School of Agrifood and Forestry Science and Engineering

(ETSEA)

University of Lleida, Spain

SOIL ORGANIC MATTER POOLS IN MEDITERRANEAN FORESTS: A MICROMORPHOLOGICAL APPROACH

MSc Thesis under Mediterranean Forestry and Natural Resources Management (MEDfOR)

Student: Olena Zaiets (MEDfOR candidate)
Supervisor: Dr. Rosa M Poch Claret

July, 2015

ACKNOWLEDGMENT

I thank the European Commission for the opportunity and financial support to attend the ERASMUS MUNDUS Master in Mediterranean Forestry and Natural Resources Management.

I am very thankful to my supervisor, Dr. Rosa Maria Poch Claret for her constant support, help, and constructive advice. Special thanks go to the Department of Environment and Soil Sciences staff, University of Lleida, for their help to conduct the preparation and chemical analysis of soil samples.

TABLE OF CONTENTS

INTRODUCTION

Soil organic matter is the important source and sink of carbon in the global biogeochemical cycling. Its transformation and accumulation in forest and grassland ecosystems happens because of diverse processes which involve complex relationships of abiotic and biotic factors. This leads to the concentration of SOM in topsoils with specific sequences of organic and organo-mineral horizons. Thus, it defines a particular humus form.

The morphological differences among diverse humus forms can give information not only about the efficiency of SOM transformation but also about the reaction of forest ecosystem on management practices and growth cycle (Bernier & Ponge, 1994; Ponge, 2009). In this sense, humus forms are thought to be the fastest and the cheapest way of indicating the level of nutrient cycling in ecosystems (Ponge, 2003; Andreeta et al., 2010). That is why several classifications of humus forms exist and are being discussed at present (Green et al., 1993; Zanella 2011), based on macromorphological observations on the field.

As soon as the humus form is classified on the field, it is investigated in a laboratory in order to find the linkage between chemical and morphological properties of topsoil. Unfortunately, the expected relationship is not always found. On the one hand, humus forms rich on biological activity may well correspond to wide range of pH (Duchaufour, 1995) and performance of enzymes (Andreetta et al., 2011); on the other, different humus orders can show the same C/N ratio and SOM content (Brethes, 1995). One of the possible solutions is to look on soil organic matter of humus forms through the microscope. Complete studies on that were made by Zachariea and Babel in the late ´60s and ´70s. Most of them were devoted to humus forms in temperate climate. However, less is known about humus forms and SOM micromorphology in Mediterranean conditions.

The purpose of this study is to examine SOM in different humus forms in Mediterranean forests and meadows using a micromorphological approach. Thus, the objectives are:

- to determine quality and quantity of humus constitutes in thin sections of topsoils;
- to find out the relationships between chemical, physical and micromorphological characteristics;
- to explore the differences between found humus forms based on macro- and micromorphological , physical and chemical observations, in order to propose guidelines for humus classification through micromorphology.

Hypothesis

The determination of the amounts of the different humus components, mineral matter and pores found in thin sections of topsoils can be used to determine their belonging to a specific type of humus form, thanks to their interrelationships with chemical and physical characteristics.

Limitation

The study is carried out only on six sampling sites of Ribera Salada basin (Catalonia, NE Spain) during May 2015. Not all thin sections for corresponding sampling sites are available.

1. LITERATURE REVIEW

1.1. First investigations of humus forms

Being the part of the topsoil strongly influenced by decomposition processes, humus forms, which are characterized by different macro-morphological and micro-morphological structure, are substantial for numerous biogeochemical processes in ecosystem (De Nicola et al., 2014). Moreover, definition of humus form in a particular ecosystem can help to distinguish various patterns of topsoil's evolution in a sense of interconnections between soil organic matter (SOM), soil biota and plants (Ponge, 2003; Brêthes, 1995). Very often depending on the humus form found, one can even judge about successional stages in terrestrial ecosystems or its perturbations caused either by human activity or by natural events (Ponge, 2003). That is why the interest of soil scientists to humus and its spatial and dynamic distribution was raised since 18th century till present.

Initially the meaning of "humus" comes back to the times of Romans, where this term was addressed to the entire soil (Senn & Kingman, 1973). Only later after with the development of natural science on the one hand, and with more prominent agriculture practices, in the other hand, the term "humus" was applied to the organic matter of soils underlying its possibility to maintain development and growth of plants by providing nutrients (Liebig, 1847; Doran et al., 1996). However, Wallerius (1761) was the first one who defined "humus" as the decomposed organic matter (Senn & Kingman, 1973). Since that time the concept of humus as the substance of decayed plant residues which is consumed and transformed by soil fauna was discussed by Darwin, Dokuchaev and others (Dokuchaev, 1883).

The first attempt to classify humus forms due to various types of humus genesis was made by P.E.Müller (1878). He stated that humus formation depends not only on climate where it is developed, but also by plants and burrowing animals (Romell & Heiberg, 1931). This idea led to perception of humus forms as a biological entity. To be more specific, he identified two main groups of humus – mull and mor (later row humus or Trockentorf[1]), which are counter in a sense of their formation. Mull is defined as a porous, loose mass with a characterized crumby structure, which represents a mixture of organic matter of different level of decay and very well integrated with mineral soil as a result of extremely high faunal activity, especially earthworms (Romell & Heiberg, 1931). In contrary, mor humus has a very high in organic matter content, it is a non-porous and compact humus form which lays directly on the mineral soil (Müller, 1878). There was also noted the third transitional group of humus forms which by its characteristics reminded mor but with more loose structure as in mull due to faunal activity. That third type was recognized by Müller as mull-like mor which is known nowadays as moder. Unfortunately later researches such as Ramann (1905), Vater (1928) and Krauss (1930) used the Müller´s definition of humus types not to the entire organic layer on topsoil, but to artificially distinguished separate layers within it, which brought a great confusion in scientific literature at the beginning of the 20th century. However, works of Juncker (1930) and Bornebush (1930) combined with Hesselman´s (1926)

[1] Trockentorf (germ.) – dry peat

stratification of humus in different horizons marked by L, F and H brought the Müller´s idea back (Romell & Heiberg, 1931).

Investigating the humus formation as the whole entity with defined horizons one can observe the successive transformation stages of fresh litter according to different levels of faunal, microbial activity and abiotic factors. Thus, Hesselman (1926) proposed to divide the humus layers into several macroscopically recognizable genetic horizons, which was later adopted by Babel (1975) for microscopical investigations of humus in thin sections. Hence, according to them the genetic horizons of humus are the following:

L horizon (litter layer): loose plant litter morphologically not changed or little changed. L horizon further can be subdivided into Ln and Lv, which stands for fresh (up till 3 months) and altered litter. It consists to the greatest extent of coniferous needles or broadleaves slightly rotten. With aging and depth a little portion of organic fine substances represented by faunal droppings can be found (Zachariae, 1967).

F horizon, also known as fermentation or decay layer, is composed of plant residues, while in microscopical observations a portion of organic fine substances can be noted. The F horizon is subdivided into Fr and Fm, depending on the proportion of plant residue to organic fine substance. Fr consists mainly of parts of plant organs or tissues falling onto the soil surface, while Fm has a medium ratio of plant rests to fine substances. In the Fr subhorizon recognizable tissues as parenchyma and lignified residues are very often colored into brown through yellow and brown-yellowish hue. In the lower Fm subhorizon beside dark brown-reddish organic fine substance numerous fungal hyphae and their resting bodies are found. Moreover, the evidence of faunal activity could be even more vivid compared to upper laying humus horizons. Typically faunal droppings can be found in chambers, channels or cavities created by rain worms, collemboles, enchytraeids and mites or inside fragments of plant organs (Schwertmann and Zachariae, 1965).

Following F horizon lays the H horizon with its subdivisions on Hr and Hf . This horizon represents higher level of decayed organic residues that means that separate plant tissues and organs are not recognized anymore. However, in Hr subhorizon some roots or their parts can be still seen, while the Hf subhorizon consists completely of organic fine substances. Organic fine substances in this horizon are dark dull, though sometimes parts of plant tissues can be recognized. Charcoal occurs typically here, maintaining the features and the structure of the former plant residue. Quartz grains occasionally appear (Bal, 1970).

Finally, the underlaying Ah horizon (mineral organic horizon) is also subdivided into Ahh and Ahu according to the richness in humus. Ahh is remarkable by its crumble structure due to formation of aggregates made of fine organic substance intensively mixed with mineral particles. The color of organic aggregates is normally very dark brown. In contrary the lower subhorizon (Ahu) appears to be lighter (Bal, 1970). Coatings of organic material on quartz grains are absent due to its dissolution and washing downwards to the lower horizons. Fungi hyphae could be seldom found, neither roots nor their fragments.

According to the presence or absence of one, or of the combination of several horizons, the organic upper part of the topsoil can be assigned to one of the humus forms. Of course, chemical, physical and biological properties are relevant as well. Until present several attempts to classify humus formation have been developed in Europe and the USA. Definitely all of them share the common principle of division set up back in 19th century by Müller, however, the difference in terms and in categories of classification of humus groups still exist.

1.2. Classifications of humus forms and their principles: French, North American and German

French classification of humus forms

French classification of humus forms was first synthetized and revised in French forests by Brêthes et al. (1995), however, according to its authors could be also successfully used for Mediterranean, tropical and mountain humus forms which are not disturbed by any human practice carried out on the land (Brêthes et al., 1995). This classification is mainly based on biological, physical and chemical properties of soil organic matter which are linked to general transformation processes under biotic and abiotic factors. Thus, the classification follows general principles of the French Pedological Reference Base of soil types (AFES, 1992) and is not hierarchically structured. Nevertheless, the subdivision into horizons is following Babel´s proposal (1975). However, additional subhorizons in litter layer (OL) and fermented layer (OF) are defined, which underline the biological origin of humus. For instance, OLt is determined as a subhorizon of poorly decomposed plant material with earthworms casts, while the OFc subhorizon is the product of high fungal activity.

Humus forms are classified by Brêthes et al. (1995) according to their chemical properties and the possibility of organic-mineral matter interaction by soil biota. Thus, three main types of organo-mineral horizon can be distinguished:

1. Biomacrostructured A horizon is characterized by clay-mineral complexes mixed with macroaggregates due to the presence of earthworms.

2. “Insolubilisation” A horizon represented by precipitation of fungal (white-rot) metabolites on clay-iron particles.

3. “Juxtaposition” A horizon developed by plant-fungal residues of high level of decay (only some parts of tissues can be recognized except roots) combined with droppings of earthworms, arthropods and enchytraeids lying beside mineral grains.

A particular humus form will be defined depending on the general features of the Ah horizon and on the absence or presence of the H horizon (OH). For example, the presence of OH horizons is a very vivid feature of mors and moders because of slower cycling of biochemical processes due to climatic constrains, recalcitrance of plant residues and lower soil biodiversity (Ponge, 2003). In contrast, mulls do not have OH horizons. Figure 1.2 is showing the classification key by Brêthes et al. (1995).

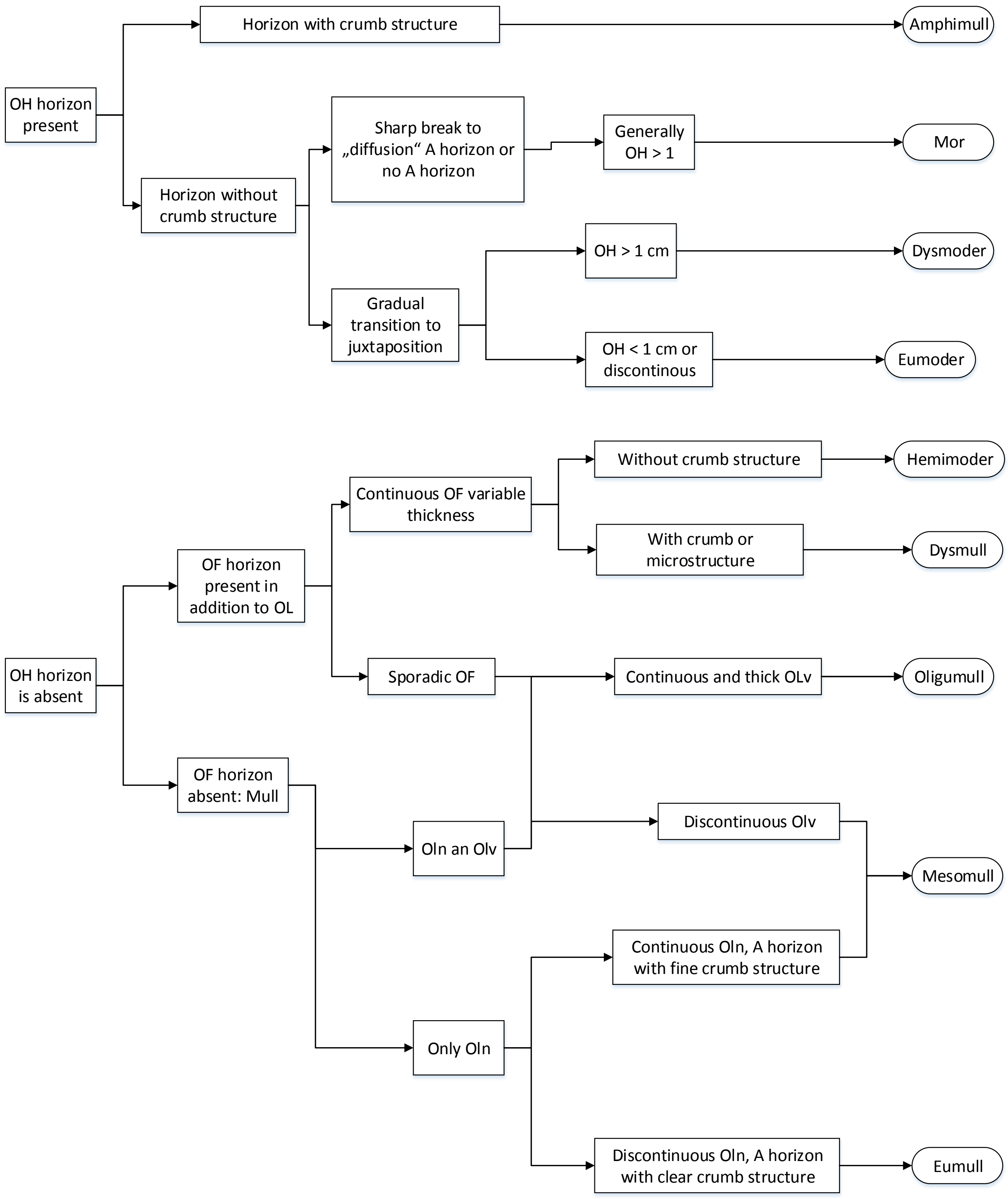


Fig. 1.2.The key to distinguish humus forms by Brêthes et al. (1995).

North American classification of humus forms

Humus forms cannot and should not be only classified according to morphological features but also to biological and chemical properties, in order to understand better the development of humus horizons and their dynamics (Brêthes et al., 1995). Similarly, Green et al. (1993) proposed classification of humus forms based on horizon sequence, thickness, fabric, color and biological activity, which was later tested in British Columbia, the U.S. and eastern Canada.

This classification is based on two-category system – order and group, with emphasizes both, the morphological features and their origin. The system has three orders based on the presence of F and A horizons which distinguishes between mull, mor and moder (the main humus forms´ orders). Moreover, there are 16 groups defined according to the observed dominated volume of organic matter, represented by decaying wood, root residues, and faunal excrements (Green et al, 1993). However, more detailed determination of a particular humus form requires the use of specific adjectives-qualifiers adapted from U.S. soil taxonomy (Soil Survey Staff, 1975). For example, charcic lignomoder means moder with abundant decaying wood within humus profile which occupies more than 35% of soil organic matter´s volume of which the majority is represented by charcoal. With 23 adjectives-qualifiers some of which could be only used for a specific humus group (mull, moder or mor) 21 possible combinations can be theoretically identified for mulls, 114 for moders and 119 for mors. Below is given a short description of the main humus form groups by Green et al., (1993).

Mull order

The mull order, as it was already underlined by numerous authors starting from Müller (1878) and until Ponge (2013) is the richest in biodiversity and is the fastest in decomposition rate form of humus, which can be found in a wide range of climates under those grassland and forest ecosystems having a favorable parent material. According to the U.S. classification of humus forms (Green et al, 1993) the main feature of mulls is discontinuous Fz or Hz horizon[2] or its complete absence. In opposite, the Ah horizon has a good incorporation of organic and mineral material, with a resulting crumby structure. In the mull order three taxonomical groups are recognized: vermimull, rhizomull and the badly drained hydromull. Vermimull and rhizomull are characterized by subangular or blocky Ah horizon which is the result of either intensive earthworm activity or fast decay of numerous fine roots by bacterias and fungi. They are found in humid temperate or subtropical and semiarid climates respectively (Green et al., 1993).

Moder order

Moders are combining features from both mulls and mors. Having the ability to accumulate organic material especially in L and F horizons they remind mors, whereas intensive decomposition of organic residues by earthworms, nematodes, springtails and enchytraeids produces that nicely mixed granular blocky structure of organo-mineral horizon which makes them similar to mulls (Kubiena, 1953; Babel, 1975). Moders typically have higher pH and lower C/N ratio compared with mors which are suitable for plant litter decomposition. That plant litter is commonly produced by deciduous broadleaves under different types of climate. Based on soil moisture, the origin of plant residues decay, thickness

[2] Lower case „z“ in horizon denotation stands for zoological origin

and the arrangement of horizons in the U.S. classification of humus forms (Green et al, 1993) moders are subdivided into 6 groups.

The first group, very close morphologically to mors, is mormoder, with a nicely developed Fa horizon which combines features of Fz and Fm horizons, and with a loose partly decomposed plant residues mixed with faunal faeces. The Fa horizon is followed by very thin Hh and Ah horizons or can even lay directly on a mineral horizon, as it happens with mors. Mormoders are typically found under dry pine forests.

Favourable climatic conditions combined with easily decomposed plant foliage and very active soil biota result in the development of leptomoder with thin Fz (till 3 cm) and comparably thicker Hh or sometimes Hz horizons.

Romell and Heiberg (1931) defined another one type of humus form which was known as "twin mull" (very similar to mulls). Later on "twin mull" was named as mullmoder by Green et al. (1993). The only difference between mullmoders and mulls is the presence of relatively thick F and H horizons (>2 cm) in the first one.

When moders are featured with abundance of decaying wood in all horizons they are classified as lignomoders. Lignomoders´ profile is generally the thickest one within the order due to greater recalcitrance of woody tissues to decomposition. Lignomoders, can indicate past disturbances in the ecosystem. The old-growth stands of even-aged structure or conversion from hardwood to softwood species can be a good example of it (Green et al., 1993).

Hydromoders and saprimoders are developed on poorly drained soils in very moist conditions and can be found at the edges of forest and swamp or riparian zones. The difference between them lays in the presence of Fz or Fa and Hh horizons (hydromoder) or direct placement of L horizon on Oh (saprimoder) (Green et al., 1993). These forms were known before as anmoor (hydromoder) (Kubiena, 1953) and humic peaty mor (Bernier, 1968), and can be considered as semi terrestrial humus forms (Zanella et al., 2011).

Mor order

The typical feature of the mor order is the accumulation of organic matter on the mineral soil through a nicely developed Fm horizon which, according to Babel (1975), reflects a slow rate of plant residue decomposition, often carried out by cellulose and lignin decomposing fungi and bacteria. The dominating fungal activity in the Fm horizon results in a compact matted structure with very poor or even no faunal activity, in contrast to moders, which have a well-developed Fz horizon with numerous voids and faunal faeces. The reaction (pH) of mors is acid. Due to that, only acid-tolerant protozoa, mites, nematodes, springtails and enchytraeids can be found (Babel, 1975). As the result of little faunal activity created by unfavorable conditions, recalcitrant organic matter with high C/N ratio tends to accumulate, thus favouring C and nutrient storage. Mor order is subdivided into 7 groups separating terrestrial and semi terrestrial humus forms (Green et al., 1993). Four groups of terrestrial humus forms are as follows:

1. Hemimors have commonly the thickest Fm horizon in comparison with the rest of mors. The absence or presence of thin H horizon can inform about the age of the hemimor and about environmental perturbations. Hemimors are characteristic of coniferous boreal forests with moisture deficiency (Green et al., 1993).
2. Humimors have a nicely developed Hh horizon which can even exceed the half of F and H horizons. This diagnostical feature of humimors is closely related to the processes of plant residue decomposition and humification of organic material. Fungal hyphae, however, also play an important role in the composition of this humus profile. Humimors are found in subalpine, mesothermal and boreal climates under climax pine stands (Green et al., 1993).
3. Resimors are separated from humimors (Bernier, 1968) because of the presence of diagnostical Hr horizon which is composed mainly of root residues and sporadically of other plant materials of reddish-brown color. That reddish-brown color indicates a lower intensity of humification compared to dark gray or black Hh horizons. The coatings of organic material can be found on mineral particles of Ah horizon due to good water penetration through the entire humus profile in humid subalpine, mesothermal and maritime climates. Resimors are dominated under coniferous climax forests by a *Ericacea* understory.
4. Lignomors have the thickest profile in the whole group which can exceed 40 cm. They are typical humus forms developed in subalpine, mesothermal and boreal conditions under old coniferous forests. According to the abovementioned climatic conditions and the abundance of wood debris Fw or Hw are formed with more than 35 % of woody residues. The faunal activity is depressed, whereas fungal is sufficient.

The three groups of semi terrestrial humus forms in mor order are developed in boreal, mesothermal and temperate climates with prolonged water saturation. That makes hydromors, fibrimors and mesimors to have badly aerated conditions that result in humus profiles with poorly decomposed plant residues. They can be recognized by Of, Om and Oh diagnostical horizons.

German classification of humus forms

The previous North American classification of humus forms proposed by Green et al. (1993) is based on biological-morphological features of a particular humus horizon but is still preserving the initial division of humus forms into mull, mor and moder. The same principle can be found in the German classification by AG Humusformen. However, the German classification is based on the separation of humus forms according to several hierarchical levels: section, order, class, and type (table 1.2.). Additionally can be used sub types and variety.

Table 1.2.The division of humus forms according to German classification. Arbeitskreis Humusformen (2014). Retrieved from http://www.humusformen.de/

Section	Order	Class	Type
Developed humus forms	Mull humus forms	Aeromorph	L-Mull
			F-Mull
		Aerohydromorph	L-Feuchtmull [3]
			F-Feuchtmull
		Hydromorph	Anmoor
	Humus forms with superimposed horizons	Aeromorph	Moder
			Rohhumus[4]
		Aerohydromorph	Feuchtmoder
			Feuchtrohhumus
		Hydromorph	F-Moor
			M-Moor
			H-Moor
Initial and non-standard humus forms	Humus forms with initial stage of humus formation	Aeromorph	Hagerhumus, F-Rohhumus
		Aerohydromorph	
		Hydromorph	

To distinguish between two sections in this classification the level of development of humus profile is used. Afterwards, the hierarchy the “Order” of humus forms is determined by the availability of humus accumulation in Oh or H horizons. Thus, when a H horizon is not found, then the humus form belongs to mulls. Consecutively, the order is split into three classes according to the moisture conditions: well aerated aeromorphs, transitional aero-hydromorphs and very moist and badly aerated hydromorphs. Classes in their turn are subdivided into types and sub types due to the particular sequence of diagnostic horizons. They are described according to the main parent material. For instance, Rhizo-F-mull which belongs to mull-order, aeromorphs class and F-mull type has nicely developed Ouf horizon which stands for great amount of fine roots that occupy more than 50% of total volume (AG Humusformen, 2014).

1.3. First European Reference Base of humus forms

The three abovementioned classifications (French, American and German) share in common the division of humus forms into mull-moder-mor ecological groups proposed by Müller (1878). Only in recent times, the European Humus Forms Reference Base (ERB) proposal developed by Zanella et al. (2011) has taken all previous works into consideration, but combining them with the IUSS Working Group WRB soil classification principles.

[3] Feucht (germ.) - moist
[4] Rohhumus (germ.) – raw humus or mor

First of all, the European classification separates terrestrial humus forms and semiterrestrial humus forms, depending on the submergence and water-saturation period. Furthermore, terrestrial forms are subdivided into Terroforms, Entiforms and Paraforms depending on the development of humus profile.

Terroforms are typical humus forms, nicely developed and never laying on the bedrock; in contrast to Entiforms, where the total thickness of OF and OH horizon hardly exceeds 5 cm. Paraforms are a result of intense biological transformation of decaying wood, where numerous living and dead roots can be found. Roots or wood occupy more than 50% of the bulk volume of OF and OH horizons, what makes them different to Terroforms and Entiforms (Zanella et al., 2011).

In all three divisions (Terroforms, Entiforms, Paraforms) mull, moder, mor, tangel and amphi are recognized.

Mull is developed under temperate climate on non-acid siliceous or calcareous parent material from easily decomposed plant litter with low C/N ratio due to activity of anecic and endogeic earthworms and bacteria. Thus, the decomposition of plant litter is very fast and does not accumulate, thus OH horizons are absent and/or the transition to organo-mineral horizons is sharp (Zanella et al., 2011).

Similarly, moders are also found in temperate climatic conditions, even though they are present in mild climates on acidic substrates. The biodegradation of plant litter is slower in comparison to mulls. Moreover, the major role in biological transformation belongs to arthropods, epigeic earthworms, enchytraeids and fungi. The OH horizon is always present and the transition to organo-mineral horizon is gradual unlike in mulls (Zanella et al., 2011).

Mors are mostly found in cold climates under unfavorable environment conditions. Recalcitrant plant residues undergo a very slow decomposition by fungi. That allows the development of OFnoz diagnostic horizon (Zanella et al., 2011).

New humus form in ERB, not included into previous classifications by Brêthes (1994), Green (1993) nor AG Humusformen, is amphi. It is distinguished from other humus forms because it is formed under Mediterranean conditions, generally on calcareous and dolomitic bedrock. However, there are some evidences of its presence in southern part of Alps (Graefe, 2007) and in Belgium forests (Ponge, 1999). Amphi was first known as twin mull under coniferous and broadleaf forests in Romell and Heiberg works (Romell & Heigberg, 1931) and later in Hartmann´s (Hartmann, 1952).

The sequence of humus horizons in amphi is represented by a litter horizon (OL); a fermented horizon (OFz) which generally consists of a great amount of droppings from enchytraeids, arthropods, and epigeic earthworms; and a OH which is developed like in typical moder and Ah horizons. Ah horizon has biomacrostructure due to intensive mixture of mineral grains and organic material by endogeic and anecic earthworms. The typical structure of amphi humus profile is shown on fig.1. 3.

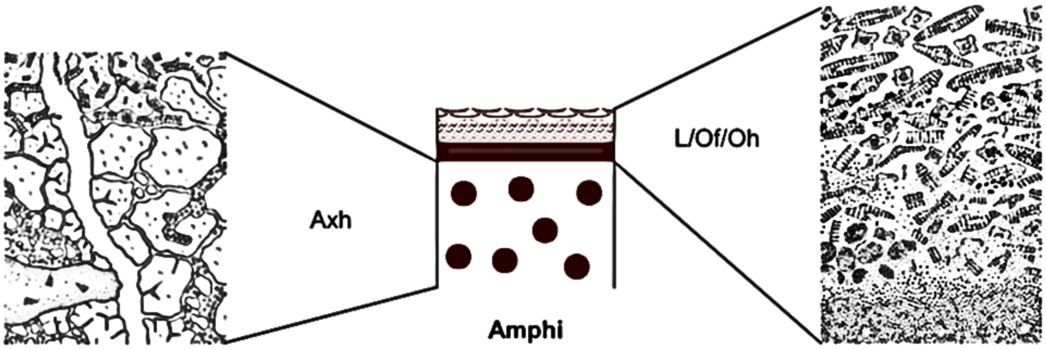


Fig.1.3. The typical amphi profile. Source: Graefe, 2007.

Tangel is the fifth humus form mentioned in ERB. As amphi, it is developed on limestone or dolomite bedrock but under cold, severe alpine and subalpine conditions. Biodegradation of plant material is very slow and maintained by epigeic earthworms, enchytraeids and arthropods which develop in the organic horizon. However, fungal activity is also present (Zanella et al., 2011). The division within the five main groups of terroforms (mull, moder, mor, amphi and tangel) is further subdivided into subgroups according to the sequence of horizons and their continuity (Table 1.3.).

Table 1.3. The division of the main humus forms according to ERB. Source: Zanella et al., 2011

Humus form	Prefix	Diagnostic horizons									
		OL	OLn	OLv	OFz	OF noz	OH	A biomacro	A biomeso	A biomicro	Anoz
Mull	Eu-		Ds[5]	-	-	-	-	Cs	-	-	-
	Meso-		Cs	Ds	-	-	-	Cs	-	-	-
	Oligo -		Cs	Cs	Ds	-	-	Cs or A biomeso	Cs	-	-
	Dys-		Cs	Cs	Cs	-	-	Cs or A biomeso	-	-	-
Moder	Hemi-	Cs[6]	-	-	Cs	-	Ds	-	-	Cs	-
	Eu-	Cs	-	-	Cs	-	Ds/1 cm	-	-	Cs or Anoz	-
	Dys-	Cs	-	-	Cs	-	Cs	-	-	Cs or Anoz	-
Mor	Hemi-	Cs	-	-	Cs	Ds	Cs	A absent (OH directly on E or AE) or if present then Ds A biomicro or Anoz			
	Humi-	Cs	-	-	Cs	Cs	Cs				
	Eu-	Cs	-	-	-	Cs	-				
Amphi	Lepto-	Cs	-	-	Cs		Ds/1 cm	Cs	-	-	-
	Eumacro-	Cs	-	-	Cs		Cs	Cs	-	-	-
	Eumeso-	Cs	-	-	Cs		Cs	-	Cs	Cs	-
	Pachy-	Cs	-	-	Cs		Ds/1 cm	-	Cs	Cs	-
Tangel	Eu-	Cs	-	-	Cs		Cs	-	Cs	-	-
	Dys-	Cs	-	-	Cs		Cs	-	-	-	Cs

5 Ds – discontinuous
6 Cs – continuous

The subdivision within mull, moder, mor, amphi and tangel is thus explained by the presence of diagnostic horizons. The organic OL, OF, and OH horizons are similar to those proposed by Babel (1975). However, the organo-mineral A horizon is subdivided into non zoogenic (Anoz) and three of zoogenic origin horizons (A biomacro, A biomeso and A biomicro). Such subdivision underlines the development of these horizons due to biological activity of either fungi or soil fauna. For example, the A biomacro horizon consists of anecic and endogeic earthworm droppings, their casts, roots and sporadic fungal hyphae, whereas the biomesostructure of A biomeso horizon is created by the activity of arthropods and enchytraeids. The biomicrostructure of the organo-mineral horizon (A biomicro) is obtained by smaller soil fauna compared to earthworms, mostly enchytraeids and microarthropods such as larvae of small insects, mites and collemboles (Zanella et al., 2011).

Another one group of terrestrial humus forms recognized by ERB is entiforms. They are developed under pioneer vegetation such as grasses, lichens and mosses and can be found on the primary stage of ecosystem succession or after strong and severe environmental perturbations (Zanella et al., 2011). The major decomposers of organic matter are bacteria and fungi, although enchytraeids, epigeic earthworms and arthropods are also present. Entiforms are subdivided into lithoforms which lay directly on Ct hard rock; peyroforms which are found among rock fragments, and psammoforms which are developed over sandy or sandy-skeletal parent material (Zanella et al., 2011). Thus, mull, moder, mor, amphi or tangel can be classified as lithomull, peyromoder, etc.

The last group of terrestrial humus forms in ERB classification is paraforms. Paraforms are subdivided into rhizoforms and lignoforms as a consequence of humus profile formation under the dominant influence of roots and their rhizosphere or decaying wood which makes 1/3 of organic horizons (Zanella et al., 2011).

Rhizoforms (mull, moder, mor, amphi, tangel) are developed under pastures. They can also be found under open coniferous forests at regeneration stage under ericaceous understory. The organo-mineral horizon consists of small aggregates which favors good aeration conditions of topsoil (Zanella et al., 2011). However, under Mediterranean conditions rhizoforms are often found under dense shrubby vegetation such as *Quercus coccifera*, *Quercus ilex, Arbutus unedo, Erica arborea* which form maquis. As a rule, under such conditions the organic horizons are nicely developed and represented by numerous root remains.

In contrast, lignoforms are developed under old forests with a great percentage of dead wood. The wood remains compose organic horizons of humus profile and could be also found in the organo-mineral A horizon as well. As woody debris is extremely recalcitrant to biodegradation the major decomposers are fungi. Soil fauna is also a decomposing agent of sapwood (Schwarze et al, 2000).

1.4. The development of humus forms

Sylvogenetic cycle and humus forms

The development of any humus form is controlled by soil-forming factors such as climate, topography, soil parent material, organisms (autotrophs and heterotrophs) and human activity (Barratt, 1969). The biological aspects of humus formation such as species composition and their richness (Ponge, 2003) together with climatic conditions and the properties of bed rock can mainly define the direction of humus form development on the scale mull-moder-mor. However, the human activity should be also taken in to account. For instance, the land use transformation such as conversion from pastoral or agricultural land to forest or other way around may shift from one humus form to another. As it was mentioned already in numerous studies (Barratt, 1964; Ponge, 1994; Willson et al., 2001; Ponge, 2013) humus forms are important components of forest ecosystems since they are able to store enormous amounts of organic carbon. Moreover, due to the fact that humus forms are the result of complex interrelations between autotrophs, heterotrophs and decomposers, they serve the habitat for microorganisms, soil invertebrates and vertebrates and provide suitable conditions for plant development and growth (Green, 1993).

Furthermore, in geological scale it does not take a long time for humus forms to develop, and because of that it has the ability to react fast after any kind of environmental perturbation. Thus, humus forms can be used as the indicators of ecosystems stability and can give important information about site nutritional characteristics. Therefore it has to be taken into account in forest management or other land use decisions.

The close tie between humus forms, soil, vegetation and soil fauna was described by Ponge (2003), Brêthes (1995), and Barratt (1967) through the prism of sylvogenetic cycles and pastoral land use.

The study of humus forms under the Norway spruce forest in French Alps (Bernier & Ponge, 1994) showed changes of the arrangement of soil organic matter in different types of humus forms depending on the stage of forest evolution. They found that the humus of the regenerated sites was a mull, with a disCt or absent OH horizon, formed by dark weathered faunal faeces. When natural regeneration of forest passes to the stage of young trees before complete canopy closure the great amount of forest floor debris consisted mainly from moss, leaves of understory species, their bud stages, pollen, and pieces of shoots and decaying roots. Such abundance of plant remains provokes extremely high diversity of soil fauna. All these materials forms mull humus similar to that of the regenerated site. In contrast, when the canopy of young spruce trees closes, the main litter source are falling needles. As it is well known, coniferous needles due to the high amount of lignin and low N are recalcitrant material for decomposition (Berg & Meentemeyer, 2002). Moreover, the residence time of coniferous litter can reach up to 10 years (Kurz-Besson, 2005). Excessive production of coniferous litter together with its slow decomposition rates lead to its accumulation on the forest floor. However, this does not influence on the activity of soil fauna (Read et al., 2004) but it presupposes the change in the proportion of bacterial and fungal community. Recalcitrant coniferous litter is the source of energy for fungal decomposers population of which may vary accordingly to the population of grazing invertebrate species and predators (Wilkinson, 2002). Under any circumstances, when the accumulation of recalcitrant litter prevails organic matter is preserved and results in the formation of mor (Stolt & Lindbo, 2010). Nevertheless, the available scientific data (Bernier & Ponge 1994; Brêthes, 1995; Salmon, 2008; Ponge, 2003; Ponge, 2013) shows the consistent change from mull type of humus to mor through moder. For instance, moders are commonly found under 50 and 60 years old spruce stands (Bernier & Ponge, 1994) where the intensity of soil faunal activity was evidenced by up to 5 cm thick OH horizon composed by holorganic faecal pellets.

Bernier (1997) and Zanella et al (2011) found out the influence of altitude on humus forms formation during forest cycle which is shown on the figure 1.4.1.

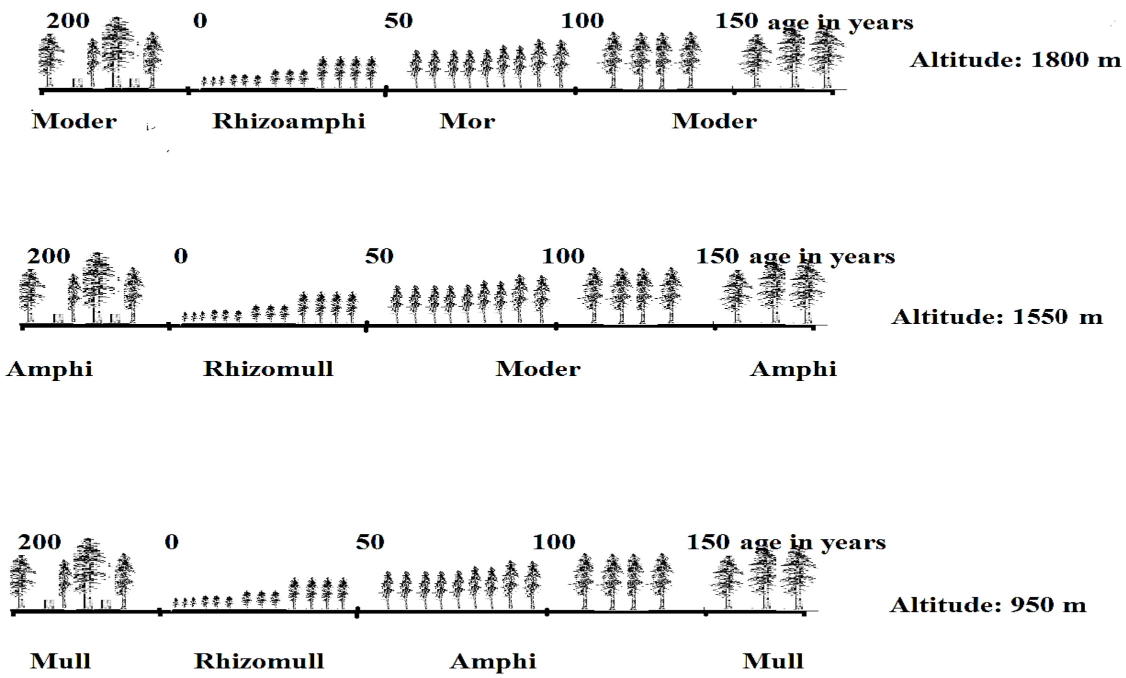


Fig.1.4.1. Humus forms development during forest cycle at different altitudes. Source: Zanella et al., 2011.

The development of moder under the mature stands at 1800 m can be explained by unfavorable conditions for faunal activity, what changes with the altitude decrease.

The shift from one humus form to another one during the forest cycle can also happen due to the development of another vegetation cover. For example, it has been noticed that when a gap opens in a forest canopy because of the death of a tree, a fast invasion of the opened space by herbaceous species occurs, together with changes in the quality of litter involving the succession of soil micro, meso and macrofauna (Bernier & Ponge, 1994). Moreover, in coniferous forests the fast and abundant development of heath and other ericaceous species in those gaps makes it difficult the future regeneration of coniferous forest. Hence, under an ericaceous cover a mor humus form develops (Bernier & Ponge, 1994), characterized by a very low faunal diversity (Ponge, 2003). The main actors of litter decomposition and forward humus formation there are fungi. However, the low amount of springtails, enchytraeids, Diptera larvae and earthworms is also found (Zachariae, 1967).

Deleporte (2001) confirms the poor diversity of earthworms (4 species with abundance 20 individuals/m^2 on average) found on mor and moder under beech forest in Fougeres (Brittany, France), compared to mull where they found 11 earthworm species with an abundance 500 individuals/ m^2.

The development of humus forms due to faunal activity

Since Darwin´s times the importance of earthworms in decomposition of plant residues and the alteration of upper horizons of humus profile was underlined (Alban et al., 1994). In numerous studies it has been observed that earthworms greatly influence soil chemical and physical properties (Swift et al., 1979; Satchell, 1983; Scheu, 1987; Alban et al., 1994). By digging tunnels in topsoil earthworms can reach up till 15 cm of depth pooling inside different plant fragments which are consumed together with finer organic material and mineral particles. That results in the development of a crumbed structured A horizon (Zanella et al., 2011).

Both anecic and endogeic earthworms are the main actors in the conversion processes from moder to mull humus forms (Bal, 1970). According to Bernier (1994), the thickness of mull humus form strongly correlates with the activity of epigeic earthworms which can burrow the topsoil until the depth of 10-15 cm. Making diverse tunnels and chambers these invertebrates cause very good mixing of organic matter with mineral particles what in its turn serves as a suitable environment for anecic earthworms. Indeed, the anecic earthworm *Lumbricus terrestris* is one of the first pioneer species after the openings of the forest canopy (Dunger, 1990). This was also proven in 50 years old spruce stand which was selectively thinned (Bernier & Ponge, 1994). In that case the populations of both anecic and endogeic earthworms strongly increased afterwards. Moreover, the species diversity and the presence of all functional groups of earthworms is a common feature of mull humus form (Berg & Staaf, 1987).

On acidic substrates under old beech stands mors and moders are found (Deleporte, 2001). The reason of their formation is a redundant accumulation of beech litter which does not decompose very fast due to poor population of epigeic, anecic and endogeic earthworms (Deleporte, 2001), even though springtails and enchytraeids help to mill the litter and incorporate it into the humus profile (Zahariae, 1967). Moreover, moder is commonly characterized by a slower rate of mineralization compared to other humus forms. The reason for that is an accumulation of organic material in lower horizons (OF and OH) which has a high C/N ratio. That was found in moder developed under 60 years old *Pinus sylvestris* stands (Bergmann & Fischer, 1999).

One of the constraints for the development of litter- and soil-dwelling earthworms is the moisture deficit in old beech forest which is very common during dry summer periods (Deleporte, 2001). In this period the litter is not protected against evaporation in stands with poorly developed understory. Deleporte (2001) observed the abundance of earthworms was twice times more under mature beech stands with good developed understory compared to stands with partial vegetation. Moreover, when the conditions are suitable for the development of earthworms moder is evolving to mull, especially during the regeneration stage. However, in some cases the diversity of soil fauna on mull can be not as high as expected. The reason for that is soil disturbance during harvesting operations, which affects soil fauna (Deleporte, 2001). The opposite effect was shown during thinning and clear cut in spruce and beech forests where silvicultural operations stimulated biological activity in the short term (Scohy et al., 1984).

The morphological change of mor to mull was also observed during the study of harvesting effects on soils in aspen forest in Minnesota (USA) (Alban, 1994). After harvest, light and nutrients had become available for herbaceous annual species, which generated easily degradable litter a with low C/N ratio. Besides, its availability prompted the expansion of microorganisms (mostly bacteria) and their predators. As a result the amount and diversity of earthworms increased just after harvest. After 14 years of mentioned above study the change of mor humus form -typical for aspen mature stands- to mull was explained by the abundant soil fauna activity. Furthermore, the decrease of litter layer was accompanied by a thickening of the A horizon. All these changes implied a redistribution of total carbon and nitrogen from upper humus profile horizons to lower ones (Alban, 1994).

The complex crumb structure of the lower horizons of mull humus profile is the result of an intense micro- and macropeds formation by soil fauna especially earthworms (Pawluk, 1987). Peds are formed by soil dwellers not only during their burrowing and digging activity, but also by consumption of organic material together with mineral grains and deposition of faunal droppings (Sandborn & Pawluk, 1989). Very often faecal pellets forming the upper organic layer of mull contain the plant tissue from mosses, grasses and other not recalcitrant plant litter. In contrast, in moder humus form partially or completely melanized plant tissues mixed with faunal droppings were found. These faunal droppings are often the result of mites´ and enchytraeids´ feeding close to the source of food which was already described by Zachariea (1967), Babel (1975) and Pawluk (1987). In enchytraeids´ droppings the rests of plant tissues are not seen but sometimes mineral particles can be recognized. They are common materials forming mull and moder profiles (Pawluk, 1987). Even though enchytraeids´ faeces may be numerous and building up F and OH horizons in a moder humus form, larger invertebrates such as larvae of Diptera, Tipulidae, Coleoptera and of course earthworms have a stronger effect on its morphology. Faeces of Diptera larvae are made of parenchymal tissues of leaves, needles and shoots which are not completely digested and can be the source of food for other invertebrates and fungi. Diptera larvae faecal material is mainly concentrated in upper horizons of moder but can be present throughout entire profile. Unlike Diptera droppings, faeces of Tipulidae larvae contain large pieces of melanized plant rests with small amount of mineral grains which are commonly found in L and F horizons. They are also the source of forage for enchytraeids which rework this material and in their turn deposit fine excreta into voids and channels (Pawluk, 1987).

The activity of Collembola is reflected through the feeding behavior on structureless moderately decomposed plant material which is found in the fermentation horizon of moder profiles. Likewise to enchytraeids Collembola also incorporate fine mineral matter with organic material, building up a loose dropping fabric in F and sometimes OH horizons (Pawluk, 1987).

Faeces of oribatid mites are found inside of big plant fragments such as decaying wood or roots where they explore cavities by consuming thin walled plant tissues. Very often mite pellets can serve as the source of food for Diptera larvae together with collapsed tissues (Pawluk, 1987). Together with enchytraeids and springtails, mite forage activity influences the development of lower horizons of moder humus profile. On the other hand, endogeic and anecic earthworms together with different kinds of larvae are the responsible for the mixing of organic matter with mineral one and for the formation of the crumbed Ah horizon, a diagnostic feature of mulls.

The drivers of soil fauna biological diversity are geology, climate, plant cover and human activity. The highest biodiversity of macro-, meso- and microfauna in forest soils was reported in mull humus form which is associated with fertile soils (Ponge, 2003). This is because mull, under grasslands and deciduous forests is rich on nutrients coming from the decaying roots and litter fall respectively, which are not used by low nutrient-use efficient understory plants and young trees (Vitousek, 1982). High nutrient availability combined with mild climate makes perfect conditions for a high diversity of soil invertebrates, which in turn maintain the high nutrient status of mull due to a fast turnover. Thus, the productivity of soil ecosystem of mull remains high even with some changes in soil reaction (pH). Due to that fact mull is suitable for successful forest regeneration. However, during the tree growth the nutrient uptake from soil sharply increases, while the litter input and nutrient availability decrease, giving rise to a change of soil fauna and biodiversity (Ponge, 1997). That is why moder humus forms are found under 40-60 years old and mature broadleaf and coniferous stands with decreased invertebrates' species richness with prevailing mesofauna (Ponge, 2003).

Both the litter input and climatic factors control the population of soil fauna (Bunting & Lundberg, 1987), which determines the way towards mor or mull humus development. If litter fall is dominated by a fast rate of consumption and decomposition the L horizon does not prevail and the transformation of fresh organics leads to formation and development of F horizons (Bunting & Lundberg, 1987). In mor the soil faunal activity is slower than in mull and moder, the acidity is higher, and this, combined with an excessive litter input builds litter horizon made of barely decomposed plant rests. Finally, the fermentation horizon (F) is made of brown-reddish plant residues penetrated by numerous fungal hyphae, typical of mors. The abundance of fungi in mor prevents the expansion of bacterial population due to strong allelopatic properties (Ponge, 2003). Thus, mor humus serves as a big pool of organic carbon as the result of accumulation of plant litter (Aerts, 1996), but it is not the optimal substrate for forest regeneration (Bernier & Ponge, 1994).

The change between humus forms during forest stand rotation can be not only explained by the abundance and richness of soil fauna and the input of plant litter, but also by vertical distribution of soil dwellers. Such vertical distribution is on its turn determined by the behavior of soil invertebrates, their physiological and ecological requirements and the source of food (Ponge, 1995). For example, the litter formed from young broadleaf trees and herbaceous understory is a good source of food for mites, enchytraeids and diptera larvae. The consumption of parenchymal tissues and thin-walled xylem by this soil fauna results in numerous faecal pellets which are serving as a source of food for microorganisms and other invertebrates (Bunting, 1987). The mixture of leaves fragments, bud scales, shoots and faunal faecal pellets as the result of enchytraeid and collembola activity frequently compose Lv and Fz horizons. Furthermore, enchytraeids can be found also in deeper horizons of the humus profile. It is commonly observed that their faecal pellets are incorporated with mineral particles and amorphous organic material forming holorganic and organo-mineral horizons of moder humus form. Sometimes when enchytraeids are not abundant in deeper horizons mites and springtails are present (Ponge, 1999).

Brêthes (1995) distinguishes between moder and other humus forms, indicating that moder has a characteristic soft transition from the OH to the A horizon, being the latter composed by holorganic faeces not incorporated into mineral material. Ponge (1999) explains this by the vertical movements of several centimeters of enchytraeids who actively consume collapsed plant tissues and faeces of other soil dwellers such as mites together with small quartz grains (5-60 µm of size) (Babel, 1968). The abovementioned behavior of enchytraeids leads to the development of the compact pelleted fabric in moders. To conclude briefly, under unfavorable conditions for earthworm activity, enchytraeids are the main ‘soil engineers’ and take a leading role in formation of moder humus form (Schwertmann & Zachariae, 1965).

In the forest cycle humus forms react relatively fast on changes induced by tree growth and senescence. In the beginning of the regeneration the easily-decomposable plant litter from the herbal cover serves as a source of food for soil fauna, dominated by earthworms (Deleporte, 2001). During tree growth the uptake of nutrients for tissue built-up is higher than its return to the soil. Thus, earthworms being sensitive to environmental changes are becoming less abundant (Ponge & Delhaye, 1995). That leads to formation of moder. Later on, during the maturation stage the trees slow down their growth rate and take fewer nutrients from soil. During this phase earthworm population recovers, especially under canopy openings due to the death of some trees. In such a way mull is recovered (Ponge, 2003).

2. MATTERIALS AND METHODS

2.1. Study area

The study area is situated in the Northern-East of Spain (Catalonia, Solsona) in the Prepyrenean mountain region within the Ribera Salada river basin. The total basin area occupies 22 250 ha covered mainly by pine and oak forests. Along the river banks riparian forest ecosystems represented by pines, willows, poplars and alders are frequently found. The agricultural area is utilized for potato crops and high mountain pasture.

The relief is tabular sometimes with steep slopes over 20 %. The altitude is between 400 – 2400 m with peacks reaching 2600 m asl. The substrate is made of massive calcareous conglomerates. The climate is typical Mediterranean with transfer to subalpine type of climate on higher altitudes with average temperature during winter 5.1°C and average summer temperature 20°C.

The average precipitation and temperature data observed in Solsona meteorological station during the period 2000 – 2012 are given in Fig.2.1.

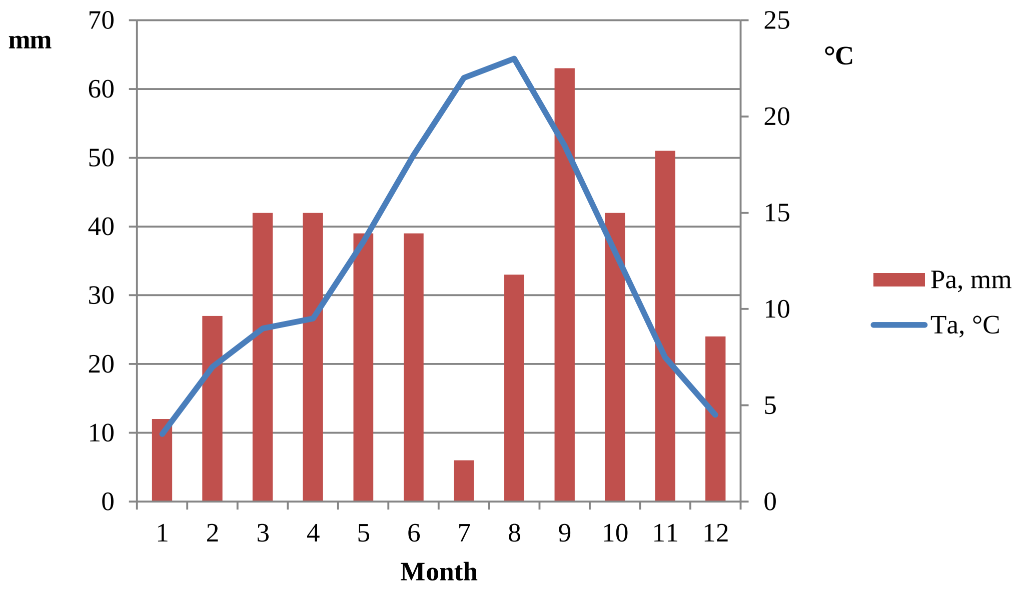


Fig. 2.1 Climatic diagram based on data from Solsona meteorological station (2000 - 2012). Pa: average monthly precipitation; Ta: average monthly temperature.

2.2. The description of sites

Within our study area four different natural forests and one meadow were chosen for topsoil sampling (Fig. 2.2.1): Torra, Canalda, Cogulers (shaded and sunny), Prat and Ramonet. These profiles were been previously studied for soil moisture monitorning, physical and soil-water analyses by Loaiza (2004 and 2007)and Martí (2007) among others, from which the main soil properties and information were obtained.

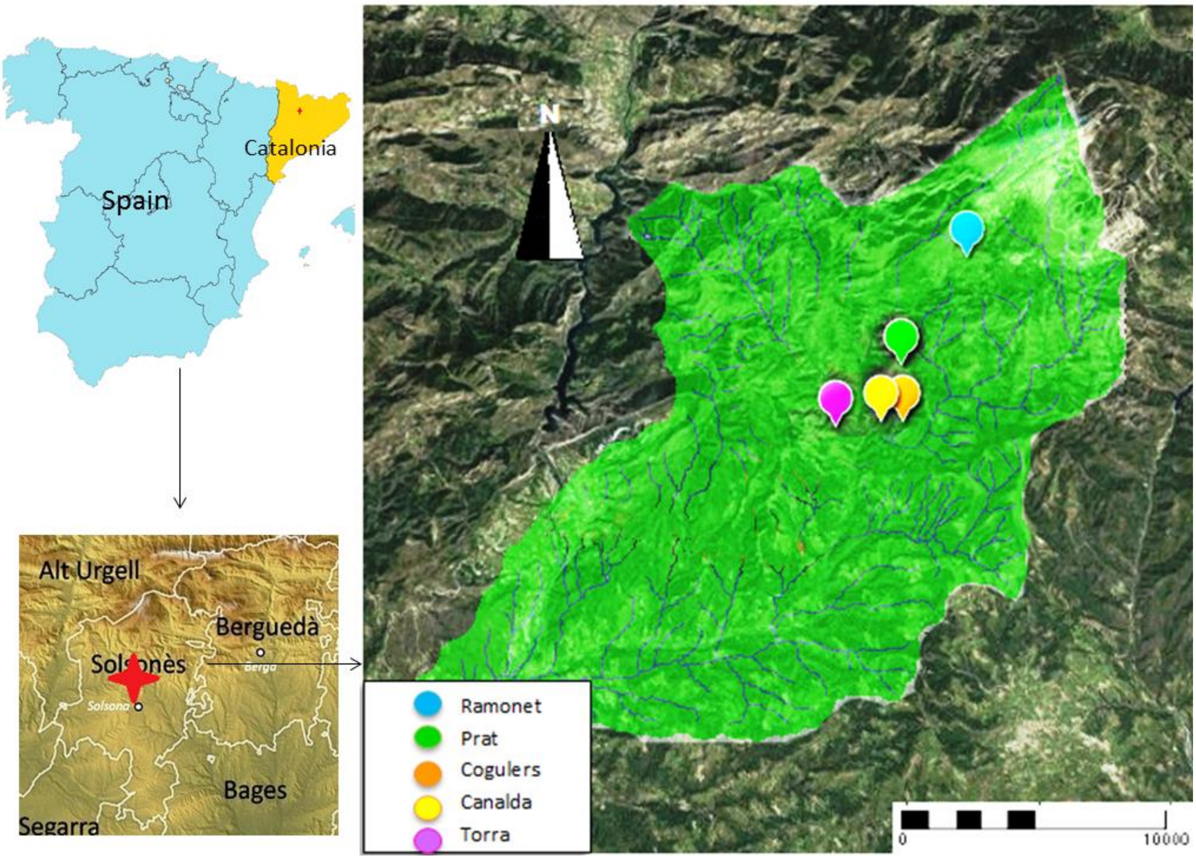


Fig. 2.2.1. Sites location

Torra

Site Torra is located in 1 km from Cal Torres farm, La Torra de Montpol, at 900 m asl level. Geomorphology of this site is upper part of a steep slope (45 %) of elongated type and convex-concave form. The site is very well moderately deep drained.

The soil is classified according to Soil Survey Stuff (1975) as Typic/Lithic Calciustept formed on conglomerate parent material. The first organic horizon is represented by sparse oak leaves with low stage of decomposition. Under the organic horizon a dark brown (7.5YR3/4), sandy loam A horizon of subangular blocky structure with abundant quartz and rock is found. This horizon is followed by a brown (7.5YR4/4) sandy loam Bw and a strong brown (7.5YR5/6) sandy loam Bwk horizon which lay upon a partly weathered conglomerates (C/R horizon). The soil profile is depicted on Figure. 2.2.2.a

The vegetation of site Torra is represented by *Quercus ilex* unmanaged forest with *Buxus sempervirens* and *Spartium junceum* understory (Fig 2.2.2.b). Forest is used for low intensity cattle pasture and wood.

Fig.2.2.2. Torra site: a) soil profile; b) Holm oak forest.

Canalda

Canalda site is situated 4 km from Cal Torra farm at 800 m asl, on a small alluvium plain of Canalda river. In spite of that, the site is very well drained.

The soil is classified as Typic Ustifluvent (Fig. 2.2.3.a). The organic horizon is made of pine needles grass residues and moss. Underlying the A horizon there is a dark brown (10YR3/3), sandy loam horizon with granular structure. Bw1 and Bw2 horizons are of dark yellowish color (10YR4/6 and 10YR3/4 respectively), sandy loam and with granular fine structure. C horizon is dark yellowish brown (10YR4/6), sandy loam and structureless. Parent material is made of conglomerates.

The vegetation is a typical riparian forest represented by young *Pinus nigra* trees with *Alnus glutinosa* and *Buxus sempervirens* understory (Fig. 2.2.3.b).

Fig.2.2.3. Canalda site: a) soil profile; b) riparian pine forest.

Cogulers

Cogulers shade site is on the left margin (NW aspect) of the Cogulers gauging station and located at 800 m asl. Geomorphology is characterized by a 45 % slope of V-shaped concave valley. The site is well drained. The soil is developed on calcareous parent material with underlying conglomerates and classified as Typic Ustorthent (Fig. 2.2.4.a). The profile is represented by Oe, A, Bw and C horizons. The top organic horizon (Oe) mainly consists of moderately decomposed pine needles and other vegetation residues. Following there is an A horizon rich on faunal activity traces and of dark yellowish brown color (10YR4/6). It is of loam texture with subangular blocky structure, slightly plastic. Bw horizon is of yellowish brown color (10YR5/6), clay loam texture and subangular blocky structure. C horizon is structureless, sandy loam and of brownish yellow color (10YR6/8).

The vegetation of this site consists of mixed unmanaged *Pinus nigra* and *Pinus sylvestris* forest. The human influence is occasional cattle pasture (Fig.2.2.4.b).

Cogulers sunny site is on the right margin (SE aspect) of the Cogulers gauging station. The altitude is 800 m asl. Geomorphology is the same as for Cogulers shaded site but with 40 % slope. The soil is classified as Typic Calciustept (Fig.2.2.4.c). The upper organic horizon (Oe) consists of grass residues, pine needles, branches and bark. The underlying A horizon is of dark yellowish brown color (10YR4/6), has sandy loam texture and subangular blocky structure. Bw horizon has dark yellowish brown color (10YR3/6), loam texture and subangular blocky structure. The last Bwk horizon is very dark grey (7.5YR3/1), loam – sandy loam, and has a subangular blocky fine structure.

The composition of the forest species is the same as for Cogulers shaded site (Fig. 2.2.4.d).

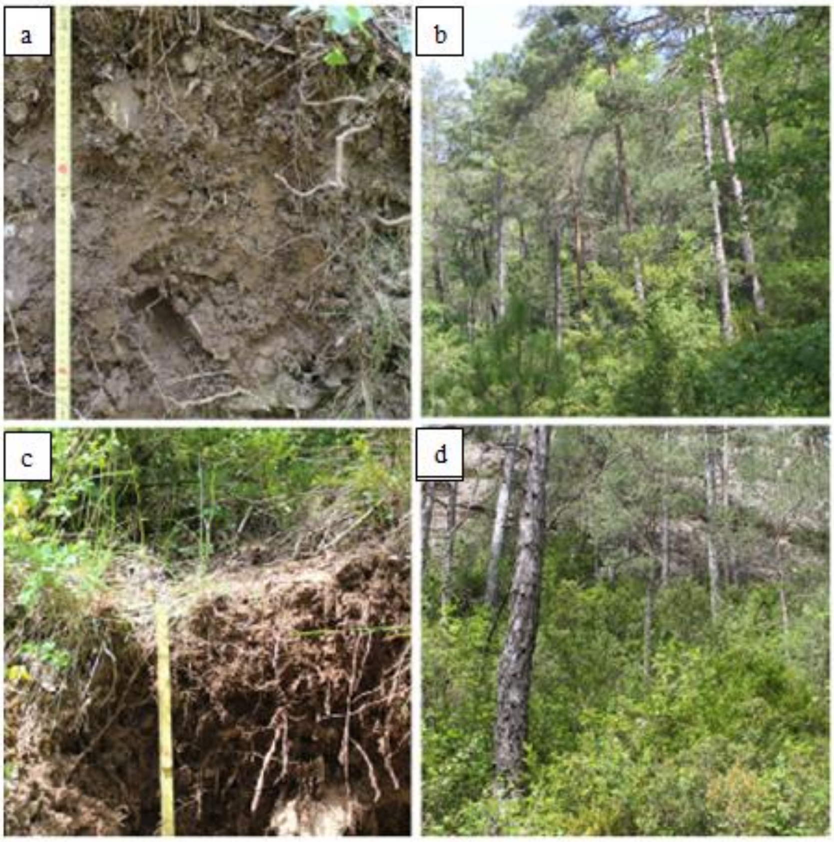


Fig.2.2.4. Sites Cogulers (shaded a-b; sunny c-d). Left (a,c) – soil profiles. Right (b,d) – forest.

Prat

Prat site is located between Cogulers and Odèn stations at 1100 m asl. It is situated on a upper position of a straight slope with 24% steepness. It is very well deeply drained.

The soil is classified as a Typic Haplustept (Fig.2.2.5. a). Soil profile is represented by A, Bw1 and Bw2 horizons. The A horizon has a dark brown color (10YR3/2), loam - sandy loam texture and subangular blocky structure. The Bw1 horizon has a brown color (10YR4/4), has a loam - sandy loam texture and a subangular blocky structure. The Bw2 horizon is dark brown (7.5YR3/4) and has a loam texture. The structure is the same as of Bw1 horizon.

The vegetation cover is represented by by *Thymus vulgaris* meadow (Fig.2.2.5.b). The human influence is occasional low intensity pasture with an oat-sanfoin rotation.

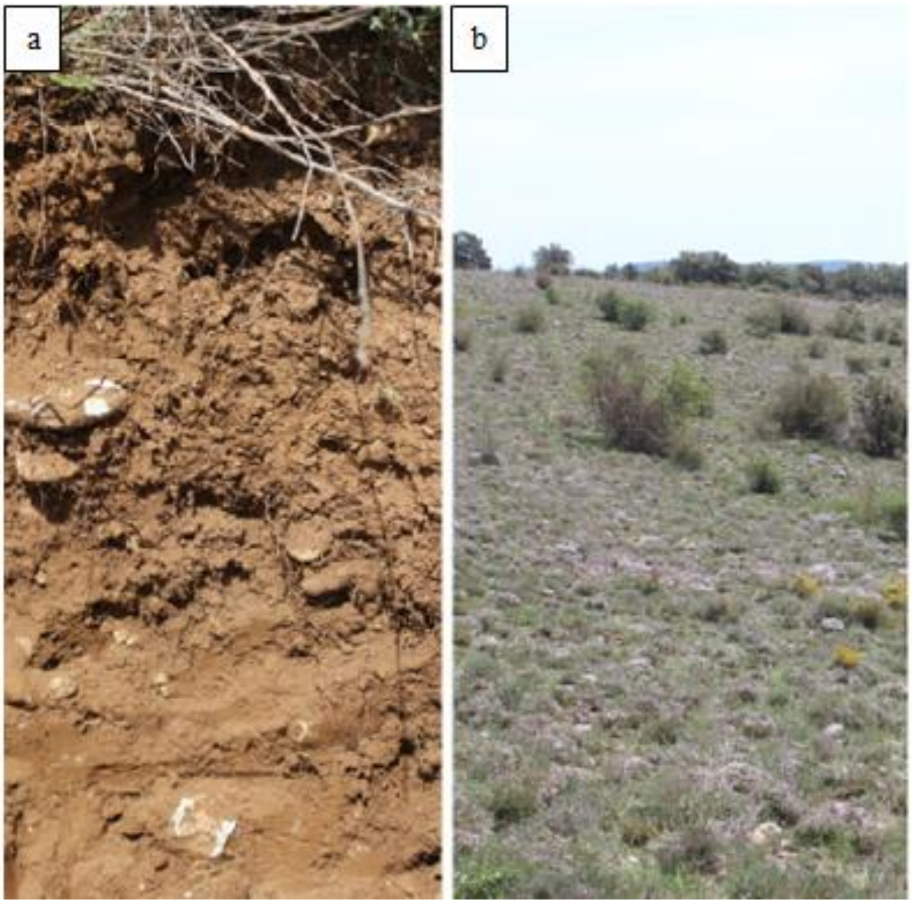


Fig.2.2.5. Site Prat: a) soil profile; b) *Thymus vulgaris* meadow

Ramonet

Ramonet site is located near Cal Ramonet farm, upper part of Odèn, at 1600 m asl. it is located at the upper part of a slope with 22 % steepness. The site is well drained. The soil is classified as Typic Udorthent (Fig.2.2.6.a). Organic horizons (Oi and Oe) consist of pine needles of different stage of decomposition, bark, branches, grass residues, and dead wood with abundant fungal hyphae. It follows an A horizon with dark brown color (7.5YR3/4), clay texture, compact and slightly plastic, with crumb structure. It overlies an AB horizon with reddish brown color (5YR5/4), with clayey texture, compact and sticky, with abundant coarse fragments. The last Bwk horizon has a red brown color (5YR5/5) and a clayey texture. It is compact, slightly plastic, structureless and with a lot of coarse fragments. The vegetation cover is *Pinus sylvestris* marginal forest (Fig.2.2.6.b).

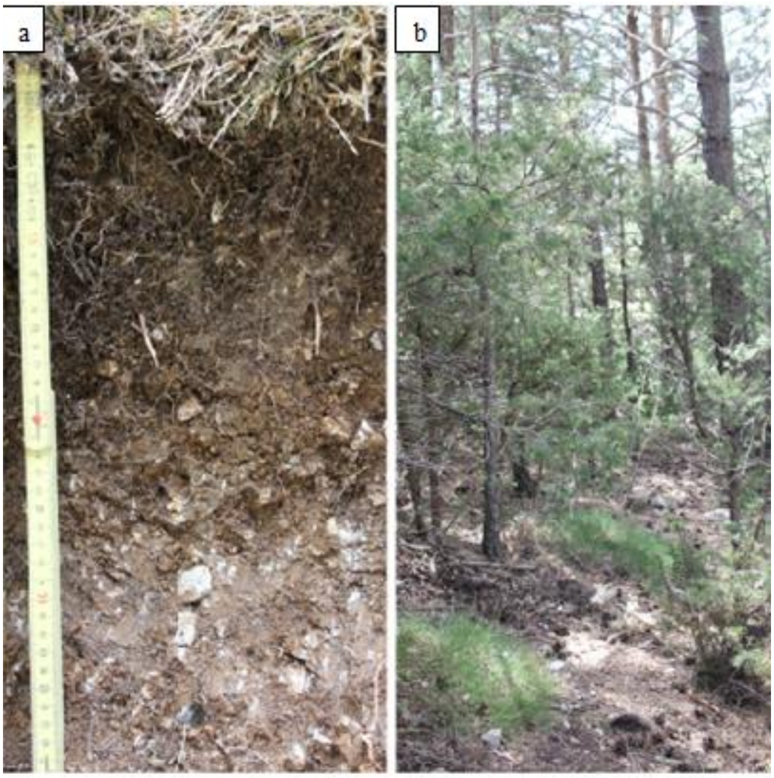


Fig.2.2.6. Site Ramonet: a) soil profile; b) Scots pine forest

2.3. Soil sampling

In each site organic, organo-mineral and mineral topsoil horizons were sampled, through the opening of a soil profile until 40 cm deep, following the macromorphological descriptions (Loaiza, 2004) in order to found the humus form. Sampling was performed at the beginning of May 2015 (Table 2.3.).

Humus forms determination was made by following the European Humus Forms Reference Base (Zanella et al., 2011). During the process of classification the sequence of organic horizons (OL, OF, OH), their continuity or discontinuity was taken into account as well as the macrostructure of organo-mineral horizon (A) and the traces of soil faunal activity. In some sampling points the litter horizon was not present or poorly developed, thus it was not sampled. Moreover, the sampling of OH horizon was provided together with OF due to the small thickness or not clear boundary with the overlying OF horizon.

Soil samples were carefully packed in polyethylene bags and transported to the soil laboratory of the Department of Environment and Soil Sciences, University of Lleida, where they were processed for analyses.

Table 2.3. Topsoil horizons and sampling scheme.

Sampling plot	Horizon	Depth, cm	Humus form
Canalda	OL	0-4	Amphi
	OF+OH	0-4	
	A	4-14	
	Bw	14-34	
Cogulers (shade)	OL	0-4	Amphi
	OF+OH	0-4	
	A	4-18	
	Bw	18-36	
Cogulers (sunny)	OL	0-3	Amphi
	OF+OH	0-3	
	A	3-17	
	Bw	17-30	
Prat	A	0-15	Mull
	Bw	15-36	
Ramonet	OL	-3-0	Amphi
	OF+OH	-3-0	
	A	0-12	
	AB	12-30	
Torra	F	0-1	Mull
	A	1-13	
	Bw	12-30	

2.4. Chemical analysis

Sample preparation

The 21 soil samples were oven dried at 40°C for 48 hours prior to analyses. Samples of organic origin were crushed with laboratory blender into thin powder, whereas samples of mineral origin were thoroughly sieved through 2 mm sieve. These sieved soil samples were ground with the help of agate pounder. The samples of OF and OF+OH horizons were treated as both soil samples of organic and mineral origins.

In all soil samples pH was measured except those from litter horizons.

Carbonate determination

The total set of 17 soil samples except those from OL horizon were checked for carbonate content with a HCl 10% solution. The intensity of reaction was noted as low, high or none, that justified the selected weight for carbonate quantification.

The quantity of carbonates was determined by the Bernard calcimeter method, based on the volumetric measurement of CO_2 which evolves during the reaction between carbonates present in soil and hydrochloric acid. From the quantity of CO_2 evolved the amount of carbonates can be calculated.

To perform the quantification of carbonates in our sampled soils we weighed 2 g of low reactive and 0.5 g of high reactive samples into the flask. Pure calcium carbonate was taken in 0.1, 0.2 and 0.3 g quantities in order to build the calibration curve and 0.5 g of standard to arbitrate the pureness of measurements. Then 4 ml of 50 % HCl was added into the small tube and was placed vertically into the flask with sample. After the connection of flask with the calcimeter the tube with acid was dropped down and intensively shacked to perform the reaction. The amount of CO_2 evolved during the reaction between hydrochloric acid and carbonates was determined by the settled level of liquid in the calcimeter. The calculation was performed with a calibration graph (Fig.2.4.1.) with the multiplication factor 2.272.

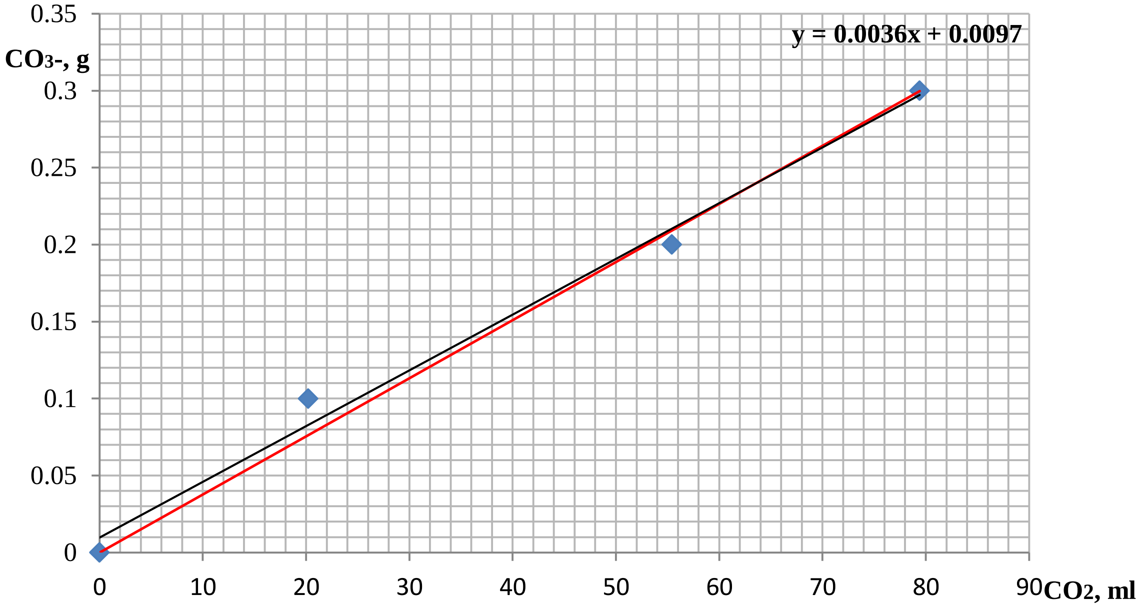


Fig. 2.4.1. Calibration graph for carbonate quantification

SOM determination with Walkley-Black wet combustion method

One of the ways of SOM quantification is based on the rapid oxidation of organic matter in the soil sample with potassium dichromate in concentrated sulfuric acid. In this procedure the amount of soil taken for analysis may vary upon expected results of SOM quantity that could be judged by the colour of the sample. The redox reaction of SOM extraction procedure is given below:

$$2Cr_2O_7^{2-} + 3C° + 16H^+ = 4Cr^{3+} + 3CO_2 + 8H_2O$$

In our study the prepared soil samples were weighed into the 250 ml flask in an amount 0.3 – 0.6 g depending on the colour of the sample and the origin. For instance, the samples taken from mineral horizons of lighter color were added in quantity of 0.6 g, whereas the samples from organic horizons (OF, OF+OH) weighed 0.3 g. Later the 5ml amount of 1N $K_2Cr_2O_7$ and 10 ml of concentrated H_2SO_4 were carefully added into the flask with samples and left for the reaction for 30 min. The digestion was stopped with deionized water.

Organic carbon was quantified by manual back titration with 0.5 N ferrous ammonium sulfate (Mohr´s Salt) solution. Ortho-phenathroline ferrous complex (Ferrosin) was added to the digestate as an indicator. The titration was stopped when the green to reddish brown color change was observed.

In calculations 1.29 correction factor was used as the result of incomplete oxidation during the digestion. To obtain the SOM percentage obtained OC result was multiplied by 1.72.

SOM determination with dry combustion method

Nine soil samples from organic horizons were weighed in amount of 5 g in porcelain crucibles and put in to the oven heated gradually to 650°C for 6 hours. After the crucibles cooled they were put to exicator approximately for 30 min. Later on the crucibles with samples were weighed. The difference in loss of weight before combustion and after was used to determine SOM percentage in samples as follows:

$$\% \text{ OM} = \frac{a-b}{a} \times 100\ \%;$$

where: *a* – weight of soil sample before combustion; *b* – weight of sample after combustion.

2.5. SOM micromorphology

Thin section preparation

Micromorphological observations were conducted on thin sections prepared in the Laboratory of Micromorphology of the University of Lleida. The thin sections belong to organic and mineral horizons of 6 sites (Torra, Prat, Ramonet, Canalda, Cogulers shaded, Cogulers sunny). The thin sections were prepared according to Murphy (1986) with polyester resin impregnation. The impregnation of undisturbed soil samples with artificial resin allows obtaining polished blocks which are sawed later with diamond saw and polished, in order to obtain a thin section.

Microscopy

Each thin section was examined with an Olympus petrographic microscope (BX51) to identify different microscopical features such as plant residues, roots, minerals, pores, humus substances etc. Observed roots, leaves and shoots were classified regarding the stage of decomposition.

Leaves and coniferous needles were divided into 4 classes (stages) according to the morphological changes during their decomposition as follows (Xing Jun Tian, 1997):

- 1st stage of decomposition – fresh intact leaves or needles of yellow-brown color with little change in mesophyll and cuticle structure;

- 2^d stage of decomposition – leaves or needles with slightly separated cuticle and epidermis from mesophyll, often with fungal hyphae colonization and penetration inside of mesophyll;
- 3^d stage of decomposition – leaves or needles are moderately decomposed, the cuticle is separated from the epidermis, mesophyll structure is disturbed due to faunal and fungal activity;
- 4^{th} stage of decomposition – leaves and needles are strongly decomposed, only 30 % of mesophyll is left, epidermis is completely separated from mesophyll, color is usually dark brown.

Decomposition of roots and shoots was characterized according to Blazejewski et al. (2005) clustered in 5 classes regarding the decomposition stage:

- 0 stage of decomposition – inner structure of root or shoot is intact, outer sheath is complete without disturbance;
- 1^{st} stage of decomposition – outer sheath is partially separated from inner portion of root or shoot, inner tissues are little disturbed by fauna;
- 2d stage of decomposition – organic matter of root or shoot is dispersed into surroundings; outer sheath is 50% separated from inner portion, inner tissues are disturbed by fauna;
- 3d stage of decomposition – visible root or shoot tissues are rarely present, the shoot or root shape is preserved and recognizable;
- 4^{th} stage of decomposition – visible tissues of root or shoot are absent, only sparse separated cells are present; root or shoot shape is indefinite.

Based on observations the list of 34 identified features was made and used in point counting:

1. broad leaf 1st stage of decomposition;
2. broad leaf 2d stage of decomposition;
3. broad leaf 3d stage of decomposition;
4. broad leaf 4th stage of decomposition;
5. calcite grain;
6. charcoal;
7. cork;
8. cuticle;
9. diptera larvae dropping;
10. earthworm dropping;
11. enchytraeid dropping;
12. epidermis;
13. moss fragment;
14. fungal hyphae;
15. limestone;
16. mite dropping;
17. organic fine substance;
18. pore;
19. parenchyma;
20. pine needle 1st stage of decomposition;
21. pine needle 2d stage of decomposition;
22. pine needle 3d stage of decomposition;
23. pine needle 4th stage of decomposition;
24. quartz grain;
25. intact root;
26. root 1st stage of decomposition;
27. root 2d stage of decomposition;
28. root 3d stage of decomposition;
29. root 4th stage of decomposition;
30. fungal sclerotia;
31. shoot 1st stage of decomposition;
32. shoot 2d stage of decomposition;
33. shoot 3d stage of decomposition;
34. wood

Point counting with Olympus microscope

Point counting was conducted with the use of 2x magnification of Olympus microscope and an eyepiece reticle. The component falling at the crosshair each moment was identified. The points were located along parallel lines on the thin section. The total number of 300 points over the various components was recorded for each thin section. The results from point counting were expressed as the percentage of a given feature (Bernier & Ponge 1994).

Image analysis with Jmicrovision software

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 a microscope. To obtain digital images suitable for forward analysis all thin sections were scanned with a high resolution Epson scanner. Three randomly chosen areas of each thin section were scanned with 4800 ppp option. Thus, were obtained images with 24 bit spectral resolution ("true color") and dimension of 3363×9051 pixels. Each pixel represented an area of 28.09 μm^2. The set of scanned images was converted to TIF format and processed with JMicroVision software. This software is useful for describing, measuring, quantifying and classifying the components depicted on the image. Its random point counting option was selected to quantify features of thin sections´ scans after image calibration. The limitation of counting grid was set to 300 points. When the image represented several horizons, then the point counting procedure was limited to the area of each one.

Due to the features´ recognition constrains the amount of classes was reduced from 34 to 8. They are as follows: plant residue, root, mineral grain, pore, big fauna droppings, small fauna droppings, and organic fine substance. The class "plant residue" includes coniferous needles, leaves, shoots, buds and their scales, and different plant tissues or separate cells, but without differenciating weathering degree. "Root" and "mineral grain" classes consisted then of roots of different stage of decomposition and diverse minerals respectively. Under "small faunal droppings" droppings of enchytraeids and mites were identified, whereas *Diptera* larvae and earthworms faeces belonged to "big faunal droppings" class. The average of all observations was calculated for every thin section.

2.6. Statistical analysis

Before conducting quantification and statistical analysis all features with the same properties were joined in several classes:

- plant residues (needles 1-4th stage of decomposition, leaves 1-4th stage of decomposition, shoots 1 -3d stage of decomposition, cork, cuticle, epidermis, parenchyma, wood);
- root (intact roots and of 1-4th stage of decomposition);
- mineral (quartz, calcite, limestone grains);
- big faunal droppings (feces of earthworms, diptera);
- small faunal droppings (feces of mites, enchytraeids);

- charcoal;
- organic fine substance;
- pores;
- fungi (fungal hyphae, sclerotia).

The Kruskal-Wallis test was used to determine the difference in SOM and carbonates content in different horizons of sampled topsoils. It followed Shapiro-Wilk normality test.

To check the relationship between chemical and physical characteristics and micromorphological features found in thin sections of sampled topsoils Pearson´s product-moment correlation was applied to organic (OL, OF+OH), organo-mineral (A) and mineral horizons (Bw).

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 correspondence analysis (CA). Sites were used as active variables, while chemical, physical and micromorphological data – as passive. The data was not transformed before analysis. CA was applied only to organo-mineral A and mineral Bw horizons. Ramonet site was not included into analysis due to the lack of data.

Statistical analysis was conducted in Statistica 12 (StatSoft Inc.) and XLSTAT 2015 (Microsoft).

3. RESULTS AND DISSCUSION

3.1. Field classification of humus forms

The macromorphological investigation of humus forms in six study plots of Ribera Salada basin which represented different ecosystem types from riparian pine forest to high mountain meadow showed that in four plots were observed amphi and in two mull humus forms.

We observed a thick OL horizon made of fresh and partly bleached pine needles, twigs, pieces of wood, grass residues and moss at the brook pine forest (study plot Canalda). The fermentation horizon had numerous white fungal hyphae throughout lightly decomposed wood and other plant rests. The underlying OH horizon was twice thinner than the previous one and of darker colour. The organo-mineral A horizon had a biomesostructure. All these observations support our classification of this humus form as **pachyamphi** (Zanella, 2011).

Under the mixed Scots and Black pine forest of the sunny part of Cogulers study plot amphi was also observed. However, the litter horizon was much poorer and consisted of fresh and old pine needles, twigs, bark, wood and grass rests. An OF horizon was present, whereas OH was discontinuous. The A horizon had biomesostructure. Thus, this humus form was classified as **eumesoamphi**.

Humus form found in shaded part of Cogulers study plot was classified as **eumacroamphi**. That was supported by presence of all organic horizons (OL, OF, OH) and biomacrostructure of the organo-mineral A horizon.

Eumacroamphi humus form was also observed at the Scots pine forest (Ramonet) next to agricultural fields All organic horizons were present and the organo-mineral A horizon had a biomacrostructure with noticeable faunal activity.

Under the Holm oak forest (Torra) we found mull humus form which belongs to **oligomull**. Fresh plant litter was not observed, however bleached old oak leaves made a thin OLv horizon. OF horizon was discontinuous and consisted of crumbled plant rests and granulated aggregates of organic matter. A horizon had biomacrostructure.

OL and OF horizons were not observed in humus form found in *Thymus* meadow (Prat). Nevertheless, A horizon had biomacrostructure. Due to the abovementioned characteristics this humus form was classified as **eumull**.

3.2. Micromorphological description of amphis and mulls

Canalda

Microscopical investigations of prepared thin sections of pachyamphi from Canalda study plot showed the presence of three organic (OL, OF, OH), one organo-mineral (A) and mineral (Bw) horizons.

Upper organic horizon OL consisted of numerous pine needles of different stage of decay, almost opaque or light yellowish plant tissues (epidermis), organic fine substance, and sparse quartz (Fig. 3.2.1.).

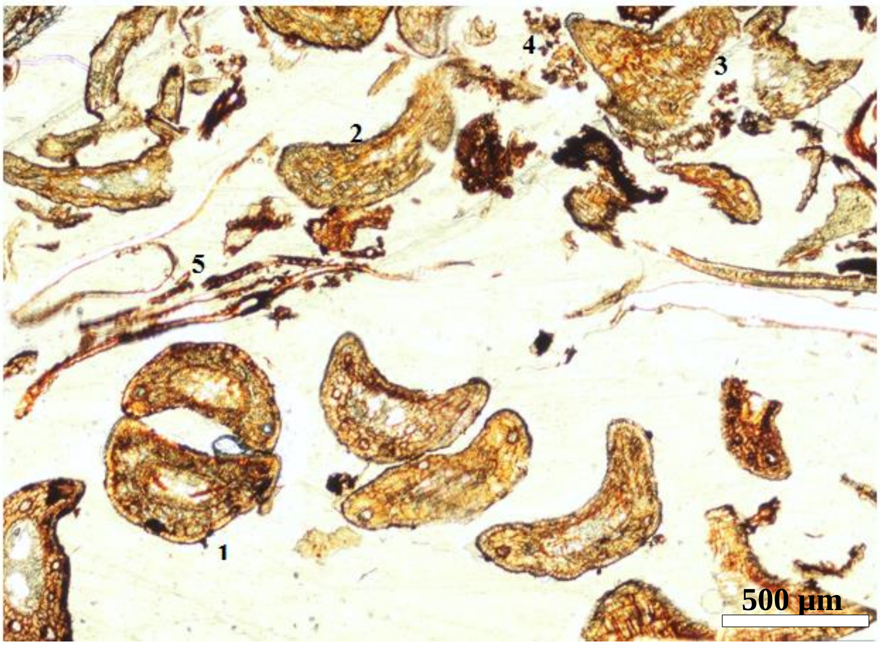


Fig. 3.2.1. Litter horizon of a pachyamphi (PPL) of Canalda. Numerous pine needles. (1) Pine needle 1st stage of decomposition: the outer sheath is complete; mesophyll and

vascular bundle are intact. (2) Pine needle 2^d^ stage of decomposition: outer sheath is broken; mesophyll is little damaged. (3) Collapsed pine needle. (4) Enchytraeid droppings of dark brown color. (5) Plant epidermis probably from grass..

Sometimes between pine needles small dark brown droppings of irregular shape were observed. More likely they belonged to enchytraeids or springtails. Mite droppings of reddish brown color were found closely or inside big pine needles or other organs.

The more or less horizontal orientation of plant residues sometimes with binding substances resulted in a laminated fabric.

The OF horizon consisted of partly decayed crumbled pine needles and leaves of dark brown color, spare fine roots, plant tissues and hardly recognizable separate plant cells with transition to organic fine substance (Fig. 3.2.2.).

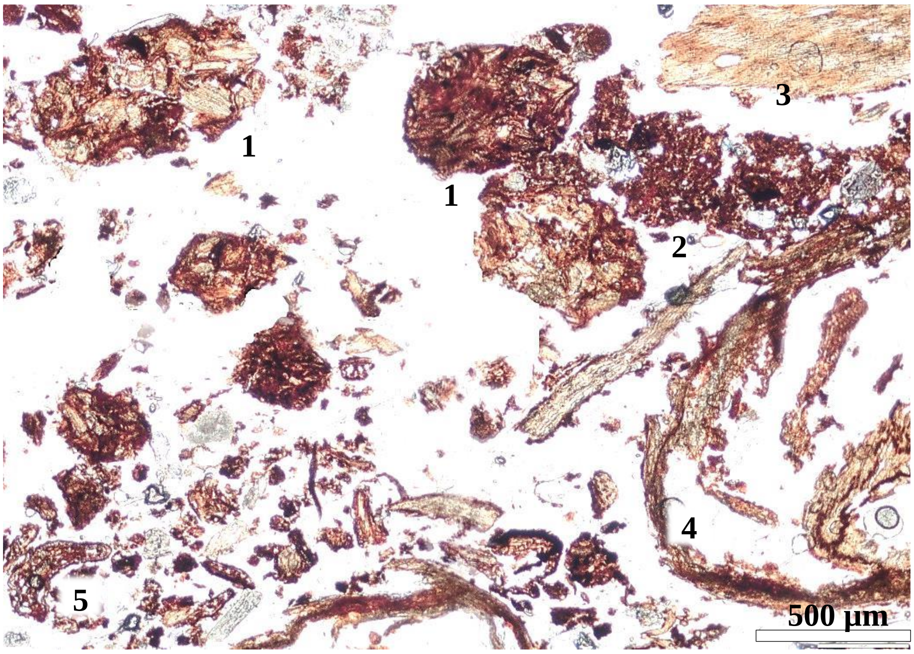


Fig.3.2.2. OF horizon of pachyamphi (PPL). (1) Diptera larvae droppings with not digested plant remain inside. (2) Dark brown organic fine substance. (3) Wood. (4) Partly decomposed plant tissue. (5) Pine needle 2^d stage of decomposition.

Among plant rests numerous dark small enchytraeids´ or springtails´ droppings (40 – 100 µm – length; 30 – 90 µm - width) are found. However large droppings (894 – 1200 µm – length; 560 – 700 µm - width) of larvae were also observed. They had close to oval shape with easily recognizable yellowish plant tissues inside. Mites´ droppings were gathered near or inside shoot rests, pine needles and roots (Fig 3.2.3.). Due to numerous faunal faeces the fabric of this OF horizon is formed by loose droppings.

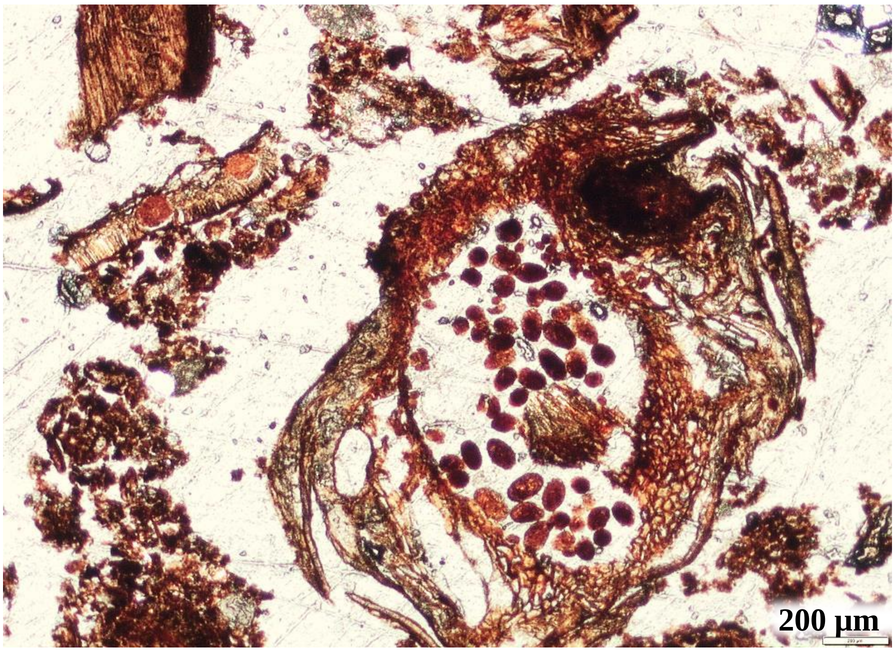


Fig. 3.2.3. OF horizon of pachyamphi (PPL). In the center shoot residue filled in with mites´ droppings. Left down: organic fine substance.

In contrary to OL and OF horizons OH horizon barely had some needles and leave residues. The most frequent features were dark brown to sometimes reddish roots of different decaying stages. They were found between organic fine substance which formed a spongy type of fabric. Near the roots fungal hyphae and sclerotia were noticed (Fig 3.2.4).

The same observations were made for the organo-mineral A horizons. Organic fine substances dominated all other organic features. Decaying roots were observed as well.

Occasionally cork tissue containing red colored phlobaphene was detected, which lay among organic fine substances. Similarly small weathered faunal droppings were found. The fabric type of this A horizon was spongy (Fig. 3.2.4).

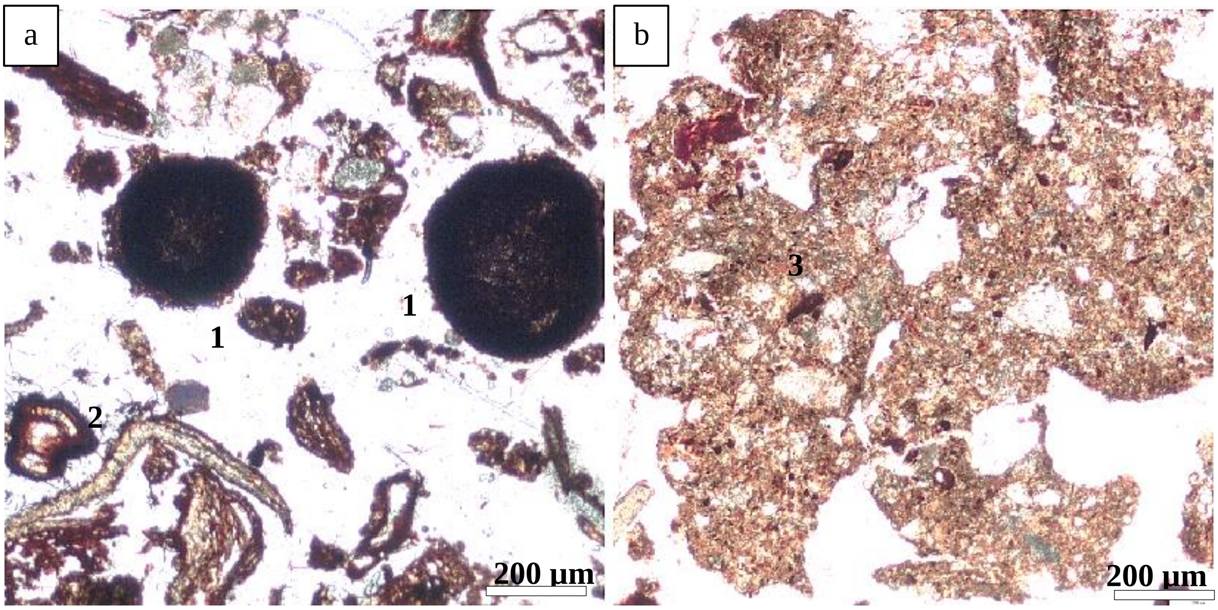


Fig. 3.2.4. OH (a) and A (b) horizons of pachyamphi (PPL). (1) Round black fungal sclerotia. (2) Root with fungal hyphae on top. (3) Spongy fabric. White spaces in it are quartz grains.

In the mineral horizon (Bw) numerous calcite and quartz grains with organic matter coating and sometimes snail shells are found. Organic fine substance together with weathered earthworms´ casts composes a dense fabric. Small fauna droppings are usually more altered than in organic horizons and can be recognized as angular aggregates (Fig 3.2.5a). Fine roots appear often hollow. However, the outer part of roots is preserved due to phlobaphene containing tissues (Fig. 3.2.5 b). Pieces of wood, cork, fungal sclerotia and fungal hyphae could be observed as well.

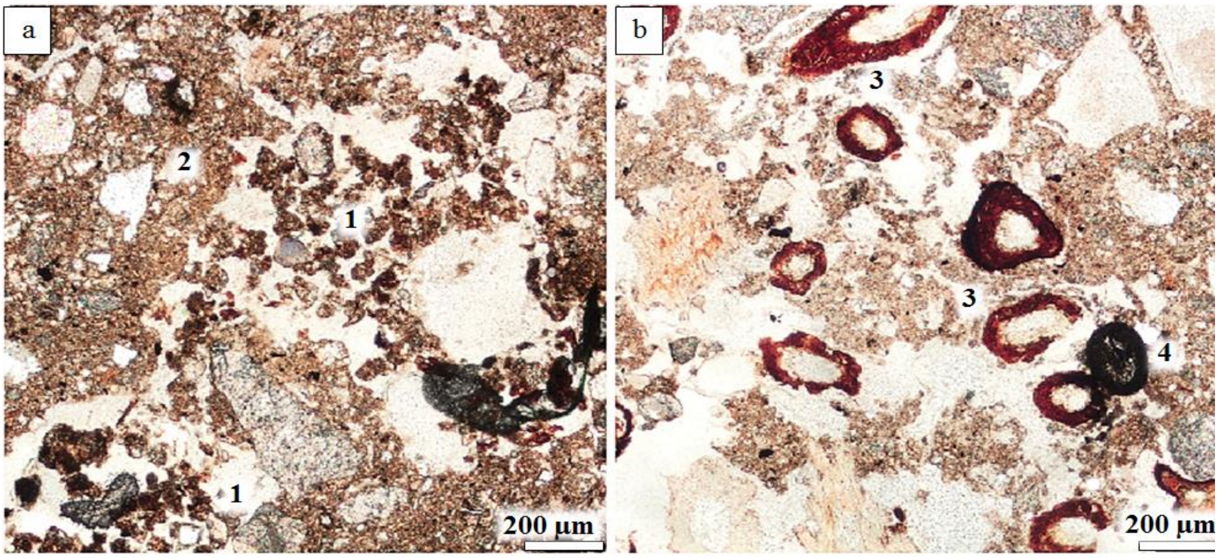


Fig 3.2.5. Mineral (Bw) horizon of pachyamphi (PPL): a) (1) dense fabric with altered droppings and (2) organic fine substance with incrusted mineral grains; b) (3) numerous fine roots with thick phlobaphene containing sheath (4) and black fungal sclerotia.

Prat

The OL horizon was not recognized in thin sections of soil sampled from Prat study plot (eumull). Instead of it an OF horizon was detected, which consisted of altered plant rests and numerous faunal droppings (Fig.3.2.6 a). Small droppings of angular shape were from enchytraeids or springtails (Fig.3.2.6 b).

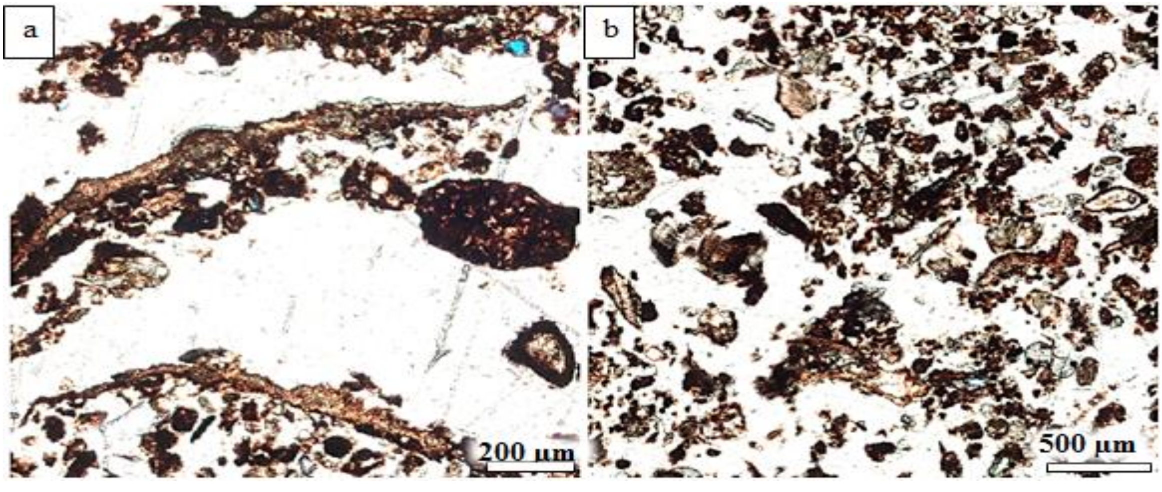


Fig. 3.2.6. OF horizon of eumull with altered plant residues (a) and numerous faunal faeces which form loose dropping fabric (b) (PPL).

Bigger aggregates of organic matter and small mineral grains were earthworms´ faeces which occurred seldom in OF horizon. All mentioned features formed a loose dropping fabric.

The A horizon consisted of earthworm´s casts, smaller faunal droppings, roots of different stage of decay and scarce plant residues that made a spongy fabric (Fig. 3.2.7 a). However, in the thin sections from the Bw horizon peds made from organic fine substance, minerals, and sometime roots were observed. Cracks in between were occasionally filled with altered droppings (Fig. 3.2.7 b).

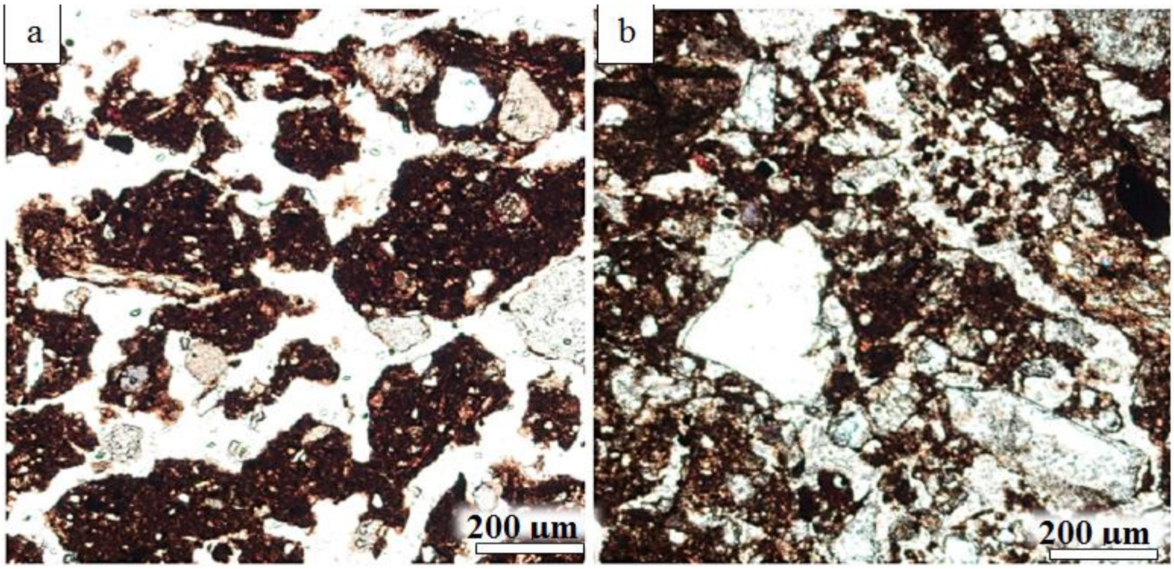


Fig. 3.2.7. A (a) and Bw (b) horizons of eumull (PPL).

Torra

Observations of thin sections of soil from Torra study plot proved that it corresponds to oligomull due to the presence of discontinuous OLv and OF horizons. The OLv horizon consisted of crumbled holm oak leaves (Fig 3.2.8), grasses and their rests. Droppings of big and small fauna were present as well.

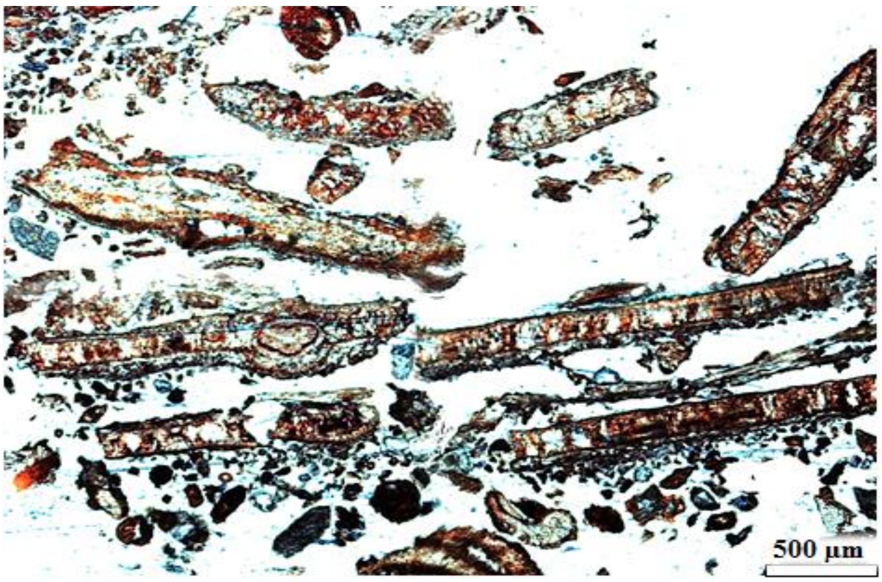


Fig. 3.2.8. Altered holm oak leaves in OLv horizon of oligomull (PPL).

Similarly to OLv, the OF horizon had abundant plant rests and faunal droppings. From the figure 3.2.9 it is seen that plant residues and faunal droppings are more altered than in the OLv horizon.

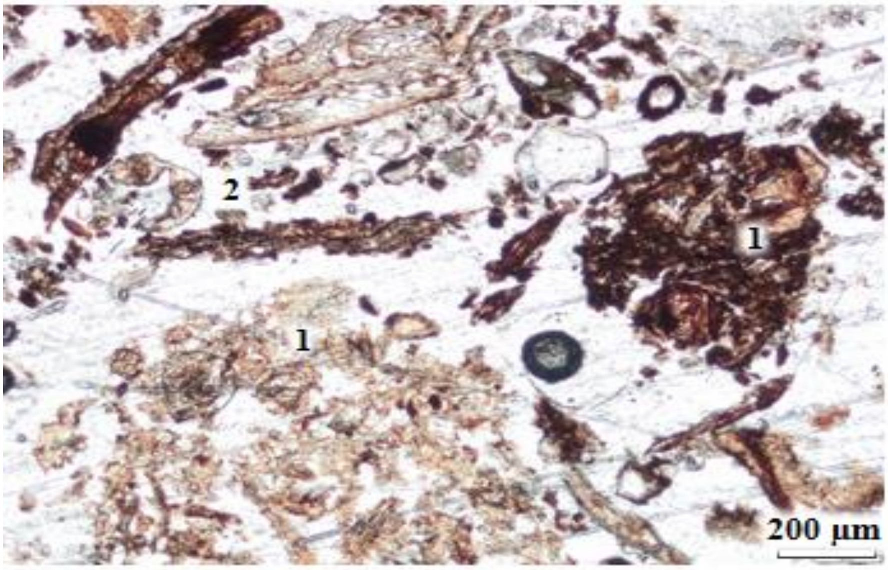


Fig. 3.2.9. (1) Altered diptera larvae droppings and (2) plant residues in OF horizon of oligomull (PPL)

Characteristic features of the A horizon were organic fine substances gathered in peds with cracks filled with angular aggregates (enchytraeids´ or springtail droppings) (Fig. 3.2.10 a). Roots of different stages of decay were observed as well as in the underlying Bw horizon. Roots as other plant residues with preserved lignin and cellulose showed interference colours (Fig. 3.2.10 b).

Bw horizon had abundant organic fine substance, minerals and roots.

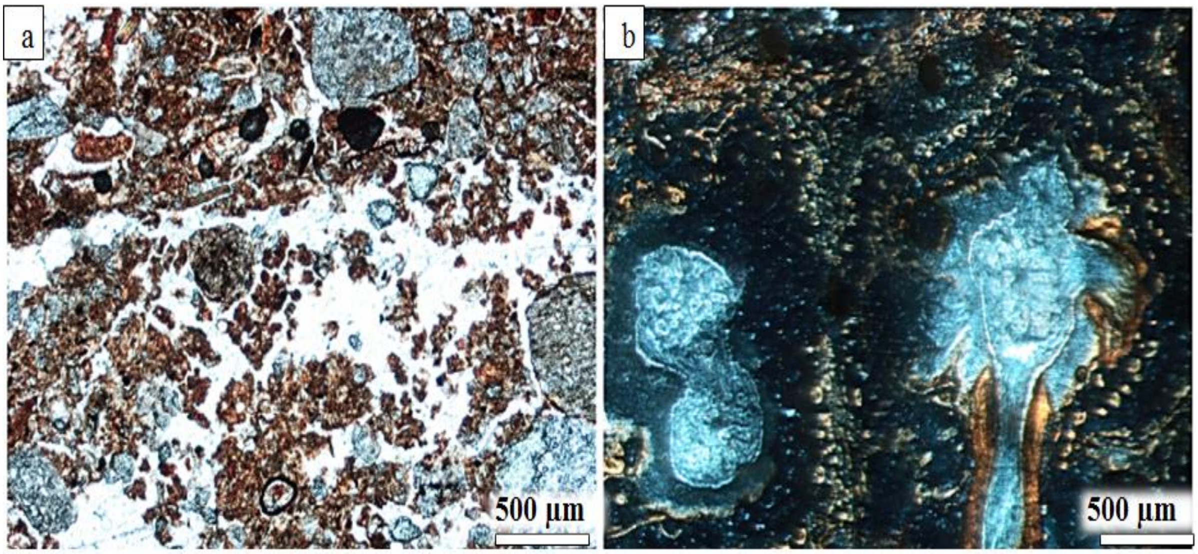


Fig. 3.2.10. A horizon of oligomull: a) faunal droppings and organic fine substance (PPL); b) root (CPL).

Cogulers

Amphi humus was observed in all thin sections from soil in both sunny and shaded parts of Cogulers. OL horizon in both sites consisted, as usual, of pine needles, pieces of wood, epidermis, residues of grasses and moss, numerous big and small faunal droppings and sparse organic fine substances. The transition between OL and OF horizons was not vividly seen. Moreover, remarkable features of OF horizon were fine roots surrounded by fungal hyphae (Fig.3.2.11 a) and diverse plant residues of dark brown color often with dropping infillings (Fig 3.2.11 b).

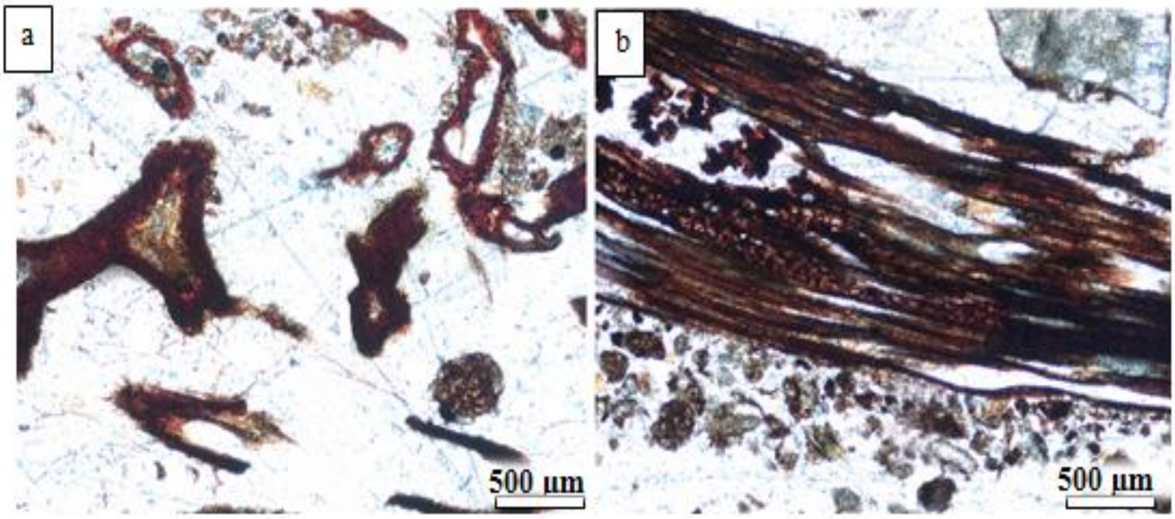


Fig. 3.2.11. OF horizons of (a) eumacro- and (b) eumesoamphi (PPL).

The A horizons were pretty similar and had a typical dense fabric made of aggregates of organic fine substance between decaying roots. Noticeable in plane polarized light holes in mentioned above aggregates were quartz grains or pores (Fig. 3.2.12.).

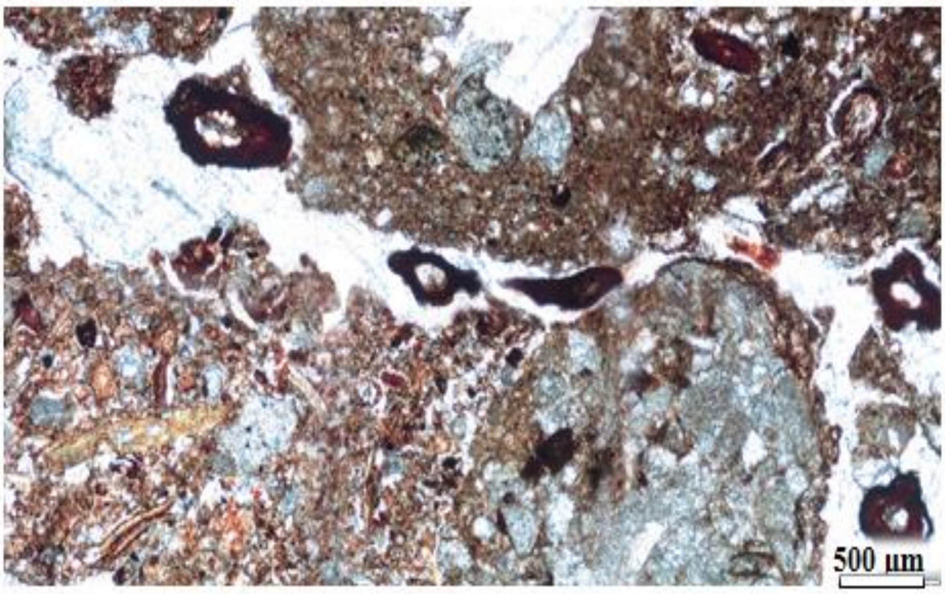


Fig. 3.2.12. A horizon (Cogulers shaded) of eumacroamphi. Roots are found between aggregates of organic fine substance (PPL).

Similarly to A horizon, organic fine substance was located in aggregates with roots filling in the cracks in Bw horizon at Cogulers shaded. Wood and charcoal were common as reddish phlobaphene containing cork tissues (Fig.3.2.13.).

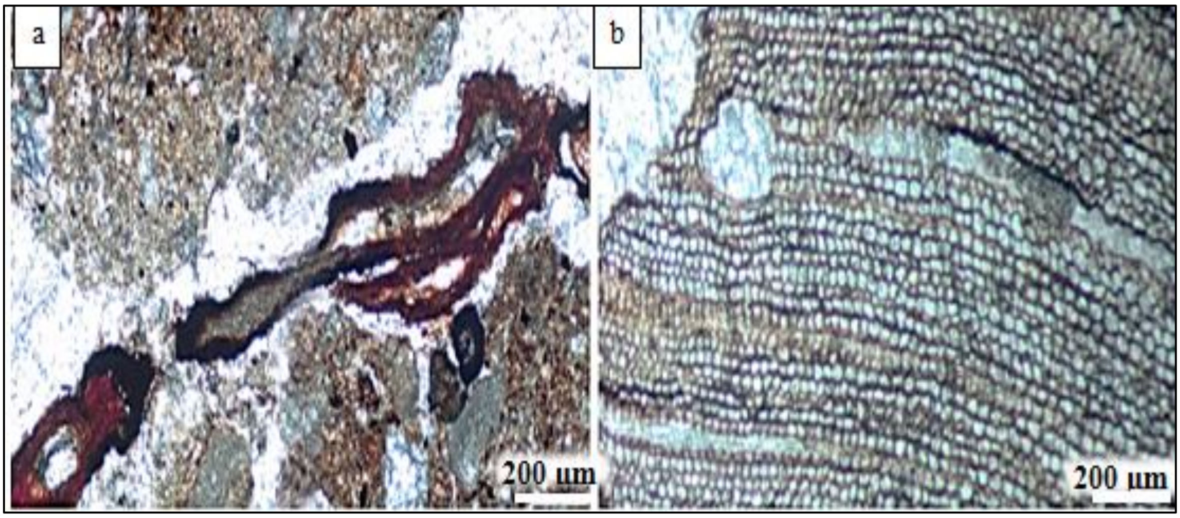


Fig. 3.2.13. Bw horizon (Cogulers shaded) of eumacroamphi (PPL): a) fine decaying roots; b) wood.

Ramonet

We had only one thin section of soil from Ramonet study plot and it belonged to the AB horizon. The horizon mainly consisted of organic fine substance aggregated in peds. They were penetrated with charcoal, mineral grains and some plant rests. A charcoal fragment with preserved structure typical for wood, surrounded by organic fine substance can be seen in figure 3.2.14.

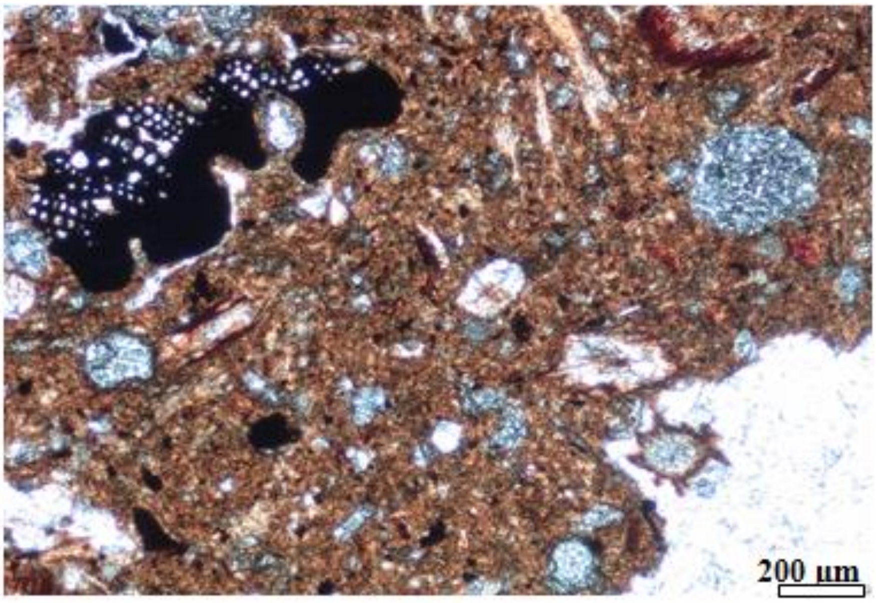


Fig. 3.2.14. Charcoal fragment (up-left) and decomposed root (down-right).in AB horizon from Ramonet (PPL).

3.3. The distribution of micromorphological features in humus forms´ horizons

Canalda

With the help of point counting conducted with microscope and thin sections scans we quantified the percentage of each feature class in topsoil of study plot Canalda. Fig. 3.3.1. shows the point counting results according to both methods (microscope and scans) as the vertical distributions of features regarding soil horizons.

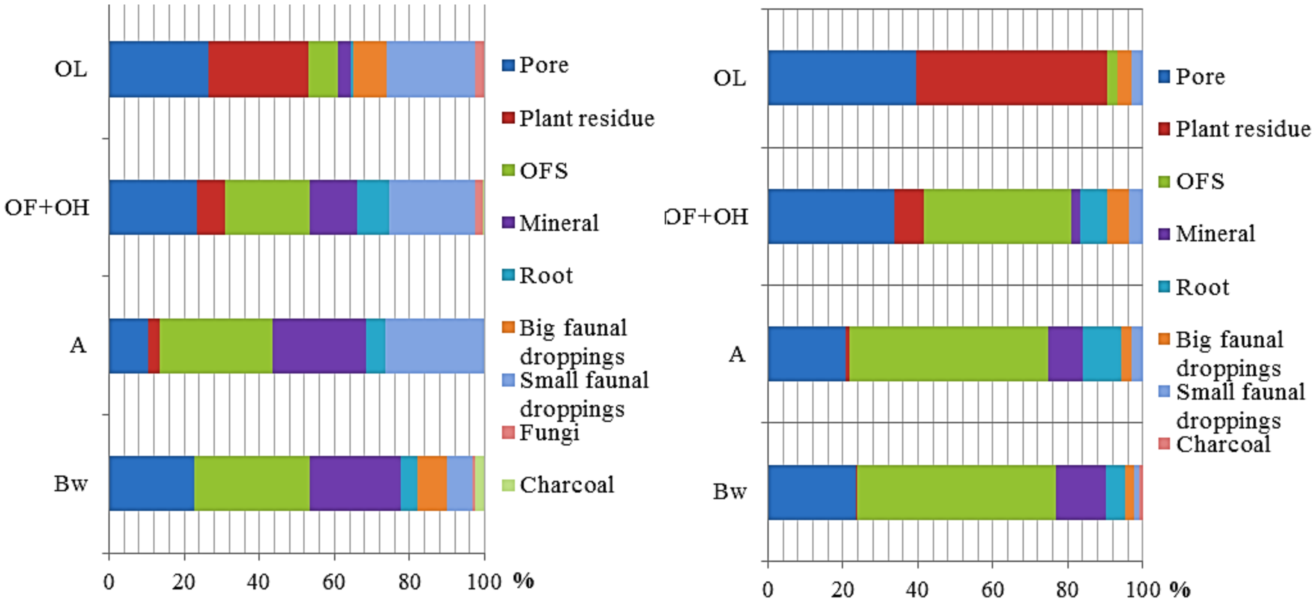


Fig.3.3.1 Percentage distribution of micromorphological features in horizons of pachyamphi from Canalda. Left: data acquired with the use of Olympus microscope. Right: data from thin sections scans.

As it is seen from the figure 3.3.1., in OL horizon the major two feature classes were plant residues (pine needles, leaves of understory, moss, plant tissues) and pores in observations with both methods. However, the quantity of plant residues acquired with the use of scans was higher compared to similar observations with the microscope (51% and 26.5 %). Small faunal droppings observed with the microscope (enchytraeids, springtails, mites) accounted for 23% in OL horizon but only 3% in scans. Moreover, the percentage of small faunal droppings detected in scans in all horizons was much smaller compared to same feature class quantified with the help of microscope.

With the increase in depth the percentage of plant residues determined with both methods declines: 7.5% in OF+OH horizon in first case and 7.8% in second. In the A horizon plant residues were almost not noticeable (3% and 1%).

The opposite trend was observed for organic fine substance which was seldom found in OL horizon and on the contrary abundant in A (30% and 51%) and Bw horizons (30% and 53%). The amount of mineral grains has also increased in lower A and Bw horizons compared to upper two.

Prat

Vertical distribution of micromorphological features found in thin sections with the two methods is shown in Fig. 3.3.2. In both cases the OL horizon was not detected.

In OF horizon the most abundant feature classes were pores, organic fine substance and plant residues in both cases. Pores were present almost in the same amount in observations with both methods – 36.7% and 30.5%. However, 19.36% small faunal droppings were found with the use of microscope, whereas only 7.4% were detected in scans. Furthermore, small faunal droppings were abundant throughout all horizons in the first case and sparse in the second.

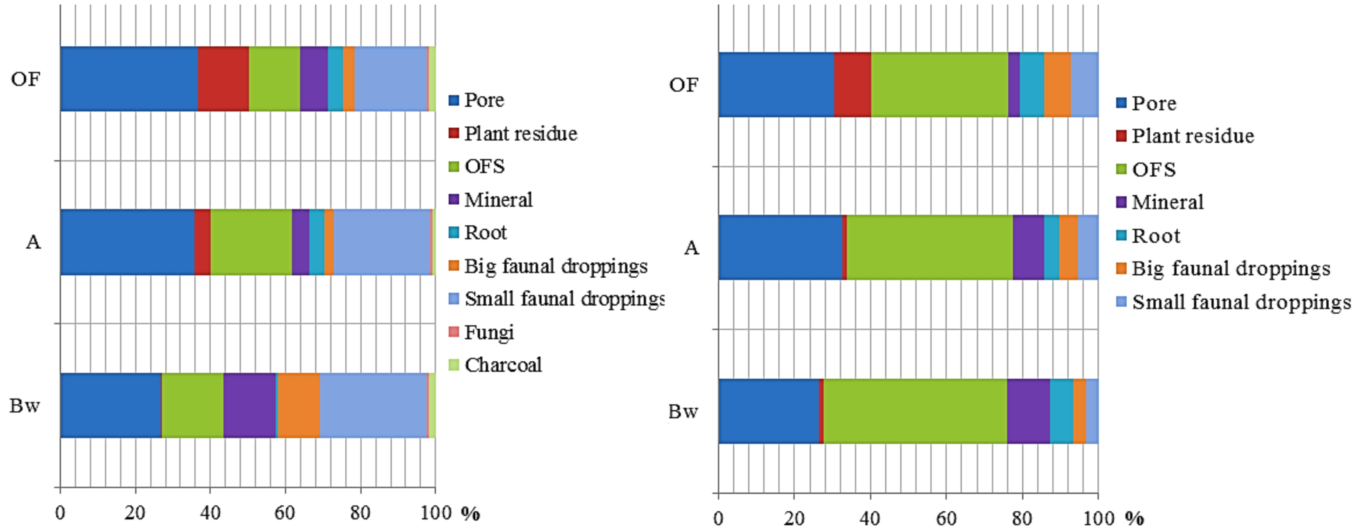


Fig.3.3.2. Percentage distribution of micromorphological features in horizons of eumull from Prat. Left: data acquired with the use of Olympus microscope. Right: data from thin sections scans.

The percentage of pores determined with both methods in A horizon did not change a lot compared to OL horizon and it was 35.7% and 32.7%. On contrary, the remarkable percentage increase was observed for organic fine substance (21.6% and 43.5%) in both cases. Opposite was observed for plant residues which had only 4.4% and 1.2%.

In Bw horizon the highest percentage from volume had pores (26.7%) and small faunal droppings (28.7%) in point counting results with microscope, while in quantification obtained from scans two main feature classes were organic fine substance (48%) and pores (26.5%).

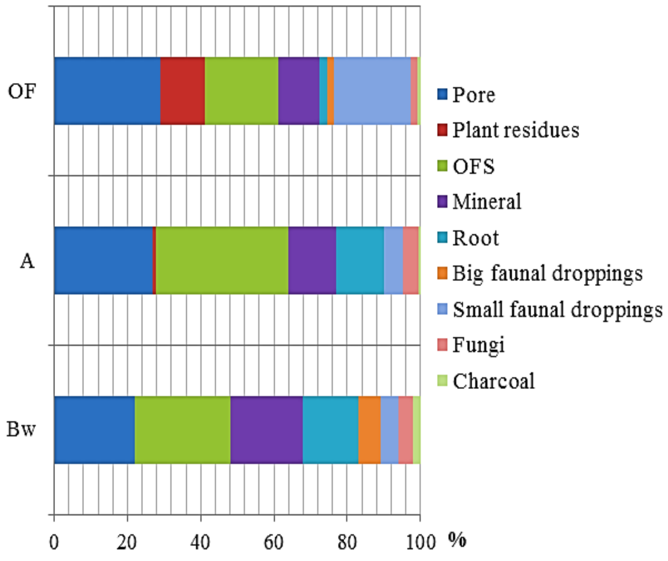
Fungi and charcoal feature classes were not detected in thin sections scans, but they had small percentages from total volume found in all horizons with the use of microscope.

Torra

According to point counting with the microscope the OF horizon of oligomull consisted of 29% of pores, 21% small faunal droppings and 20% of organic fine substance. In the organo-mineral horizon the percentage of organic fine substance increased until 36%, while in Bw horizon it dropped off (26%) (Fig.3.3.3). Plant residues counted up only 12.2% in OL horizon; moreover, they almost disappeared in A horizon and were not observed in Bw.

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

Fungi, charcoal and big faunal droppings had very small percentage acquired by microscopy in all horizons but with the trend to increase with the depth.



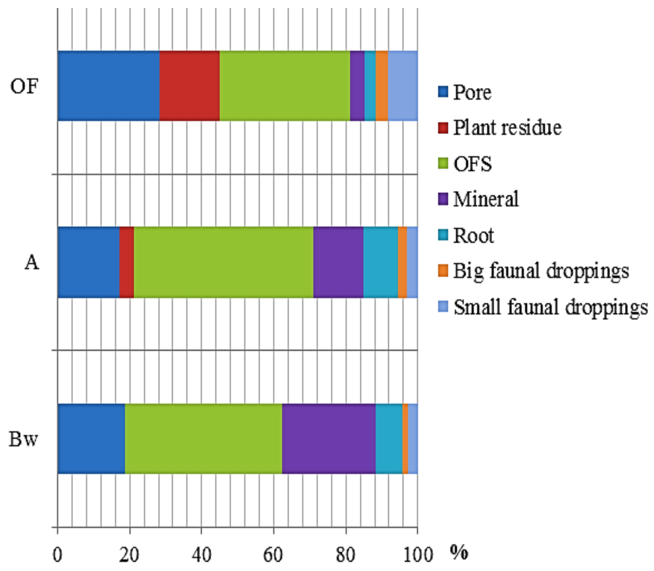


Fig.3.3.3. Percentage distribution of micromorphological features in horizons of oligomull from Torra. Left: data acquired with the use of Olympus microscope. Right: data from thin sections scans.

Cogulers

The percentage amount and distribution of features observed in topsoils sampled in sunny and shaded parts of Cogulers study plot is shown on figures 3.3.4 and 3.3.5 .[7] The results are acquired by two methods: point counting with microscope and thin sections scans.

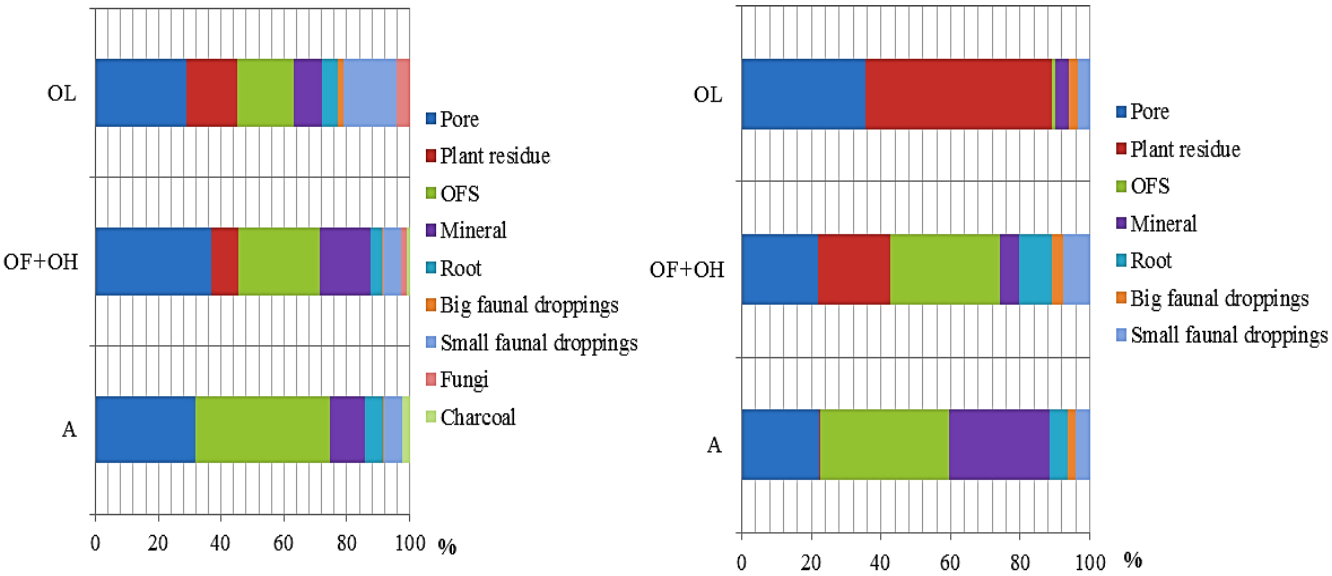


Fig.3.3.4. Percentage distribution of micromorphological features in horizons of eumesoamphi (Cogulers sunny part). Left: data acquired with the use of Olympus microscope. Right: data from thin sections scans.

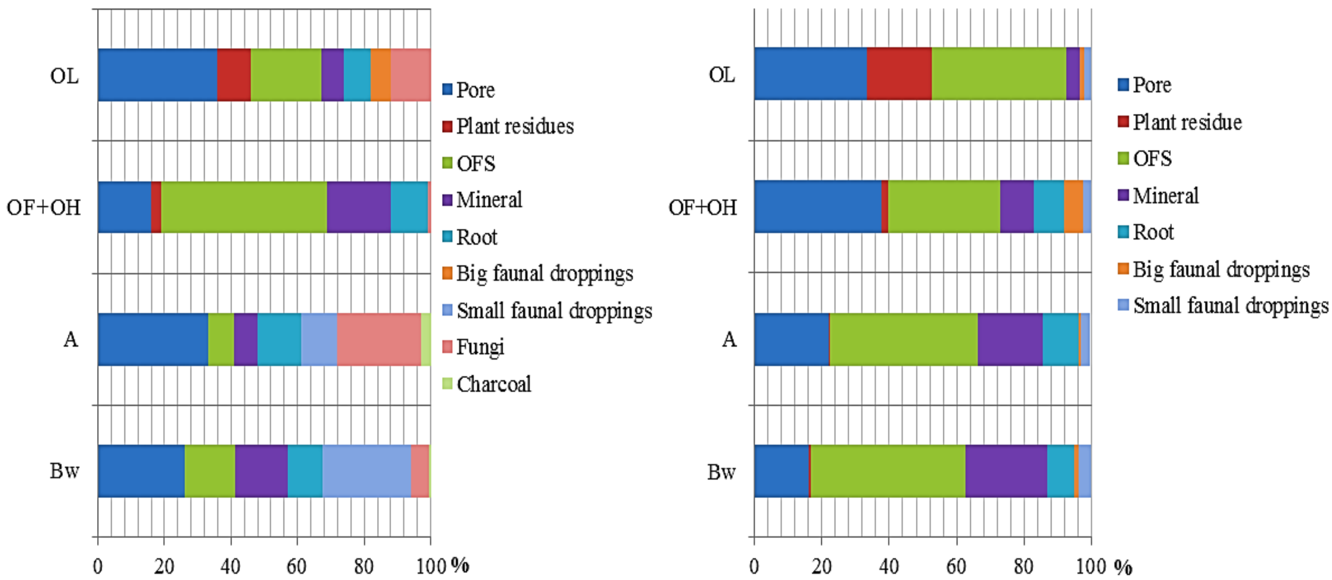


Fig.3.3.5. Percentage distribution of micromorphological features in horizons of eumacroamphi (Cogulers shaded part). Left: data acquired with the use of Olympus microscope. Right: data from thin sections scans.

---

[7] Thin section of Bw horizon was available only for shaded part of Cogulers study plot.

Point counting with microscope showed that in OL horizons of sunny and shaded parts of Cogulers pores accounted for 29 and 36 % over total volume. In both cases the second abundant feature class was organic fine substance which occupied 18 and 21% respectively. Plant residues and mineral grains were observed in higher amount in sunny part of Cogulers study plot (16% and 9%) compared to shaded one (10% and 7%). On the other hand, OL horizon of shaded part of Cogulers was rich in fungi (12%) and poor in small faunal droppings.

In OF+OH horizons the amount of organic fine substance increased until 26 and 50% from total volume in sunny and shaded parts respectively. The same was observed for mineral grains which counted up 16% in both.

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

In Bw horizon of shaded part of Cogulers pores and small faunal droppings had nearly the same percentage (26 and 26.7%), likewise organic fine substance and mineral grains which counted 16 %. Fungi occupied only 5.3 % from total volume

The results obtained from thin section scans demonstrated that OL horizon of Cogulers sunny study plot had higher percentage of plant residues (53.5%) compared to the same of Cogulers shaded (19.3%). However, it decreased with the depth in both. In OL horizon of Cogulers sunny the amount of organic fine substance was poor (1%), while in Cogulers shaded site it was the main feature class (40%). Organic fine substance increased until 44% and 46% in A and Bw horizons of Cogulers shaded site. It was also abundant in A horizon of Cogulers sunny site (37%).

Ramonet

Only the thin section of AB horizon was available for micromorphological investigations. Nevertheless, the distribution of micromorphological features according to point counting results with microscopes and scans is given below (Fig.3.3.6)

Point counting with the first method showed that the most abundant three feature classes of AB horizon were organic fine substance (31.7%), roots (20.7%) and pores (17%). Small faunal dropping and minerals counted up 12 and 10.3% respectively. Plant residues, fungi and charcoal were scarce (6; 1.7; 0.7%), while big faunal droppings were not detected at all.

Organic fine substance and pores were also two main feature classes according to point counting results in thin section scans (60.6%, 24%), while minerals and roots had 9.5% and 5%. Small faunal droppings as plant residues and charcoal were not recognized with this method.

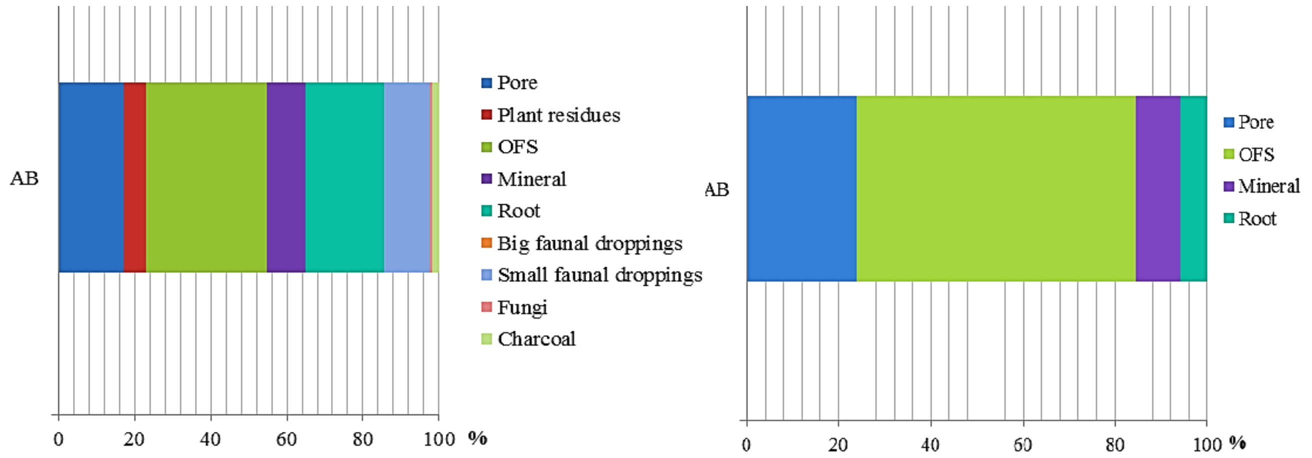


Fig.3.3.6. Percentage distribution of micromorphological features in AB horizon (Ramonet study plot). Left: data acquired with the use of Olympus microscope. Right: data from thin sections scans.

3.4. Chemical analysis

SOM

Soil organic matter content was different in the horizons of all examined topsoils of our study area ($p \leq 0.05$). In all observations was noted higher amount of SOM (%) in upper organic horizons (OF+OH) with its decrease in lower organo-mineral and mineral horizons (Fig 3.4.1.).

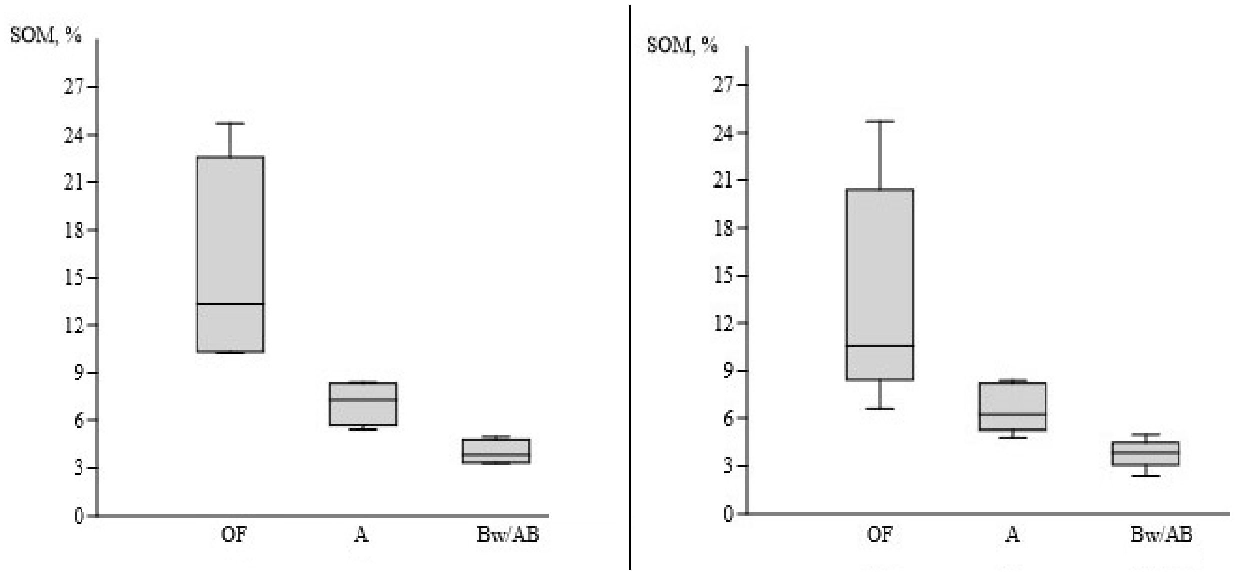


Fig. 3.4.1 Application of Kruskal-Wallis test to content of SOM (%) in different horizons of amphi (left), $p=0.007$, and amphi and mull (right), $p=0.002$.

The amount of SOM (%) found in soil horizons of different sites is shown on the figure 3.4.2.

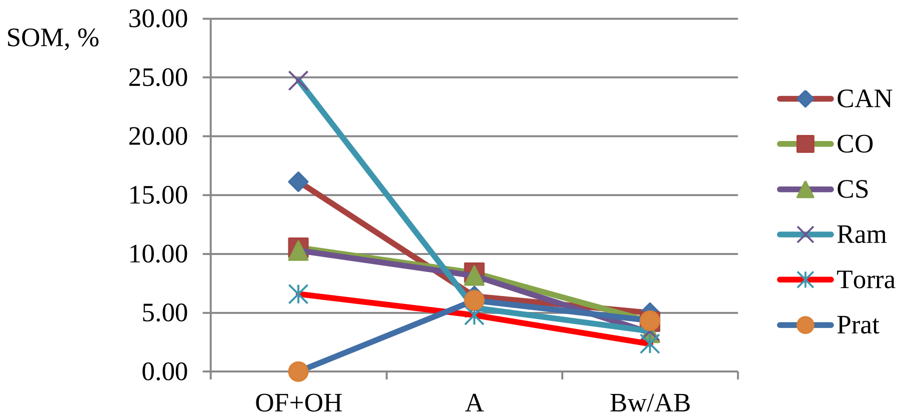


Fig.3.4.2. Soil organic matter content in soil horizons of different sites: CAN – Canalda; CO – Cogulers shaded; CS – Cogulers sunny; Ram – Ramonet.

The highest content of SOM in OF+OH horizon was observed in eumacroamphi sampled in *P. sylvestris* marginal forest (Ramonet) and it was 24,7%, while the lowest (6.6%) was found in oligomull of *Q. ilex* forest (Torra).

The A horizon of both eumeso- and eumacroamphi in mixed *P. nigra* and *P. sylvestris* forest (Cogulers) was the richest on SOM (8.2% and 8.4%). On the other side, the lowest record 4.8% of SOM was found in the A horizon of oligomull (Torra).

Regarding mineral horizons, SOM content was highest in the soil of *P.nigra* brook forest, Canalda site (5%) and lowest (2.4%) in the soil of *Q. ilex* forest (Torra).

Carbonates

The amount of carbonates in all samples of amphi humus form (CAN, CO, CS, Ram, figure 3.4.3) was higher in comparison with mull (Torra, Prat). Moreover, the content of carbonates in topsoils´ samples increased with the depth. However, this difference among organic, organo-mineral and mineral horizons is not significant ($p \geq 0.05$). In one observation (organic horizon of Ramonet) carbonates were not detected at all.

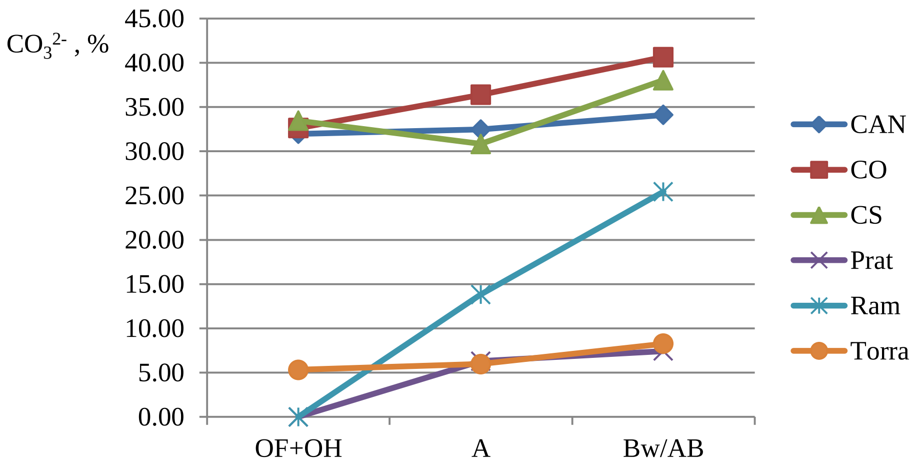


Fig. 3.4.3. Carbonate contents in soil horizons of different sites: CAN – Canalda; CO – Cogulers shaded; CS – Cogulers sunny; Ram – Ramonet

pH

The results of pH are given in table 3.4.1. Most of the organic horizons had a weakly basic pH reaction. Only in one case was observed weakly acidic close to neutral (Ramonet). Moreover, the general trend of increasing pH values in depth is present in all observations.

Table 3.4.1. pH values in soil horizons of different sampling sites: CAN – Canalda; CO – Cogulers shaded; CS – Cogulers sunny; Ram – Ramonet

Site code	Humus form	OF+OH	A	Bw/AB	Altitude, m
CAN	Pachyamphi	7.5	8	8.1	800
CO	Eumacroamphi	7.9	8.1	8.5	800
CS	Eumesoamphi	8	7.9	8.4	800
Ram	Eumacroamphi	6.4	8	8.2	1600
Prat	Eumull	---	7.8	8.2	1100
Torra	Oligomull	7.3	7.5	8	900

Pearson´s correlation coefficient was computed to assess the relationship between the pH, amount of SOM (%), carbonates content (%) and altitude (m asl) in all soil samples (table 3.4.2.). As the result, there was a significant negative correlation between pH and SOM percentage ($p = 0$). In contrast, pH and carbonates amount had strong positive relationship ($p = 0.007$). pH did not have a significant correlation with the altitude at which soil sample was found.

Table 3.4.2. Pearson´s correlation matrix between measured chemical parameters in all soil samples: showed results are significant at $p \leq 0.05$; ns – not significant;

	SOM%	Carbonates%	pH	Altitude, m
SOM%	1			
Carbonates%	ns	1		
pH	-0.77	0.63	1	
Altitude	ns	-0.53	ns	1

3.5. Micromorphological features, chemical and physical parameters relationship in horizons of humus forms

To check if there is any relationship between the observed micromorphological features (in volume percentage), SOM, carbonates, pH and soil texture (silt, clay and sand percentage) 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 3.5.1.

In organic horizons a significant negative correlation between the amount of plant residues on one side, and roots, organic fine substance and mineral grains on the other side was detected. This relation was positive in case of big faunal droppings. On the one hand, big faunal dropping had also a positive relationship with SOM percentage; on the other hand, negative with mineral grains content. Organic fine substance correlated negatively with plant residues, and positively with roots and mineral grains

Table 3.5.1. Pearson´s correlation matrix between found amount of micromorphological features (% of volume) and SOM (%) in organic horizons: Plr – plant residues, Rt – root, OFS – organic fine substance, Min – mineral grains, BFD – big faunal droppings, SFD – small faunal droppings, ns – not significant.

	Plr (r/*p*)	**Rt (r/*p*)**	**OFS (r/*p*)**	**Min (r/*p*)**	**BFD (r/*p*)**	**SOM (r/*p*)**
Plr	1					
Rt	**-0.81**/0.027	1				
OFS	**-0.82**/0.022	**0.82**/0.023	1			
Min	**-0.85**/0.015	ns	**0.87**/0.01	1		
BFD	**0.81**/0.027	ns	Ns	**-0.88**/0.008	1	
SOM	ns	ns	Ns	**-0.86**/0.001	**0.92**/0.003	1

In the organo-mineral A horizons significant positive correlations were found between the amount of plant residues and small faunal droppings. Meanwhile a negative relationship was found between percentage of pores and mineral grains, clay and small faunal droppings (table 3.5.2.).

Table 3.5.2. Pearson´s correlation matrix between found amount of micromorphological features (% of volume) and physical parameters in A horizon: Plr – plant residues, Min – mineral grains, SFD – small faunal droppings, ns – not significant.

	Plr (r/*p*)	**SFD (r/*p*)**	**Sand (r/*p*)**	**Silt (r/*p*)**	**Clay(r/*p*)**	**Min (r/*p*)**	**Pore (r/*p*)**
Plr	1						
SFD	**0.89**/0.04	1					
Sand	ns	ns	1				
Silt	ns	ns	**-0.97**/0.04	1			
Clay	ns	**-0.95**/0.013	Ns	ns	1		
Min	ns	ns	Ns	ns	ns	1	
Pore	ns	ns	Ns	ns	ns	**-0.99**/0.002	1

In the lower mineral horizons (Bw or AB) the amount of organic fine substance and mineral grains as pores and small faunal droppings correlated positively (r=0.97, *p*=0.03; r=0.99, *p*=0.003). The same relationship was observed between the percentage of fungal elements and clay (r=0.98, *p*=0.02). In contrast, we found negative correlations between big faunal droppings and clay (r=-0.95, *p*=0.045).

3.6. Correspondence analysis

In order to perform correspondence analysis (CA) we used only the dataset of organo-mineral A horizon and mineral horizon Bw of all sampled soils.

The independence test between rows and columns of used for analysis contingency table showed that the computed p-value is lower than the significance level ($p<0.0001$, $\alpha=0.05$), thus we rejected null hypothesis and stated that there is a link between rows and columns.

The CA suggested eight factors (dimensions) which explain 100% of variability. However, for forward performance and explanation of results only two first factors were used as they explain in total 67% of variability. The eigenvalues and percentages of inertia are given in table 3.6.1. Their variable composition is given in Table 3.6.2.

Table 3.6.1. Eigenvalues and inertia of 8 dimensions performed by CA

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	0.005	0.003	0.002	0.001	0.00	0.00	0.00	0.00
Goodman and Kruskal tau (%)	43.09	24.05	17.89	7.81	3.52	3.13	0.47	0.045
Cumulative %	43.09	67.14	85.03	92.84	96.36	99.49	99.96	100.00

The interpretation of CA in our case can be made on two ways: humus forms can be explained in micromorphological, chemical and physical characteristics, or vice versa. On the figure 3.6.1 the distribution of humus forms in the axis space is shown. Due to the contribution of values of each column (micromorphological features, physical and chemical parameters, table 3.6.2.) the horizontal axes (F1) reflected the opposition of sand, organic fine substance and big faunal droppings contents (negative side of the axis) to amount of carbonates (positive side of the axes) in humus forms. The vertical axis (F2) was defined by the amount of carbonates and organic fine substance (negative side of the axes), pores and small faunal droppings (positive side of the axes). Thus, on the positive side of the axes appeared contents of pores and small faunal droppings, whereas the negative one associated with the amount of carbonates and organic fine substance found in humus forms.

The position of different humus forms along the horizontal axes (F1) gives a separation between mull (TA, TBw, PratA, PratBw) and amphi forms of humus (COA, COBw, CANA, CANBw). It can be associated with the texture of humus forms (mull has in both cases higher percentage of sand), organic fine substance and big faunal droppings percentage.

The vertical axes (F2), on the one hand, allowed associating the high amount of small faunal droppings and pores with eumull humus form (PratA, PratBw) and eumacroamphi (COA, COBw), on the other hand, oligomull (TA, TBw), pachyamphi (CANA, CANBw) and eumesoamphi (CSA) with organic fine substance and carbonates amount.

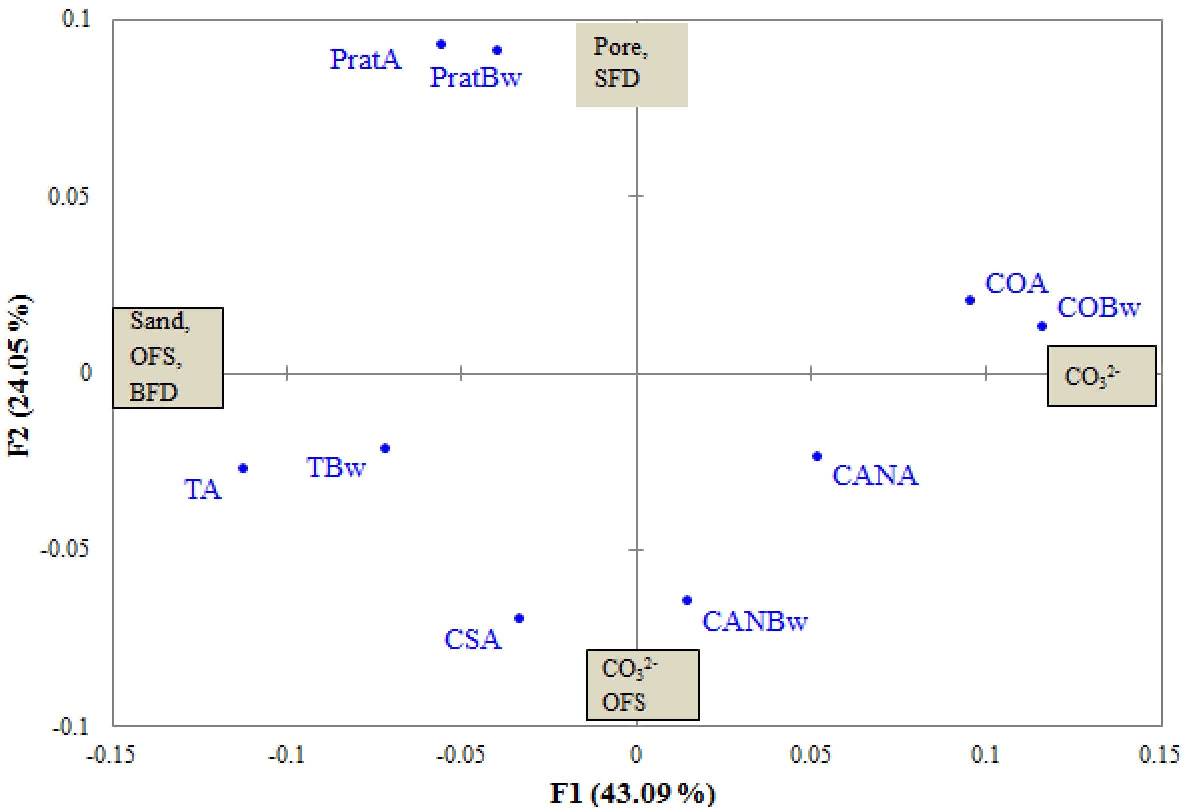


Fig. 3.6.1. Projection of humus forms in the plane of two main axes of correspondence analysis: CANA, CANBw – pachyamphi, COA, COBw – eumacroamphi, CSA – eumesoamphi, PratA, PratBw – eumull, TA, TBw – oligomull, BFD – big faunal droppings, OFS – organic fine substance, SFD – small faunal droppings.

Table 3.6.2. Contribution of column values to the definition of axes

	F1	F2
Plant residues	0.000	0.003
Root	0.000	0.009
OFS	0.164	0.257
Mineral	0.000	0.062
Pore	0.022	0.069
Big faunal droppings	0.006	0.007
Small faunal droppings	0.027	0.365
Fungi	0.034	0.005
Carbonate %	0.403	0.192
Sand	0.295	0.003
Silt	0.047	0.027

If we locate the micromorphological features found in humus forms, together with the chemical and physical characteristics in the plane of two main axes (Fig. 3.6.2.) it can be seen that the distribution of micromorphological features changes on the two axes from mull to amphi (horizontal) and amphi to mull (vertical). Thus, percentage of sand, pores, and big faunal droppings can be associated with mull (along F1), while quantity of fungal elements, silt, and clay, minerals, carbonates and SOM percentage with amphi. Along F2 both classes of faunal droppings together with pores are associated with mull, although clay, carbonates, SOM, OFS, charcoal and root percentages – with amphi.

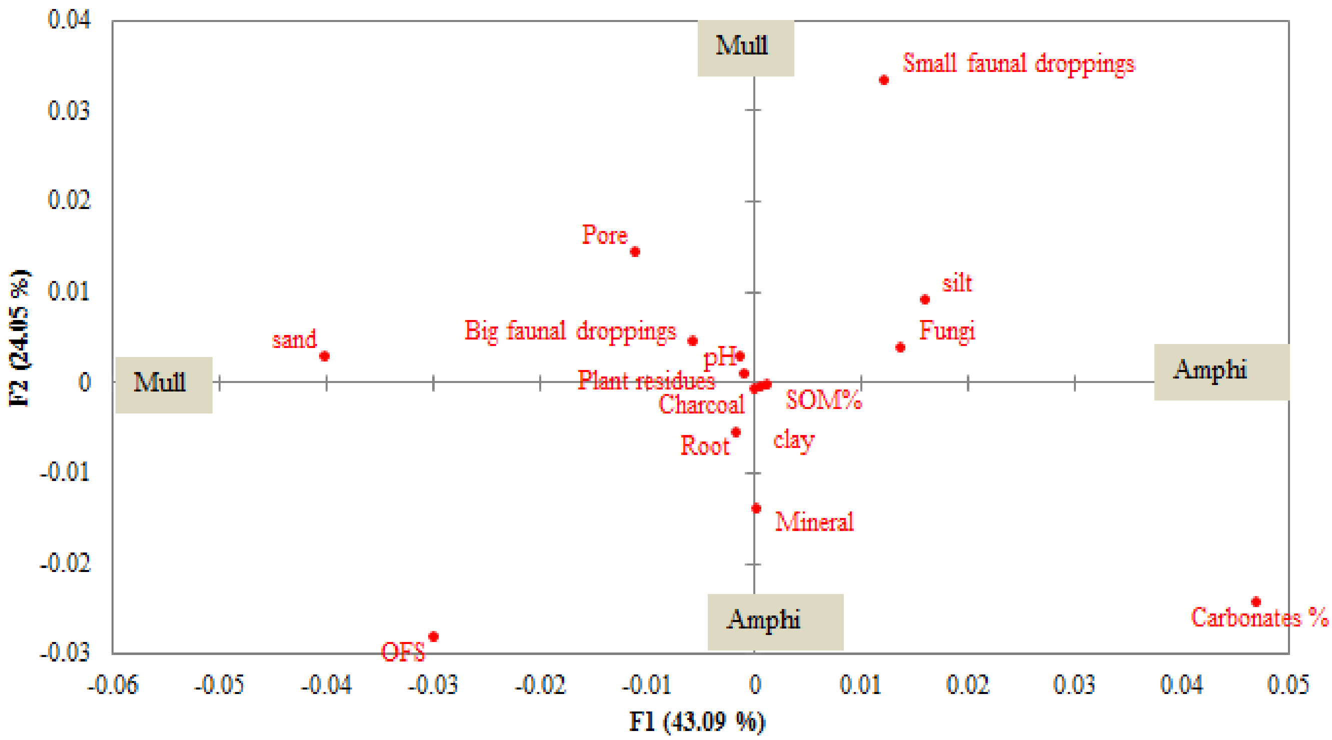


Fig. 3.6.2. Projection of micromorphological features, chemical and physical parameters in the plane of two main axes of correspondence analysis.

3.7. Discussion

Micromorphological differences between humus forms

The difference between mull and amphi humus forms can be detected macroscopically due to the presence or absence of particular horizons and their sequence. Moreover, we demonstrate that micromorphology gives additional information which can help to judge about the development of particular humus form, and can even replace the diagnosis of such humus forms. In our study all amphis have developed organic horizons (OL, OF, OH). Their formation can be explained through the properties of litter. Recalcitrant litter which has slow rate of biodegradation is accumulated in OL horizon and composes a laminated fabric. Babel (1975) observed the development of that fabric type in moder humus and related it to build-up of coniferous needles. However, other humus-forming constitutes are present in organic horizons besides needles or leaves. For instance, numerous macrofaunal droppings especially those from diptera larvae, 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 we found between big faunal droppings with plant residues and SOM in organic horizons of humus forms. Within some time span plant residues lose their coherence and transform into organic fine substance which does not have any preserved structure from the original material (Babel, 1975). It occurs very often in OF and OH horizons compared to upper lying OL in all amphi samples. A similar process was described by Babel (1970) in the profile of moder under the beech forest. According to his observations OL horizon contains up to 90% of plant rests and only 10% of organic fine substance. Furthermore, with depth the content of organic fine substance increases until 70% in OH horizon (Babel, 1970). The only one category of plant residues which do not completely lose their structure and can be easily recognizable among organic fine substance in lower organic and organo-mineral horizons is that one that contains phlobaphene. Babel (1975) showed that phlobaphene containing tissues are not prone to degradation and they can be preserved in a humus profile for a long time. In our study phlobaphene containing tissues were associated with root cortex and separated cork which had a bright reddish-brown color.

In the OF horizons of amphi samples the formation of a loose fabric which consists of angular oval aggregates was noticed. These aggregates are faeces of enchytraeids or springtails. The droppings of these animal groups is sometimes difficult to separate. That was demonstrated in studies by Zachariae (1964), Babel (1975) and Pawluk (1987). Nevertheless, the formation of dropping fabric for the whole horizon is underlined in Babel´s work (1975) by the dominance of enchytraeid activity in organic horizons as they feed not only on slime films and microorganisms but also on droppings of primary consumers. Enchytraeid faeces can also appear in lower A and Bw horizons were they fill in cracks between peds formed by organic matter and mineral grains. These cracks are usually the result of faunal activity or growing roots. For instance, Babel (1975) observed the arrangement of enchytraeids droppings in lateral rows in OH horizon in numerous humus forms in central Europe, whereas in mineral horizons their infillings followed the channels created by roots.

Organo-mineral A horizons in amphi as in mull humus forms are abundant on organic fine substance. It is often mixed with mineral grains forming spongy fabric. Numerous cracks and holes among aggregates which are the result of faunal borrowing activity are very evident in mull humus forms found in meadow and holm oak forests. The burrowing activity of soil fauna especially earthworms promotes the increase of pore volume which was noted by Babel (1969, 1975). Thus, it is not unusual that pores in A horizons of mulls are one of the main feature classes observed in micromorphological investigations.

To summarize, the main difference between amphi and mull humus forms according to our micromorphological investigations is associated with the abundance of plant residues especially pine needles and leaves which form laminated fabric in OL horizon in amphi, whereas in mull (if litter horizon is detected) it has loose fabric composed of crumbed plant rests and faunal droppings. However, the arrangement of humus components into loose dropping fabric in OF horizon and spongy fabric in A may be similar for both amphi and mull. The only difference between these two humus forms is a gradual transition from dropping fabric type to spongy in amphi due to the presence of additional OH horizon.

The influence of chemical, physical and biotic factors on humus forms development

Component Analysis demonstrated a clear association of topsoil from sites Prat and Torra with the higher amount of big faunal droppings and organic fine substance found in thin sections. At the same time other topsoils (Canalda, Cogulers shaded) were associated with elevated content of carbonates. Moreover, the position of separate horizons along the horizontal axis may support the trend of carbonates content increase with the depth.

The opposition of Prat and Torra to Canalda and Cogulers in the dimension of two main axes of CA is not unexpected. In fact, they belong to different humus forms. According to our macromorphological examination topsoils from Prat and Torra belong to mull, whereas the others (Canalda, Ramonet, Cogulers) to amphi. Furthermore, qualitative micromorphological analysis of thin sections showed prevailing typical crumb structure of A horizons in Prat and Torra due to earthworms' casts. This phenomenon is supported by numerous studies (Ponge, 2003; Waez-Mousavi & Habashi, 2012; Zanella, 2011; Ponge, 2013, De Nicola et al, 2014) which underline the fast transformation of plant debris into soil organic matter and an intensive mixing with minerals as it passes the gut of earthworms in mull. Moreover, the turnover of nutrients in mull is thought to be faster compared to other humus forms (Ponge, 2003). Thus, the accumulation of SOM in mull is less than in amphi, moder or mor. That explains the observation of little amount of SOM in topsoils of Prat and Torra which is supported by the position of SOM on vertical and horizontal axes of CA. However, the opposite results were presented in study by De Nicola et al. (2014) that suggested mull can store more organic matter in the whole profile compared to other humus forms.

The difference between mull found in Prat (eumull) and Torra (oligomull) is underlined by absence of OLv and discontinuous OF horizons the first case. The accumulation of recalcitrant litter in holm oak forest (Torra) as seen in macromorphological and micromorphological observations allowed to develop a weak OLv horizon. The same was observed by De Nicola et al. (2014) under sclerophyllous evergreen forest represented by *Q. ilex* and *Viburnum tinus*. This allows concluding that recalcitrant plant litter together with macrofaunal activity in Mediterranean conditions leads to the formation of oligomull humus form. At the same time easily degradable litter from meadow herbs does not form a thick litter layer what results in the formation of eumull without upper organic horizons and with noticeable biomacrostructured A horizon.

The abundance of small faunal droppings in the A horizon of eumull (Prat) was unexpected because of the common concept of antagonism among earthworms and enchytraeids (Ponge, 2003). Galvan et al. (2008) found positive correlation between percentages of enchytraeid droppings in organic horizon and the droppings incorporated with minerals of the same animal group in organo-mineral A horizon in humus forms developed on acidic substrates (moder). Thus, they concluded that the abundance of enchytraeids activity in upper horizon led to their domination in underlying horizon. The same was stated for earthworm activity. However, we observed enchytraeids feces mainly in cracks between macroaggregates formed by earthworms in A and Bw horizon. They correlated significantly with the amount of pores. Similar observations were made by Zachariae (1967) and Babel (1969) where they noticed infillings of enchytraeid droppings between earthworm casts. In fact, earthworm faeces could serve as the source of food for enchytraeids (Babel, 1975).

The horizontal axis of CA helped to associate the percentage of SOM, fungal elements, minerals, carbonates and silt with amphi humus form sampled in *P. nigra* and/or *P. sylvestris* forests (except one from Cogulers sunny site). In fact, all of mentioned amphi samples (A horizon) belong to loam texture class and only one (Cogulers sunny) to sandy loam. The medium soil textural groups are thought to be richer in nutrients compared to coarse ones and to promote the activity of soil fauna (Galvan et al., 2008). However, in our study we have seen the strong negative correlation between the percentage of small fauna droppings (enchytraeids, springtails and mites) and fine clay particles. In addition, Ponge (2003) argues that soil mesofauna species require less rich substrates for their activity. In our case it is also true if we consider the dominance of enchytraeid activity in A horizons of amphi compared to mull.

The activity of mesofauna can also be explained through the build-up of humus horizons in amphi. Amphi humus, in contrast to mull, has thicker and continuous organic horizons (OL, OF, OH) (Brethes et al., 1994; Graefe, 2007; Zanella, 2011). The formation of these horizons in our study is linked to the recalcitrant litter produced by coniferous trees. Pine needles, having lower decomposition rate (van Wesemael, 1993), can induce the decrease of bacteria and the increase of fungal activity. Meanwhile the rates of litter decomposition are slowed down. As a consequence the accumulation of organic matter occurs (Sadaka & Ponge, 2003; Seeber & Seeber, 2004) which is assembled in OL, OF and OH horizons. The main actor in crumbing and transforming organic matter in these horizons is mesofauna. Moreover, in dry Mediterranean conditions they can dig deeper into organo-mineral A horizons where they can find fine roots and plant rests. This fact explains the significant positive correlations between percentage of small faunal droppings and plant residues found in A horizons under coniferous forests obtained in the present study.

The formation of amphi is similar to the one of moder in terms of biological activity as the main two drivers are litter input and its transformation by soil organisms (Galvan et al., 2008). However, the chemical properties of amphi ressemble more to mull due to its development on basic substrates. Besides, the transition from mull to amphi happens while the consumption of litter by earthworms decreases which promotes the formation of continuous organic horizons (Brethes et al., 1994). This phenomenon can be related to the difference in macro- and micromorphology between mull humus forms developed under meadow herbs and holm oak forest and amphis formed in pine forests. 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 occurred.

Quantification of micromorphological features in scans and its difficulties

The distribution of micromorphological features in soil horizons can be determined with the help of point counting. Traditionally it is done with the use of microscope and an eye piece grid (Kooistra, 1991). However, nowadays the scans of thin sections with the forward image processing can be applied (Aydemir et al., 2004). In our study we used both methods for the quantification of micromorphological features, in order to determine if the use of the microscope could be disregarded, using scans as an alternative, more available method. The obtained results showed some differences in the percentage and vertical distribution of humus components. For instance, the amount of organic fine substance found with the use of scans was always higher in all samples compared to the same determination with the microscope. However, percentage of pores remained almost the same. The higher quantity of organic fine substance can be interpreted as a mix-up with other features due 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).

Small features such as enchytraeids droppings, fungal hyphae and especially separate plant tissues were hardly or not recognized at all. The main reason for that is the resolution of analyzed image. In our study each pixel represented an area of 28.09 μm^2 which was not enough for accurate detection of mesofaunal faeces or tissues in our case. However, Protz et al. (1987) noted that the pixel size for mesofaunal faeces recognition should lie at least within 10^{-4} m scale. The only way to omit the errors in features´ misinterpretation 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. On the other hand, increasing the resolution of the images results in extremely long scanning times and very heavy images.

The comparison of the results obtained from point counting with the microscope and with thin section scans in our study showed that even if the percentages of features are different their vertical distribution keeps the same trend. For example, organic fine substance amount tends to increase with the depth while the opposite is observed for plant residues. Furthermore, the percentage of pores obtained in thin sections with both methods was pretty the same. Thus, the features with bigger size such as big pores, organic fine substance aggregates, needles, leaves, shoots and wood can be recognized using lower resolution (10^{-3} – 10^{0} m) (Protz et al., 1987), but for finer identification the microscope should be used.

CONCLUSION

The study of the topsoils of 6 sampling sites at the Ribera Salada basin shows that:

1. The classification of humus forms in the frame of macromorphological observations corresponds to different assemblages of micromorphological features along a sequence of organic and organo-mineral horizons, in particular excrement size, structure and porosity and types of organic remains.

2. The studied mull humus forms are lacking upper organic horizons and have intensive faunal activity expressed in mixing of SOM with minerals. This results in a spongy type of fabric in topsoils from meadow and holm oak forest. In contrast, amphi humus forms have developed organic horizons where the main actor of SOM transformation is mesofauna. Hence, laminated and dropping fabric types are common in the organic horizons of topsoils with amphi humus from coniferous forests. The latter is characterized by higher amounts of plant residues, roots and SOM content.

3. The micromorphological differences between mull and amphi humus are mainly their aggregation by macrofaunal faecal pellets (basically earthworms) and pores (mull) versus organic fine substance and carbonates (amphi). This concludes that mull which is claimed to be the humus form with intensive nutrient turnover and low SOM accumulation has morphological and chemical differences compared to amphi.

4. The variation inside humus forms orders that leads to second level of classification (Zanella, 2011) is due to local conditions of humus formation, which is reflected by the position of eumesoamphi (CSA) opposite to eumacroamphi on the CA graph. The same separation was detected between eumull and oligomull, where the last one has developed an organic layer due to the recalcitrance of *Q. ilex* debris.

5. Mull humus form on calcareous soils under Mediterranean conditions that undergo a change in plant composition to one that generates more resistant litter (pine species or sclerofilous oaks), is susceptible to evolve to amphi.

6. The scans of thin sections as the alternative to microscope can be applied for identification of micromorphological features with the bigger size (earthworms´ and larval faeces, organic fine substance aggregates, pores, minerals and plant organs), while finer (enchytraeids´ and springtails´ droppings, fungal hyphae) require higher resolution of the image or the use of microscope.

7. Our study demonstrates that the micromorphological investigation can clear the doubts in humus forms classification based only on macromorphology. Moreover, it may explain the transformation processes of organic matter through interactions of climate, geology, plants and soil organisms. That is why the micromorphological approach in SOM examination is very useful and should be recommended for soil survey and management in rangelands.

REFERENCES

Aerts, R. (1996). Nutrient resorption from senescing leaves of perennials: are there general patterns? Journal of Ecology, 597-608.

AFES (1992) Référentiel pédologique. Principaux sols d´Europe. INRA, Paris, France

AG Humusformen. Arbeitskreis Humusformen (2014). Retrieved from http://www.humusformen.de/

Alban, D. H., & Berry, E. C. (1994). Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. Applied Soil Ecology, 1(3), 243-249.

Aydemir, S., Keskin, S., & Drees, L. R. (2004). Quantification of soil features using digital image processing (DIP) techniques. *Geoderma*, *119*(1), 1-8.

Babel, U. (1968). Enchytraeen-losungsgefuge in löss. Geoderma, 2(1), 57-63.

Babel, U. (1975). Micromorphology of soil organic matter Soil components (pp. 369-473): Springer.

Bal, L. (1970). Morphological investigation in two moder-humus profiles and the role of the soil fauna in their genesis. Geoderma, 4(1), 5-36.

Barratt, B. C. (1964). A classification of humus forms and micro-fabrics of temperate grasslands. Journal of Soil Science, 15(2), 342-356.

Barratt, B. C. (1967). Differences in humus forms and their microfabrics induced by long-term topdressings in hayfields. Geoderma, 1(3), 209-227.

Barratt, B. C. (1969). A revised classification and nomenclature of microscopic soil materials with particular reference to organic components. Geoderma, 2(4), 257-271.

Berg, B., & Meentemeyer, V. (2002). Litter quality in a north European transect versus carbon storage potential. Plant and Soil, 242(1), 83-92.

Berg, B., Staaf, H., & Wessen, B. (1987). Decomposition and nutrient release in needle litter from nitrogen-fertilized scots pine (Pinus sylvestris) stands. Scandinavian Journal of Forest Research, 2(1-4), 399-415.

Bergmann, C., Fischer, T., & Hüttl, R. F. (1999). Significance of litter-and humus-layer quality for rates and forms of N cycling in moder-to rawhumus-moder profiles under Scots pine (Pinus sylvestris L.). Plant and Soil, 213(1-2), 11-21.

Bernier, B. (1968). Descriptive outline of forest humus-form classification. Paper presented at the Proc. of Seventh Meeting of the National Soil Survey Committee of Canada, Univ. Alberta, Edmonton.

Bernier, N., & Ponge, J.-F. (1994). Humus form dynamics during the sylvogenetic cycle in a mountain spruce forest. Soil Biology and Biochemistry, 26(2), 183-220.

Blazejewski, G. A., Stolt, M. H., Gold, A. J., & Groffman, P. M. (2005). Macro-and micromorphology of subsurface carbon in riparian zone soils. *Soil Science Society of America Journal*, *69*(4), 1320-1329.

Bornebusch, C. H. (1930). The fauna of forest soil: Skovbundens dyreverden. Nielsen & Lydiche.

Brêthes, A., Brun, J.-J., Jabiol, B., Ponge, J., & Toutain, F. (1995). Classification of forest humus forms: a French proposal. Paper presented at the Annales des Sciences Forestières.

Bunting, B., & Lundberg, J. (1987). The humus profile-concept, class and reality. Geoderma, 40(1), 17-36.

De Nicola, C., Zanella, A., Testi, A., Fanelli, G., & Pignatti, S. (2014). Humus forms in a Mediterranean area (Castelporziano Reserve, Rome, Italy): classification, functioning and organic carbon storage. Geoderma, 235, 90-99.

Deleporte, S. (2001). Changes in the earthworm community of an acidophilous lowland beech forest during a stand rotation. European journal of soil biology, 37(1), 1-7.

Doran, J. W., Sarrantonio, M., & Liebig, M. (1996). Soil health and sustainability. Advances in agronomy, 56, 1-54.

Dunger, W., & Voigtländer, K. (1990). Succession of Myriapoda in primary colonization of reclaimed land. Paper presented at the Minelli, A.: Proceedings of the 7th international congress of Myriapodology. EJ Brill, Leiden, New York, København, Köln.

Emeis, C. (1875). Waldbauliche Forschungen und Betrachtungen.

Galvan, P., Ponge, J. F., Chersich, S., & Zanella, A. (2008). Humus components and soil biogenic structures in Norway spruce ecosystems. *Soil Science Society of America Journal*, *72*(2), 548-557.

Graefe, U. (2007). Gibt es in Deutschland die Humusform Amphi? *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft, 110*, 459-460.

Green, R., Trowbridge, R., & Klinka, K. (1993). Towards a taxonomic classification of humus forms. Forest Science, 39(Supplement 28), a0001-z0002.

Hesselman, H. (1926). Studien über die Humusdecke des Nadelwaldes. Meddelanden fran Statens Skogsförsöksanstalt, 516f.

Juncker, F. (1930). Rødgranskovens Jordbundsproblem i praktisk forstlig Belysning. *Dansk Skovforenings Tidsskrift*, 15: 37-84.

Kooistra, M. J. (1991). A micromorphological approach to the interactions between soil structure and soil biota. *Agriculture, Ecosystems & Environment*, *34*(1), 315-328.
Krauss, G. (1930). Utterance in discussion. *Proc. Intern. Congr. Of For. Exp. Stockholm*, 1929, p. 506.

Kubiena, W. L. (1953). The soils of Europe.

Kurz-Besson, C., Coûteaux, M.-M., Thiéry, J. M., Berg, B., & Remacle, J. (2005). A comparison of litterbag and direct observation methods of Scots pine needle decomposition measurement. Soil Biology and Biochemistry, 37(12), 2315-2318.

Liebig, J., & Playfair, L. P. B. (1847). Chemistry in its application to agriculture and physiology: TB Peterson.

Loaiza, J.C. (2004). Validación de sensores ECH_2O para medida continua de la humedad del suelo y Caracterización Física e Hidrológica de Suelos en la Ribera Salada - Unpubl. Msc thesis. Univ. de Lleida. 93p.
Loaiza, J.C. (2007). Soil hydrology in the Ribera Salada Catchment (Catalan Pre Pyrenees). Application of simulation models for the estimation of hydrologic transitional regimes – Unpubl. PhD. Thesis. Universitat de Lleida. 264p.(In Spanish).91 p.

Müller, P.E. (1878). Studien über die natürlichen Humusformen (1-124). Berlin

Murphy, C. P. (1986). *Thin section preparation of soils and sediments*. AB Academic.

Pawluk, S. (1987). Faunal micromorphological features in moder humus of some western Canadian soils. Geoderma, 40(1), 3-16.

Ponge, J.-F. (1999). Horizons and humus forms in beech forests of the Belgian Ardennes.

Ponge, J.-F. (2003). Humus forms in terrestrial ecosystems: a framework to biodiversity. Soil Biology and Biochemistry, 35(7), 935-945.

Ponge, J.-F. (2013). Plant–soil feedbacks mediated by humus forms: a review. Soil Biology and Biochemistry, 57, 1048-1060.

Ponge, J.-F., & Chevalier, R. (2006). Humus Index as an indicator of forest stand and soil properties. Forest Ecology and Management, 233(1), 165-175.

Ponge, J.-F., & Delhaye, L. (1995). The heterogeneity of humus profiles and earthworm communities in a virgin beech forest. Biology and fertility of soils, 20(1), 24-32.

Ponge, J.-F., Arpin, P., Sondag, F., & Delecour, F. (1997). Soil fauna and site assessment in beech stands of the Belgian Ardennes. Canadian Journal of Forest Research, 27(12), 2053-2064.

Protz, R., Shipitalo, M. J., Mermut, A. R., & Fox, C. A. (1987). Image analysis of soils-present and future. *Geoderma*, *40*(1), 115-125.

Ramann, E. (1905). Bodenkunde, 2 Aufl. Berlin

Read, D. J., Leake, J. R., & Perez-Moreno, J. (2004). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, *82*(8), 1243-1263.

Romell, L.-G., & Heiberg, S. O. (1931). Types of humus layer in the forests of northeastern United States. Ecology, 12(3), 567-608.

Sadaka, N., & Ponge, J. F. (2003). Climatic effects on soil trophic networks and the resulting humus profiles in holm oak (Quercus rotundifolia) forests in the High Atlas of Morocco as revealed by correspondence analysis. *European journal of soil science, 54*(4), 767-777.

Salmon, S., Artuso, N., Frizzera, L., & Zampedri, R. (2008). Relationships between soil fauna communities and humus forms: response to forest dynamics and solar radiation. Soil Biology and Biochemistry, 40(7), 1707-1715.

Sanborn, P., & Pawluk, S. (1989). Microstructure diversity in Ah horizons of black chernozemic soils, Alberta and British Columbia (Canada). Geoderma, 45(3), 221-240.

Satchell, J. E. (1983). Earthworm ecology-from Darwin to vermiculture: Chapman and Hall.

Scheu, S. (1987). Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). *Biology and fertility of soils, 5*(3), 230-234.

Schwarze, F. W., Engels, J., & Mattheck, C. (2000). *Fungal strategies of wood decay in trees*: Springer Science & Business Media.

Schwertmann, U. (1965). Zachariae, G.: Spuren tierischer Tätigkeit im Boden des Buchenwaldes. Forstwissenschaftliche Forschungen. Beiheft z. Forstw. Centralbl. Heft 20. Verlag P. Parey, Hamburg u. Berlin 1965. 68 Seiten, 20 Abbildungen. Kart. DM 16,—, f. Bezieher d. Forstw. Centralbl. DM 12, 80. *Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde, 109*(1), 51-51.

Scohy, J.-P., Andre, P., & Lebrun, P. (1984). Influence des intensités d'éclaircie sur les populations de macroarthropodes et d'annelides dans les litières d'épicéa. *Pedobiologia, 26*(3), 179-184.

Seeber, J., & Seeber, G. U. H. (2005). Effects of land-use changes on humus forms on alpine pastureland (Central Alps, Tyrol). *Geoderma*, *124*(3), 215-222.

Senn, T., & Kingman, A. R. (1973). A review of humus and humic acids. Research Series(145).

Soil Survey Staff (1975). Soil taxonomy: abasic system of soil classification for making and interpreting soil surveys. USDA Soil conservation Service, Washington, DC, USA. Retrieved from http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/class/taxonomy/

Stolt, M., & Lindbo, D. (2010). Soil organic matter. *Interpretation of micromorphological features of soils and regoliths. Elsevier, Amsterdam*, 369-396.

Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems* (Vol. 5): Univ of California Press.

Tian, X. J., Takeda, H., & Ando, T. (1997). Application of a rapid thin section method for observations on decomposing litter in mor humus form in a subalpine coniferous forest. *Ecological Research*, *12*(3), 289-300.

van Wesemael, B. (1993). Litter decomposition and nutrient distribution in humus profiles in some Mediterranean forests in southern Tuscany. *Forest Ecology and Management*, *57*(1), 99-114.

Vater, H. (1928). Beiträge zur Kenntnis der Humusauflage von Fichte und Kiefer. *Mitt. a. d. sächs. forstl. Versuchsants. zu Tharandt*, 3:129-181.

Vitousek, P. (1982). Nutrient cycling and nutrient use efficiency. *American Naturalist*, 553-572.

Wilkinson, S., Anderson, J., Scardelis, S., Tisiafouli, M., Taylor, A., & Wolters, V. (2002). PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. *Soil Biology and Biochemistry*, *34*(2), 189-200.

Wilson, S. M., Pyatt, D., Malcolm, D., & Connolly, T. (2001). The use of ground vegetation and humus type as indicators of soil nutrient regime for an ecological site classification of British forests. *Forest Ecology and Management, 140*(2), 101-116.

Zachariae, G. (1967). Der einsatz mikromorphologischer methoden bei boden-zoologischen arbeiten. *Geoderma, 1*(3), 175-195

Zanella, A., Jabiol, B., Ponge, J.-F., Sartori, G., De Waal, R., Van Delft, B., Hager, H. (2011). European humus forms reference base.

Докучаев,В.В. (1936). Севеврная граница черноземной полосы. В *Русский чернозем* (сс. 53-187). Москва, Россия: СЕЛЬХОЗГИЗ.