

Article

The Conversion of Abandoned Chestnut Forests to Managed Ones Does Not Affect the Soil Chemical Properties and Improves the Soil Microbial Biomass Activity

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Abstract: Recently, several hectares of abandoned chestnut forests (ACF) were recovered into chestnut stands for nut or timber production; however, the effects of such practice on soil mineral horizon properties are unknown. This work aimed to (1) identify the better chestnut forest management to maintain or to improve the soil properties during the ACF recovery, and (2) give an insight into the effect of unmanaged to managed forest conversion on soil properties, taking in consideration sweet chestnut (*Castanea sativa* Mill.) forest ecosystems. The investigation was conducted in an experimental chestnut (*Castanea sativa* Mill.) forest located in the northern part of the Apennine chain (Italy). We identified an ACF, a chestnut forest for wood production (WCF), and chestnut forests for nut production with a tree density of 98 and 120 plants ha⁻¹ (NCF_L and NCF_H, respectively). WCF, NCF_L and NCF_H stands are the result of the ACF recovery carried out in 2004. After 15 years since the ACF recovery, generally, the effects on the main soil chemical properties were negligible. Some differences occurred for the water-soluble organic carbon (WSOC) and microbial biomass and its activity. NCF_L showed the highest WSOC content in the uppermost soil horizon likely due to higher amount of roots which are source of labile organic compounds. The higher WSOC amount might explain the greatest amount of microbial biomass in the A horizon of NCF_L. Furthermore, the microbial biomass harboring in the A horizon of NCF_L has also shown both a better C use efficiency and a larger soil organic carbon immobilization in the microbial biomass itself. Our data would indicate that the ACF recovery into pure chestnut forests did not have negative impacts on soil chemical and biochemical properties, though chestnut stands for nut production with a low plant density are the most suitable ones.

Keywords: mountain soil; soil organic matter; soil microbial biomass; soil profiles; forest management

1. Introduction

In Europe, forest ecosystems cover more than one billion hectares [1]. Forests provide numerous services to humans such as the supply of fuel, raw materials and food, and play an important role for biodiversity protection, climate regulation, landscape preservation, etc. In this context, although the aboveground biomass is considered fundamental for life and landscape quality [2], soil plays a crucial role because, beyond to support the aboveground biomass functions, it preserves watersheds, hosts a wide variety of microorganisms and of meso- and macrofauna, regulates the biogeochemical cycling of nutrients, mitigate climate change, etc. [3]. Since soil is a key component of the forest

ecosystems and soil properties are highly influenced by the forest management systems [4], several research papers focused on the evaluation of the influence of management on forest soils. For example, wood harvesting reduces the amounts of macro nutrients as N, P, K, Ca and Mg [5] but it does not affect soil organic C [6,7]. Grüneberg et al. [8], in a study conducted in unmanaged and managed beech forests, found higher amount of soil labile organic C pool in the former than in the latter. Goldmann et al. [9], in a study carried out in managed and unmanaged beech forests and coniferous forests in southwest, central and northeast Germany, revealed the absence of differences in soil pH and C:N ratio. Wic Baena et al. [10], investigating maritime pine forest in central–eastern Spain, observed a higher amount of soil organic C and soil microbial biomass in unmanaged forests than in those subjected to thinning. Borges et al. [11], in north eastern Portugal, found out a higher soil labile organic C content and organic C stock in non–tillage chestnut orchards than in those characterized by conventional tillage.

Another common management practice in forest ecosystems is the conversion of mixed forests to pure ones and vice versa which is driven by economic and ecological reasons [12,13]. The conversion from one type of forest vegetation to another can affect the soil properties [14]. For example, after 40 years from the conversion of a natural mixed forest to a pure forest in China, Yang et al. [15] found lower amounts of soil organic C, including the most labile ones, in pure forests than in native ones. Hizal et al. [16] reported that, after around 30 years from the conversion of a native broadleaf forest to a pure coniferous plantation, the native forest had a higher organic matter and total N contents and lower amounts of exchangeable potassium and calcium compared to planted stands. Similarly, the conversion of evergreen broad-leaved forests to plantations in the subtropical area of Eastern China decreased the soil C and N contents as well as soil pH [17]. Conversely, the study conducted by Haghdoost et al. [18] on the conversion of degraded natural forest to planted stands in Iran highlighted an increase in organic C, total N and available P in the latter than in the former. Similar findings were observed in Northeast Brazil by da Silva et al. [19], who observed higher concentrations of soil organic matter and total P in regenerated than in native forests, while no differences occurred for soil microbial biomass and respiration.

Hence, the aforementioned literature would indicate how the human interventions in forest ecosystems have generally negative impacts on soil properties unless they have the aim of restoring degraded forests.

Sweet chestnut (*Castanea sativa* Mill.) forests represent an important landscape component in the European forest ecosystems covering an area of more than 2.5 million hectares [20]. Sweet chestnut cultivation has a long tradition in Europe; in fact, some evidence demonstrates chestnut tree spreading due to human activity since the IV century BC [21]. Sweet chestnuts' wide distribution all over Europe is mainly related to the multipurpose of the chestnut trees because they can be used for wood and food production and landscape conservation [22,23]. During the 20th century, many chestnut stands were abandoned due both to the spread of the Asian chestnut gall wasp and chestnut blight [24,25] and to the rural depopulation [26]. Because of the abandonment, these chestnut stands are generally invaded by those tree species that persisted on such lands before chestnut tree spreading by humans [27–29] and progressively develop the features of natural woodland. However, in recent decades, thanks to the increased prices of the chestnut nuts [30] and the increasing interest both in eco-friendly wood biomass-derived products [31] and chestnut timbers in the structural sector [32], old chestnut stands are being restored.

Given the possible extension of chestnut stands in the European forest ecosystems [33] and the very few researches carried out on soil chestnut forests, the aim of the present work was to give an insight into the effects of conversion from unmanaged to managed chestnut forest on soil properties. Furthermore, the present study aimed also to identify the best chestnut forest management method to maintain or to improve the soil quality during the restoration of abandoned chestnut forests. We speculated that the recovery of abandoned chestnut forests reduces the concentrations of both soil organic matter and major nutrients and negatively affects the soil microbial biomass and its activity.

Furthermore, we hypothesized that, among the managed chestnut forests, stands used for wood production purposes could affect the soil properties less negatively because of their higher tree density.

2. Materials and Methods

2.1. Study Area and Soil Sampling

The study area is the experimental chestnut forest located in Granaglione (Italy) in the northern part of the Apennine chain ($44^{\circ}08' \text{ N}$, $10^{\circ}57' \text{ E}$) at an altitude ranging from 650 to 750 m above sea level (Figure 1).

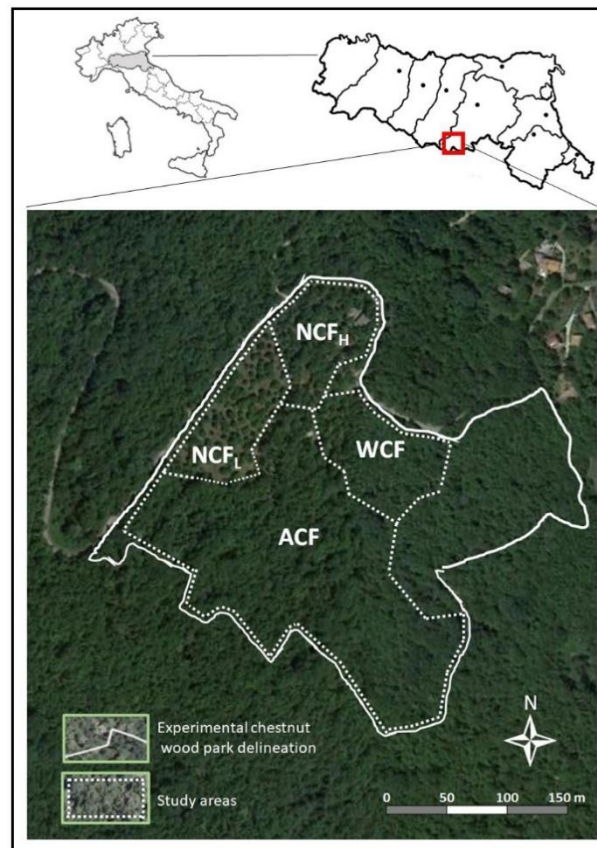


Figure 1. Location of study sites in the northern part of the Apennine chain, Italy. ACF: abandoned chestnut forest; chestnut forest for wood production (WCF); chestnut forest for nut production with a tree density of 98 plants ha^{-1} (NCF_L); a chestnut forest for nut production with a tree density of 120 plants ha^{-1} (NCF_H).

Owing to the cold, temperate climate, the rainfall in this area has an annual average of 905 mm with July as the driest month (42 mm) and November as the wettest one (113 mm). The mean annual air temperature is 12.2°C with July as the warmest month (22.0°C) and January as the coldest one (2.5°C). The parent material is sandstone, which belongs to the Miocene period, with feldspars, micas and quartz as the main minerals [34]. Within the study area, four study sites were identified: an abandoned chestnut forest with a tree density of about 370 plants ha^{-1} (ACF), a chestnut forest for wood production with a tree density of 151 plants ha^{-1} (WCF), a chestnut forest for nut production with a tree density of 98 plants ha^{-1} (NCF_L) and a chestnut forest for nut production with a tree density of 120 plants ha^{-1} (NCF_H). The ACF study site is composed of uneven-aged chestnut trees (73% of the total tree numbers), with ages up to 200 years, and *Abies alba* Mill., *Populus alba* L., *Populus nigra* L., *Prunus avium* L. and *Quercus pubescens* Will. WCF, NCF_L and NCF_H stands are the result of the recovery of abandoned chestnut forests carried out in 2004. Specifically, in WCF and NCF_L the abandoned chestnut forests

were clear-cut and the chestnut stumps of the clear-cut trees were grafted. In NCF_H the recovery actions included the selection of the healthiest trees to maintain a tree spacing of about 10 × 10 m. Moreover, with the exception of ACF, in each study site, since 2016, the cut branches obtained by pruning together with organic residues accumulated on the soil surface are chopped yearly and left on the soil surface. The herb-layer vegetation of the selected stands covered less than 5% of the soil surface and it was composed of fern plants. All the study sites have a northwest exposition with a slope ranging from 12 to 18%. The soils were classified as Leptic Skeletic Dystric Regosol (Loamic, Humic) according to the World Reference Base [35].

2.2. Soil Sampling and Analysis

Soil sampling was conducted in November 2019. In each study site, three soil pits, arranged according to the vertices of an equilateral triangle with sides 20 m long, were dug up to the BC horizon. Afterwards, for all pits, each identified soil mineral horizon was characterized according to Schoeneberger et al. [36] and sampled. The mean thickness of soil horizons is 3.75 cm for A horizon, 9 cm for AB, 12 cm for Bw and 12 cm for BC (Table 1). The soil colors of our soil profiles showed yellow-red hues, reflecting limited weathering, and the values increase from the A to BC horizons due to the reduction in the organic matter content. The A horizons had a weak developed granular structure, while the deeper horizons showed a blocky structure with aggregate dimensions less than 10 mm. The fine earth content ranged from 86% in the A horizon of ACF to 55% in the BC of WCF with rock fragments increasing along the soil depth (Table 1). After soil collection, soil samples were air dried and passed through a 2-mm mesh to remove roots, visible plant debris, and stones. An aliquot of each soil sample was also finely ground.

Soil pH was measured at 1:2.5 soil-to-water ratio using a pH meter electrode. The particle size distribution was determined by pipette method [37]. The determination of total organic carbon (TOC) and total nitrogen (TN) concentrations was carried out by a CHN elemental analyzer (EA 1110 Thermo Fisher, Waltham, MA, USA) without pre-treatment with hydrochloric acid due to the absence of carbonates. The total amount of Ca, Mg, Na, K, Fe, Mn, P and S were measured using the inductively coupled plasma optical emission spectrometer (ICP-OES, Ametek, Spectro Arcos, Kleve, Germany) after aqua regia extraction [38]. Total organic P (TOP) was determined according to Kuo [39]. Specifically, for each sample 2 g of finely ground soil were ignited at 550 °C for 1 h. After, the ignited soil samples and 2 g of non-ignited ones were extracted in 1 M H₂SO₄ (1:50 soil-to-solution ratio) for 16 h. Phosphorus inside the extracts was determined by blue colorimetric method at 880 nm using a UV-visible spectrophotometer (V-530, Jasco, Hachioji, Tokyo, Japan). The TOP content was calculated by difference between the amount of P measured in ignited and non-ignited soil samples. The available P was extracted in 0.5 M NaHCO₃ (pH = 8.5) and measured by the blue colorimetric method at 720 nm [40]. The cation exchange capacity (CEC) and the exchangeable cation contents were determined according to the method proposed by Orsini and Remy [41] and modified by Ciesielski and Sterckeman [42] using 0.017 M hexamminecobalt(III)chloride as extracting solution and the amounts of Co and exchangeable cations were measured by ICP-OES.

Microbial biomass C and N were estimated by the fumigation-extraction method using 0.5 M K₂SO₄ as extracting solution [43,44]. Specifically, for each sample 10 g of 2-mm air dried soil was adjusted to 50% of field capacity and pre-incubated for 5 days. The soil samples were fumigated with CHCl₃ for 24 h at 25 °C. After, the fumigated and non-fumigated samples were mixed with 40 mL 0.5 M K₂SO₄ for 30 min on a horizontal shaker. The suspensions were filtered through 0.45 μm membrane filter and C and N contents in the filtered solution were determined by a TOC-V CPN total organic carbon analyzer (Shimadzu, Kyoto, Japan). The microbial biomass C was calculated as EC/k_{EC}, where EC = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC} = 0.45. Microbial biomass N was calculated as EN/k_{EN}, where EN = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and k_{EN} = 0.54.

Table 1. Morphological characteristics of representative soil profiles dug in an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L).

Forest	Horizon	Depth (cm)	Boundary D/T	Color (Munsell)		Structure G/S/T	Bulk Density g/cm ³	Consistence wp/ws	Roots Q/S	Rock Fragments S/V%/R
				dry	moist					
ACF	Oi	2.5–0	A/S							
	Oe/Oa	0–1.5	A/S	10YR 3/2	10YR 2/2					fgr/1%/2
	A	1.5–6	A/W	10YR 5/3	10YR 4/3	1/f/gr	0.90	po/so	2/f-m	fgr/14%/2
	AB	6–15	C/W	10YR 5/6	10YR 5/8	1/f/sbk	0.97	ps/ss	2/f-m	fgr/20%/2
	Bw	15–22	A/W	10YR 6/4	10YR 5/8	1/f/sbk	0.96	p/s	0/m	fgr/21%/2
	BC	22–30+	U	10YR 6/4	10YR 5/4	2/f/sbk	0.99	p/s		mgr/31%/1
WCF	Oi	2–0	A/S							
	Oe/Oa	0–0.7	A/S	10YR 3/3	10YR 3/1				2/vf-f	fgr/3%/2
	A	0.7–3	A/W	10YR 4/3	10YR 3/4	1/f/gr	0.79	ps/ss	2/f	fgr/20%/2
	Bw	3–18	C/W	10YR 6/4	10YR 5/6	1/f/sbk	1.01	p/s	1/f-m	mgr/32%/1
	BC	18–30+	U	10YR 6/6	10YR 5/8	1/f/sbk	1.15	p/s	0/m	cgr/45%/1
NCF _H	Oi	2–0	A/S							
	Oe/Oa	0–1.5	A/S	10YR 3/3	10YR 3/2					fgr/4%/2
	A	1.5–6	A/W	10YR 5/3	10YR 3/4	1/f/gr	1.01	ps/so	2/f	fgr/25%/2
	Bw	6–20	C/W	10YR 6/4	10YR 5/6	2/f-m/sbk	1.15	p/s	1/f-m	mgr/28%/2
	BC	20–30+	U	10YR 6/8	10YR 5/4	1/f/sbk	1.25	p/s	0/m	mgr/32%/1
NCF _L	Oi	2.5–0	A/S							
	Oe	0–1	A/S	10YR 4/2	10YR 2/2				3/vf	FGR/2%/2
	A	1–4	C/S	10YR 5/2	10YR 3/1	1/f-m/gr	1.02	po/so	3/f	FGR/16%/2
	AB	4–13	C/W	10YR 6/3	10YR 3/4	2/f-m/sbk	1.08	ps/so	1/m	MGR/30%/1
	BC	13–30	U	10YR 6/4	10YR 4/6	2/f-m/sbk	1.19	ps/ss	0/f-m	MGR/36%/1

Horizon Boundary. (D) Distinctness: A = abrupt, C = clear–(T) Topography: S = smooth, W = wavy, U = unknown Structure. (G) Grade: 0 = structureless, 1 = weak, 2 = moderate–(S) Size: f = fine, m = medium–(T) Type: gr = granular, abk = angular blocky, sbk = subangular blocky. 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, (w) sv = very sticky Roots. (Q) Quantity: 0 = very few, 1 = few, 2 = common, 3 = many–(S) Size: vf = very fine, f = fine, m = medium. Rock fragments. (S) Size: fgr = fine gravelly, mgr = medium gravelly; cgr = coarse gravelly–(V%) Fragment content% by volume–(R) Roundness: 1 = angular, 2 = subangular.

According to Chantigny et al. [45], C and N inside the filtered solution obtained from non-fumigated soil samples were considered as water-soluble organic C (WSOC) and water-soluble N (WSN).

Basal respiration was determined by quantifying the CO₂ released in the process of microbial respiration during 28 days of incubation at 25 °C of incubation according to Vittori Antisari et al. [46] after conditioning of the samples at 50% of their field capacity and a pre-incubation of 5 days. In particular, 10 g of 2-mm air dried soil sample was placed in 0.5 L jars with hermetic lids and after 1-3-7-10-14-21-28 days the beginning of incubation, the amount of CO₂ emitted from incubated soils was measured by alkali (1 M NaOH solution) absorption of the evolved CO₂ and titration of the residual OH⁻ with a standardized HCl solution. While the soil basal respiration (SBR) of each soil sample was computed as the average of the values measured during the incubation period, the cumulative amount of CO₂-C (RCUM) was expressed as the total amount of CO₂ evolved during the 28 days of incubation. Then the RCUM:Cmic, SBR:Cmic, RCUM:WSOC and Cmic:TOC ratios were also calculated.

2.3. Statistical Analysis

The statistical analyses were performed through R software 3.5.2. In order to monitor the effect of chestnut stand managements on the considered soil chemical and biological properties, for each pedogenic soil horizon one-way analysis of variance was performed. The normality and homogeneity of variances of residuals were tested by graphical analysis. If these assumptions were violated, the data were transformed according to the Box and Cox procedure. A comparison of the means was carried out using Tukey's HSD post-hoc test ($p < 0.05$).

3. Results

3.1. Chemical Properties

The soils under investigation had a silt-loam/loam texture and an acid pH without significant differences among the study sites (Table 2).

Table 2. Mean \pm standard error of sand, silt and clay contents, and pH of the soils under an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L).

Horizon	Forest	Sand	Silt	Clay	pH
		g kg ⁻¹			
A	ACF	429 \pm 8	482 \pm 15	89 \pm 24	4.35 \pm 0.13
	WCF	585 \pm 24	356 \pm 12	59 \pm 17	4.15 \pm 0.13
	NCF _H	529 \pm 71	416 \pm 58	54 \pm 13	4.41 \pm 0.26
	NCF _L	335 \pm 21	537 \pm 20	129 \pm 12	4.48 \pm 0.12
AB	ACF	387 \pm 64	505 \pm 25	108 \pm 39	4.62 \pm 0.08
	NCF _L	342 \pm 12	515 \pm 14	143 \pm 17	4.75 \pm 0.08
Bw	ACF	357 \pm 37	528 \pm 6	115 \pm 32	4.64 \pm 0.05
	WCF	376 \pm 7	503 \pm 13	121 \pm 19	4.69 \pm 0.05
	NCF _H	425 \pm 64	469 \pm 39	107 \pm 29	5.00 \pm 0.15
BC	ACF	358 \pm 52	526 \pm 32	116 \pm 20	4.75 \pm 0.13
	WCF	368 \pm 36	491 \pm 31	141 \pm 13	4.75 \pm 0.05
	NCF _H	358 \pm 46	498 \pm 11	144 \pm 36	4.99 \pm 0.14
	NCF _L	325 \pm 10	564 \pm 14	111 \pm 8	4.91 \pm 0.06

Taking in account the organic C and the most important soil nutrients (N and P), no differences in TOC, TN and TOP concentrations occurred among the forests (Figure 2a,b,e). For the most labile forms of these elements (i.e., available or soluble), both WSOC and WSN showed some differences in A

horizon (Figure 2c,d), while the available P did not change among the stands (Figure 2f). In particular, NCF_L showed the highest WSOC and WSN contents.

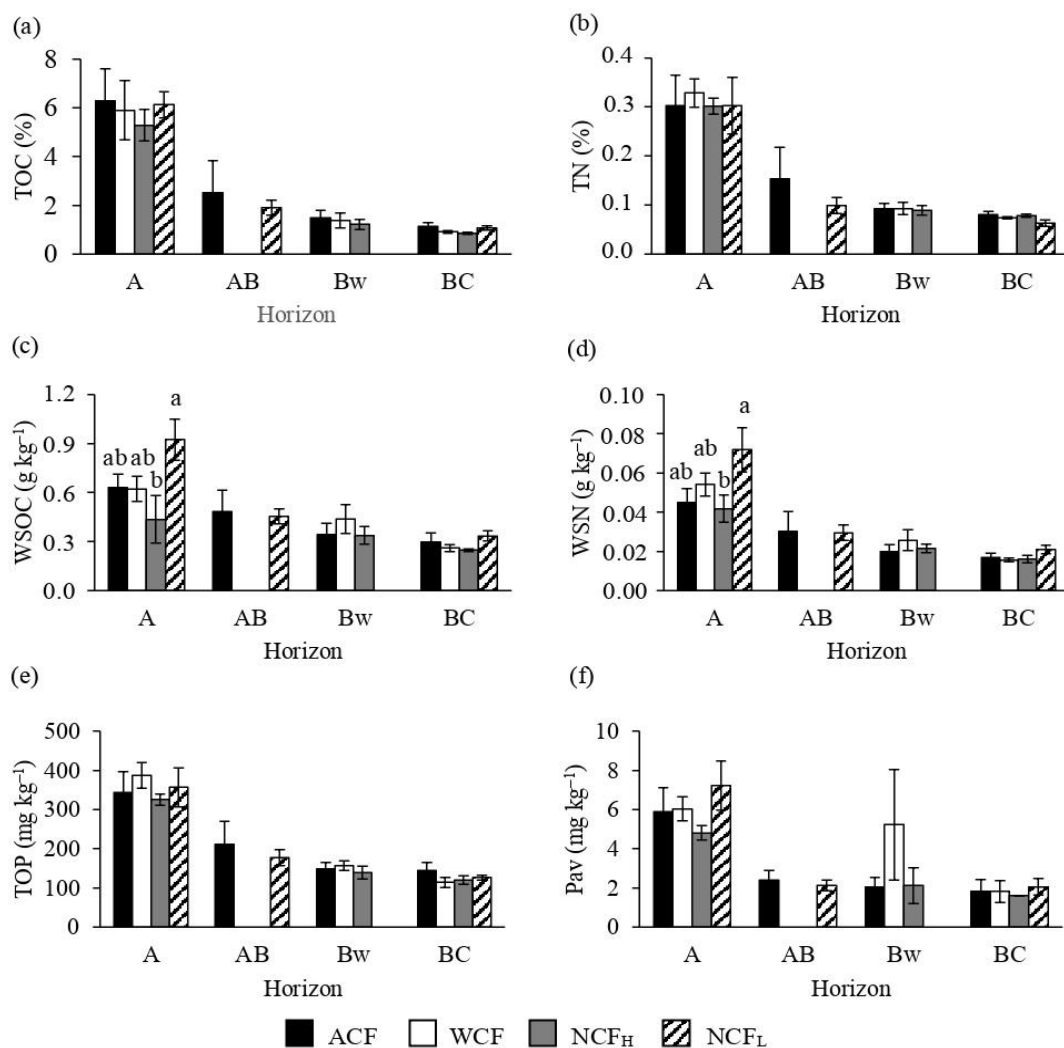


Figure 2. Concentrations of total organic carbon (TOC; a), total nitrogen (TN; b), water-soluble organic carbon (WSOC; c), water-soluble nitrogen (WSN; d), total organic phosphorus (TOP; e) and available P (Pav; f) of soils under an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L). Error bars are the standard errors. Within each horizon, different letters indicate significant differences by Tukey's *t*-test $p \leq 0.05$.

As expected from the similar soil texture (Table 2) and TOC content (Figure 2a), the study sites showed similar CEC values with exception of AB horizon where ACF had a higher CEC compared to NCF_L (Figure 3a). The exchangeable Ca and Mg did not show differences among the stands (Figure 3b,c) with exception of B horizon which showed the highest exchangeable Mg values in ACF and the lowest ones in WCF. With regard to exchangeable K, NCF_L displayed always the lowest values (Figure 3d). Furthermore, it is to notice that while in A horizon the highest exchangeable K values were observed in ACF, in B horizon WCF showed lower exchangeable K values compared to ACF and NCF_H. For the exchangeable Na, some differences occurred in A and B horizons (Figure 3e). Specifically, while in A horizon NCF_L had higher exchangeable Na content compared to WCF, in B horizon NCF_H showed lower exchangeable Na values compared to WCF and ACF. Because of the few differences found for

exchangeable bases and the similar CEC values, negligible are the differences found for the percentage base saturation (Figure 3f) with exception of B horizon where a lower BS was observed in NCF_H than in ACF.

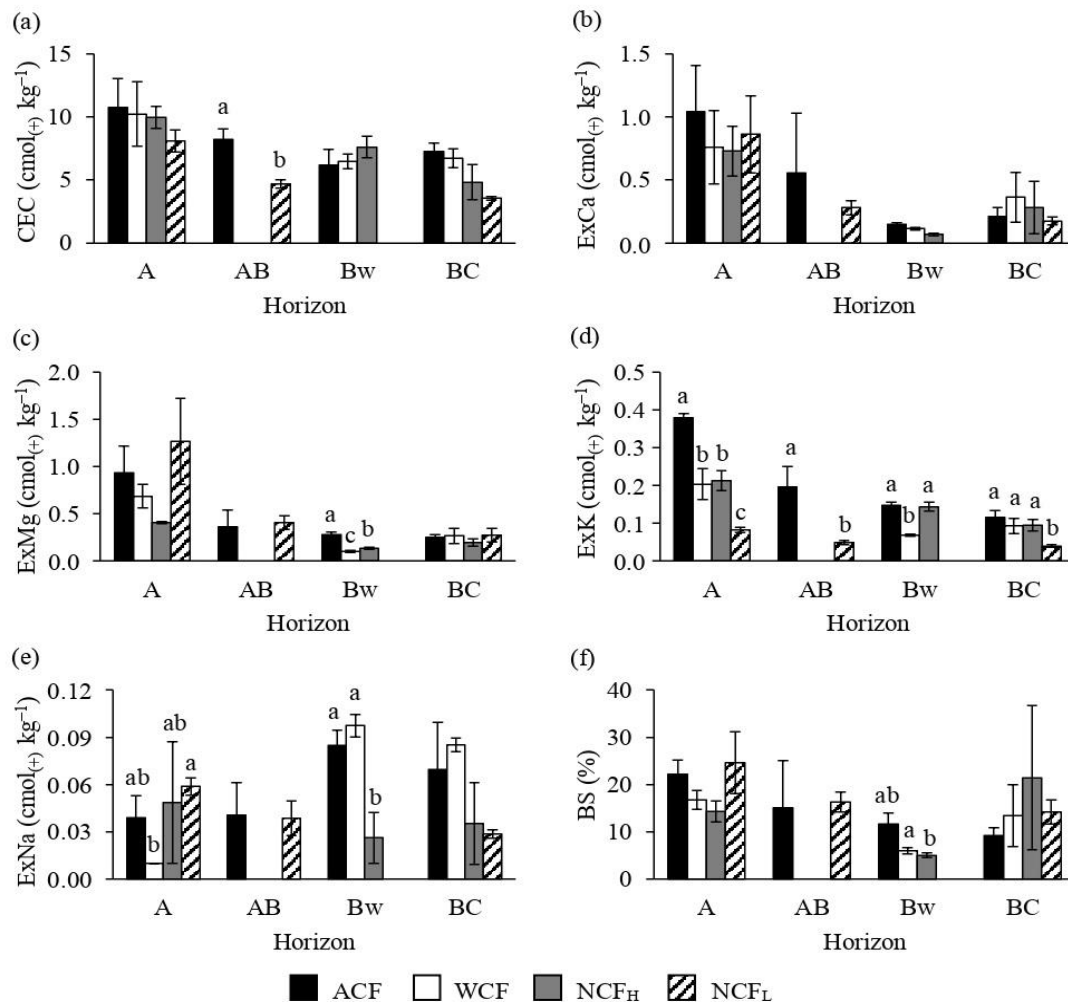


Figure 3. Cation exchange capacity (CEC; **a**), concentrations of exchangeable calcium (ExCa; **b**), exchangeable magnesium (ExMg; **c**), exchangeable potassium (ExK; **d**), exchangeable sodium (ExNa; **e**) and base saturation (BS; **f**) of soils under an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L). Error bars are the standard errors. Within each horizon, different letters indicate significant differences by Tukey's *t*-test $p \leq 0.05$.

Given the similar parent material of the soils, the differences are limited for the total element concentrations (Figure 4a–f). Generally, ACF exhibited the highest total Ca, Mg and K contents (Figure 4a–c). Furthermore, in BC horizon WCF displayed the lowest total K content among the study sites. For the total amounts of Na and P (Figure 4d,e), generally NCF_L showed the highest values along the whole soil profile among the forests. Finally, a lack of differences was found the total S content.

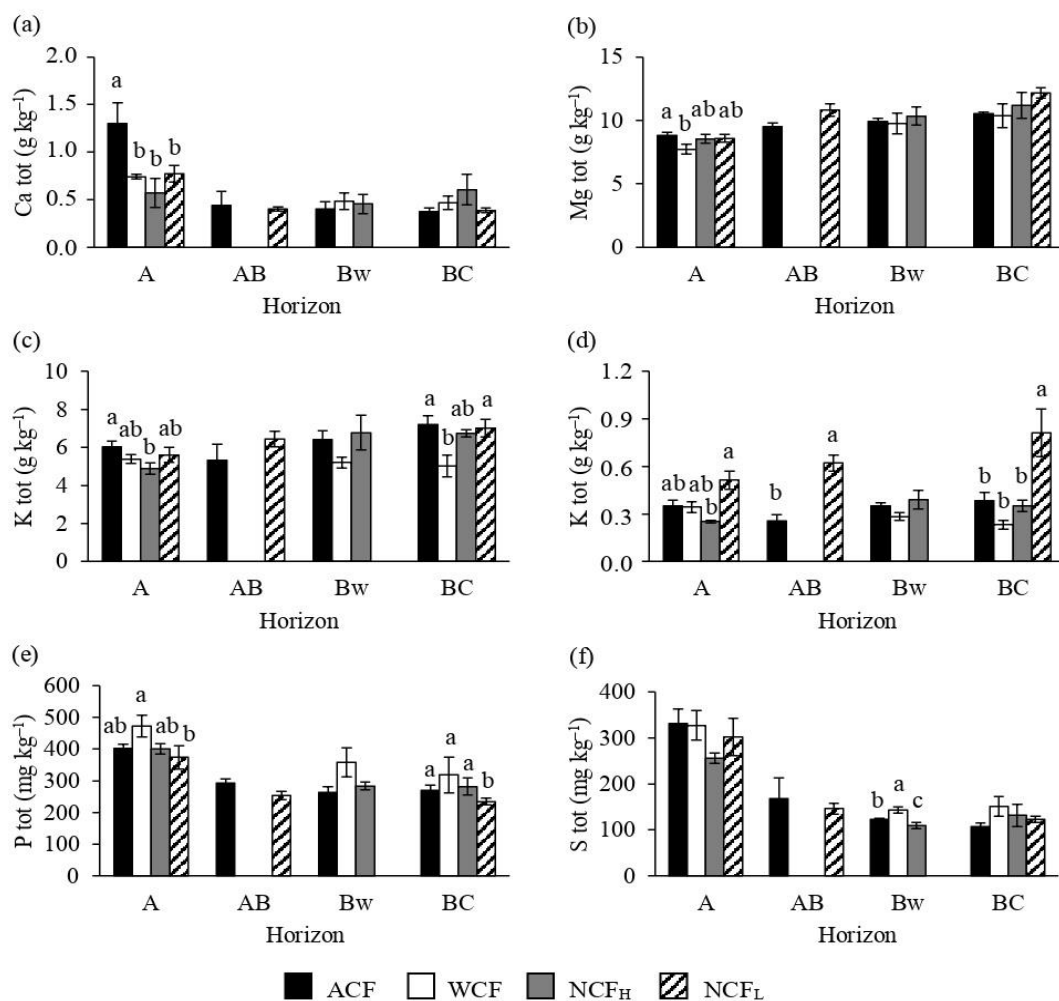


Figure 4. Concentrations of total calcium (Ca tot; a), magnesium (Mg tot; b), potassium (K tot; c), sodium (Na tot; d), phosphorus (P tot; e) and sulfur (S tot; f) of soils under an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L). Error bars are the standard errors. Within each horizon, different letters indicate significant differences by Tukey's *t*-test $p \leq 0.05$.

3.2. Biochemical Properties

In A horizon, the highest microbial biomass C and N (Figure 5a,b) were found in NCF_L, while for the BC horizon the lowest microbial biomass C was observed in NCF_H. For the soil microbial respiration, some differences occurred only in BC horizon (Figure 5c,d). Specifically, the highest values of total amount of CO₂-C and basal respiration were found in NCF_L, while the lowest ones in WCF.

Data of RCUM:Cmic and SBR:Cmic ratios (Figure 6a,b) for the A horizon displayed the lowest values in NCF_L. However, for the deepest soil horizons, NCF_L and NCF_H had the highest RCUM:Cmic and SBR:Cmic ratios. Regarding to RCUM:WSOC ratio, in A horizon, the lowest values were found in NCF_L, while in B horizon the highest RCUM:WSOC ratio was found in NCF_H. In BC horizon, instead, the highest RCUM:WSOC ratio was observed in NCF_L, whereas the lowest one in WCF. For the Cmic:TOC ratio, in A horizon NCF_L showed the highest value among the study sites, while in B horizon the Cmic:TOC ratio followed the following trend WCF ≤ ACF ≤ NCF_H.

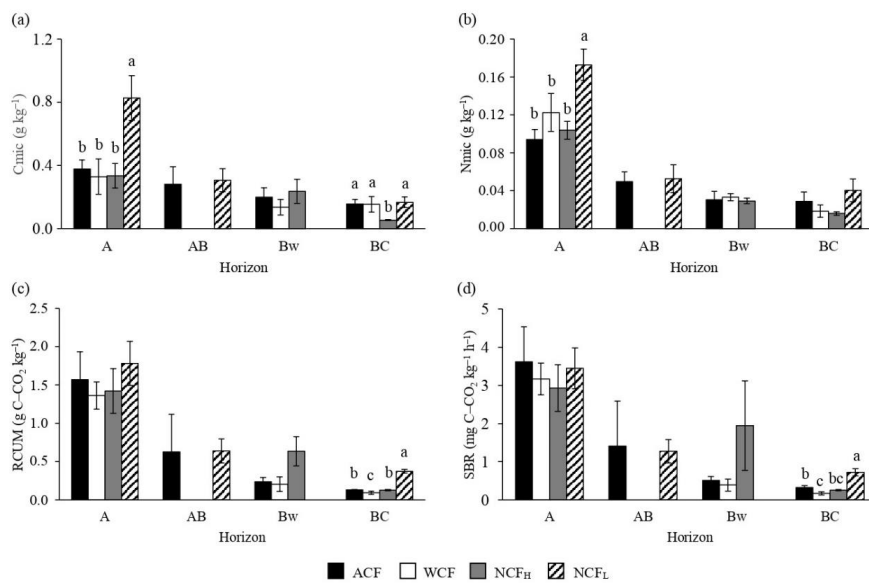


Figure 5. Concentrations of microbial biomass carbon (Cmic; **a**), microbial biomass nitrogen (Nmic; **b**), cumulative amount of CO₂-C evolved during 21-days incubation experiment (RCUM; **c**) and soil basal respiration (SBR; **d**) of soils under an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L). Error bars are the standard errors. Within each horizon, different letters indicate significant differences by Tukey’s *t*-test $p \leq 0.05$.

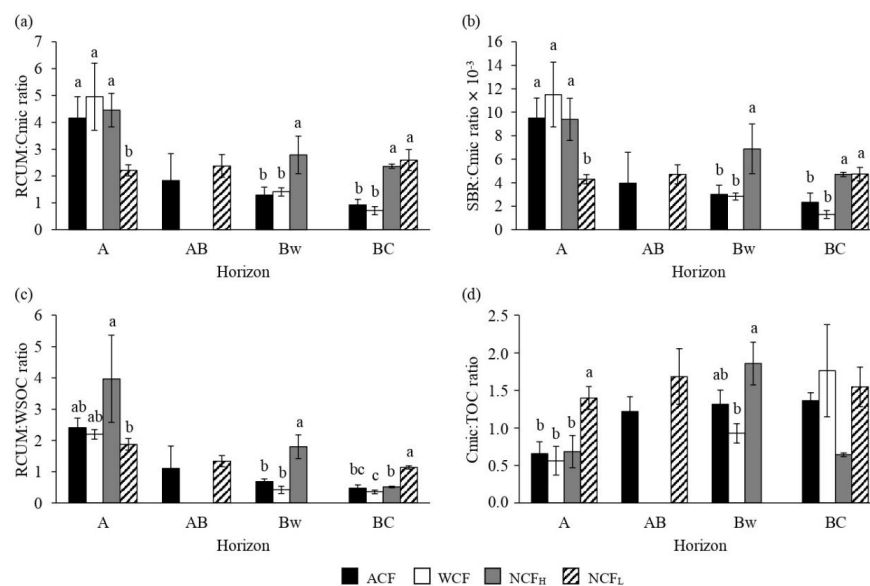


Figure 6. RCUM:Cmic (**a**), SBR:Cmic (**b**), RCUM:WSOC (**c**) and Cmic:TOC (**d**) ratios of soils under an abandoned chestnut forest (ACF), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H) and a chestnut forest for nut production with a tree density of 98 plants ha⁻¹ (NCF_L). Error bars are the standard errors. Within each horizon, different letters indicate significant differences by Tukey’s *t*-test $p \leq 0.05$. RCUM = cumulative amount of CO₂-C evolved during 21-days incubation experiment; SBR = soil basal respiration; WSOC = water-soluble organic carbon content; TOC = total organic carbon content.

4. Discussion

Our research provides a unique opportunity to evaluate the effect of the conversion of an abandoned European chestnut forest to pure chestnut forests with different managements on some soil chemical and biochemical properties. In fact, it is rare to perform a field study with very similar pedo-climatic conditions that allows us to reduce the interference of uncontrolled factors.

The present study displayed how the major differences concerned the biochemical properties. Our findings are in accordance with previous studies, which reported a higher sensibility of soil biological properties compared to the chemical ones to the management practices both in agricultural [47–49] and forest ecosystems [10,50,51].

Taking in account the soil chemical properties, the similar concentrations of organic C among the study sites would suggest how the recovery of the abandoned chestnut forests into pure chestnut stands does not affect the most important indicator of soil quality [52]. Although WCF and ACF study sites have a greater tree density and, as a consequence, a larger aboveground biomass compared to NCF_L and NCF_H all the study sites showed similar values of organic C. The results are in accordance with previous forestry studies [53,54] which detected unaltered soil OC amounts among forests characterized by a different tree density and tree biomass. The lack of differences in OC could be assigned to the generally similar tree composition (100% chestnut trees in WCF, NCF_H and NCF_L and 73% in ACF). Indeed, the vegetation types play a pivotal role in the control of the soil organic C [55,56] because of the different litter quality which is species-specific [57]. Another possible reason for the similar OC values among the study sites can be attributed to the high amounts of sand (always higher than 33%) and the low clay content (always lower than 15%), which does not allow OC accumulation in our investigated soils [58,59]. Specifically, the predominance of a coarser soil texture implies a limited formation of organo-mineral complexes and, therefore, prevents physical protection against a microbial attack [60].

Despite the generally similar CEC values among the study sites, NCF_L showed the lowest exchangeable K content for all soil horizons. The low exchangeable K values in NCF_L can be assigned to the low amount of the element in the litter floor (Table S1 of Supplementary Materials). In fact, this is recognized in the litter floor nutrient recycling in the underlying mineral horizons [61–63]. Conversely, the rationale of the low amount of K in the litter floor is unclear because of the absence of data about the plant tissues.

Despite the similar parent material, and in no one of the study sites fertilizers are applied as well as no removal of plant residues is performed, the total amount of Ca showed the highest values in ACF. The different concentration of total Ca between ACF and the pure chestnut forests could be attributed to the Ca loss that likely occurred at the time of clear-cut practices due to the occurrence of soil erosion processes. Although we do not have data related to the possible occurrence of soil erosion, in literature it is recognized that clear-cut practices in mountainous forest areas cause the acceleration of the soil erosion processes [64] with consequent loss of nutrients accumulated in the eroded soil [65]. However, our hypothesis about the occurrence of soil erosion processes might be confirmed by the study conducted in our same experimental field by Vittori Antisari et al. [34] which found the loss of the surface soil mineral material after the clear-cut practices. The higher total Ca content in ACF than in pure chestnut forests can be also attributed to the scarce or lack of a forest canopy after clear-cut practices, which prevented the formation of the litter floor and, therefore, the soil Ca restoration through the organic material degradation and incorporation in the mineral soil. In fact, the pivotal role of the forest floor on soil nutrient cycles is well known [66]. However, this process was not observed for Mg, K, Na and P, likely due to their much lower concentrations in the Oi horizon compared to Ca. With regard to soil erosion, noteworthy, although the erosion processes are known to cause the loss of organic matter from the soils [67], in our case, the lack of differences in TOC content among the study sites would indicate rather efficient chestnut forest ecosystems in relation to the recovery of soil organic matter.

Although the study sites had a similar amount of TOC, which is considered an important source of labile organic C [68], in the uppermost soil mineral horizon, NCF_L showed the highest WSOC

content. This high value can be assigned to the lower tree density. Specifically, since chestnut trees are sensitive to the competition with other trees [69,70], the high spacing among the plants could promoted plant growth [71,72] and, therefore, root development. However, we cannot exclude that the low plant density could have also promoted a wider distribution of the root system [73]. In fact, as reported in Table 1, in A horizon of NCF_L we found a greater number of fine roots compared to the other study sites. In both cases, the larger root system could have promoted the increased presence of water-soluble organic compounds provided through the rhizodeposition processes [74,75].

The lack of differences for the labile organic substances among the study sites in subsurface horizons might be assigned due to the superficial distribution of root chestnut trees [76,77]. Because the labile organic substances are a source of energy and nutrients readily accessible for soil microbial communities [78,79], the higher WSOC content in the A horizon of NCF_L can also explain the greatest amount of microbial biomass (C_{mic} and N_{mic}). Despite the higher microbial biomass in A horizon of NCF_L, the microbial respiration did not show differences among the stands for the A horizon. Conversely, it was interesting to observe how the A horizon of NCF_L showed the lowest values of RCUM:C_{mic} and SBR:C_{mic}, which would indicate how NCF_L management favored a more efficient microbial growth [80,81]. Furthermore, NCF_L also showed a better use efficiency of the heterotrophic microbes of the easily available substrates, as highlighted by the lowest values of RCUM:WSOC ratio [82]. Hence, the lowest RCUM:C_{mic}, SBR:C_{mic} and RCUM:WSOC ratios together with the highest C_{mic} content would suggest that the soil microbes, harbored in A horizon of NCF_L, require less energy for their maintenance and address the assimilated C to their growth. In addition, the higher C_{mic}:TOC ratio in NCF_L compared to the other managements indicates a larger C immobilization in the microbial biomass [83]. Although for the most surface mineral horizon NCF_L showed the best microbial C use efficiency, in the deepest soil horizons (B and BC horizons) both chestnut stands for nut production showed faster C turnover rates, indicating the development of a microbial community with a high catabolic activity and turnover [84].

Although the sweet chestnut trees generally grow in environments very similar to that investigated in the present work, we are aware that our results need to be interpreted with caution because our study took into account an area with specific pedo-climatic conditions. Therefore, in order to obtain a clearer picture about the effects of the studied managements on soil properties, similar investigations in other pedo-climatic environments are needed.

5. Conclusions

This study provides novel information on the effects of different recovery strategies on some soil chemical and biological soil properties in a representative abandoned chestnut forest located in a mountainous area. In particular, the present paper pointed out how the recovery of an abandoned chestnut forest to a pure chestnut one for agricultural purposes (timber and nut production) did not negatively affect the indicators of soil quality (TOC, WSOC, C_{mic}, microbial activity, soil fertility). Therefore, view of the re-evaluation of mountainous areas, the establishment of chestnut forests could strike the right balance due to the environmental and socio-economic services provided by chestnut forests. Among the tested pure chestnut stand managements, the forest used for nut production and with a plant spacing of 98 plants ha⁻¹ m seems to be the best one, at least from the biological point of view. In fact, NCF_L showed the lowest energy costs for metabolic maintenance and resource acquisition by soil microbial population. However, in the deeper soil horizons, both chestnut forests for nut production have shown a microbial community characterized by a low C use efficiency. Overall, our findings highlight that an ecosystem like our studied pure chestnut forests should be promoted in order to synergistically combine their yields and environmentally beneficial attributes within a landscape unit.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/11/8/786/s1>, Table S1: Mean ± standard error of calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphorus (P) and sulfur (S) contents in Oi horizon of a chestnut forest for nut production with a tree density of 98 plants ha⁻¹

(NCF_L), a chestnut forest for wood production with a tree density of 151 plants ha⁻¹ (WCF), an abandoned chestnut forest (ACF) and a chestnut forest for nut production with a tree density of 120 plants ha⁻¹ (NCF_H).

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