Micromorphology of organic matter and humus in Mediterranean mountain soils

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A B S T R A C T

Humus classification is one of the most useful approaches when studying the dynamics of SOM in an ecosystem. Until now, soil micromorphology has seldom been applied to the determination of humus types, in spite of the close relationship between soil organic matter and soil structure. Micromorphological techniques were applied to the study of humus soils under forest and pasture in the Catalan Pre-Pyrenean region in order to characterize particulate organic matter, its degree of decomposition and its relationship with the physico-chemical and biotic properties of topsoil. Fourteen thin sections were studied from the humus profiles of 5 soils (2 Typic Ustorthent, 2 Typic Calcisudent and 1 Typic Ustorthent). Nine fabric units related to organic matter were identified and counted through a petrographic microscope and in scanned images of the thin sections. The results helped to classify two humus forms (Amphi and Mull) that had statistically different qualities and quantities of micromorphological features and assemblages. Amphi humus forms were characterized by a laminated fabric in the OL horizon and by a loose dropping fabric in the OF and OH horizons; with droppings of mesofauna (enchytraeids, springtails and mites), and fungal elements (sclerotia, hyphae). Mull humus forms had larger amounts of earthworm casts and feces of Isopods/Arthropods/Diptera together with faunal pores. The use of scanned thin sections proved to be useful for the general determination of the humus forms, although for a detailed organic matter study the use of the optical microscope is essential.

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1. Introduction

The transformation and accumulation of soil organic matter (SOM) in terrestrial ecosystems occur mainly in the topsoil. The humus form is the part of the topsoil that is strongly influenced by organic matter and coincides with the sequence of organic and underlying organo-mineral horizons. The specific sequences and properties of these horizons, formed under given environmental factors, determine the type of humus form.

Humus as a decomposed organic matter was first described by Wallerius in 1761 (Senn and Kingman, 1973). However, the first one who defined humus forms in terms of the organization of soil organic matter in the horizons was P.E. Müller (1878). At the time, two main groups of humus – Mull and Mor – were identified. Mull humus appears in biologically active soils with well humified organic matter, while Mor humus develops in poorly active, acidic environments, with a slow humification. Moder forms represent an intermediate stage between them.

In the works of Zachariae (1964) and Bal (1970) the important role of soil fauna in soil organic matter disintegration was discussed. However, Babel (1975) described the most common humus components and fabric types of several topsoils found in Central Europe. Further work on humus forms led to their perception as indicators of ecosystem nutrient status through the reflection of SOM dynamics (Brethes et al., 1995; Ponge, 2003; Ponge et al., 1997; De Nicola et al., 2014). At the same time, field morphological studies and descriptions resulted in the revision of previous attempts at humus form classification and the development of several new ones, as the Amphi (Zanella et al., 2011), also called Amphimull (Brethes et al., 1995) humus type, appearing in Mediterranean, eutrophic environments where humification is not complete due to a seasonal water deficit or other factors. This type of humus forms corresponds to “Amphi humus” in the Référentiel Pédocologique (Baize and Girard, 2008). According to some authors, Amphi is the result from the co-evolution of Mull and Moder, as a response to a periodically dry sub-Mediterranean climate (Zanella et al., 2001; Galvan et al., 2008; Ponge et al., 2014). Nevertheless, Bottnner et al. (2000) indicate that it is common to find a “Xeromoder” overlying the A horizon of an earthworm Mull in Mediterranean forests, which is a typical feature of the Amphi humus type. Exhaustive characterization of Mediterranean humus forms including Amphi, related to the ecosystem functioning are given by Andreetta et al. (2011) and Andreetta et al. (2016).

All present-day classifications (North American, French, German, and European) are based on humus characterization in the field. Nevertheless, field observations cannot always give complete information on SOM dynamics, and additional analytical data is needed (Brethes et al., 1995). Although microscopy-assisted observations of disturbed humus...
have been applied to the quantification of some components (Galvan et al., 2008), the micromorphology of humus, through the study of thin sections of undisturbed samples is at present seldom applied.

The most comprehensive studies on the micromorphology of humus forms date from last century (Kubiśna, 1955; Zachariae, 1964; Babel, 1968; Babel, 1975; Pachuk, 1987). Mainly all of them are dedicated to Moders and Mors that are found in temperate climates and are based on their qualitative description, while little is known about humus micromorphology of Mediterranean region. Moreover, the micromorphology of SOM components is restricted to four basic forms depending on the degree of decomposition in the most recent guidelines for soil thin section description (Stoops, 2003). Thus, it would be necessary to enlarge and complete the methods of description and quantification of SOM forms through the microscope, so that they could be used to characterize the humus forms, as indicators of ecosystem health status, and help model the dynamics of soil organic matter and its turnover (Jeffery et al., 2010). Moreover, the interpretation of micromorphological features found in thin sections reflects the history of the site and its evolution (Bunting and Lundberg, 1987), which is essential in the evaluation of the management practices carried out on the soil (Pagliai, 1993).

The aim of the present paper is to propose a qualitative and quantitative method of studying soil organic matter in thin sections and its contribution to humus classification, in particular to Amphi humus forms, through its application to selected humus profiles with known chemical and physical properties from a Pre-Pyrenean region in the NE Iberian Peninsula.

2. Materials and methods

2.1. Study sites

The study area is situated in the northeastern Iberian Peninsula, in the Pre-Pyrenean mountain region within the Ribera Salada river basin, belonging to the Ebro river basin. The area is covered mainly by pine and oak forests.

The relief is tabular, sometimes with steep slopes over 20%. The altitude is between 800 and 1100 m with peaks reaching 2500 m asl. The substrate is made of massive calcareous conglomerates. The climate is typical Mediterranean with a transition to subalpine at higher altitudes. The average temperature during winter is 5.1 °C and average summer temperature is 20 °C. The annual precipitation is 731 mm, with two peaks in spring and autumn. The soils in the region are stony and calcareous. Topsoils from 5

<table>
<thead>
<tr>
<th>Site name and code</th>
<th>Vegetation</th>
<th>Altitude, m asl</th>
<th>Soil type (SSS, 2014/1USS 2014)</th>
<th>Humus form (Zanella et al., 2011)</th>
<th>Characteristics of the sampled soils.</th>
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<td>Natural riparian Pinus nigra forest</td>
<td>800</td>
<td>Typic Ustifluent/Orthofluvic Fluvisol</td>
<td>Pachyamphi OL</td>
<td>SOM (%) (1) 69.6 39.6 6.4 5 62.7 22.3 8.4 4.2 39.3 9.4 8.2 3.3 19.4 4.8 2.4 6.1 4.3</td>
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<td>Natural not managed Pinus nigra and Pinus sylvestris mixed forest</td>
<td>800</td>
<td>Typic Ustorthent/Calcaric Regosol</td>
<td>Eumacroamphi OL</td>
<td>Carbonates (%) – 32 32.5 34.1 – 32.6 36.4 40.6 – 33.4 30.8 38 7.9 8.1 8.5 8 8.3 8.1 8.3 8.3 7.8 8.2</td>
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<td>Typic Calciustep/Lepptic Calcisol</td>
<td>Eumesoamphi OL</td>
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<td>Oligomull OF</td>
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<td>Thymus vulgaris high meadow</td>
<td>1100</td>
<td>Tropic Haplustept/Lepptic Calcaric Cambisol</td>
<td>Eumull A</td>
<td>Bulk density (kg/m³) (1) 1200 1100 1300 1300 1400</td>
</tr>
</tbody>
</table>


2.2. Chemical analysis

The organic, organo-mineral and mineral horizons of the profiles were analyzed following the methods described by Porta et al. (1986). Seventeen soil samples (one per horizon, taking composite samples for OF and OH, see Table 1) were oven dried at 40 °C for 48 h prior to analyses. Samples from OL horizons were crushed in a mortar into thin powder, whereas the others were sieved through a 2 mm sieve following grinding. Carbonates were determined by the Bernard calcimeter. SOM in the OL horizons was analyzed by combustion, and in the rest of the samples it was analyzed by the Walkley-Black method (wet oxidation).

2.3. Humus sampling and classification

Observation and sampling of humus were conducted at the beginning of May 2015 in all selected study sites through the opening of a topsoil profile. Humus forms were classified following the European Humus Forms Reference Base (Zanella et al., 2011). During the process of classifying the sequence of organic horizons (OL, OF, OH), their continuity or discontinuity was taken into account as well as the macrostructure of the organo-mineral horizon (A) and the traces and types of soil faunal activity. At some points the litter horizon was not present or poorly developed, thus it was not sampled. Moreover, the sampling of OH horizons was done together with OF due to their thinness and gradual boundary with the overlying OF horizon.

2.4. SOM micromorphology

Micromorphological observations were conducted on 14 horizontal thin sections, 5 × 13 cm in size, following the methods of Benyarku and Stoops (2005). They were distributed as follows: 3 CS, 2 CO, 3 P, 2
CAN. 4 T. Due to their size, and to the irregularity of the horizon boundary, two or three horizons (often the organic ones) were found in the same section. Each thin section was examined with an Olympus petrographic microscope (BX51) to identify different microscopic features such as plant residues, roots, minerals, pores, humus substances, or faunal excrements.

Organic fine material, as defined by Stoops (2003) comprises organic material with no recognizable origin, including groups of five or less interconnected cells, amorphous organic fine material, punctuations and organic pigment in the inorganic micromass. These fabric units correspond to Babel’s (1975) “organic fine substance” and “organic matter as pigment of the mineral fine substance”. At the scale of the observation, it was difficult to distinguish the different degrees of mixing between amorphous organic fine material and organic pigment in the micromass, therefore it was decided to include all of them in one single fabric unit named organic fine material, following Stoops’ (2003) terminology, that would partly include inorganic micromass.

Based on these preliminary observations, the following 9 fabric units were used in point counting:

- plant residues other than roots: needles, leaves, shoots and tissues with different degrees of decomposition.
- roots with different degrees of decomposition
- fungi: sclerotia and hyphae
- big faunal droppings (0.2–1 mm or larger)
- small faunal droppings (20–200 μm, typically 50–100 μm)
- charcoal
- organic fine material (OFM)
- mineral components (quartz, limestone, calcite)
- pores.

It was conducted using an Olympus microscope with 2× magnification and an eyepiece reticle. The points were located along the parallel lines on the thin section. The component under the crosshairs at each point was identified. A total number of 300 points over the various components were recorded for each thin section. The results from point counting were expressed as the percentage of a given feature (Bernier and Ponge, 1994) for each horizon type.

In order to explore an easier, non-microscope assisted humus classification, all thin sections studied with the help of light microscopy were also used for computer-performed point counting, using a scan instead of the microscope. To obtain digital images suitable for further analysis all thin sections were scanned with a high resolution Epson scanner. Three randomly chosen areas, 1.8 × 4.8 cm, of each thin section were processed with the 4800 ppp (dots per inch) option. Thus, images with a 24 bit spectral resolution (“true color”) and dimension of 3363 × 9051 pixels were obtained. Each pixel represented an area of 28.09 μm². The set of scanned images was converted to TIFF format and processed with JMicroVision® software. Its random point counting option was selected to quantify features of thin section scans after image calibration. The limitation of the counting grid was set to 300 points. As with the microscope, when the image represented several horizons, then the point counting procedure was limited to the area of each one.

2.5. Statistical analysis

One-way ANOVA with Tukey–Kramer post-hoc test was used to determine the difference in SOM and carbonate contents in different horizons of sampled topsoils. Pearson’s product-moment correlation was applied to organic (OL, OF + OH), organo-mineral (A) and mineral horizons (Bw) to check the relationship between chemical and physical characteristics and micromorphological features found in thin sections of sampled topsoils.

In order to compare the difference between sampled topsoils according to collected chemical, physical and micromorphological data and to support macromorphological classification on the field we used Principal Component Analysis (PCA) based on the calculation of Spearman’s correlation. Micromorphological, chemical and selected physical variables (silt, clay and sand percentage) were designated as active, while bulk density, infiltration rates and coarse fragment percentage were inputted as supplementary variables, in order to omit the influence of missing data on the calculation of axes. PCA was performed on the dataset of organo-mineral A and mineral Bw horizons of the sampled soils and data from the previous physical investigations of the same topsoils (Loaiza-Usuga and Poch, 2009). Statistical analysis was conducted in Statistica 12 (StatSoft Inc.) and XLSTAT 2015 (Microsoft).

3. Results

3.1. Field classification of humus forms

The humus form which was found under holm oak forest (T) was classified as Oligomull. Bleached old oak leaves made a thin DLv horizon. The OF horizon was discontinuous and consisted of crumbled plant rests (mainly oak leaves) and granulated aggregates of organic matter. The OH horizon was not observed at all. The A horizon had a biomacrostructure.

The OL horizon was not present and the OF horizon was discontinuous in the humus form found in Thymus meadow (P). The OH horizon was absent. Nevertheless, the A horizon had a biomacrostructure due to the conspicuous earthworm casts. The abovementioned characteristics allowed the classification of this humus form as Eumull.

Amphi humus forms were found in Pinus nigra riparian forest (CAN), and on sunny and shaded aspects of mixed P. nigra and Pinus sylvestris forest (CS and CO). All these three humus forms had a well-developed litter horizon (OL), and underlying fermented and humified horizons (OF and OH), however the thickness of the latter was different. CAN, CO and CS humus were classified as Pachyamphi (OH ≥ 3 cm), Euamicoamphi (biomesostructured A and OH < 3 cm) and Eumesoamphi (biomacrostructured A and OH ≥ 1 cm) respectively. OL horizons of all Amphi were composed of fresh and old pine needles, bark and twig residues, pine cones, and moss.

In OF and OH there were pieces of deadwood with noticeable white mycelia on them. Fungal hyphae were observed on crumpled plant residues as well. The biostucture of the A horizon allowed us to distinguish the three humus Amphi subforms (Table 1) in the second level of classification according to ERB (Zanella et al., 2011).

3.2. Chemical characteristics

Table 1 shows the SOM, carbonate content and pH of the studied topsoils. Soil organic matter content was different in the horizons of all examined topsoils (p < 0.05), and is consistent with the horizon differentiation done in the field. A higher amount of SOM (%) was present in upper organic horizons (OF + OH), decreasing in the lower organo-mineral and mineral horizons for all profiles. In contrast, no statistical differences were observed between OM content of horizons of Amphi and Mull forms.

The carbonate content of all the Amphi form samples (CAN, CO, CS) was higher than in the Mull form (T, P). Moreover, the carbonate content in topsoil samples tended to increase with depth. However, this difference among organic, organo-mineral and mineral horizons was not significant (p ≥ 0.05).

3.3. Micromorphology of the Mull humus forms

The structural features of Oligomull were reflected in the thin sections of topsoils from the Torra site. They showed the presence of
discontinuous OLv and OF horizons. The OLv horizon consisted of crumpled holm oak leaves (Fig. 1a), grasses and their rests. Droppings of big and small fauna were also present. Similar to OLv, the OF horizon also had abundant plant rests and faunal droppings (Fig. 1b–c). According to the microscope point counts, the OF horizon of Oligomull consisted of 29% pore space, 21% small faunal droppings and 20% organic fine material (Fig. 2).

A characteristic feature of the A horizon was that the organic fine material was gathered in peds with cracks filled with angular aggregates (enchytraeid or springtail droppings) (Fig. 1d). The percentage of organic fine material was 36% while small faunal droppings were only 5%. Roots in different stages of decay were observed in A and also in the underlying Bw horizon.

The vertical distribution of all features found in the thin section scans had the same tendency as the microscopy results, even though the percentages were different. Nevertheless, the feature class “fungi” were not recognized in scans.

In the thin sections of the Prat (P) OF horizon, altered plant rests and numerous faunal droppings were observed. According to the point counts, plant rests were 13.4% while mite and enchytraeid droppings were 19.36%. Earthworm feces consisted of larger rounded aggregates of organic matter and small mineral grains, which occurred seldom in the horizon. All mentioned features formed a loose dropping fabric, with pores amounting to 36.7% of the volume of all features in the thin section (Fig. 2). The biomacrostructure of the A horizon observed with the naked eye was recognized in thin sections as organic fine material peds and numerous earthworm casts. With higher magnification enchytraeid feces and sporadic plant residues could be observed.

The Bw horizon was made of organic fine material and mineral grains. Cracks between peds were occasionally filled with altered droppings and root rests.

3.4. Micromorphology of the Amphi humus forms

Our micromorphological observations of prepared thin sections of topsoils from these three locations coincided with those made in the field, except for the Pachyamphi, since in thin section the thickness of the OH horizon was less than 3 cm, probably due to sampling problems. Micromorphology helped us to understand the biological conditions of SOM transformation, reflected in a layering of recalcitrant plant litter in the OL horizon with sporadic penetration of fungal hyphae, together with numerous enchytraeids and mite droppings in OF and OH horizons. The typical feature of all sampled Amphi humus forms was the laminated fabric of the OL horizon, which is the result of the overlying pine needles of different stages of decay, almost opaque or light yellowish plant tissues (epidermis), organic fine material, and sparse quartz (Fig. 3a). That is why in the OL horizon the major two feature classes were plant residues (pine needles, leaves of the understory, moss, plant tissues) and pores that made up of 26.5% of the total volume (Fig. 2). Partly decayed crumbled pine needles and leaves of dark brown color, sparse fine roots, plant tissues and hardly recognizable separate plant cells with a transition to organic fine material, together with dark small enchytraeid or springtail droppings (typically 40–100 μm long and 30–90 μm wide) and mite feces resulted in the loose dropping fabric often found in OF and OH horizons (Fig. 3b). The major feature classes were pores (23.5%), small faunal droppings (23%) and organic fine material (22.5%). Fig. 4 shows the high faunal activity as worm channels and chambers over the entire Amphi humus profiles on three scanned images of the thin sections. The organic horizons of the Pachyamphi (CAN) are thick and continuous, although in the scans the OH did not reach 3 cm. In the OL of CO frequent pine needle sections can be observed. The main differences are seen at the level of A horizon: biomesostructured in CAN and biomacrostructured in CO. The finer

Fig. 1. Oligomull under Quercus ilex forest. Brl — broad leaf rest from Q. ilex; Prl — aerial plant rest; D — feces of Isopoda/Diplopoda/Arthropoda type; and ED — enchytraeid droppings.
structure of the Pachyamphi is made of a finer crumb microstructure due to small droppings. On the contrary, the Eumacroamphi the crumb microstructure is coarser, due to large droppings. Large faunal droppings of Diptera, Isopoda or Arthropoda were found all over organic horizons in small amounts (8–9%). They had an approximately oval shape with easily recognizable yellowish plant tissues inside.

Fungal hyphae and sclerotia were found in all thin sections of Amphi samples, but were abundant, in particular, in organic horizons of Eumacroamphi (CO) (Fig. 3c) and Pachyamphi (CAN). They were gathered mainly near pine needles, large faunal droppings and roots.

The A horizon of the sunny part of Cogulers had 43% (CS) organic fine material, whereas the shaded part had only 8%. On the contrary, the A horizon of the shaded part (CO) was relatively abundant in fungi and roots (25 and 13%) (Fig. 2). Plant residues were absent in both.

The most typical feature of the A horizons of all the Amphi samples was the dark brown to reddish colored roots of differing decay stages. They were found between the organic fine materials which formed a spongy fabric. Within the organic fine material aggregates, reddish infillings of phlobaphene-containing plant tissues could be noticed. Cracks situated between organic fine material aggregates were sometimes filled with enchytraeid feces.

The mineral Bw horizon of all Amphis had a dense fabric made of mineral grains, roots, sporadic phlobaphene-containing plant tissues, charcoal, wood and organic pigment (Fig. 3d).

3.5. Relationships between micromorphological, chemical and physical characteristics

To check if there was any relationship between the frequency of the micromorphological features, SOM, carbonates, pH and soil texture (silt, clay and sand percentages), Pearson’s correlation coefficients were calculated for each horizon of all sampled humus forms. In the correlation analysis for organic horizons (OL, OF + OH) all feature classes in volume percentage were used, together with SOM. The correlation matrix is given in Table 2.

In the organo-mineral A horizons significant positive correlations were found between the amount of plant residues and small faunal droppings. Negative relationships were found between percentage of pores and mineral grains, clay and small faunal droppings (Table 3).

3.6. Principal Component Analysis

The PCA suggested eight factors (dimensions) that explained 100% of the variability using the microscope data. However, for the interpretation of results only the two first components (factors) were used as they explain in total 54.67% of the variability (Fig. 5).

The calculation of the first component (F1) was influenced significantly by pH, carbonate and silt percentage on the negative side of the axis; and by OFM and sand percentage — on the positive side of the axis. The second component (F2) was determined by plant residues, small faunal droppings and SOM percentages on the negative side of the axis. Roots, fungi and clay percentages represented the positive side of F2.

The position of sampling sites (humus forms) in the dimension of first two components shows clear separation between Amphi (COA, COBw, CAN, CABw) and Mull (PtA, PBw, TA, TBw). Amphi humus forms are situated on the left side of the graph showing close association with higher pH, carbonates, silt, clay, fungi, root and small faunal dropping percentage, whereas Mull humus forms are gathered on the right and are linked to OFM, plant residues and sand percentage. Nevertheless, Eumesoamphi (CSA) situated on the right side of the graph showing more similarity with Mull humus forms, even though it was classified as Amphi.

The first two axes (F1 & F2) explained 60.77% of the variability in PCA for the data acquired from scans. Similar groupings as with the microscope data are obtained. In this case Mull horizons are
associated to sand, bulk density, faunal droppings and high infiltration rates, while Amphiphorizon is associated with silt, carbonates, pH, organic fine substance and roots. Again, the exception is the A horizon of Eumesoamphi (CSA) that is grouped with the Mulls (Fig. 5).

4. Discussion

4.1. Micromorphological differences between humus forms

In our study all the Amphis developed organic horizons (OL, OF, OH). Their formation can be explained through their litter properties. Recalcitrant litter, which has a slow rate of biodegradation, is accumulated in the OL horizon and forms a laminated fabric. Babel (1975) observed the development of that fabric type in Moder humus and related it to the build-up of coniferous needles. However, other humus-forming constituents are present in organic horizons besides needles or leaves. For instance, numerous macrofaunal droppings especially those from Diptera, Isopods or Diplopods could be often found mainly in OL and OF horizons due to their feeding habit (Babel, 1975). This fact explains the positive correlation that we found between large faunal droppings with plant residues and SOM in organic horizons. Roots and OFS are also positively correlated, since OFS is a nutrient supplier for plants. On the contrary, when mineral grains increase (coarse material), there is a decrease in faunal activity reflected in less SOM and less big droppings. After some time, plant residues lose their coherence and transform into organic fine material that does not have any preserved structure from the original material (Babel, 1975). It occurs very often in OF and OH horizons compared to upper OL in all Amphihorizon samples. A similar process was described by Babel (1968) in a Moder profile under beech forest. The only category of plant residues that does not completely lose its structure and can be easily recognized among the organic fine material in lower organic and organo-mineral horizons is the one containing phlobaphene, a component of the suberine that is derived from pine bark, which is mostly present in Amphis. Babel (1975) showed that these residues can be preserved in a humus profile for a long time.

Organo-mineral A horizons in Amphim and Mull humus forms contain abundant organic fine material. It is often mixed with mineral grains forming a spongy fabric. The positive correlation of plant residues and small faunal droppings are again associated to meso- and micro-faunal activity. The negative correlation between small faunal droppings and clay could be due to the lack of enough aeration. Numerous cracks and holes among aggregates which are the result of faunal burrowing activity are evident in the Mull humus forms found in meadow and holm oak forests. The burrowing activity of soil fauna especially earthworms promotes the increase of pore volume that was noted by Babel (1968, 1975). Thus, it is not unusual that pores (mainly biogenic as channels and chambers) in A horizons of Mulls are one of the main feature classes observed in the thin sections.

**Fig. 3.** Amphi humus forms. Pachyamphi under *P. nigra* brook forest (a–c). Eumacroamphi under *P. nigra* and *P. sylvestris* mixed forest (d). ED — enchytraeid droppings; EP — epidermis; Fh — fungal hyphae; MD — mite droppings; Plr — aerial plant residue; Pn1 — pine needle first stage of decomposition; Pn2 — pine needle second stage of decomposition; Pn3 — pine needle third stage of decomposition; R — root; and Sc — fungal sclerotia.
4.2. Relationships between topsoil properties and humus form classification

The opposition of Mulls (P and T) and Amphi humus forms (CO and CAN) in the dimension of the two main axes of PCA using microscope data is influenced mainly by physicochemical and biological properties of the sampled topsoils. The first component of PCA (F1) reflects the physicochemical environment which creates the conditions of organic matter accumulation and transformation.

Mull humus forms appear to have better aeration due to the higher sand percentage and infiltration rate. Their opposition to Amphi humus forms is also associated with the percentage of organic fine material in the A horizons of Mulls and positively correlates with big faunal droppings. The latter is supported by qualitative micromorphological analysis of thin sections which showed a typical crumb structure of A horizons in PA and TA (Mulls) due to earthworm casts. This phenomenon is described in numerous studies (Ponge, 2003; Zanella et al., 2011; Ponge, 2013, De Nicola et al., 2014) which underline the rapid transformation of plant debris into soil organic matter and an intensive mixing with minerals as it passes the gut of earthworms in Mull. Furthermore, the burrowing activity of soil fauna results in a higher porosity of the substrate, thereby creating suitable conditions for the development of bacteria (Kubiéna, 1970) which in turn leads to faster decomposition of organic material (Ponge, 2003).

The abundance of small faunal droppings in the A horizon of Eumull (PA) was unexpected because of the commonly accepted concept of antagonism between earthworms and enchytraeids (Ponge, 2003). Galvan et al. (2008) found a positive correlation between percentages of enchytraeid droppings in organic horizons and the droppings incorporated with minerals of the same animal group in organo-mineral A horizons in humus forms developed on acidic substrates (Moder). The same was stated for earthworm activity. However, we observed enchytraeid feces mainly in cracks between macroaggregates formed by earthworms and root growth in the A and Bw horizons. They correlated significantly with the amount of pores. Similar observations were made by Zachariae (1964, 1965) and Babel (1968). In fact, earthworm feces could serve as the source of food for enchytraeids (Babel, 1975).

Being developed on a shallow calcareous substrate, Amphi humus forms have high amounts of carbonates, higher percentage of silt and higher pH. The PCA also shows that big faunal droppings have a negative correlation with high carbonate percentage and high pH. This implies the low representation and performance of earthworms, diplopods and arthropods in A horizons of all Amphis of our study area. Nevertheless, other mesofaunal traces (collembola, mites and enchytraeids) are present in different amounts depending on the specific soil type.

### Table 2

<table>
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<tr>
<th>Plr (r/p)</th>
<th>Rt (r/p)</th>
<th>OFS (r/p)</th>
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### Table 3

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<th>SFD (r/p)</th>
<th>Sand (r/p)</th>
<th>Silt (r/p)</th>
<th>Clay (r/p)</th>
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<tr>
<td>SFD 0.89/0.04</td>
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<tr>
<td>Sand ns</td>
<td></td>
<td>ns 0.97/0.04</td>
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<tr>
<td>Silt ns</td>
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<tr>
<td>Clay ns</td>
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<td>ns 0.97/0.04</td>
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present in the organo-mineral horizons of Amphi humus forms, which underline the role of these mesofaunas in the transformation of recalcitrant plant rests. An exception is the Eumacroamphi (CO), which is formed by macrofauna other than earthworms (e.g. Diptera, Isopods) in highly carbonated environments. The SOM decomposition by mesofauna takes place already in the O horizons, ensuring a functional connection between A and O horizons (Galvan et al., 2008), as can be seen in the scanned images of the thin sections (Fig. 5).

Fungal traces, which were noticed in all organic horizons of the Amphis, also contribute to the separation between Mull and Amphi in PCA together with clay and root percentages. This means that fungal activity flourishes on the sites rich in organic residues in A horizons, especially in the shadow, humid conditions of the soils with higher clay contents.

However, our Eumesoamphi (CSA), with a sunny aspect under a mixed pine forest, has similarities with Mull along the F1 axis. Its position between Mull and Amphi could be explained through the local conditions of humus formation. On the one hand, the A horizon has a high sand percentage and also a high infiltration rate. On the other hand, due to its sun-facing (drier) location, it has probably a higher litter input compared to Mulls, but lower than in other Amphis. These three factors contribute, to some extent, to a higher macrofaunal activity which resulted in higher percentage of OFM. As soon as the consumption of organic material by macrofauna (mainly earthworms) increases, the continuity between organic and organo-mineral horizons is prone to become vague, and vice versa (Brethes et al., 1995). This phenomenon can be related to the difference in macro- and micro-morphology between Eumull developed under meadow (Prat), Oligomull in holm

Fig. 5. Projection of the variables and the humus forms in the plane of two main axes of Principal Component Analysis: CANA, CANBw — Pachyamphi; COA, COBw — Eumacroamphi; CSA — Eumesoamphi; PA, PBw — Eumull; TA, TBw — Oligomull. Data acquired with the microscope and with scans.
oak forest (Torra) and Eumesoamphi formed in mixed pine forest (CS). Thus, it is possible to infer the shift from Mull to Amphi on basic substrates if an excessive input of recalcitrant litter and slower biological activity has occurred; and conversely from Amphi to Mull, which happens when litter is intensively transformed by earthworms.

The second axis (F2), positively correlated with roots and coarse fragments, and negatively correlated with SOM and plant residues does not correspond to any humus or biostructure classification, but rather to the capability of SOM storage. In our case, rock fragments would prevent the incorporation of organic matter to mineral matter by fauna (Callaham and Blair, 1999), but would not result in a different humus form. Indeed, A horizons of P (Mull) and CAN (Amphi) are the ones with less coarse fragments and higher SOM. These relationships also stress the role of plant residues as SOM source in these soils.

4.3. Quantification of micromorphological features in scans

Another method of quantifying the micromorphological constituents in thin sections of diverse humus forms is the use of scanned true color images with the forward processing in a specialized computer program (Aydemir et al., 2004). In the present study we applied this method in order to see if thin sections could be used for humus and SOM investigations without the use of microscopes.

Scan data can successfully be used to classify humus forms, although the variables slightly differ. The differences in the results of two analyses (microscope-assisted and scans) are due to the methodological problems when recognizing and counting features in scans, since they work at a lower magnification. Small features such as enchytraeid droppings, fungal hyphae and especially separate plant tissues were hardly or not recognized at all in the scans. The main reason for that is the resolution of the analyzed image. In our study each pixel represented an area of 28.09 μm² which was not enough for accurate detection of mesofaunal facies or tissues in our case. However, Protz et al. (1987) noted that the pixel size for mesofaunal facies recognition should lie at least within the 10⁻⁴ m scale. Also, the amount of organic fine material found with the use of scans was always higher than with the microscope in all samples. This is interpreted as a mix-up with other features due to their similar spectral characteristics and the resolution size. In addition, errors in feature quantification may be due to the quality of acquired image. Distortions, which are made while scanning, are one of the main sources of errors (Kooistra, 1991).

The only way to omit the errors in features’ interpretation is to reduce the pixel size, thus, enlarging the resolution. Unfortunately, with the increase of image’s resolution its size also increases, which involves the problem of image processing and analyzing, i.e. long scanning times and very large images (in our case the average size of a single image was already 720 kb). Thus, the larger features such as big pores, organic fine material aggregates, needles, leaves, shoots and wood could be recognized using these lower resolutions (10⁻³ – 10⁻⁵ m) (Protz et al., 1987), but for a finer identification the microscope should be used.

5. Conclusions

Despite the limited number of samples, the microscopic approach is a powerful tool that can be successfully applied to the description, examination and classification of Mull and Amphi humus forms, which can be related with some physicochemical and micromorphological characteristics. These relationships can be explained by the types of transformation of SOM by soil biota under specific microenvironments.

Mull humus forms are found in meadows as well as under forests. Under our Mediterranean conditions, annuals and perennials form an easy degradable litter that can be quickly transformed by soil fauna (especially earthworms), therefore the characteristic micromorphological features are big faunal droppings, organic fine material and high porosity.

In contrast, the Amphi humus forms, having a slower turnover rate (formed in more carbonated, less permeable environments), are micromorphologically characterized by plant residues in organic horizons that form a laminated fabric (OL horizon), as well as droppings of mesofauna (enchytraeids, springtails and mites), which are gathered in OF and OH horizons forming a loose dropping fabric, and fungal elements (sclerotia, hyphae). The latter are found in greater amounts compared to the studied Mull humus forms.

Regarding the methodology, both microscope assisted and scanned images could be used for the humus form classification. However, more accurate results are obtained from microscopy, while scans can only give the approximation of the amount of micromorphological humus components, mainly overestimating organic fine material. With the higher resolution images and more powerful computers it will be possible, in the future, to proceed with image classification techniques which will save time in detection and quantification of micromorphological features.

While only 9 different fabric units related to organic matter and biological activity were used for the micromorphometric analyses, it would be necessary to test whether using other groupings, like those distinguishing different degrees of plant tissue decomposition, could be useful for the analysis of the SOM dynamics and humus classification.

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