Humus forms, organic matter stocks and carbon fractions in forest soils of northwestern Italy

DOI:10.1007/s00374-011-0568-y

Bonifacio E., Falsone G., Petrillo M. 2011. Humus forms, organic matter stocks and carbon fractions in forest soils of North-western Italy. Biology and Fertility of Soils, 47, 555-566. DOI: 10.1007/s00374-011-0568-y

Humus forms, organic matter stocks and carbon fractions in forest soils of North-western Italy

Eleonora Bonifacio[1], Gloria Falsone[12] & Marta Petrillo[1]

[1]Università degli Studi di Torino, DIVAPRA, Grugliasco (TO), Italy

Email: Eleonora.Bonifacio@unito.it

[2] *present address: Università di Bologna, DISTA*

Abstract

Humus forms may be the first tool to assess qualitatively organic matter turnover in soils; as such they should be related to the stocks of organic C a soil can store, to the characteristics of organic matter that affect its stability and, more generally, to the factors of soil formation. In this work we tested these hypotheses in 27 forest soils of north-western Italy. Site variables representing the pedogenic factors allowed to classify the plots into 3 clusters, which were significantly different for soil and humus types. The average stocks of organic C in the humic episolum (organic and top mineral horizons) ranged from 2.7 in Eumulls to 9.5 kg m^{-2} in Amphimulls. A clear trend in C stocks was visible and related both to the increasing presence of organic layers where the environmental conditions do not favour a rapid turnover of organic matter, and to the good mixing of organics and minerals in "bio-macrostructured" A horizons. The characteristics of organic matter were also linked to humus forms: the proportion of humified complex substances was the highest in the most active forms and, conversely, non-humified extracted substances formed a considerable part of organic matter only where the environmental conditions limit organic matter degradation. Humus forms seem therefore to reflect several mechanism of organic matter stabilization and are clearly related to the capacity of the soil to store C.

Keywords: organic matter stability, Alpine areas, soil types, carbon storage

Introduction

Humus forms are good indicators of soil functionality, as they encompass biological, biochemical and chemical factors. The importance of humus forms and the need for humus form classifications have been recognized since the beginnings of soil science (Berthelin et al. 2006) but the emphasis given nowadays to the capacity that forest soils have to store C originated a new interest in this traditional field of soil science. This is well demonstrated by the revisions and developments of humus classification systems in several European countries (Broll et al. 2006), and by the proposal of European systematics of humus forms (Jabiol et al. 2004).

The variability of humus forms reflects the varying degradation rate of organic matter in different environments (e.g. Duchaufour 1997), thus humus forms should provide a first tool to assess the capacity of the soil to act as a carbon sink. They are described in the field, with no use of chemical analyses and may thus offer an inexpensive tool to optimise sampling strategies for the evaluation of C stocks, or to have a preliminary assessment of organic matter transformation. In fact, in Mor humus forms the complete sequence of organic horizons is visible, testifying the low turnover of organic matter. On the opposite, Mulls only show the most recent litter inputs, as older organic C has already been oxidised to CO_2 or incorporated into the organo-mineral horizon.

Moders evidence an intermediate situation. When more detailed classifications are used, the sequence in the degradation of organic matter is respected also within a single class of humus forms. For example, according to the French humus form classification (Brêthes et al. 1995), Eumulls show only the most recent inputs of litter (OLn), in Mesomulls an old OL layer (OLv) may sometimes be present, but it becomes continuous and thicker only in Oligomulls, which may also have additionally an OF layer. Dysmulls are the least active mull humus forms and the OF layer is always present. Similar trends can be found in other humus classes, and in other humus classification systems (e.g. Green et al. 1993).

Because of the relation between humus forms and organic matter degradation, many Authors have investigated the effects vegetation and soil biota have on the occurrence of humus forms, in several environments. Cassagne et al. (2004) found that replacing beech stands with spruce, whose litter has a lower turnover rate, caused a shift from Mull to Moder in the French Pyrenées. In Mediterranean environments, Peltier et al. (2001) found an increasing thickness of OF horizons with increasing Aleppo pine influence, although little effect of vegetation was visible in the A horizon. In tropical forests, the chemical characteristics of litter were the main factors leading to the formation of Amphimulls or Dysmulls (Loranger et al. 2003). Vegetation dynamics also induce changes in soil biota, and the composition and diversity of invertebrate communities is correlated to humus forms (Salmon et al. 2008).

The quality of organic matter in litterfall is however only one of the factors influencing its degradation and, basically, the development of one humus form or another should depend on the same factors that affect soil formation (climate, relief, organisms, parent material and time, Jenny 1941). All these factors interact and drive the processes of accumulation and degradation of organic matter. Climatic conditions influence soil biota populations, dynamics and activity, and a low degradation of otherwise easily decomposable organic substrates is visible upon changes in temperature (e.g. Wetterstedt et al. 2010) or in the presence of water stress (e.g. Andresen et al. 2010). As a result, humus forms differ on south and north-facing slopes of otherwise similar sites (Salmon et al. 2008), or with altitude (Bernier 1996), or when drought is frequent (Descheemaeker et al. 2009). A good water storage, as in the case of footslope positions in toposequences, favours the presence of Moder with respect to Mor (Sevink et al. 1989), thus indirectly testifying the effect of relief, which drives water flows. The direct effect of soil parent material has been demonstrated in a few cases; more frequently the variability in humus forms that develop on soils with different lithology has been ascribed to indirect effects, such as the intensity of drought stress caused by different soil textures (Kooijman et al. 2005), or element content of litter (Ponge et al., 1999). The time of soil formation is probably less important for humus types, as the humus profile develops quickly following vegetation succession (e.g. Frouz and Novakova 2005); changes in humus form with time have been however documented also when no changes in tree species occurred (Turk et al. 2008).

Several methods are available to assess in detail organic matter stability, and they are either chemically or physically-based. In the last years, much emphasis has been given to the mechanisms influencing the stability of organic matter (e.g. Sollins et al. 1996; Jastrow et al. 2007; Kögel-Knabner et al. 2008); besides the intrinsic recalcitrance of organic compounds, stability is enhanced by the interactions between organics and minerals and by the inclusion of organic matter into aggregates, which precludes accessibility to enzymes and microbial cells (Sollins et al. 1996). As a consequence, physical fractionation methods are often preferred over older chemical methods that take into account organic matter dispersibility and solubility in acidic solutions. Due to the emphasis on the stabilisation of organic matter by interaction with minerals, physical methods are however poorly suitable to the humic episolum (i.e. the portion of the profile that includes all organic horizons and the top mineral one), where so many horizons are organic. Chemical methods are suitable both for organic humified and mineral horizons and may still offer some important information; while of little significance from the molecular point of view, the basic hydrolysis with NaOH or pyrophosphate, coupled with acid solubility of organic fractions (Schnitzer 1982), extracts recalcitrant substances hence providing information about SOM stability (von Lützow et al. 2007). Furthermore, also recently, the evaluation of humic substances contributed to understand the effects of vegetation and other pedogenic factors on soils (e.g. Iimura et al., 2010; Bonifacio et al., 2008; Cerli et al., 2008; Zanelli et al., 2006), thus this technique may help in differentiating humus forms.

Very few papers relating humus forms and C stocks are available to our knowledge, even if such a relationship would provide a link between low-cost qualitative information and capacity of soils to act as C sink, if the stability of organic matter is also evaluated. In this work we wanted to 1) evaluate the distribution of humus forms in forest soils of North-western Italy and to correlate them to the factors of soil formation; 2) assess the stability of soil organic matter through fractionation in the different types of humus forms; 3) calculate the stocks of organic matter in forest stands and relate them to humus forms.

Materials and methods

Twenty-seven plots were selected in the Piemonte region (NW Italy). The general characteristics of the study areas and the soil classification according to the World Reference Base (IUSS, ISRIC, FAO 2006) are reported in Table 1. The annual rainfall and the mean annual temperature of the sites were obtained from the Atlante Climatologico del Piemonte (Cagnazzi and Marchisio 1998); slope and aspect were recorded in the field, as well as the dominant tree species and the soil parent material.

At each plot, 4 sites were selected within a radius of 12.5 m from the centre of the plot, according to ICP Forests sampling procedures (UN/ECE 2006). At each site a pit was dug and samples were taken from the organic horizons and from fixed-depth mineral layers (0-10, 10-20, 20-40 and 40-80 cm). A representative soil profile was also described in the plot and samples taken from each genetic horizon, including organic layers. Thus samples from 5 different pits were available for each plot. The bulk density was measured using the core method (Blake and Hartge 1986). Organic layers were sampled using a frame, whose size varied depending on thickness of organic horizons.

Humus forms were described in the field according to Zanella et al. (2001) who adopted basically the French system (Brêthes et al. 1995), although adapted to Italian situations. This system was the standard Italian one taken into account in the proposal of European systematics of humus forms (Jabiol et al. 2004). Practically, the system discriminates among humus forms on the basis of the presence and characteristics of organic layers and of top mineral horizon. Several properties of O horizons are considered, such as: the degradation level of organic matter (OH, OF, OL), the presence of fresh or old litter (e.g. OLn, OLv), the continuity of the horizon, etc... The degree of mixing between organics and minerals is instead used to subdivide mineral horizons: the “bio-macrostructured” A horizon has a good granular structure that derives, not only from chemical reactions between organic and mineral components, but also from physical mixing of particles by soil biota. In the “juxtaposition” A horizon instead the mineral and organic components show little or no interaction.

The pH was determined potentiometrically in a 1:2.5 or 1:20 soil:deionised water suspension, for mineral or organic horizons, respectively. Organic C (OC) and total N (TN) contents were measured by dry combustion (CE Instruments NA2100 elemental analyser, Rodano, Italy). The stocks of organic C in the fine earth fraction (< 2 mm) were calculated by taking into account fine earth bulk density and OC concentration, with the exception of the OL layer, whose C stock was calculated by weighting the sample and dividing the total weight of organic matter by 1.72. For organic soil horizons (OF and OH) the bulk density was calculated according to the pedotransfer function of Hollis and Woods (1989). Eleven plots were then selected as representative of the variety of humus forms and on OH and A horizons of the selected profiles the total extractable C (TEC) was determined using NaOH as reported by Schnitzer (1982). Humic (HA) and fulvic acids (FA) were separated from the extract by bringing the pH to 1 with HCl. FA were purified by eluating them on PVP resins to separate non-humic extracted material (NH). The C content in the extracts of the different fractions was determined by wet oxidation.

All statistical data treatments were carried out using SPSS v. 17.0. Lithology and vegetation cover were divided into three groups and ranked in order of increasing acidity (basic rocks, mixed lithology, acid rocks), or of increasing presence of conifers (broadleaf species, mixed vegetation, pure conifer stands). The aspect was taken as an indicator of microclimatic conditions, it was divided into four groups ranked from the coldest to the warmest sites (N to E; E to SE and NW to N; SE to S and W to NW; S to W). Hierarchical clustering was used to classify the sites, using Ward's agglomeration method after variables were standardised by Z-scores. Differences between groups were evaluated by oneway Anova. Before analysis, the homogeneity of variances was checked by the Levene test. In case of non quantitative variables, their distribution into groups was compared with a Chi-square distribution by Montecarlo test.

Results

The sequence of diagnostic horizons for humus forms is reported in Table 2. Within plot variability was low and only in three cases a different sequence of diagnostic layers was found in the plot. Mull forms were the most commonly found (14 plots), followed by Moders (8 plots). Dysmulls dominated among Mulls, while the most frequent Moder form was Hemimoder. Only in two plots a Mor type of humus was present. Amphimulls dominated in 2 plots and were associated to Dysmulls in another one.

The trend in variability of humus forms as a function of elevation and vegetation type is visible in Figure 1a. Eumulls, Mesomulls and Dysmulls were found only below 900 m asl, while Oligomulls were also present at higher elevations. Moder humus forms showed a wide range of variability, with Dysmoders and Eumoders even above 1500 m. The two Mor humus forms were found between 1200 and 1500 m asl. Pure conifer stands always originated Moder or Mor humus, while in the case of mixed conifer-broadleaf forests, the least active types of Mulls were often present. Amphimulls were under broadleaf forest cover, generally at an elevation ranging from 700 to 1100 m asl, although they were found at 300 m asl when associated to Dysmulls. No clear trends with rainfall or with parent material were visible (Figure 1b); in particular, Dysmulls and Amphimulls showed an extreme variability.

Categorical and numerical site variables allowed to classify the plots into 3 clusters (Table 3). Cluster 3 grouped the coldest stands at the highest elevation, with low annual rainfall, with conifers or mixed vegetation. Cluster 1 included only soils that developed on acidic rocks, located around 900 m asl, with very abundant rainfall. Eighteen sites were included in Cluster 2, which shared a relatively low annual rainfall with Cluster 3, but the mean annual temperature was higher and the elevation lower. No significant differences in parent material were found among clusters (Table 3). The aspect was significantly different and reflected temperature data: 15 of the 19 warmest sites (from 135° to 315°N) were grouped in Cluster 2. All pure conifer stands were in Cluster 3, and broadleaved sites in Clusters 1 and 2; mixed forests were either in Cluster 2 or in Cluster 3.

The clusters built by using site variables reflected soil development, with significant differences ($p<0.01$) in the distribution of WRB groups (Table 4): Luvisols could only develop in the site conditions of Cluster 2, Regosols were in Clusters 2 and 3, as well as Cambisols, while the majority of Umbrisols was in Cluster 1. At a more detailed classification level, i.e. using WRB qualifiers, no differences were found; cambic and cutanic soils were all grouped in Cluster 2, but the most common leptic and haplic soils were distributed over the three clusters. Significant differences among clusters ($p<0.05$) were also found in dominant humus types (i.e. Mull, Moder, Mor and Amphimulls); Mor humus forms were all in Cluster 3, Moders shared between Clusters 2 and 3, and Cluster 1 mainly comprised Mulls, as well as Cluster 2. Amphimulls were found both in Cluster 1 and in Cluster 2 (Table 4). However, when humus forms were taken into account, i.e. when a greater detail in classification was used, no differences were visible (Table 4).

The organic soil horizons of all humus forms had similar organic C concentrations and C to N ratios (Table 5), and no differences among humus types were found either. The mineral horizons of Amphimulls always had the highest C concentration and the highest C/N ratio ($p<0.01$). All Moder forms were similar both in C concentration and C to N ratio at all depths and showed C concentrations ranging between 36 and 43 g kg^{-1} in the first 10 cm of soil. Mull forms were more variable, with Eumulls and Mesomulls having lower OC concentrations than Oligomulls and, to some extent, Dysmulls. Eumulls had the lowest C/N, but were not significantly different from Mesomulls. Mor humus were rather similar to Dysmoders and Hemimoders. In general, differences were less marked with increasing depth and, in the deepest layers (40-80 cm), only Amphimulls were significantly different from the other humus forms. No differences were found in pH (data not shown).

The average stocks of OC contained in the humic episolum ranged from 2.7 in Eumulls to 9.5 kg m^{-2} in Amphimulls, and within Mulls they increased from Eu- to Meso- to Oligo- and to Dysmulls (Figure 2). Among Moders, low contents where found in Eumoders (3.6 kg m^{-2}), but the stocks were rather high in Dysmoders (6.4 kg m^{-2}). The humic episolum of Mors stored, on the average, 6.0 kg C m^{-2} (Figure 2). Eumulls, Mesomulls and Eumoders ($p<0.05$) had significantly lower OC stocks than Amphimulls. The amounts of OC stocked in the OL layer were always below an average value of 1.7 kg m^{-2}, with maximum contents in Dysmoders and Hemimoders (Figure 2), but without any significant difference. As a consequence, when the litter layer was excluded from the analysis, the trends in C stocks obtained for the whole humic episolum were still present, but Hemimoders, as well as Oligomulls, fell into the low OC stock group. When all organic layers were excluded and the whole depth of the mineral soil profile was considered, the stocks of C were significantly higher in Amphimulls than in all other humus forms (Figure 2), although not significantly different from those of Oligomulls. Large OC stocks where also found in Dysmulls and Mors (8.3 and 8.6 kg m^{-2}, respectively). The kind of mineral horizon in the humic episolum (i.e. bio-macrostructured or juxtaposition) affected the stock of organic C in the horizon, although no differences were visible by considering the whole humic episolum (Figure 3). Marked differences were instead visible when considering OL and A horizons separately. The stocks of C in OL were much lower in humus forms having a bio-macrostructured A horizon than in the case of juxtaposition, while the opposite was true for C stocks in A horizons (Figure 3). When the OL layer was discarded from the analysis of the humic episolum, soils having a bio-macrostructured A horizon showed an OC stock of 3.4 kg m^{-2}, while in the case of juxtaposition lower contents were found (2.8 kg m^{-2}, $p<0.05$). The soils with bio-macrostructured A horizons had higher OC stocks also when all mineral horizons were considered ($p<0.01$).

The total extractable C (TEC) corresponded to 45% of OC on the average both in OH and A horizons (Table 6). Non-humic extractable substances (NH) heavily contributed to TEC, up to 62% in Dysmull A horizons. Fulvic (FA) and humic acids (HA) were present in similar amounts in the organic horizons of Mors and Amphimulls, while HA dominated in Dysmoders. In A horizons, HA always dominated over FA, with the exception of Mor humus forms that showed a FA to HA ratio of 1.2 (Table 6). The sum of HA and FA, i.e. the total humified extracted substances, decreased from the most active to the least active form in Mulls and the same trend was observed for Moder types, mainly because of the variation in the proportion of HA. They represented in fact 45% of TEC in Eumulls and Mesomulls and only 25% in Dysmulls.

Discussion

A great variability was found in humus forms, as expected from the variability of study sites, which differed in vegetation, parent material, relief and climate conditions. Soil variability was related to differences in factors of soil formation, and site variables were helpful in classifying the sites into three clusters, with the exception of parent material. Clusters reflected the distribution of WRB soil groups, with the most developed Luvisols found at lower elevation, mainly on south-facing slopes (Cluster 2). One cluster (Cluster 1) was only made by Umbrisols of the rainiest zones, with broadleaf vegetation, while Regosols were under conifer or mixed vegetation, in the coldest areas of high elevations (Cluster 3). This subdivision well corresponds to the main pedogenic processes that occur in forest areas of temperate environments (Chesworth 1992): leaching of cations and the subsequent process of clay eluviation-illuviation are possible only where soil erosion and mass wasting do not interrupt pedogenesis and where vegetation is not limited by climate conditions. For the formation of Umbrisols, an important input of biomass from leaf litter is needed, and the pH should be acidic either because of cation leaching, or thanks to the acidity of the parent material. This last case was depicted by Cluster 1, with thin Umbrisols (leptic) on acidic rocks. Regosols are typical soils of the highest mountain areas, where all factors together concur in limiting soil formation, and finally Cambisols represent an intermediate situation, where still no specific soil process dominates. Although site variables well described soil forming processes and the type of soil that consequently originated, they were efficient in discriminating humus forms only when the broad classes (Mulls, Moders, Mors and Amphimulls), were considered. Where no limitation in pedogenesis was present and Cambisols or Luvisols may develop, as in Cluster 2, Mulls and Moders dominated. The two Mors developed on Regosols and they were grouped in Cluster 3, where soil forming factors were the most limiting. Amphimulls, in agreement with their dual character (Brêthes et al. 1995), were found both in the Mull-prevailing Cluster 1 and in Cluster 2. However, the more detailed classification, which takes into account the accumulation/degradation of organic matter within a humus form class, indicated that humus forms were scattered in the three clusters and their distribution was not significantly different as ascertained by Chi-square. Beside the presence of different humus forms within a single stand, indicating the effects of micro-site factors, a wide within-humus form variability was found even in relation to the site variables that allowed to individuate the clusters. In particular nutrient-poor Mull forms (Oligomulls) showed a wide range in elevation (from 300 to 1500 m) encompassing both beech forests and less productive downy oak stands. An even higher variability was found in Dysmoders, which were present both at 400 m and above 1800 m asl. This variability has been found in several Alpine areas, where Moder humus frequently developed after the abandonment of pasture or for the presence of light management of previously pastured or forested land (Seeber and Seeber 2004), and whose distribution is therefore rather scattered. The presence of a relationship between humus and factors of soil formation only when broad classes were considered, suggests that other variables, besides those we used, are necessary to fully account for humus variability. Among the factors that are known to influence humus forms, the activity of soil biota and the groups of biota that are present deeply affect litter degradation and incorporation of organic materials into mineral soil horizons (e.g. Wolters 2000; Chauvat et al. 2007), but no direct information was available for this study. Other factors that may determine the presence of specific humus forms such as management practices and the regeneration stage of the forest stand (e.g. Salmon et al. 2008; Salmon et al. 2006; Podrazsky 2006) are probably not important in affecting the distribution of humus forms. All sites are lightly managed, and even if they may derive from tree plantations, the management practices are nowadays at a minimum. Wood harvesting for timber, if any, occurs by selecting trees and regeneration is always natural. As a consequence, a mosaic of trees of different species and at different growth stages was always found in the studied region, giving rise to a sort of steady-state, and probably contributing to the scarce within-plot variability in humus forms. Indeed, Aubert et al. (2006) after the evaluation of the effect of hornbeam in beech stands, concluded that a dispersed mixture of species induced a more homogeneous distribution of Mull forms than a clumped mixture.

Although the C stocks of the humic episolum ranged from 3 to about 10 kg m^{-2}, the within-group variability was rather high and the differences were more evident when the OL horizon was excluded from the evaluation. The higher C stocks of Amphimulls with respect to all other humus forms are probably related to the action of two preserving mechanisms: the slow decomposition of litter, documented by the presence of different O layers, enhanced by the binding of organic matter to mineral phases and the inclusion of organic compounds into soil aggregates, which is typical of bio-macrostructured A horizons. The effects of the two mechanisms are visible by examining the trends in C stocks. The presence of a good mixing between minerals and organics, as in the bio-macrostructured A horizons, induced higher C stocks, not surprisingly as the organo-mineral interaction is one of the main mechanisms of organic matter stabilisation in soil (e.g. Sollins et al. 1996). The slow degradation of organic matter is instead visible when the type of A horizon is kept constant. All Mull humus, including Amphimulls, show a bio-macrostructured A horizon, whose thickness was similar in all humus forms ($p=0.716$); the increasing trend in C stocks from Eumulls to Amphimulls is therefore likely to be caused by the increasing presence of OF and OH layers. Moders and Mors lack instead a well structured A horizon and mainly differ in the presence and thickness of the OH horizon. Also in this case, the thickness of the A horizon was not significantly different among humus forms ($p=0.136$), and therefore the trend in C stocks, which showed on one side Hemimoders and Moders, and on the other Dysmoders and Mors, is related to the presence of thicker and more continuous OH layer. These results contrast with those obtained by Andreetta et al. (2010), who suggested that the higher stocks of Amphimulls and Mulls were mainly related to the presence of a well structured A horizon. They have however worked in Italian Mediterranean environments, while soils of the present study belonged either to temperate or Alpine Italian areas, where the presence of OH and OF layers is expected to be more important.

The quality of organic matter reaching the soil surface and stored into organic layers was similar in all humus forms, as indicated by the C to N ratio. This characteristic is plant specific (e.g. Versterdal et al. 2008), but the admixture of different trees sharply decreases differences in mineralisation (e.g. King et al. 2002), as probably occurred in the study areas, when no monospecific stands were present. The *a priori* resistance to degradation of organic matter can therefore be assumed to be of little importance for its transformations. An evaluation of these transformations in mineral horizons is given by fractionation results. The sum of HA and FA represents the total amount of extracted humified substances that are formed through complex biochemical reactions. Conversely, non-humified extracted substances (NH) are mainly proteinaceous residues and saccharides solubilised by the alkaline extractant (Cheshire 1979) or other dissolved organic compounds such as low molecular weight organic acids. They are easily decomposed, thus should be present in the soil only when biological activity is limited. Within bio-macrostructured A horizons, the proportion of total humified substances decreased from Eu- and Meso- to Oligo- to Dysmulls, in agreement with the sequence of decreasing biological activity (Brêthes et al. 1995). In Amphimulls, the quality of organic matter was very similar to that of the most active forms of Mulls, further stressing the good biological activity that originates their biomacrostructure. In A horizons that lack the biomacrostructure, a marked difference in the sum of HA and FA was visible between Mor and Moder types. In Mors the proportion of humic substances was lower and, consequently, the NH/TEC ratio increased. Low molecular weight organic acids, together with FA, are among the compounds responsible for podzolisation (Lundström et al. 2000) and although no Podzols were found in the study sites, incipient podzolation was visible in the Humimor plot where the concentration of OC was slightly higher in the 10-20 cm layer than in the 0-10 cm (44 and 42 g kg^{-1} respectively). The slight migration of humic substances at depth may contribute to explain the low TEC/OC ratio we found in the A horizon of Mors, in addition to the low biological activity of this type of humus. Another typical feature of the transformations of organic matter is reflected in humus forms: in Mors the FA/HA ratio was close to 1 in the OH horizons and 1.2 in the A horizons. Humic acids are considered the most stabilised compounds (von Lützow et al. 2007), thus they should provide information about an additional mechanism of organic matter stabilisation. Their proportion was systematically higher in the most biologically active humus forms within a single class. The rank order was: Eumull > Mesomull > Oligomull > Dysmull and Hemimoder ~ Eumoder > Dysmoder. Amphimulls were similar to Eumulls and Mesomulls in the proportion of HA, thus they are enriched in the most complex and recalcitrant compounds; this may be an additional mechanism contributing to higher C stocks in Amphimull humus form.

Conclusions

All aerobic humus forms were found in the forest soils of North-western Italy and, although Mulls prevail, Moders were present in a wide range of environmental conditions. Site variables allowed a good discrimination among areas and the clusters were significantly different in the presence of soil groups and of humus types (Mulls, Moders, Mors and Amphimulls). The full classification of humus forms was however too detailed to highlight significant differences in their distribution in the three clusters found in our study. In particular, the large variability in the distribution of Moders, suggest that other factors besides those considered in this study are necessary to fully link them to site conditions. A clear trend in C stocks was visible in humus forms, due to the increasing presence of organic layers where the environmental conditions do not favour a rapid turnover of organic matter, and the good mixing of organics and minerals in bio-macrostructured A horizons. When both factors are present, as in Amphimulls, the stocks of C are the highest. The quality of organic matter, as assessed through fractionation, also vary with humus forms: the highest proportion of the most stable substances was in fact found in the most active forms of each humus class. This trends suggest that humus forms are good indicators not only of organic matter turnover in specific environmental conditions, but also provide a first indication of the transformations leading to the formation of stable organic compounds.

Acknowledgements

The data of this paper were collected within the CONECOFOR programme (Programma Nazionale per il Controllo degli Ecosistemi Forestali), co-funded by the European Commission following the EU Regulation Forest Focus (n. 2152/2003).

References

Andreetta A, Ciampalini R, Moretti P, Vingiani S, Poggio G, Matteucci G, Tescari F, Carnicelli S (2010) Forest humus forms as potential indicators of soil carbon storage in Mediterranean environments. Biol Fertil Soils. DOI 10.1007/s00374-010-0499-z

Andresen LC, Michelsen A, Jonasson S, Schmidt IK, Mikkelsen TN, Ambus P, Beier C (2010) Plant nutrient mobilization in temperate heathland responds to elevated CO_2, temperature and drought. Plant Soil 328:381-396.

Aubert M, Margerie P, Ernoult A, Decaëns T, Bureau F (2006) Variability and heterogeneity of humus forms at stand level: comparison between pure beech and mixed beech-hornbeam forest. Ann For Sci 63:177-188

Bernier N (1996) Altitudinal changes in humus form dynamics in a spruce forest at the montane level. Plant Soil 178:1-28

Berthelin J, Babel U, Toutain F (2006) History of soil biology. In : Warkentin BP (ed) Footprints in the Soil. People and Ideas in Soil History. Elsevier, Amsterdam, pp 279-306

Blake GR, Hartge KH (1986) Bulk density. In: Klute A (ed) Methods of Soil Analysis. Part 1, Second edition. Agron Monogr 9: 363-375

Bonifacio E, Santoni S, Cudlin P, Zanini E (2008) Effect of dominant ground vegetation on soil organic matter quality in a declining mountain spruce forest of Central Europe. Boreal Env Res 13: 113-120

Brêthes A, Brun JJ, Jabiol B, Ponge JF, Toutain F (1995) Classification of forest humus forms: a French proposal. Ann Sci For 52:535-546

Broll G, Brauckmann HJ, Overesch M, Junge B, Erber C, Milbert G, Baize D, Nachtergaele F (2006) Topsoil characterization—recommendations for revision and expansion of the FAO-Draft (1998) with emphasis on humus forms and biological features. J Plant Nutr Soil Sci 169:453–461

Cagnazzi B, Marchisio C (1998) Atlante climatologico del Piemonte. In: Precipitazioni e Temperature, Collana di Studi Climatologici in Piemonte, CD produced by Regione Piemonte – Università di Torino, Torino

Cassagne N, Bal-Serin MC, Gers C, Gauquelin T (2004) Changes in humus properties and collembola communities following the replanting of beech forests with spruce. Pedobiologia 48:267-276

Cerli C, Celi L, Kaiser K, Guggenberger G, Johansson M-B, Cignetti A., Zanini E (2008)
Changes in humic substances along an age sequence of Norway spruce stands planted on former agricultural land. Organic Geochemistry 39:1269-1280.

Chauvat M, Ponge JF, Wolters V (2007) Humus structure during a spruce forest rotation: quantitative changes and relationship to soil biota. Eur J Soil Sci 58:625-631

Cheshire MV (1979) Nature and Origin of Carbohydrates in Soil. Academic Press, London

Chesworth W (1992) Weathering systems. In: Martini IP and Chesworth W (eds) Weathering, Soils and Paleosols. Elsevier, Amsterdam, pp 19-40.

Descheemaeker K, Muys B, Nyssen J, Sauwens W, Haile M, Poesen J, Raes D, Deckers J (2009) Humus form development during forest restoration in exclosures of the Tigray highlands, Northern Ethiopia. Restor Ecol 17:280-289

Duchaufour P (1997) Abrégé de pédologie. 5th edition, Masson, Paris

IUSS, ISRIC, FAO (2006) World Reference Base for Soil Resources—a Framework for International Classification, Correlation and Communication. World Soil Resources Report 103, FAO, Rome.

Frouz J, Novakova A (2005) Development of soil microbial properties in topsoil layer during spontaneous succession in heaps after brown coal mining in relation to humus microstructure development. Geoderma 129:54-64

Green RN, Trowbridge RL, Klinka K (1993) Towards a taxonomic classification of humus forms. For Sci 39:1-49

Hollis JM, Woods SM (1989) The measurement and estimation of saturated soil hydraulic conductivity. SSLRC Report for MAFF Project, Soil Survey and Land Research Centre, Silsoe

Iimura Y, Fujimoto M, Hirota M, Tamura K, Higashi T, Yonebayashi K, Fujitake N (2010) Effects of ecological succession on surface mineral horizons in Japanese volcanic ash soil. Geoderma 159: 122-130

Jabiol B, Zanella A, Englisch A, Hager H, Katzensteiner K, de Waal RW (2004) Towards an European Classification of Terrestrial Humus forms. http://www.bodenkunde2.uni-freiburg.de/eurosoil/abstracts/id372_Jabiol_full.pdf. Accessed 14 December 2010

Jenny H (1941) Factors of Soil Formation. McGraw-Hill, New York

Jastrow JD, Amonette JE, Bailey VE (2007) Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Clim Change 80:5–23

King RF, Dromph KM, Bardgett RD (2002) Changes in species evenness of litter have no effect on decomposition processes. Soil Biol Biochem 34:1959–1963

Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B, von Lützow M (2008) An integrative approach of organic matter stabilization in temperate soils: Linking chemistry, physics, and biology. J Plant Nutr Soil Sci 171:5-13

Kooijman AM, Jongejans J, Sevink J (2005) Parent material effects on Mediterranean woodland ecosystems in NE Spain. Catena 59:55-68

Loranger G, Ponge JF, Lavelle P (2003) Humus forms in two secondary semi-evergreen tropical forests. Eur J Soil Sci 54:17-24

Lundström US, Van Breemen N, Bain D (2000) The podzolisation process. A review. Geoderma 94:91-107

Peltier A, Ponge JF, Jordana R, Arino A (2001) Humus forms in Mediterranean shrublands with Aleppo pine. Soil Sci Soc Am J 65:884-896

Podravsky V (2006) Logging and forest decline effects on the surface humus horizons in the Sumava Mts. J For Sci 52:439-445

Ponge JF, Patzel N, Delhaye L, Devigne E, Levieux C, Béros P, Wittebroodt R (1999) Interactions between earthworms, litter and trees in an old-growth beech forest. Biol Fertil Soils 29:360-370

Salmon S, Mantel J, Frizzera L, Zanella A (2006) Changes in humus forms and soil animal communities in two developmental phases of Norway spruce on acidic substrate. For Ecol Manag 237:47-56

Salmon S, Frizzera L, Camaret S (2008) Linking forest dynamics to richness and assemblage of soil zoological groups and to soil mineralization processes. For Ecol Manag 256:1612-1623

Schnitzer M (1982) Organic matter characterization. In: Page AL et al. (eds) Methods of Soil Analysis. Part 2. 2nd edn. Agron. Monogr. 9. ASA and SSSA, Madison, pp 581-594

Seeber J, Seeber GUH (2004) Effects of land-use changes on humus forms on alpine pastureland (Central Alps, Tyrol). Geoderma 124:215-222

Sevink J, Verstraten JM, Jongejans J (1998) The relevance of humus forms for land degradation in Mediterranean mountainous areas. Geomorphology 23:285–292

Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter: Mechanisms and controls. Geoderma 74:65-105

Turk JK, Goforth BR, Graham RC, Kendrick KJ (2008) Soil morphology of a debris flow chronosequence in a coniferous forest, Southern California, USA. Geoderma 146:157-165

UN/ECE ICP Forests (2006) Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests. 2006 edition. Manual part IIIa on Sampling and Analysis of Soils. Programme co-ordinating centre. http://www.icp-forests.org/pdf/Chapt_3a_2006(1).pdf. Accessed 14 December 2010

Vesterdal L, Schmidt IK, Callesen I, Nilsson LO, Gundersen P (2008) Carbon and nitrogen in forest floor and mineral soil under six common European tree species. For Ecol Manag 255:35-48

von Lützow M, Kogel-Knabner I, Ekschmittb K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biol Biochem 39: 2183-2207

Wetterstedt JTM, Persson T, Ågren GI (2010) Temperature sensitivity and substrate quality in soil organic matter decomposition: Results of an incubation study with three substrates. Glob Change Biol 16:1806-1819

Wolters V (2000) Invertebrate control on soil organic matter stability. Biol Fertil Soils 31:1-19

Zanella A, Tomasi M, De Siena C, Frizzera L, Jabiol B, Nicolini G (2001) Humus Forestali. Manuale di Ecologia per il Riconoscimento e l'Interpretazione. Applicazione alle Faggete. Centro di Ecologia Alpina, Trento

Zanelli R, Egli M, Mirabella A, Giaccai D, Fitze P (2006) Influence of laurophyllous species, Castanea sativa and Quercetum-Betuletum vegetation on organic matter in soils in southern Switzerland and northern Italy. Geoderma 136:723-737

Table 1. Characteristics of the study areas and soil types

Plot	Prevailing tree species	Elevation (m asl)	Slope (%)	Aspect	Parent material	Soil classification
U114	*Fagus sylvatica, Prunus avium*	1174	65	SE	Gneiss	Leptic Umbrisol
U116	*Fagus sylvatica, Quercus petraea,*	782	70	NE	Gneiss	Folic Umbrisol
U147	*Quercus petraea, Sorbus aria*	836	55	SW	Granite	Leptic Umbrisol
U179	*Castanea sativa, Corylus avellana*	495	85	SW	Gneiss	Cambic Umbrisol
U254	*Quercus robur, Carpinus sp., Corylus sp.*	365	60	SW	Pre-würm moraines	Haplic Cambisol
U255	*Castanea sativa, Quercus sp.*	417	20	S	Moraines (Würm)	Haplic Regosol
U279	*Fagus sylvatica, Betula sp., Alnus sp.*	1051	60	NW	Greenstones	Leptic Regosol
U305	*Pinus sylvestris*, *Larix decidua*	1219	45	NW	Calcschists	Haplic Regosol
U306	*Fraxinus excelsior*, *Larix decidua*	1405	30	N	Moraines (Würm)	Haplic Umbrisol
U334	*Castanea sativa*, *Carpinus sp.*	935	20	S	Gneiss	Leptic Regosol
U360	*Castanea sativa*	793	35	N	Gneiss	Haplic Cambisol
U368	*Castanea sativa, Robinia pseudoacacia*	240	10	NW	Sandstones	Haplic Cambisol
U385	*Alnus viridis, Larix decidua*	2027	55	N	Slope deposits	Leptic Regosol
U387	*Castanea sativa, Alnus glutinosa*	490	37	S	Gneiss	Haplic Cambisol
U391	*Pinus strobus, Quercus robur*	480	35	E	Flysch	Leptic Regosol
U392	*Quercus pubescens, Sorbus aria*	372	75	S	Marls	Leptic Cambisol
U393	*Pinus strobus, Quercus petraea*	365	35	NW	Marls	Cutanic Luvisol
U394	*Castanea sativa, Quercus pubescens*	490	45	W	Claystones	Cutanic Luvisol
U411	*Larix decidua, Acer pseudoplatanus*	1630	55	W	Calcschists	Leptic Cambisol
U412	*Fagus sylvatica, Pinus sylvestris*	834	8	E	Carbonates	Haplic Luvisol
U439	*Castanea sativa, Fraxinus excelsior*	620	15	S	Pre-Würm alluvium	Haplic Regosol
U441	*Castanea sativa, Pinus sylvestris*	697	45	S	Conglomerates	Haplic Luvisol
U459	*Picea abies*	1185	58	S	Quartzites	Leptic Regosol
U2001	*Quercus pubescens*	553		SW	Moraines	Leptic Cambisol
U2002	*Pinus sylvestris, Betula sp.*	786	50	SW	Moraines	Leptic Regosol
U2006	*Picea abies, Larix decidua*	1585	65	E	Gneiss	Leptic Regosol
U2007	*Castanea sativa*	746	50	SW	Micaceous schists	Haplic Regosol

Table 2. Humus forms of the study areas

Plot	Diagnostic horizons of the humus episolum[a]	Humus form[b]	OL[c] (cm)	OF[c] (cm)	OH[c] (cm)	A[c] (cm)
U114	OLn, OLv, (OF) //A_{bio}	Oligomull	2	<0.5		13
U116	OLn, OLv, OF, OH//A_{bio}	Amphimull	1	3	9	33
U147	OLn, OLv, OF//A_{bio}	Dysmull	3	1		29
U179	OLn, OLv, OF//A_{bio}	Dysmull	5	2		12
U254	OLn, (OLv), (OLt)//A_{bio}	Mesomull/Oligomull (1)	5			12
U255	OL, OF, OH~A_{jux}	Dysmoder	5	2	5	14
U279	OLn, OLv, OF, OH//A_{bio}	Amphimull	1	1	1	7
U305	OL, OF, OH//A	Fibrimor	2	4	2	14
U306	OLn, OLv,(OF)//A_{bio}	Oligomull	1.5	<0.5		4
U334	OL, OF~A_{jux}	Hemimoder	5	2.5		6
U360	OLn, OLv, OF//A_{bio}	Dysmull	5	2		9
U368	OLn, OF~A_{jux}	Hemimoder	2	4		3
U385	OL, OF, OH~A_{jux}	Dysmoder	0.5	1.5	6	7
U387	OL, OL, OF, (OH)~A_{jux}	Hemimoder	2	5	<0.5	9
U391	OLn, OLv, OF//Abio	Dysmull	2	1		2
U392	OLn, OLv, OF//Abio	Dysmull	3	0.5		3
U393	OLn, OLv, OF//Abio	Dysmull/Amphimull (2)	2.5	0.5		7
U394	OL, OL, OF (OH)~A_{jux}	Eumoder	2	0.5	<0.5	3
U411	OL, OL, OF, OH~A_{jux}	Eumoder	1	0.5	0.5	15
U412	OLn, OLv,(OF)//A_{bio}	Oligomull	2	<0.5		3
U439	OLn// A_{bio}	Eumull	1.5			11
U441	OLn, OLv, OF// A_{bio}	Dysmull	1.5	1		2
U459	OL, OL, OF~A_{jux}	Hemimoder	2	1		3
U2001	OLn// A_{bio}	Eumull	2			13
U2002	OLn, Olv, OF//A_{bio}	Dysmull	0.5	2.5		3
U2006	OL, OL, OF, OH//A	Humimor	1	0.5	2	18
U2007	OL,OF,OH ~A_{jux}	Eumoder/Dysmoder (2)	5	1.5	0.5	3

[a] ~ indicates a gradual boundary and // an abrupt boundary; A_{bio}: bio-macrostructured A horizon, A_{jux}: juxtaposition A horizon

[b] in case of more than one humus form, the number in parentheses indicate how many times the second form was found. The sequence of diagnostic horizons and their thickness are not reported for the second form.

[c] the average thickness is reported

Table 3. Results of Cluster Analysis and differences in site variables among clusters

		Cluster 1	Cluster 2	Cluster 3	p[a]
n of cases		3	18	6	
Mean and oneway analysis of variance					
Slope	%	63	39	51	ns
Elevation	m asl	931^b	596^c	1508^a	<0.01
Annual rainfall	mm	1882^a	1140^b	959^b	<0.01
Mean annual temperature	°C	9.3^a	10.9^a	5.7^b	<0.01
P/T	mm °C^{-1}	201^a	106^b	187^a	<0.01
n of cases in each Cluster and Chi-square significance					
Parent material					ns
	Acidic rocks	3	4	2	
	Mixed lithology	0	9	2	
	Basic rocks	0	5	2	
Vegetation					<0.01
	Broadleaf forests	3	13	0	
	Mixed forests	0	5	3	
	Conifer forests	0	0	3	
Aspect					<0.05
	N to E	1	3	3	
	E to SE & NW to N	1	0	0	
	SE to S & W to NW	0	10	3	
	S to W	1	5	0	

[a] Different letters indicate significant differences (p<0.05, Duncan's test); ns: non significant

Table 4: Distribution of soil types and humus forms in Clusters

	Cluster 1	Cluster 2	Cluster 3	p
Regosols	0	7	4	<0.01
Cambisols	0	6	1	
Umbrisols	3	1	1	
Luvisols	0	4	0	
Cambic	0	1	0	n.s.
Cutanic	0	2	0	
Folic	1	0	0	
Haplic	0	9	2	
Leptic	2	6	4	
Mull	2	11	1	<0.05
Moder	0	6	3	
Mor	0	0	2	
Amphimull	1	1	0	
Eumull	0	2	0	n.s.
Mesomull	0	1	0	
Oligomull	1	1	1	
Dysmull	1	7	0	
Hemimoder	0	3	1	
Eumoder	0	2	1	
Dysmoder	0	1	1	
Mor	0	0	2	
Amphimull	1	1	0	

Table 5: Variability in organic C (in g kg^{-1}) and C to N ratio according to humus forms

		Eumull	Mesomull	Oligomull	Dysmull	Hemimoder	Eumoder	Dysmoder	Mor	Amphimull
C	OL	260.9	443.8	285.3	426.8	298.3	338.1	447.1	408.9	442.9
	OF			187.2	356.6	408.9	478.7	315.5	348.2	399.3
	OH						122.0	154.0	182.1	212.4
	A	23.1	29.7	60.3	54.8	44.8	48.0	26.7	23.2	44.9
	0-10 cm	22.4^{d}	27.0^{cd}	63.9^{b}	47.9^{bc}	35.6^{cd}	43.4^{bcd}	35.5^{cd}	34.8^{cd}	105.8^{a}
	10-20 cm	13.0^{c}	13.1^{c}	37.9^{b}	25.5^{bc}	18.8^{bc}	24.6^{bc}	20.6^{bc}	28.0^{bc}	69.8^{a}
	20-40 cm	10.1^{c}	6.7^{c}	32.2^{ab}	21.2^{bc}	11.0^{c}	12.6^{c}	8.3^{c}	18.1^{bc}	45.9^{a}
	40-80 cm	8.3^{b}	3.5^{b}	12.7^{b}	8.8^{b}	7.5^{b}	7.8^{b}	6.5^{b}	7.3^{b}	36.4^{a}
C/N	OL	27.9	31.0	19.7	32.1	24.8	23.2	24.7	30.6	34.9
	OF			20.3	24.0	24.5	22.0	19.2	24.2	22.9
	OH						18.5	19.3	21.7	17.6
	A	12.1	13.5	14.0	15.4	16.5	17.1	16.0	14.4	17.2
	0-10 cm	11.7^{c}	13.5^{bc}	14.3^{abc}	14.5^{abc}	15.8^{ab}	14.7^{abc}	14.8^{abc}	12.5^{c}	17.4^{a}
	10-20 cm	10.7^{d}	13.1^{bcd}	15.0^{abc}	13.0^{bcd}	15.0^{abc}	15.7^{ab}	13.0^{bcd}	12.3^{cd}	17.9^{a}
	20-40 cm	10.7^{c}	12.1^{bc}	16.0^{b}	12.6^{bc}	13.1^{bc}	15.0^{bc}	10.8^{c}	11.1^{c}	22.6^{a}
	40-80 cm	10.6^{b}	10.5^{b}	16.9^{b}	11.1^{b}	10.6^{b}	12.9^{b}	10.4^{b}	11.8^{b}	25.8^{a}

Different letters indicate significant differences ($p<0.05$, Duncan test)

Table 6: Proportion of fulvic acids (FA), humic acids (HA) and non-humic extracted substances (NH) with respect to total extractable carbon (TEC), contribution of TEC to total organic C stocks, and FA to HA ratio in OH and A horizons of humic episola

		Eumull	Mesomull	Oligomull	Dysmull	Hemimoder	Eumoder	Dysmoder	Mor	Amphimull
OH	TEC/C							0.54	0.41	0.36
	FA/TEC							0.11	0.28	0.26
	HA/TEC							0.51	0.35	0.27
	NH/TEC							0.38	0.36	0.48
	FA/HA							0.21	0.81	0.96
A	TEC/C	0.47	0.45	0.36	0.48	0.54	0.44	0.54	0.26	0.47
	FA/TEC	0.20	0.22	0.13	0.13	0.17	0.12	0.21	0.24	0.19
	HA/TEC	0.46	0.44	0.30	0.25	0.39	0.42	0.31	0.20	0.43
	NH/TEC	0.34	0.33	0.57	0.62	0.44	0.47	0.48	0.56	0.38
	FA/HA	0.43	0.50	0.46	0.50	0.43	0.28	0.65	1.20	0.44

Figure 1. Distribution of humus forms according to elevation and vegetation (a) and rainfall and lithology (b)

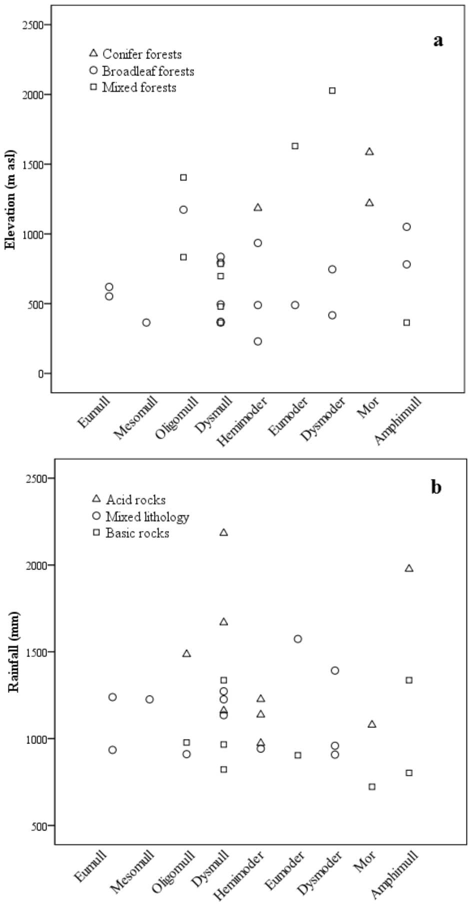

Figure 2. Organic C stocks in relation to humus forms. Different letters indicate significant differences (p<0.05 Duncan's test); bars represent standard errors

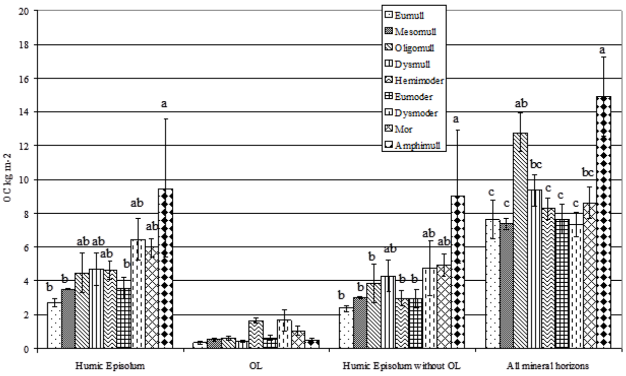

Figure 3. Organic C stocks in relation to the presence of bio-macrostructured or juxtaposition A horizons. Different letters indicate significant differences (p<0.05 Duncan's test)