

Soil processes related to organic matter modifications following Douglas-fir mature reforestation

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Received: 13 June 2014 / Revised: 30 September 2014 / Accepted: 3 October 2014 / Published online: 15 October 2014
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Abstract The aim of the study was to analyse the pedological, chemical and biochemical properties of soil in order to assess the effect of plant cover species (chestnut, Douglas-fir and mixed vegetation) on soil processes. The selected area under Douglas-fir reforestation was homogeneous for climate, morphology and parent material. The study involved three soils: under chestnut forest (CS), Douglas-fir reforestation (DF) and the transition zone (T). A soil profile differentiation occurred after 50 years of Douglas-fir plantation. The thickness of soil horizons at the surface varied, and the OH horizon in T site was fourfold thicker than that in DF site. The A1 mineral horizon in T site was also thicker than that in DF site. The Munsell value of mineral horizons was significantly lower in DF than CS site. Morphological differences (thickness and colour) probably reflect soil organic matter quality changes: in DF higher values of humification index were found in surface layers (OF/OH or OH and A1) and lower in deep organo-mineral and transition horizons (A2 and BA or AB) with respect to CS site. Also, a slight increase of weathering process was observed under Douglas-fir reforestation. The morphological, chemical and biological properties of soil were effective to assess the impact

of various vegetation types on soil organic matter properties related to pedogenetic process. The various methodological approaches allow studying soil processes using a small–medium spatial scale sampling scheme.

Keywords Humification · Soil microbial activity · Spodicity index · Chestnut forest · Apennines

Introduction

The acquisition of precise soil data, representative of the entire survey area, is a critical issue for many study cases. The spatial analyses of the soil data are usually performed geographically to examine the nutrient (available K, Mg and P) and analyse the soil (Baxter et al. 2006; Oliver et al. 2006). Nevertheless, the influence of the geographically differentiated pedogenic factors on soil properties is usually studied along a transect stretching between the north and south or east and west zones of a vast area (Degórski 2003). In fact, soil surveys have traditionally used the representative pedon concept as a basis for site selection, determined from the evaluation of soil morphology and component landforms within mapping units (Burt et al. 2006). However, the variability of soil cover can be affected by the micro-habitat differentiation and expressed through the variability of thickness of the genetic horizons for individual pedon (Degórski 2003). It is known that plant cover is one of the pedogenetic factors which has a marked effect on soil organic matter properties over a short-term period and at a small-to-medium spatial scale (Vittori Antisari et al. 2010, 2011). For instance, Douglas-fir plantations generally have a very fast initial height growth, which usually reaches breast height during the first year after planting (2-year-old nursery seedlings). For this reason, Douglas-fir plantations provide a rapid increase of soil organic matter content, but differences in soil organic matter (SOM) composition and quality may also occur (Jaffrain et al. 2007). In particular, a

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significant reduction in the aromatic substance content of dissolved organic C was found in litter leachates of the Douglas-fir specie compared to that of beech plantations (Jaffrain et al. 2007). Significant differences in aromatic substance content were also found in capillary soil solutions from the two planted stands (Jaffrain et al. 2007). Moreover, Vittori Antisari et al. (2011) reported that the plant cover affects the soil profile development of Cambisols with different expressions of the Eutric qualifier (IUSS Working Group WRB 2007) thus changing SOM stability and soil pH. The soil organic matter quality of epipedon under coniferous species proved to have a more recalcitrant and hydrophobic composition than that of epipedon under beech. The Douglas-fir forest floor showed the highest relative nitrification and the largest contribution of nitrate to the anionic charge; therefore, Douglas-fir might induce a specific signature in terms of proton donors at forest level, which may potentially affect pedogenetic processes in the mineral horizon beneath (Trum et al. 2011). In this paper, an integrated approach is proposed in order to study soil processes related to organic matter changes which occurred at a small–medium spatial scale over a relatively short period of time (50 years) from Douglas-fir reforestation in the *Castanetum* zone. The Douglas-fir plantations in the Apennines were established in quite different environmental conditions, from 400 to 1000 m above the sea level, on sites geologically characterized by sandstone, siltstone and loam stone; the climate varies between sub-humid and humid Mediterranean conditions, with mild to cool winters and a more or less pronounced dry period in the summer. Almost all plantations were established in order to recover degraded agricultural and forest lands, because of the fast growth of Douglas-fir and the consequent increase of soil organic matter content along soil profile. However, there were no actual expectations concerning the changes of soil organic matter properties and the related soil processes. The extensive study can be effective to assess the impact of vegetation on soil organic matter properties related to pedogenic processes: morphological parameters and chemical and biochemical properties can be able to identify pedogenic processes such as soil organic matter translocation/accumulation and soil weathering. For this kind of study, the selected area was homogeneous for climate, morphology and parent material. The aim of the study was to analyse the pedological, chemical and biochemical properties of soil in order to assess the effect of plant cover species (chestnut, Douglas-fir and mixed vegetation) on organic matter content changes as a driving force for soil processes.

Material and methods

The study area

The study area is located in the Northern Apennines, Italy (Fig. 1). The area is at 925–935 m above sea level (a.s.l.),

northwest oriented (between 308° and 317° N) on a gentle slope of 11–15 % (Fig. 1). The parent material belongs to the sandstone facies of the Monghidoro formation, with variable grain size from coarse to fine (Valloni and Zuffa 1984). The siliciclastic component is dominant, and it is composed of quartz clasts (36 %), feldspars (orthoclase, plagioclase and microcline) (23 %) and micas (muscovite, biotite and chlorite) (8 %). The accessory phases mainly consist of opaque (pyrite framboïdale) (8 %) and transparent (20 %) minerals with a prevalence of garnet, staurolite, tourmaline and subordinated anatase, kyanite, zircon and monazite. The interstices among the clasts are filled with siltitic components and carbonate mineral (calcite) (5 %).

The annual mean temperature of the area is 10 °C, while the mean rainfall ranges between 1110 and 1460 mm. The soil temperature regime is *mesic*, and the soil moisture regime is *udic* according to the Newhall Simulation Model (Newhall 1972; Van Wambeke 2000). The soil regimes are typical of the Northern Apennines (Vittori Antisari et al. 2010) in the transition zone between the phytoclimatic unit of the *Castanetum* (500–900 m a.s.l.) and *Fagetum* (900–1200 m a.s.l.) (De Philippis 1951).

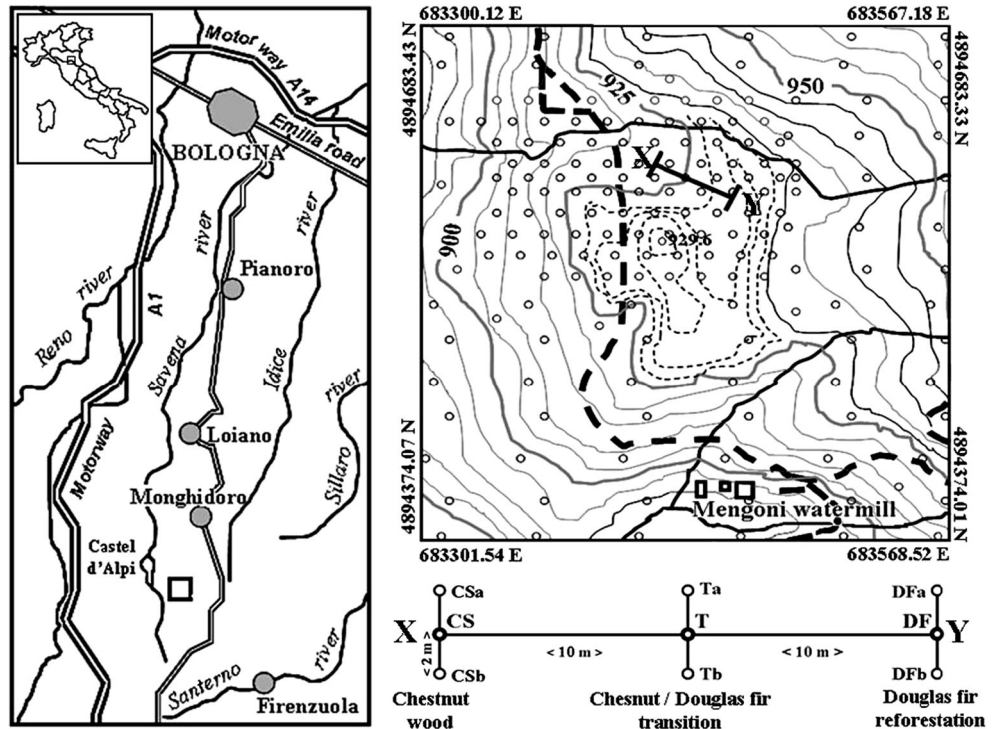
The study area is dominated by European chestnut (*Castanea sativa* Mill.) forests, and in 1963–1964, about 3.5 ha was reforested with Douglas-fir, characterized by high productivity and timber quality (Tarrant and Miller 1963; Whiteside et al. 1977; Page-Dumroese et al. 1990; Marshall and Curtis 2005). The age of the Douglas-fir trees was confirmed by collecting drill cores from six plants using a Pressler borer. The chestnut forests were replaced due to the attacks of pathogenic fungi (*Phytophthora cambivora* and *Phytophthora cinnamomi*) causing ink disease in the chestnut trees, as occurred at the beginning of the 1960s in a large area at a higher altitude than 600 m a.s.l. where the chestnut wood had suffered a sharp decline. The holes into which the seedlings were inserted were manually opened without using heavy mechanical equipment in order to minimize soil disturbance and compaction during the planting operation. The holes were opened at 2×2 m triangular spacing.

Three sites were clearly distinguished on the basis of the dominant tree cover and litter composition: a chestnut-dominated wood whose litter was composed of leaves, branches, burrs and other plant residues which clearly came from chestnut trees (CS site); a transitional zone on the border of the reforestation dominated by Douglas-fir trees, with litter composed of both chestnut and Douglas-fir residues (T site); and a Douglas-fir-reforested zone with a litter layer composed of needles, branches and other Douglas-fir residues (DF site).

Soil sampling

In the summertime, a preliminary soil investigation was performed in the study area by random pit opening and auger

Fig. 1 Cartographic localization of the survey area and soil sampling, properties scheme (left Regione Emilia Romagna technical map at scale 1:5000; geographic coordinates: WGS84 UTM-32T reference system)



drilling. Due to the limited size of the area and the effect of trees on the thickness of surface soil horizons, soil sampling was carried out according to the cluster sampling system rather than random sampling. Therefore, a transect crossing the whole study area was selected with northwest–southeast orientation and avoiding sites close to trees (Fig. 1). In each site, a soil profile was opened along the transect and taken as reference soil (CS, T and DF soil profiles). The location of each soil profile was georeferenced using GPS precision and WGS84 UTM-32T as a reference system. The three representative soil profiles were dug at 10-m intervals, measured with a long metric tape ruler, along a 20-m transect. Furthermore, in order to assess soil morphology variability, two additional sampling spots (A and B) were dug in each site 2 m from the representative soil profile along a 4-m secondary transect, orthogonal to the 20-m transect crossing the whole study area and parallel to the border of the Douglas-fir reforestation. Data from the survey were recorded on map at scale of 1:5000 (Fig. 1).

The representative soil profiles were described (Table 1), and samples were taken from each genetic horizon. In each spot, the thickness of the organic layers, the organo-mineral (A) and the transitional horizons (AB or BA) were recorded and sampled. Both the organic and A horizons were taken into account in order to study the processes related to the soil organic matter changes, and the sub-horizons were collected

since SOM characteristics may greatly influence the pedological processes (Rumpel and Kögel-Knabner 2011). The Munsell value colour of each mineral horizon of the profiles and spots was recorded, since the content of the organic matter of mineral horizons can be estimated from the value colour as established in the Munsell soil colour charts of a dry and/or moist soil (FAO 2006). The Munsell value is the lightness or darkness of colour and ranged from 1 (dark) to 8 (light). The estimation of the organic matter content is based on the assumption that the soil colour value is due to a mixture of dark-coloured organic substances and light-coloured minerals.

Soil morphology

Soil pedons are commonly differentiated into a succession of horizons based on observable differences in soil colour, texture, structure or other characteristics, which are essentially associated with or determined by the chemical composition of soil materials. In this study, the thickness and Munsell colour value of dry and moist soil in each genetic horizon were compared among the soil profiles.

Soil chemical properties

The soil samples were air-dried, milled and hand screened to 2 mm prior to analysis. The pH was determined

Table 1 Morphological description of investigated soil profiles. Codes according to Schoeneberger et al. (2012)

Profile	Horizon	Depth (cm)	Boundary ^a	Colour (Munsell)		Structure ^b	Texture ^c	Consistence	Roots ^d	Rock fragments ^e
				D/T	Dry					
CS	OL	1.5/1–0.5								
	OF/OH	0.5–0	A/S							
	A1	0–3	A/W	10YR 5/3	10YR 3/2	2/f/sbk	ls	(w)po/(w)so	3/vf	0
	A2	3–11	A/S	10YR 6/4	10YR 4/3	1/f/abk	sl	(w)ps/(w)so	2/f	0
	BA	11–15/17	A/W	10YR 6/4	10YR 6/6	1/m/abk	sl	(w)p/(w)s	1/m	0
	CB	15/17–30+	U	10YR 6/6	10YR 5/6	0	sl	(w)p/(w)s	1/f	FGR/3/2
T	OL	6/4.5–4/3.5								
	OF	4/3.5–3/1								
	OH	3/1–0	A/S							
	A1	0–2/4	A/W	10YR 4/3	10YR 3/3	2/f/sbk	ls	(w)po/(w)so	3/m	0
	A2	2/4–12	C/S	10YR 5/3	10YR 5/4	3/f/abk	ls	(w)po/(w)so	2/m	FGR/3/1
	AB	12–32	C/S	10YR 6/4	10YR 4/3	3/f/abk	sl	(w)p/(w)ss	1/m	FGR/2/1
	Bw	32–60	C/S	10YR 6/4	10YR 5/4	3/m/abk	sl	(w)ps/(w)so	1/f	MGR/3/1
	CB	60–70+	U	10YR 7/4	10YR 5/4	0	sl	(w)p/(w)ss	1/f	MGR/5/1
DF	OL	3.5–1.5								
	OF	1.5–0.5								
	OH	0.5–0	A/S							
	A1	0–1/1.5	A/W	10YR 4/3	10YR 3/3	1/f/gr	ls/sl	(w)po/(w)so	3/f	0
	A2	1/1.5–16/18	C/S	10YR 4/4	10YR 3/3	2/m/abk	sl	(w)ps/(w)so	2/m	0
	AB	16/18–36	C/S	10YR 5/4	10YR 3/3	2/m/abk	sl	(w)p/(w)so	1/f	MGR/3/1
	Bw	36–80	C/S	10YR 5/4	10YR 4/4	1/m/abk	sl	(w)ps/(w)ss	0	MGR/3/1
	CB	80–100+	U	10YR 6/4	10YR 4/4	0	sl	(w)ps/(w)ss	0	MGR/7/1

^a Horizon boundary: *D* distinctness (*A* abrupt, *C* clear, *G* gradual, *D* diffuse) and *T* topography (*S* smooth, *W* wavy, *I* irregular, *U* unknown)

^b *G* grade (0=structureless, 1=weak, 2=moderate, 3=strong); *S* size (*vf* very fine, *f* fine, *m* medium, *co* coarse); *T* type (*gr* granular, *abk* angular blocky, *sbk* subangular blocky, *sg* single grain)

^c Field estimation: *s* sand, *ls* loamy sand, *l* loam, *sil* silt loam, *sl* sandy loam; consistence: *P* plasticity [(w)po=non-plastic, (w)ps=slightly plastic, (w)p=moderately plastic]; *S* stickiness [(w)so=non-sticky, (w)ss=slightly sticky, (w)s=moderately sticky]

^d *Q* quantity (0=very few, 1=few, 2=common, 3=many); *S* size (*vf* very fine, *f* fine, *m* medium, *co* coarse)

^e *S* size (*FGR* fine gravely, *MGR* medium gravely, *CGR* coarse gravely, *VGR* very gravely, *XGR* extremely gravely); *V*% fragment content percent by volume; *R* roundness (1=angular, 2=subangular, 3=subrounded)

potentiometrically in a 1:2.5 (*w/v*) soil-deionised water suspension for mineral or organic horizons (Van Reeuwijk 2002). The total organic C (TOC) and total N (TN) contents were measured by dry combustion (EA-1110 Thermo Scientific Lab). The exchangeable cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Ametek, Arcos Spectro) after exchange with 1 M NH_4^+ acetate at pH 7 (Summer and Miller 1996). The effective cation exchangeable capacity (ECEC) was calculated by the sum of the exchangeable cations and the exchange acidity by mixing soil with 1 M KCl (Van Reeuwijk 2002). Amorphous iron oxide (Feo) and aluminium oxide (Alo) forms were estimated by extraction with acid ammonium oxalate (Schwertmann 1964), and Fe and Al in the extracts were measured by ICP-OES. The amount of amorphous Al and Fe oxides allowed to calculate the spodicity index (SI;

IUSS Working Group WRB 2007) as $\text{Alo}+1/2\text{Feo}$. The SI was calculated in order to evaluate if cheluviation/chilluviation of organo-metal complexes was enhanced by the presence of Douglas-fir. Pedogenic iron oxides (Fed) were estimated through extraction with Na-dithionite–citrate–bicarbonate (Mehra and Jackson 1960), and Fe of the extracts was analysed by ICP-OES.

The total extractable C (TEC) and the carbon of humic acid (CHA) as well as the carbon of fulvic acid (CFA) were determined by wet oxidation at 160 °C with 1/3 M $\text{K}_2\text{Cr}_2\text{O}_7$, according to Springer and Klee (1954). The TEC was extracted with a solution of 0.1 M NaOH and 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ at 65 °C for 24 h. The CHA fraction was separated from the CFA by precipitation at $\text{pH}<2$; then the CFA was separated from the non-humified organic material by chromatography with solid resin of polyvinylpyrrolidone (PVP) according to

Ciavatta et al. (1990). The total humification index (HU) was calculated using the following equation: $HU\% = [(TOC - TEC) + (CHA + CFA)] \cdot 100 / TOC$ (Dell'Abate et al. 2002).

Microbial indices and enzyme activities

Microbial biomass C (C_{mic}) was measured by the fumigation–extraction method (Vance et al. 1987) carried out on soil samples previously conditioned by incubation for 1 week at 60 % water holding capacity (WHC) at 20 °C. The organic C from fumigated and unfumigated samples extracted with 0.5 M K_2SO_4 was measured using a TOC analyser (TOC-VCPN, Shimadzu). K_2SO_4 -extractable C from unfumigated soil samples was used to estimate the soluble C fraction (Badalucco et al. 1992). The respiration of soil microflora was determined using the slightly modified Isermeyer method (1952). Soil samples (10 g) were conditioned at 60 % of the WHC and pre-incubated for 3 days at 4 °C; then each sample was placed in an hermetically sealed container (500 ml) containing a beaker with 4 ml 0.5 M NaOH to trap the evolved CO_2 ; the basal respiration (BR) of each soil sample ($\mu g C-CO_2 kg^{-1} soil h^{-1}$) was computed as the average of values measured during the 28-day incubation period at 1, 3, 7, 10, 14, 21 and 28 days. The basal respiration was expressed per unit of microbial biomass C, and the microbial biomass C was expressed per unit of total organic C in order to calculate the metabolic quotient (qCO_2) and the microbial quotient (the percentage of organic C present as microbial biomass C; C_{mic}/TOC), respectively. The metabolic and microbial quotients were calculated as follows (Dilly and Munch 1998; Anderson and Domsch 1989):

$$qCO_2 = (\mu g C-CO_{2BR} h^{-1} \times \mu g \text{ biomass } C^{-1}) \cdot 10^3$$

$$C_{mic}/TOC = \mu g \text{ of biomass } C \mu g \cdot \text{Total organic } C^{-1} \cdot 100$$

The activity of enzymes involved in the C cycle was measured according to Marx et al. (2001) and Vepsäläinen et al. (2001) using fluorogenic methylumbelliferyl (MUF) substrates. β -Cellulohydrolase (EC 3.2.1.91), α -glucosidase (EC 3.2.1.21), β -glucosidase (EC 3.2.1.20) and xylosidase (EC 3.2.2.27) activities were determined using 4-MUF- β -D-cellobioside, 4-MUF- β -D-glucoside, 4-MUF- β -D-glucoside and 4-MUF-7- β -D-xyloside as substrates, respectively. A moist sample (equivalent weight to 1 g oven-dried material) was weighed inside a sterile jar, and 50 ml of 0.5 M Na acetate buffer at pH 5.5 was added. A homogenous suspension was obtained by homogenising with the Ultra-Turrax

at 9600 rev min^{-1} for 3 min. Aliquots of 100 μl were withdrawn and dispensed into a 96-well microplate (3 analytical replicates $sample^{-1} substrate^{-1}$). Finally, 100 μl of 1 mM substrate solution was added thus obtaining a final substrate concentration of 500 μM . Fluorescence (excitation 360 nm; emission 450 nm) was measured after 0, 30, 60, 120 and 180 min at 30 °C with an automated fluorimetric plate reader (Fluoroskan Ascent).

The enzyme-specific activity (per unit of TOC) was calculated in order to maintain the amount of organic matter as an internal control (Trasar-Cepeda et al. 2008).

Statistical analysis

Differences among soils in morphological characteristics and organic C pools of horizons were evaluated by one-way ANOVA. Before analysis, the homogeneity of variances was checked using Levene's test. Discriminant function analysis (DFA) was performed using organic C pools and SI as grouping variables. Discriminatory functions were derived, and the results of DFA were graphically presented. The statistical significance of the discriminant function was checked with Wilk's lambda test. The standardized canonical discriminant coefficient (SCDC) was used to rank the importance of each variable. All data treatments were carried out using SPSS software package (SPSS Inc., Chicago, IL).

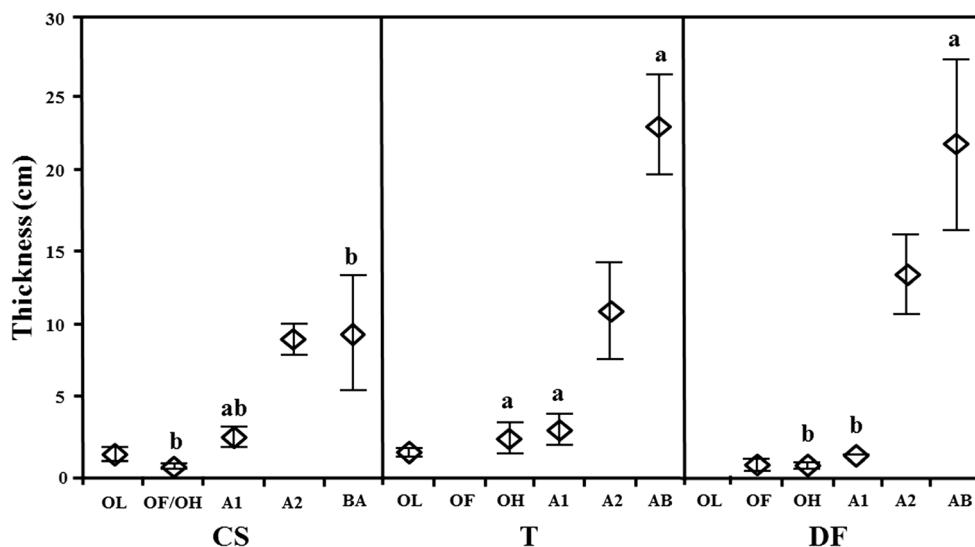
Results

Morphological characteristics of the soil profiles

The preliminary survey showed that there were no differences in physiographic variables or soil horizon sequence (i.e. soil morphology) within each site (CS, T and DF); only the thickness of soil horizons at the surface varied, especially close to plants. Similarly in all sites, the soil texture of mineral horizons was loamy sand and sandy loam in the surface and deep horizons, respectively (Table 1). In fact, the organic horizon was moderately decomposed (OF/OH) below the litter (OL) in the CS site. The underlying organo-mineral horizon was differentiated in A1 and A2, and the deepest horizons were BA-CB. The soil profiles of T and DF sites were characterized by the OL-OF-OH organic horizon sequence, followed by both A1 and A2 organo-mineral and transitional AB horizons, and the Bw-CB sequence was found in depth (Table 1).

In all the sites, the OL and OF horizons were thin, 1.4 and 0.6 cm on average, and no differences were found in their thickness among the sites (Fig. 2). On the other hand, the thickness of the OH horizon in T site was fourfold thicker than

Fig. 2 Thickness of organic and mineral horizons in CS, T and DF sites based on the mean of three replicate soil pits (i.e. one representative soil profile and two additional sampling spots). Error bars are the standard deviations. The letters show the significant differences at p level <0.05 (Duncan’s test) between sites



that observed in DF site. The mineral A1 horizon in T site was also significantly thicker than that in DF site (2.8 and 1.2 cm, respectively). CS had an intermediate A1 thickness value (2.4 cm; Fig. 2). The transitional AB horizons in T and DF sites were significantly ($p < 0.05$) thicker than the BA in CS site (Fig. 2). As regards to the colour of mineral horizons, the Munsell value in dry conditions (Fig. 3(a)) varied from around 4 (dark grey) to 6 (grey), while in moist conditions (Fig. 3(b)), it varied from 3 (black grey) to 5 (grey). The Munsell values of soil horizons in DF site were generally lower than those in CS site. Particularly, the values of dry A2 and moist AB in DF site

were significantly lower than those observed in CS site (Fig. 3(a, b)).

Soil chemical properties

In the organic horizon pH value, total organic C (TOC), total N (TN) content and TOC/TN ratio were lower in CS than those in DF and T sites (Table 2). The pH values of the mineral soil were very similar among the sites, and the acidity level decreased along the profiles. Both TOC and TN contents in DF were higher than those found in CS and T sites. In all soil

Fig. 3 The Munsell colour value of dry (a) and moist (b) mineral horizons in CS, T and DF sites based on the mean of three replicate soil pits (i.e. one representative soil profile and two additional sampling spots). Error bars are the standard deviations. The letters show the significant differences at p level <0.05 (Duncan’s test) between sites

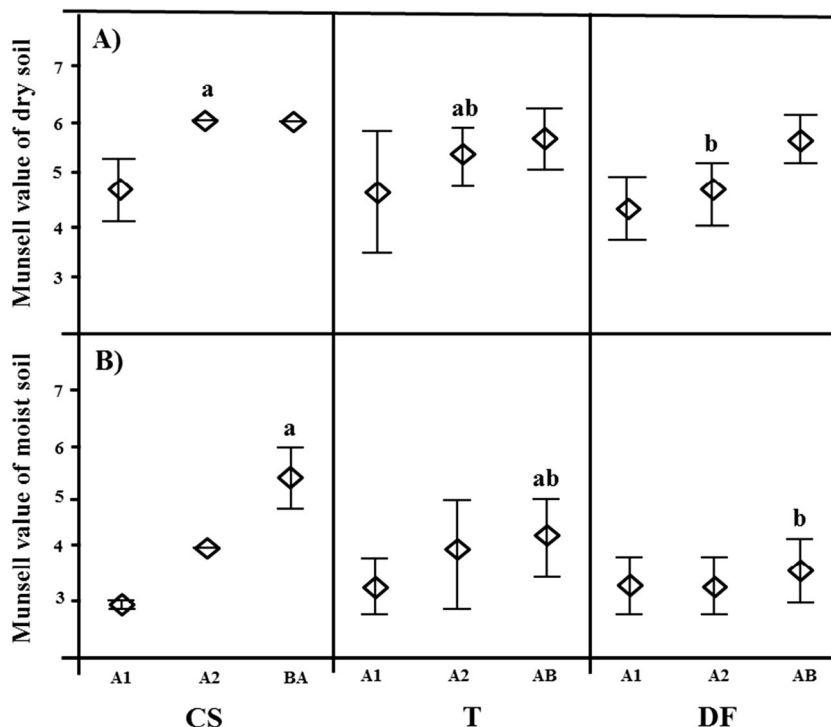


Table 2 Selected chemical properties of soils based on the mean of three replicate soil pits (only one for Bw and CB horizons)

Site	Horizon	Number	pH	(H ₂ O)	TOC (g kg ⁻¹)		TN (g kg ⁻¹)		TOC/TN		BS (%)		Feo (g kg ⁻¹)		Fed (g kg ⁻¹)		SI (%)	
CS	OL	3	4.7	<i>0.1</i>	438.5	<i>5.7</i>	13.5	<i>1.5</i>	32	<i>4</i>								
	OF/OH	3	5.2	<i>0.1</i>	214.3	<i>66.0</i>	11.1	<i>2.5</i>	19	<i>1</i>								
	A1	3	4.6	<i>0.2</i>	59.8	<i>2.8</i>	4.0	<i>0.3</i>	15	<i>1</i>	69.2	<i>3.0</i>	2.2	<i>0.2</i>	6.5	<i>0.5</i>	0.27	<i>0.02</i>
	A2	3	4.9	<i>0.2</i>	16.0	<i>1.9</i>	1.7	<i>0.3</i>	9	<i>1</i>	59.6	<i>2.6</i>	2.6	<i>0.3</i>	7.1	<i>0.3</i>	0.32	<i>0.03</i>
	BA	3	5.0	<i>0.1</i>	8.1	<i>2.4</i>	1.0	<i>0.2</i>	8	<i>3</i>	59.2	<i>3.3</i>	2.5	<i>0.2</i>	7.1	<i>0.3</i>	0.30	<i>0.02</i>
	CB	1	5.2		2.1		0.5		4		82.6		1.7		8.5		0.19	
T	OL	3	4.9	<i>0.1</i>	467.3	<i>8.0</i>	12.2	<i>0.1</i>	38	<i>1</i>								
	OF	3	5.9	<i>0.1</i>	399.5	<i>18.5</i>	15.7	<i>1.2</i>	25	<i>1</i>								
	OH	3	5.5	<i>0.2</i>	315.5	<i>26.7</i>	15.7	<i>1.8</i>	20	<i>1</i>								
	A1	3	4.4	<i>0.1</i>	53.7	<i>22.9</i>	3.7	<i>1.4</i>	15	<i>3</i>	64.4	<i>2.2</i>	2.4	<i>0.1</i>	6.8	<i>0.2</i>	0.31	<i>0.01</i>
	A2	3	4.8	<i>0.1</i>	18.0	<i>3.4</i>	1.8	<i>0.2</i>	10	<i>2</i>	57.7	<i>2.7</i>	2.6	<i>0.2</i>	7.7	<i>0.3</i>	0.37	<i>0.02</i>
	AB	3	4.8	<i>0.2</i>	15.7	<i>3.3</i>	1.4	<i>0.1</i>	11	<i>1</i>	53.8	<i>1.1</i>	2.8	<i>0.2</i>	8.3	<i>0.3</i>	0.40	<i>0.03</i>
	Bw	1	4.8		7.1		0.9		8		53.5		3.2		8.2		0.42	
	CB	1	4.8		4.4		0.6		7		55.3		1.7		5.9		0.24	
DF	OL	3	5.0	<i>0.1</i>	471.8	<i>13.2</i>	11.4	<i>1.0</i>	42	<i>4</i>								
	OF	3	6.0	<i>0.1</i>	346.7	<i>31.9</i>	14.4	<i>0.6</i>	24	<i>2</i>								
	OH	3	5.7	<i>0.2</i>	246.4	<i>5.1</i>	11.8	<i>0.4</i>	21	<i>1</i>								
	A1	3	4.8	<i>0.4</i>	62.4	<i>10.0</i>	4.2	<i>0.1</i>	15	<i>2</i>	68.6	<i>4.0</i>	2.7	<i>0.2</i>	9.2	<i>0.9</i>	0.37	<i>0.04</i>
	A2	3	5.0	<i>0.4</i>	26.2	<i>0.7</i>	2.7	<i>0.1</i>	10	<i>1</i>	59.7	<i>0.5</i>	2.9	<i>0.1</i>	10.0	<i>0.4</i>	0.43	<i>0.04</i>
	AB	3	5.0	<i>0.1</i>	16.6	<i>2.5</i>	1.7	<i>0.3</i>	10	<i>1</i>	59.2	<i>2.1</i>	3.0	<i>0.3</i>	9.1	<i>0.4</i>	0.42	<i>0.03</i>
	Bw	1	5.1		10.2		1.2		8		55.6		3.2		9.2		0.42	
	CB	1	5.3		6.4		0.7		9		56.0		3.0		8.2		0.34	

Numbers in italics showed the standard deviation. Values are determined as a mean of a single sample

TOC total organic C, TN total N, BS base saturation calculated as Σ exchangeable bases/effective cation exchange capacity, Feo oxalate-extractable Fe, Fed dithionite-extractable Fe, SI spodicity index calculated as (oxalate-extractable Al%+0.5·oxalate-extractable Fe%); IUSS Working Group WRB 2007)

profiles under study, the degree of base saturation (BS) was higher than 50 %, ranging from 53.5 to 69.2 %. A higher amount of pedogenic Fe oxides, both amorphous (Feo) and well-crystalline (Fed), was found in DF and T soils rather than in CS, and the vertical distribution of Feo in DF and T soils progressively increased from A1 to Bw horizons (Table 2). This difference in the amorphous oxides was also highlighted by the high SI value under Douglas-fir plantation (Table 2).

Soil organic matter quality and specific enzyme activity

The percentage of extractable C to total organic C (TEC/TOC) varied from 59 to 76 % with the exception of the value obtained for the A2 and AB horizons in DF site which reached 94 % (Fig. 4(a)). The trend of total humification index (HU) varied among sites. CS showed a different level of humification compared to DF site; in particular, the HU value was higher in the surface layers (OF/OH or OH) and transition horizons (BA or AB) (Fig. 4(b)). No statistical differences were observed in A1 horizons among the sites, while the HU index for A2

in T was higher than that found in CS and DF. A general increase of K₂SO₄-extractable C, expressed as a percentage to total organic C, was found along the soil profiles in all sites (Fig. 4(c)). The microbial quotient (C_{mic}/TOC) that is the percentage of TOC present as C_{mic} decreased along CS and T soil profiles, while an unclear trend was observed in DF. This percentage was significantly higher in surface layers (OH and OF/OH) in CS and T than in DF site. On the other hand, the highest value was registered in DF soil profile in the deepest horizon (Fig. 4(d)). The metabolic quotient (qCO_2) was highly variable along soil depth (Fig. 5(a)). The highest value was found in BA/AB horizons for CS and T (15 and 19 $\mu g C-CO_2 mg^{-1} C_{mic} h^{-1}$, respectively), and in A2 horizon for DF (9.3 $\mu g C-CO_2 mg^{-1} C_{mic} h^{-1}$), even if no statistical differences were observed with respect to the other sites. The qCO_2 was significantly higher in organic layer in DF than CS and T. Conversely, in A1 horizon, this quotient was lower in DF than CS and T sites.

The activity of enzymes involved in the C cycle, expressed as a specific activity (per unit of TOC), was significantly higher in CS than in DF; soil at T site

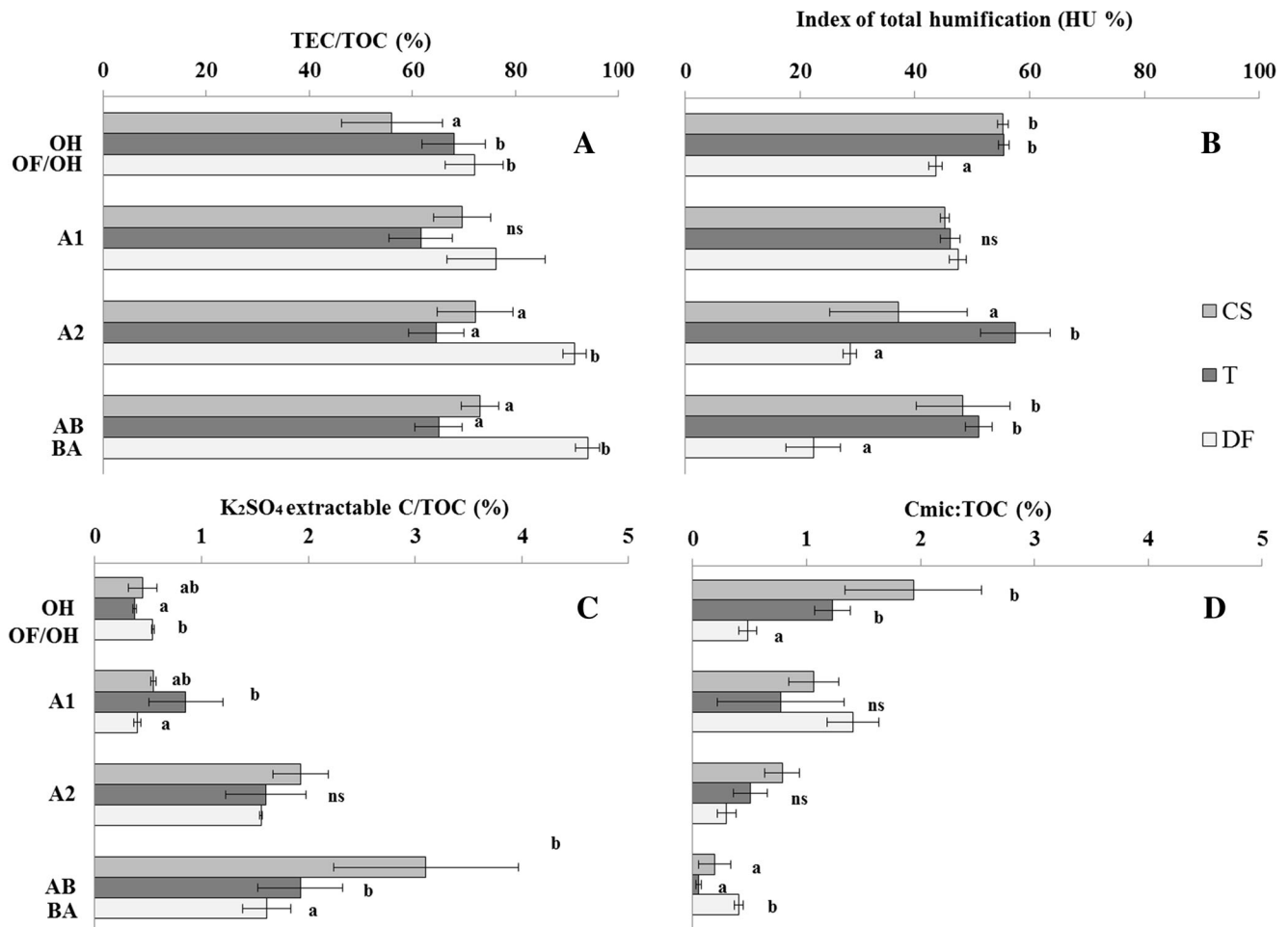


Fig. 4 Soil C pools: percentage of total extractable to organic C (a), total humification index (HU) (b), percentage of K₂SO₄ extractable to total organic C (c) and microbial quotient (d) along the soil profiles in chestnut (CS), transition zone (T) and Douglas-fir (DF), respectively. Error bars

are the standard deviation. Different letters indicate significant differences at p level <0.05 (Duncan's test) among sites (CS, T and DF); ns not significant

showed similar values to CS and DF in OH and A1/A2 horizons, respectively (Fig. 5(b)).

The discriminant function analysis was used in order to understand which variables related to soil organic C cycles and SI are discriminated among the three groups (CS, T and DF). The discriminant function analysis was determined with a linear combination of discriminating variables and mostly discriminated the CS, T and DF groups according to function 1, while no trend was found in vertical direction (function 2). Wilk's lambda test (0.10 with chi-square statistic test $p < 0.05$) showed a statistical significance of the discriminant function. The SCDC was used to rank the importance of each variable; humic acid (CHA) and spodic index (SI) showed the highest negative SCDC, while specific enzymatic activity of C cycle and K₂SO₄-extractable C showed positive SCDC. The canonical score plot, shown in Fig. 6, highlighted the points and multivariate means in the two dimensions that best distinguish the groups.

Discussion

The sampling scheme proposed in this study (three soil profiles at 10-m intervals along a 20-m transect) was a representative of organic matter content and quality changes, across sites under different tree covers (chestnut, CS, and Douglas-fir, DF, including the transition vegetation zone, T). Furthermore, the two additional sampling spots (A and B) at 2 m from the typical soil profile enabled us to take into account the variability of soil morphology at each site. According to this sampling scheme, the morphological and chemical properties of soils proved to be significantly different between sites under the different tree covers (chestnut, CS, vs. Douglas-fir, DF). According to the WRB (IUSS Working Group WRB 2007) and considering these characteristics, the CS soil was classified as Haplic Regosol (humic, eutric), while the DF and T soils were classified as Haplic Cambisols (humic, eutric). The variables linked to organic matter changes and the SI, considered as an index of cheluviation/

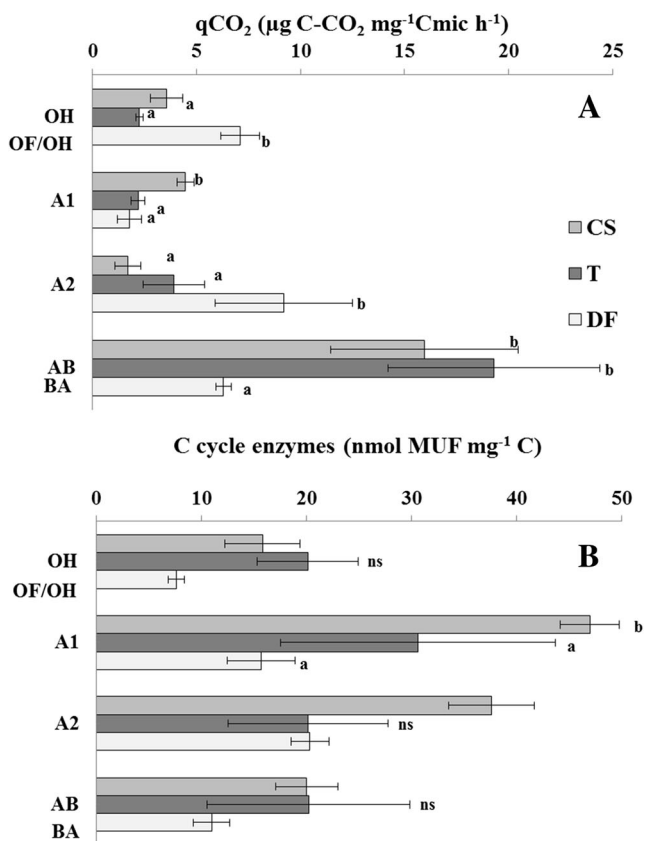


Fig. 5 Metabolic quotient (a) and specific activity of enzyme involved in carbon cycle (b) along the soil profiles in chestnut (CS), transition zone (T) and Douglas-fir (DF), respectively. Error bars are the standard deviation. Different letters indicate significant differences at *p* level <0.05 (Duncan’s test) among sites (CS, T and DF); *ns* not significant

chilluviation of organo-metal complexes, discriminate the different sites under the different tree covers. As regards to soil organic matter properties, substantial differences among CS, T and DF sites were found in terms of both total humification and amounts of C pools, such as soluble organic compounds (K₂SO₄ extractable-C) and microbial biomass

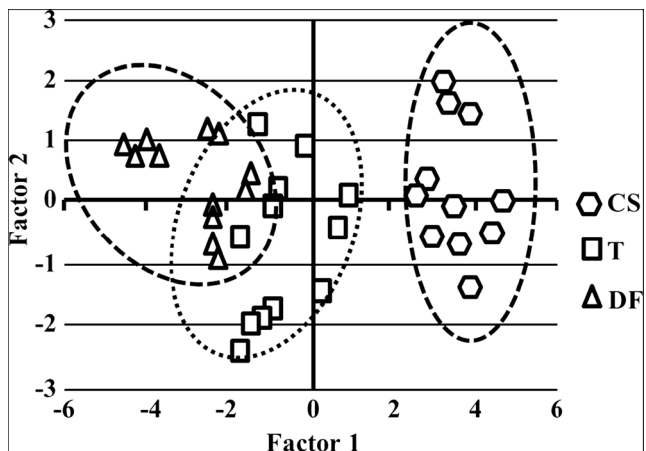


Fig. 6 Scatter plot of discriminant analysis scores

expressed as percentages of total organic C. No effect of Douglas-fir, with respect to the native broadleaf vegetation, was found in soil pH and humification degree of SOM (Andreux et al. 2002). On the other hand, Prietzel and Bachmann (2012) observed changes of organic matter turnover after the partial replacement of indigenous coniferous (*Picea abies* L. Karst.; *Pinus sylvestris* L.) with Douglas-fir (*Pseudotsuga menziesii* spp. *menziesii*) and European beech (*Fagus sylvatica* L.). It is known that the litter from various tree species has different levels of biodegradability. In fact, the decomposition rates of plant residues depend on its composition: (i) shoot and root residues usually have different decomposition rates; (ii) as shown by Shi et al. (2013), the number of plant species, from a single or mixture of plant species, can affect decomposition rate; and (iii) the litter content of specific compounds, such as phenylpropanoid, can affect the relative litter degradation by fungi (Tilston et al. 2013). Wittich (1961) and Kubartová et al. (2009) reported that litter decomposition rates increased in the following order: Scots pine<Norway spruce<Douglas-fir<European broadleaf. In our study, differences in soil organic matter properties under Douglas-fir were already observed as morphological features: the OH and A1 horizons were thinner in DF site, while the A2 and AB horizons had a lower colour value than those of CS and T sites. The SOM data confirmed changes under Douglas-fir. Indeed, differences were found in terms of C pool amounts and humification level. In particular, the percentage of extractable to total organic C was higher in both surface and deep horizons of DF site than those of CS site. The HU was lower in the surface layers (OH and A1 horizons) and higher in the deep soil (A2 and AB horizons) of DF than that of CS site. Moreover, SOM under Douglas-fir resulted less biodegradable than that under chestnut, since lower values of metabolic quotient qCO₂ and specific activity of enzymes involved in C cycle were found in the organo-mineral horizons. The qCO₂ (the respiration per unit of microbial biomass) can measure C use efficiency by soil microbial communities as well as the degree of substrate limitation for these communities (Wardle and Ghani 1995; Dilly and Munch 1998), while the specific activity of enzymes involved in C cycle may represent the potential hydrolytic activity per unit of soil organic compound (Marinari et al. 2013). According to the less available C forms under Douglas-fir, the labile C pool, such as soluble organic compound, was lower in the organo-mineral horizon of DF site than in that of CS site. According to Vittori Antisari et al. (2010), the soil organic matter under conifers was recalcitrant, while the weathering of soil appeared more intense than in broadleaves covered soil. The presence of Bw horizons and the increase of pedogenic Fe oxides along the soil profile of DF and T sites suggest a more expressed weathering process in these soils than in CS, also supported by the high value of the SI. Mineral weathering is deemed to be very slow and unlikely to change over the decades. Fifty years after

reforestation, we observed some mineral transformations that were, therefore, apparently striking. However, they actually implied weak chemical weathering (formation of Fe oxyhydroxides) and incipient cheluviation. In fact, under sub-alpine vegetation, much longer periods are normally required for the establishment of soil weathering than in Castanetum zone, but the development of Cambisols from Leptosols was observed after 120 years of soil evolution driven by vegetation (Egli et al. 2001, 2006). Mineralogical transformation may occur rather quickly in the presence of organic ligands (Kelly et al. 1998), and in situ experiments have demonstrated that over a period of 3 years, significant dissolution of feldspars occurs under field conditions (Nugent et al. 1998; Augusto et al. 2000).

Firstly, this study has clearly detected changes in SOM quality and in the weathering process over a 50-year period after replacing the chestnut wood and planting the Douglas-fir species. The second relevant result is that our integrated approach identified a complex set of processes in the soil located in the transition zone between the two types of plant cover. In fact, our results showed that the surface horizons (i.e. organic and A1 horizons) of T site were similar to those of CS, while the deeper horizons (i.e. A2 and AB) were more similar to those of DF site, regarding both their morphological and chemical properties. This may indicate that even under mature reforestation of Douglas-fir, the presence of chestnut residues influences the concentration of C pools and quality of SOM at least in the surface layers. Broadleaf litter fall is known to spread throughout the stands (Rothe and Binkley 2001); therefore, the differences between sites may be due to the direct effect of C input from leaf litter. However, chestnut leaf litter is mainly formed of low mobile organic components, and therefore, its effect may be attenuated along the soil profile. Besides the effect of leaf litter, the influence of roots should also be considered. Root turnover typically provides more than 30 % of organic matter inputs in coniferous stands (Godbold et al. 2003). Therefore, in the deeper horizons of T site, organic matter inputs from Douglas-fir were of primary importance in the soil development rather than those produced by chestnut.

Conclusions

In the Castanetum zone of Apennines, soil profile differentiation occurred after 50 years of Douglas-fir plantation, mainly due to differences in (i) organic matter deposition, (ii) humification process and (iii) translocation of soluble organic compounds along soil profile. Also, a slight increase of weathering process was observed under Douglas-fir reforestation. The plausible hypothesis is that different properties of organic layers are the main trigger of weathering under Douglas-fir mature reforestation.

The morphological, chemical and biological properties of soil can allow studying soil organic matter properties related to pedogenetic process. The various methodological approaches are active to study soil processes using a small-medium spatial scale sampling scheme.

Acknowledgments We thank Enrico Cappelloni commander of the Forest Service of Italian State, Monghidoro District (Northern Italy), and his staff for information and dendrometric measures relating to the survey site.

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