Humus Components and Soil Biogenic Structures in Norway Spruce Ecosystems

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Whether the structure of Oa and A horizons varies according to animal activity is still a matter of conjecture, especially in amphi, a humus form with mixed features of mull and moder, which has been described in environments with strong seasonal contrasts. The Oa and A horizons of spruce [*Picea abies* (L.) Karst.] coniferous forests of the Province of Trento (Italy) were sampled in six sites with a total of 134 humus profiles along transect lines, embracing the variety of parent rocks, climate, and vegetation conditions that prevail at the upper montane level in this region. The distribution of humus components (plant debris, roots, animal feces, minerals) was assessed by an optical method and analyzed with correspondence analysis (CA). Moder humus forms were characterized by enchytraeid activity, with concomitant deposition of organic and mineral-organic feces in Oa and A horizons, respectively. Conversely, amphis were characterized by the concomitant deposition of organic and mineral-organic earthworm feces in Oa and A horizons, respectively. We conclude that Oa and A horizons of moders and amphis differed only quantitatively in the content of mineral matter in animal feces. The fine-grained mineral-organic structure that is mostly found in the A horizon of amphis results from the alimentary activity of small epigeic earthworms that mix organic matter with mineral matter, like anecic earthworms do in mulls.

Abbreviations: AN, Acid North; AS, Acid South; BN, Basic North; BS, Basic South; CA, correspondence analysis; IN, Intermediate North; IS, Intermediate South.

Coniferous forests, in particular Norway spruce stands, are known for the accumulation of organic matter in thick litter horizons (Ovington, 1954; Nihlgård, 1971). Space-for-time substitution (Pickett, 1989) studies on indigenous multilayered forest stands showed that this accumulation occurs during the pole stage of tree development and is reversible under mature trees (Bernier and Ponge, 1994; Bernier, 1996; Sagot et al., 1999), whereas studies dealing with even-aged plantations show a continuous increase until mature trees are cut (Chauvat et al., 2007). The influence of stand development on soil nutrient pools and decomposer communities (Nilsson et al., 1982; Miller, 1984; Ulrich, 1994) and the patchy structure of coniferous forests (Bernier and Ponge, 1998; Ponge et al., 1998; Ponge, 1999a) are thought to be responsible for the observed patterns.

Moders, mulls, and mors are the dominant humus forms in the topsoil of forest ecosystems (Ponge, 2003). They differ by the distribution of organic and mineral-organic horizons, called Oi (organic, slightly decomposed), Oe (organic, moderately decomposed), Oa (organic, highly decomposed), and A (mineral mixed with humus, decomposed) in the most complete case (Schaetzl and Anderson, 2005). Mulls are characterized by a crumby structure (mostly created by earthworms) in the mineral-organic A horizon. Moders are characterized by the accu-

mulation of organic invertebrate feces in the overlying Oa horizon. Amphimulls (Brêthes et al., 1995; Ponge et al., 2002; Galvan et al., 2005), now called amphis (Jabiol et al., 2004; Graefe and Beylich, 2006), exhibit mixed features of moders and mulls, and are widely represented in Mediterranean as well as mountain and tropical forest ecosystems (Loranger et al., 2003; Jabiol et al., 2004). They are characterized by a crumby structure in the mineral-organic A horizon, like in mulls, and by the concomitant accumulation of organic invertebrate feces in the overlying Oa horizon, like in moders. The objective of the current study was to investigate whether the two horizons should be considered as two largely independent compartments of the topsoil, or whether the same kind of animal activity could explain their common occurrence. In other words, when lumbricids (typical of mulls) are present, are they able to process the organic matter accumulated in Oa horizons? When earthworms are replaced by animals of a smaller size, such as enchytraeids (typical of moders), does the latter animal group create the structures observed in the A horizon? Is there any influence of the age of trees on these patterns? Answering these questions may improve our knowledge of the biological processes by which organic matter may accumulate or not in mountain and boreal coniferous forests, known worldwide as active carbon sinks (Vetter et al., 2005).

MATERIALS AND METHODS
Study Sites

Six Norway spruce sites were chosen in the northern Italian Alps, Province of Trento, embracing the wide range of environmental conditions (substrate, elevation, aspect, rainfall) that prevail in the western European part of its present-day natural distribution area (Scotti et al., 2000).

Three geological substrates (referred to as Acid, Intermediate, and Basic), each with two aspects (North, South), were selected at an altitude ranging from 1650 to 1870 m, that is, at the upper montane level (Odasso, 2002) (Fig. 1). Table 1 summarizes climatic and geological fea-
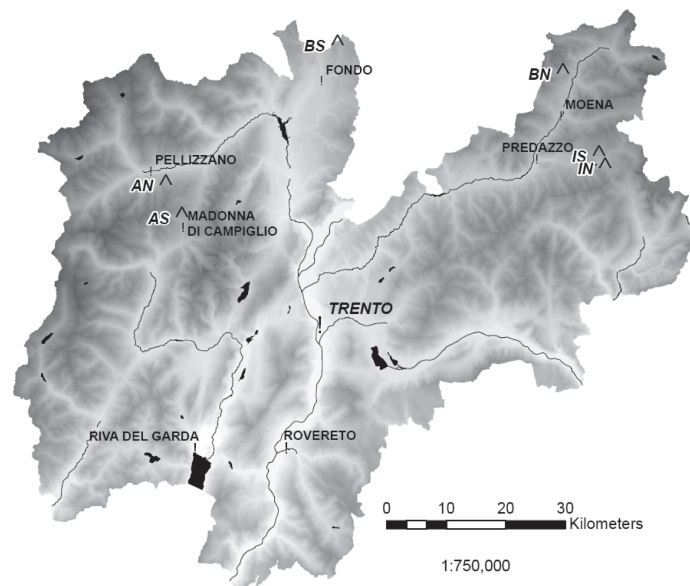

Fig. 1. Geographical position of the six study sites Acid North (AN), Acid South (AS), Intermediate North (IN), Intermediate South (IS), Basic North (BN), and Basic South (BS) in the Province of Trento.

tures of the six sites. All study sites were managed with natural regeneration (selection system) as the prevailing system for successive croppings. At each site the selected transect crossed a mosaic of trees or clumps of trees of different ages.

Sampling

A transect line was drawn in six spruce stands, representing a wide variety of environmental conditions at the site. At each site the transect crossed the widest possible variation in vegetation, in particular the four main stand developmental phases: 'gap', 'regeneration', 'intermediate', and 'mature'. Oa and A horizons were sampled every 3 m, thus totalling 134 samples (19 in AN, 27 in AS, 18 in IN, 25 in IS, 22 in BN, 23 in BS). They were analyzed microscopically according to the 'small volume' method (Sadaka and Ponge, 2003). The sampling method for humus profiles was described in detail by Bernier et al.

Table 1. Main features of the six study sites. Soil types according to World Reference Base (Bridges et al., 1998). Humus forms according to Brêthes et al. (1995).

Site code	AN	AS	IN	IS	BN	BS
Site name	Pellizzano	Madonna di Campiglio	Paneveggio	Paneveggio	Gardeccia	Fondo
Soil types	Podzols, Histosols, Umbrisols	Umbrisols, Cambisols, Podzols	Cambisols, Podzols, Leptosols	Cambisols, Podzols, Regosols	Cambisols, Regosols	Cambisols, Leptosols, Regosols
Humus forms	Mor, dysmoder, dysmull, amphimull	Mor, dysmoder, eumoder, oligomull, amphimull	Mor, dysmoder, dysmull, amphimull	Mor, dysmoder, dysmull, oligomull, amphimull	Eumoder, dysmull, oligomull, amphimull	Eumoder, dysmull, mesomull, amphimull
Parent material	Acid boulder clay overlying granitic stones	Plutonic granite often covered by late-Wurm silica-rich deposits	Permian rhyolite	Mixed volcanic/dolomitic moraine	Dolomitic moraine with granitic erratic boulders	Dolomitic moraine with granitic erratic boulders
Exposure	North	South	North	South	North	South
Elevation, m	1650–1870	1720–1865	1660–1780	1620–1818	1620–1820	1640–1690
Mean slope, %	27	32	16%	15	18	15
Mean annual temperature,°C	4.2	4.0	9.2	4.4	4.3	5.3
Mean annual rainfall, mm	962	1303	782	1103	1049	863

(1993) and Bernier and Ponge (1994). A block, 25 cm^2 in area and with such a depth as to reach the B horizon, was prepared directly in the field with a sharp knife. Each Oa and A horizon was isolated and fixed immediately in 95% (v/v) ethyl alcohol in a 400-mL hermetic seal polypropylene box, with as little disturbance as possible, then transported to the laboratory until analysis. Several layers may be sampled separately wihin the same Oa or A horizon, providing they exhibit clear differences (to the naked eye) in their apparent composition.

Laboratory Analyses

In the laboratory, each sampled horizon was studied under a dissecting microscope, after spreading it out as gently as possible in a 15-cm diam. Petri dish, filled with 95% (v/v) ethyl alcohol. The different components were quantified by the point-count method first described by Jongerius (1963) for the study of soil sections. Under the dissecting microscope, humus components under each point of a transparent film with a 157-points grid were recorded, resulting in a

table showing the distribution (percentage volume) of 84 categories of humus (Table 2) among the 375 layers investigated. In a given layer the relative volume of each component of the soil matrix was estimated by the number of times it has been recorded divided by the total number of points of the grid.

Chemical analyses were performed on a selection of soil samples taken along each transect line. Only data for the top 1 cm of the A horizon were shown (Table 3). Organic C and total N were measured in 10 to 20 mg of dried soil. Air-dried soil samples were sieved to 200 µm and homogenized, then C and N were dosed in a CHN atomic analyzer (PerkinElmer CHNS/O Analyzer 2400 Series II, PerkinElmer, Waltham, MA). Calcium carbonates were removed with HCl before analysis. Soil pH-H$_2$O and pH-KCl were measured with a glass-electrode in a 1:2.5 w/v suspension of soil in deionized water or KCl solution, mixed for 2 h, pH being measured on the supernatant. The cation-exchange capacity (CEC) was measured in BaCl$_2$ (pH 8.2). A successive addition of MgSO$_4$ allowed the complete exchange between Ba and Mg, followed by analysis of supernatant for Mg by

Table 2. List of categories used to describe the composition of Oa and A horizons.

1	Brown entire spruce needle
2	Grey entire spruce needle
3	Entire spruce needle with Lophodermium
4	Browsed entire spruce needle with Lophodermium
5	Red entire collapsed larch needle
6	Brown browsed entire spruce needle
7	Brown fragmented spruce needle
8	Dark fragmented spruce needle
9	Brown fragmented spruce needle with Lophodermium
10	Red fragmented collapsed larch needle
11	Brown browsed fragmented spruce needle
12	Grey fragmented spruce needle
13	White fragmented spruce needle
14	White collapsed entire spruce needle
15	White collapsed fragmented spruce needle
16	Brown collapsed entire spruce needle
17	Brown collapsed fragmented spruce needle
18	White collapsed browsed spruce needle
19	Brown empty fragmented spruce needle
20	White empty fragmented spruce needle with Lophodermium
21	Entire leaf
22	Fragmented leaf
23	Intact leaf base of grass
24	Fragmented leaf base of grass
25	Intact grass leaf
26	Fragmented grass leaf
27	Fragmented grass stem
28	Fragmented forb leaf
29	Twig
30	Intact moss
31	Fragmented moss
32	Bark
33	Intact flower scale
34	Fragmented flower scale
35	Wood
36	Intact seed
37	Fragmented seed
38	Cone scale
39	Spruce cone
40	Pollen
41	Unidentifiable plant fragment
42	Brown intact fine root

Table 2 continued.

43	Brown fragmented fine root
44	Brown intact mycorrhiza
45	Brown fragmented mycorrhiza
46	Brown collapsed fine root with white mycelium
47	White intact fine root
48	White fragmented fine root
49	Brown intact large root
50	Brown fragmented large root
51	White large root
52	Fine organic matter
53	Charcoal
54	White mycelium
55	Dark mycelium
56	Yellow mycelium
57	Rhizomorphs
58	Cenocccum mycorrhiza
59	Cenococcum sclerotium
60	Cenococcum mycelium
61	Fragmented fungal carpophore
62	Organic epigeic earthworm feces
63	Mineral-organic epigeic earthworm feces
64	Mineral-organic anecic earthworm feces
65	Millipede feces
66	Organic enchytraeid feces
67	Mineral-organic enchytraeid feces
68	Mite feces
69	Red unidentified feces
70	Insect feces
71	Gravel
72	Stone
73	Mineral mass
74	Mica
75	Quartz sand
76	Epigeic earthworm
77	Snail
78	Insect larva
79	Mite
80	Springtail
81	Enchytraeid
82	Dead scissiparity enchytraeid parts
83	Insect moult
84	Mite moult

Table 3. Main physicochemical features of selected samples of A horizons in the six study sites (mean ± SE).

Chemical analyses	Site code					
	AN 8 samples	**AS** 15 samples	**IN** 11 samples	**IS** 15 samples	**BN** 17 samples	**BS** 18 samples
Organic C (g kg^{-1})	186 ± 36	200 ± 16	126 ± 21	124 ± 19	97 ± 9	135 ± 20
Total N (g kg^{-1})	10.2 ± 2.1	9.9 ± 0.7	7.5 ± 1.4	6.0 ± 0.8	6.0 ± 0.4	8.0 ± 0.9
C/N	18.6 ± 0.9	20.3 ± 0.5	17.2 ± 0.9	20.2 ± 0.8	16.2 ± 0.8	16.5 ± 0.8
Carbonates (g kg^{-1})	–	–	3.6 ± 0.1	–	23.0 ± 4.3	24.4 ± 4.1
pH KCl	3.4 ± 0.1	3.6 ± 0.1	4.3 ± 0.1	4.2 ± 0.3	5.8 ± 0.2	5.5 ± 0.4
pH H$_2$O	4.2 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	5.1 ± 0.2	6.3 ± 0.2	6.2 ± 0.3
CEC pH soil (cmol kg^{-1})	98 ± 13	92 ± 4	63 ± 5	86 ± 8	85 ± 4	90 ± 9
CEC pH 7 (cmol kg^{-1})	68 ± 6	51 ± 6	44 ± 4	57 ± 7	57 ± 3	64 ± 6
Exchangeable Ca (cmol kg^{-1})	3.7 ± 1.6	10.4 ± 1.4	5.2 ± 1.2	28.6 ± 8.5	46.0 ± 3.4	41.6 ± 7.2
Exchangeable Mg (cmol kg^{-1})	1.9 ± 1.3	1.6 ± 0.2	0.8 ± 0.1	5.8 ± 1.9	13.4 ± 1.3	13.5 ± 2.1
Exchangeable K (cmol kg^{-1})	0.5 ± 0.2	1.0 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Base saturation (%)	5.2 ± 1.5	13.8 ± 2.1	10.1 ± 1.7	38.4 ± 8.5	70.6 ± 4.2	62.2 ± 9.6
Exchangeable acidity (cmol kg^{-1})	11.2 ± 0.6	7.3 ± 0.9	7.0 ± 1.8	3.6 ± 1.2	0.3 ± 0.02	4.2 ± 0.7
Exchangeable Al^{+++} (cmol kg^{-1})	8.9 ± 0.7	4.7 ± 0.7	5.3 ± 1.5	2.8 ± 0.9	0.2 ± 0.02	2.8 ± 0.5
Exchangeable H$^+$ (cmol kg^{-1})	2.3 ± 0.2	2.5 ± 0.3	1.6 ± 0.4	0.8 ± 0.2	0.1 ± 0.01	1.4 ± 0.4
Total acidity (cmol kg^{-1})	121 ± 8	99 ± 7	54 ± 7	34 ± 6	25 ± 3	54 ± 11
Particle size distribution	5 samples	8 samples	8 samples	8 samples	8 samples	8 samples
Coarse sand (g kg^{-1})	175 ± 33	307 ± 35	330 ± 53	190 ± 41	35 ± 4	61 ± 13
Fine sand (g kg^{-1})	169 ± 12	233 ± 20	162 ± 29	148 ± 15	132 ± 8	145 ± 13
Coarse silt (g kg^{-1})	156 ± 15	136 ± 11	102 ± 14	146 ± 21	230 ± 20	140 ± 11
Fine silt (g kg^{-1})	262 ± 19	201 ± 13	232 ± 29	252 ± 11	391 ± 19	334 ± 17
Clay (g kg^{-1})	238 ± 32	123 ± 13	174 ± 15	264 ± 43	211 ± 12	321 ± 9

complexometry (West, 1969). Exchangeable Ca^{2+}, Mg^{2+}, and K^+ were determined by atomic absorption spectrophotometry (PerkinElmer Analyst 200, PerkinElmer, Waltham, MA). Base saturation was calculated as the ratio Σ(exchangeable Ca^{2+}, Mg^{2+}, K^+)/CEC × 100. Total acidity was determined by titrating with HCl the solution obtained by treating the soil sample with $BaCl_2$, buffered at pH 8.2. To obtain exchangeable acidity, KCl was added to the soil sample and the solution was titrated with NaOH. By adding NaF (10 mL) to this solution and titrating with HCl we obtained exchangeable Al^{+++}. H^+ was calculated by subtracting exchangeable Al from exchangeable acidity.

Statistical Treatment

Correspondence analysis or CA (Greenacre, 1984) was used to see whether clusters with similar distributions of humus components could be distinguished within the whole set of layers. Raw data (percentage volumes of categories) were considered as active (main) variables and were not transformed before analysis. The six sites (AN, AS, IN, IS, BN, BS) and the Oa and A horizons were included as passive (additional) variables, to show whether there was a correspondence between the horizons, the sites and the animal groups.

The product-moment correlation coefficient (Sokal and Rohlf, 1995) was used to test the correlation between either the same humus component sampled in different horizons, or between different humus components sampled in the same horizons. When several successive layers of the same horizon (Oa or A) had been sampled, they were averaged by weighting them according to thickness.

Multivariate statistics and other statistical treatment of the data were performed using the Statbox Pro software package (Grimmersoft, France).

RESULTS
Were There Different Kinds of Oa and A Horizons?

The 84 categories of humus components identified under the dissecting microscope are listed in Table 2, together with their numerical codes in the CA. They were comprised mainly of plant debris (mostly spruce needles and roots but other plant parts were identified, too) at varying stages of transformation by microbes, and animal feces. Feces were attributed to animal groups (epigeic and anecic earthworms, millipedes, enchytraeids, mites, insects) according to their sizes, shapes, and colors (Galvan et al., 2005). Only one category (69) was left unidentified. Mineral particles and assemblages, as well as animals (bodies, moults) were also included in the analysis. Miscellaneous humus components were plant debris not attributable to any plant parts (Category 41) or too transformed and without any recognizable plant or animal origin (Category 52).

The first four axes explained 11.4, 10.2, 7.9, and 7.0% of the total variance, respectively (eigen values 0.49, 0.44, 0.34, 0.3). Although they explained only 36% of the total variance, the first four factorial axes allowed to discern clearly interpretable clusters in both Oa and A horizons, which was the criterion used for the detection of significant trends according to Benzécri (1969). The projection of categories of humus components in the plane of the first two axes (Fig. 2) displayed three branches, one corresponding to Oa horizons, the others to two different kinds of A horizons. Axis 1 reflected the opposition between Oa (positive side) to A (negative side) horizons. As expected, Oa horizons were characterized by organic feces (Categories 62, 65, 66, 68, and 70), bodies of epigeic animals (Categories 76 and 77) and numerous plant debris (Categories 1 to 41, with a few exceptions), while A horizons were characterized by mineral-organic
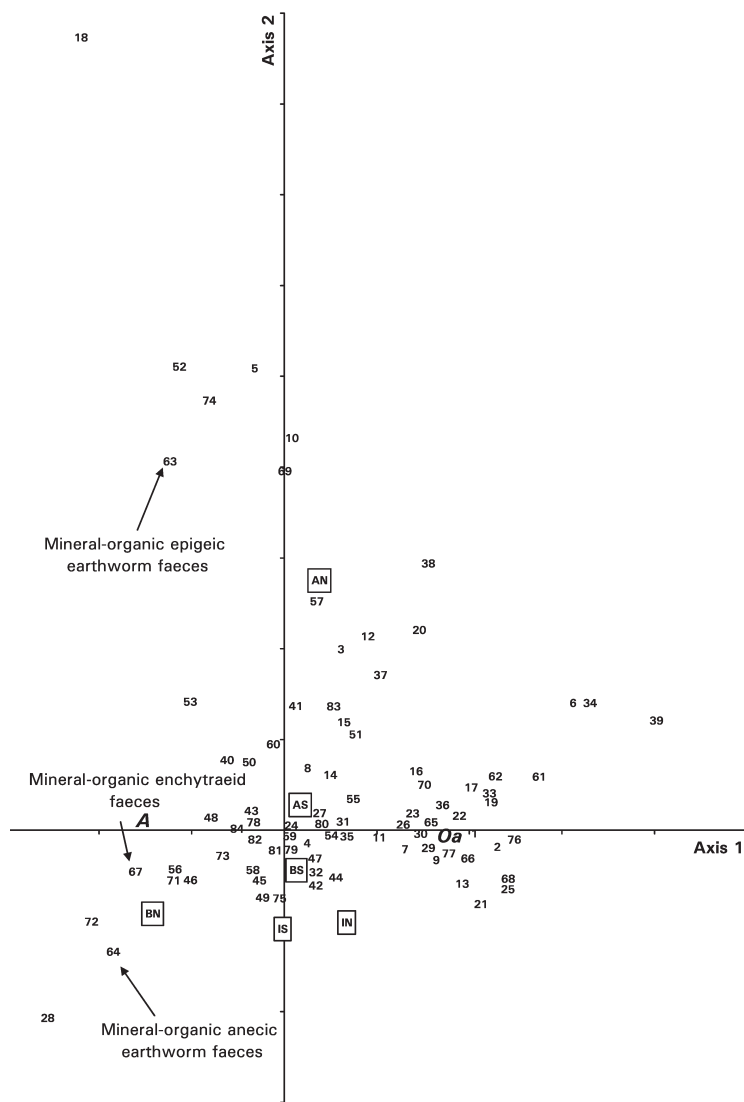
Fig. 2. Projection of the categories of humus components (main variables) in the plane of the first two axes of correspondence analysis, together with additional variables (horizons, sites). Most prominent animal feces in A horizons were arrowed

feces (Categories 63, 64, and 67), roots (Categories 42 to 51), and mineral particles (Categories 71 to 75). Axis 2 showed the contrast between two kinds of A horizons, the one (positive side) with mineral-organic epigeic earthworm feces (Category 63), the other (negative side) with anecic (all mineral-organic) earthworm feces (Category 64) and mineral-organic enchytraeid feces (Category 67). The projection of layers on the same two axes (Fig. 3) showed that the distinction between Oa and A horizons, as done by naked eye in the field as organic or mineral-organic, respectively, was fairly good, except in BN where most layers exhibited intermediary features (corresponding points were projected not far from the origin, indicating the paucity of characteristic features). A few A horizons had been misidentified as Oa horizons, in particular in AS. Conversely, a few Oa horizons had been misidentified as A horizons, in particular in AN. The two kinds of A horizons (with either anecic/enchytraeid or epigeic worm feces) were not equally distributed in the six sites. While A horizons with epigeic worm feces were absent from Intermediate North (IN), Intermediate South (IS) and near absent from Basic North (BN) and Basic South (BS), they were dominant in Acid

North (AN) and present (although as a minor part of the samples) in Acid South (AS).

The projection of the categories in the plane of Axes 1 and 3 (Fig. 4) showed the same clustering of Oa horizons on the positive side of Axis 1 (same as in Fig. 2), but now two categories of A horizons were separated along Axis 3, the one with mineral-organic enchytraeid feces (Category 67), on the positive side, the other with anecic earthworm feces (Category 64) on the other side. These two categories belonged to the same branch in the plane of Axes 1 and 2 (Fig. 2). The projection of layers in the plane of Axes 1 and 3 (Fig. 5) shows that A horizons with mineral-organic enchytraeid feces were mostly present in AS, but some horizons of this kind were also present, although less frequently, in AN and IS, and practically not in IN, BN, and BS. If we combine results from the first three axes, taking into account only the three main groups of mineral-organic feces (epigeic and anecic earthworms, enchytraeids), it appears that the A horizon of AN was mostly characterized by epigeic earthworm activity, whereas AS was mostly characterized by enchytraeid activity and the other sites by anecic earthworm activity, IS being in an intermediate position between AS and BN/BS.

While Axes 2 and 3 did not distinguish clusters within the Oa horizon, Axis 4 (Fig. 6) opposed Oa horizons with organic earthworm feces (projected on the positive side of this axis) to another cluster, characterized by organic enchytraeid and mite feces (projected on the negative side). The projection of layers (Fig. 7) showed that both clusters of Oa horizons were present in AN and AS, only the cluster with enchytraeid and mite activity was present in IN and IS, and poor differentiation (but rather on the earthworm side) occurred in BN and BS.

Biotic Relationships between Oa and A Horizons

We further investigated whether earthworm and enchytraeid activity occurred both in Oa and A horizons, or whether different animal groups were living in these horizons. The percentage volume of organic enchytraeid feces in the Oa horizon was positively correlated with that of mineral-organic feces of the same animal group in the corresponding A horizon ($r = 0.50$, $P < 0.0001$). The correlation of organic enchytraeid feces in the Oa horizon with organic feces of the same animal group in the A horizon was insignificant ($r = 0.11$, $P = 0.26$). The correlation of organic enchytraeid feces in the Oa horizon with mineral-organic epigeic earthworm feces in the A horizon was negative ($r = -0.36$, $P < 0.001$). Thus, Oa horizons with high enchytraeid activity (in the form of organic deposits) were associated with high enchytraeid activity (in the form of mineral-organic deposits) in the underlying A horizon. Similarly, organic epigeic earthworm feces in the Oa horizon were positively correlated with mineral-organic epigeic earthworm feces in the A horizon ($r = 0.50$, $P < 0.0001$). Thus, the two kinds of Oa horizons that had been depicted by CA, the one dominated by enchytraeid activity and the other by earthworm activity,

were associated with A horizons showing dominance of the same animal groups.

Although poorly represented in the studied layers (at most 2% of the matrix against 57% for organic enchytraeid feces), mite feces were positively correlated with organic enchytraeid feces (r = 0.20, P < 0.001), indicating the coincidence of these two animal groups, with mites largely subordinate to the other animal group.

Anecic earthworm feces in the A horizon were positively correlated with the same category of feces in the overlying Oa horizon (r = 0.41, P < 0.0001) and were negatively correlated with organic epigeic earthworm feces in the Oa horizon (r = −0.22, P < 0.05). Thus, anecic earthworm activity was concomitant in Oa and A horizons, but these animals deposit mineral-organic feces in both horizons, contrary to enchytraeids and epigeic earthworms, the feces of which

Fig. 3. Projection of the samples in the plane of the first two axes of correspondence analysis. (black dots = Oa horizons, empty dots = A horizons)

were in agreement with the organic or mineral-organic nature of the horizon.

We further investigated whether the depth of the Oa horizon was related to biogenic structures. Organic enchytraeid feces exhibited by far the highest (and positive) correlation with depth of the Oa horizon (r = 0.42, P < 0.0001). Thus, the thicker the Oa horizon, the more enchytraeid feces it contained.

Influence of the Age of Trees on the Composition of Oa and A Horizons

Mineral-organic enchytraeid feces in the A horizon exhibited a positive significant correlation with age of trees (r = 0.41, P < 0.0001). Conversely, anecic earthworm feces in A horizons exhibited a negative significant correlation with age of trees (r = -0.21, P < 0.05). This points to a change in the balance between enchytraeid and anecic earthworm activity during stand development. The correlation between age of trees and depth of the Oa horizon was insignificant (r = 0.06, P = 0.51).

DISCUSSION
Enchytraeid Activity and the Formation of Moder

Correspondence analysis and correlation studies showed that a few distinct assemblages of Oa and A horizons were present in our study sites, and that each of them could be characterized by a particular kind of animal activity. Awaiting for experimental studies, it can be considered that the common occurrence of the same category of feces in both Oa and A horizons is an indirect evidence that the same organisms are involved in the building of their structures, according to the
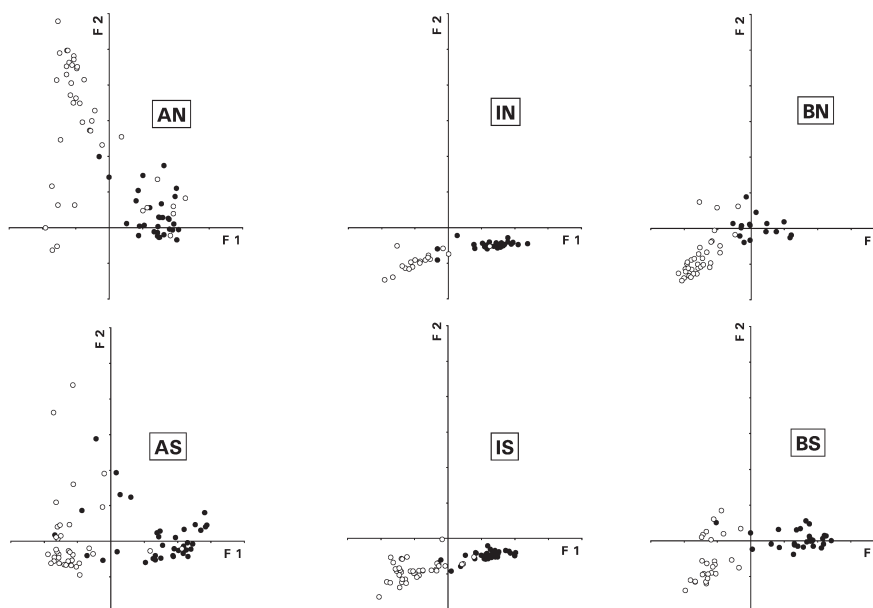
view stressed by Faber (1991) that the same species may exert different roles in different horizons. It can also be considered that the distribution of fecal categories reflected the activity of different animal groups. Oa horizons with high enchytraeid activity were associated with A horizons with high enchytraeid activity, too, although these animals deposited organic feces in
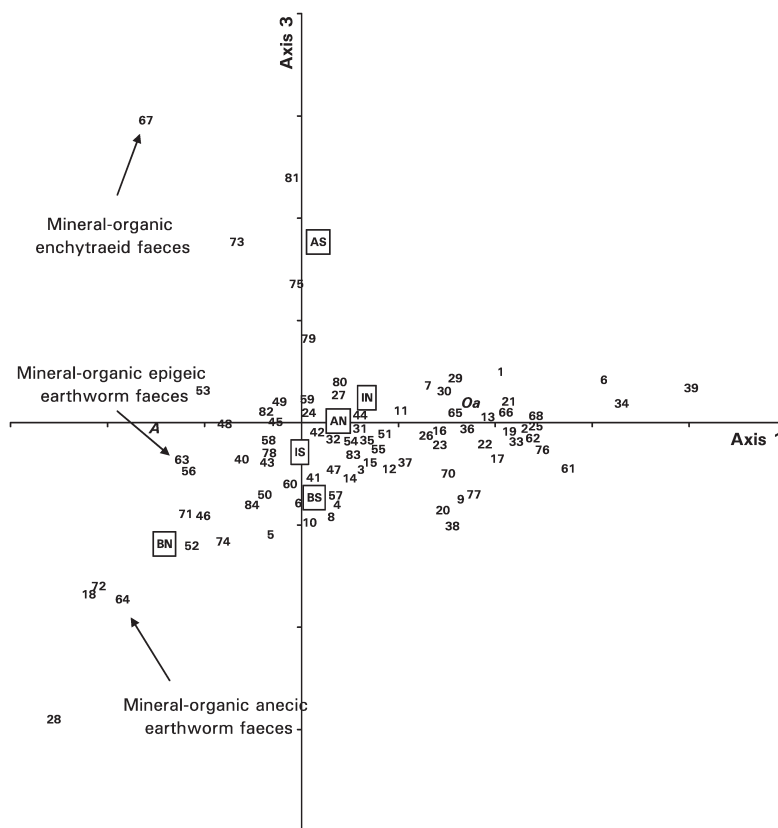

Fig. 4. Projection of the categories of humus components (main variables) in the plane of Axes 1 and 3 of correspondence analysis., together with additional variables (horizons, sites). Most prominent animal feces in A horizons were arrowed.
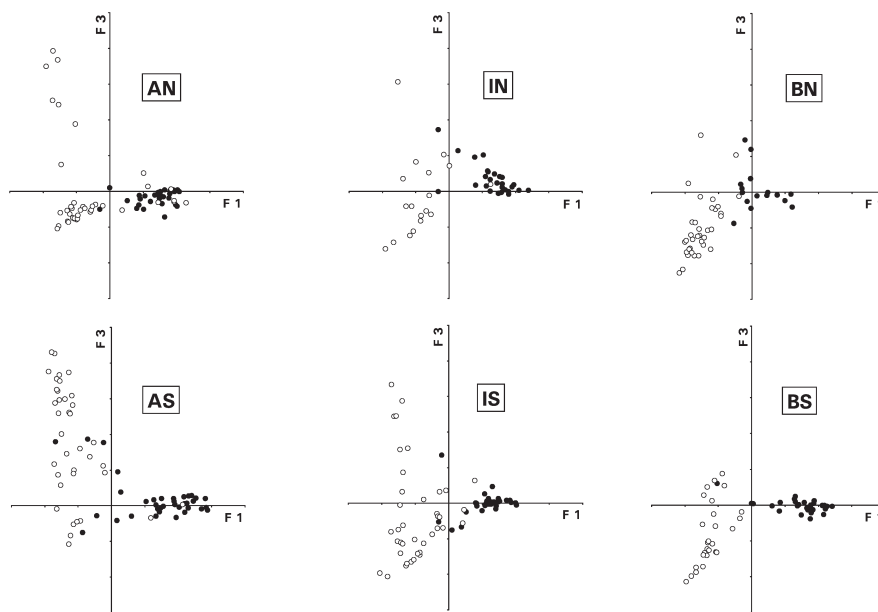
Fig. 5. Projection of the samples in the plane of Axes 1 and 3 of correspondence analysis. (black dots = Oa horizons, empty dots = A horizons).

the Oa horizons and mineral-organic feces in the underlying A horizons. Different species could be involved in this process but, as an animal group, these tiny annelid worms seem to characterize a dysmoder sensu Brêthes et al. (1995) or mormoder sensu Green et al. (1993), that is, a moder with a thick Oa horizon (>1 cm). Among our study sites, this humus form was absent on dolomitic substrates (BN, BS), but it was present from place to place on the more acidic substrates (Fig. 4–7). In the latter case, this points to a possible influence of overlying vegetation, if we consider the positive correlation between the amount of enchytraeid feces in the A horizon and the age of the trees at the sampling point. In their study of 13 beech forest stands growing under various environmental conditions, Ponge et al. (1997) showed that, among mesofaunal and macrofaunal invertebrates, enchytraeid worms were the dominant animal group in dysmoder. Other studies showed that enchytraeid worms create a microgranular structure (at the scale of 20 to 100 μm, not visible to the naked eye) in agricultural fields with poor earthworm activity (Didden, 1990; Van Vliet et al., 1993; Chan and Heenan, 1995) as well as in the A horizon of moder (Babel, 1968; Pawluk, 1987; Ponge, 1999b). Thus, these small annelids, known to ingest humified organic matter, pure or mixed with mineral matter (Ponge, 1991; Dawod and FitzPatrick, 1993), and to perform vertical movements over several centimeters (Springett et al., 1970), could be considered as mainly responsible for the continuous passage from an Oa to an A horizon, without any abrupt transition, which has been considered as a prominent feature of moder (Green et al., 1993; Ponge, 1999b). This contradicts the contention that the A horizon of moders, contrary to that of mulls, would be made of a side-by-side assemblage of organic and mineral matter, resulting from leachates of dissolved substances and suspended faunal droppings (Bal, 1970; Babel, 1975; Brêthes et al., 1995). This might hold for sandy or gravelly soils, where the size of mineral grains exceeds the silt size (2–50 μm) required for their passage through an enchytraeid gut, as shown in soil sections

by Zachariae (1965), but not for finer-grained soils where these animals are able to mix organic matter with mineral matter (Didden, 1990; Topoliantz et al., 2000).

Regarding the increased role played by enchytraeid worms in Oa horizons of increasing thickness, some authors argued that enchytraeids did not play a significant part in the degradation of organic matter, and that their abundance could not contribute to the decomposition of litter nor to the mixing of organic matter with mineral matter (Zachariae, 1964; Toutain et al., 1982). The observations made by Ponge (1984, 1985, 1988) on *Pinus sylvestris* L. litter showed that these animals, in particular the acidophilic *Cognettia sphagnetorum* Vejd., ingested well-decayed coniferous needles but digested only the fungi they hosted, and that they consumed (and degraded in their guts) a great amount of mycorrhizal fungal hyphae. It has also been shown that enchytraeids increased the mineralization of O horizons by stimulating the microflora, if not overgrazed (Standen, 1978; Wolters, 1988; Briones et al., 1998). We suggest that these animals may find more food, and a moister environment in the organic matter accumulated in coniferous forests, especially when a rich fungus flora is present, which is the case in particular in moder humus forms (Ponge, 2003). This may also explain their dominance (as ascertained by fecal deposits) under older trees. In the course of time, the ectomycorrhizal root system of coniferous trees is known to densify (Persson, 1980), thus increasing the resource available for enchytraeids through the development of mycorrhizal flora. However, we showed that the amount of enchytraeid feces in the A horizon increased with the age of trees, to the detriment of anecic earthworm feces (a feature indicating a change from mull to moder), but without any relationship with the depth of the Oa horizon. This points to the existence of two kinds of Oa horizons, one associated with moders (and enchytraeid activity), the other with amphis (and earthworm activity).

The fact that mite feces seemed to be largely subordinate to enchytraeid feces should not be considered to reflect the respective population sizes of these animal groups. Oribatid mites are abundant in thick O horizons where they reach population sizes (but not biomasses) higher than those of enchytraeids (Petersen and Luxton, 1982; Hartmann et al., 1989; Ponge et al., 1997). However, Ponge (1991, 1999a) observed that enchytraeids ingested oribatid mite feces, where the reverse phenomenon was not recorded. We infer that in the course of time, and with increasing depth in the soil, mite feces are progressively replaced by enchytraeid feces even where both animal groups are present at similar densities.

Earthworm Activity and the Formation of amphi

If we disregard a few samples of mull, near all humus profiles sampled in the study transects belonged either to moder

or to amphi humus forms. In the latter case, earthworm activity was prominent in both Oa and A horizons. However, the two ecological categories of earthworms we identified (anecic and epigeic earthworms) were not distributed equally among the sites, judging from the part played by their excrements in the composition of A horizons (Table 4). Epigeic earthworm activity was dominant in AN (Fig. 3), while anecic earthworm activity was dominant in BN and BS (Fig. 5), and enchytraeids and anecic earthworms were present in IN and IS (Fig. 3 and 5). It seems that two distinct forms of amphi were present in the study region, according to the presence or the absence of anecic earthworms (Table 4). A fine-grained structure in the A horizon (size of aggregates < 5 mm in diameter) is typical of epigeic earthworm activity, forming the Form 1, which is present in AN and AS, while anecic activity (even when epigeic earthworms are present) results in bigger aggregates (Galvan et al., 2005), forming the Form 2, which is present in BN, BS, IN, and IS.

We may wonder whether epigeic earthworms, known to live in litter, are able to mix organic matter with mineral matter in the same way as anecic earthworms do. *Dendrobaena octaedra* (Savigny) was the dominant epigeic species in our study sites (Guella, 2006, personal communication) and was near the only earthworm species living in AN, where all amphis were of the epigeic form. This small, slender acidophilic species (10–60 mm, width 1 mm) is known to feed not only on litter components, such as coniferous needles, but also on humified mineral matter, and to burrow in the mineral soil (Ponge, 1988). However, vertical distribution data differ according to authors. Abrahamsen (1972) never found it below 5 cm in Norwegian coniferous forest soils while Martinucci and Sala (1979), working not too far from our study sites (in the Venetian Alps) considered this species to be a weak burrower, which was also found in the mineral soil, especially in the fall.

Moder versus Amphi and the Balance of Annelid Communities

The difference between moders and amphis was depicted by a strong negative correlation between enchytraeid and epigeic earthworm feces. This could be ascribed to the competitive interactions that have been observed, first by co-occurrence data, second by laboratory experiments, between the lumbricid *D. octaedra* and the enchytraeid *C. sphagnetorum* (Huhta and Viberg, 1999), showing suppression of the enchytraeid population by earthworms. The growth of spruce may influence this balance in favor of the less nutrient-consuming animal group (enchytraeids) through an increase in the net nutrient uptake of trees, which impoverishes the soil (Miller, 1984). A reversal of this phenomenon in favor of the earthworm population has been observed under old trees (~200 yr) in the French northern Alps (Bernier and Ponge, 1994; Ponge et al., 1998).

Our observations in different upper montane spruce forests of the Trentino revealed the gradual passage from moder to amphi humus forms, the former more typical for acid, the latter for basic domains (Table 4). Despite our lack of knowledge on the conditions favoring amphi, the role played by soil fauna in the building of this humus form can be established by the quantitative analysis of animal feces. Our results point to the need to reconsider and to precise the juxtaposition of mineral
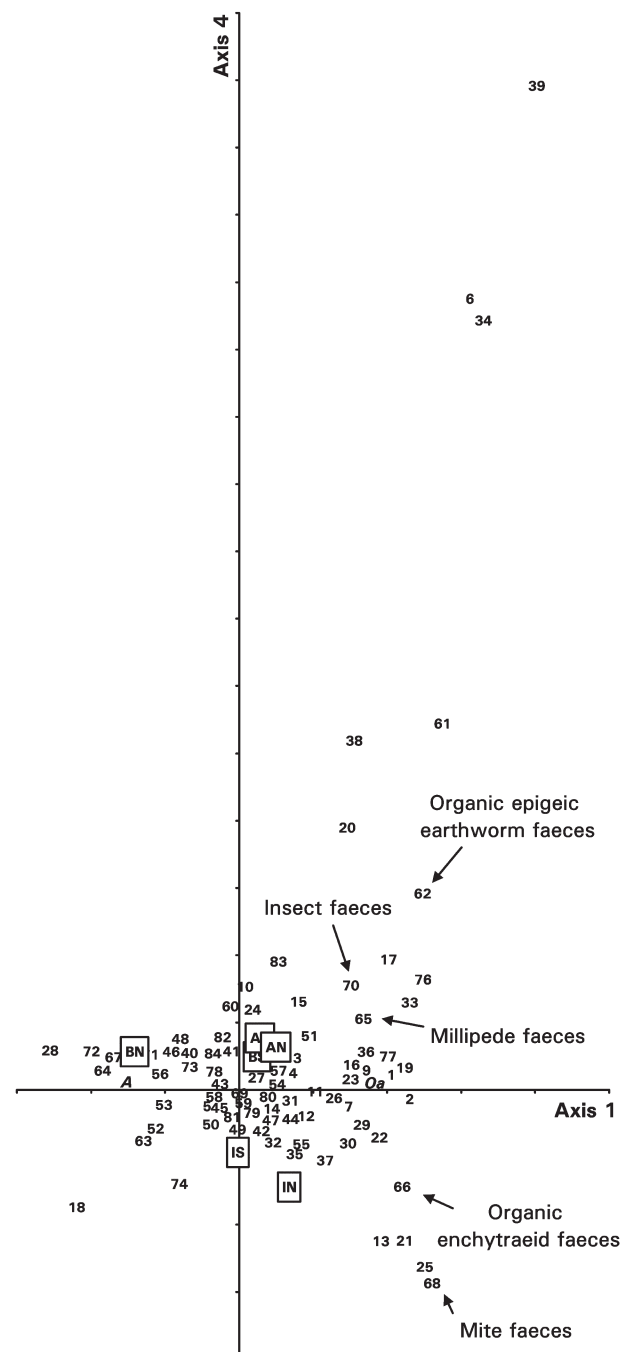

Fig. 6. Projection of the categories of humus components (main variables) in the plane of Axes 1 and 4 of correspondence analysis., together with additional variables (horizons, sites). Most prominent animal feces in Oa horizons were arrowed.

matter and organic matter in the A horizon as a diagnostic feature of moders (as opposed to mulls). We showed that the structure created by enchytraeid activity in the A horizon of moders was similar to that of earthworm mulls and amphis (i.e., a mixing of mineral and organic matter), but it was much finer. The widely reported assemblage of coarse mineral grains, covered or mixed with organic deposits, in moders, results more from the coarse texture of sandy soils (more prone to the formation of moders because of their poor nutrient availability) than from the absence of burrowing activity in the A horizon. Amphis differ from moders by the coarser grain of the structure in the A horizon (earthworm feces instead of enchy-
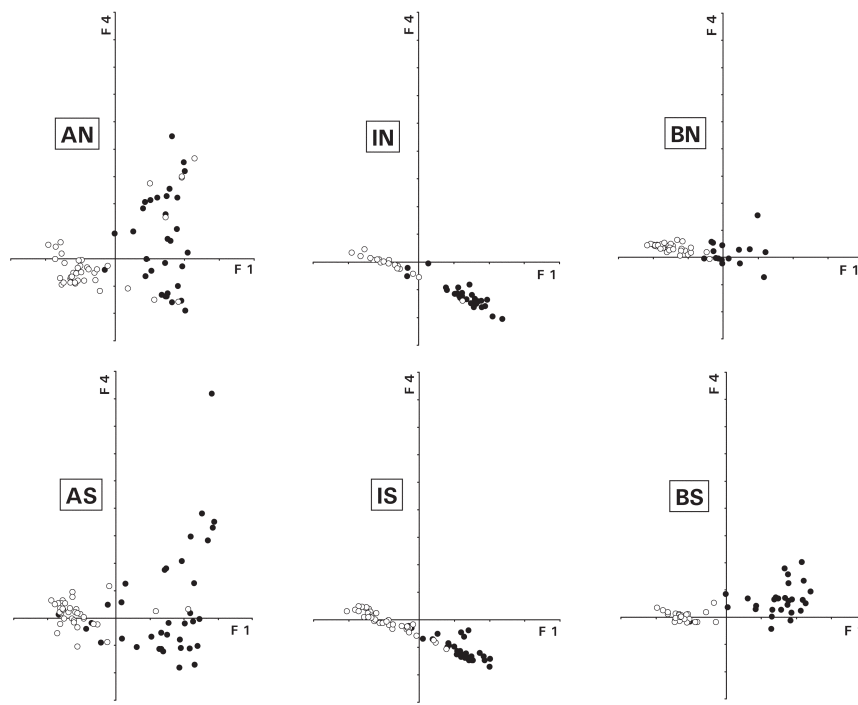
Fig. 7. Projection of the samples in the plane of Axes 1 and 4 of correspondence analysis. (black dots = Oa horizons, empty dots = A horizons).

traeid feces) but also in the Oa horizon, which is comprised of earthworm feces. In that sense, the Oa horizon of amphis does not differ from that of eumoders (Brêthes et al., 1995), which is thin (≤1 cm) and comprised of fecal pellets visible to the naked eye (>100 µm).

Awaiting further observations and laboratory experiments, it can be hypothesized that the main difference between moders and amphis lies in the important part played by epigeic earthworms (compared with other macro-invertebrates) in the saprophagous community of the latter, combined with seasonal influences on their vertical distribution: earthworms are known to live deeper in the ground than most other litter-dwelling macro-invertebrates when the cold (or dry) season becomes unfavorable to epigeic activity (Schaefer, 1991).

Concerning the independence of Oa and A horizons, it can be concluded that they are inhabited by the same animal groups: when enchytraeid or earthworm activity is dominant in the A horizon, it also dominates in the Oa horizon. The same animal group (maybe the same species, not proved in the present study) ingests organic matter in Oa or a mixture of mineral and

organic matter in A. This had been already shown to occur in moders in beech forests of Belgium (Ponge, 1999b). Reasons for the incomplete incorporation of organic matter (Oa horizon) within the A horizon of amphi is still a matter of conjecture, even though climatic reasons are highly probable, given its geographical location in European Alpine and Mediterranean régions.

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Table 4. Dominant humus forms and biogenic structures in the six study sites.

Site codes	AN	AS	IN	IS	BN	BS
Dominant humus forms	moder, amphi 1	moder, amphi 1	amphi 2, moder	amphi 2, moder	amphi 2	amphi 2
Fecal deposits in Oa horizon						
epigeic earthworms	†	†	nil	nil	‡	‡
enchytraeids and mites	†	†	‡	‡	§	§
Fecal deposits in A horizon						
anecic earthworms	nil	§	‡	‡	‡	‡
epigeic earthworms	‡	†	nil	nil	§	§
enchytraeids	†	‡	§	†	§	§

† abundant

‡ very abundant

§ few

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