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## Tree or soil? Factors influencing humus form differentiation in Italian forests

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### Abstract

We aim to investigate the occurrence of forest humus forms (Moder, Amphi and Mull) in relation to environmental factors describing parent material, climate and tree species. Boosted regression trees (BRTs) were applied as modeling tool to analyze data of 238 plots of the BioSoil database covering the whole Italian forest territory. Though predictive ability was not very high, especially for the Amphi form, we could gain significant insight into factors controlling humus form differentiation. In the BRT analysis, the diversity of tree species was the most important predictor for Moder and Mull models and specific plant effects were evidenced. However, our results showed that the geographic distribution of Italian forest species was influenced by soil and climate conditions, partly explaining the high weight of tree species as factor. The importance of the soil nutritional status, due to parent material properties, in driving humus form differentiation was stated, highlighting the key role played by pH and calcium content, with the hitherto understated importance of phosphorus. This study further clarified the functioning of the still poorly understood Amphi form. Reduced effective soil volume (EfVol) combined with seasonality appeared to constrain pedofauna activity in otherwise favorable and nutrient rich systems, favoring the evolution of Amphi instead of Mull forms.

### 1. Introduction

Humus forms are the morphological expression of the pathways through which organic debris is either incorporated within the mineral topsoil or accumulated on top of it, to form ectorganic horizons (Ponge, 2003; Zanella et al., 2011). Numerous studies (see Ponge, 2003, 2013 for reviews) have established that humus forms result from composition, biomass, activity and behavior of soil meso- and micro-fauna; in turn, these biotic factors are controlled by nutrient availability and pedoclimate conditions (Wall et al., 2008). Nutrient availability is conditioned by soil fertility, but also by complex feedbacks involving soil microbiota, climate (Aerts, 2006) and plants (Hooper et al., 2000), through litter quality and quantity. Further feedbacks were identified in relation to forest stand life cycle and development (Mulder et al., 2013; Ponge and Chevalier, 2006; Schaefer and Schauerermann, 1990; Scheu and Falca, 2000), influencing sign and magnitude of soil-plant nutrient transfers and, also, pedoclimate, through control on soil insolation and temperature exerted by stand density, LAI, continuity, etc. Extensive knowledge of these relationships brought Ponge (2003) to point to humus forms as keys to soil biodiversity and as indicators of ecosystem nutrient management strategies. More recently, humus forms have been found to be significant indicators of soil organic carbon storage (Andreetta et al., 2011; De Vos et al., 2015).

Existing data demonstrate how humus forms react punctually and rapidly to even small changes in soil nature, forest life cycle and forest management (Ponge et al., 2014) and this, given their easy experimental access, leads them to be proposed as practically useful keys to forest eco- system surveys (Andreetta et al., 2011; Ponge et al., 2014). In more general terms, humus forms might have a potential to represent what soils, in their entirety, were expected to represent at the dawn of Pedology, i.e., a faithful “impression” of the environment (climate, biota, drainage) on a portion of the Earth's crust, and are responsive enough to change accordingly to environmental changes, thus offering an “integrating” view of ongoing environmental processes and their results.

Recently, analysis of the factors driving humus form differentiation has received increasing attention (Labaz et al., 2014; Ponge et al., 2011, 2014), but a basic issue in these analysis still requires more investigation. This is the relative weight of different kinds of factors, which may be alternately defined as “distal” (geology, climate, main tree species) vs. “proximal” (topsoil conditions, litter quality, microbiota) or as “truly independent” vs. “co-varying with humus”. Ponge et al. (2011) showed that, for the French territory, geology and climate were the major determinants of humus forms, while the influence of forest canopies was negligible. Labaz et al. (2014) found that bedrock geology was the least influencing factor on humus forms, though they pointed out that this result was possibly influenced by relatively homogeneous geology in their sample. In Veneto (Northern Italy), Ponge et al. (2014) showed that the first canonical component of the projection of environmental variables could be interpreted as a composite factor embracing both geological, climate and soil gradients. They also showed that geology, climate, soil and vegetation exert a prominent influence on the distribution of humus forms.

This study takes advantage of the existence of a database covering the entirety of Italy as produced by the BioSoil project, to identify the environmental factors that mostly influence the occurrence of Moder, Amphi and Mull forms, treated in three separated models, at national level. Differently from comparable studies (Cools et al., 2014; Ponge et al., 2011; Ponge, 2013) the central aim of this study is not to select covariates for upscaling humus form observations at national or continental scale, but rather to get deeper into elucidating factors controlling humus form development, involved processes, and soil-humus feedbacks.

Studies that have previously applied the same model tool of the present work to determine the main factors explaining forest floor parameters such as C/N ratio (Cools et al., 2014) and C stock (De Vos et al., 2015), found that the diversity of tree species was clearly the most important predictor. Due to the close link between humus forms and carbon-related parameters (Andreetta et al., 2011, 2013a, 2013b; Bonifacio et al., 2011; De Nicola et al., 2014), we hypothesized that tree species were also associated with humus forms with high relative influence score. A specific objective was then to deeply analyze interactions between tree species and other environmental factors.

## 2. Materials and methods

### 2.1. Study area

Studied sites were made up by the Level I sites of the European ICP- Forests network, based on a 16 km × 16 km grid (Van Ranst et al., 1998), modified to 15 by 18 km in Italy. Sites are located across the whole Italian territory. Sampling was carried out according to standard ICP- Forests protocols (FSCC, 2006). At each site, composite samples were made from samples collected at five different points. Organic horizons OF and OH were sampled together by a 25 × 25 cm frame, as OFH layer, due to their inconsistent and, in some cases, small thickness. Mineral soil was sampled to represent fixed soil depth intervals (0–10 cm; 10–20 cm; 20–40 cm; 40–80 cm).

## 2.2. Humus form classification

Humus forms were classified according to the structure (IUSS Working Group WRB, 2006) of the first mineral horizon (Fao, 2006) and the presence/absence of the OH horizon. Classification corresponds to the higher hierarchical level of the European Humus Group proposal (Zanella et al., 2011), namely:

- Moder, with massive E-AE or bio-microstructured (peds  $\varnothing \leq 1$  mm) A horizon and organic horizons (OL, OF and OH) present;
- Amphi, with either bio-mesostructured (1 mm  $b \varnothing \leq 5$  mm) or bio-macrostructured ( $\varnothing \geq 5$  mm) A horizon and the presence of organic horizons (OL, OF and OH);
- Mull, with bio-mesostructured (1 mm  $b \varnothing \leq 5$  mm) or bio-macrostructured ( $\varnothing \geq 5$  mm) A horizon and OH horizon absent.

Due to their rare occurrence in Italy, Mor forms were not considered in this study.

## 2.3. Soil analysis

Analytical methods followed the ICP Forests Manual on sampling and analysis of soil (FSCC, 2006; ICP Forest, 2010). Specifically, soil pH was measured in the supernatant suspension of a 1:2.5 soil:water mixture, exchangeable cations were determined after exchange with an unbuffered 0.1 M BaCl<sub>2</sub> solution, while extractable elements were determined in aqua regia extracts.

## 2.4. Statistical analyses

In order to evaluate differences in all parameters between humus forms and tree species populations, a non-parametric statistical test (Kruskal–Wallis) was applied due to non-normal distribution of some properties.

### 2.4.1. Predictor variables

Selection of predictor variables was derived from the aforementioned main objectives. Environmental factors such as climate/ pedoclimate, parent material and vegetation were selected as primary predictor variables as they have a one-way relation to humus forms, i.e. they are true “independent” variables. Tree species has been considered as partially dependent (Ponge et al., 2011) but there is a shortage of physical hypotheses on such dependence.

Climatic data were obtained from the WorldClim database (<http://www.worldclim.org/current>), a gridded climate database with the very high resolution of 30 arc sec ( $\approx 1$  km<sup>2</sup> or  $\sim 0.09^\circ$ ). Data layers are generated through interpolation of average monthly climate measurements from 1950 to 1990, using thin plate splines with climate data from meteorological stations and a digital elevation model to spatially model various climatic variables (Hijmans et al., 2005). For our models, we selected those variables that may affect biological activities, such as the mean temperature of warmest quarter seen as climatic limiting factor, and the range between the precipitation of the wettest quarter and the precipitation of the driest quarter, to represent seasonality.

Data from the ICP Forests database do not allow full model estimates of soil water availability; as a proxy data, we used effective soil volume, i.e. the plant- (and earthworm-) available soil volume, in m<sup>3</sup>·m<sup>-2</sup> of surface area, obtained by subtracting coarse fragment percent volume from soil depth. This parameter is referred to as EfVol.

Parent material (p.m.) was recorded according to FSCC (2006); this is a simplified way, often derived from available geological maps. As such, it is equivalent to “Geology” as in Ponge et al. (2011) and De Vos et al. (2015); it is one of the most useful variables for upscaling geographical distribution of humus forms and carbon stocks, but not as much to understand relationships between p.m. and humus forms. In the models,

we included subsoil extractable Ca (sub.Ca), subsoil total P (sub.P) and pH (sub.pH) as properties indicative of p.m. These parameters were those obtained from the deepest samples. Sub.Ca and sub.P were included in the model after being log transformed to improve readability of the partial dependence plots.

The ‘Tree’ variable was taken from the dominant tree species record- ed in ICP Forests crown condition survey (Lorenz et al., 2004). Frequen- cy of individual tree species was quite variable. According to Cools et al. (2014), species were grouped to obtain groups of no less than 20 sites (Table 1). The most frequent species (Norway spruce, *Picea abies* (Pabi), European beech, *Fagus sylvatica* (Fsyl), sweet chestnut, *Castanea sativa* (Csat), Turkey oak, *Quercus cerris* (Qcer)) were analyzed as pure groups. Other species were grouped according to physiological and ecological similarities, as follows: Conif included all conifers except *P. abies*; this group is dominated by black pine (*Pinus nigra*) and European larch (*Larix decidua*). “Other” grouped all broadleaved trees except *F. sylvatica*, *C. sativa* and oaks. Qpub included all strictly decidu- ous oaks, i.e. excluding *Q. cerris*; this group is dominated by downy oak (*Quercus pubescens*). Med grouped all sclerophyll oaks, mostly holm oak (*Quercus ilex*); in this group we also included other Mediterranean species such as *Pinus halepensis* and *Eucalyptus* spp.

Table 1: Frequency table of tree species and humus forms.

	Conif (n = 32)	Csat (n = 27)	Fsyl (n = 49)	Med (n = 25)	Other (n = 20)	Pabi (n = 27)	Qcer (n = 25)	Qpub (n = 33)
Moder (n = 57)	14 (0.24)	10 (0.18)	6 (0.11)	4 (0.07)	0 (0.00)	13 (0.23)	7 (0.12)	3 (0.05)
Amphi (n = 92)	10 (0.11)	7 (0.08)	24 (0.26)	8 (0.08)	6 (0.07)	12 (0.13)	11 (0.12)	14 (0.15)
Mull (n = 89)	8 (0.09)	10 (0.11)	19 (0.21)	13 (0.15)	14 (0.16)	2 (0.02)	7 (0.08)	16 (0.18)

#### 2.4.2. Model building

Boosted regression trees (BRTs) were applied as a modeling tool to explore the influence of environmental factors on the occurrence (as presence/absence) of humus forms. BRTs are a relatively new statistical method, based on techniques from both statistical and machine learning methods (Friedman et al., 2000; Elith et al., 2008), and are increasingly applied in spatial modeling of species or environmental variables, including soil parameters. BRTs are very flexible and capable of dealing with complex responses, includ- ing nonlinearities and interactions (Elith et al., 2008). BRTs performed well in soil modeling (Ciampalini et al., 2014; Cools et al., 2014; De Vos et al., 2015) and have been applied in various study domains, from predicting the distribution of organisms (Elith et al., 2008) to comparing the factors of cropland abandonment (Müller et al., 2013). Regression trees predict a response from obser- vations and one or more continuous or categorical predictor variables, and boosting uses a forward stagewise procedure to gradually add regression trees to the model. BRTs show several advantages, since they can accommodate any type of variable (con- tinuous, categorical, nominal), missing and non-independent data and can also deal with many distribution types (Gaussian, binomial, Poisson, etc.). Additionally, a BRT model allows the derivation of par- tial dependence plots, which indicate how the response is affected by a certain predictor after accounting for the average effects of all other predictors in the model. These plots can be used for interpreting model behavior (Elith et al., 2008).

We built three different models, one for each humus form, apply- ing the BRT using the R version 3.0.2 (R Core Team, 2013), package “dismo” version 0.9-3 (Hijmans et al., 2013). We applied the `gbm.step` function of the `dismo` package, which assesses the optimal number of boosting trees using cross validation. We tested

various combinations of the learning rate (0.01 to 0.001), which controls the contribution of each tree to the growing model, and tree complexity (3 to 5), the number of split levels of each tree. By changing these two parameters the number of trees (nt) required for optimal model fit was calculated. Bag fraction, used to control model stochasticity, was set at 0.5. The most effective parameters for our data set were 0.001 for the learning rate and 5 for the tree complexity. The model was fit with a Bernoulli distribution, since we evaluated the presence–absence of each humus form. Cross-validation was performed to estimate the optimal number of trees producing the best predictive performance, which was evaluated by predictive deviance and area under the receiver operator characteristic curve (ROC AUC).

## 2.5. Soil variables

“Soil” variables, relative to actual topsoil properties, which, of course, are linked to humus forms by two-way interactions, were not included as predictors in the models, but were analyzed statistically and considered in the discussion. The selected parameters were those linked to the subsoil properties: topsoil (0–10 cm) pH, Ca and P contents; exchangeable aluminum (Exc\_Al) was also included due to its effects on biotic activity.

Topsoil P and Ca contents (0–10 cm) were related to litter (P\_OL and Ca\_OL) and subsoil P and Ca contents (sub.P and sub.Ca) through single linear regressions, after log<sub>10</sub> transforming.

## 3. Results and discussion

The surveyed humus forms (n = 238) were classified as Moder (n = 57), Amphi (n = 92) and Mull (n = 89). Existing studies (Cools et al., 2014; Ponge et al., 2011; Ponge, 2013) concentrate on the Mull–Moder–Mor series, Amphi forms representing minor proportions of the populations they examined. However, it is clear how Amphi is a major humus form throughout Italy (Andreetta et al., 2011, 2013b; De Nicola et al., 2014; Ponge et al., 2014). The issue of the conditions determining the appearance of Amphi forms is then primary to the understanding of humus ecology in Italy.

### 3.1. Boosted regression trees

The relative importance of the predictors in BRT models (Table 2), and the correspondent partial dependence plots for each predictor and probability of humus form occurrence (Fig. 1, 2, 3) were identified. The best model for Moder included 1900 trees and showed a cross-validated ROC AUC score of 0.825 and a training ROC AUC of 0.972 (55% of the variance explained). The selected model for Amphi included 1100 trees and showed a cross-validated ROC AUC score of 0.65 and a training ROC AUC of 0.882 (25% of the variance explained). The best model for Mull included 2250 trees and showed a cross-validated ROC AUC score of 0.742 and a training ROC AUC of 0.95 (40% of the variance explained). The Moder model reached thus better performance than Mull and Amphi models. Grouping together macro- and meso-structured forms likely led to highly variable populations of these two main forms, as meso- and macro-groups were reported to be differentiated in terms of soil organic carbon storage (Andreetta et al., 2011), soil organic matter properties and enzyme activities (Andreetta et al., 2013b) while, on the opposite, meso-structured forms, especially of Mull, show analogies with Moder forms (Andreetta et al., 2011, 2013a, 2013b).

### 3.2. Climate

There was a high probability to find a Moder where the mean temperature of warmest quarter (tmax) was below 15 °C (Table 3 and Fig. 1); in contrast, Mull prevailed where tmax was higher than 15 °C (Table 3 and Fig. 3). A trend similar to Mull, though less marked, was found for Amphi forms (Table 3 and Fig. 2). Climate

exerts a strong control on litter decomposition rates, both directly and indirectly, through effects on litter chemistry (Aerts, 1997) and soil organisms (Ascher et al., 2012). It is generally agreed that higher temperatures lead to increased decomposition and faunal activity, and decreased mass of organic horizons (Aerts, 2006; Ponge et al., 2011). A different effect on soil organisms may be relevant in Mediterranean conditions; Sadaka and Ponge (2003) and Andreetta et al. (2011) proposed that summer high temperatures and drought force most faunal groups into endogeic behavior, thus allowing for Mull forms even when other conditions would lead to accumulation of OH horizons. A mirror effect, i.e. an increasing epigeic behavior with lower temperatures, was reported by Ascher et al. (2012). Climate–tree species interactions should also be influential. Significant differences in occurrence of tree species were associated with MAT and tmax (Table 4), species favoring Mull differentiation often being associated with higher temperatures. On the other hand, mean annual precipitation (MAP) showed no significant differences between tree species except for Mediterranean species (Med) for which it was significantly lower. Rainfall seasonality, as expressed by pmax.min, evidenced that *P. abies* and the Mediterranean species tend to grow in sites where seasonality is more marked, while for all the other species no significant differences were recorded. Pmax.min was a significant variable in both Moder (Fig. 1) and Amphi (Fig. 2) models. Moder was clearly associated with low pmax.min, while Amphi showed an opposite behavior. Seasonality seemed to have no effect on the evolution of Mull (Fig. 3). Seasonal precipitation affects pedofauna composition, density and activity. It is to be noted that seasonality in this dataset is not to be intended as a synonymous of Mediterranean conditions, as wide areas of the Alpine chain show strong seasonality in precipitation, marked by dry winter conditions.

Although we found evidence for climate as a driving force in humus form differentiation, the actual pathways of climate influence and their direct or indirect nature are still unclear, notably with respect to the relations between Mull and Amphi. It is likely that a more detailed analysis of climate and pedoclimate conditions would be more effective.

Table 2: Relative influence (RI%) of the factors on Moder, Amphi and Mull occurrence following a boosted regression tree analysis. Site factors: tree: tree species; pmax.min: the range between the precipitation of the wettest quarter and the precipitation of the driest quarter; tmax: mean temperature of warmest quarter; EfVol: effective soil volume; sub.Ca: subsoil calcium content; sub.P: subsoil phosphorus content; sub.pH: subsoil pH.

Moder		Amphi		Mull	
Factor	RI (%)	Factor	RI (%)	Factor	RI (%)
Tree	20.1	EfVol	25.0	Tree	24.4
Sub.pH	16.3	Tree	17.5	EfVol	23.6
Sub.Ca (log)	15.9	Sub.P (log)	14.8	Sub.pH	15.8
Tmax	14.3	Pmax.min	14.4	Sub.Ca (log)	10.9
Pmax.min	13.3	Sub.Ca (log)	11.1	Tmax	9.4
EfVol	12.2	Tmax	8.8	Sub.P (log)	9.1
Sub.P (log)	8.0	Sub.pH	8.3	Pmax.min	6.8

Fig. 1. Partial dependence plots from Moder BRT model for environmental factors: tree: tree species; pmax.min: the range between the precipitation of the wettest quarter and the precipitation of the driest quarter; tmax: mean temperature of warmest quarter; EfVol: effective soil volume; Ca.sub (log): subsoil calcium content (log-transformed); P.sub (log): subsoil phosphorus content (log-transformed); pH.sub: subsoil pH.

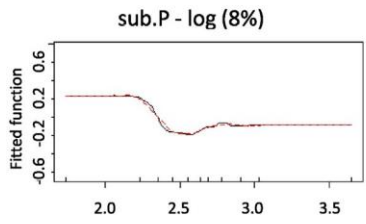
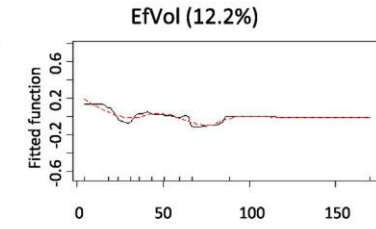
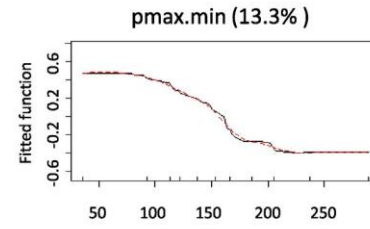
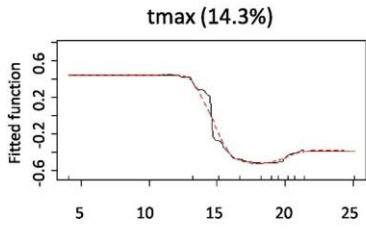
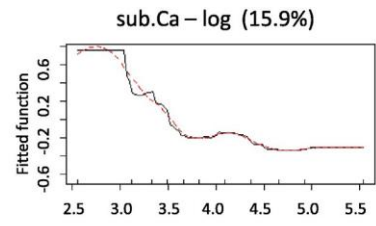
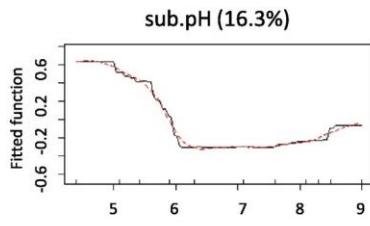
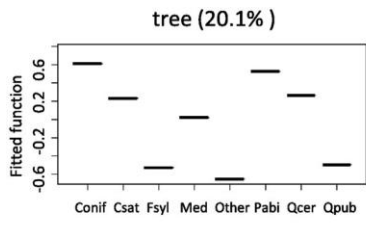




Fig. 2. Partial dependence plots from Amphi BRT model for environmental factors: tree: tree species; pmax.min: the range between the precipitation of the wettest quarter and the precipitation of the driest quarter; tmax: mean temperature of warmest quarter; EfVol: effective soil volume; Ca.sub (log): subsoil calcium content (log-transformed); P.sub (log): subsoil phosphorus content (log-transformed); pH.sub: subsoil pH.

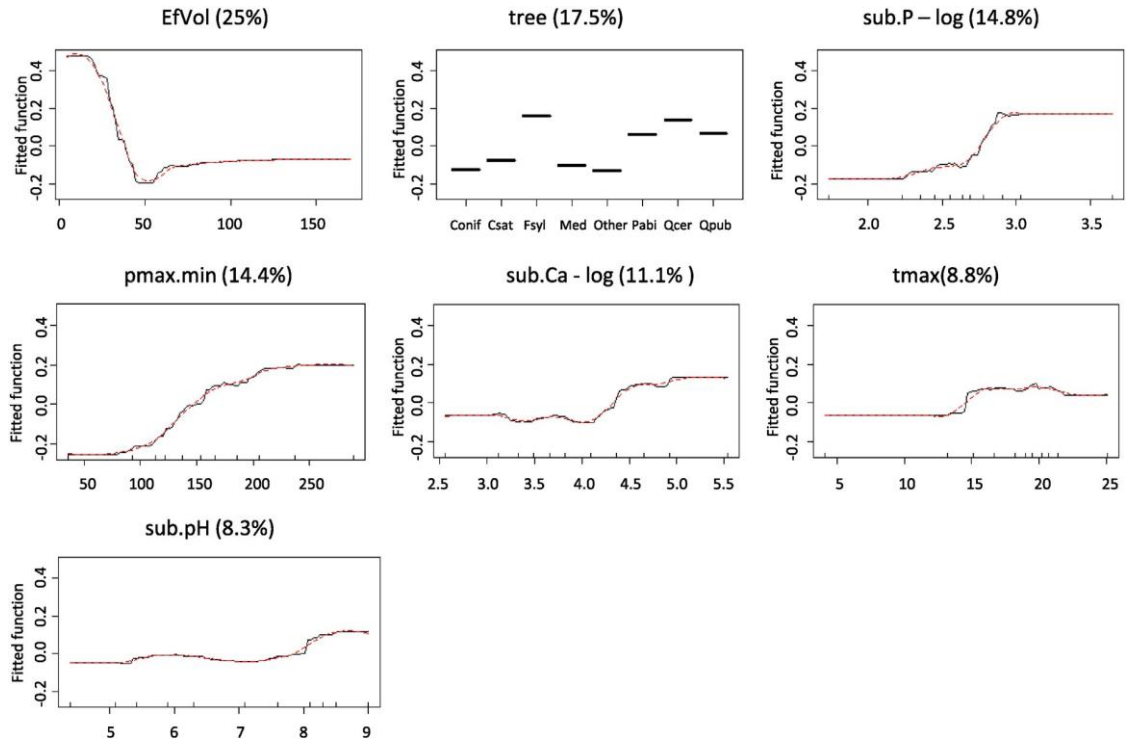


Fig. 3. Partial dependence plots from Mull BRT model for environmental factors: tree: tree species; pmax.min: the range between the precipitation of the wettest quarter and the precipitation of the driest quarter; tmax: mean temperature of warmest quarter; EfVol: effective soil volume; Ca.sub (log): subsoil calcium content (log-transformed); P.sub (log): subsoil phosphorus content (log-transformed); pH.sub: subsoil pH.

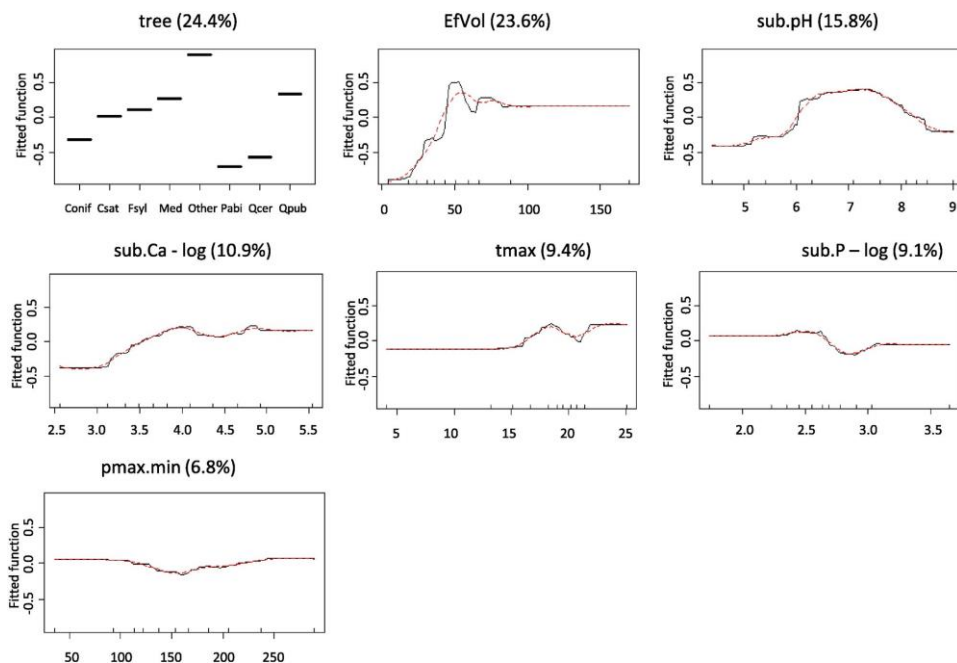


Table 3: Summary statistics: mean, standard deviation (sd), minimum (min) and maximum (max) value for each predictor among humus forms. EfVol: effective soil volume; pmax.min: the range between the precipitation of the wettest quarter and the precipitation of the driest quarter; Tmax: mean temperature of warmest quarter; P.sub: subsoil phosphorus content; Ca.sub: subsoil calcium content. Soil factors: topsoil (0–10 cm) C/N; topsoil pH; Exc\_Al: top soil exchangeable aluminum; Ext\_Ca: topsoil calcium content; P: topsoil phosphorus content; Ca\_OL: litter Ca content.

	Moder				Amphi				Mull			
	Mean	sd	Min	max	Mean	sd	Min	Max	Mean	sd	Min	Max
EfVol (cm)	44.2a	23.3	7.5	110.0	46.6a	36.0	4.5	170.0	57.8b	31.8	14.0	150.0
Tmax (°C)	16.5a	4.1	7.2	22.4	17.9a	3.4	4.1	23.8	19.0b	2.6	10.8	25.1
Pmax.min (mm)	149a	51	42	247	168a	51	75	289	155a	58	36	289
pH.sub	6.2a	1.4	4.4	8.7	7.1b	1.3	4.6	8.9	7.2b	1.15	5	9
P.sub (mg/kg)	421a	275	65	1256	633b	564	55	4449	560b	525	97	3363
Ca.sub (mg/kg)	17,274a	50,142	825	344,539	34,010b	49,435	366	266,419	29,320b	47,762	802	329,178
pH	5.6a	1.4	3.9	8.6	6.5b	1.2	4.4	8.6	6.6b	1.1	4.1	8.6
Ext_Ca (mg/kg)	16,308a	54,42	939	395,04	26,257b	38,01	635	202,01	25,263b	40,60	108	269,77
P (mg/kg)	450a	250	109	1196	765b	559	67	3591	698b	489	126	2675
Exc_Al (cmol+/kg)	4.5a	4.7	0.01	18.1	1.3b	2.4	0.01	11.9	0.9b	2.0	0.01	9.8
Ca_OL (mg/kg)	15,534a	8176	1973	44,475	23,155b	11,367	3805	68,694	22,134b	11,620	1082	62,126

Lower-case letters indicate significant differences ( $p < 0.05$ ) between humus forms with Kruskal–Wallis test.

Table 4: Summary statistics: mean, standard deviation (sd), minimum (min) and maximum (max) value for climate parameters (MAP: mean annual precipitation; pma.min: range between the precipitation of the wettest quarter and the precipitation of the driest quarter; Tmax: mean temperature of warmest quarter; MAT = mean annual temperatures) among tree species. Conif: coniferous; Csat: C. sativa; Fsyl: F. sylvatica; Med: Mediterranean species; Other: broadleaves other than those represent as single group; Pabi: P. abies; Qcer: Q. cerris; Qpub: Q. pubescens.

	MAP (mm)				Pmax.min (mm)				Tmax (°C)				MAT (°C)			
	Mean	sd	Min	Max	Mean	sd	Min	Max	Mean	sd	Min	Max	Mean	sd	Min	Max
Conif	939b	185	512	1332	161bc	70	36	286	14.8b	4.3	4.1	21.3	7.2b	3.6	-1.2	12.2
Csat	929b	139	746	1267	155ab	45	71	277	19.6c	1.2	16.4	21.7	11.1c	1.6	7.5	14.2
Fsyl	909b	161	607	1296	148a	49	72	288	17.0b	1.9	13.9	21	8.4b	1.9	4.9	12.7
Med	725a	152	454	1019	188cd	49	77	280	21.2e	1.7	17.7	25.1	13.5d	1.7	10.7	17.5
Other	869b	186	483	1327	137a	61	51	289	19.1cd	2.6	10.8	22.9	11.0c	2.0	6.9	15.0
Pabi	890b	137	738	1276	201d	30	125	247	13.4a	2.3	8.1	17.5	5.5a	1.8	2.8	9.5
Qcer	805b	102	596	953	138a	44	75	266	20.5de	1.2	17.7	22.6	12.2b	1.5	9.3	14.8
Qpub	822b	111	512	1094	148ab	52	71	289	20.1ce	1.9	18.3	22.8	11.9b	1.8	7.4	15.4

Lower-case letters indicate significant differences ( $p < 0.05$ ) between tree species using Kruskal–Wallis test.

### 3.3. Tree species

Tree diversity was the most important predictor for Moder and Mull models, with a relative importance of 20.1% and 24.4%, respectively (Table 2). The effect of tree species was then further investigated; to better elucidate causal chains running from tree to humus, various soil and litter properties were analyzed in relation to tree species. In order to discover potential interactions producing such weights of tree species as predictors, correspondences between tree species and other significant predictor variables were analyzed, too.

A dependence between tree species and soil fauna, through litter quality, might explain the strong predictive performance of the tree factor. The probability of finding Moder humus was high under coniferous trees, both mixed and pure spruce, and also, though with a weaker influence, under chestnut (Fig. 1). Schwarz et al. (2015) found strong and consistent negative effects on earthworms, and consequently on Mull formation, of European larch (*L. decidua*) and Norway spruce (*P. abies*). They suggested that such negative effects might be due to rather low litter palatability and to the dense canopy structure reducing soil moisture and temperature. Rajapaksha et al. (2013) found that chestnut leaves were the least selected, among various broadleaved trees, by typical anecic earthworms.

General trends in litter Ca concentration, according to tree species (Table 5), agreed with trends in humus form probability. Species groups with the lowest litter Ca, i.e. "Conifers", spruce and chestnut, were the most associated with Moder (Fig. 1); species groups with high litter Ca, such as "Other" and "Qpub" were, conversely, associated with Mull (Fig. 3). Groups with intermediate litter Ca were either associated with Amphi forms, as beech and Turkey oak, or not significant, as for "Med" (Fig. 2). The effect of chestnut on Moder formation appears weaker than could be expected from Ca litter contents (Table 5). This could result from other litter characters, chestnut litter being frequently reported as containing significantly low lignin (Cortez et al., 1996; Sariyildiz and Anderson, 2005).

Table 5

Table 5: Ca (Ca\_OL) and P (P\_OL) contents in the OL layer among different tree species.

	Ca_OL (mg/kg)				P_OL (mg/kg)			
	Mean	sd	Min	Max	Mean	sd	Min	Max
Conif	16,173ab	7800	4908	44,475	803ab	289	319	1496
Csat	15,611a	7352	1973	32,830	740a	340	165	1558
Fsyl	20,395bc	10,408	3011	68,694	915bc	338	326	1947
Med	22,367b	10,700	8868	42,980	727ab	283	277	1583
Other	31,097d	12,634	11,755	62,126	1062cd	324	551	1742
Pabi	15,948ab	6495	6400	36,518	1031d	256	537	1693
Qcer	22,886cd	11,015	4309	50,009	906bc	312	350	1449
Qpub	26,375cd	13,842	1082	54,573	765ab	247	255	1498

Lower-case letters indicate significant differences ( $p < 0.05$ ) between tree species with Kruskal-Wallis test.

Reich et al. (2005) established that plots of tree species with Ca-rich litter had greater density, diversity and biomass of earthworms than plots of trees with Ca-poor litter. Species such as *Fraxinus excelsior*, *Acer pseudoplatanus* and *Prunus avium*, grouped in our dataset as "Other", were considered as mull-forming (Neiryck et al., 2000) and under them earthworm biomass was found to be conspicuously higher than under *Quercus robur* and *F. sylvatica* (De Schrijver et al., 2012; Neiryck et al., 2000). Relations between tree species and topsoil C/N ratio (Table 6) were similar, if at lower absolute values, to those reported by Vesterdal et al. (2008) and Cools et al. (2014). Though differences were of low statistical significance, there was a general similarity, C/N decreasing from species associated with Moder to species associated with Mull. Spruce and

“Other” stood out at the two extremes, being significantly different from all others. A further significant characteristic of litter was P content (Table 5); trends in this parameter were not in direct agreement with either Ca or topsoil C/N, the most notable findings being the low P content of chestnut and “Med” litter and the very high P content of spruce. This last appears as a specific plant effect, well different from other conifers and not influenced by site factors, as there appears to be no significant differences in subsoil P for spruce (Table 8), and may explain the more favorable effect of spruce on Amphi differentiation.

Topsoil pH associated with the different tree species (Table 6) showed a clear increasing trend in the order: Pabi b Csat b Conif b Fsyl b Med ≈ Qcer b Qpub ≈ Other. Trees likely influence soil habitat by modifying soil pH (Augusto et al., 2002; Mueller et al., 2012), but differences in topsoil pH are also due to differences in parent material. Topsoil and subsoil pH (Table 9) were found to be well correlated for both the whole dataset and for each humus form. The pH differences between subsoil and topsoil, for sites with subsoil pH higher than 6.9, evidenced a mean decrease in pH at least of 0.5 unit for all species and groups, with significant differences (Table 7). This suggests that topsoil pH, a most important soil factor driving towards Moder instead of Amphi and Mull, was also related to tree species. The strongest acidification impact appeared for *P. abies*, in agreement with Augusto et al. (2002), followed by *C. sativa* and *F. sylvatica*, while *Q. pubescens* and “Other” had the lowest acidification impact.

Although comparison between species evidenced clear specific plant effects, tree/site interactions must also be considered. Conifers and chestnut tend to be found on sites with low subsoil Ca (Table 8); low Ca content of litter (Table 5) may then be at least partly ascribed to low-Ca soils. There is also a trend towards sites with low subsoil pH, but this is not very strong for mixed conifers. On the other hand, “Other” thrives on subsoil with high Ca and P, associated with similar litter properties.

Interactions with subsoil and climate parameters also help to elucidate specific pathways that lead to Amphi formations under species such as beech. Beech, although having high acidification impact (Table 7) and intermediate values of litter Ca, shows high litter P content and clearly concentrates on sites with neutral subsoil and high subsoil P (Table 8). Thus, beech combines with site effects in favoring Amphi over Moder formation.

Table 6: Summary statistics: mean, standard deviation (sd), minimum (min) and maximum (max) value for topsoil and subsoil pH values among tree species. Conif: coniferous; Csat: *C. sativa*; Fsyl: *F. sylvatica*; Med: Mediterranean species; Other: broadleaves that are not grouped in other classes; Pabi: *P. abies*; Qcer: *Q. cerris*; Qpub: *Q. pubescens*.

	Topsoil pH				Subsoil pH				Topsoil C/N			
	Mean	sd	Min	Max	Mean	sd	Min	Max	Mean	sd	Min	Max
Conif	6.2b	1.3	4.0	8.2	6.7abc	1.4	4.4	8.6	13.6bc	3.46	7.5	21.7
Csat	5.3a	0.9	3.9	7.7	5.8a	1.0	4.6	7.9	13.4b	2.92	9.6	22.1
Fsyl	6.1b	1.3	4.3	8.0	6.9bcd	1.2	4.8	8.7	13.1b	2.4	5.7	18.8
Med	6.9c	0.9	5.1	8.6	7.2cd	1.2	5.1	9.0	13.0abc	3.6	5.8	21.6
Other	7.0ce	1.2	4.8	8.2	7.5de	1.2	5.2	8.6	11.0a	2.4	7.3	15.9
Pabi	5.2a	1.1	4.0	7.9	6.2ab	1.4	4.8	8.6	14.5d	3.06	9.9	20
Qcer	6.7bc	1.2	5.0	8.6	7.3de	1.2	5.3	8.7	12.0c	2.1	5.2	15.8
Qpub	7.3e	1.2	4.4	8.6	7.7e	1.3	5.2	8.9	11.8ac	2.3	4.8	15.9

Lower-case letters indicate significant differences ( $p < 0.05$ ) between tree species using Kruskal–Wallis test.

The tree species effect which was more difficult to explain concerned some oaks. The association with humus forms of Turkey oak, rather favorable to Amphi and Moder, and of the downy oak-dominated “Qpub” group,

clearly favorable to Mull, is strong. Analysis of both litter quality and site interactions failed to point out significant differences. Cools et al. (2014) also evidenced very similar leaf and litter C/N ratios, in agreement with similar topsoil C/N in our result (Table 6). Certain parameters showed high variability, as acidifying power for Turkey oak or litter Ca content for the “Qpub” group, and strong statistical tails may influence BRT results in the absence of overall significant differences. Data on the organic litter fraction of these species are exceedingly hard to find. Difference in tannin content was shown to influence humus form differentiation under two different *Quercus* species (Bonifacio et al., 2015). Hints to high polyphenol and tannin content in *Q. cerris* leaves (Al-Masri and Mardini, 2013) and to much higher tannin content in *Q. cerris* than in *Q. pubescens* acorns (Ancillotto et al., 2015) were previously found, representing an interesting suggestion for further investigation.

### 3.4. EfVol

In the BRT analysis, EfVol was the most significant driver of Amphi/ Mull differentiation (Figs. 2 and 3). Significant differences between Amphi and Mull were detected (Table 3). Amphi forms tend to be found when soil volume for fauna, especially burrowing earthworms, is constrained by either reduced depth or high content of rock fragments. Several studies support the hypothesis that carbon incorporation in mineral soil by annelids is conditioned by effective soil volume for burrowing. Lee (1985) suggested rock fragment abrasiveness as the limiting factor for faunal activity. Ponge et al. (2014) found a positive correlation between thickness of organic layers and rock fragment content in the A horizon of Italian Amphi humus. Loranger-Merciris et al. (2007) and Xu et al. (2013) pointed to deep and stone-free soils offering better conditions for earthworms to endure summer drought, a likely explanation for the frequency of Amphi forms in Mediterranean conditions.

Table 7: Summary statistics: mean, standard deviation (sd), minimum (min) and maximum (max) value for pH.range, pH difference between subsoil and topsoil for sites with pH value higher than 6.9, among tree species. Conif: coniferous; Csat: *C. sativa*; Fsyl: *F. sylvatica*; Med: Mediterranean species; Other: broadleaves that are not grouped in other classes; Pabi: *P. abies*; Qcer: *Q. cerris*; Qpub: *Q. pubescens*.

	Mean	sd	Min	Max
Conif	0.7c	0.5	0.2	1.9
Csat	1.2cd	1.0	0.2	2.9
Fsyl	1.1c	0.9	0.1	4.4
Med	0.6b	0.2	0.4	1.0
Other	0.6b	0.4	-0.2	1.5
Pabi	1.5d	0.8	0.5	3.0
Qcer	0.8abc	0.9	0.1	3.6
Qpub	0.4a	0.5	-0.3	1.9

Lower-case letters indicate significant differences (p < 0.05) between tree species with Kruskal–Wallis test.

### 3.5. Subsoil pH and Ca content

Among variables describing soil buffering and mineral nutrient availability, subsoil pH and extractable Ca revealed the strongest predictive ability (Table 2), especially with respect to Moder (Fig. 1) and Mull forms (Fig. 3). Known relations for these two forms were generally confirmed; Moder probability was much enhanced by low subsoil pH and Ca, while Mull occurrence was positively affected by high subsoil Ca content and subsoil pH between 6.5 and 7.5. It then appears that such conditions, and the resulting humus forms, are heavily influenced by parent material. A clear association existed between subsoil and topsoil conditions,

statistical differences being strong, significant and consistent for both subsoil and topsoil parameters (Tables 9 and 10). There are several reports that Ca content is directly influential on faunal diversity and activity, beyond the effect of pH. According to Ponge (2013) macro-invertebrates have higher Ca demand than other fauna, while Ponge et al. (2014) state that Ca availability is a major factor of earthworm activity, pH rather playing the proxy in this context, and that its influence is more marked on anecic species. Ponge et al. (1999) found that small differences in parent material, acting on Ca availability, promoted humus form differentiation under an otherwise homogeneous forest stand.

Table 8

Subsoil Ca (sub.Ca) and P content (sub.P) among different tree species. sub.Ca (mg/kg) sub.P (mg/kg)

	Mean	sd	Min	Max	Mean	sd	Min	Max
Conif	35,82 <sup>5ab</sup>	67,26 <sup>3</sup>	95 <sup>6</sup>	344,63 <sup>9</sup>	450 <sup>b</sup>	273	65	1256
Csat	516 <sup>1a</sup>	573 <sup>7</sup>	82 <sup>5</sup>	21,71 <sup>2</sup>	390 <sup>a</sup>	357	55	1546
Fsyl	24,91 <sup>1c</sup>	39,42 <sup>5</sup>	36 <sup>6</sup>	186,77 <sup>2</sup>	701 <sup>bc</sup>	606	132	3364
Med	23,63 <sup>2bc</sup>	36,76 <sup>0</sup>	107 <sup>4</sup>	144,23 <sup>7</sup>	495 <sup>ab</sup>	501	96	2457
Other	48,14 <sup>3cd</sup>	73,63 <sup>0</sup>	204 <sup>1</sup>	329,17 <sup>8</sup>	917 <sup>c</sup>	951	225	4449
Pabi	13,81 <sup>5ab</sup>	22,93 <sup>6</sup>	95 <sup>9</sup>	82,99 <sup>9</sup>	465 <sup>abc</sup>	280	73	1039
Qcer	30,12 <sup>3cd</sup>	39,34 <sup>0</sup>	54 <sup>1</sup>	159,07 <sup>5</sup>	516 <sup>bc</sup>	280	127	1117
Qpub	46,25 <sup>8cd</sup>	63,55 <sup>0</sup>	80 <sup>2</sup>	266,41 <sup>9</sup>	504 <sup>bc</sup>	328	119	1620

Lower-case letters indicate significant differences (p < 0.05) between tree species using Kruskal-Wallis test.

Table 9: Single linear regression models for pH of the mineral topsoil (0–10 cm) as dependent on pH of the subsoil (sub.pH), for the entire dataset (tot) and for each humus form (Moder, Amphi and Mull).

	R2	Intercept	p
tot	0.77	0.42	<0.001
Moder	0.78	0.16	<0.001
Amphi	0.86	0.52	<0.001
Mull	0.62	1.00	<0.001

A further topsoil condition associated with Moder humus was found to be topsoil exchangeable Al (Table 3). The pH range favoring Moder was clearly superimposed with the field of presence of soluble Al<sup>3+</sup> (cf. Ponge et al., 2014); studies on Al effect on soil fauna agree that it is toxic for earthworms (Bilalis et al., 2013; Van Gestel and Hoogerwerf, 2001; Tejada et al., 2010; Zhang et al., 2013). Exchangeable Al then appears as a significant proximal cause for Moder formation; Ponge et al. (2002) found a positive correlation between aluminum content and humus index (from Eumull to Dysmoder).

Concerning Amphi forms, these parameters were clearly of less impact. Population statistics (Table 3 and Fig. 2) suggest that the typical field of occurrence of Amphi forms, concerning nutrients, tends to overlap with that of Mull forms, soil effective volume being more influential on the differentiation of these two form groups.

### 3.6. Subsoil P

Subsoil P was among the most important predictors for all humus forms (Table 2). Both subsoil and topsoil P contents in Moder were significantly lower (p < 0.001) than in Amphi and Mull (Table 3), while little difference appeared between the last two forms. Phosphorus is present in both organic and inorganic forms (Frossard et al., 2000; Condon and Newman, 2011), and its dynamics are controlled by multiple chemical and biological processes. Topsoil phosphorus content was better correlated with P content in the subsoil than

with P content of litter (Table 10). The trend holds very well for Amphi and Mull. In Moder forms, correlations were much poorer, but followed the same trend. In Amphi and Mull sites, topsoil P content was then likely mostly related to soil parent material and geochemical evolution, a result consistent with the study of Marichal et al. (2011) that found no relationship between litter and soil stoichiometry (C:N:P). In Moder, greater complexity was injected by the frequency of podsolization. For- mation of Al<sup>3+</sup> and Fe<sup>3+</sup> chelates can reduce the availability of Al<sup>3+</sup> and Fe<sup>3+</sup> for P fixation, increasing P solubility (Šantrůčková et al., 2004), while P ions can be immobilized as Fe- and Al-phosphates when Al<sup>3+</sup> and Fe<sup>3+</sup> are present in high concentration (Hinsinger, 2001). The first process is more likely in topsoil and the second in soil B horizons; this can foster some P transportation.

Table 10: single linear regression models for the P and Ca contents in the mineral topsoil (0–10 cm) as dependent on P and Ca in the litter (P\_OL) and P and Ca contents in the subsoil (sub.P and sub.Ca), for the entire dataset (tot) and for each humus form (Moder, Amphi and Mull).

		R2	Intercept	p
tot	P_OL	0.27	0.30	<0.001
	sub.P	0.70	0.74	<0.001
Moder	P_OL	0.15	1.13	<0.01
	sub.P	0.45	1.26	<0.001
Amphi	P_OL	0.30	-0.09	<0.001
	sub.P	0.73	0.78	<0.001
Mull	P_OL	0.31	0.26	<0.001
	sub.P	0.80	0.60	<0.001
tot	Ca_OL	0.43	-2.34	<0.001
	sub.Ca	0.81	0.88	<0.001
Moder	Ca_OL	0.36	-1.71	<0.001
	sub.Ca	0.79	0.80	<0.001
Amphi	Ca_OL	0.51	-4.04	<0.001
	sub.Ca	0.84	0.96	<0.001
Mull	Ca_OL	0.32	-0.79	<0.001
	sub.Ca	0.76	0.96	<0.001

These findings suggest that P might represent a limiting factor for faunal activities. Biological stoichiometry suggests that high biomass P content and low C: P ratio reflect increased allocation to P-rich ribosomal RNA, which in turn enables increased protein synthesis and growth rates, of both individuals and populations (Elser et al., 2000, 2003; Mulder and Elser, 2009). P limitation was reported for many soil invertebrates (Mulder and Elser, 2009; Bishop et al., 2010; Huang et al., 2012; Lemoine et al., 2014), evidencing that P-limitation is potentially as strong as N-limitation (Lemoine et al., 2014; Vonk and Mulder, 2013) and that N availability for arthropods is P-limited (Bishop et al., 2010). Mulder and Elser (2009) suggested that microfauna (e.g., nematodes) copes better with P scarcity than mesofauna (micro-arthropods), as stoichiometric theory predicts that fauna with higher P demand suffers a competitive disadvantage in low-P soils, due to poorer resource quality. Marichal et al. (2011) found that earthworms show strict tissue homeostasis, i.e., they maintain their stoichiometry independently from food resources. This would lead to limitation of earthworm activity by the most deficient nutrient, a hypothesis that was also supported by a positive correlation between earthworm density and soil P content. Moder forms are dominated by micro-invertebrates (Schaefer and Schauermaun, 1990; Scheu and Falca, 2000), which are likely better able to cope with constrained nutrition.

### 3.7. Moder, Amphi and Mull models

Moder (Fig. 1 and Table 3) evolved in sites with low temperature, associated with tree species with Ca-poor detritus and high acidification impact. Acidic condition was often associated with high level of  $Al^{3+}$ , with toxicity effect on pedofauna, and seemed to contribute to a decreased P content in topsoil, further reducing meso- and macro-fauna activities. Thus, Moder forms should be associated with a plant– pedofauna–soil feedback where a nutrient poor environment, due to poor inputs from litter decomposition and parent material weathering, is the habitat for a low and localized biological activity that, in combination with low temperature, slows down nutrient release and allows for organic horizons' accumulation.

Amphi (Fig. 2) and Mull (Fig. 3) evolved in similar, nutrient rich, systems with favorable conditions for pedofauna activity. The optimal ranges of soil acidity and fertility of Amphi and Mull are not fully overlapping, Amphi being better predicted by higher P and Ca contents, Mull by near neutral pH values and very low  $Exc\_Al$ . Altogether, these data suggest that Amphi and Mull mostly tend to appear in the range of well buffered and fertile soils, differentiation being often driven by soil physical conditions. This last hypothesis is also supported by the weight of rainfall seasonality in the Amphi model, high  $p_{max.min}$  being physically synergic with low  $EfVol$  in creating unfavorable, dry, seasonal conditions for faunal activity. A non-monotonic trend is visible in climate effects; Amphi is clearly associated with high rainfall seasonality but definitely not with high summer temperature ( $T_{max}$ ). Population statistics show that no significant differences between the three humus forms can be ascertained for  $p_{max.min}$ , while Mull populations are clearly differentiated for  $T_{max}$ . This complex relation can be explained by a vanishing of the Amphi-favoring effect of soil dryness when high summer temperatures force all faunal populations to endogeic behavior (Andreetta et al., 2011, 2013a).

### 3.8. Inconsistent and peculiar conditions

Various peculiar conditions occurred within the database, which help to explain the somewhat limited performance of the BRT models. First comes the issue of certain calcareous soils, showing either Moder or Amphi humus, irrespectively of them being in Mediterranean environments. To our best knowledge, association of high pH with Moder forms has never yet been reported, even on highly calcareous mountain soils. High pH topsoils with Moder or Amphi humus were found in Central Italy soils, developed on non-coherent marine sands and clays, while in the many sites on hard calcareous rock, with high subsoil pH, topsoil pH very rarely exceeded 8, irrespectively of total carbonate content. The difference can be explained by finely divided lime, commonly present on non-coherent parent materials but not on hard limestone. Finely divided lime is then likely a factor having strong negative influence on faunal activity. Studies of agroecosystems suggest that high pH can affect pedofauna activity as much as low pH. Tripathi and Bhardwaj (2004) found that maximum biomass for *Eisenia fetida* and *Lampito mauritii* was reached at pH 6.5 and 7.5, respectively, then decreasing at higher values. In neutral to alkaline soils, fertilization and amendments decreasing soil pH were found to increase faunal biomass (McCormack et al., 2013; Wang et al., 2015), the opposite effect holding for practices increasing soil pH (Liesch et al., 2010; Wang et al., 2015). In forest soils, such stressful effects may drive a shift of soil fauna to the organic layers which very rarely, if ever, reach pH values  $\geq 8$ . This poorly known effect would justify more investigations.

A similar case concerns Andosols; in these soils, Amphi humus is clearly dominant, as can be expected by causal hypotheses. Andosols tend to be too acid and Al-rich for Mull development, but their properties of organic matter stabilization and eluviation suppression (Cecchini et al., 2002) make highly unlikely the absence of a substantial A horizon. A final group of inconsistencies concerns Mull forms developing in very acid conditions, not uncommon in mountain regions and even found in Al-rich soils; this condition is closely associated with the development of Umbrisols, and the small number of samples in this database does not allow further investigation. It appears likely that the composition of pedofauna in both Andosols and



Umbrisols may be quite different from established knowledge, and that it would well warrant further investigation.

#### 4. Conclusions

The statistically based analysis of the recurrence of humus forms in Italian forests pointed out the relative roles played by various environmental factors as drivers of humus form differentiation; at least in part, the processes through which such factors influence humus development were also clarified.

The role of soil nutritional status, as conditioned by parent material properties, was found to be fundamental; the major role played by calcium was further evidenced, together with the hitherto understated importance of phosphorus. Soil nutritional status appears to influence pedofauna composition and activity both directly and through litter quality.

It can be stated, from our results, that Italian forest species are geographically distributed with a major influence of soil and climate conditions. This partly explains the weight of tree species as a factor in humus differentiation; nonetheless, various specific plant effects were evidenced. Specific characters of soil–plant–litter nutrient flows, acting on litter quality, were evidenced for such major species as Norway spruce, European beech and Turkey oak. These specific characters represent a direct effect of tree species on humus form development. Given the weight of these species in the sample population, it is likely that similar effects could be evidenced for other species, if suitably large samples could be studied. Our results evidence how vegetation and humus form can, when considered together, supply highly detailed insights on the status and evolving trend of a forest ecosystem, especially about availability and management strategies for nutrients. For the practical application of soil nature prediction, it clearly appears that joint analysis of vegetation and humus is a potentially powerful means of disentangling soil geography, at all scales.

The distinctive variability of Italian climate conditions allowed to better evidence climate effects on humus formation that were either recently proposed or not yet studied in detail. It was thus confirmed that conditions leading to seasonal soil dryness, due either to rainfall pattern or to limited soil water storage, have a primary role in inducing Amphi formation in soils with fair to good nutrient status. On the other hand, our data suggest that, in sites with really high average temperature, the Mull form is dominant. Other peculiar humus-forming conditions were evidenced in Andosols and in soil containing finely divided lime, and this ensemble of results suggests that Mediterranean forest soils are habitats for unique assemblages of pedofauna and microflora. As reported by previous studies, environment–humus modeling produces significant results in terms of process understanding, but overall predictive performance is not necessarily very high. In our opinion, our results further evidence that this is due to humus being somewhat sensitive and fast-responding. Changes in nutrient flow and soil conditions driven by such lower-order factors as forest growth cycle, forest management, specific soil chemical and physical characters, can overprint the larger-scale environment “signature” on humus form. If this reduces the accuracy of region-wide pictures, it does evidence the potential of humus as an indicator of specific ecosystem conditions and as a tool to assess the effects of lesser and man-induced perturbations. This appears to be quite an interesting perspective, also given the strong relationship between humus forms and soil carbon storage already evidenced by various studies.

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