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## Humic acid characteristics in podzol soil chronosequence

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Humic acid (HA) characteristics were studied in a natural revegetation chronosequence of 10-, 20-, 60-year-old and mature podzol soils under a pine forest, along the spoil heaps of a sand quarry. The elemental composition of HAs showed a trend towards carbon increase and hydrogen decrease with soil age. Essential differences in the atomic ratios between humic acids extracted from the different soils may be due to the intensity of the humification process as related to soil age. Compared with HAs extracted from organic horizons, those from mineral horizons showed lower C/N and H/C ratios, whereas O/C ratios were higher. Electrophoretic data showed that the proportion of the more-mobile fraction (L-MS) was higher in the mineral horizons than in the organic horizons and, among organic layers, in the Oe and Oa horizons compared with Oi. Intensive transformation of pine remains may have occurred and led to HAs with an increasing degree of humification in only 60 years. Moreover, progressive accumulation of the L-MS fraction in the E and Bs horizons with soil age might be the result of the ongoing migration of the most-polar organic compounds down through the soil profiles.

**Keywords:** podzol chronosequences; humus formation; humic acids

### 1. Introduction

Soil chronosequences have long been considered a classic object of study in evolutionary soil science [1,2], and are particularly useful in following changes in soil chemical properties over time. Chronosequences of soils, especially on sandy parent materials, are powerful instruments for pedological and pedochemical investigations because of the unique possibility to follow soil development from the very beginning [2–7]. This is particularly valuable because the pedogenesis rate is reported to be rapid in the first 50–60 years and then to decrease, whereas the quasi-equilibrium stage emerges after 1500–2000 years [1].

In the taiga forest zone, various forms of soil chronosequences, developed on different types of Quaternary sand, have been studied: continental coastal dunes [8], surfaces with lake transgressions of different ages [9], quarry spoil-heaps of different ages [10] and substrates of military construction [11]. Under such climatic conditions, the combination of coniferous vegetation and

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sandy substrate usually leads to the formation of podzols, as pointed out in some reviews on chronosequences studies [1,8].

The main mechanism of podzolisation is the mobilisation of organic compounds and sesquioxides from the O and E horizons of acidic soils and their precipitation into a Bs horizon [12]. Evidence of Al–Fe–humus complex formation and its role have been detected analytically [9,13] and the rates of thickness increase have been estimated for the whole soil profile and for each horizon [10]. However, the rate of C accumulation [14,15] and podzol development on a sandy substrate are still under debate [9,16] and little attention has been paid to organic matter dynamics in podzol chronosequences [4,8,17–19]. Soil organic matter transformation and movement along the profile are among the major mechanisms of podzol formation, because organic compounds act as acidifying and chelating agents during mineral weathering, leading to eluviation processes [14]. Illuviation processes may follow because of the increasing pH or the progressive occupation of organic Al/Fe binding sites. This in turn may activate the formation of polynuclear hydroxyl Al ions in solution and a shift of the majority of metal (Me) ions into the inorganic form for the synthesis of allophane/imogolite in the Bs horizon [20]. However, this theory has been contested [21], and a mechanism for the *in situ* formation of imogolite/allophane in the Bs horizon by a carbonic acid weathering process and precipitation of fulvic–Al and Fe chelates at the top of Bs has been proposed. Additional studies on podzolisation [4,17,18] have shown that organic matter in the Bs horizon may derive from illuviation processes and root turnover and that the latter may be dominant in well-drained boreal systems.

The aim of this study was to investigate the characteristics and dynamics of organic matter in podzolic soils developed under natural vegetation with Scots pine (*Pinus sylvestris* L.) in a former sand quarry in north-west Russia. Because organic matter consists of a continuum of components at different degrees of decomposition and in a dynamic state, we concentrated on the more altered portion, i.e. humic acids (HAs), which is an operationally defined fraction useful for characterising and evaluating the dynamics of organic matter in soil. Because mineral weathering and Me–organic chelate migration are related to humic substance characteristics, we tracked modifications in the composition of HAs extracted from soils of different ages. We used polyacrylamide gel electrophoresis (PAGE) in the presence of denaturing agents as a promising tool to determine molecular size and the electrophoretic mobility of HAs, with the aim of evaluating both their transformation and relative tendency to migrate down the different profiles.

## 2. Materials and methods

### 2.1. Description of the study sites

The chronosequence of sandy soils within the large Maluksa sand quarry (Kirovsky district, Leningrad oblast, Russia; Supplementary Figure S1 – available online only) was studied [10]. The quarry is situated in the south taiga zone, with an annual precipitation of ~600–700 mm and a mean annual temperature of ~3.5 °C.

The quarry, ~10 km long and up to 2 km wide, is a former esker composed of glaciofluvial sands. Exploitation of the quarry started in 1930–1933 and resulted in the formation of lakes, which now occupy up to 70% of the area (Supplementary Figure S2 – available online only). The extracted sands were near the surface, therefore, the very thin new-soil layer is not mixed with material forming the spoil heaps. The soil parent material is composed of glaciofluvial sands that are very homogeneous in chemical composition and particle-size distribution. This allows soils developed at different times after the cessation of quarrying to be considered as a chronosequence of pedogenesis. In this study, we sampled sandy 10-year-old primitive soil, 20- and 60-year-old embryopodzols and the mature (benchmark) podzol under a mature pine forest.

Table 1. Morphological features and chemical properties of soils.

Horizon	Depth cm	Colour	TOC	Clay content	Fe oxalate	pH
			g · kg <sup>-1</sup> soil			
10 years						
O	2	10YR3/2	100.8	nd	nd	4.5
E	1	2.5 Y 3/2	3.5	7	0.5	5.7
Bs	1	5Y 4/2	1.7	8	0.6	5.9
20 years						
O	3	5Y3/2	420	nd	nd	4.7
E	2	5YR 3/2	3.7	7	0.5	5.3
Bs	2	10YR4/2	2.1	9	0.8	5.4
60 years						
O	8	7.5YR2.5/2	382.6	nd	nd	4.2
E	7	5YR 3/2	9.6	8	3.2	4.2
Bs	12	10YR4/2	4.8	10	3.8	4.8
Mature						
O	8	7.5YR2.5/2	413.3	nd	nd	4.5
E	13	5YR 3/2	0.69	8	3.1	4.2
Bs	12	10YR4/2	0.46	12	4.5	4.7

Embryopodzols are situated on the flat surfaces of former spoil heaps that have been naturally revegetated by Scots pine (*Pinus sylvestris* L.) since the cessation of quarrying. The mature forest is Scots pine, present before quarrying began and not subjected to any clear cutting over the previous 70 years. Some patches of the mature forest still exist near the quarry and one was included in the sampling. The management of and technology used in quarrying were the same for the whole mining period and there is no current significant anthropogenic impact. Therefore the zero-point of soil development is considered to be the same for all plots.

Data on the morphological features of these soils and their main chemical properties have been reported previously [10] and the general characteristics of the soils are given in Table 1. These data show that the investigated B horizons are really spodic (Bs) according to WRB criteria [22]. Soil profile morphology (Figure 1) shows that podzolisation starts at the 20-year-old site with the formation of a weak E horizon under the forest floor. In older soils, the thickness of the forest floor increases, as does the thickness of the E and Bs horizons. With age, the forest floor differentiates into the sub-horizons of accumulation (Oi), fermentation (Oe) and humification (Oa). The chronosequence also shows a gradual increase in organic matter, in both the organic and mineral horizons. The soil properties of the profiles have been published previously [10,23].

At each site, organic and mineral horizons were sampled at two randomly chosen locations, the subsamples pooled into a composite sample and oven dried at 40 °C. Organic materials were ground and sieved at 2 mm, the mineral samples were dry sieved at < 2 mm and large root debris was picked out manually.

## 2.2. Humic acid preparation and characterisation

Humic acids were extracted from each soil sample according to the following procedure [24]. Briefly, HAs were extracted with 0.1 M NaOH solution (soil/solution ratio 1:10) under N<sub>2</sub>. After 24 h of shaking, the alkaline supernatant was separated from the soil residue by centrifugation at 1516 g for 20 min and acidified to pH 1 with 6 M HCl solution to induce the precipitation of HAs. Supernatant containing FA was separated from the precipitate (HAs) by centrifugation at 1516 g for 15 min. The HAs were then re-dissolved in 0.1 M NaOH solution, shaken for 4 h under N<sub>2</sub> and the suspended solids were removed by centrifugation. The HAs solution was acidified

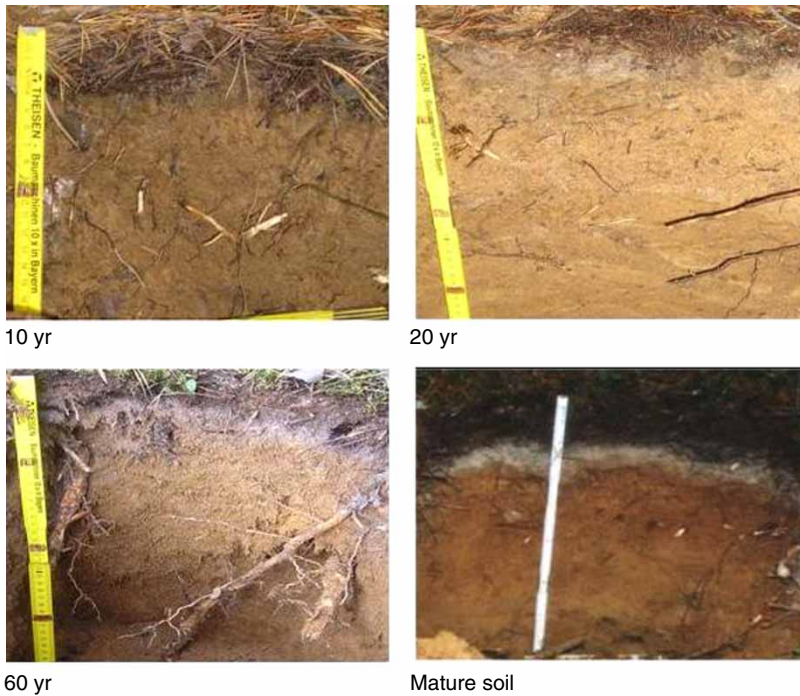


Figure 1. Soil profile morphology of the four sites.

again with 6 M HCl to pH 1, and the HAs separated by centrifugation. HAs were demineralised by shaking overnight with a 0.1 M HCl/0.3 M HF solution (solid/solution ratio 1:1) and then repeatedly washed with deionised water until pH 3 was reached, then freeze-dried. Humic acids were characterised for their elemental composition (C, N, H, and S) using a CHN 185B-Hewlett Packard analyser. Data were corrected for water and ash content. Oxygen content was calculated by difference.

The electrophoresis method for the fractionation of HAs [25–29] was used to simultaneously estimate the electrophoretic mobility of HAs and their molecular mass. Acrylamide 9.7% and bisacrylamide 0.3% were dissolved in a buffer solution containing 89 mM Tris–borate, at pH 8.3, with 1 mM EDTA and 7 M urea. The apparatus comprised a vertical electrophoresis device (LKB 2001 Vertical Electrophoresis, Sweden) with a gel slab (20 × 20 cm). Electrophoresis was carried out for 1 h at a current of 50 mA. On each gel, 0.4 mg of freeze-dried HAs was applied after complete dissolution in 100  $\mu$ L of buffer solution. Using this, the distribution of apparent molecular size and electrophoretic fractions were affected only by the properties of HAs and not by their concentration in solution or content in the different horizons.

### 3. Results and discussion

The HAs in the 10-year-old soil were rich in C and H (Table 2). The C content was higher in HAs from the organic horizon than in HAs from the E horizon. This may be because the organic horizon is not yet well-developed and is still rich in relatively fresh organic remains, and because of the low incorporation of oxidised material into the mineral soil and its HAs.

With soil age, HAs from both the organic and mineral horizons showed an increased C content, following a change in the composition of the forest floor and its development. At the 10-year-old

Table 2. Elemental composition of HAs (element content in atomic percent) and atomic ratios.

Horizons	C	H	N	O	C/N	H/C	O/C
10 years							
Oi	28.0 ± 0.1	50.0 ± 0.1	1.6 ± 0.0	20.4 ± 0.1	17.4	1.78	0.72
E	27.4 ± 0.3	43.5 ± 0.1	2.7 ± 0.1	26.4 ± 0.1	10.2	1.58	0.96
20 years							
Oi	36.4 ± 0.2	49.1 ± 0.2	0.6 ± 0.1	13.9 ± 0.2	60.7	1.35	0.38
Oe	28.7 ± 0.2	41.4 ± 0.2	1.7 ± 0.1	28.2 ± 0.2	16.7	1.44	0.46
E	36.6 ± 0.1	47.5 ± 0.2	0.9 ± 0.0	15.0 ± 0.1	39.4	1.30	0.41
60 years							
Oi	36.4 ± 0.1	50.2 ± 0.4	0.7 ± 0.0	12.7 ± 0.1	66.2	1.38	0.35
Oe	35.4 ± 0.2	45.1 ± 0.3	1.5 ± 0.0	18.1 ± 0.1	23.9	1.27	0.51
Oa	34.8 ± 0.1	42.0 ± 0.3	1.3 ± 0.0	22.0 ± 0.2	26.1	1.21	0.63
E	35.5 ± 0.1	40.0 ± 0.2	1.1 ± 0.0	23.4 ± 0.1	32.9	1.12	0.66
Bs	33.1 ± 0.1	38.0 ± 0.2	1.9 ± 0.1	26.9 ± 0.1	17.6	1.15	0.81
Mature							
Oi	36.8 ± 0.2	49.2 ± 0.3	0.5 ± 0.1	13.5 ± 0.2	70.8	1.34	0.36
Oe	36.7 ± 0.1	40.8 ± 0.2	1.6 ± 0.1	21.0 ± 0.1	23.1	1.11	0.57
Oa	35.7 ± 0.1	44.4 ± 0.1	1.7 ± 0.0	18.3 ± 0.2	21.5	1.24	0.51
E	35.6 ± 0.1	38.1 ± 0.1	1.3 ± 0.0	25.0 ± 0.3	26.5	1.07	0.70
Bs	32.4 ± 0.1	41.0 ± 0.1	2.4 ± 0.0	24.2 ± 0.2	13.4	1.27	0.74

site, the forest floor was made up of some herbaceous remains with pine needles, whereas an almost homogenous layer of exclusively pine residues was present in the 20-year-old and older sites. Furthermore, HAs extracted from 10-year-old soil appeared O and N rich, especially in the E horizon. This may also be explained by a higher contribution of herbaceous species, present more in the young site than in the older sites. In fact, N depletion in the HAs occurred in the O and E horizons when the amount of litterfall input increased intensively and the organic horizons were well developed and mainly composed of needles and branches. The dominance of coniferous material likely caused the high C/N ratio and the decrease in H/C due to the progressive incorporation of lignin residues in the HA structure [16], although further investigations are required to better understand the contribution of lignin-derived phenols in the humification processes.

During soil formation and chronosequence development, the organic material appeared to be subjected to progressively intensive decomposition processes that caused oxidation, as deduced by the increase in the O/C ratio of the HAs. As a result, the C/N ratio of the HAs from the Oi horizon at the 10-year-old site was the lowest, although it increased with age. The forest floor appeared to be well developed at the 60-year-old site with ratios very similar to those obtained in the HAs extracted from the forest floor at the mature forest site.

The H/C ratio was generally > 1, which possibly indicates the abundance of the aliphatic component [30]. It was shown that the composition of organic matter in organo-mineral associations is highly variable [31], with loamy soils generally having a higher contribution of polysaccharides, whereas mineral-associated organic matter in sandy soils is often more aliphatic. With soil age, the H/C ratio decreased more in HAs from mineral horizons than in those from organic ones. These trends have been discussed in a spruce chronosequence of Haplorthods in the south of Sweden [16].

The humic fractions extracted from the Bs horizons of the 60-year-old soil and of the mature soil were richer in nitrogen than HAs from the E layers and showed higher H/C and O/C. Essential differences in the elemental composition and in the consequent atomic ratios between the humic substances from the E and downwards Bs horizons may be related to the migration of more-polar material from the eluvial into the Bs horizon. Humus material illuviation in these profiles has previously been reported to be evident from monochromic organic coatings on the surface of mineral grains in thin sections [23].

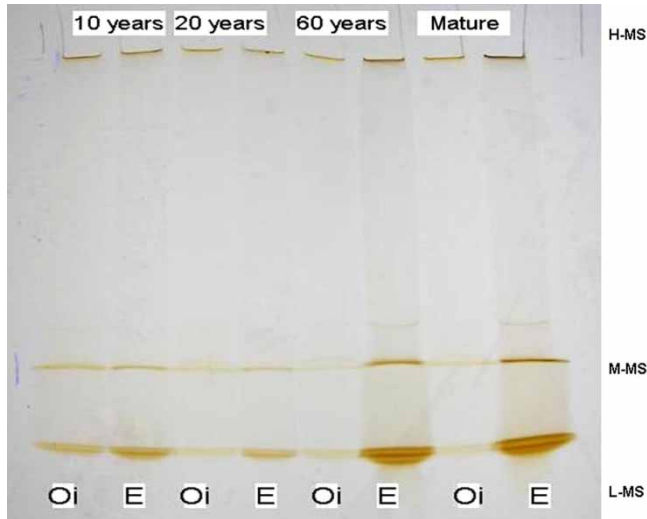


Figure 2. Electrophoretic patterns of HAs extracted from Oi and E horizons of the soil chronosequence.

To better understand the molecular properties of organic matter, HAs from Oi and E horizons of different ages were fractionated using polyacrylamide gel electrophoresis (PAGE) [27,28] in the presence of denaturing agents (Figure 2). This method has previously been used successfully for the comparison of: (i) soil HAs and model phenolic polymers [25]; (ii) soil HAs before and after acid hydrolysis [26]; and (iii) soil, river and lake humic substances [29].

Figure 2 shows electrophoretic patterns of HAs extracted from the Oi and E horizons of the chronosequence. During electrophoresis, all samples were separated into three discrete fractions based on their molecular size (MS): H-MS, the starting fraction; M-MS, the mid-gel fraction; and L-MS, the fraction at the bottom of the gel. It has previously been shown that the molecular size of electrophoretic fractions decreased with increasing electrophoretic mobility [27]. Thus, the H-MS fraction had the highest MS and L-MS the smallest. These data showed that in the Oi horizons the intensity of all HA fractions (especially L-MS) decreased with soil age. This might be the result of the presence of smaller humified fractions in the organic layers of older soils. By comparison, in the E horizon, the intensity of all fractions decreased slightly between the 10- and 20-year-old soils and then increased to the 60-year-old soil, with features comparable with HA fractions derived from the mature soil. This suggests a rapid transformation of the organic material present in the mineral horizons and progressive accumulation of more polar and small molecules in the E horizon, in agreement with elemental analysis. Accumulation of the L-MS fraction in the Bs horizon might be due to the preferential migration of the smallest compounds from the organic and E horizons and precipitation onto the mineral phase in the Bs horizon. This is in line with previous work at the same site [23] where thin section images of B horizons showed coatings of monomorphic organic matter on the surfaces of mineral grains and in cracks. Thus it could be hypothesised that illuviation is the main process accounting for the occurrence of organic material in the Bs horizon, although there are data that podzol B horizons in well-drained and boreal systems are dominated by root derived organic matter [18].

Total profile electropherograms of the 60-year-old and mature soil are shown in Figure 3. The intensity of H-MS, M-MS and L-MS humic fractions in the E and Bs horizons of the 60-year-old soil was similar to the pattern in the mature soil but showed much less intense colour compared with the Oi, Oe and Oa sub-horizons of forest floor. The beginning of the humification processes had evidently occurred in the Oe and Oa horizons, as deduced by the increasing intensity of H-, M- and especially L-MS fractions.

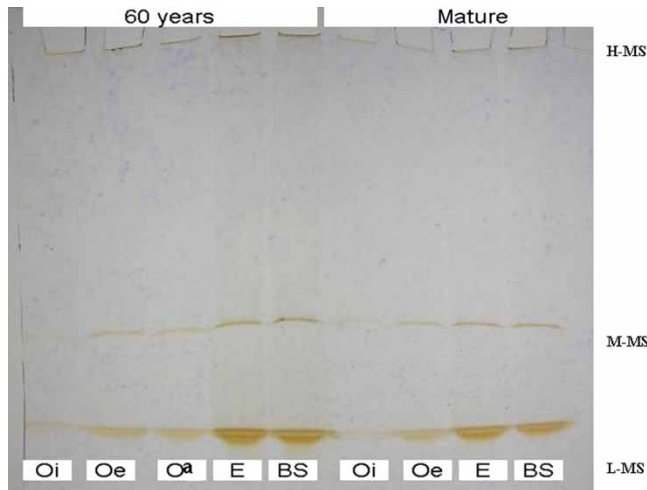


Figure 3. Electrophoretic patterns of HAs extracted from all horizons of the 60-year-old and mature soil profiles.

#### 4. Conclusions

HAs in soils of different ages, forming under natural revegetation of sandy dumps, show essential changes in their elemental composition and electrophoretic mobility. Comparing the elemental composition of 10-year-old soil HAs with older ones shows that essential changes begin to appear in the 20-year-old soil, when the beginning of the illuviation of soil organic material and its redistribution along the soil profile were observed. The fractions with the smallest molecular size were more abundant in the Oe horizon than in the Oi layer, indicating the transformation of plant remains into humic materials. The high values of L-MS fractions in the E and Bs horizons supported the hypothesis of the migration of small compounds along the profile. The L-MS fraction accumulated intensively in the mineral horizons of the older soil as a result of the downward migration of the smallest, more polar and mobile compounds.

The data obtained show that soil on the sandy spoil heaps under a pine forest in south taiga starts to differentiate within 20 years not only morphologically, but also in the composition of the HAs. This gives evidence that podzolic processes in embryonic soils start to develop very early, although further investigations are needed to better understand mineral weathering and the transformations of the whole organic matter pool that drive soil formation and evolution in such a short time. The data obtained confirm the monochromic coatings accumulation in spodic horizons of young podzols and suggest that in the investigated chronosequence, organic material illuviation is a key process.

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