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# The impact of parent material, climate, soil type and vegetation on Venetian forest humus forms: a direct gradient approach

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## ABSTRACT

The impact of geology, climate, soil type and vegetation on forest humus forms was studied in the Veneto Region (northern Italy). A total of 352 study sites were compared by Redundance Analysis (RDA). Humus forms were described by the structure (micro-, meso-, or macro-aggregated) of the organo-mineral A horizon, by the thickness of litter horizons and by their nomenclature, which followed the morpho-functional classification recently proposed for inclusion in the WRB-FAO. The size of aggregates within the A horizon was distributed along a common gradient embracing geology,

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climate, soils and vegetation. Macro-aggregation (as opposed to micro-aggregation, meso-aggregation being intermediate) was favoured by carbonated (as opposed to silicated) parent rocks, warmer climate associated to lower elevation, lower soil acidity, deciduous (as opposed to coniferous) forest vegetation and relatively high clay content. The amphi group of humus forms, associated with carbonated substrates in Esalpic and Mesalpic climate districts, was distributed according to thickness of litter horizons along a gradient of soil stoniness. Biological reasons for the observed environmental influences on the size of soil aggregates, a criterion of humus form classification, were discussed to the light of knowledge on annelid (earthworm and enchytraeid) ecology. Humus forms can be easily identified and classified on the field, using a table included in the article. Our results can be used for mapping the distribution of forest humus forms in the Veneto Region, implying a better understanding of carbon cycling processes in the frame of present-day global warming.

*Keywords:* Veneto, humus forms, mull, moder, amphi, aggregate size

## 1. Introduction

Following the United Nations Framework Convention on Climate Change (UNFCCC, 1992), there is an urgent need to characterize organic carbon pools both quantitatively and qualitatively worldwide, in order to (*i*) optimize models of global climate warming (Bottner et al., 1995; Marland et al., 2003; Thum et al., 2011), and (*ii*) discern trends in C turnover/sequestering and nutrient availability/retention at ecosystem level (De Deyn et al., 2008; Zhang et al., 2008; Egli et al., 2010). Some countries, such as the Netherlands, implemented soil organic carbon (SOC) inventories, based on forest standing crops (Nabuurs and Mohren, 1993) and agricultural soil maps (Kuikman et al., 2003). However, in forests soils carbon stocks vary to a great extent according to the development of organic layers (Schulp et al., 2008; Andreetta et al., 2011; Bonifacio et al., 2011), although more stable C stocks in the mineral horizons should not be neglected (Garlato et al., 2009; Rumpel and Kögel-Knabner, 2011). Humus forms, patterning the way organic matter is distributed and transformed within the forest soil profile (Bal, 1970; Klinka et al., 1990; Kindel and Garay, 2002), and known as key components of plant-soil relationships (Ponge, 2003, 2013), are easy to identify directly on the field without expensive laboratory analyses. They also could be mapped with the aid of digital mapping techniques (Aberegg et al., 2009), taking into account their local variability by standardized protocols (Ponge et al., 2002; Lalanne et al., 2008). The systematic census of humus forms could allow in a near future a clearer assessment of the amount and distribution of fast-recycling (organic horizons) and stable (organo-mineral horizons) carbon at scales varying from local to regional then to global levels (Thornley and Cannell, 2001; Hedde et al., 2008; Andreetta et al., 2011). In parallel, searching proxies for humus forms in geology, climate, soils and vegetation, mapped for a long time or easily accessible by remote sensing, and known as main determinants of forest soil conditions (Egli et al., 2009, 2010; Li et al., 2010), would help to achieve this goal even more rapidly.

A recent study by Ponge et al. (2011) showed that at national level (France) forest humus forms were mainly ‘explained’ (in statistical sense) by geology and climate. Tree canopies (coniferous vs deciduous) exhibit only a minor additive influence. The present study was undertaken in the mountain and hilly part of the Veneto Region (northern Italy), embracing a wide array of climate and geologic conditions on a relatively small area (6,000 km$^2$) owing to its particular position on the south-eastern side of the Alps arch and on the northern border of the Adriatic Sea (Del Favero and Lasen, 1993). The aim was to assess whether the influence of (*i*) components of climate (temperature, precipitation), elevation, aspect, soil type and substrate (carbonated versus silicated parent materials), known to affect forest humus forms in other North Italian contexts (Carletti et al., 2009; Bonifacio et al., 2011; Ascher et al., 2012), and (*ii*) vegetation, whose effects have been well-established at a very local level (Schulp et al., 2008; Trap et al., 2011, 2013), could be retrieved in the regional context of Veneto. For that purpose we used for the first time a new classification of humus forms, based on morpho-functional principles previously defined at European level by Zanella et al. (2011), then refined by Jabiol et al. (2013) in the frame of the World Reference Base for Soil Resources (IUSS Working Group WRB, 2007.

## 2. Materials and methods

### *2.1. Climate, geology and distribution of forest and soil types in Veneto*

Veneto is an Alpine Italian Region, displaying an altitudinal gradient from the Mediterranean Sea (Gulf of Venice) up to the summits of the eastern Alps (Carnic Alps, Austrian boundary) (Fig. 1). Four climatic districts have been described by Del Favero and Lasen (1993).

The Avanalpic climatic district (numbered 1 in Fig. 1) concerns the lowland area between the Mediterranean Sea and the Alpine reliefs. The area is comprised of the wide Veneto plain and a hilly zone at the piedmont of the Alps, with a climatic gradient between them. On the whole, the Mediterranean district is characterized by a mean annual precipitation of 900–950 mm with a maximum in spring, and a mean annual temperature of 12–13°C with July as the warmest month but without any arid summer season. Ericaceous species and sweet chestnut (*Castanea sativa* Mill.) are common on silicated substrates or on acid soils as opposed to oak (often downy oak, *Quercus pubescens* Willd.), hornbeam (often hop hornbeam, *Ostrya carpinifolia* Scop.) and ash (often Manna ash, *Fraxinus ornus* L.), which grow preferably on carbonated substrates. Remains of the natural oak-hornbeam forest which covered the Po plain between 6000 BC and a few centuries ago (Susmel, 1994), are nowadays managed as hedgerows in a mechanized agricultural landscape or as small private woods.

The Esalpic district is characterized by Mediterranean-type temperatures but mean annual precipitation is higher (ca. 1500 mm) owing to wet-warm air coming from the sea and raising on pre-alpine reliefs (Fig. 1). Carbonated substrates (calcareous and dolomitic), largely dominant, are favourable to the development of pure or mixed forests of hop hornbeam, with Manna ash, downy oak, field maple (*Acer campestre* L.), which occupy about one third of the forest area in Veneto (Del Favero, 2010). Above 800 m altitude, hop hornbeam is replaced by common beech (*Fagus sylvatica* L.) in thermophile beech forests which constitute the second most important element of forest vegetation.

The more internal Mesalpic district is characterized by a high mean annual precipitation (ca. 1400 mm, snowfall included), which is mostly distributed from April to November. Temperatures are much lower than in the Esalpic district, averaging 7-8 °C. The whole district is covered with mountain beech forests, pure or mixed with Norway spruce [*Picea abies* (L.) Karst.] and silver fir (*Abies alba* Mill.), and by mixed spruce-fir forests. The composition of these forests varies according to substrate (95 % carbonated), slope and exposure.

The Endalpic climatic district corresponds to the highest part of the region. The mean annual precipitation is lower than in Mesalpic and Esalpic districts (ca. 1000 mm) and is distributed according to a continental regime with a single peak in July. Mean annual temperature is 5 °C, being too cold for many broadleaved tree species. Above 1500 m altitude, Swiss pine (*Pinus cembra* L.) is common, while Norway spruce and European larch (*Larix decidua* Mill.) grow at the same altitude but also lower, often favoured by human activities (pasture or forest management). Spruce forests cover silicated substrates in the high north-eastern part of the district.

In short, there is a gradient of decreasing temperature from the Mediterranean Sea (Avanalpic district) to the higher Carnic Alps (Endalpic district), while precipitation is distributed in a humped back fashion (Fig. 1), maximum precipitation occurring at mid elevation (Esalpic and Mesalpic districts).

Geologically, the study area can be divided, from North to South, into Alpine, Prealpine and Hilly areas. The Alpine sector displays a remarkable lithological variability, from metamorphic acid rocks of the crystalline basement of the Dolomites to the overlying stratigraphic succession (mainly calcareous, dolomitic and terrigenous rocks). In the Prealpine area limestone and marly limestone are common, but in the eastern part basalts of Tertiary volcanism are widespread, while the area of Recoaro and the Piccole Dolomiti (Small Dolomites), due to particular tectonic conditions, show a Permo-Triassic stratigraphic succession, similar to Dolomite reliefs. The Hilly Area is characterized by extremely heterogeneous geological substrates, ranging from limestone of the Berici to volcanic rocks of the Euganean hills with a prevalence of carbonated substrates (Antonelli et al., 1990).

Lithological variability results in prominent soil heterogeneity (Antonelli et al., 1990) in mountain areas. On carbonated rocks, shallow soils with a high content in skeleton and a low differentiation of profiles, classified by the World Reference Base (IUSS Working Group WRB, 2007) as Rendzic Leptosols, prevail besides deeper and moderately developed soils with cambic (Bw) horizons (Endoleptic Cambisols [Calcaric, Skeletic]). On silicated rocks with cold climate and high altitude the podzolization process is dominant (Podzols). At lower altitude this process is less intense with translocation of iron and aluminium sesquioxides (Bs horizons) but not of organic matter (Haplic Cambisols [Dystric]).On marly limestone, especially in the Prealpine area, well-differentiated and deep soils free of carbonates dominate even on very steep slopes, with a horizon of clay accumulation (Cutanic Luvisols). In the Hilly Area sandy and marly lithotypes are associated with moderately deep soils, partially decarbonated (Haplic Cambisols [Eutric] and [Calcaric]). In Euganean hills acid soils are prevalent (Haplic Cambisols [Dystric]).

### *2.2. Selection, description and classification of humus profiles*

Study sites (N = 348) were selected in order to embrace the widest possible variety of geological, climate, soil and vegetation conditions found in forests of the Veneto region, in the framework of the Soil Map of Veneto Region at 1:250,000 scale (ARPAV, 2005). Humus profiles were described according to the thickness of OL (not retained for data analysis due to strong seasonal variations), OF and OH organic horizons (litter s. l.) and to the structure of the A horizon: organo-mineral aggregates were classified in macro- (> 4 mm), meso- (2–4 mm), and micro- (< 2 mm) aggregates (Zanella et al., 2011). Humus profiles were then assigned to humus forms according to Zanella et al. (2011). Table 1 summarizes main features useful for the identification of humus forms found during the survey. Given the scarcity of acid silicated substrates in the study region (Fig. 1) only a restricted array of acidic humus profiles were found, all of them classified as dysmoder, while mull and amphi were represented by all described humus forms. Amphi is a recently acknowledged group of humus forms (mostly carbonated), displaying a combination of moder (in organic horizons) and mull features (in organo-mineral horizons), which were first described under the collective name amphimull (Jabiol et al., 1994; Brêthes et al., 1995; Jabiol et al., 1995, 2007), then separated from mull under the name amphi (Graefe, 2007; Galvan et al., 2008) or amphimus (AFES, 2008; Gobat et al., 2010) and more recently shared in several humus classification systems (Zanella et al., 2009, 2011; Jabiol et al., 2013). Considered (explained) variables were then the thicknesses of OL, OF, OH and A horizons and the presence of macro-, meso- and micro-aggregates.

### *2.3. Explanatory data (Table 2)*

Among distal factors (not affected by humus forms), parent rocks were classified in silicated (most of them acid) and carbonated rocks (limestone, dolomite and marls). At each site, the elevation (a.s.l.) was recorded, as well as the aspect according to the nearest cardinal direction. The climate variables considered were: mean annual precipitation (precipitation, in mm), mean annual temperature (temperature in °C), annual and summer (June-July-August) potential evapotranspiration (in mm) calculated according to Thornthwaite and Mather (1957), Gams continentality index (precipitation/elevation), simplified Lang aridity index (precipitation/temperature), and the site's occurrence in one of the four climate districts.

Among proximal or site factors (in mutual relationship with humus forms, Ponge, 2003, 2013) forest types were classified in 8 groups: beech, chestnut, other deciduous trees, spruce-beech, spruce-pine, spruce-larch, silver-fir and larch forests. A vegetation index, varying along a scale from 1 to 3 expressed the dominance of coniferous versus deciduous canopies within forests. Herbaceous cover was measured as a dummy variable (presence > 50% cover; absence < 50% cover). Soils were classified in six groups based on the World Reference Base (IUSS Working Group WRB, 2007): rzLP = Rendzic Leptosols, CMca = calcareous Cambisols, CMeu = non calcareous Cambisols with high base saturation, LVhe = Luvisols with high base saturation, LVdy = very acid Luvisols, CMdy/PZ = aggregate group embracing Podzols and very acid Cambisols. Several soil analyses were done in the A horizon of humus profiles: $pH_{water}$ (glass electrode method in 1:5 soil:water suspension, ISO

10390:2005), presence of carbonates (effervescence to cold dilute hydrochloric acid, scale 0–4 according to USDA Soil Survey Manual), clay, sand and rock fragments in mass percentages (sieving and sedimentation method, ISO 11277:2009), organic C and N in mass percentages (dry combustion method, ISO 10694:1995 and 10878:2009, respectively), and C/N ratio. The $\mathrm{pH_{water}}$ of the topsoil was also measured with the same method in a bulk sample from 0 to 40 cm, including the A horizon but not the O horizon.

### *2.4. Data analysis*

Data were analyzed by Redundancy Analysis (RDA), a direct gradient multivariate method (Van den Wollenberg, 1977; Kenkel, 2006), allowing discerning trends in a set of explained variables (humus form categories and humus profile features) which could be explained by a set of explanatory variables (geology, climate, soil, vegetation). For the sake of clarity, we projected independent (explanatory) and dependent (explained) variables in separate bi-plots, and dependent variables were arbitrarily subdivided (for easier visual interpretation) in climate and geology in one bi-plot, and vegetation and soil in another bi-plot. All three bi-plots belong to the same analysis.

Major trends detected by visualizing RDA bi-plots were checked for statistical significance by chi-square test. Quantitative variables to be analyzed by chi-square test were classified in two or three categories on the basis of arbitrary class limits, taking into account the need for having balanced class sizes among categories, to the exception of pH, which was divided in two classes with a limit at pH 5.2, separating soils with ($\mathrm{pH} < 5.2$) or without ($\mathrm{pH} \geq 5.2$) soluble aluminum (Walker et al., 1990), a threshold known to affect the composition of annelid communities (Graefe and Beylich, 2003). All statistical calculations were performed under XLSTAT® version 2013.4.05 (Addinsoft, Paris, France).

## 3. Results

The main features of the 9 recorded humus forms are presented in Table 2. Most represented humus forms were Oligomull (84 sites) and Eumacroamphi (61 sites), and the least represented ones were Mesomull (8 sites) and Pachyamphi (15 sites). The four mull humus forms (Eumull – Mesomull – Oligomull – Dysmull) were more often recorded on carbonated substrates, with a dominance of macro- and meso-structures in the A horizon. Dysmoder was found exclusively on silicated (mostly acid) substrates with very acid and podzolic soils (CMdy/PZ) and at high altitude. Within the amphi group, which was mostly represented (but not exclusively) on carbonated substrates, there was a trend of increasing dominance of carbonated substrates, from 60 to more than 90%, along the sequence Leptoamphi → Eumesoamphi → Eumacroamphi → Pachyamphi, which was associated with increasing thickness of OF horizon from 1.1 to 2.5 cm and OH horizon from 0.5 to 6.4 cm (Fig. 2). This trend was concomitant with an increase in the representation of shallow or very shallow calcareous soils (rzLP and CMca), from 30 to 80% (Table 2). Amphi humus forms were found near exclusively in Esalpic and Mesalpic climate districts and were absent from Endalpic and Avanalpic districts. Mor and Tangel have not been observed in forest soils of Veneto.

The first canonical component F1 extracted 37% of the explained variance. Mull and moder were projected on opposite sides of this component. Humus forms were distributed along F1 mainly according to the structure of the A horizon, with a gradient from macro- to meso- then to micro-aggregates (Fig. 3a). The thickness of fragmented and humified O horizons (OF and OH, respectively) varied in a direction opposite to that of OL and A horizons. The ends of the gradient were characterized by Oligomull and Dysmoder, respectively, other humus forms being projected in an intermediary position.

The projection of environmental variables showed that the first canonical component could be interpreted as a composite factor embracing both geological, climate and soil gradients. Among distal factors (Fig. 3b) the positive side of F1 was characterized by silicated rocks (as opposed to carbonated rocks), high elevation, low temperature, low summer and to a lesser extent annual potential evapotranspiration, high Lang index (low aridity), and Mesalpic district (as opposed to Esalpic and Mediterranean districts on left side). Among site factors (Fig. 3c), the positive side of F1 was characterized by most acidic soils (CMdy/PZ: Podzols and dystric Cambisols), low pH (whether A horizon or first top 40 cm), high C/N and organic C, high sand content (as opposed to clay content) and high vegetation index (dominance of coniferous trees), exemplified by spruce-pine forests (right side) opposed to other deciduous forests (left side) with opposite characters (soil types CMeu, LVhe, CMca).

Mean annual precipitation, organic N, exposure (four cardinal directions), Endalpic district, rock fragments content and soil effervescence were projected not far from the origin along F1, thus did not seem to contribute to a great extent to the gradient depicted by F1. Thus temperature and potential evapotranspiration (in particular during summer months) for climate, and pH, organic C and C/N for soil, are given more importance in this gradient than mean annual precipitation and organic N, respectively.

We tested the significance of the gradient from macro- to meso- then to micro-aggregated structure of the A horizon according to some possible proximal (soil, vegetation) and distal (geology, climate) factors (Fig. 4). The impact of geology (silicated vs. carbonated rocks, Fig. 4a), elevation (three classes, < 800m, 800-1500m, > 1500m, Fig. 4b), temperature (three classes, < 6°C, 6–10°C, > 10°C, Fig. 4c), aridity (three classes of Lang index, < 150, 150–250, > 250, Fig. 4d) and climate district (four districts, Fig. 4e) was verified, with a very high significance level ($P < 0.0001$ for all tests, to the exception of $P < 0.001$ for Lang index). The impact of vegetation (three classes of vegetation index, Fig. 4f), soil pH (two classes, pH < 5.2, pH ≥ 5.2, Fig. 4g) and clay content (three classes, < 15%, 15–25%, > 25%, Fig. 4h) was verified with a significant level for clay content ($P < 0.05$) and a highly significant level for vegetation and pH ($P < 0.0001$). The variation along geological, climate, soil and vegetation gradients of the proportion of meso-aggregated A horizons was much less pronounced than that of micro- and macro-aggregated A horizons, which exhibited strongly contrasted trends, exemplifying the intermediate nature of meso-aggregates.

The second canonical component F2 extracted 21% of the explained variance. Along F2 amphi (negative side) was opposed to both mull and moder (positive side). Humus forms were distributed along this factor mainly according to the thickness of OF and OH horizons, in particular within the amphi group, Pachyamphi (with thickest OF and OH horizons, see Table 2) being projected far from the origin on the negative side of F2, followed by Eumacroamphi then by Eumesoamphi and at last Leptoamphi (Figs. 2 and 3a). Within the mull group, Dysmull was opposed to Oligomull along F2, Mesomull and Eumull being in an intermediate position, thus not depicting a similar gradient of thickness of OF (OH absent in mull) horizon (see Table 2). Among distal and site environmental factors, F2 mainly depicted an opposition between silicated and carbonated rocks (Fig. 3b), and a gradient in soil effervescence, clay and rock content (Fig. 3c): very shallow calcareous soils (rsLP) with low clay and high rock fragment content, developed over carbonated substrates are on the negative side of F2 (Fig. 3c).

The thickness of OH horizons, the % of carbonated substrates among recorded profiles (Fig. 2) and the rock fragments content of A horizons (Fig. 5) increased along the gradient Leptoamphi – Eumesoamphi – Eumacroamphi – Pachyamphi. The thickness of the OH horizon and the effervescence level (varying from 0 to 4) were positively correlated with the rock fragment content of the soil on carbonated substrates ($r_s = 0.26$ and 0.37, respectively, $P < 0.0001$).

## 4. Discussion and conclusions

We showed that a large variety of humus forms, mainly associated with carbonated substrates (hard limestone, dolomite and marl), were recorded in Veneto. We also showed that geology, climate, soil and vegetation exert a prominent influence on the distribution of humus forms, especially when considering the micro-, meso- or macro-aggregated structure of the A horizon. The coldest climatic district in the Veneto region is situated in areas with the highest elevation, the most acidic substrate (acid silicated rocks), the most recalcitrant litter (spruce and pine), and a finer structure in the A horizon. This means that all factors (low temperature, high acidity, litter recalcitrance), known to decrease litter decomposition and soil biological activity (reviewed in Ponge, 2003, 2013), may affect the size of soil aggregates, whether these factors co-occur or not. In the study region, substrate acidity and litter recalcitrance (spruce and pine) are correlated. Coniferous forest vegetation, especially spruce, is ubiquitous and man-induced for a large part, being mostly established on poorest soils as commonly observed in other regions (Willis et al., 1997; Wilson et al., 2001). On the contrary elevation and soil acidity are not correlated in Veneto (Fig. 1), where the highest mountain summits and cliffs are of carbonated (dolomitic) nature (ARPAV, 2005; Viero et al., 2010), while in eastern and western parts of the Alps acidity increases with altitude, since carbonated rocks are eroded at a higher rate than core metamorphic and igneous silicated rocks during orogeny (Frisch et al., 2008), among many other causal factors (Körner, 2007).

The structure of the organo-mineral horizon is mainly governed by the activity of soil organisms, among which annelids, after roots, take a prominent position (Wolters, 1991, 2000; Zangerlé et al., 2011). Among annelids, large worms (generally earthworms) and small worms (generally enchytraeids) diverge in their tolerance to low temperatures (Holmstrup et al., 2007), acidity (Graefe and Beylich, 2003), and litter quality (Ponge et al., 1997). Annelids create different structures according to their size, macro- (> 4 mm) and meso-structures (2–4 mm) of mull and amphi humus forms resulting from the burrowing and defecating activity of earthworms (Galvan et al., 2008), while microstructures (< 2 mm) of moder humus forms result mainly from the activity of enchytraeids (Didden, 1990; Dawod and FitzPatrick, 1993; Ponge, 1999). Enchytraeids, in particular the common, asexual *Cognettia sphagnetorum*, replace earthworms when conditions become more severe along latitudinal and altitudinal gradients (Bernier et al., 1993; Briones et al., 2007). Exclusion mechanisms between enchytraeids and earthworms, whether competitive or behavioral in advance of competition, have been demonstrated (Haukka, 1987; Ponge, 2003), in addition to abovementioned differences in tolerance, which might explain observed gradients of annelid composition and resulting changes in humus forms (Ponge, 2003).

As expected, main groups of humus forms present in the Veneto region, namely mull, moder and amphi, are distributed according to their main morphological features and their contrasted responses to environmental factors (Ponge, 2003; Graefe and Beylich, 2003; Graefe, 2007; Ponge, 2013). Our results show a strong contrast between (*i*) Dysmoder (as the unique representative of the moder group) in environmental conditions favoring fungal and mesofaunal activity (aggregates < 2 mm, mainly done by enchytraeids), as in very acid podzolic soils, and (*ii*) mull and amphi, representatives of soils with intense bacterial and macrofaunal activity (aggregates > 2 mm, mainly done by earthworms). We hypothesize that the gradient depicted by the first canonical factor of RDA (embracing climate, geology, soil and vegetation) corresponded to the balance between fungal-based and bacterial-based soil trophic networks, a balance largely controlled by the environment (Hedlund et al., 2004). The impact of environmental gradient on humus forms is mainly mediated by the activity of annelids (enchytraeids and earthworms) which transform the litter into humus and incorporate it (or not) to mineral horizons, with strong variations according to their size and behavioral (burrowing) type. Low temperature, acidity and poor litter quality favor small-sized annelids (enchytraeids) to the detriment of big-sized annelids (anecic earthworms), medium-sized annelids such as epigeic earthworms being intermediary in their environmental requirements (Ponge, 2003, 2013). Awaiting a study embracing both animal communities and humus profiles in a wide range of environmental conditions, this may contribute to explain the observed gradient of aggregate size distribution.

The contrast between mull and amphi was mainly associated with physicochemical properties of parent materials, in particular texture (clay → sand → rock fragments) and calcium content (soil effervescence). Our results do not point to a great importance given to climate in the contrast between amphi and mull, although climate has been suspected to be a driving factor of amphi build-up (Graefe, 2007). Amphi, being characterized by thick organic horizons with a well-developed OH horizon (otherwise typical of moder and mor) overlying a macro- or meso-aggregated A horizon (otherwise typical of mull), can be explained by the combination of (*i*) large earthworm populations with (*ii*) some factors preventing them to incorporate litter at least during part of the year. The former is linked to calcium availability (here approximated by soil pH and presence of carbonates), a prominent factor of earthworm activity (Piearce, 1972), while the latter is linked to climate harshness (summer drought and/or winter frost) and hardness of the parent rock (here approximated by percent rock fragments), known to be stressful in shallow soils (Aussenac, 2002). According to present results, and given our still incipient knowledge on amphi humus forms (Andreetta et al., 2011), high calcium availability, shallow soils and associated poor water storage capacity seem to be driving factors in the genesis of the most typical humus form of this group: Pachyamphi. In some sense it can be said that on carbonated substrates with summer and/or winter climate stress for earthworm communities the development of a thick OH horizon saturated in Ca may compensate soil shallowness.

Galvan et al. (2008) observed that the size of aggregates reflected the size of species which were dominant in the earthworm community, in particular the balance between epigeic (small earthworms) and anecic (big earthworms) species. According to their records in subalpine forests of the Trento Province (northern Italy), the group they called amphi 1 (meso-structured, embracing Eumesoamphi and Pachyamphi in the present classification) was restricted to acid crystalline substrates, while the group they called amphi 2 (macro-structured, embracing Leptoamphi and Eumacroamphi) was found on dolomitic and mixed substrates. This means that substrates poor in nutrients, in particular calcium, are unfavorable to more exacting litter-feeding earthworms, i.e. anecics, a result supported by studies on earthworm communities (Nordström and Rundgren, 1974; Satchell, 1980; Ponge et al., 1999). Here we found that the gradient Leptoamphi (macro) → Eumesoamphi (meso) → Eumacroamphi (macro) → Pachyamphi (meso) did not reflect the structure of the A horizon (macro- versus meso-aggregates) but rather the thickness of organic layers, and was positively correlated with the percentage of carbonated substrates among samples (from 60% for Leptoamphi to 90% for Pachyamphi) and the percentage of rock fragments in the A horizon (from 14% for Leptoamphi to 38% for Pachyamphi). This suggests that the richer is the topsoil in rock fragments (in calcareous context) the more difficult the incorporation of organic matter to mineral matter by burrowing earthworms (Callaham and Blair, 1999). In the absence of earthworm records we cannot go deeper in elucidating this causal relationship. The balance between unincorporated carbon and that incorporated by annelids in the mineral soil would not be entirely driven by climate, as suspected so far (Bottner et al., 1995; Graefe, 2007; Ascher et al., 2012), although other influences such as phosphorus availability have been suspected (Tagger et al., 2008), but also by the restricted soil volume available for burrowing due to the abundance of rock fragments. This ‘rock fragment effect’, typical of shallow calcareous soils (rzLP) on dolomite and hard limestone substrates might have important consequences on carbon sequestration (Wironen and Moore, 2006; Andreetta et al., 2011; Bonifacio et al., 2011), beside climate effects (Marland et al., 2003; Egli et al., 2010; Thornley and Cannell, 2001) and their interactions with vegetation (Paré et al., 2006; De Deyn et al., 2008; Podwojewski et al., 2011) and mineral composition (Rasmussen et al., 2006).

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## Figure captions

**Figure 1.** The distribution of climate and geology over the Veneto region, with the distribution of climate districts according to Del Favero and Lasen (1993), modified for the Mediterranean district, which has been updated to Avanalpic.

**Figure 2.** Distribution of amphi humus forms and thickness of organic horizons according to geological substrate. Error bars for OH thickness indicate standard errors of the means.

**Figure 3.** Redundancy Analysis (RDA). Projection of explained variables (Fig. 1a) and explanatory variables (Fig. 1b) in the plane of the first two canonical factors (see list in Table 1).

**Figure 4.** Distribution of micro- (< 2 mm), meso- (2–4 mm) and macro-aggregates (> 4 mm) according to geology (Fig. 4a), climate (Figs. 4b to 4e), vegetation (Fig. 4f), pH (Fig. 4g) and clay content (Fig. 4h) of the A horizon.

**Figure 5.** Distribution of amphi humus forms and thickness of organic horizons according to soil stoniness. Error bars for OH thickness and % rock fragments indicate standard errors of the means.

**Table 1.** Criteria used for the classification of terrestrial humus forms from Veneto Region. Never = horizon never present; always = horizon always present; transition O-A < 3 mm = thickness of the junction zone between O (organic) and A (organo-mineral) horizons < 3 mm; bracketed horizon = discontinuous or in pockets; OH < 1cm = thickness of OH horizon < 1 cm; zo = zoogenic; noz = non zoogenic; maA = biomacrostructured A horizon; meA = biomesostructured A; miA = biomicrostructured A; maA ≥ OH/2 = thickness of maA ≥ half that of OH; * = not included in Veneto dataset.

| Main morpho-functional types | Humus Form Reference Groups (Jabiol et al. (2013) | Diagnostic horizons |
|---|---|---|
| **MULL:** never: OH, OFnoz, Anoz, miA; always: maA or meA; transition O-A < 3 mm | Eumull | (OLn), maA |
| | Mesomull | OLn, (OLv), maA |
| | Oligomull | OLn, OLv, (OFzo) maA or meA |
| | Dysmull | OLn, OLv, OFzo, maA or meA |
| **MODER:** never: maA, OFnoz; always: Anoz or miA; transition O-A ≥ 5mm | Hemimoder* | OLn, OLv, OFzo, (OH), miA |
| | Eumoder* | OLn, OLv, OFzo, OH≤1cm, (Anoz) or miA |
| | Dysmoder | OLn, OLv, OFzo, OH>1cm, (Anoz and OH?2A) or miA |
| **MOR***: never: maA, meA; never: maA, meA; always: OFnoz; transition O-A < 3 mm | Hemimor* | OLn, OLv, OFzo, (OFnoz), (OH), miA or Anoz or E |
| | Humimor* | OLn, OLv, OFzo, OFnoz, (OH), miA or Anoz or E |
| | Eumor* | OLn, OLv, OFzo, OFnoz, OH, Anoz or E |
| **AMPHI:** never: OFnoz, Anoz; always: OH, maA or meA; always: A ≥ OH/2 | Leptoamphi | OLn, OLv, OFzo, OH < 1cm, maA ≥ OH/2 |
| | Eumacroamphi | OLn, OLv, OFzo, OH ≥ 1cm, maA ≥ OH/2 |
| | Eumesoamphi | OLn, OLv, OFzo, OH < 3cm, A ≥ OH/2, miA and/or meA or only meA |
| | Pachyamphi | OLn, OLv, OFzo, OH ≥ 3cm, A ≥ OH/2, miA and/or meA or only meA |
| **TANGEL*:** never: OFnoz; always: OH > 2A | Eutangel* | OLn, OLv, OFzo, OH > 2A, Anoz or meA, transition OH-A < 5 mm |
| | Dystangel* | OLn, OLv, OFzo, OH > 2A, Anoz, transition OH-A ≥ 5 mm |

**Table 2.** Mean features of Veneto humus forms according to morphology of humus profiles, geology, topography, climate, vegetation and soil characteristics. When data are of analytical nature (measures), values are given as means with standard deviations. When data are of typological nature (occurrences), they are indicated as percent occurrence of the different categories.

| | **EUMULL (n = 48)** | **MESOMULL (n = 8)** | **OLIGOMULL (n = 84)** | **DYSMULL (n = 55)** | **DYSMODER (n = 24)** | **LEPTOAMPHI (n = 10)** | **EUMESOAMPHI (n = 43)** | **EUMACROAMPHI (n = 61)** | **PACHYAMPHI (n = 15)** |
|---|---|---|---|---|---|---|---|---|---|
| OF (cm) | 0±0 | 0±0 | 0.08±0.19 | 2.6±1.9 | 3.0±1.8 | 1.1±1.0 | 1.5±1.6 | 2.2±2.6 | 2.5±2.0 |
| OH (cm) | 0±0 | 0±0 | 0±0 | 0±0 | 3.7±3.2 | 0.5±0.0 | 1.3±0.5 | 3.5±4.3 | 6.4±3.7 |
| A (cm) | 10.3±5.7 | 17.6±9.9 | 14.0±10.0 | 9.9±7.7 | 7.2±4.7 | 7.6±3.9 | 14.6±9.8 | 14.0±10.9 | 12.7±7.5 |
| Macro-aggregates | 24% | 38% | 61% | 52% | 0% | 100% | 0% | 100% | 0% |
| Meso-aggregates | 74% | 50% | 38% | 48% | 0% | 0% | 100% | 0% | 100% |
| Micro-aggregates | 2% | 13% | 1% | 0% | 96% | 0% | 0% | 0% | 0% |
| Silicated rock | 36% | 25% | 32% | 28% | 100% | 40% | 37% | 21% | 7% |
| Carbonated rock | 64% | 75% | 68% | 72% | 0% | 60% | 63% | 79% | 93% |
| Elevation (m) | 1325±443 | 931±712 | 640±396 | 1087±465 | 1494±329 | 817±387 | 1073±521 | 981±412 | 1186±311 |
| South aspect | 18% | 25% | 30% | 31% | 46% | 30% | 30% | 26% | 40% |
| West aspect | 28% | 0% | 23% | 14% | 8% | 20% | 5% | 13% | 13% |
| East aspect | 28% | 25% | 23% | 28% | 8% | 40% | 51% | 45% | 40% |
| North aspect | 26% | 50% | 25% | 26% | 38% | 10% | 14% | 16% | 7% |
| Annual precipitation (mm) | 1321±277 | 1225±259 | 1292±311 | 1304±242 | 1258±195 | 1540±348 | 1212±208 | 1349±262 | 1453±146 |
| Mean annual temperature (°C) | 6.1±2.4 | 8.4±4.0 | 10.0±2.3 | 7.4±2.6 | 5.2±1.6 | 8.8±2.2 | 7.4±2.9 | 7.9±2.3 | 6.9±1.6 |
| Annual potential evapotranspiration (mm) | 549±68 | 604±122 | 645±75 | 582±69 | 518±48 | 635±77 | 591±97 | 606±76 | 564±57 |
| Summer potential evapotranspiration (mm) | 116±15 | 129±26 | 140±15 | 124±15 | 108±10 | 132±13 | 123±19 | 127±15 | 120±11 |
| Gams index | 1.2±0.6 | 3.1±3.5 | 2.6±1.3 | 2.6±6.5 | 0.9±0.3 | 2.4±1.3 | 1.7±1.5 | 1.9±2.1 | 1.3±0.4 |
| Lang index | 241±79 | 193±122 | 142±59 | 200±77 | 261±80 | 182±51 | 189±74 | 185±57 | 226±74 |
| Mediterranean climate district | 2% | 38% | 27% | 10% | 0% | 0% | 12% | 5% | 0% |
| Esalpic climate district | 36% | 13% | 62% | 48% | 8% | 80% | 35% | 55% | 60% |
| Mesalpic climate district | 44% | 50% | 8% | 33% | 79% | 20% | 51% | 35% | 40% |
| Endalpic climate district | 18% | 0% | 2% | 9% | 13% | 0% | 2% | 5% | 0% |
| Vegetation index | 2.4±0.7 | 2.0±0.9 | 1.5±0.7 | 2.2±0.8 | 2.5±0.6 | 1.7±0.7 | 2.2±0.8 | 1.9±0.8 | 2.3±0.5 |
| Spruce-pine forests | 34% | 25% | 8% | 38% | 42% | 10% | 37% | 19% | 27% |
| Beech forests | 6% | 0% | 8% | 22% | 13% | 30% | 14% | 19% | 40% |
| Chestnut forests | 8% | 25% | 15% | 7% | 0% | 10% | 12% | 3% | 0% |
| Other deciduous forests | 14% | 38% | 60% | 21% | 4% | 40% | 23% | 32% | 0% |
| Spruce-beech forests | 8% | 0% | 5% | 9% | 13% | 10% | 9% | 15% | 33% |
| Subalpine spruce-larch forests | 18% | 13% | 4% | 2% | 8% | 0% | 2% | 5% | 0% |
| Silver fir forests | 6% | 0% | 0% | 2% | 17% | 0% | 2% | 5% | 0% |
| Larch forests | 6% | 0% | 0% | 0% | 4% | 0% | 0% | 2% | 0% |
| Herbaceous cover | 73% | 80% | 37% | 35% | 32% | 40% | 51% | 38% | 53% |
| rzLP (WRB classification) | 17% | 25% | 14% | 25% | 0% | 10% | 33% | 31% | 47% |
| CMca (WRB classification) | 27% | 38% | 29% | 13% | 0% | 20% | 14% | 26% | 33% |
| CMeu (WRB classification) | 15% | 13% | 17% | 9% | 0% | 10% | 16% | 3% | 0% |
| LVhe (WRB classification) | 15% | 13% | 23% | 31% | 0% | 20% | 12% | 16% | 13% |
| LVdy (WRB classification) | 10% | 13% | 8% | 9% | 0% | 20% | 14% | 5% | 0% |
| CMdy/PZ (WRB classification) | 17% | 0% | 10% | 11% | 100% | 20% | 12% | 18% | 7% |
| pH water (A horizon) | 6.2±1.3 | 7.2±0.9 | 6.7±1.2 | 6.3±1.3 | 4.5±0.5 | 6.1±1.2 | 6.7±1.3 | 6.6±1.4 | 7.2±0.9 |
| pH water (0-40 cm) | 6.6±1.2 | 7.4±0.4 | 6.7±1.2 | 6.5±1.1 | 4.8±0.4 | 6.5±1.3 | 6.8±1.2 | 6.7±1.2 | 7.1±1.0 |
| Effervescence | 0.6±1.3 | 0.8±1.4 | 0.7±1.3 | 0.5±1.1 | 0±0 | 0.4±0.8 | 0.9±1.3 | 1.0±1.4 | 1.9±1.7 |
| Clay% | 19±9 | 22±11 | 26±12 | 21±11 | 16±8 | 21±9 | 20±11 | 20±12 | 13±7 |
| Sand% | 31±13 | 28±15 | 27±17 | 32±16 | 41±12 | 26±12 | 34±18 | 30±18 | 33±17 |
| Rock fragments% | 11±14 | 5±4 | 15±15 | 16±16 | 12±10 | 14±13 | 17±16 | 24±22 | 38±19 |
| Organic C% | 4.9±2.5 | 4.7±3.6 | 5.3±3.8 | 5.4±2.0 | 8.1±3.9 | 5.9±2.3 | 5.4±3.5 | 5.2±2.8 | 5.0±2.8 |
| Organic N‰ | 4.6±2.5 | 3.3±1.6 | 5.1±3.4 | 4.9±2.2 | 5.2±2.4 | 4.2±1.1 | 4.7±2.7 | 4.7±2.0 | 4.4±2.1 |
| C/N | 11.3±8.1 | 9.5±2.5 | 11.0±2.4 | 11.9±3.0 | 14.9±2.6 | 15.8±5.6 | 13.6±2.7 | 11.9±2.8 | 11.5±2.5 |

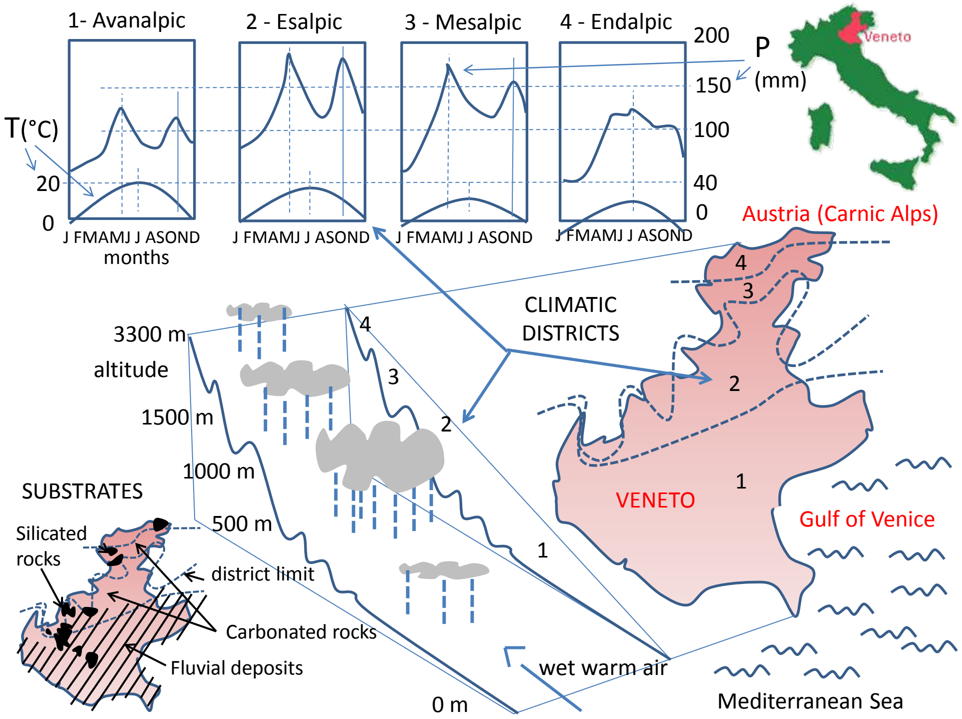
1- Avanalpic
2 - Esalpic
3 - Mesalpic
4 - Endalpic
T(°C)
20
0
200
150
100
40
0
P
(mm)
J FMAMJ J ASOND
months
J FMAMJ J ASOND
J FMAMJ J ASOND
J FMAMJ J ASOND
Veneto
Austria (Carnic Alps)
CLIMATIC
DISTRICTS
3300 m
altitude
1500 m
1000 m
500 m
0 m
4
3
2
1
SUBSTRATES
Silicated
rocks
district limit
Carbonated rocks
Fluvial deposits
wet warm air
VENETO
Gulf of Venice
Mediterranean Sea
4
3
2
1

Fig. 1

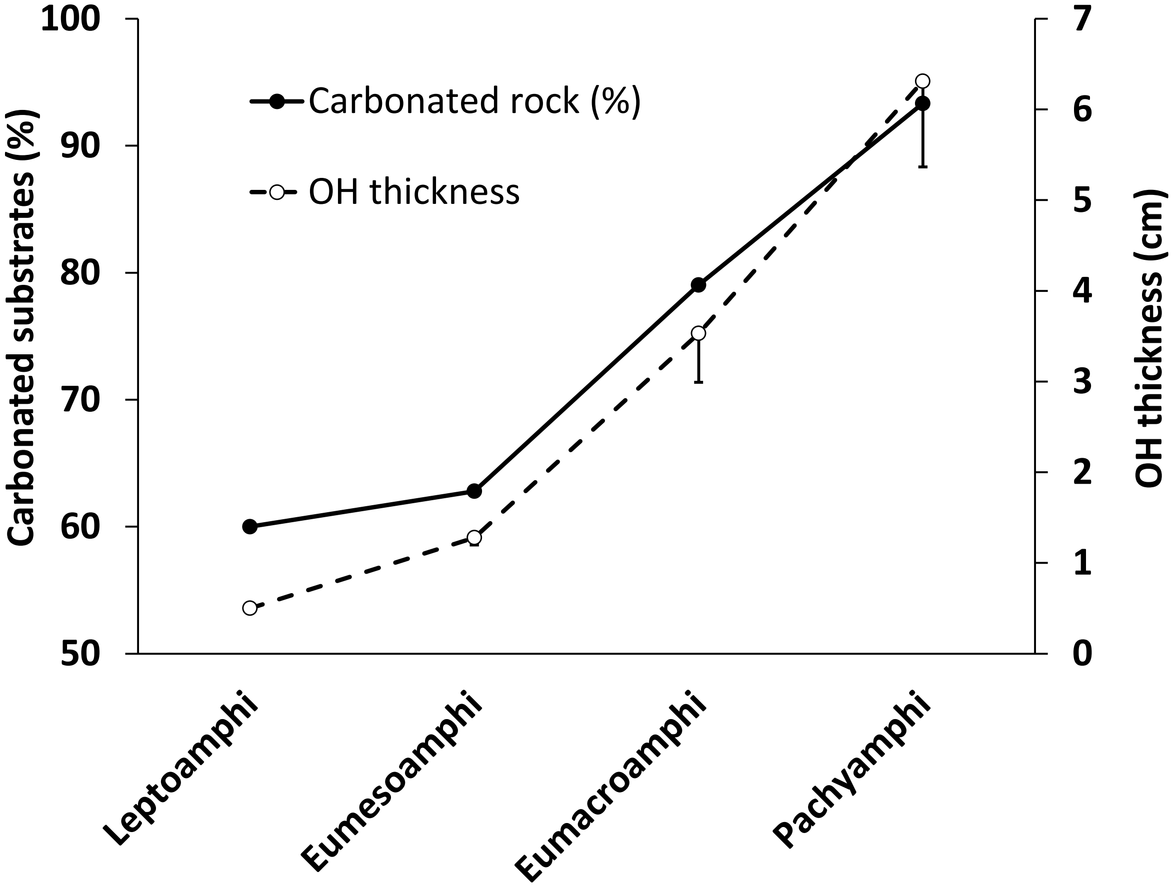
100
90
80
70
60
50
Carbonated substrates (%)
7
6
5
4
3
2
1
0
OH thickness (cm)
Carbonated rock (%)
OH thickness
Leptoamphi
Eumesoamphi
Eumacroamphi
Pachyamphi

Fig. 2

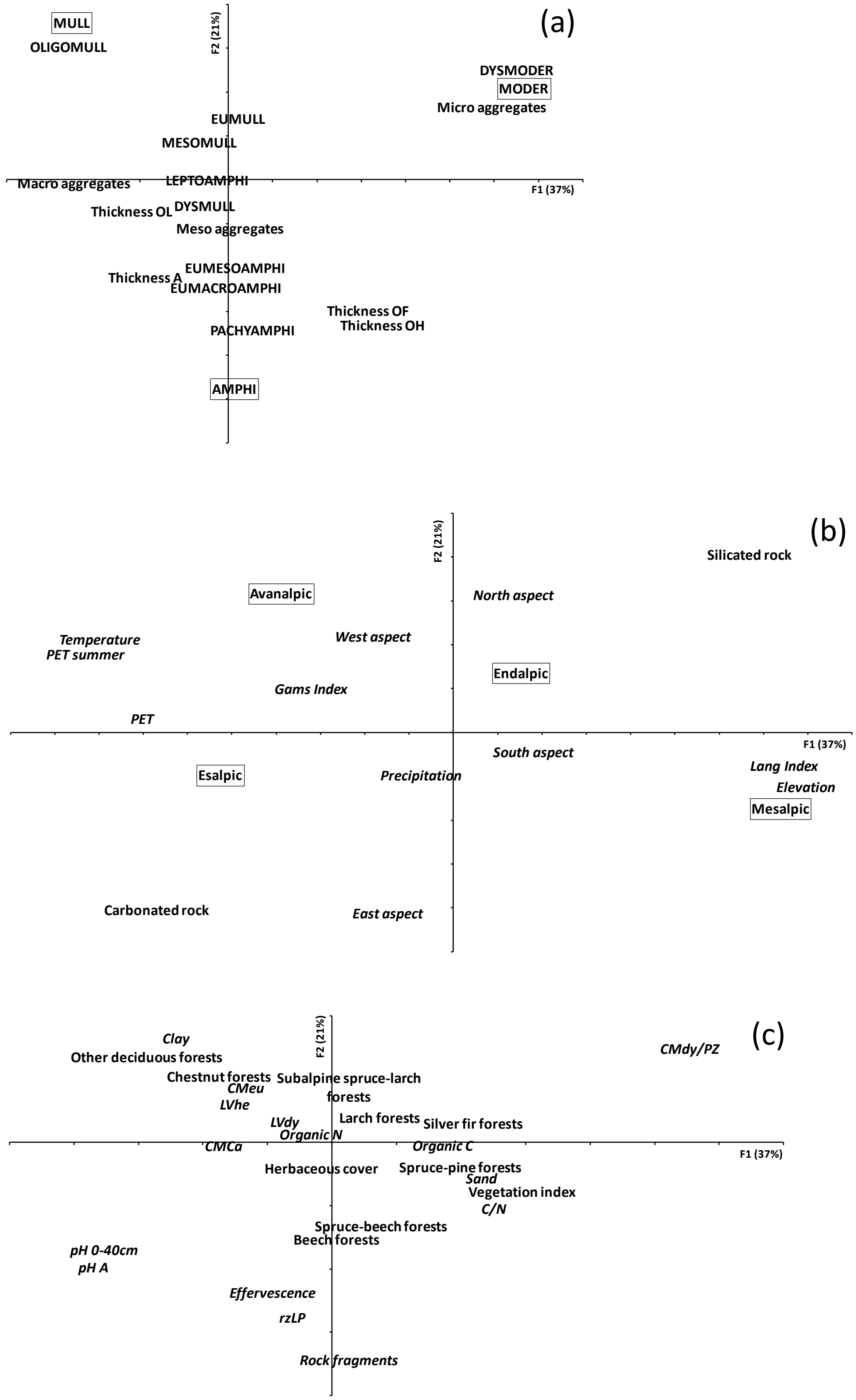
(a)
MULL
OLIGOMULL
F2 (21%)
DYSMODER
MODER
Micro aggregates
EUMULL
MESOMULL
Macro aggregates
LEPTOAMPHI
F1 (37%)
Thickness OL
DYSMULL
Meso aggregates
Thickness A
EUMESOAMPHI
EUMACROAMPHI
Thickness OF
Thickness OH
PACHYAMPHI
AMPHI
(b)
F2 (21%)
Silicated rock
Avanalpic
North aspect
Temperature
PET summer
West aspect
Endalpic
Gams Index
PET
F1 (37%)
South aspect
Esalpic
Precipitation
Lang Index
Elevation
Mesalpic
Carbonated rock
East aspect
(c)
F2 (21%)
Clay
CMdy/PZ
Other deciduous forests
Chestnut forests
Subalpine spruce-larch forests
CMeu
LVhe
LVdy
Larch forests
Silver fir forests
Organic N
CMCa
Organic C
F1 (37%)
Herbaceous cover
Spruce-pine forests
Sand
Vegetation index
C/N
Spruce-beech forests
Beech forests
pH 0-40cm
pH A
Effervescence
rzLP
Rock fragments

Fig. 3

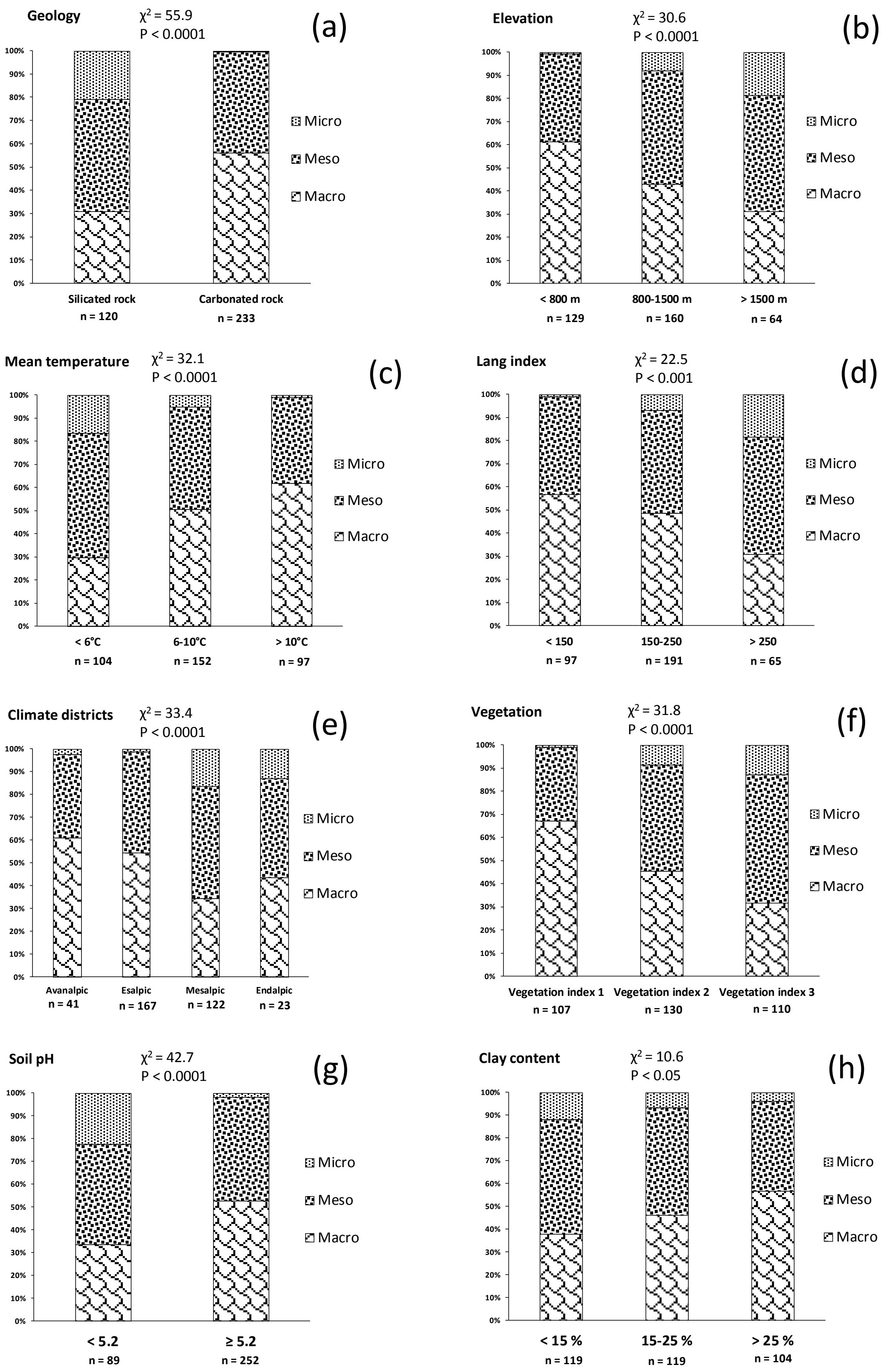
Geology
χ² = 55.9
P < 0.0001
(a)
Silicated rock
n = 120
Carbonated rock
n = 233
Elevation
χ² = 30.6
P < 0.0001
(b)
< 800 m
n = 129
800-1500 m
n = 160
> 1500 m
n = 64
Mean temperature
χ² = 32.1
P < 0.0001
(c)
< 6°C
n = 104
6-10°C
n = 152
> 10°C
n = 97
Lang index
χ² = 22.5
P < 0.001
(d)
< 150
n = 97
150-250
n = 191
> 250
n = 65
Climate districts
χ² = 33.4
P < 0.0001
(e)
Avanalpic
n = 41
Esalpic
n = 167
Mesalpic
n = 122
Endalpic
n = 23
Vegetation
χ² = 31.8
P < 0.0001
(f)
Vegetation index 1
n = 107
Vegetation index 2
n = 130
Vegetation index 3
n = 110
Soil pH
χ² = 42.7
P < 0.0001
(g)
< 5.2
n = 89
≥ 5.2
n = 252
Clay content
χ² = 10.6
P < 0.05
(h)
< 15 %
n = 119
15-25 %
n = 119
> 25 %
n = 104
Micro
Meso
Macro
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Fig. 4

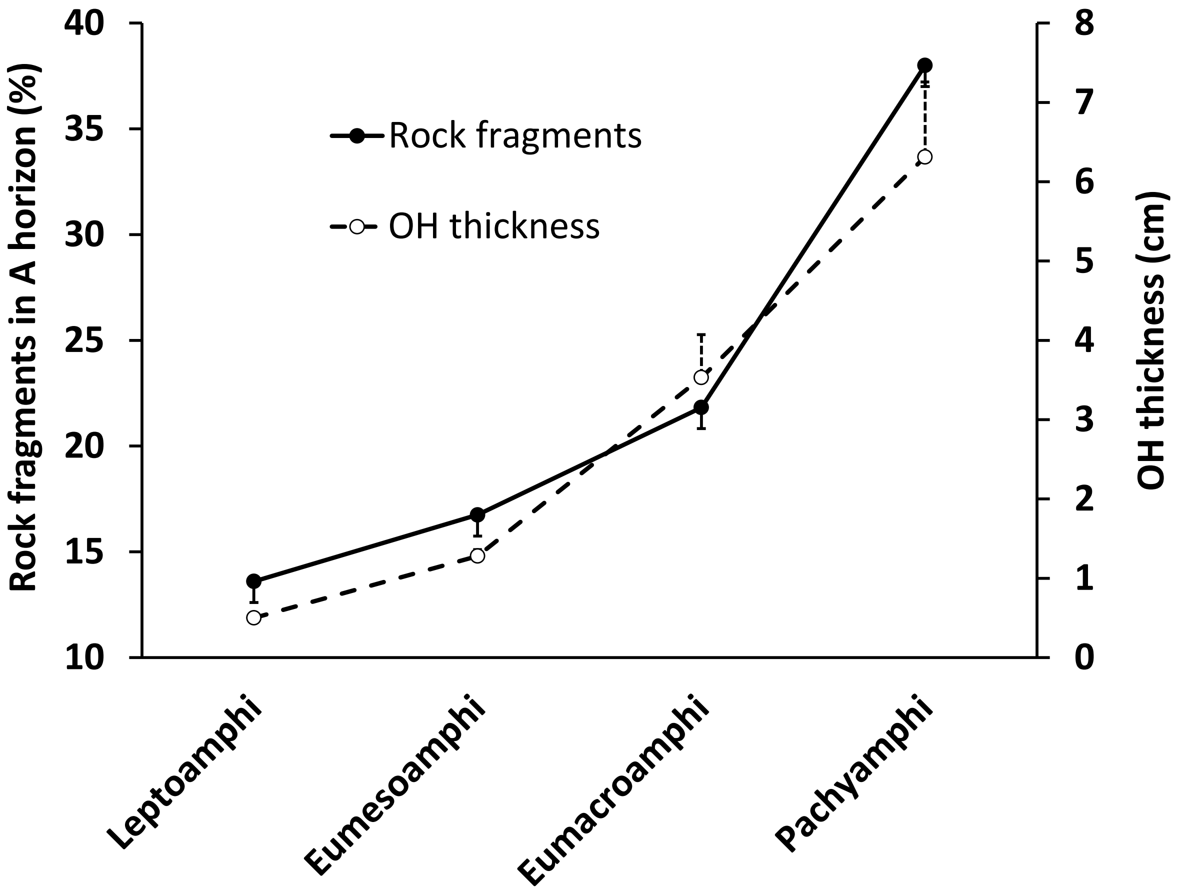
Rock fragments in A horizon (%)
40
35
30
25
20
15
10
OH thickness (cm)
8
7
6
5
4
3
2
1
0
Rock fragments
OH thickness
Leptoamphi
Eumesoamphi
Eumacroamphi
Pachyamphi

Fig. 5