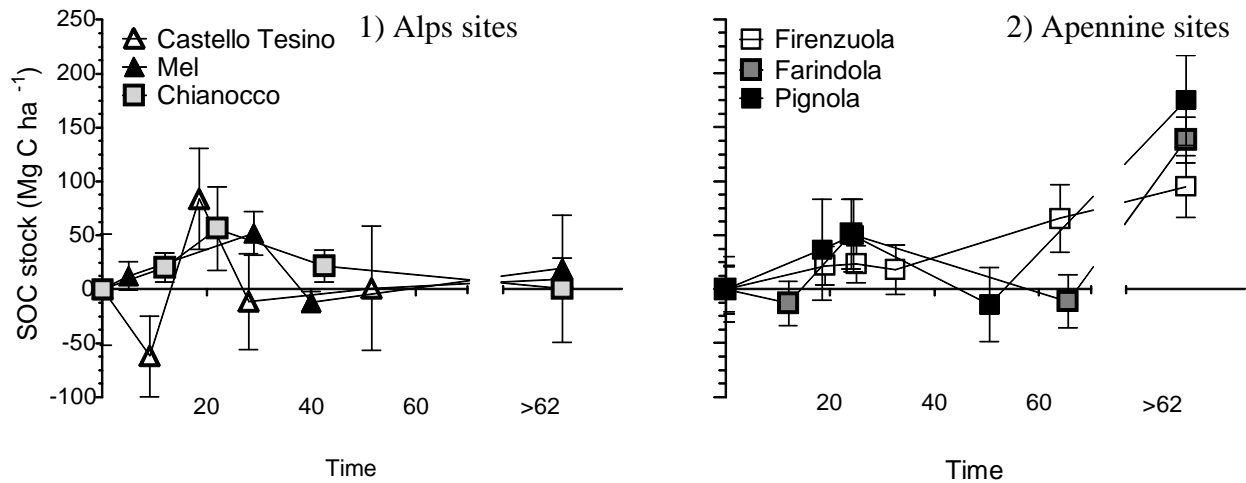


Figure 29: SOC stock amount as difference from the previous pasture / grassland value. The SOC stock is considered in the whole soil profile (0-70 cm of depth). Graph 1 refers to the sites in the Alps, while graph 2 refers the sites in the Apennine. Triangle and square symbols refer to conifer and broadleaves stands, respectively. Each value represents the mean and the bars the sd (n=3).

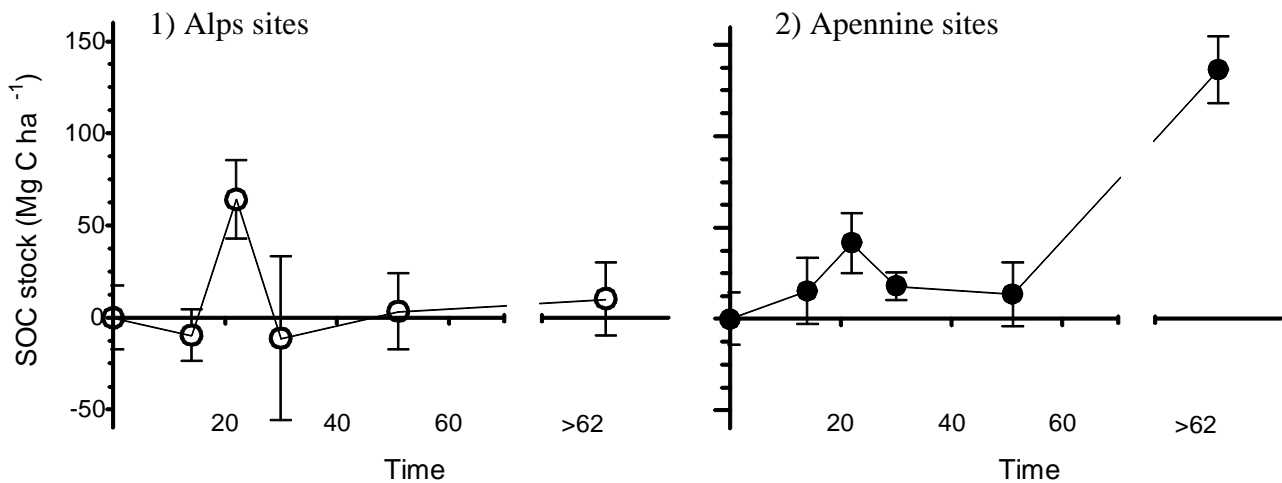


Figure 30: SOC stock variation (difference) from pasture / grassland values along the woody encroachment. The sites are grouped in Alps sites (on the left), Apennine sites (on the right). All the graphs in the figures refer to the whole soil depth profile (0-70 cm). Each value represents the mean and the bars the sd, which was estimated on the data included in Fig. 29.

9.4. SOC and climate

In the next part of this chapter, only the pasture and forest stages are considered in order to summarise the whole woody encroachment effect on SOC stocks. More specifically, both the forest to pasture SOC stock ratio and the difference between forest and pasture SOC stock are discussed. The effects of main climatic variables on SOC changes were investigated in light of to the main literature on this topic (Guo and Gifford, 2002 and Jackson *et al.*, 2002, Alberti *et al.*, 2008, 2011). According to these authors, the mean annual precipitation (MAP) of each study area is the main driver

of the woody encroachment effect on SOC stock changes. Therefore, MAP was the first variable to be tested on the SOC forest-to-pasture ratio and on forest-to -pasture difference by mean of Pearson's correlation test. Surprisingly, no statistically significant correlation was detected between these two variables (see Tab. 18). Therefore, the next step was to test a possible correlation between SOC stock changes from pasture to forest (expressed both as a ratio or as a difference) and the mean annual temperature (MAT). Spearman correlation P values are included in Tab. 18 and they show that both the ratio and the difference in SOC stock are strongly correlated with MAT, although a statistically significant correlation ($P < 0.05$) was found only for the ratio. Graph A in Figs. 31 and 32 show the linear regression plotted on the ratio and the difference, respectively. The next step was test the effect of precipitation and temperature parameter combinations adopting the most common Aridity Indices (AIs) used for ecological studies. All the climatic indices, with the relative formulae and P values, are summarised in Tab. 18. Again, it was surprising to find that all the indices that include the precipitation variable are not statistically correlated to the SOC stock variation (either as a ratio and a difference). A marked statistically significant correlation was estimated only for the Mitrakos Winter Cold Stress (WCS), an index that takes into account only the mean minimum temperatures of the winter months (December, January and February). In graph B of Figs. 31 and 32, a linear regression was drawn to describe the correlation between the Mitrakos WCS index and the ratio or the difference in SOC stock respectively.

Table 18: SOC ratio and SOC variation correlated on climate variables (MAP, MAT and some of the most common Aridity Indices). SOC stock ratio is value of forest SOC stock divided by that of previous pasture / grassland. SOC variation was estimated as the difference in SOC stock (in MgC ha^{-1}) between forest and pasture / grassland stages. The aridity indices are based on the following organised literature: Gaussen (1957); Lang (1915, 1920); De Martonne (1926); De Martonne (1942); Emberger (1930); Mitrakos (1980). Terms in the Formula column are: P = mean annual precipitation, T = mean annual temperature, p = precipitation of the driest month, t = mean temperature of driest month, M = mean of the maximum temperatures of the warmest month and m the mean of the minimum temperatures of the coldest month, P_s = mean of the mean cumulative precipitations of June, July and August, T_{\min_w} = mean of the mean lower temperatures of December, January and February.

Variable/Aridity Index	Formula	Pearson's correlation	
		P value	
		F / P	F - P
MAP		0.6103	0.9569
MAT		0.0401 *	0.0615
Gaussen	$AI = P / 2T$	0.1640	0.2989
Lang	$AI = P / T$	0.1541	0.2813
De Martonne pluviometer	$Pf = P / (T + 10)$	0.2580	0.4855
De Martonne – Gottmann	$AI = [(P / (10 + T)) + 12 * (p / (10 + t))] / 2$	0.0864	0.1630
Emberger pluviometric quotient	$Q = (P / (M^2 - m^2)) * 100$	0.1658	0.3150
Mitrakos Summer Dry Stress	$MDS = 2 * (50 - P_s)$	0.0685	0.1278
Mitrakos Winter Cold Stress	$WCS = 8 * (10 - T_{\min_w})$	0.0142 *	0.0209 *

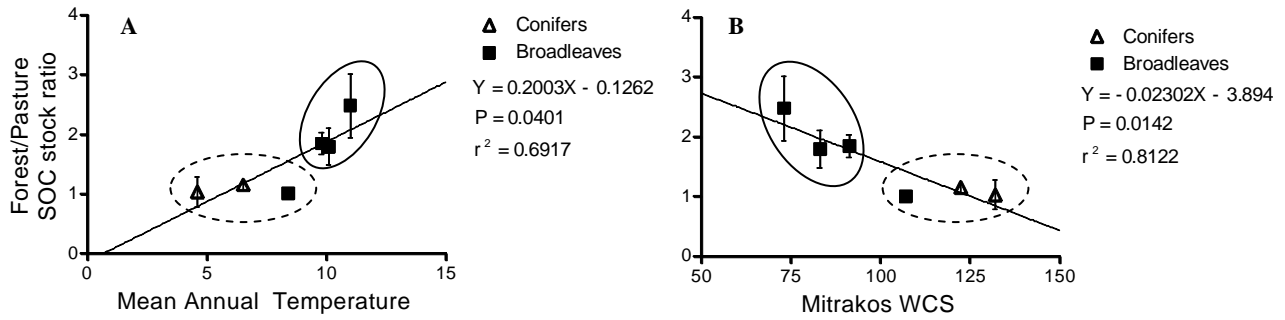


Figure 31: Linear regression of forest SOC stock to pasture SOC stock ratio plotted against climatic variables: A) Mean Annual Temperatures (MAT of Tab. 3); B) Mitrakos Winter Cold Stress. Triangle and square symbols refer to conifer and broadleaves stands, respectively. Each value represents the mean and the bars the sd (n=3). The meaning of dotted and solid line circles is explained the main text.

Fig. 31 clearly shows the presence of the two groups of study areas previously described: the Alps, with a SOC stock ratio close to one (i.e. a SOC stock that does not change substantially between pasture and forest stages) and the Apennines, with a ratio markedly higher than one. The two groups are included in the dotted and solid line circles respectively.

Graphs A and B of Fig. 32 show the same grouping: Alps, with a forest to pasture difference close to zero, and the Apennine, with a clearly positive difference. The two groups are included in the dotted and solid line circles respectively. In graph C and D of Fig. 32 the amount of the forest stage litter C pools (expressed as a difference from the previous pasture value) are plotted against the MAT and Mitrakos WCS respectively. In both cases, the correlation is statistically significant. Alps and Apennines do not clearly segregate in groups any more.

In Fig. 32, comparing the two pair of graphs which take into account the same climatic variable (A-C versus B-D), it appears that a positive correlation for the SOC stock is associated with a negative correlation for the litter C pool, and vice-versa.

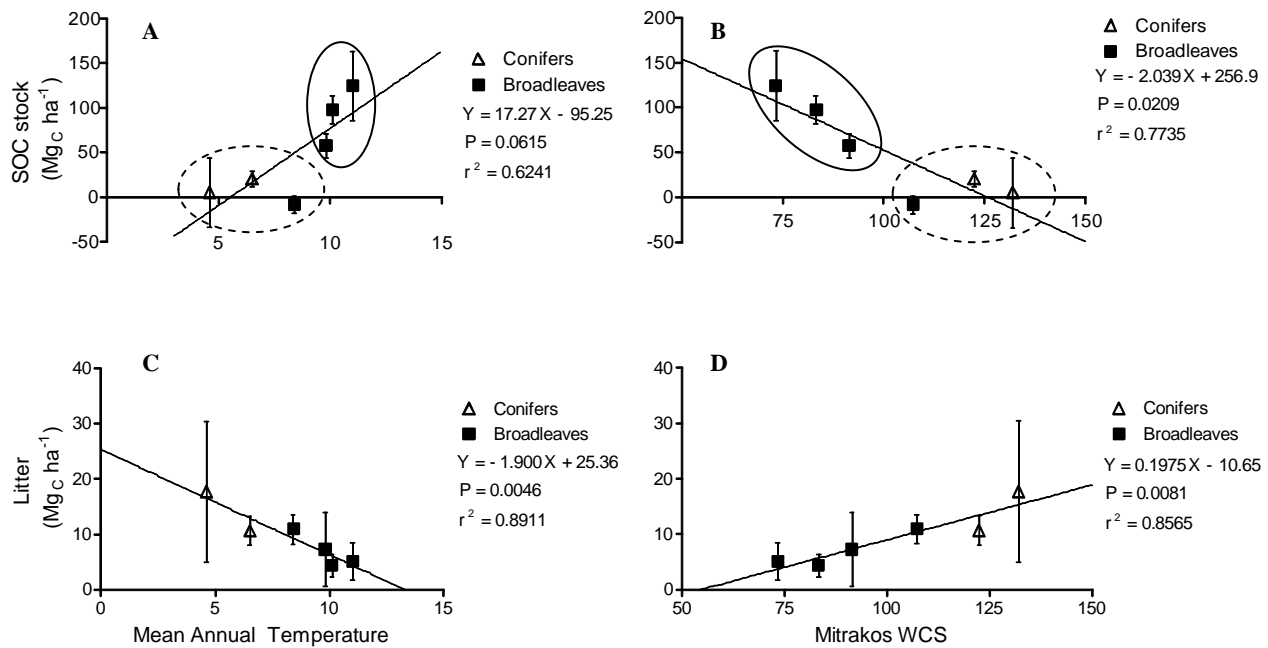


Figure 32: Linear regression of forest vs. pasture SOC stock variation plotted against (A) Mean Annual Temperatures (MAT) and (B) Mitrakos Winter Cold Stress (WCS), and of variation in the C stock in the litter accumulation along the entire woody encroachment process against (C) MAT and (D) Mitrakos WCS. Variations are expressed as forest values minus pasture values. Triangle and square symbols refer to conifer and broadleaves stands, respectively. Each value represents the mean and the bars the sd ($n=3$). The meaning of dotted and solid line circles is explained in the main text.

9.5. Living biomass, dead organic matter and ecosystem C stocks

The C stock estimation for the above- and belowground biomass and dead organic matter are summarised in Tab. 19. As described in sections 8.6 and 8.7, these variables were directly measured for three stages: a) pasture, b) the intermediate stage and c) forest. The total biomass and necromass C stock of the other stages (those not summarised in Tab. 19) were estimated by linear interpolation. More precisely, given the age of the forest was left-censored but otherwise not known, for this linear interpolation procedure it was set to 80 years for all chronosequences. For all the other chronostages, their mean estimated age (Tabs. 2-8, Section 8.2) was used. The linear interpolation was performed separately between the pasture and the intermediate stage and between the intermediate stage and the forest. Although Alberti *et al.* (2008) noted that a generalised logistic function (or Richards' curve) was more appropriate to describe the biomass C stock over time than a piecewise linear function, the three data points available were not enough to fit Richards' curve (4 parameters).

Table 19: *C* stock in the Above-Ground Biomass (ABG), Below-Ground Biomass (BGB), and Necromass: Litter, Fine Woody Debris (FWD) and Coarse Woody Debris (CWD). Values are the mean ($n = 3$) and *sd* (in brackets). All the values are expressed in $Mg_C ha^{-1}$.

Study area	Stage	ABG			BGB			Necromass		
		Grass	Shrubs	Trees	Grass	Shrubs	Trees	Litter	FWD	CWD
Castello Tesino	Pasture	2.26 (0.62)	0	0	9.54 (2.77)	0	0	0	0	0
	Intermediate	3.34 (0.68)	1.24 (1.04)	1.30 (1.11)	14.10 (2.87)	2.28 (1.91)	0.26 (0.22)	0	0	0
	Forest	0.34 (0.19)	0	167.07 (38.96)	1.44 (0.80)	0	33.58 (7.83)	17.72 (12.74)	2.75 (0.73)	0.43 (0.36)
Mel	Pasture	2.98 (0.98)	0	0	12.60 (4.15)	0	0	0	0	0
	Intermediate	2.54 (0.32)	0.18 (0.16)	0.17 (0.30)	10.71 (1.36)	0.33 (0.29)	0.03 (0.06)	0	0	0
	Forest	0	0	156.04 (4.28)	0	0	31.36 (0.86)	10.71 (2.63)	2.85 (1.21)	0.70 (0.00)
Chianocco	Pasture	3.36 (0.11)	0	0	14.21 (0.46)	0	0	0	0	0
	Intermediate	1.37 (0.67)	1.42 (1.23)	32.52 (26.97)	5.80 (2.81)	2.61 (2.26)	7.84 (6.50)	0.95 (1.17)	1.96 (1.20)	0
	Forest	0	0	94.96 (16.77)	0	0	22.89 (4.04)	11.01 (2.68)	2.30 (0.32)	0
Firenzuola	Pasture	0.29 (0.06)	0	0	1.20 (0.24)	0	0	0	0	0
	Intermediate	1.47 (0.06)	9.38 (8.47)	0.53 (0.53)	6.22 (0.24)	17.23 (15.56)	0.13 (0.81)	0	0	0
	Forest	0.00 (0.01)	1.14 (1.62)	113.38 (24.25)	0.02 (0.04)	2.09 (2.98)	27.32 (5.84)	4.42 (2.05)	1.42 (0.57)	0
Farindola	Pasture	0.22 (0.10)	0	0	0.94 (0.44)	0	0	0	0	0
	Intermediate	2.11 (0.81)	0.80 (0.51)	0.83 (1.43)	8.91 (3.41)	1.47 (0.93)	0.20 (0.34)	0	0	0
	Forest	0.05 (0.01)	0	121.25 (26.93)	0.22 (0.04)	0	29.23 (6.49)	7.36 (6.70)	0.74 (0.85)	0.67 (0.83)
Vastogirardi	Pasture	0.99 (0.65)	0	0	4.19 (2.73)	0	0	0	0	0
	Intermediate	0.09 (0.08)	0.27 (0.00)	23.77 (3.78)	0.36 (0.32)	0.49 (0.01)	5.73 (0.91)	3.14 (0.68)	0.86 (0.08)	0
	Forest	0.02 (0.03)	0.09 (0.05)	163.59 (70.01)	0.10 (0.12)	0.16 (0.09)	39.42 (16.87)	7.71 (3.21)	1.08 (0.10)	0.01 (0.01)
Pignola	Pasture	1.40 (0.29)	0	0	5.92 (1.24)	0	0	0	0	0
	Intermediate	0.92 (0.23)	1.45 (0.47)	0.78 (1.35)	3.87 (0.98)	2.66 (0.86)	0.19 (0.33)	0	0	0
	Forest	0.11 (0.07)	0.13 (0.13)	135.53 (30.01)	0.48 (0.30)	0.24 (0.24)	32.66 (7.23)	5.20 (3.30)	2.30 (0.50)	2.05 (0.60)

According to the IPCC (2006) the ecosystem C pool can be estimated as the sum of soil, above- and belowground biomass and necromass pool. The change in the percentage of each pool along the woody encroachment is described by the Fig. 33 for each study area. Although the mean age estimated for each stage varies between chronosequences, to simplify the exposition of the following results, corresponding stages across all chronosequences are plotted against the average of their mean age estimates: e.g. the first stage after the pasture is identified with an age of 14 years, the average of the mean age estimates of this first stage in all chronosequences.

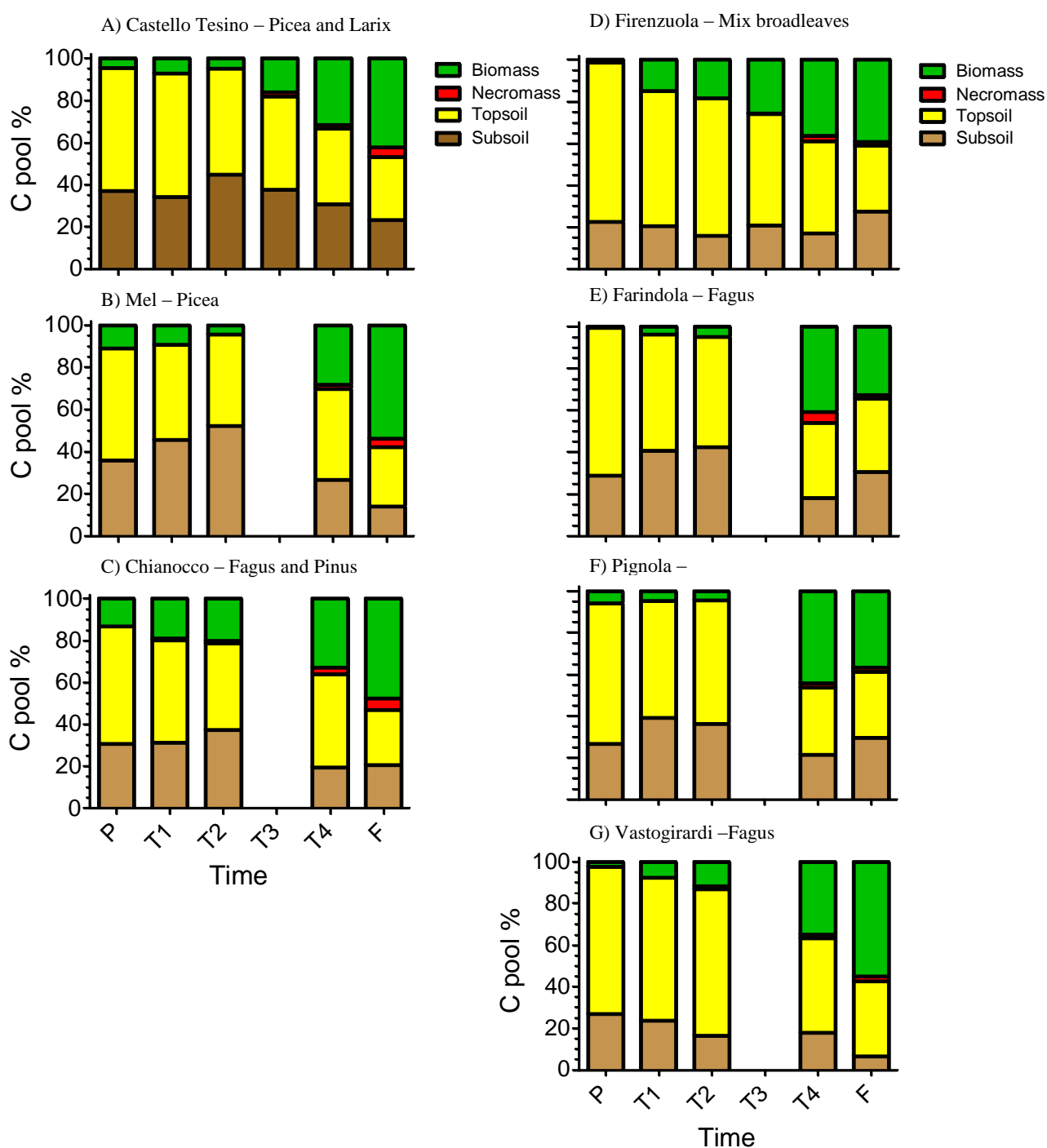


Figure 33: Percentage of C stock in the major pools at the ecosystem level. Biomass pool includes aboveground biomass (ABG) and belowground biomass (BGB), while the necromass includes litter, fine woody debris (FWD) and coarse woody debris (CWD). Biomass and necromass pools were estimated by direct measurements only in the pasture/grassland stage, in the intermediate stage (around 22 years old) and in the forest. In all the other stages the biomass and necromass were estimated by linear regression, as described in the main text. Graphs are subdivided in the two main groups: the left column (graphs A, C, E) refers to Alps sites, while the right column (graphs B, D, F, G) refers to Apennine sites.

Fig. 33 highlights some important observations:

1. The total biomass C increased along the woody encroachment and in particular in the last part of the process. Moreover, when all the study areas were considered together, in the forest stages its percentage ranges between 40 and 60 % of the total.
2. The C stock in the living biomass always increases. However, when expressed in percentage, it may appear to decrease along part of the process because of a more pronounced increase of the SOC stock.
3. The whole soil profile C stock was almost always the most important pool at the ecosystem level.
4. The subsoil was almost always a significant C pool and usually it reached its maximum values in the intermediate stage of the woody encroachment.

Fig. 34 is the ecosystem C stock equivalent of what Fig. 29 was for the soil. It shows the variation of the total ecosystem C stock expressed as the difference of each stage with respect to the previous pasture stage. The most interesting results are that:

1. In all the study areas, independently of the group they are included in, the woody encroachment process leads to an increase in the ecosystem C stock. Therefore, in the Italian peninsula, the woody encroachment acts as a C sink, especially in the oldest stages of the process. Moreover, when the different chronosequences are considered together the ecosystem C stock increased from the initial pasture value by +12, +42, +32, +66, +163% in T1, T2, T3, T4 and forest stages, respectively. Similar values were estimated with the addition of Vastogirardi (+147% in the forest stage).
2. In the Alps sites (graph 1) the biomass and necromass pools of the oldest stages are fundamental in the increment of the ecosystem C stocks (compare Fig. 29 and Fig. 34).
3. Given the biomass and necromass pools in the initial stages of the process are negligible, the SOC stock plays a much larger role early on than in the older stages (see also Fig. 33). Therefore, oscillations or reductions of the SOC stock in these initial stages could lead to a temporary or prolonged decrease of ecosystem C stock from the previous pasture values.

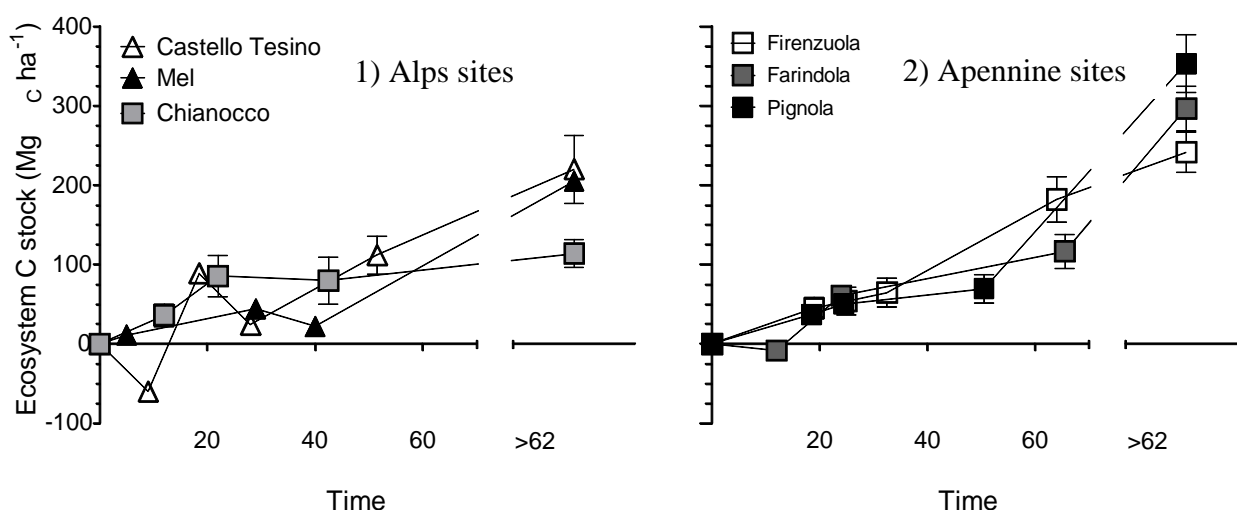


Figure 34: Total ecosystem C stock variation from the previous pasture / grassland. The sites were grouped by location: Alps (graph 1, on the left) and Apennine (graph 2, on the right). Triangles and squares symbols refer to conifer and broadleaves stands, respectively. The whole soil profile (0-70 cm depth) was considered as one of the pools. Each value represents the mean and the bars the sd ($n=3$).

The mean values for each group of study areas are plotted in Fig. 35. Looking at this figure, two main observations can be made:

1. The increase in the ecosystem C stock proceeds more steadily in the Apennine study areas (graph 2), compared to that of the Alps group (graph 1). Indeed, in the Apennine group, a progressive increment in the ecosystem C stock was estimated on the basis of the mean percentage values, with respect to the initial pasture stage. It increased by 21, 41, 53, 92, 220% in T1, T2, T3, T4 and F stage respectively.
2. In the Alps sites (Graph 1), the peak in the intermediate stage observed in the soil figures is still consistently present. Indeed, in the Alps group, the ecosystem C stock increased by 4 and 43% with respect to P in the stage T1 and T2, respectively. Then it markedly decreased in T3 (to +11% more than P). Finally, it progressively tended to increase to 40 and 105% more than P in the T4 and forest stages.

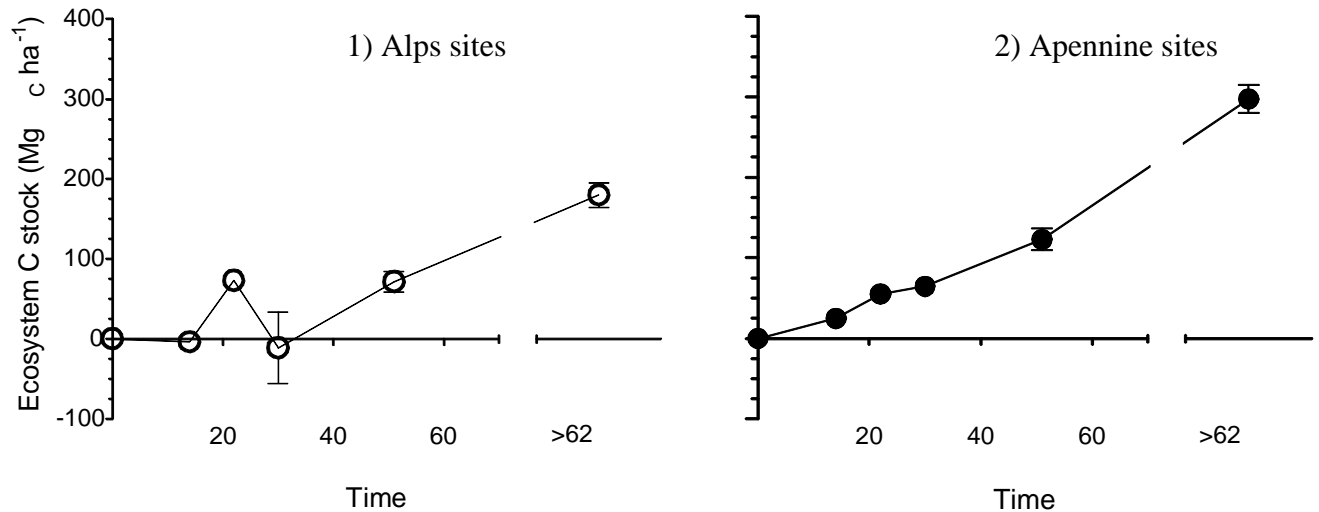


Figure 35: Ecosystem C stock variation (difference with the initial pasture stage) along the woody encroachment, from pasture/grassland to forest. The sites are grouped in Alps sites (graph 1) and Apennine sites (graph 2). Both graphs include the soil pool as the C stock of the whole soil profile (0-70 cm). Each value represents the mean and the bars the sd, which was estimated on the data included in Fig. 34.

The changes in the ecosystem C stock indicate that the intermediate stage is particularly important. Indeed, when all the study areas were considered together, an intermediate ecosystem C peak was estimated in stage T2 (see section 9.4). Therefore, it was decided to examine this fact more in detail on the basis of the information that was recorded on the field. In particular, it was observed that the intermediate stage was usually more heterogeneous in the plant species distribution and more diverse in the plant species composition with respect to the relative pasture and forest stages. These results were unexpected and could not be foreseen so, unfortunately, no specific data were collected to estimate the real plant diversity. However, this information could be indirectly derived for the woody plant species (shrubs and trees separately) because of the more refined biomass estimation procedure for these plant types. On the contrary, no species richness and species abundance were estimated for the grasses, even if their biomass was calculated. It was then decided to estimate an aboveground functional type diversity index as described in the section 8.9.2. Given the different impact that the forest vegetation has on the soil microbial community, and in particular the very low diversity value typical of the conifer forest, rather than a distinction based on geographical location (Alps versus Apennines), the study areas were grouped in Conifer and Broadleaves. The results are summarised in Tab. 20 and shown in Fig. 36. By means of this index, a maximum diversity value was confirmed for both the groups in the intermediate stage. The Tukey test confirms that the differences between the intermediate stage and the others are statistically significant.

Table 20: Above-ground functional-type diversity index. Grass biomass was estimated in Mg ha^{-1} . Above-ground woody plant species diversity (shrubs and trees) is expressed by Shannon's H diversity index.

Study area	Stage	Aboveground			Normalised values			Sum
		Grass biomass	Shrubs diversity	Trees diversity	Grass biomass	Shrubs diversity	Trees diversity	
Conifers	Pasture	5.52 (1.76)	0.00	0.00	0.81 (0.23)	0.00	0.00	0.81 (0.23) A
	Intermediate	6.19 (1.37)	0.70 (0.14)	0.38 (0.33)	0.91 (0.17)	1.00 (0.00)	1.00 (0.00)	2.57 (0.62) B
	Forest	0.36 (0.47)	0.00	0.06 (0.09)	0.05 (0.07)	0.00	0.18 (0.22)	0.18 (0.26) C
Broadleaves	Pasture	2.78 (2.81)	0.00	0.00	0.57 (0.44)	0.17 (0.39)	0.00	0.74 (0.71) A
	Intermediate	3.09 (1.34)	0.76 (0.49)	0.25 (0.49)	0.78 (0.30)	0.85 (0.28)	0.43 (0.53)	1.88 (0.43) B
	Forest	0.09 (0.12)	0.53 (0.61)	0.35 (0.43)	0.03 (0.04)	0.58 (0.28)	0.77 (0.33)	1.06 (0.74) A

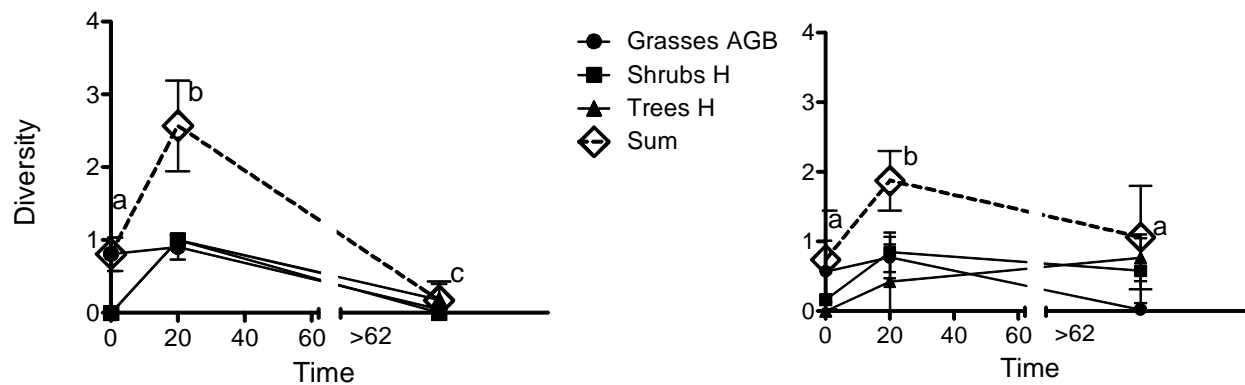


Figure 36: Above-ground functional-type diversity index (empty diamonds) and normalised values of each functional type as described in Tab. 5. The data points refer to pasture, intermediate stage and forest, and are positioned in time at their average age (as previously described in the main text).

9.6. Microbial biomass and soil enzyme activity

The values of the most important biochemical parameters are summarised in Tabs. 21 and 22, for the depth intervals of 0-5 and 5-15 cm, and are grouped by study area and chronostage.

Part of these data is also shown in the Figs. 37-43. In particular, this group of images include the microbial biomass (C_{mic}), the synthetic enzymatic index for the C cycle (SEIc) and the chitinase activity data. All figures are subdivided in two columns: the left one include the data referring to the 0-5 cm and the right one include the data of the 5-15 cm layer.

Fig. 44 summarises the results included in Tab. 21 and Tab 22 and shown in Figs. 37-43. Moreover, Fig. 44 shows what was expected, that is:

1. The upper part of the soil profile (the 0-5 cm layer) is the richest in microbes and the most biochemically active. Indeed, all the biochemical indexes reach their maximum values in this part of the soil.
2. The upper part of the soil (the 0-5 cm layer) is the part that is most subject to the biochemical variability among the chronostages.

Microbial biomass (C_{mic}) values show a general decrease – although this is not always statistically significant – along the woody encroachment process in both the 0-5 cm and 5-15 cm layer. Microbial nitrogen (N_{mic}), also showed a general decrease along the woody encroachment, but only in the 0-5 cm layer. On the other hand, the derived C_{mic}/N_{mic} ratio did not show significant trends along the transect that were consistent among study areas.

Minimum values in the forest stage were also found for enzyme activities (SEIc and chitinase). However, two groups of study areas can be delimited considering these indices, at least in the 0-5 cm depth layer: the chronosequences characterised by a conifer-dominated forest stage showed a common peak in activities at the intermediate successional stage, while soil enzymatic activity in the chronosequences characterised by a broadleaves-dominated forest was maximal in the pasture stage.

More interesting is the information linked to the microbial biomass and enzymatic activities when normalised by the organic carbon concentration at each layer. In particular, the microbial biomass per unit of organic carbon (C_{mic}/C_{org}) significantly decreases in nearly all the study areas along the woody encroachment process in the 0-5 cm layer. In the layer 5-15 cm layer, statistically significant differences were also measured but no common patterns were observed among different study areas. A similar trend along the secondary successional process, leading to a progressive reduction, was also observed regarding specific enzyme activities, $SEIc/C_{org}$ and $chitinase/C_{org}$. The only difference was that, generally, the maximum values are registered in the intermediate stage. This is the same stage which is characterised by both a high SOC stock and high plant diversity and plant spatial heterogeneity.

Table 21: Mean biochemical parameters in the 0-5 cm soil layer. Values are the mean over the field measurement replicates ($n = 3$), and values in brackets represent the standard deviation. Bold capital letters show the groups resulting from the Tukey's test.

Study Area	Stage	C_{mic} ($\mu g_C g_{soil}^{-1}$)	SEIc ($nmol_{NDF} g_{soil}^{-1}$)	Chitinase ($nmol_{NDF} g_{soil}^{-1}$)	N_{mic} ($\mu g_N g_{soil}^{-1}$)	C_{mic} / N_{mic} ($\mu g_C \mu g_N^{-1}$)	C_{mic} / C_{org} ($g_{Corg} g_{Corg}^{-1}$)	SEIc / C_{org} ($nmol_{NDF} \mu g_C^{-1}$)	Chitinase / C_{org} ($nmol_{NDF} \mu g_N^{-1}$)
Castello Tesino	Pasture	2378.96 (167.43) A	2510.26 (224.66) AB	457.23 (173.60)	197.57 (8.48) A	12.04 (0.99)	0.23 (0.01) A	258.35 (43.85) AB	44.65 (15.48)
	Intermediate	1929.31 (146.06) AB	4037.64 (340.69) A	627.45 (238.46)	96.56 (28.71) B	19.98 (6.13)	0.13 (0.01) B	282.73 (58.51) A	41.70 (14.95)
	Forest	1048.54 (611.31) B	2000.41 (269.85) B	399.14 (126.91)	31.61 (15.30) C	33.17 (25.14)	0.08 (0.04) B	151.87 (28.71) B	30.24 (8.20)
Mel	Pasture	874.79 (126.64)	1955.05 (251.42) B	328.14 (106.51) B	103.00 (51.49)	8.49 (4.42)	0.08 (0.01)	179.10 (34.83) A	28.86 (8.86) A
	Intermediate	1287.33 (392.40)	3920.81 (398.46) A	605.09 (32.78) A	156.17 (90.41)	8.24 (5.39)	0.13 (0.04)	380.80 (37.09) B	61.90 (5.95) B
Chianocco	Forest	1405.75 (1122.92)	942.07 (153.53) C	185.24 (97.84) B	63.72 (35.11)	22.06 (21.41)	0.10 (0.08)	69.40 (11.04) C	13.87 (8.02) A
	Pasture	761.87 (202.05)	2042.10 (229.41)	372.64 (131.84)	94.84 (15.02) A	8.03 (2.48)	0.08 (0.02) AB	206.82 (41.51) A	37.43 (17.58)
	Intermediate	1361.25 (561.93)	2220.06 (257.57)	350.86 (50.32)	169.31 (17.43) B	8.04 (3.42)	0.11 (0.05) A	167.88 (64.39) AB	30.15 (42.31)
	Forest	472.54 (220.49)	902.10 (131.35)	259.05 (109.89)	125.92 (21.95) A	3.75 (1.87)	0.03 (0.01) B	57.67 (2.77) B	14.61 (12.62)
Firenzuola	Pasture	1728.32 (85.46) A	3495.32 (206.20) A	616.22 (73.37) AB	165.32 (12.41) A	10.45 (0.94)	0.15 (0.02) A	264.49 (54.62) A	45.52 (10.62) A
	Intermediate	1509.24 (134.75) A	3065.03 (126.07) A	832.15 (92.30) A	133.33 (9.70) A	11.32 (1.30)	0.13 (0.02) A	263.54 (27.19) A	58.85 (14.56) A
	Forest	711.53 (174.03) B	1463.40 (115.68) B	226.95 (45.21) B	40.40 (25.58) B	17.61 (11.95)	0.06 (0.02) B	115.18 (10.83) B	18.81 (3.31) B
Farindola	Pasture	2055.40 (428.90)	2962.85 (232.72) A	754.12 (197.30) A	122.83 (31.87)	16.73 (5.57)	0.21 (0.05) A	284.42 (52.06) A	76.03 (22.23) A
	Intermediate	1372 (400.72)	2461.18 (233.03) AB	460.66 (141.43) AB	115.01 (59.22)	11.9 (7.06)	0.15 (0.04) AB	239.71 (62.00) AB	48.98 (14.54) AB
	Forest	123.49 (101.38)	1444.74 (194.63) B	352.30 (106.03) B	131.13 (50.05)	9.44 (3.68)	0.11 (0.02) B	128.82 (24.81) B	31.29 (8.59) B
Vastogirardi	Pasture	2614.37 (667.43) A	3642.60 (372.67) A	1080.94 (214.79) A	146.82 (19.52) A	17.81 (5.13) A	0.26 (0.06) A	394.78 (67.38) A	108.54 (50.83) A
	Intermediate	2637.25 (899.93) A	3319.95 (93.92) A	559.45 (46.17) B	37.05 (13.86) B	71.18 (36.04) B	0.23 (0.05) A	306.72 (22.65) A	48.94 (19.56) B
	Forest	598.40 (183.82) B	953.89 (118.07) B	368.92 (110.54) B	45.52 (23.23) B	13.15 (7.83) A	0.05 (0.01) B	81.23 (10.13) B	31.37 (3.66) B
Pignola	Pasture	1567.53 (291.90)	2816.61 (240.34)	752.11 (144.89)	158.85 (27.22) AB	9.87 (2.50)	0.12 (0.02) AB	245.70 (59.13) A	56.33 (6.33) A
	Intermediate	1825.75 (313.45)	4575.13 (228.96)	774.96 (99.77)	256.49 (64.28) A	7.12 (2.16)	0.15 (0.03) A	382.78 (35.67) B	72.75 (18.32) B
	Forest	1210.74 (170.03)	3486.16 (199.03)	724.22 (100.97)	142.13 (6.10) B	8.52 (1.25)	0.08 (0.01) B	233.43 (5.83) A	48.70 (8.85) A

Table 22: Mean biochemical parameters of the 5–15 cm soil layer. Values are the mean over the field measurement replicates ($n = 3$), and values in brackets represent the standard deviation. Bold capital letters show the groups resulting from the Tukey's test.

Study Area	Stage	C_{mic} ($\mu g\ g_{soil}^{-1}$)	Enzyme C ($nmol_{\mu UF}\ g_{soil}^{-1}$)	Enzyme N ($nmol_{\mu UF}\ g_{soil}^{-1}$)	N_{mic} ($\mu g\ g_{soil}^{-1}$)	C_{mic} / N_{mic} ($\mu g\ \mu g_N^{-1}$)	C_{mic} / C_{org} ($g_{Corg}\ g_{Corg}^{-1}$)	Enzyme C / C_{org} ($nmol_{\mu UF}\ \mu g_C^{-1}$)	Enzyme N / C_{org} ($nmol_{\mu UF}\ \mu g_N^{-1}$)
Castello Tesino	Pasture	890.56 (181.34) B	967.13 (79.63) B	169.79 (53.57)	54.05 (13.08)	16.48 (5.21)	0.09 (0.01)	99.28 (17.71)	17.55 (6.37)
	Intermediate	1883.90 (666.06) A	2189.93 (237.98) A	278.63 (64.42)	119.76 (94.91)	15.73 (13.65)	0.14 (0.04)	157.17 (39.31)	19.92 (5.62)
	Forest	735.40 (405.32) B	1359.71 (241.80) B	311.53 (115.96)	44.65 (10.20)	16.47 (9.83)	0.06 (0.03)	113.62 (34.66)	25.98 (9.15)
Mel	Pasture	432.75 (379.75) AB	747.32 (143.44) AB	116.96 (60.53) AB	54.31 (46.26)	7.97 (9.74)	0.04 (0.02) A	63.87 (25.90) AB	10.06 (5.74) AB
	Intermediate	719.92 (115.89) A	145.22 (142.60) A	227.24 (92.12) A	62.98 (15.97)	11.42 (3.43)	0.08 (0.01) B	142.70 (49.57) A	24.90 (10.26) A
	Forest	259.35 (101.65) B	374.39 (39.71) B	41.00 (10.27) B	18.51 (14.58)	14.01 (12.33)	0.02 (0.01) A	33.58 (4.18) B	3.68 (0.91) B
Chianocco	Pasture	411.33 (14.31)	761.03 (80.42) AB	138.18 (67.62) AB	86.63 (23.08) AB	4.75 (1.28)	0.04 (0.00) AB	75.26 (26.01) A	14.92 (7.12) AB
	Intermediate	703.10 (296.96)	986.34 (62.51) A	189.32 (8.24) A	123.82 (28.24) A	5.68 (2.73)	0.06 (0.02) A	85.62 (9.85) A	16.53 (5.3) A
	Forest	301.45 (36.94)	384.21 (46.55) B	83.12 (21.04) B	57.22 (14.32) B	5.27 (1.47)	0.02 (0.00) B	23.97 (4.06) B	5.22 (1.46) B
Firenzuola	Pasture	116.97 (384.69)	106.92 (182.35)	222.60 (1.20)	97.14 (37.55)	11.50 (5.95)	0.12 (0.04) A	143.78 (52.72)	28.22 (6.54)
	Intermediate	600.29 (315.22)	908.18 (72.77)	225.12 (41.41)	48.27 (33.25)	12.44 (10.77)	0.06 (0.02) AB	139.86 (83.32)	36.47 (22.64)
	Forest	418.53 (118.10)	849.12 (117.62)	156.26 (41.43)	44.91 (11.53)	9.32 (3.56)	0.04 (0.01) B	65.93 (28.10)	14.01 (4.15)
Farindola	Pasture	719.21 (73.51)	913.70 (92.60)	251.55 (54.81)	32.70 (7.10)	21.99 (5.28)	0.08 (0.01)	99.22 (18.15)	27.33 (5.89)
	Intermediate	736.52 (174.87)	1203.59 (154.51)	294.92 (58.85)	62.92 (26.72)	11.71 (5.69)	0.08 (0.02)	129.44 (23.77)	31.97 (8.83)
	Forest	801.29 (173.95)	1004.59 (167.67)	244.17 (132.42)	73.44 (12.39)	10.91 (3.00)	0.08 (0.02)	101.10 (30.79)	24.54 (13.28)
Vastogirardi	Pasture	1101.76 (212.18) A	2222.52 (173.02) A	831.11 (170.99) A	75.07 (26.39) A	14.68 (5.88)	0.11 (0.02) A	239.18 (15.97) A	86.39 (2.67) A
	Intermediate	637.68 (242.33) AB	1545.36 (267.12) B	390.58 (127.20) B	43.11 (15.34) AB	14.79 (7.70)	0.06 (0.03) B	141.92 (27.73) B	36.42 (15.62) B
	Forest	436.82 (48.74) B	544.24 (26.56) C	37.26 (3.42) B	12.37 (18.58) B	35.31 (53.19)	0.04 (0.00) B	71.45 (8.17) C	23.55 (2.05) B
Pignola	Pasture	741.54 (103.34)	1417.19 (388.70)	421.34 (226.97)	84.51 (17.56)	8.77 (3.11)	0.08 (0.03)	160.25 (105.38)	48.51 (37.06)
	Intermediate	785.95 (140.81)	2267.03 (168.98)	572.37 (117.66)	108.23 (34.28)	7.26 (2.64)	0.07 (0.01)	206.67 (25.24)	52.27 (12.18)
	Forest	574.89 (92.19)	1501.10 (164.73)	373.71 (38.47)	85.01 (41.11)	6.76 (3.45)	0.04 (0.01)	114.06 (18.74)	28.45 (4.03)

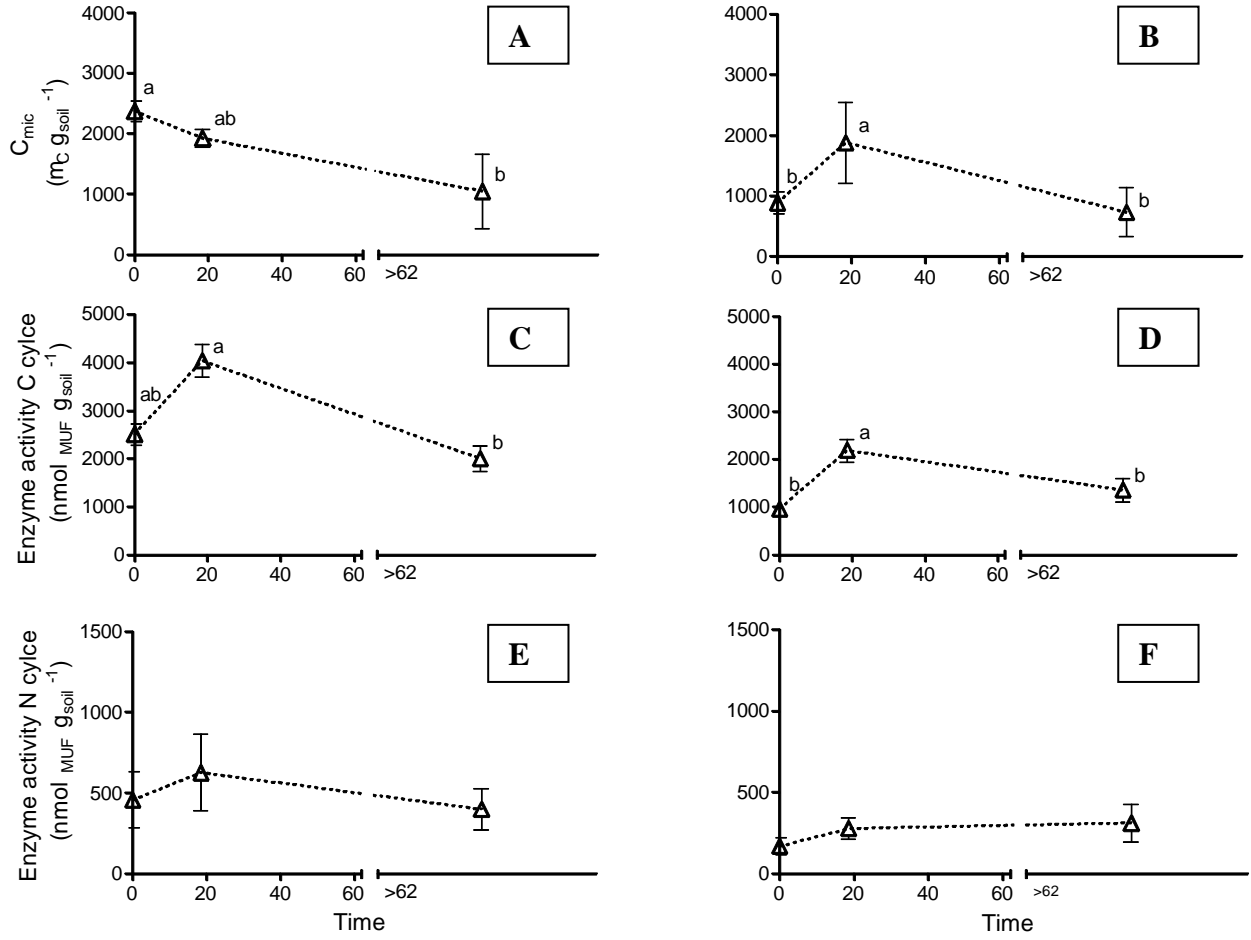


Figure 37: *Castello Tesino site. Changes in microbial carbon C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N cycle. The figure is subdivided in two columns: the left one (graphs A, C, E) refers to the 0-5 cm layer, while the right one (B, D, F) referred to the 5-15 cm layer. The horizontal axis represents the chronostages' "age", i.e. the time since the recolonisation process started, measured in years before present. The data points refer to pasture, intermediate stage and forest, and are positioned in time at the average age of these three stages (see main text above Fig. 25). Means and standard deviations ($n = 3$) were connected by a dotted line in order to roughly describe changes over time. When present, a,b and ab labels refer to statistically significantly different ($P < 0.05$) groups resulting from the Tukey's post-hoc test.*

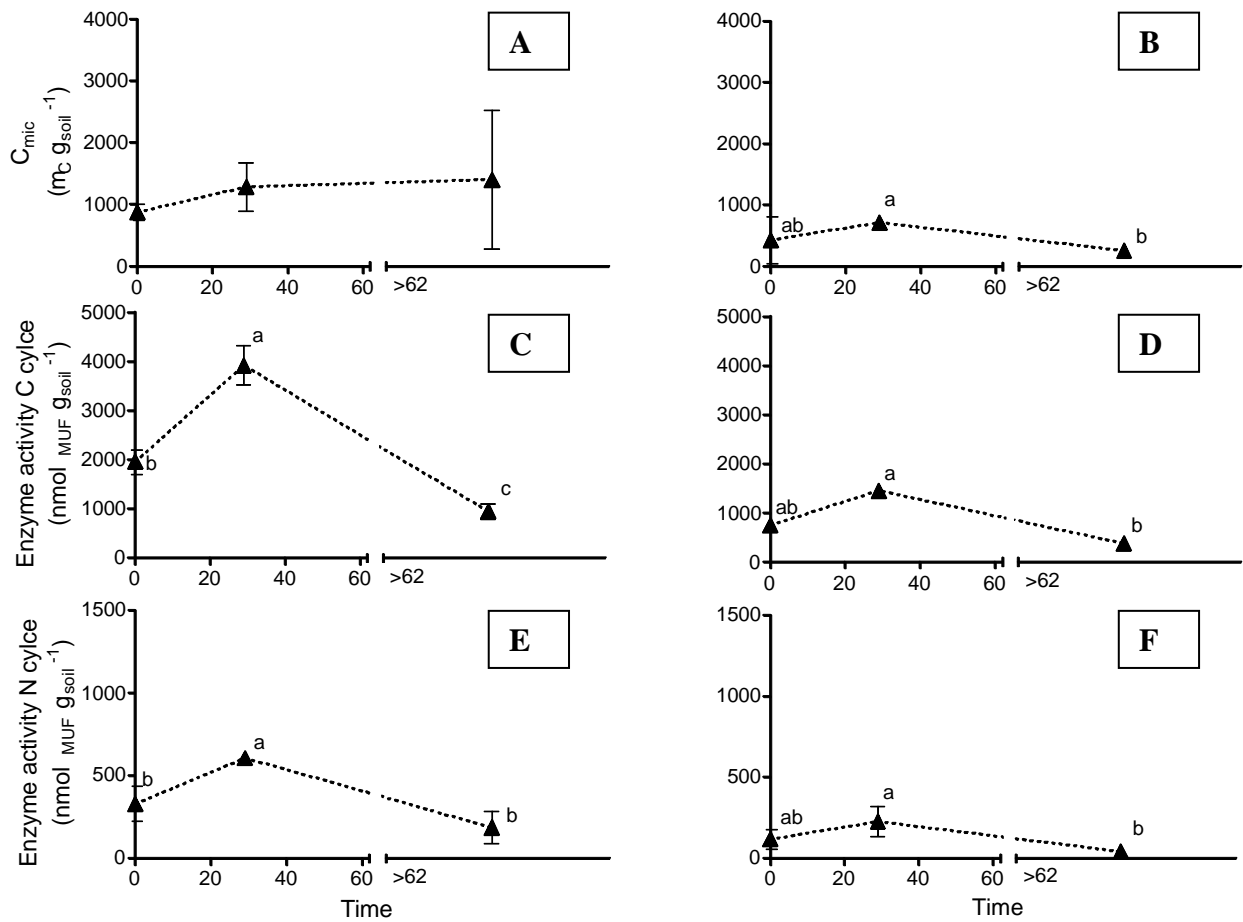


Figure 38: *Mel* site. Changes in the values for C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N one (E, F). Other details are as in Fig. 37.

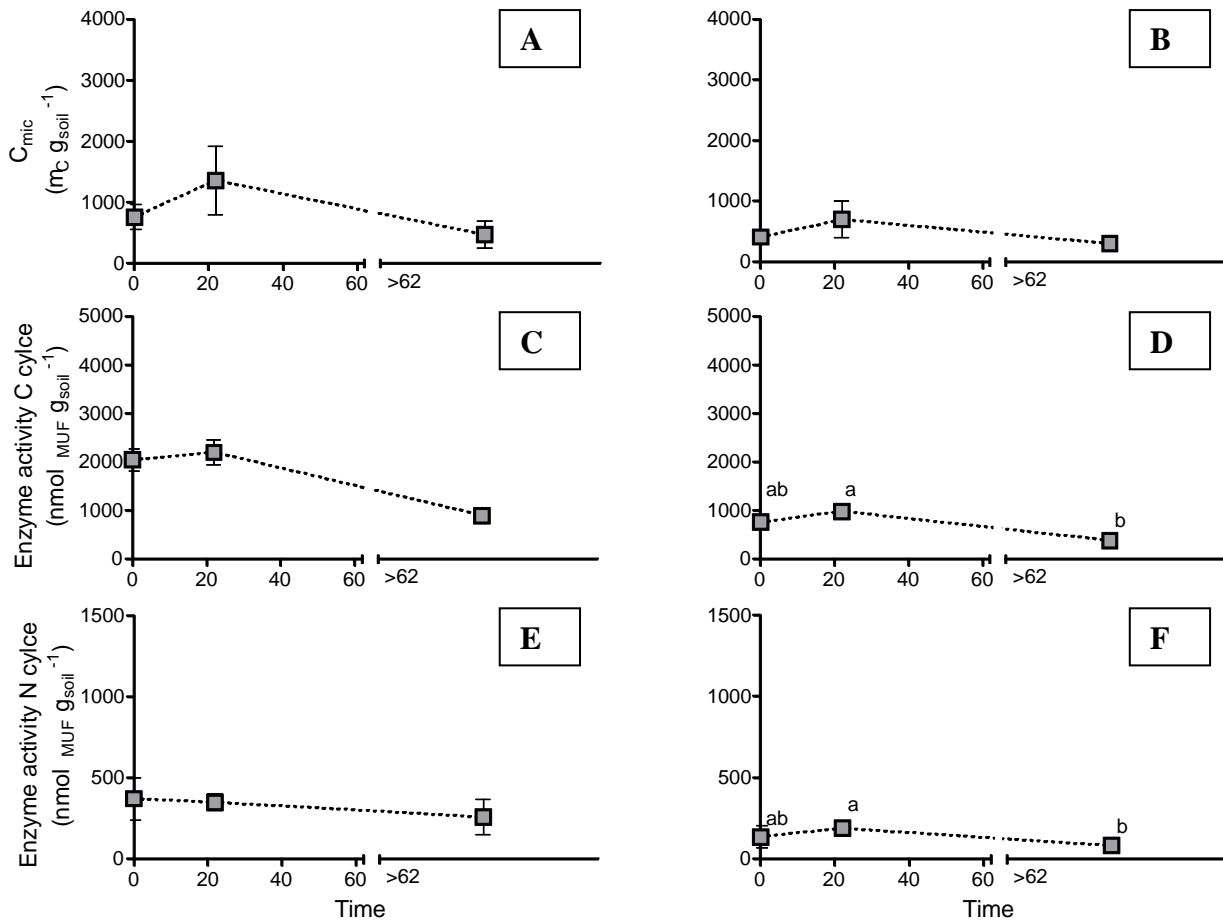


Figure 39: Chianocco site. Changes in the values for C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N one (E, F). Other details are as in Fig. 37.

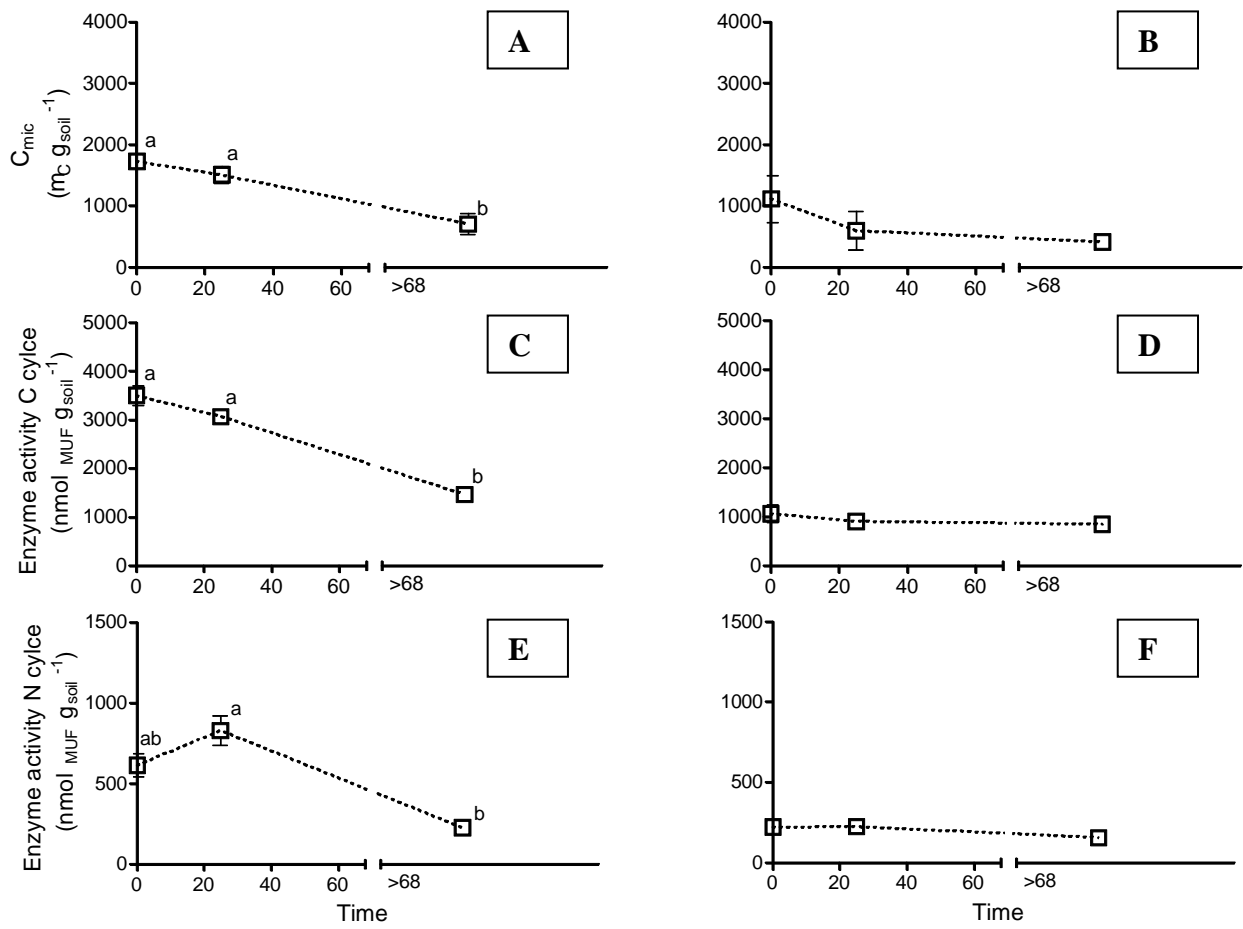


Figure 40: Firenzuola site. Changes in the values for C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N one (E, F). Other details are as in Fig. 37.

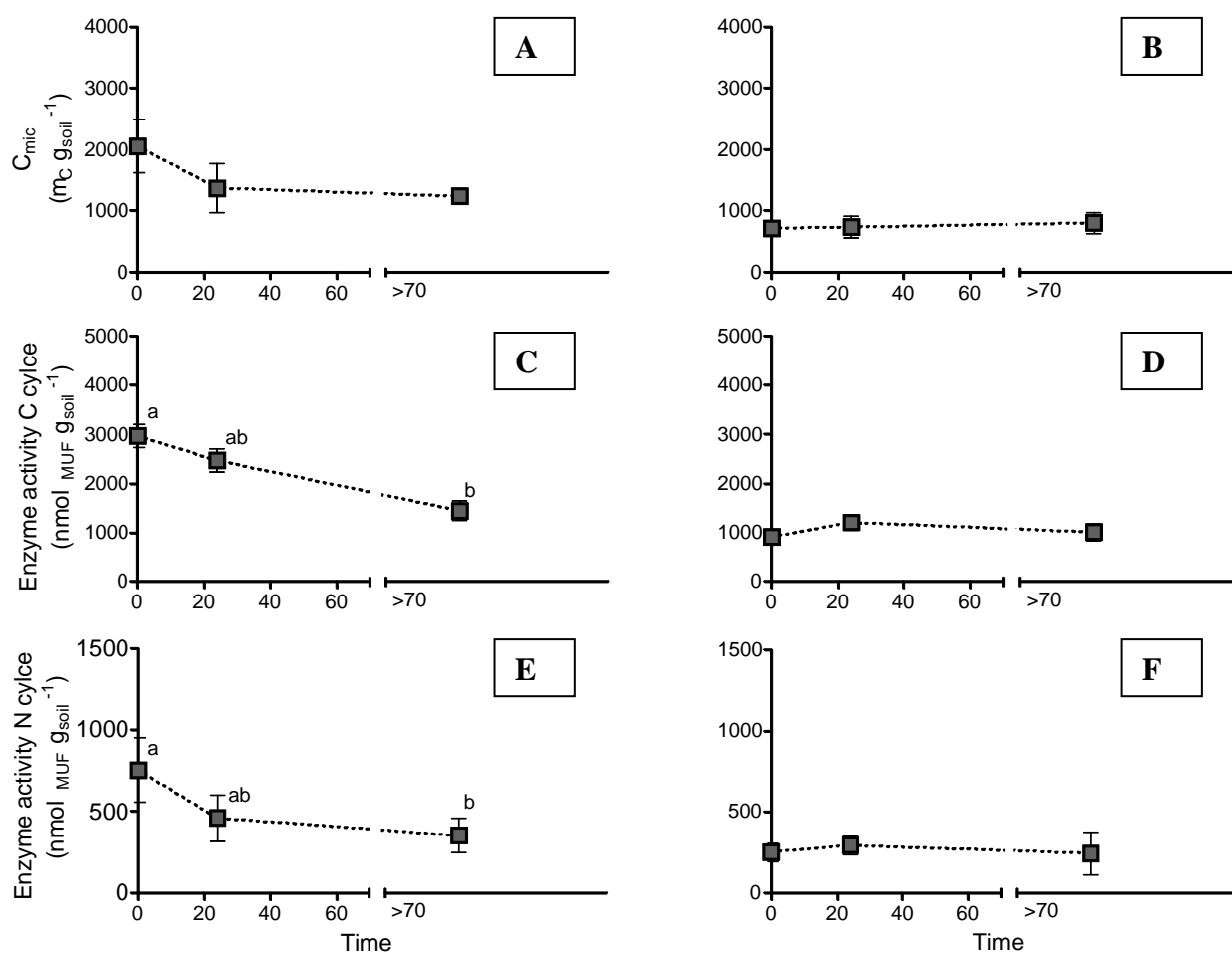


Figure 41: Farindola site. Changes in the values for C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N one (E, F). Other details are as in Fig. 37.

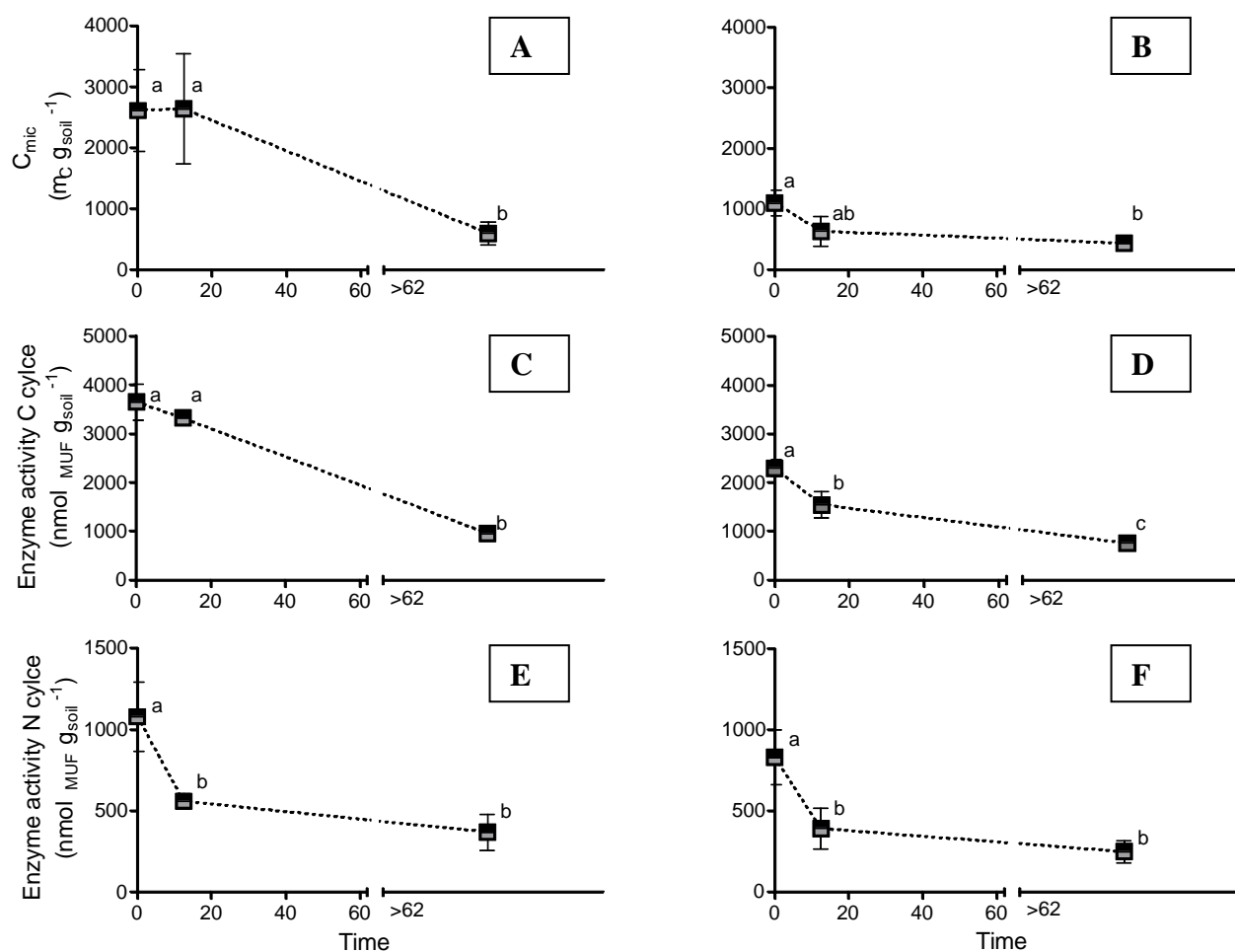


Figure 42: Vastogirardi site. Changes in the values for C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N one (E, F). Other details are as in Fig. 37.

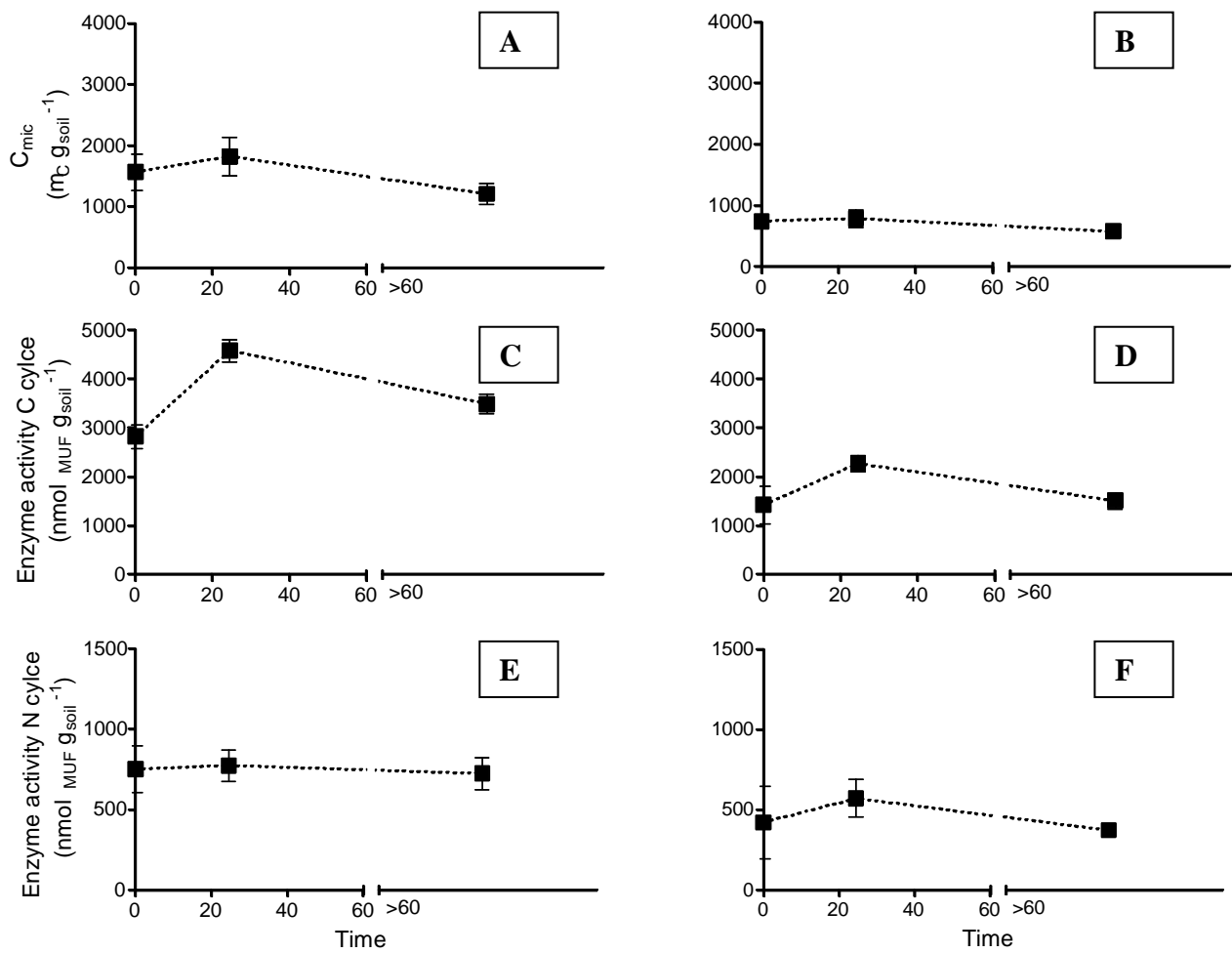


Figure 43: Pignola site. Changes in the values for C_{mic} (A, B), enzyme activity linked to the C cycle (C, D) and enzyme activity linked to the N one (E, F). Other details are as in Fig. 37.

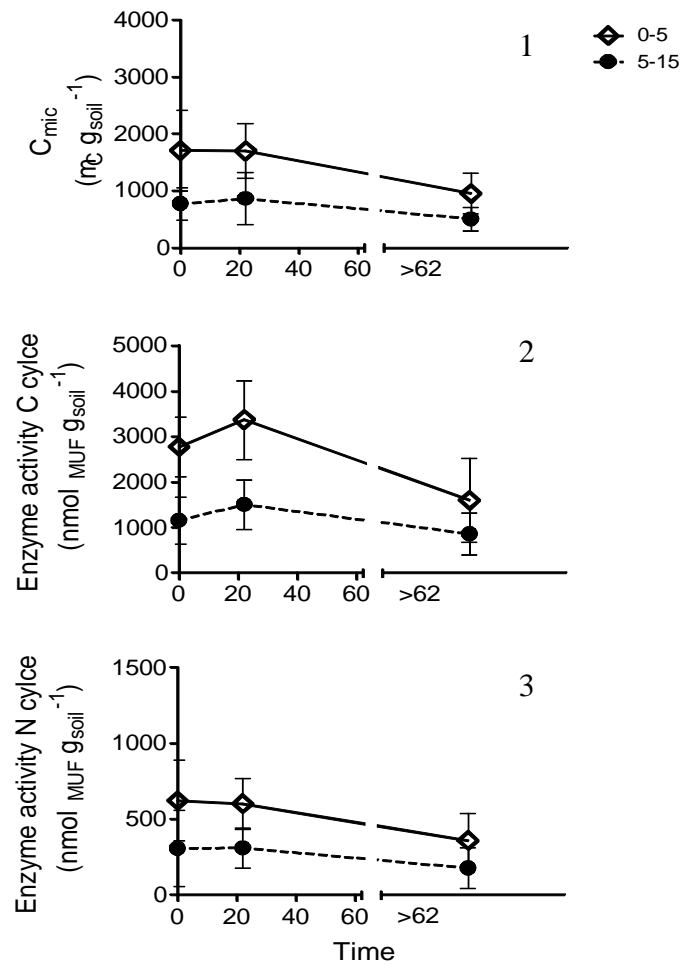


Figure 44: Mean changes in the microbial biomass (Graph 1), in the enzyme activity linked to the C and N cycle (Graphs 2 and 3, respectively). In each graph, both 0-5 cm (empty diamonds) and 5-15 (black dots) are shown. The data shown in the graphs are mean and standard deviations, over all chronosequences, of the mean values of each study area over the 3 replicate measurements.

No significant correlations were observed when each biochemical index included in the Tabs. 21 and 22 was plotted against each soil chemical index in Tab. 1 separately. However, maximum values for enzyme-specific activities have been generally observed in the intermediate stage – which is characterised by the maximum woody plant species diversity – while minimum values were measured in the forest stages (minimum woody plant diversity, see Tab. 20 and Fig. 36).

Fig. 45 includes a series of graphs, divided in the two groups of study areas (conifer and broadleaves) adopted for the functional type diversity index introduced in Tab. 20 and corresponding test description. In each column (group) of Fig. 45, the changes over time in the functional-type diversity index and the other biochemical indices are shown. In particular, the biochemical indices considered refer only to the 0-5 cm depth because it is the most sensitive to changes in the vegetation. A remarkable similarity between the functional type diversity index (empty diamonds in Fig. 45, graphs 1A and 1B) and the specific enzymatic indices, SEIc/Corg (graphs 3A and 3B) and chitinase/Corg (graphs 4A and 4B) are visible for both the conifers and broadleaves.

The final pair of graphs in Fig. 41 (5A and 5B) shows the changes in the litter and 0-5 cm SOC stocks considered together. The change in this variable is an effect of the decomposition of the soil organic matter (SOM) essentially driven by the enzyme activity. It was then reasonable to expect that, when the specific enzyme activity reaches its minimum values (forest stages), the C stock accumulation is higher. Indeed, Fig. 41 confirms this expectation, as the marked accumulation in the litter plus 0-5 cm layer SOC stock (5A and 5B) between the intermediate and forest stages corresponds to a dramatic decrease in specific enzyme activities (3A-B and 4A-B). Furthermore, a steeper variation in SOC stock corresponds to steeper variations in enzyme activities, as visible by comparing conifers (3A, 4A and 5A) versus broadleaves (3B, 4B and 5B).

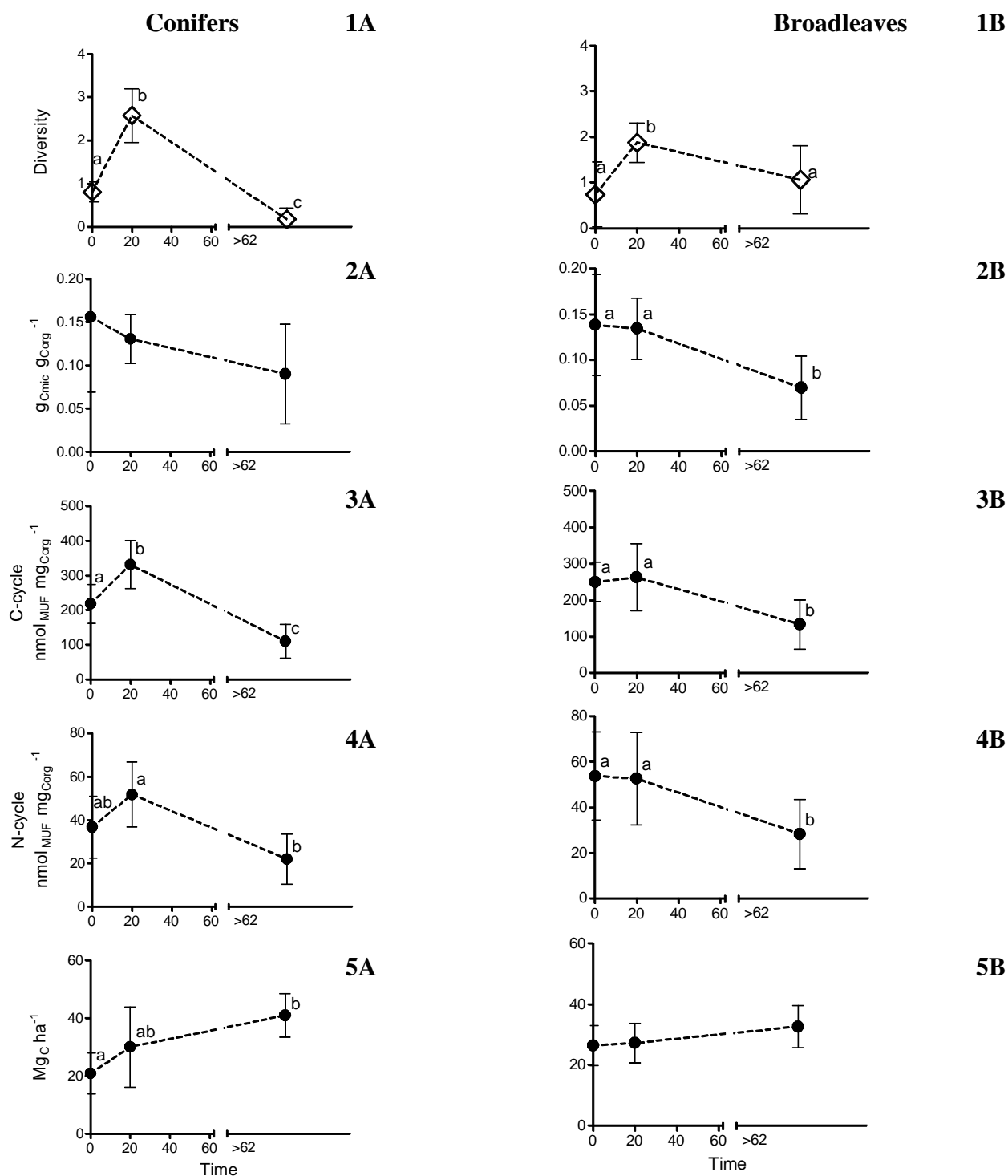


Figure 45: Above-ground and below-ground carbon content per surface area ($MgC ha^{-1}$) in conifers (column A) and broadleaves (column B) sites. In all the graphs, the horizontal axis is time, in years before present (YBP), and refers to the average age of each stand (Pasture, Intermediate and Forest). Graphs 1A and 1B depict the above-ground functional type diversity index, estimated as described in Tab. 21 and the relative text. All the other graphs (except for 5A and 5B) take into account, separately, the main biochemical indices only in the 0-5 cm soil depth interval. In particular, graphs 2A and 2B show the change in microbial biomass per unit of soil organic carbon; 3A and 3B show the change in the SEIc (synthetic enzymatic index for the C-cycle) per unit of soil organic carbon; and 4A and 4B the variation in Chitinase activity per unit of soil organic carbon. Graphs 5A and 5B show the C stock trend in litter and upper soil layer considered together. Significant differences are marked with lowercase labels next to the data points.

Correlations between the aboveground functional type diversity index and the specific enzymatic index for SEI c and chitinase are tested and graphically shown in Fig. 46. Statistically significant correlations were always detected in the conifer study areas both for the C and for the N cycle (SEI c and chitinase specific enzymatic index, respectively). This was not the case in the broadleaves study areas, even though the correlation is statistically significant for the SEI c when all the study areas were considered together.

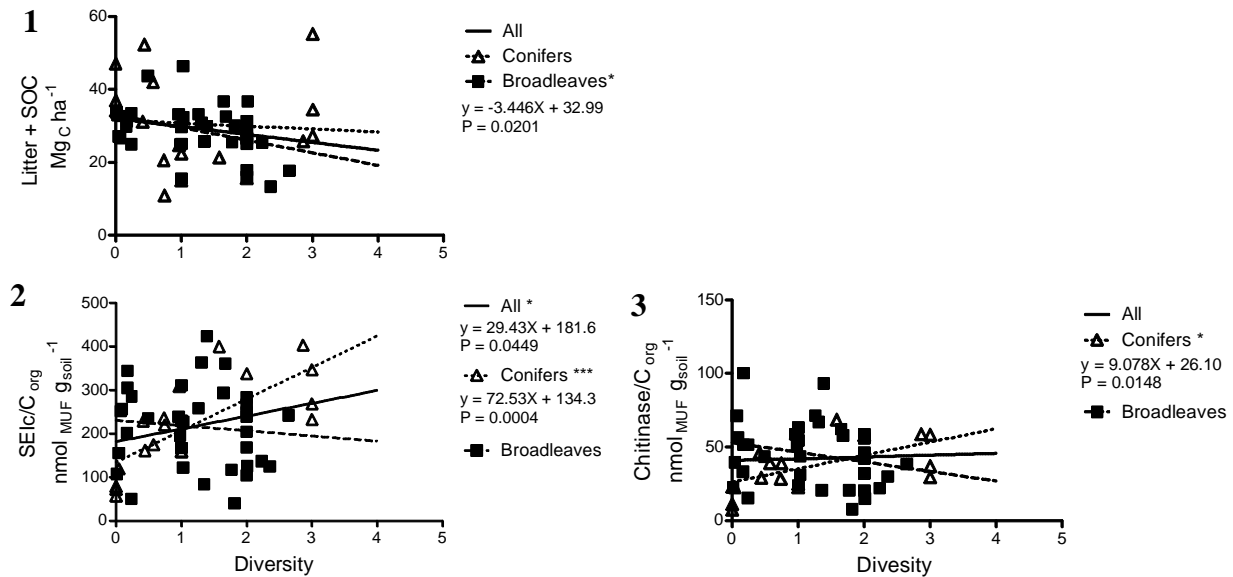


Figure 46: Correlation between the functional type diversity index and (1) C stock accumulation in litter and upper mineral soil layer, and (2) SEI c and (3) chitinase enzyme activity per unit of soil organic carbon. The effect of functional type diversity on each variable is considered for all the stand type (solid line) and separately for conifers (dotted line) and broadleaves (dashed line). Significant correlations are marked with one or more * near to the line type in the legend of each graph. In case of significant linear regression, line equation and P value are reported in the legends. In this case, all the raw data (rather than just mean and sd) are shown because the three replicates of each stage differ both for the x and y graph variables.

10.DISCUSSION

10.1. SOC and N concentrations

The progressive decrease in C_{org} concentration along the soil profile observed in all studied sites is in line with the results of Jobbagy and Jackson (2000), Montané *et al.* (2007), Hiltbrunner *et al.* (2013) and Guidi *et al.* (2014a), Zhao *et al.* (2016). Although a full statistical analysis of the variation in C_{org} concentration at different soil depths was not carried out, a gradual and marked decrease in each stage of all chronosequences is clearly visible. According to Hiltbrunner *et al.* (2013), this is indicative of the fact that the stages selected for this work in each study area are effectively characterised by a pedological profile which was not disturbed by human activities, erosion and landslide processes.

The ranges of C_{org} concentration values measured at each soil depth interval are comparable with what estimated in similar studies in the Alps (Chiti, 2007; Hiltbrunner *et al.* 2013; Guidi *et al.*, 2014a; Fino, 2015). Indeed, the largest part of the results shown in Fig. 23 ranges between the 7 and 120 gC kg_{soil}⁻¹, and with highest values in the 0-5 cm interval. However, in this layer, extremely high C_{org} concentrations were measured in the intermediate stage of Castello Tesino study area. The C concentrations are much higher than those of similar studies on SOC conducted on the Alps and are close to the threshold of 200 gC kg_{soil}⁻¹ considered as a typical value for the H soil horizon. Therefore, it is possible that a part of the soil samples that were analysed were inadvertently collected, at least partially, from the organic layer. This is however deemed improbable, as the sample was a mixture of 25 different subsamples collected separately and it is therefore unlikely for a large number of them to be simultaneously affected by potential inaccuracies.

The same trend as that of the C_{org} concentration was also observed for the total N in the soil. A similar total N reduction along the soil profile was described by Guidi *et al.* (2014a) in the study area they selected in the Southern Alps and by Montané *et al.* (2007) in mountain encroached grasslands in the Iberian Peninsula. However, differently from what was observed by Guidi *et al.* (2014a), not all the forest stages investigated in this work show a statistically significant reduction in N concentration with respect to the younger stages of the woody encroachment process.

When comparing the changes of C_{org} and N (shown in Fig. 23 and Fig. 24, respectively) along the secondary succession, results seem to be more in line with what described by Fino (2015), where C and N were also observed to change simultaneously (both in time and depth). In our case, this is also confirmed by the small variability (though statistically significant) in the C/N ratio results (see Tab. 15). Overall, the C/N ratios observed in the forest stages are unexpectedly low, as similar values generally characterise grasslands or grazing areas, where there is a manure input to the soil. Although similar results have been found in other studies (e.g. Guidi *et al.*, 2014a), such low values, at least in the present case, could be attributed to either a relatively young forest, or to some grazing activity that may have been, or to some extent may still be, occurring. In almost all study areas, the C/N ratio tends to increase from the pasture to the forest. The opposite trend was generally observed for the pH, which gradually tends to reach more acid values in the oldest part of each chronosequence. Similar C/N and pH changes over time are described in Thuille *et al.* (2000), Seeber and Seeber (2005), Gamper *et al.* (2007), Hiltbrunner *et al.* (2013) and Guidi *et al.* (2014a). According to Guidi *et al.* (2014a), the changes of C/N ratio and pH can be due to the change in both litter quality (Hiltbrunner *et al.*, 2013) and the decomposition process in the organic layers (Gamper *et al.*, 2007).

As far as the soil texture is concerned, in some of the study areas, in particular Farindola, Vastogirardi and Pignola, a variation in the percentage of clay, silt and sand was observed when comparing pastures / grasslands and the relative forest stages (a marked increase in Vastogirardi and a marked decrease in Farindola and Pignola). Such a large variability was unexpected, and could have a non-negligible effect on the results obtained in this study. Indeed, according to Feng *et al.* (2013) and Doetterl *et al.* (2016), soil texture can have a key role in the stabilisation of the C_{org} in the soil through the formation of organo-mineral associations. In particular, the clay phyllosilicates and hydroxides mineral particles are the most important component involved in this process. Jobbagy and Jackson (2000) also obtained similar results on the effect of clay content, and in addition they observed that the positive correlation between clay percentage and SOC is more statistically significant in the deeper part of the soil (below 40 cm) compared to the upper part. Instead, closer to the surface (at 0-40 cm of depth), higher correlations were measured between SOC concentration and climatic variables (MAT and MAP). In order to test if, in the present study, the difference in clay content between forest and pasture could explain the observed changes in the SOC among the stages, a Spearman correlation analysis was performed (Fig. 22). A positive correlation could have been indicative of a potentially strong impact of the role of soil texture on the results obtained here. However, a negative correlation was observed (the SOC decrease in Vastogirardi and increases in Farindola and Pignola), and this was consistent when considering separately the topsoil, subsoil and the whole profile (Fig. 22). Therefore, the correlation found between clay content and SOC was deemed spurious, and was attributed to the change in the vegetation composition. This was reassuring, as it indicates that the high variability in soil texture is unlikely to weaken the conclusions of this study.

Excluding the case of Vastogirardi, whose characteristics, as explained before (Sections 8.2.6 and 9.2), were somewhat anomalous compared to the other sites, a possible explanation for the marked decrease in the clay content between pasture and forest stages, especially in Farindola and Pignola, was the following. In mountain ecosystems, it is reasonable to assume that areas of low productivity were the first to have been abandoned, or even not to have been used at all for pasture activities, and that are therefore now in the forest stage. These areas are likely to be the steepest ones, both because less accessible and less able to retain clay content when affected by erosion processes, or those that, for any other reason, have limited clay content. Indeed, according to what described by Tasser *et al.* (2007) and to Tappeiner *et al.* (2008) for the Alps, inaccessible and steep areas have been increasingly abandoned in the last 150 years. In addition, the less intensively the land was formerly used (less productive areas), the longer ago the area was abandoned (Tasser *et al.*, 2007). On the contrary, the soil rich in clay content has likely been use for grazing until recently, especially in the central and Southern part of Italy.

10.2. SOC and N stocks

Generally, the SOC stock measured in all study areas in the whole soil profile varied between 100 and 220 $MgC\ ha^{-1}$. (see Fig. 25 and Tab. 16, Section 9.3). However, some study areas were characterised by soils that, at least in one of the encroached stages, had extremely high SOC stock values. In particular, in the Castello Tesino chronosequence, the whole profile SOC stock ranged between 180 and 330 $MgC\ ha^{-1}$, while the Farindola and Pignola forest stages had a SOC stock amount of approximately 300 $MgC\ ha^{-1}$. Overall, the study areas investigated in this work were characterised by a higher SOC stock amount with respect to the previous studies mostly conducted in the Alps ridge. Indeed, SOC stock values in the ranges 10-50, 40-100, 30-156, 40-90 $MgC\ ha^{-1}$ were estimated by Thuille *et al.* (2000) Thuille and Schulze (2006), Risch *et al.* (2008) and Guidi *et al.* (2014a), respectively, for woody plant species establishments over pasture and grasslands. However, these authors generally considered only the upper part of the soil

(not more than 50 cm deep), which is a thinner soil portion compared to the one was considered in this work. Therefore, it was reasonable to expect here values higher than those estimates. Nevertheless, as the SOC stock tends to decrease progressively when the soil depth increases, it was expected that the differences between the values measured by these authors and the values obtained here would be less pronounced. The results of the present study, when focusing only on the 0-30 cm depth interval were approximately in line with the SOC stock values range of 110-157 MgC ha⁻¹ estimated by Alberti *et al.* (2008) (see Tab. 16, Section 9.3). An overall soil profile comparison could be done with the results of Hiltbrunner *et al.* (2013) and Fino (2015), who estimated the SOC stock in the 0-80 and 0-60 cm layers. Their results were, respectively, lower (101-140 MgC ha⁻¹) and slightly higher (158-223 MgC ha⁻¹) than those obtained here.

All the study areas were characterised by a very high N stock amount in the soil. The mean amount of N over the whole soil profile was of 20 MgN ha⁻¹, a quantity that appeared much higher than the about 12 MgN ha⁻¹ estimated by Fino (2015) in the Belluno province, who however focussed only on the 0-60 cm depth interval. When restricting the attention to the topsoil layer only (0-30cm), the present results, approximately in the 5-15 MgN ha⁻¹ range (Tab. 17), also appear to be higher than those in the 3-9 MgN ha⁻¹ range described by Guidi *et al.* (2014a).

The distribution of the SOC stock along the soil profile was extremely interesting. The results of this work suggest that the subsoil is an important reservoir of organic C in all the considered stages. On average, 36.5% of the total soil profile stock was found in the subsoil. This result is particularly important because usually this part of the soil is not considered for total SOC stock estimations. In fact, the largest part of the literature consulted on the LUC effect on SOC stock did not take the subsoil into account. This was even the case in the original form of the protocol that was adopted in this work. Despite this marked capacity of the soil to store C also in the deeper layer being known (see for example Jobbagy and Jackson, 2000), the work on the effect of land-use change on SOC stock tends to focus of the most dynamical part of the soil (the topsoil). Indeed, in this part of the soil both the C_{org} input and C_{org} mineralisation are highest because of leaves, woody litter and fine root litter deposition, and because of microbial activity, respectively. Moreover, according to Rey *et al.* (2008), the higher contribution to the total heterotrophic respiration is due to the microbial population that inhabits the top few centimetres of soil. The same authors suggested that the contribution to the mineralisation process tends to decrease with increasing soil depth. For this reason, the soil data collection for the biochemical analyses was restricted to the 0-5 and 5-15 cm depth intervals.

Finally, the N stock distribution in the soil profile followed that described for the SOC stock. Again, it is important to highlight the necessity to take into account the whole soil profile for a correct estimation on the total SOC and N stocks.

10.3. SOC and N stock changes along the chronosequences

The chronosequence approach gives the possibility to compare the SOC and N stocks among the different stages of the woody encroachment or the afforestation. The same methodology was adopted in similar studies (see Thuille *et al.*, 2000; Thuille and Schulze, 2006; Montané *et al.*, 2007; Alberti *et al.*, 2008; Guidi *et al.*, 2014a) in order to understand the effect of the woody encroachment or afforestation on SOC stock over time.

As previously described when discussing the microbial activity and litter deposition, the fastest SOC turnover was observed in the upper part of the soil profile (Conant *et al.*, 2001). For this reason, according to Harrison *et al.* (2011), Hiltbrunner *et al.* (2013) and Poeplau and Don (2013), most of the studies that consider the land-use change effect on SOC stock investigated only the upper part of the soil profile (generally the topsoil).

Fig. 27 shows a difference in the topsoil SOC stock changes between the Alps and Apennines study areas. This part of the soil is generally mostly affected by high fine root system activity and turnover, and by litter deposition input. In addition, in the topsoil there is the highest microbial activity, which, on the basis of climatic condition and organic matter recalcitrance, is involved both in mineralisation and humification of the dead organic matter (La Mantia *et al.*, 2013). In the forest stages, the quantity and quality of aboveground litter input tended to become particularly different from those of the previous stages, especially in comparison to the grasslands / pastures. Therefore, the differences in the topsoil SOC stock among the Alps and the Apennines study areas (see Fig. 27) were probably linked both to the different climatic conditions (soil temperature and humidity) and to the litter quality due to plant species composition. Indeed, in the Alps sites, where colder temperatures were registered compared to the Apennines, the conifers were at least present – if not dominant – in the forest stands and these plants are characterised by a higher litter C/N ratio with respect to broadleaves (Rey and Jarvis, 2006). Therefore, in the Alps sites the litter is also more resistant to the mineralisation process (Bruke *et al.*, 1989). More specifically, Hobbie *et al.* (2006) estimated average C/N ratio values of 49, 65 and 82 for *P. abies*, *L. deciduas* and *P. sylvestris*, respectively. On the opposite, 56, 37, 74, 49 and 31 are the average C/N ratio values for *F. sylvatica*, *T. cordata*, *Q. cerris*, *A. pseudoplatanus* and *F. excelsior*, respectively (Gahrooe, 1998; Hobbie *et al.*, 2006; Jacob *et al.*, 2010), the most common tree species in the Apennines, where higher temperatures were registered.

Even if the subsoil is generally not considered in SOC stock studies along LUC (Harrison *et al.*, 2011; Hiltbrunner *et al.*, 2013), it was recognised as a significant C pool (Jobbagy and Jackson, 2000; Poeplau *et al.*, 2011; Fino, 2015). This was confirmed by the results obtained in the present work (see Fig. 28). In addition, according to Hiltbrunner *et al.* (2013), the changes in the SOC stock of the subsoil seemed to follow those of the topsoil compartment. Although the process involved in the stabilisation of the SOC in the deeper soil are still unclear (Lützow *et al.*, 2006), it seems that the SOC stock, especially in that layer, can be related to the roots characteristics of the aboveground plant species (Jobbagy and Jackson, 2000). In line with this, in the present work the highest increase in the subsoil SOC stock was observed in the stage where the dominant vegetation is characterised by a deeper root system, which is therefore able to storage the organic matter released by root activities and root turnover directly in the deeper part of the soil. Indeed, both the broadleaves and the shrubs species tend to produce a deeper root system with respect to conifers and grasses (Jackson *et al.*, 1996; Jobbagy and Jackson, 2000). Furthermore, in addition to the subsoil input provided by deep roots, earthworms can also contribute in transporting organic matter from the upper to the deeper soil (Muys *et al.*, 1992; Seeber *et al.*, 2005), where lower levels of heterotrophic respiration of the microbial component are recorded (Rey *et al.*, 2008). The higher acidity and larger C/N values in the soil covered in conifers compared to broadleaves is known to enhance the suppression of earthworms' activity, which in fact Muys *et al.* (1992) suggests is less marked at least under some broadleaves than under conifers. Finally, the translocation of organic matter from upper to lower soil could also facilitate organo-mineral interactions that have a positive impact on the stabilisation of the organic matter.

Therefore, once more, these considerations highlight an urgent need to take into account the whole soil profile for a correct estimation on the total SOC and N stocks. This is crucial, as the subsoil compartment is typically ignored not only by most of the scientific research, but also by the majority of studies aiming to quantify the C stocks to evaluate the impact of mitigation policies.

10.4. SOC stock variation in the whole profile

Despite the numerous papers published on this argument an extensive evaluation of the SOC changes occurring during a secondary forest succession is still lacking, particularly for the central Italian territory (Alberti *et al.*, 2011). Moreover, as described in the introduction, no common trend in SOC stock change along secondary successions or afforestation processes can be identified among all the sites examined in the literature. In particular, the SOC stock might decrease (Thuille *et al.* 2000; Thuille and Schulze, 2006; Alberti *et al.* 2008; Pinno and Wilson, 2011; La Mantia *et al.*, 2013; Guidi *et al.* 2014a), increase (Brejda, 1997; Feldpausch *et al.*, 2004; Montané *et al.*, 2007; Fonseca *et al.*, 2011, La Mantia *et al.*, 2013, Chiti *et al.*, 2016) or show no statistically significant differences (Hiltbrunner *et al.*, 2013; Risch *et al.*, 2008; La Mantia *et al.*, 2013).

More in detail, Alberti *et al.* (2008) described a linear decrease in the SOC stock in the Italian Eastern Prealps ridge with a trend of $-0.69 \text{ MgC ha}^{-1} \text{ yr}^{-1}$. Hiltbrunner *et al.* (2008) also described a decrease in the SOC stock considering a *P. abies* afforestation in the Alps. Guidi *et al.* (2014a) measured a progressive reduction in the SOC stock over time under a natural woody encroachment in the Italian Alps. All these trends were statistically significant in the 0-5 and 5-15 cm depth intervals. In addition, Thuille *et al.* (2000) measured a general decrease in the SOC stock in the A horizon along the whole process, even if a positive peak was observed in an intermediate stage around 25 years from the beginning of the process. Thuille and Schulze (2006) observed a decrease in the SOC stock after the afforestation or the woody encroachment in several sites. Generally, the decrease they found was more pronounced at the beginning of the process and less in the final part, with a possible final gain of C. The authors observed that a possible consistent gain towards the last stages is possible and can lead the process to reach, in the forest stage, C stock values similar to those initially present in the pasture. Pinno and Wilson (2011) observed a statistically significant decrease in the SOC stock but only during the shrubland vegetation conversion into forest.

On the contrary, Montané *et al.* (2007) showed that the shrubs encroachment (both conifers and N-fixing species) in the mountain ecosystem in the Iberian Peninsula can lead to a SOC stock increase in the 0-15 cm depth interval. The increase is constant, with values ranging between 28 and 452 $\text{g m}^{-2} \text{ yr}^{-1}$ (0.28 and 4.52 $\text{MgC ha}^{-1} \text{ yr}^{-1}$). In addition, Fonseca *et al.* (2011) observed a constant increase in the SOC stock of 1.09 MgC ha^{-1} in the first 20 years from the secondary forest encroachment over abandoned pastures in a humid tropical forest in Costa Rica. Feldpausch *et al.* (2004) found an increase in the SOC stock of 65% in the 12-14 years between pasture and forest stages in Amazonia (MAT 26.7 °C, MAP 2200 mm yr^{-1}) in the 0-45 cm depth interval. Brejda (1997) observed that SOC stock at 3.8 cm of depth increased when grazing had been eliminated and that the grazing areas had been encroached by woody plant species in subtropical Arizona chaparral. Rather than an increase or decrease in SOC stock, Risch *et al.* (2008) did not measure any statistically significant change along the process in the 0-20 cm depth interval. Finally, La Mantia *et al.* (2013) described different trends of SOC stock content in the 0-30 cm depth interval among secondary successions over pastures in different climatic condition in Sicily. In particular, a reduction in SOC stock was measured for wettest and coldest sites, an increase in the SOC stock was measured for the hottest and driest sites and a null effect of the woody encroachment on SOC stock was measured for intermediate climatic conditions.

The results obtained in the present work showed marked differences among the study areas. This was not unexpected, given the inconsistency of the results in the cited literature. Concerning the Alps ridge, it was worth highlighting that the results of this work did not fit perfectly with what was described by Thuille *et al.* (2000), Thuille and Schulze (2006), Alberti *et al.* (2008) and Guidi *et al.* (2014a). Indeed, the lack of a statistical difference among the pasture and

forest stage in the study areas selected in the Alps ridge seemed to be more in line with the results of Risch *et al.* (2008) and e Hiltbrunner *et al.* (2013). In addition, in the present work, a common (albeit not always statistically significant) SOC stock increase was estimated in the intermediate stage of these study areas. Among all studies considered in the literature, only Thuille *et al.* (2000) observed a similar increase in the 25-year-old stage. The authors suggested that the results they estimated were due to the extensive grazing that was observed in this stage and that could have added a significant amount of C to the soil through animal faeces. However, even if in the present study a grazing pressure was observed in the intermediate stage of Castello Tesino and Mel, it cannot be considered extensive. Therefore, it was hypothesised that in the intermediate stage – which was characterised by an higher woody plant species diversity and species heterogeneity compared to the other stages – the different plant functional types were able to release simultaneously organic matter of different quality and at different depths in the soil.

Paired-site studies were performed by McKinley and Blair (2008), Jackson *et al.* (2002), Alberti *et al.* (2011), Poeplau and Don (2013) comparing one or more pairs of stages (grasslands or croplands versus encroached stages). In the first study, the authors observed an increase of 12% in the C_{org} in a *Juniperus virginiana* encroached stage compared to the pasture. Both Jackson *et al.* (2002) and Alberti *et al.* (2011) estimated the SOC stock changes among encroached stages and nearby managed areas in different climatic conditions. They highlighted a marked negative correlation between MAP and SOC stock changes from the grass vegetation to the encroached one, with SOC stock variations inverting direction, from increasing to decreasing, as the MAP rises. In particular, Jackson *et al.* (2002) measured – for the North America abandoned grasslands – a threshold of nearly 500 mm yr⁻¹, which separates positive and negative effect of the woody encroachment on SOC stock. On the contrary, Alberti *et al.* (2011) estimated this threshold to be around 900 mm yr⁻¹ for the Italian peninsula. Poeplau and Don (2013) estimated a reduction in the SOC stock of -10 ± 7 Mg ha⁻¹ comparing grassland and afforested stages for a wide mean annual precipitation range, without observing a correlation with this variable.

Similarly, Guo and Gifford (2002) showed, in their meta-analysis, that both secondary successions and afforested stands lead to a decrease in the SOC stock with respect to the previous pasture land-use. Moreover, they considered separately the land-use change *pasture to plantation* – for which they have a database of 83 case studies – from the *pasture to secondary forest* – for which have only 6 cases. For the first LUC, they estimated a statistically significant loss of C from the soil, while for the latter they observed a more pronounced mean loss of C but with a higher uncertainty, which cannot confirm that the reduction is statistically significant. In addition, considering the *pasture to plantation* LUC they showed that both tree type (broadleaf and conifer) and mean annual precipitation value can affect the SOC change. Concerning the former factor, while the conifer plantation led always to a statistically significant reduction in the SOC, this was not the case for the broadleaves. Concerning the latter factor, a positive correlation between precipitation and SOC loss was detected.

Because of the attention that the mean annual precipitation has received in the literature on SOC stocks, this was the first climatic parameter taken into account in this work, even if several studies in different parts of the world (as for example Pinno and Wilson, 2011; Fonseca *et al.*, 2011; Chiti *et al.*, 2016) found the SOC stock changes due to the secondary succession process not to depend significantly on mean annual precipitation. In line with these last studies, the results of this work did not detect a significant correlation between forest to pasture SOC stock ratio (or difference) and mean annual study areas precipitations (see Tab. 18). However, it has to be noted that the precipitation range considered in this work (957-1670 mm yr⁻¹) is much more limited than the 230 – 1070 mm yr⁻¹, the <900 – >1500 and

the 650 – 2415 m yr⁻¹ considered by Jackson *et al.* (2002), Guo and Gifford (2002) and Alberti *et al.* (2011), respectively.

The limited precipitation range observed in this study is mainly due to the geographically restricted area in the Italian peninsula where the study sites were selected, i.e. along the Alps and Apennine mountains ridges, where the pasture abandonment and subsequent woody encroachment process are more frequent and hence easier to observe. In addition, a similar choice was also originally made in response to the lack of similar studies investigating secondary successions in particular in the Central Apennines (Alberti *et al.*, 2011).

However, although no correlation with mean annual precipitation was found, a statistically significant correlation was observed both for forest to pasture SOC stock ratio and difference, and both mean annual temperature and a winter cold stress index (see Tab 18 and Figs. 31, 32 A and B in section 9.4). In this case, the latitudinal gradient of the Italian peninsula led to a rather wide range of values for these indices. These results were also strengthened by the elevation variability among the study areas, which led to mean annual temperature estimates varying, among study areas, between 4.6 °C and 11 °C. Even if this interval might not appear particularly wide, it has to be taken into consideration both that it refers to an average of the whole annual temperatures and that all the study areas are restricted to mountain territories. In the scientific literature, the role of temperature is also considered as an important parameter that can affect the SOC stock changes along the secondary succession process (see Jobbagy and Jackson, 2002; Alberti *et al.*, 2011; Guidi *et al.*, 2014a, Thuille and Schulze, 2006, La Mantia *et al.*, 2013), but much less than the mean annual precipitation, in particular after the strong correlation between encroached to grasslands SOC stock values and mean annual precipitation found by Jackson *et al.* (2002), Guo and Gifford (2002) and Alberti *et al.* (2011).

Given the universally recognised role of climatic parameters as the main driver in the SOC stock changes along the secondary succession process, the clearly different trend in SOC stock changes in two study area groups that was found in this work (corresponding to Alps and Apennine) should not be considered as a result of the mountain chains orogenic processes themselves, but rather a consequence of the different climatic conditions between the two mountain ranges. Moreover, thanks to the chronosequence concept, which allows excluding the confounding effect of most environmental variables, it is reasonable to assume that the geolithological variables are also not influencing the changes observed in the results. However, because of the limited number of study areas investigated, it was not possible to categorically exclude that different soil types could affect the SOC stock trends over time from the pasture abandonment. Indeed, according to the fundamental equation of soil-forming factors of Jenny (1941), several such factors could play an important role. Among them, in addition to climate, living organisms and time, the most relevant ones are topography and parent material. Therefore, even if climate is the factor on which most of the literature on this topic focuses, the lack of a clear picture emerging from all available studies suggests a more complex scenario, where other factors could play a similarly important role and should therefore not be ignored in future studies.

Similarly to what was observed for the SOC stock differences when plotted against temperatures (Fig. 32A and 32B), strong correlations among temperatures and litter amount (Fig. 32C and 32D) were also detected. Indeed, the temperature not only affects the organic matter mineralisation (Rey and Jarvis, 2006) but also the distribution range of plant species, which in turn produce a more or less decomposable organic matter. Indeed, the higher C stock amount in the litter in the conifer study areas (empty triangles) with respect to what was observed for the broadleaves (fill squared) shown in Fig. 32C and 32D are due to:

1. The lower temperature conditions which characterise the distribution range of these conifer stands;
2. The higher C/N ratio of needle-like leaves; and
3. The ability of the gymnosperms to produce allelochemical compounds (phenolic substances) which reduce the decomposition rate of their decaying tissues (Singh *et al.*, 1999).

10.5. Living biomass, dead organic matter and Ecosystem C stocks

The results summarised in Tab. 19 showed that the living biomass (aboveground and belowground) varies substantially among the three stages considered. Such a result was not unexpected and it is in accordance with several studies on this topic (see Thuille *et al.*, 2000; Thuille and Schulze, 2006; Alberti *et al.*, 2008; Risch *et al.*, 2008; Fonseca *et al.*, 2011; Hiltbrunner *et al.*, 2013; Guidi *et al.*, 2014a).

According to Piussi (2000) and Alberti *et al.* (2008), the biomass increment along a successional process follows a logistic function, i.e. it manifests an initially slow increase, which speeds up, growing significantly steeper, and then slows down again before reaching an approximately stable value in the oldest phases of the process. The reason for slowing in growth after an intermediate peak is the emergence of negative feedback processes. Among them, the most common are the competition for the limited resources and the stresses. In addition, the living biomass increment rates do not only vary on the basis of the stand age, but also on the basis of abiotic and biotic parameters that each forest stand is subject to, especially climate, soil fertility and plant species composition.

The results obtained for the living biomass were similar to those described by Pinno and Wilson (2011), where only three stages were observed and the age of the forest stands was not known. Although here, like for Pinno and Wilson (2011), only three stages are considered, the forest stages were at least guaranteed to be older than the oldest airborne photograph collected to arrange the chronosequence in each study area. Given the larger number of time points in their studies, Thuille *et al.* (2000), Thuille and Schulze (2006) and Alberti *et al.* (2008) were able to describe more carefully how the aboveground biomass changes over time in Alpine environments, and found that the forest has reached the biomass increasing rate plateau around 60-80 years since the start of the secondary succession (or afforestation). Therefore, given that the only age information available was that the forest stages in all chronosequences had at least 60 years, it was not possible to prove beyond any reasonable doubt that the forest biomass had reached a steady state. However, it is reasonable to assume that the forest stages observed had at least occurred after the peak of maximum biomass increasing rate, and that therefore were not too far off the stable biomass final equilibrium state.

The belowground biomass is a C pool that is more difficult to measure because available direct methods are usually expensive and time consuming. In addition, standardised methods to estimate root biomass are much less common in the literature than those used for the aboveground biomass (Cairns *et al.*, 1997). Therefore, this pool is generally not considered in similar studies that focus on estimating the ecosystem C stocks. Here, the belowground biomass was estimated approximately by applying the root to shoot ratio as suggested by Moganay *et al.* (2006) and as also adopted by the IPCC (2006). It is important to notice that the ratio between aboveground and belowground that resulted from the work of Pinno and Wilson (2011) was significantly higher than that used in this work. However, it refers to a region with precipitations no larger than 380 mm yr⁻¹, where it is arguable that plants might have invested more in the production of root biomass in order to absorb water from deeper soil levels. This potential explanation for the strong difference in the root to shoot ratio between this study and that of Pinno and Wilson (2011) was compatible with the fact that, even if Cairns *et al.* (1997) did not find any statistical correlation between the root to shoot ratio and climatic

variables, these variables – in addition to the plant species – were in some way considered in the vegetation types listed by the IPCC (2006).

Considering the necromass (litter, fine woody debris and coarse woody debris), the trend observed followed what was described for the aboveground biomass. The differences among the study areas were marked, especially when considering the litter amount and the coarse woody debris. Generally, the study areas in the Alps ridge were characterised by a higher litter C pool with respect to those of the Apennines, but the coarse woody debris C pool did not seem to vary following a precise rule and it was probably linked to the actual age of the forest stand and to its management. An increase in the necromass C pool along the secondary succession was not unexpected, as it was also described Alberti *et al.* (2008). The same trend was also described in other studies on the Alps (see Thuille *et al.*, 2000; Thuille and Schulze, 2006; Risch *et al.*, 2008 and Hiltbrunner *et al.*, 2013), although in these papers only the litter and the organic layer were considered. It was challenging to compare the results obtained in this work with those of others because of the lack of information concerning the actual age of the forest stages, the difference in plant species composition and other variables that can affect both the litter and dead wood accumulation, as for example the slope steepness and forest management. However, the mean value estimated for the necromass C pool of all the forest stages is of 11.53 MgC ha⁻¹, which is comparable to the 10.7 MgC ha⁻¹ estimated by Alberti *et al.* (2008) for a 75-year-old forest stage studied in the Eastern Prealps of Italy.

The sum of the C stock stored in the living biomass (aboveground and belowground), in the dead organic matter (litter and dead wood) and in the soil was considered as the ecosystem C stock (IPCC, 2003). As observed also by Thuille and Schulze (2006), the relative contribution of the biomass increases along the secondary succession in all the study areas considered. More specifically, the living biomass acted as a significant C sink mostly in the last part of the process, while in the initial stages its contribution was limited. The same observation can be made concerning the dead organic matter. Similar results were observed by Thuille *et al.* (2000), Risch *et al.* (2008), Alberti *et al.* (2008) and Hiltbrunner *et al.* (2013) considering only the aboveground biomass. Unlike the results of Thuille and Schulze (2006), the results of the present study showed a marked contribution of the SOC pool in all the stages in each study areas. Indeed, while Thuille and Schulze (2006) observed a percentage of the SOC in the total ecosystem C of nearly 20% in the oldest forest stages, Fig. 33 showed that it never decreased below the 40% threshold in this work. This was due to both the generally high SOC content of the areas studied here, and to the deeper soil profile considered for the SOC stock estimation with respect to what investigated by the cited authors.

Although Jackson *et al.* (2002) suggested the possible loss of soil organic carbon from the soil could be substantial enough to offset the increase in plant biomass, all the study areas considered in the present work showed an increase in the whole ecosystem C stock. The main reason was that all the investigated woody encroachments (with the exception of the Vastogirardi study area) did not even show a reduction of the SOC stock along the process. In addition, even if SOC stock losses were detected, the work of Thuille *et al.* (2000), Thuille and Schulze (2006), Risch *et al.* (2008), Alberti *et al.* (2008) and Hiltbrunner *et al.* (2013) showed that even in the case of SOC stock decrease over time, the climax vegetation to which the woody encroachment tend in the Alps ridge – i.e. a forest – is significantly more able to store C with respect to the shrub / woodland communities studied by Jackson *et al.* (2002) in the South-Western part of the USA territory. Therefore, it is possible to conclude that in the Italian Peninsula, the land-use change from pastures / grasslands to forest acts as a sink of organic carbon. For this reason, the process of woody encroachment could represent an important strategy to reduce the amount of CO₂ in the atmosphere.

10.6. Soil biochemical analyses

It is widely accepted that a LUC, as the woody encroachment is, can affect the microbial community composition and, therefore, its activity in the soil. Indeed, the variation in the plant community composition, with an increase of woody plant species, leads to an alteration in the quantity and quality of the organic matter that reach the soil system (Wedin and Tillman, 1990; Trasar-Cepeda *et al.*, 2008). Fig. 44 highlights how the major part of the microbial biomass and its activity is confined to the 0-5 cm soil layer. The same conclusion was reached by Rey *et al.* (2008), who observed that 50-70% of the CO₂ mineralization, which is strictly connected to the microbial activity, is confined to the upper 5 cm of soil. For this reason, the following part of the discussion will focus only on the upper part of the soil profile.

The results of Figs. 37-43 shown that, in general, both the microbial biomass (C_{mic}) and soil enzymatic activities linked to the C and N cycle are lower in the forest compared to the pasture. This is probably due to the synergic effect of two factors. The first one is the lower soil temperature of the forest stages compared to those of the pastures, especially in the summer and vegetative period. Indeed, according to Smith and Johnson (2004), an increasingly closer tree plant cover can reduce the solar radiation reaching the ground. A reduction in the soil temperature, in turn, determines a consequent reduction in soil organic matter mineralisation by the heterotrophic respiration (Smith and Johnson, 2004; Yannarell *et al.*, 2014), and therefore in the microbial activity.

The second factor potentially at play is that, as it was described by Chabrerie *et al.* (2003) and Trasar-Cepeda *et al.* (2008), the woody plant species produce an higher amount of lower quality organic matter substrate (mainly aboveground litterfall and roots litter) that reach the soil (Wedin and Tillman, 1990). Indeed, in the forests, this organic matter is characterised by a higher amount of chemically complex and recalcitrant molecules as, for example, lignin (Miles, 1985; Condrón and Newman, 1998; Lucas-Borja *et al.*, 2010).

The observed lower microbial biomass and enzymatic activities in forest-dominated stages compared to the pasture stages could be, therefore, explained by a reduced efficiency of microbial communities in using this lower-quality substrates for anabolic (e.g. production of biomass or complex molecules such as enzymes) processes (Cotrufo *et al.*, 2013).

Although this overall reduction between pasture and forest is observed in almost all the study areas, some differences can be pointed out concerning the transient dynamics of microbial biomass and enzyme activities. Indeed, an essentially progressive decrease along the woody encroachment is visible in the study areas characterised by a broadleaves forest vegetation (Figs. 39-43). On the contrary, in the conifers study areas (Figs. 37-38), an unexpected and marked increase in enzymatic activities (especially of those linked to the C cycle) in the intermediate stage (T2) is visible, even when microbial biomass is still progressively decreasing. Furthermore, a marked peak simultaneous to that of the enzymatic activity is also estimated in the aboveground functional-type diversity index of the conifer-dominated study areas (Tab. 20 and Fig. 36). Therefore, on the base of both the different trend observed in enzyme activities and in the aboveground functional-type diversity index of chronosequences with conifer- versus broadleaf-dominated forests, these two groups of study areas are considered separately in Fig. 45. The different behaviour observed here between conifer and broadleaf forests are not surprising: in fact, although not comparing conifers and broadleaves, Rutigliano *et al.* (2004) and Lucas-Borja *et al.* (2010) observed that the microbial community composition is affected by the plant species present, although they suggest the plant cover type could be even more important factor than the species themselves.

Fig. 45 shows the previous biochemical indices (C_{mic} and enzyme activities related to C and N cycles), but this time normalised on the unit of SOC (graphs 2-4 A and B). Again, simultaneous peaks are observed both for the enzymatic activities per unit of soil organic carbon and the aboveground functional type diversity index, with graphs 3 and 4 of Fig. 46 confirming the correlation between the two is statistically significant only in the conifer-dominated study areas. These results suggest that enzyme production and activity increase in the intermediate stage even when substrate quality, and hence substrate use efficiency (SUE), are expected to decrease due to increasing dominance of recalcitrant litter associated with woody shrubs and tree species in this intermediate stage. It could therefore be hypothesised that the unexpected increase in enzymatic activity is linked to the observed increase in functional-type diversity at this intermediate stage (Fig 45 A and B), resulting from the partial colonization of the grasslands by a diverse guild of shrubs and tree species. This increase in functional diversity is more pronounced in conifer-dominated chronosequences, where both shrubland and tree diversity peaked in this intermediate stages.

While there is a limited number of studies to support these observed links between aboveground diversity and belowground activity (Steinauer *et al.*, 2015), functional diversity has been historically linked to increasing exploration of niches and optimization of the productivity of ecosystems (Tilman *et al.*, 1997). Unfortunately, no data on productivity are available for this study. However, despite a decrease in SUE due to vegetation change, a stronger increase in ecosystem productivity could be a plausible explanation for this observed intermediate peak in soil activity. In fact, usually the intermediate stages of a secondary succession in mesic climate conditions are characterised by a higher level of net primary productivity compared to both the pasture / grassland and mature forest stages (Kimmins, 1987). Several studies have shown how plant diversity may strongly affect soil functioning: for example, Stephan *et al.* (2000), Zak *et al.* (2003) and Steinauer *et al.* (2015) observed that plant diversity can have a positive effect on microbial communities, with Stephan *et al.* (2000) additionally observing that the increase in the plant functional-type diversity can influence positively the overall microbial catabolic activity. However, all these studies were concerned with grassland environments and none of them focussed on the woody encroachment process. Nevertheless, the results shown in Fig 45 seem to confirm that these conclusions can be valid also when woody plant species are considered. A further support to this hypothesis comes from the work of García-Morote *et al.* (2012), who observed a high soil enzymatic activity under shrubs species in a patchy woody vegetation, and it could be argued that, although not measured directly here, the intermediate stage of a woody encroachment are also expected to display high spatial heterogeneity (Guidi *et al.*, 2014a).

However, although the considerations above and the discussed literature suggest how an increase plant diversity could explain an increase in the enzymatic activity, such a trend is typically associated also with an increase in microbial biomass. Surprisingly, though, this is not what observed in the present work, with the former showing a marked peak in the intermediate stage, while the latter gradually reducing over time. Therefore, the enzymatic activity values expressed per unit of microbial biomass are also characterised by a sharp peak in the intermediate stage (Fig. 45, graph 5A and B). This pattern was highly unexpected, all the more so when considering that a statistical increase in the C stock of the sum of soil (0-5 cm depth) and litter pools was not detected.

It is possible to speculate that the observed increase in enzymatic activity, with no increases in microbial biomass, could be associated with microbial population stress (Trasar-Cepeda *et al.*, 2008), and in particular that it could be a reaction of a "grassland-adapted" microbial community to the scattered encroachment of the first conifer plants, which are able to produce allelopathic substances (Singh *et al.*, 1999). The results are supported by the fact that a strong correlation

between the aboveground functional type diversity and the belowground activity was observed only for the study areas characterised by conifers forest stands (see Fig. 46).

The potential explanations suggested in this section, however, cannot be proved on the basis of the results obtained in this study. Although interesting, they remain speculative at this stage, especially given the number of studies on soil microbial and biochemical analyses linked to woody encroachment or secondary successions over pastures and grassland is extremely limited and usually confined to arid or semiarid ecosystems (see for example Liao and Button, 2008; Cable *et al.*, 2009; Hollister *et al.*, 2010; García-Morote *et al.*, 2012).

11. CONCLUSIONS AND FUTURE PERSPECTIVES

Strategies for mitigating the impact of climate change require a deeper understanding of the interactions between greenhouse gases circulation and biogeochemical cycles. Among these interactions, an important one is the impact of land-use change on greenhouse gases emissions and removals, which is in urgent need for a more accurate quantification in all countries, as requested both at the European and at international level, in documents such as:

- The 529/2013/UE decision “on accounting rules on greenhouse gas emissions and removals resulting from activities relating to land use, land-use change and forestry and on information concerning actions relating to those activities”;
- The EUR-Lex - 52008PC0017 entitled “Proposal for a Decision of the European Parliament and of the Council on the effort of Member States to reduce their greenhouse gas emissions to meet the Community’s greenhouse gas emission reduction commitments up to 2020”.
- The United Nations Framework Convention on Climate Change of Rio (1992)
- The articles 3.3, 3.4 and 7 of the Kyoto Protocol (1997, which entered in force in the 2005).

Among all land-use changes, this thesis was concerned in particular with secondary successions over abandoned pastures/grasslands, as the impact of such processes on terrestrial C and N pools are still incompletely understood and qualitatively and quantitatively contrasting results appear in the literature. Given its extension (7.64% in the Southern Europe between 1950 and 2010, and 1.07% in Italy between 1990 and 2008), a more refined estimation of the changes in C and N stocks along this process is needed. Identifying a secondary succession over abandoned pastures/grasslands is challenging, especially when other stages – in between the two extremes of pastures/grasslands and forest – need to be considered. Even if the chronosequence approach has several limitations in heterogeneous landscapes, this methodology can be a helpful tool in the investigation of the woody encroachment process.

On the basis of the consulted literature, this is the first study that estimated the ecosystem C stock changes along the woody encroachment in the Central and Southern Italian mainland territory. Moreover, among the Italian studies concerning secondary successions over pastures and grasslands, it is the first one that considered all terrestrial ecosystem C pools over a latitudinal (and climatic) gradient along the peninsula. In addition, differently from existing pair-site studies, the analyses of several intermediate stages along the process allowed to gain understanding of the temporal dynamics of the process.

The results confirmed that, in general, the soil is the main C pool at the ecosystem level. The changes in the SOC stock are not consistent among all study areas. First, the results suggest that the study areas in the Alps tend to accumulate C in the soil over the first 20-25 years and then tend to lose it. The SOC stock increase in the intermediate stages of the woody encroachment process is consistent among study areas, although not always statistically significant, and reaches, on average, a value 41% (range: 34 – 49%) larger than that of the previous pasture. The decrease in the second part of the process leads to SOC stocks in forests not significantly different from those of the previous pasture / grassland, with an average increment of only 7% (range: +0.5 – +16%). Therefore, at the end of the whole process, the soil does not act either as a C sink or as a C source.

Second, the results for the study areas in the Apennine ridge are different from those of the Alps. Indeed, in the central and Southern part of the Italian peninsula, the woody encroachment leads to an increase in the SOC stock also beyond the intermediate stage. The differences among the forest stages and the previous pastures / grasslands are substantial in all the study areas, with an average increment of +104% (range: 80 – 147%). The increment seems to be gradual, even if the SOC stock might experience a decrease in one of the intermediate stages of the chronosequence. Therefore, at the end of the whole process in the Apennine study areas, the soil does act as a C sink.

Third, according to the previous literature, climate appears to be a key factor responsible for different trends on SOC stock among study areas. Therefore, the grouping of study areas obtained here on the basis of similarities in SOC stock trends, which separated Alps from Apennines, should be attributed, rather than to the different orogenies, to the differences in the climatic conditions. Even if in the literature the mean annual precipitation is declared as the best climatic predictor, our results identify the temperature as the main factor. Moreover, the forest to pasture SOC stock ratios are statistically significantly correlated both to mean annual temperature and to the Mitrakos winter cold stress index. The higher the temperatures, the larger the organic C stock accumulating in the soil. Similar statistically significant correlations were pointed out when plotting the forest to pasture SOC stock differences against the same temperature parameters. No other statistically significant results were observed in correlating SOC stocks (either ratios or differences) on any of the most common aridity indices that consider, simultaneously, temperature and precipitation values.

In all study areas, high levels of N stock were estimated both in the topsoil and in the subsoil, even in the forest stages. The trends observed of the N stock were similar to those estimated for the SOC stock everywhere.

Unexpectedly, the deeper part of the soil (subsoil) is found to be a substantial pool, which in some cases can contain an organic C stock comparable to that of the topsoil. In addition, the changes in the subsoil organic C stocks are, in general, larger than those of the topsoil. However, to date, the quantifications requested by the IPCC are compulsory for the topsoil only. Similarly, the original form of the protocol used for this study, the one specifically designed by the EU Joint Research Centre to quantify the SOC stock change due to land-use change processes, also focuses only on the topsoil. This thesis suggests that, for an accurate estimation of the impact of secondary successions on terrestrial ecosystem C stocks, extending such requests to the subsoil is paramount.

As far as the living biomass and dead organic matter C pools are concerned, the results of this research confirmed that both C pools increase along the process in all the study areas considered. Furthermore, the living biomass C pool can reach values that are comparable or higher than those of the soil, although only in the forest-dominated stages.

Overall, summing all pools (SOC, above- and belowground living biomass, and dead organic matter) the whole ecosystem C stock increases along the entire process by +163% (range: 86 – 280%) – excluding Vastogirardi study area – or +147% (range: 57 – 280%) – including it – of the initial pasture value: therefore, the woody encroachment in Italy acts as a sink of C in each latitudinal and climatic condition.

An analysis of the soil microbiota was also carried out. Generally, both the microbial biomass and soil enzyme activity linked to the C and N cycles tend to decrease in the upper part of the mineral soil when transitioning from the pasture / grassland to the forest stages. Even if the response of the microbial community to the secondary succession have not received much attention in the previous literature, these results were expected and are linked to the reduction of the

litter and soil organic matter quality. However, an unexpected increase in the specific enzyme activity was observed in the intermediate stage of the succession, especially in the conifer-dominated study areas. This stage is generally characterised both by a high spatial heterogeneity and a high plant functional diversity. A statistically significant correlation among plant functional diversity and specific enzyme activities was observed, but only in the conifer-dominated chronosequences.

The microbial biomass per unit of carbon does not follow the same pattern, as no statistically significant change in the microbial biomass per unit of carbon was observed. The increase in the specific enzyme activity and without significant change in the microbial biomass per unit of carbon could be associated with microbial population stress. However, it was not possible to provide a solid explanation to the trends observed in this study, because of the limited number of scientific work on this topic, especially those focused on the intermediate and transitional stages of the secondary succession process in temperate and alpine ecosystems. Therefore, the results of this work suggest that the transitional stages could be promising for biodiversity and ecosystem function research investigations.

A general and consistent result of this work is that in the intermediate stage of all chronosequences show an increase in C stock and surprisingly high levels of enzymatic activity. Although no clear explanation could be provided, it was observed that they occur in stage of high plant diversity and spatial heterogeneity. Previous studies suggest that an increase in biodiversity could have important effects on ecosystem properties such as the net primary productivity, but are limited to grassland areas. Therefore, despite the higher challenges involved in the study of shrub species, future work should focus on these shrub-rich transition phases, to better understand their ecosystem functions and biodiversity.

In conclusion, the results of this work highlight that the changes in C and N stocks along secondary successions over abandoned pastures / grasslands might depend on climate and the characteristics of the local climax vegetation. However, they consistently stress the importance of the subsoil as a substantial and dynamic C and N pool. Furthermore, the results point out that, between the stable conditions of both pasture/grassland and forest, there is a transient phase with interesting dynamics in terms of SOC and N stocks, living biomass, dead organic carbon, microbial biomass and enzymatic activity, which is as yet poorly understood. This work is of particular interest not only for future scientific research, but also for political and decision-making future planning at the Italian national level. Indeed, these results should be taken into account when refining the quantification of the land-use change C sink / source effects, as requested both at the European and international level.

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