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Pedodiversity and Organic Matter Dynamics in the North Apennines (Italy): Relationships among Soil Types, Biodiversity, and Ecological Functionality

Livia Vittori Antisari , Mauro De Feudis *, William Trenti, Gloria Falsone , Federico Puliga ,
Alessandra Zambonelli , Giulia Tabanelli  and Fausto Gardini

Department of Agricultural and Food Sciences, Alma Mater Studiorum-University of Bologna, Viale Fanin 40, 40127 Bologna, Italy; livia.vittori@unibo.it (L.V.A.); william.trenti2@unibo.it (W.T.); gloria.falsone@unibo.it (G.F.); federico.puliga2@unibo.it (F.P.); alessandr.zambonelli@unibo.it (A.Z.); giulia.tabanelli2@unibo.it (G.T.); fausto.gardini@unibo.it (F.G.)

* Correspondence: mauro.defeudis2@unibo.it

Abstract: Pedodiversity is generally neglected in studies concerning soil organic carbon (SOC). Therefore, this investigation aimed to explore the effect of soil types on the following: (1) soil processes related to organic matter (OM) dynamics along the profile; and (2) the microbial community and functionality within the uppermost horizon. Humic Dystrudepts (HD), Typic Dystrudepts (TD), and Humic Lithic Dystrudepts (HLD) were selected in beech forests of the Apennine ridge in the Emilia-Romagna Region (Italy). Soils were sampled by horizons until parent material, and physico-chemical and functional analyses were performed. The results showed that both HD and HLD soils had a higher SOC accumulation than TD, particularly within the deeper horizons. Such accumulation might be due to the lower turnover rate of soil OM forms, namely fulvic acid-like substances, humic acid-like substances, and non-extractable OM. Noteworthy, the A horizons showed slight differences in SOC among the soil types, suggesting similar SOC decomposition processes. This fact was confirmed by the lack of differences in microbial DNA-based diversity and functionality. This study highlighted the importance of combining pedodiversity and microbial diversity for a wider perspective on SOC dynamics.

Keywords: dystrudept; microbial functionality; soil horizons; mountain soils; beech forests



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1. Introduction

Soils are a part of the world's natural heritage, and they are ecosystems in which biological (biodiversity) and geological (geodiversity) resources coexist [1]. Soil represents a fundamental component of the natural environment and is responsible for many essential ecosystem services that contribute to water regulation and supply, climate regulation, biodiversity conservation, plant productivity, and carbon sequestration [2].

Pedodiversity, also known as soil diversity within an area, could further improve and enhance such ecosystem services due to its positive influence on biodiversity, food production, water conservation and transport, and many other ecological processes [3,4]. Because of the diverse soil properties between different soil types and the consequent inter-relationship with the soil ecological functions [3], the spatial variation of soil properties according to soil types was also described with the term “functional pedodiversity”, which indicates the land's versatility to provide ecosystem functions [5]. Unfortunately, pedodiversity is not a stable land characteristic since soil degradation processes due to accelerated erosion [6,7]. This fact would highlight how much soils are complex and dynamic bodies whose properties are driven by the numerous processes that occur contemporary within them and by various biotic and abiotic factors and their interactions affecting the pedodiversity itself [3,8–12]. Despite the importance of pedodiversity on soil functioning and

ecosystem service provision [13], soil classification and, therefore, soil horizon sequence identification are still usually neglected in studies concerning nutrient cycling, organic matter dynamics, and more in general soil properties evaluation.

In the context of pedodiversity and its functions, previous studies reported the pivotal role of soil biodiversity on the performance of the soil ecosystem [14,15]. In fact, the soil biota is involved in most of the key functions that the soil provides by driving the nutrient cycling processes, soil structural dynamics, degradation of pollutants, gas exchange, and regulation of plant communities [16]. In this sense, earlier studies highlighted the positive relationship between soil functionality and soil quality [17,18]. Soil organic matter (SOM) is widely recognized as the major soil component supporting the multiple soil ecosystem functions [19] and is used as the most important soil quality indicator [20–22]. This is because several physical, chemical, and biological processes and properties are modulated by the SOM amount and its forms [23]. SOM mainly derives from plant litter residue input and is degraded, transformed, and respired by microbial communities [24]. Consequently, the SOM degradation by-products are mainly composed of microbial-derived molecules [25], which in the uppermost soil layer could be up to 62% of total SOM [26]. Other than SOM, soil quality can be described by eco-physiological indices such as the metabolic quotient, the microbial quotient, and the Dilly index [27–30]. However, the specific functioning of soil biodiversity within soil is still unclear [31], probably due to pedodiversity and its effect on the soil microbiome. In this sense, to fill this gap currently, there is much interest in linking microbiome composition and diversity to their functions within soil [32–34] and also through the investigation of the microbial functional gene composition [35].

Hence, the main aims of the present investigation were as follows: (a) to evaluate the effect of soil types on soil processes related to nutrients and organic matter dynamics along the profile; and (b) to explore if soil type influences the microbial community and functionality within the uppermost horizon.

2. Materials and Methods

2.1. Characterization of the Study Areas: Location, Climate, Land Use, and Vegetation

Three study areas (BAC, TAS, and PIA) were identified within the Frignano Regional Park, a protected area of 15,000 ha covering the Apennine ridge in the Emilia-Romagna Region (Italy), mainly covered by European beech (*Fagus sylvatica* L.) forests (Figure 1). The study areas were beech forests managed as high forests in TAS and PIA and as coppice in BAC.

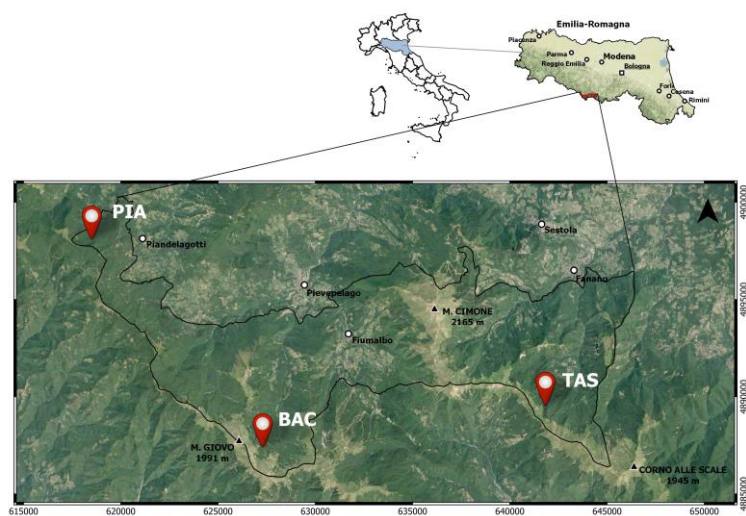


Figure 1. Localization of the study areas within the Frignano Regional Park (Northern Italy).

The study areas, at approximately 1500 m above sea level, are characterized by an average air temperature of 6.6 °C and mean annual precipitation of 1550 mm, mainly in autumn and spring.

The identification of the study areas was based in accordance with the “brown soils” characterization [8] of high North Apennine. Soils develop on flysch of turbiditic origin, formed and developed on Modino, Cervarola (TAS and PIA), and Macigno (BAC) sandstone formations, as described by Vittori Antisari et al. (2022), which were the most frequent in the park [36]. Humic Dystrudepts (HD), Typic Dystrudepts (TD), and Humic Lithic Dystrudepts (HLD) were selected in a soil survey opening profile as representative soils of the investigated sites (Table 1).

Table 1. Location, topographical features, and soil type of soil profiles. The X and Y coordinates (Xcoor and Ycoor, respectively) are based on the WGS84 UTM32 coordinate reference system. The soil type is based on the Soil Survey Staff (2014) classification system.

Profile	Xcoor mE	Ycoor mN	Elevation m a.s.l.	Slope °	Aspect	Soil Type
TAS 1	641,818	4,889,485	1517	25	E	Humic Dystrudept, coarse loamy over coarse loamy skeletal, frigid (HD)
TAS 2	641,876	4,889,540	1487	14	E	Humic Dystrudept, coarse loamy over coarse loamy skeletal, frigid (HD)
PIA 1	618,341	4,898,077	1501	2	NW	Typic Dystrudept, coarse loamy, skeletal, frigid (TD)
PIA 2	618,311	4,898,094	1484	31	NW	Typic Dystrudept, coarse loamy, skeletal, frigid (TD)
PIA 3	618,558	4,898,111	1457	9	N	Typic Dystrudept, coarse loamy, skeletal, frigid (TD)
PIA 4	618,491	4,898,090	1465	16	NE	Typic Dystrudept, coarse loamy, skeletal, frigid (TD)
BAC 1	627,259	4,887,352	1602	8	N	Humic Lithic Dystrudept, coarse loamy, skeletal (HLD)
BAC 2	627,165	4,887,374	1598	30	NW	Humic Lithic Dystrudept, sandy over coarse loamy, skeletal (HLD)
BAC 6	627,358	4,887,628	1601	5	W	Humic Dystrudept, coarse loamy, skeletal (HD)
BAC 7	627,298	4,887,626	1593	18	W	Humic Lithic Dystrudept, coarse loamy, skeletal (HLD)

2.2. Soil Survey

The survey took place at the end of September 2020 by digging soil profiles until at least the transitional pedogenetic horizon with the parent material (BC). The soil depth was less than 50 cm for those with lithic contact and between 65 and 75 cm for the other soil profiles. For each soil profile, the genetic horizons were described and sampled, as indicated by Schoeneberger et al. [37]. For metabarcoding analyses performed on A horizons, three replicates for each site were taken using a sterilized soil core sampler that is 5 cm in diameter. The samples were transported in a sterile plastic bag, stored at 4 °C, and immediately processed upon return to the laboratory. The location of each soil profile is summarized in Table 1.

2.3. Soil Analysis

2.3.1. Physicochemical Soil Characteristics

The soil samples, including organic horizons, were air-dried and passed through a 2 mm sieve, determining the skeletal percentage for each horizon. An aliquot of sieved samples was finely ground using a steel ball mill. For the 2 mm sieved soil samples, the pH was determined potentiometrically in a 1:2.5 soil-to-deionized water ratio (pH-meter Crison, Barcelona, Spain), while the particle-size distribution was measured by the pipette method [38].

2.3.2. Total Element Concentration

Briefly, 250 mg of soil samples were mineralized with aqua regia solution (2 mL 65% HNO₃ plus 6 mL 37% HCl, suprapur grade, Carlo Erba, Milano, Italy) using a microwave oven (Milestone 2100, Sorisone, Bergamo, Italy). After digestion, the soil sample solution

was made up to 20 mL with Milli-Q ultrapure distilled water and filtered with Whatman 42 filter paper. The total amount of P, S, Ca, Mg, Fe, Al, and K was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Arcos II, Kleve, Germany, Ametek Spectro). Each soil sample was analyzed three times, and the data were calibrated using International Reference Materials (BCR) and internal laboratory standards [39].

2.3.3. Organic C and Total N Determination and Stable Isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

The organic carbon (OC) and total nitrogen (N) contents of the finely ground samples were determined by a CHN elemental analyzer (Flash 2000, Thermo Fisher Scientific, Waltham, MA, USA) coupled with an isotope ratio mass spectrometer (IRMS Delta C or DELTA+XL, Thermo Finnigan MAT, Bremen, Germany) for the stable carbon and nitrogen isotope (^{13}C and ^{15}N) determination, expressed as $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) with respect to the V-PDB universal reference standard for ^{13}C and air for nitrogen.

2.3.4. Total Element Concentrations

The soil organic matter of each horizon was chemically fractionated based on Agnelli et al. [40], with some adjustments. A sample of 10 g of soil was treated with 100 mL of deionized water and shaken on a horizontal shaker for 16 h at 25 °C, after which it was centrifuged, thus separating the supernatant from the precipitate. The first was sieved at 53 μm , and the particles retained by the sieve represented the POM (particulate organic matter). The precipitate was treated with 100 mL of NaOH 0.1 M, shaken for 24 h at 25 °C, and centrifuged again. The extract was filtered with a 0.45 μm polycarbonate filter, and the precipitate (the NEOM, non-extractable organic matter) was rinsed with deionized water to achieve a $\text{pH} \leq 7$. The filtered extract was brought to a pH of about 1.5 with a solution of HCl 6M and left to settle overnight, after which it was centrifuged to separate the supernatant FS (fulvic acid-like substances) from the precipitated humic acid-like substances (HA). The first was neutralized with a solution of NaOH and placed in 1000 Da cutoff dialysis membranes (Spectra/Por[®] Dialysis membrane) against deionized water, while the latter was rinsed with deionized water. The obtained FS and HA were freeze-dried, while the POM and NEOM were dried at 40 °C. For each fraction, organic carbon, total nitrogen, and their isotopic ratios were determined using the CHN elemental analyzer coupled with an isotope ratio mass spectrometer.

2.3.5. Biochemical Soil Parameters

Basal respiration was determined by quantifying the $\text{CO}_2\text{-C}$ released in the process of microbial respiration during 28 days of incubation at 25 °C, according to Vittori Antisari et al. [41]. Incubation was preceded by bringing the samples to 60% of their water-holding capacity and by a pre-incubation of 3 days. The amount of $\text{CO}_2\text{-C}$ emitted after 1-3-7-10-14-21-28 days from the beginning of incubation was measured by alkali [41]. 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 $\text{CO}_2\text{-C}$ (RCUM) was expressed as the total amount of $\text{CO}_2\text{-C}$ evolved during the 28 days of incubation.

Microbial biomass C and N (C_{mic} and N_{mic}) were estimated by the fumigation-extraction method using 0.5 M K_2SO_4 as the extracting solution [42,43]. Specifically, for each sample, 10 g of 2-millimeter air-dried soil was adjusted to 60% of the water holding capacity and pre-incubated for 5 days. The soil samples were fumigated with CHCl_3 for 24 h at 25 °C. Then, the fumigated and non-fumigated samples were shaken, filtered through a 0.45 μm membrane, and extracted with 40 mL of 0.5 M K_2SO_4 for 30 min. The 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/kEC , where EC = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and kEC = 0.45. Microbial biomass N was calculated as EN/kEN , where

EN = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and kEN = 0.54.

According to Chantigny et al. [44], C and N inside the filtered solution obtained from non-fumigated soil samples were considered water-extractable organic C (WEOC) and water-extractable N (WEN).

Then the mineralization quotient (qM; RCUM/OC), metabolic quotient (qCO₂; SBR/Cmic), and microbial quotient (qMIC; Cmic/OC) were also calculated, according to Anderson and Domsch [45] and Mocali et al. [28]. Dilly's index was calculated as qCO₂/OC, according to Dilly [30]. The ratio indicated the efficiency of using the OC by soil microbial biomass (from 100 efficiency to >400 inefficiency).

2.3.6. DNA Extraction and Taxonomic Assignment

Total DNA extraction was performed on all organo-mineral horizons using DNeasy Power-Soil (Qiagen), following the manufacturer's instructions, and for each soil sample, three technical replicates were made.

For microbial and fungal communities, the 16S rDNA V3-V4 region using the locus-specific polymerase chain reaction (PCR) primers 16S 341F/805R [46] and the internal transcribed spacer (ITS) region using the primer pairs ITS1F/ITS4R [47,48] were amplified with the integration of relevant flow-cell binding domains and unique indices. The libraries obtained were sequenced on a MiSeq Illumina instrument using 300-bp paired-end mode, and the FASTQ sequences generated were analyzed using DADA2 version 1.12.1 [49] in the R 3.5.1 environment. The reads were filtered and trimmed using the filterAndTrim function set to (270, 230).

The UNITE database was used to perform taxonomic assignments for fungi. For microbial communities, the taxonomic assignment was performed using SILVA nr database version 138, updated according to the reclassification of the genera *Bacillus* and *Lactobacillus*, and applying the assign Taxonomy and add Species functions.

2.4. Data Elaboration and Statistical Analysis

Statistical analyses were performed with RStudio 2023.03.0.

To compare soil horizons, cluster analysis using the Ward method (Packages: Factoextra e ggplot2) was performed on the dataset, grouping into three categories (O—organic horizons; A—organo-mineral horizons, which also included some statistical units like Oe and AB horizons; ENDO—mineral horizons). Furthermore, a principal component analysis (PCA) to evaluate the main parameters driving the variability among the statistical units was carried out (packages: FactoMineR). Because of the diverse horizon sequence among the investigated soil profiles and the clusters suggested by the cluster analysis, the data were grouped as organic (O; Oe and Oa horizons), organo-mineral (A; A horizons), and mineral (ENDO; AB, Bw, BC, C, and C_R horizons) layers. To evaluate differences among soil types and soil layers, a non-parametric ANOVA (Kruskal–Wallis test) was performed at $p < 0.05$ using the Agricolae package. Fisher's least significant difference post hoc test was used for comparing the means.

The relationship between the prevalent vertical decrease of OC and the increase of $\delta^{13}\text{C}$ in depth profiles was used as a natural indicator of SOC turnover [50]. For each soil type and each SOM fraction, the slope (β) of the linear regression ($y = a + \beta x$) between the mean $\delta^{13}\text{C}$ values and their respective log-transformed C concentrations (g C kg^{-1}) was calculated and is referred to as the $\beta_{\delta^{13}\text{C}}$ value [50]. In particular, "y" was the $\delta^{13}\text{C}$, "a" was the intercept of the liner regression, and "x" was the ln of SOC content. The distribution of ^{15}N along soil depth was compared among the investigated areas using the soil enrichment factor ($\epsilon_{\text{soil}^{15}\text{N}}$). It is defined as absolute enrichment between the litter (Oi horizon) and the other soil deeper horizons (e.g., organic other than Oi, A, and other mineral horizons here called ENDO) [51] and was calculated as follows:

$$\epsilon_{\text{soil}^{15}\text{N}} (\text{‰}) = \delta^{15}\text{N} (\text{O, A, and ENDO horizons}) - \delta^{15}\text{N} (\text{Oi horizon}) \quad (\text{Lorenz et al., 2020 [52]}).$$

The $\delta^{15}\text{N}$ of Oi was -5.2 ± 0.0 , -5.3 ± 0.0 , and $-5.2 \pm 0.0\%$ for Humic, Humic Lithic, and Typic Dystrudepts, respectively.

Shannon and Simpson diversity indices were calculated to assess microbial and fungal biodiversity within the A horizon. Furthermore, fungal guilds were predicted using FungalTraits [53].

3. Results

3.1. Pedodiversity

The soil profiles were classified (Table 1), according to Soil Taxonomy classification [54], as Humic Dystrudepts (HD, which includes TAS1, TAS2, and BAC6), Humic Lithic Dystrudepts (HLD, which includes BAC1, BAC2, and BAC7), and Typic Dystrudepts (TD, which includes PIA1, PIA2, PIA3, and PIA4). A certain pedodiversity in the three sites was therefore already visible in subgroup soil classification based on soil color (upper 18 cm of soil depth darker in Humic subgroup) and depth of lithic contact (within 50 cm in Lithic subgroup).

3.2. Principal Component Analysis

The two main dimensions of PCA explained 71.1% of the variability of the statistical units (Figure 2). Such statistical units were distributed from left to right of the scatterplot, separating organic (Oe and Oa), organo-mineral (mostly A horizons and, to a lesser extent, AB horizons), and mineral (AB, Bw, BC, C, and C_R horizons) horizons, as aforesaid by cluster analysis.

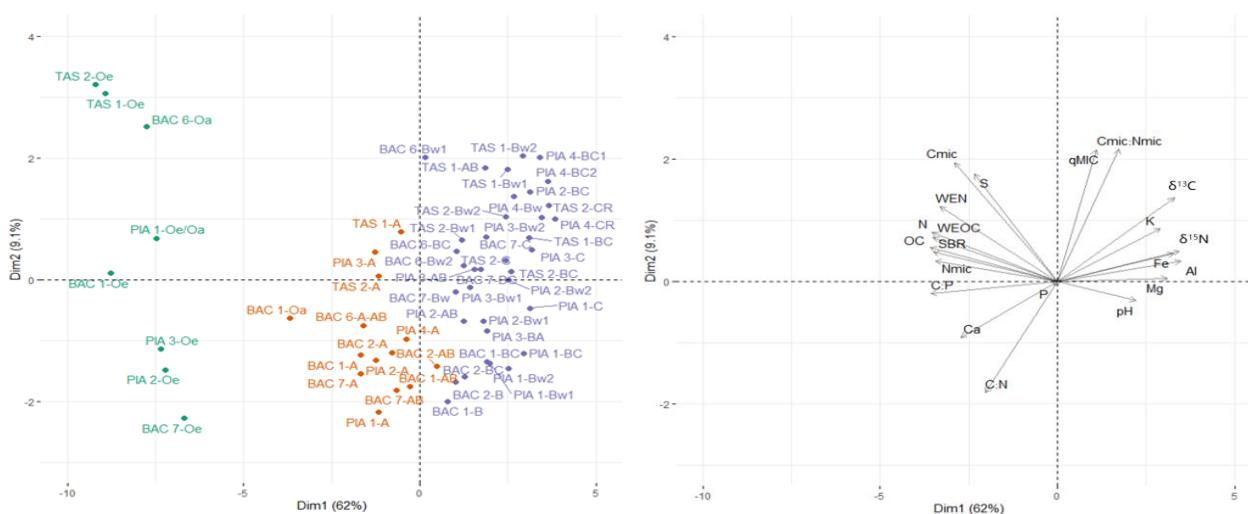


Figure 2. Principal component analysis: plot of statistical units grouped according to cluster analysis results (left); plot of variables (right).

As expected, the variables linked to biogeochemical cycles (OC, N, S, and P concentrations and C:P and C:N ratios), microbial biomass content (Cmic and Nmic), and its activity (SBR) characterized the organic horizons and, to a lesser extent, the organo-mineral horizons. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as well as terrigenous element (Fe, Al, Mg, and K) contents were positively correlated to mineral horizons.

3.3. Soil Profiles, Features, and Organic Matter Characterization of Bulk Soil

Considering the different soil types (i.e., Humic Dystrudept—HD, Humic Lithic Dystrudept—HLD, and Typic Dystrudept—TD), the amount of both nutrients and total elements found in the O, A, and ENDO layers is shown in Table 2.

Table 2. Mean and standard deviation of the chemical properties of the organic (O; Oe and Oa horizons), organo-mineral (A; A horizons), and mineral (ENDO; AB, Bw, BC, C, and C_R horizons) layers of the investigated soils. Within each column, means shearing similar letters indicate non-significant differences according to the Kruskal–Wallis test.

	pH	N	OC	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Al	Fe	Ca	Mg	P	S	OC:N	OC:P
		%	%	‰	‰	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹		
Humic Dystrudept													
O	4.0 c (0.30)	1.8 a (0.11)	37.9 (2.23) a	-27.66 de (0.60)	-2.82 c (1.30)	7.5 c (1.22)	6.0 d (0.97)	3.9 ab (0.12)	1.5 d (0.09)	0.7 a (0.10)	1.2 a (1.21)	20.9 a (0.44)	512 a (103)
A	3.7 c (0.02)	0.6 a (0.30)	9.2 (3.45) a	-26.79 cd (0.29)	1.45 bc (1.73)	27.2 bc (9.83)	15.9 c (0.83)	1.1 c (0.44)	3.5 cd (0.23)	0.7 ab (0.43)	0.6 a (0.56)	18.5 ac (6.07)	185 ab (96)
ENDO	4.5 b (0.24)	0.2 b (0.07)	3.3 b (0.97)	-25.57 a (0.27)	5.48 a (0.51)	37.9 a (5.79)	27.9 a (0.07)	0.7 d (0.76)	7.5 b (0.54)	0.6 ab (0.25)	0.2 bc (0.26)	15.8 c (4.24)	75 b (59)
<i>p</i> value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05	<0.05
Humic Lithic Dystrudept													
O	4.3 bc (0.35)	1.2 a (0.32)	27.8 a (8.79)	-28.13 e (0.46)	-3.64 c (1.15)	6.4 c (5.4)	5.1 d (4.23)	7.5 a (3.64)	1.8 d (0.02)	0.6 ab (0.10)	0.8 a (0.14)	22.6 a (1.17)	475 a (111)
A	4.4 bc (0.11)	0.5 (0.05) a	9.4 a (0.79)	-27.29 de (0.07)	-1.56 c (0.07)	23.3 bc (0.03)	14.9 c (0.09)	3.9 ab (0.04)	4.4 cd (0.04)	0.4 ab (0.04)	0.4 ab (0.07)	18.9 ac (0.34)	228 ab (6)
ENDO	4.6 ab (0.27)	0.2 b (0.08)	3.7 b (1.33)	-26.55 c (0.49)	3.60 b (2.00)	32.4 b (5.9)	23.6 b (5.61)	2.7 b (0.52)	7.1 b (0.24)	0.3 b (0.07)	0.2 cd (0.03)	20.5 a (2.38)	119 ab (40)
<i>p</i> value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05	<0.05
Typic Dystrudept													
O	4.2 bc (0.53)	1.5 a (0.23)	36.2 a (6.41)	-28.57 e (0.72)	-3.89 c (0.92)	9.9 c (0.40)	8.0 d (1.22)	3.6 ab (0.69)	2.4 cd (0.21)	0.6 ab (0.06)	0.9 a (0.11)	23.5 a (0.72)	587 a (60)
A	3.9 c (0.11)	0.4 a (0.18)	8.7 a (2.76)	-27.25 de (0.42)	-0.11 c (0.72)	28.2 bc (1.45)	17.4 c (0.73)	1.2 c (0.57)	5.7 bc (0.32)	0.5 ab (0.36)	0.5 ab (0.16)	20.4 ab (5.07)	175 ab (99)
ENDO	4.7 a (0.19)	0.1 c (0.05)	2.1 c (0.07)	-26.01 b (0.52)	5.43 a (1.28)	39.4 a (5.79)	25.3 b (3.85)	0.8 c (0.35)	10.2 a (0.27)	0.6 ab (0.27)	0.2 d (0.08)	16.6 bc (3.67)	59 b (79)
<i>p</i> value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05	<0.05

N, OC, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, Al, Fe, Ca, Mg, P, and S are the concentrations of total nitrogen, organic carbon, ^{13}C , ^{15}N , total aluminum, total iron, total calcium, total magnesium, total phosphorus, and total sulfur, respectively; ns = non-significant.

The investigated soils showed acidic conditions without significant differences among the soil types, with the exception of the ENDO layer, which showed slightly higher pH values in TD than in HD. Along the soil depth, lower pH values in the O and A layers compared to ENDO were observed for both HD and TD (Table 2). Concerning the total amount of elements (i.e., N, P, S, OC, Al, Ca, Fe, and Mg), neither O nor A layers showed differences among the investigated soil types. The only difference occurred for Ca in the A layer, whose amount was more than three times higher in HLD than in HD and TD. Unlike the O and A, the ENDO layer showed some more differences among the soil types. In particular, the concentrations of N and OC had the lowest values in the TD (0.1 and 2.1%, respectively). The highest amounts of Fe and Mg were observed in HD and TD, respectively, while the lowest Al content was found in HLD. Finally, the Ca content decreased in the order HLD > HD > TD. Similar to the total amount of elements, the C:N and C:P ratios did not show differences among the soil types for the O and A layers. The ENDO layer showed the highest C:N ratio (20.5) within the HLD. As expected, the amount of nutrients related to the SOM cycle (i.e., N, OC, Ca, Mg, and S) decreased with depth; conversely, those related to the parent material (i.e., Al and Fe) increased. Noteworthy, the A layer generally had similar element contents to the O layer (i.e., OC, N, Al, Mg, and S). The C:N and C:P ratios showed some differences between the O and ENDO layers of HD and TD, with higher values in the most superficial layer than in the deepest layer. Regarding the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, some differences among the soil types occurred only within the ENDO layer, where the lowest $\delta^{15}\text{N}$ value was observed within the HLD, while the $\delta^{13}\text{C}$ values decreased in the order HD > TD > HLD. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ increased with depth, but with similar values between the O and A layers.

Concerning the stable isotope fractionation in biological processes (-0.97, -0.88, -0.83 with R2 0.86, 0.90, and 0.96 for HD, HLD, and TD, respectively) within the bulk soil, $\beta_{\delta^{13}\text{C}}$ values showed an increasing trend as follows: TD > HLD > HD, as well as high values, mainly in the A layer, of $\epsilon_{\text{SOIL}}^{15}\text{N}$ in HD (Figure 3).

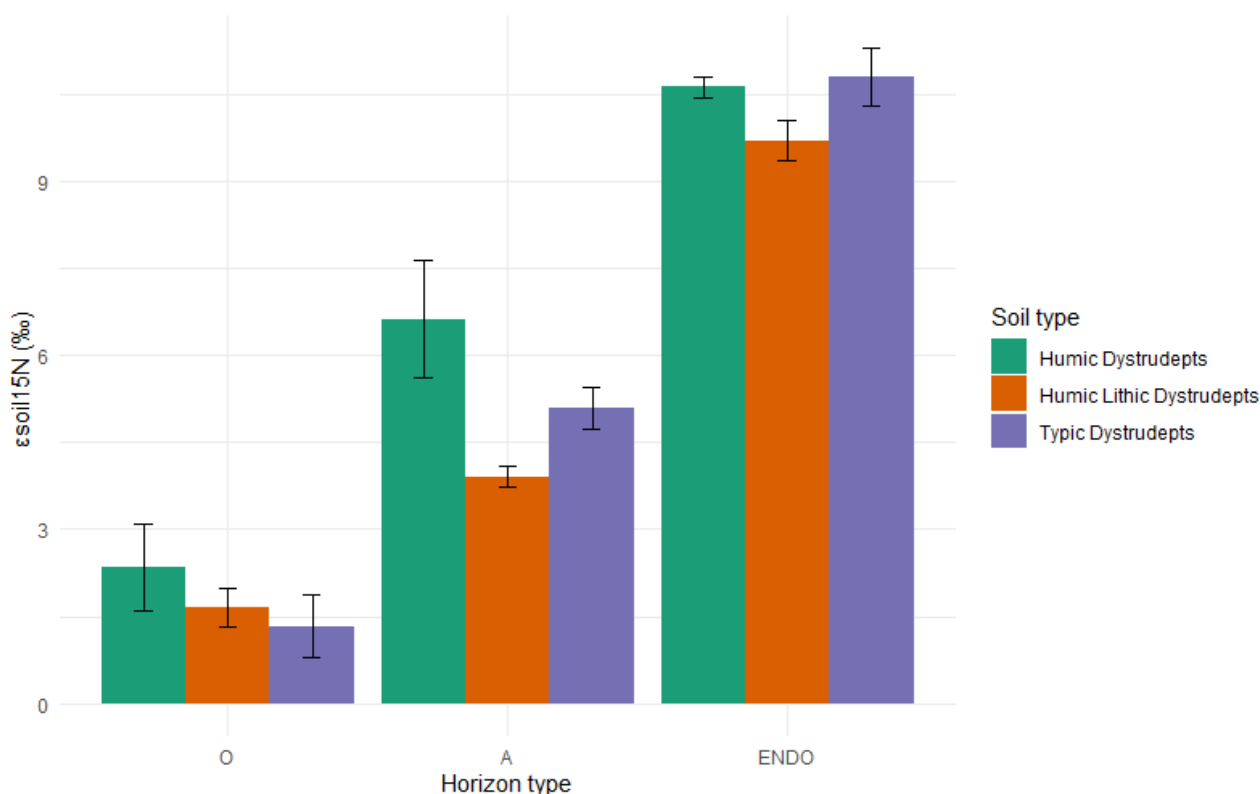


Figure 3. $\epsilon_{\text{soil}}^{15}\text{N}$ values for organic (O; Oe and Oa horizons), organo-mineral (A; A horizons), and mineral (ENDO; AB, Bw, BC, C, and C_R horizons) layers. Error bars are the standard deviation.

3.4. Soil Organic Matter Fractions

The chemical characteristics of SOM fractions are shown in Table S1.

In all soil types, the $\beta_{\delta^{13}\text{C}}$ of the obtained SOM fractions (Table 3) showed higher values for the POM fraction, while the humic substances (FA and HA) showed the more negative ones.

Table 3. $\beta_{\delta^{13}\text{C}}$ and R2 values resulted from the linear regression between $\delta^{13}\text{C}$ and log C for the humic acid-like substances (HA), fulvic acid-like substances (FS), non-extractable organic matter (NEOM), and particulate organic matter (POM).

	HA		FS		NEOM		POM	
	$\beta_{\delta^{13}\text{C}}$	R2	$\beta_{\delta^{13}\text{C}}$	R2	$\beta_{\delta^{13}\text{C}}$	R2	$\beta_{\delta^{13}\text{C}}$	R2
Humic Dystrudepts	−1.11	1.00	ns	ns	−0.61	0.87	−0.17	0.98
Humic Lithic Dystrudepts	−1.04	0.88	−1.09	0.48	ns	ns	0.27	0.97
Typic Dystrudepts	−1.41	0.64	−1.38	0.98	−0.63	0.98	−0.09	0.80

ns = non-significant.

Through the comparison of soil types, the major differences occurred for POM and humic substances. Specifically, while the $\beta_{\delta^{13}\text{C}}$ values of POM followed the order HD < TD < HLD, for the humic substances, the $\beta_{\delta^{13}\text{C}}$ values showed TD < HD = HLD.

The pattern of $\delta^{15}\text{N}$ values in relation to the C:N ratio of each humic fraction along the profiles' horizons is shown in Figure 4. Generally, the $\delta^{15}\text{N}$ values of POM in the deep soil were more negative compared to other fractions, indicating a lower SOM transformation, with the exception of HLD, where a great transformation in the ENDO layer was found. In HLD, thinner soils occurred as lithic contact was within 50 cm of the soil surface. Therefore, the shallower depth of ENDO compared to other types of soil must be considered. The C:N ratio in deeper horizons increased, losing nitrogen between 40 and 60. In the ENDO

Table 4. Cont.

	WEOC	WEN	Cmic	Nmic	Cmic:Nmic	qCO ₂	qM	qMIC	Dilly's Index
	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹		µgC-CO ₂ /µgCmic	%	%	
Typic Dystrudept									
O	1770 a (20)	153 ab (25)	523 ab (125)	310 a (30)	1.7 c (0.87)	1.01 ab (0.35)	2.9 a (1.21)	0.16 c (0.03)	167 ac (34.1)
A	696.3 ab (211)	54 b (13)	297 cd (87)	83 bc (46)	3.7 bc (2.11)	3.05 a (0.21)	2.6 a (0.67)	0.35 bc (0.04)	344 ab (67.2)
ENDO	336 c (183)	18 d (10)	118 e (74)	12 e (11)	14.4 a (11.01)	0.57 b (0.08)	1.8 bc (0.34)	0.58 b (0.01)	589 a (111,1)
<i>p</i> value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

WEOC, WEN, Cmic, and Nmic are the concentrations of the water-extractable organic carbon, water-extractable nitrogen, microbial biomass C, and microbial biomass N, respectively. qCO₂, qM, and qMIC are the metabolic, mineralization, and microbial quotients, respectively.

Regarding the indicators of soil microbial functionality, the qCO₂ showed differences among the soil types only for the O layer, with the highest values within the TD (1.0 µgC-CO₂/µgCmic). Instead, the qMIC showed some differences within the ENDO layer, with the highest value (9.8%) in HD. Considering the trend with depth, the qCO₂ showed a dissimilar trend among the considered soil types. Unlike qCO₂, the qM was similar between the O and A layers, which were higher compared to the ENDO layer. The qMIC, instead, showed some differences in HD and TD, with lower values in O than in the ENDO layer. The Dilly's index grew with increased depth, and HD showed along the soil profiles a great efficiency to use the OC by soil microbial biomass.

3.6. Functional and Genetic Biodiversity of Organo-Mineral Horizons

Figures 5 and 6 show the phyla of bacterial and fungal populations, and no significant differences were found among the A horizons of soil types. This is congruent with the lack of significant differences in functional parameters of microbial biomass in the organo-mineral horizons. In fact, no differences in microbial parameters were found (Table 4) except for the amount of Cmic in HD, which was significantly higher than in HLD and TD.

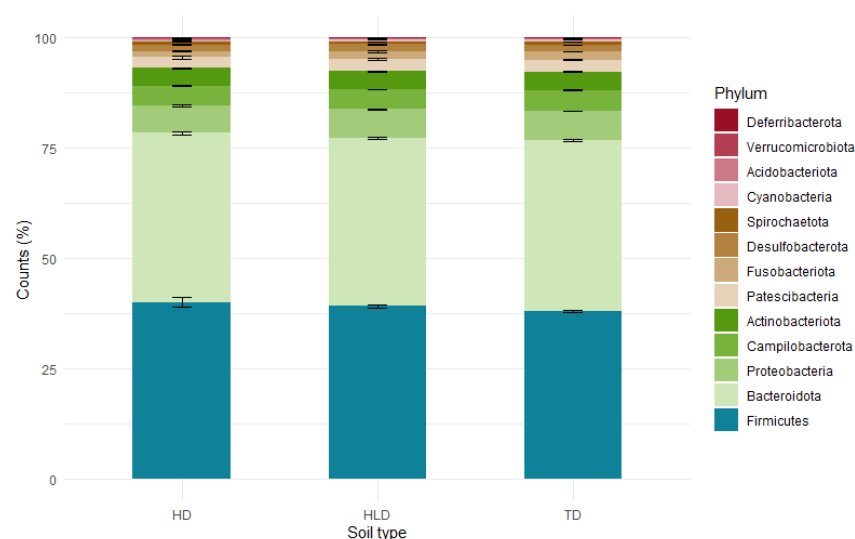


Figure 5. Phyla of bacterial populations characterizing A horizons in Humic, Humic Lithic, and Typic Dystrudepts. The error bars are the standard deviations.

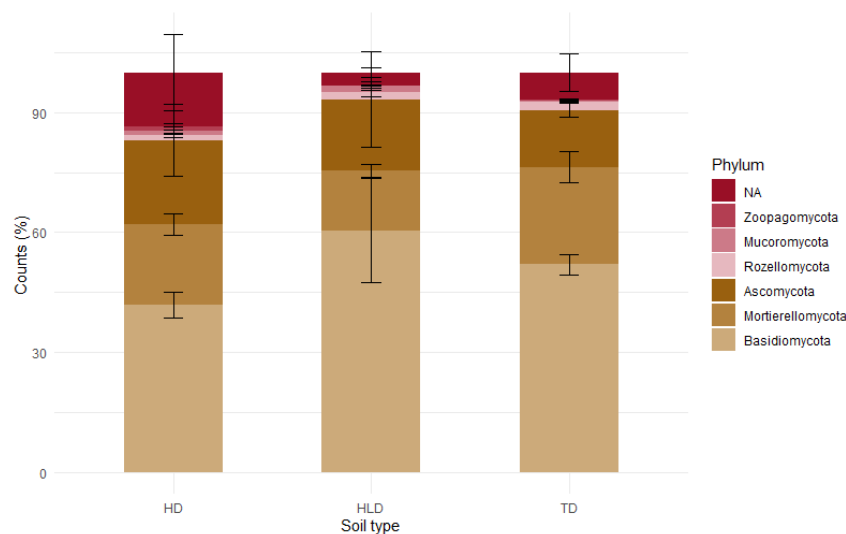


Figure 6. Phyla of fungi populations characterizing A horizons in Humic, Humic Lithic, and Typic Dystrudepts (HD, HLD, and TD, respectively). The error bars are the standard deviations.

In all soils, Firmicutes and Bacteroidota accounted for more than 75% of ATS, followed by Proteobacteria and Campylobacterota (Figure 5). Concerning fungal communities, Basidiomycota varied from 41% to 61%, followed by Ascomycota (from 14% to 22%) and Mortierellomycota (from 15% to 24%) (Figure 6).

Both Simpson's and Shannon's indexes highlighted a similarity between the bacterial populations in the three soil types at the phylum level (Simpson: 0.684, 0.692, and 0.696 in HD, HLD, and TD, respectively; Shannon: 1.47, 1.50, and 1.52 in HD, HLD, and TD, respectively). Similar values were found for fungal community at species level, for Simpson (0.8181, 0.8138, and 0.8564 in HD, HLD, and TD, respectively), and for Shannon index (2.667, 2.506, and 2.53 in HD, HLD, and TD, respectively).

Figure 7 shows the distribution of different fungi categories like ectomycorrhizal, litter-saprotroph, and soil-saprotroph fungi, highlighting a significantly higher prevalence of ectomycorrhizal fungi in all soil types. Litter saprotrophs are the least abundant group and appear to be absent in HLD. Despite that, no significant differences were found between soil types by considering fungal guilds.

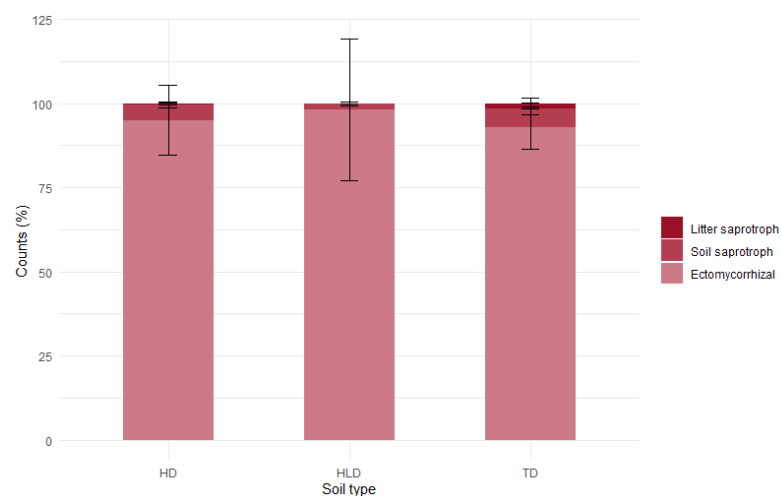


Figure 7. Distribution of ectomycorrhizal, litter saprotroph, and soil saprotroph within the A horizons in Humic, Humic Lithic, and Typic Dystrudepts (HD, HLD, and TD, respectively). The error bars are the standard deviations.

4. Discussion

4.1. Chemical and Biochemical Features of the Investigated Soils

The investigated soil types (i.e., HLD, HD, and TD) are some of the most frequent in European beech forests in southern parts of Europe [8,55,56]. In our study area, the lithic contact occurring within the 50 cm of soil depth for the HLD soil might be mainly due to the weaker alterability of the Macigno Massif sandstone formation, from where such soils developed (BAC), than the other sandstone formations [8]. However, at the BAC study site, the lack of lithic contact within the 50 cm of soil depth for the BAC6 soil profile could be attributed to its position. Specifically, the BAC6 soil profile was located on the ridge, which allowed the deepening of the soil [56,57]. Considering the soil profiles as a whole, a darker soil color observed within the upper 18 cm for the HLD and HD soils than TD [54] indicated an overall higher organic matter accumulation in HLD and HD compared to TD [58,59]. This fact might be attributed to the slightly lower plant biomass amount in the forests of TD than in those of HD and HLD due to the diverse beech forest management, which affects the organic matter input into the soils [60,61].

The clustering of the soil horizons was similar for the investigated soil types, and it was driven by the chemical and biochemical features of soil horizons linked to organic C and N cycles. Specifically, the cluster grouping of the O horizons was positively related to the biochemical soil properties (i.e., Cmic, Nmic, SBR, and WEOC), suggesting the role of plant residue quality on both O horizon features and the degradation processes of organic matter [62,63]. Since the organic matter degradation byproducts get into the underlying A horizon [64], this fact might explain the similar features observed among the A horizons. The clustering of O horizons positively driven by biochemical properties was in accordance with previous studies conducted in forest ecosystems [65,66]. Also, it was interesting to observe that the distinction of the cluster containing the O horizons was strongly affected by Ca content, indicating the occurrence of an intense biocycling of such a nutrient [67]. Calcium is a very scarce element in acidic soils, but beech trees can absorb it from the subsoil and transfer it to the soil surface via litterfall [68,69]. In accordance with early studies (e.g., [70]), the Cmic content decreased with soil depth. However, it was interesting to observe that, considering each soil profile, the HD showed the highest soil microbial biomass and the Cmic represented between 4 and 10% of organic carbon (qMIC). All the investigated soil profiles showed a slight increase in the Cmic:Nmic ratio from the O to the ENDO layer, suggesting a shifting of the microbial community to a fungi-enriched community with depth [71].

The qCO₂ values were higher in the A horizons than the ENDO ones in both HD and TD soils, indicating a higher energy demand by the microbial communities in topsoil than in ENDO [72]. However, using Dilly's index, it was interesting to observe higher values in TD than in HD and HLD, indicating a lower carbon use efficiency of the soil microbial community in the former than in the latter [30], which further might have prevented the melanization process in the upper 18 cm of TD soil, as depicted by soil color.

4.2. Soil Organic Matter Turnover

The highly negative $\beta_{\delta^{13}\text{C}}$ values of bulk soil suggest a rapid turnover of the organic matter in all the investigated soil types [50], likely due to the coarse texture and the low interaction with the mineral phase [73,74]. Although HD and HLD showed slightly more negative $\beta_{\delta^{13}\text{C}}$ values compared to TD, the higher organic matter accumulation defined by the darker soil color might be attributed to the long-lasting higher organic matter input due to the greater plant biomass amount [75,76]. In accordance with early studies, the $\epsilon_{\text{soil}^{15}\text{N}}$ value increased with soil depth [52] due to the increase of degraded and ¹⁵N-enriched organic matter [51,52].

Looking at the extracted organic carbon fractions, the organic carbon related to POM generally showed the highest $\beta_{\delta^{13}\text{C}}$ values and therefore the lowest transformation rate. The POM fraction is recognized to be a labile organic matter fraction because of the absence of any physical protection, making it easily degradable by the microbial community [77].

Despite this, the highest $\beta_{\delta^{13}\text{C}}$ values of POM might be attributed to the low degradation of such a fraction. In fact, the POM fraction showed the highest C:N ratio, which could indicate its low mineralization by the soil microbial community. Noteworthy, the similar POM C:N ratio between O and A layers would confirm the great influence of organic matter in O horizons on that of the underlying A horizon.

The highest $\delta^{13}\text{C}$ values of the humic substances (FS and HS) suggest how such compounds can be the results of microbial activity [78] and, therefore, they can be considered the most relevant by-product due to microbial SOM degradation and humification processes. In fact, early studies found that the humic substances could be easily degraded due to their low C:N ratio [79–81] especially if not protected from the soil microbial community [82]. The relative low C:N ratio and high $\delta^{15}\text{N}$ value would confirm the transformation of humic substances by microbial biomass. The higher N content in HS than in FS is in accordance with early studies that demonstrated that FS are small N-enriched molecules [83] coming from the depolymerization and transformation of organic matter and therefore the most degraded organic molecules [84,85].

Our findings generally showed that FS, HA, and NEOM had a lower turnover in HD and HLD compared to TD, which is in accordance with Ukalska-Jaruga et al.'s [83] finding that the type of soil can determine the direction of chemical organic matter's transformation. In particular, the NEOM fractions, which include highly aliphatic compounds [86] such as cutin and suberin, which are abundant in beech plant residues [87,88], showed differences about $\beta_{\delta^{13}\text{C}}$ values among the soils.

4.3. Genetic and Ecophysiological Biodiversity of the A Horizons

The soil biochemical properties of organic-mineral horizons were greatly affected by the edaphic properties [35,89]. In this sense, because of both the lack of differences in the chemical properties of topsoil among the investigated soil types and the greater influence of organic matter quality compared to its quantity [63,90], within the topsoil no differences in both biochemical properties and microbial community were observed. In fact, low variability in bacterial phyla among the investigated soils was found. Firmicutes and Bacteroidota, which accounted for approx. 75% of the total ASV detected, are considered root-inhabiting communities and thus prevailing in the root endosphere (in this case up to 95% of ATS), and their prevalence is not affected by soil type. In addition, many of the genera belonging to these phyla are commonly part of the animal microbiota and, in particular, of the gut microbiome [91,92]. Many of the remaining phyla (including Acidobacteriota) are signaled as members of the rhizosphere and excluded from the root endosphere niche. Within the soil microbiota, the *Bacteroidetes* tend to be a dominant phylum, just like in human and animal intestines, because of their ability to secrete diverse arrays of carbohydrate-active enzymes (CAZymes) that target the highly varied glycans in the soil [93], which can be considered precursors of humic substances [94]. Firmicutes have been found as dominant phyla in many forest soils around the world [95–97].

In our type of soils, much variability was detected in fungi communities, and Ascomycota, Basidiomycota, and Mortierellomycota can be considered the major microorganisms in forest soils, mostly involved in cellulose decomposition [98]. Ma et al. [99] showed that Ascomycota were the dominant communities in the early and late stages of residue decomposition. Both litter and soil saprophyte communities were mainly detected in TD, suggesting a greater role for dead wood in SOM turnover and, therefore, for the fungal communities that degrade it. Indeed, saprophytic fungi degrade cellulose and lignin, suggesting different sources, in addition to litter, of SOM supply in TD compared to other soils.

5. Conclusions

In weakly developed Dystrudepts in mountain areas, soil organic matter quality seemed to be the main driving factor regulating soil biodiversity. Some differences in soil functionality, however, appeared related to pedodiversity, at least as detectable by the

subgroup taxonomic level. In fact, lower melanization occurred in the upper part of the soil profile, where the plant density was lower and Typic Dystrudepts were present. Here, Dilly's index showed a lower efficiency of microbial biomass, and the highest presence of saprophytic fungi suggested the relevance of dead wood.

Because of the complex interlinks among pedodiversity, biodiversity, and soil functionality, our findings did not explain if the differences in biodiversity and soil functionality were the results of pedodiversity, or rather, if pedodiversity was their result. The proposed combined approach, considering soil diversity, however, appears to be very promising in investigations on soil C dynamics and in future research, and it could provide new insights into the complexity of the soil ecosystem.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15020353/s1>. Table S1: Concentrations of nitrogen (N), carbon (C), ^{13}C and ^{15}N , and C:N ratio in humic acid-like substances (HA), fulvic acid-like substances (FS), non-extractable organic matter (NEOM), and particulate organic matter (POM) for organic (O; Oe and Oa horizons), organo-mineral (A; A horizons), and mineral (ENDO; AB, Bw, BC, C, and C_R horizons) layers.

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