1 Humic fractions as indicators of soil organic matter responses to clear-cutting in the

2 mountain and lowland conditions of south-western Poland

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4 Effect of forest removing on soil organic matter in the lowland and mountain conditions

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17 Abstract

18   Clear-cutting means forest removing (stem only) and is the most common type of
19 forest harvesting but undoubtedly has a negative impact on the C budget in soils. This work
20 aimed to describe responses of soil organic matter in the forest soils to forest removing under
21 temperate climate conditions of lowland and mountain regions in south-western Poland.
22 Using advanced instrumental analysis, like EPR, ^{1}H NMR and FT-IR spectroscopy it has been
23 found that clear-cutting, alters C cycling and accelerates decomposition in the forest floor
24 leading to loss of humic fractions in the investigated soils. In the mountain forests the more
25 labile, low-molecular fulvic fraction decreased as the effect of harvesting practice. The

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26transformation of organic matter after clear-cutting resulted in the loss of less humified

27organic matter containing humic substances of less polymerised molecules. Analysis of the

28semiquinone radical structures and concentrations showed a decrease in radical concentration

29observed for HA from mountain clear-cut areas compare to the undisturbed forest. Results

30presented in this paper have proved less aliphatic character of humic acid molecules from the

31lowlands, compared to the mountain forest as the effect of clear-cutting. Harvesting practices

32in mountain regions should be approached with particular care due to the risk of erosion of

33exposed surfaces and soils containing less humified and less stable organic matter than in the

34lowlands. Humic fractions of higher solubility, less stability and tendency to migrate through

35the soil profile may favour the leaching of nutrients and consequently cause the eutrophication

36of waters.

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38**Key words:** humic fraction**,** soil organic matter, humic acids, EPR, ^{1}H NMR spectroscopy,

39forest ecosystems

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411. Introduction

42   Soils are a huge source of terrestrial carbon, storing several times more C than there is

43in the atmosphere (Mayer et al., 2020). Particularly important in this matter are forest soils,

44due to their storing over 40% of the global total organic carbon (Cerli et al., 2008; Falsone et

45al., 2012; Mayer et al., 2020). Most of the soil organic carbon, about 70%, occurs as humic

46substances (De Nobili et al., 2020; Loffredo and Senesi, 2006), whose properties can vary

47depending on not only environmental, natural factors such as soil properties, plant species,

48climate but also anthropogenic factors, such as type of management. Humic fractions, both

49humic and fulvic acids, have been used to study the properties and function of soil organic

50matter (SOM) for hundreds of years (Abbt-Braun and Frimmel, 2002; Olk et al., 2019).

51According to many authors, changes in forest cover can alter microbial community and 52accelerate the degree of organic matter decomposition, leading to C losses (Ishikawa et al., 532007; Jamroz et al., 2014; Lan et al., 2020; Prescott, 2005; Ussiri and Johnson, 2007). Clear-54cutting, forest removing (stem only), is the most common type of forest harvesting worldwide 55but undoubtedly has a negative impact on the C budget in soils (Mayer et al. 2020). 56Understanding the effect of practices like clear-cutting on the soil environment is critical for 57the postharvest management of sites.

58   Forests in Poland occur mainly in areas with the weakest soils; they are a significant 59part of the geographic space, 30.4% of the country's area, and they are mainly publicly owned 60(Jamroz 2014). Mountain habitats occupy 8.7% of the forest area. In 2018 the clear-cutting 61constituted about 23% of the total timber production. The total area of clear-cutting in Poland 62was 40.6 thousand ha. The increase in this area in the last two years was mainly due to the 63necessity from hurricane (2017) liquidation (FinP 2019).

64   Forest harvesting, especially practices like clear-cutting, is described in the literature 65for both deciduous and coniferous forests (Bergholm et al., 2015; Falsone et al., 2012; 66Fuzukawa et al., 2006; Ishikawa et al., 2007; Jamroz et al., 2014; Jussy et al., 2004; Piirainen 67et al., 2002; Ussiri and Johnson 2007). The articles, however, mainly characterise the effect of 68clear-cutting on nutrient releases, nitrogen and total organic carbon fluxes. There is in the 69literature still a lack of information describing the effect of clear-cutting on the soil organic 70matter properties and direction of soil organic matter transformation.

71   Clear-cutting is removing all tree stems in a stand. It causes a strong disturbance of the 72forest ecosystem by disruption of the biogeochemical cycle of elements (Mayer et al., 2020; 73Jussy et al., 2004). The disadvantages of clear-cutting for the environment are, among others, 74unfavourable growth conditions for species requiring shelter when they are young, the threat 75of wind and water erosion to the surface, especially in the mountain areas, the risk of

76secondary swamping in wetlands, drying of the topsoil layers and a strong expansion of

77herbaceous vegetation (Borelli et al., 2017; Ussiri and Johnson 2007). This type of practice

78induces wide biogeochemical changes from the change in soil moisture and temperature,

79which affects the microbiological activity through rapid accelerated organic matter

80decomposition, leading to high C losses and the reduction of the organic horizon thickness

81although the results from the literature are not consistent across forest sites (Prescott et al.,

822000; Smolander et al., 2019; Valenzuela and Cervantes, 2021). Efforts should be made to

83long-term protection of soil carbon from losses. The C stabilisation in soils requires not only

84practices that can lead to the diminishing of organic matter decomposition but equally

85importantly enhancing the transformation of organic matter into more stable humic

86substances, which can form complexes with a lower turnover rate (Barancikova et al., 2018;

87De Nobili et al., 2020; Jamroz 2012; Jandl et al., 2007; Ojeda et al., 2015; Weber et al., 2018).

88   Decomposition of litter is a process that is very important for determining the

89sustainability of managed forest ecosystems (Rocha et al., 2016). Forest litter consists mainly

90of a mixture of polysaccharides, lignin, aliphatic biopolymers and tannins (Kögel-Knabner,

912002). Humic fractions, as a significant part of soil organic matter, are universally recognised

92as the most reactive soil components maintaining the soil fertility and productivity status and

93are involved in most physical, chemical and biological processes within the soil environment

94(Jerzykiewicz et al., 2018; Senesi et al., 2003, Stevenson 1994, Weber et al., 2018).

95   Ussiri and Johnson (2007) found that accelerated organic matter decomposition after

96clear-cutting resulted firstly in the decomposition of O-alkyl C compounds and the decrease in

97the humic substances in the soil. In some experiments in mountain conditions, it has been

98found that complete removal of trees causes increased humic acid internal oxidation states,

99and the increase of aromatic structures and carboxylic compounds share in the molecules as

100well (Jamroz 2009). The processes of organic matter transformation in the area after clear-

101cutting also reduces the share of aliphatic compounds in humic acid structures.

102   Using advanced instrumental analysis, like EPR, ^{1}H NMR and FTIR spectroscopy, we

103can describe not only the detailed chemical properties of humic substances and follow the

104direction of humification processes but also predict a change in the impact of organic matter

105after serious disturbance in the plant cover on the soil environment and the entire ecosystem.

106Thus, knowing how to apply modifications to the species composition of new plantings on the

107post-harvested areas to improve the quality of the organic matter will be much easier.

108   This work aimed to describe the direction of humification processes and properties of

109humic substances in the forest soils after clear-cutting in the lowland and mountain regions in

110south-western Poland.

111   The hypotheses were that (1) the direction of soil organic matter transformation after

112clear-cutting does not differ in the lowland and mountain soils and (2) the organic matter from

113the soils after clear-cutting, both in the lowland and mountain regions, transform into humic

114substances of simple molecules with low-molecular-weight and more aliphatic structure.

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116**2. Materials and methods**

117*2.1. Site description*

118   The objects of the investigations were areas two years after clear-cutting (CC), both in

119the lowland and in the mountain region. The type of humus was mor.

120*2.1.1. Lowland study site*

121   The study area was located in the Oborniki Slaskie Forest District (51° 17.98' N, 19°

12249.059' E), south-western part of Poland. The main site parameters are presented in Table 1.

123Soils from the study area are Brunic Arenosols derived from sand (FAO, 2015). The forest

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124habitat before forest harvesting was a mature mixed coniferous forest with dominant mature

125trees of *Pinus silvestris, Larix decidua, Quercus petraea* and *Acer pseudoplatanus*. At the

126same time, investigations were carried out on the sites after clear-cutting (LF CC) and the

127sites where trees were not cut (LF F). Types of forest habitat soils were the same.

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129*2.1.2. Mountain study site*

130   The mountain study area (50° 14.669' N, 16° 50.071'E) was located at an altitude of

131970 m a.s.l. in the Zmijowiec Range, East Sudety Mountains, south-west Poland. The main

132characteristics are presented in Table 1. Soils were described as Dystric Cambisols (FAO,

1332015). The forest habitat was mountain mixed coniferous forest with dominant mature trees:

134*Picea abies*, *Abies alba*, *Acer pseudoplatanus* and *Fagus sylvatica*. The study area was

135located on the site after clear-cutting (MF CC) and at the same altitude under forest cover

136without any harvesting practice (MF F).

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138*2.2. Soil sampling*

139   Two replicated soil profiles were located on each site, both after clear-cutting and

140without any harvesting practice, on the same soil type in the lowland – Brunic Arenosols and

141in the mountain – Dystric Cambisols. Two samples from each profile and each horizon were

142prepared for chemical analysis. The texture was determined only in mineral horizons. Soil

143samples from Oa and AE soil horizons in the lowland and Oa and AB soil horizons in the

144mountain area were taken for detailed structural analysis of humic acids.

145   Mineral horizons of lowland soils had a sandy texture, with a very low content (0–4%)

146of particle sizes > 2 mm and a very low content of clay (3–4%). Soils from the mountain area

147had a sandy texture as well but with a much higher (15–23%) content of particles > 2 mm and

148a higher content of silt (27–34%) and a low content of clay (5–7%). Very low pH values 149throughout the whole profile were found for soils investigated in both regions (Table 1).

150   Humic substances were extracted from organic and mineral horizons and detailed 151analyses were performed on humic acids from Oa and AE (AB) horizons.

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153*2.3. Extraction and purification of humic and fulvic acids*

154   Humic fractions were extracted from the genetic horizons using the procedure 155described by Swift (1996), recommended by the International Humic Substances Society. 156Humic acids (HA) gel was purified with a 0.1 M HCl/0.3 M HF solution, left overnight and 157centrifuged; this procedure was repeated three times. The precipitate was then transferred to a 158Visking dislysis tube (Spectra/Por 7 MWCO 10,000, Spectrum Europe B.V., Breda, The 159Netherlands) and dialyzed against distilled water until a Cl$^-$ test was negative. Then, the HA 160were freeze-dried.

161   Fulvic acid (FA) extracts were passed through the XAD-8 column and H$^+$-saturated 162cation exchange resin. The eluate was freeze-dried.

163   Elemental analysis of HA and FA was performed with a Perkin-Elmer 2000 164instrument. The O was calculated from the mass balance.

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166*2.4. Spectroscopic analysis*

167*2.4.1 EPR*

168   Semiquinone radicals, which are stabilised in humic substances, are highly sensitive to 169various factors, both physical (temperature and humidity) and chemical (redox and/or acid-170base reactions, etc.) (Senesi and Schnitzer, 1977). The concentration of radicals and their g-171parameters (related to the magnetic moment of the unpaired electron of radicals) depends on

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172the type and origin of the substances in which these radicals are formed (Swift 1996; Senesi

173and Loffredo, 2001). Thus, the studies of the structure and concentration of these radicals

174could be indicative of the whole structure of the organic fraction (Jerzykiewicz et al., 2019).

175The electron paramagnetic resonance EPR method, as the best-known method for studies of

176paramagnetic substances, is a good choice for using for the investigation of structural changes

177occurring in radicals within soil organic matter. EPR analysis is known as an important

178method for determining humic substance characteristics of various origins (Senesi 1990;

179Jerzykiewicz et al., 1999).

180　　　All EPR spectra of humic acids were recorded at room temperature using a Bruker

181Elexsys E500 spectrometer equipped with an NMR Teslameter (ER 036TM) and standard

182Bruker frequency counter. X-band spectra were measured using a double rectangular cavity

183resonator ER 4105DR operating in the TD104 mode at a microwave power of 20 mW and

184modulation amplitude of 1G. For quantitative measurements, the radical species concentration

185standards were placed in the double resonator's first cavity, while the analysed sample was

186placed in its second cavity. After tuning, the spectra were recorded separately for each of the

187two cavities without changing any of the measurement parameters. As the references of

188radical concentration, the standards distributed by IHSS (peat and Leonardite HA; 2.0×10^{17}

189and 4.4×10^{17} spins per gram, respectively) and Bruker (alanine pill, 1.7×10^{17} spins per pill)

190were used. Double integrations were conducted using the *WinEPR 2.22 rev.12* programs

191(developed by Bruker).

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193*2.4.2 $^1H – NMR$*

194　　　^{13}C-NMR and/or ^1H-NMR spectroscopy is one of the most useful methods for the

195characterisation of soil organic matter (Schnitzer 1994; Kögel-Knabner 1997; Conte et al.,

1962004). This spectroscopy is commonly used in the study of humic substances to describe the

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197concentration of functional groups (Jamroz et al., 2014). ^{1}H-NMR measurements were 198performed on a Bruker Avance III at 500 MHz. Samples of humic acids were dissolved in a 199solution of NaOH in D_2O and transferred to standard glass NMR tubes.

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201*2.4.3 FT-IR*

202   Fourier transformed infrared (FTIR) spectroscopy provides information about nature 203and functional groups as well as structural information indicated the presence of both aliphatic 204and aromatic components. FTIR spectroscopy gives us also information about what 205degradation products are derived from, e.g. proteins and polysaccharides (Senesi et al., 1991; 206Traversa et al., 2008). Fourier transformed infrared spectra of humic and fulvic acids were 207recorded with a Bruker Vertex 70 FT-IR spectrometer on KBr pellets (an approximately 1 mg 208sample in 400 mg of KBr). The spectra were integrated into the oscillations rages of the 209characteristic group.

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211*2.5 ICP-OES*

212   Humic and fulvic acid digestion was carried out on the reactor's Ertec Magnum and 213using HNO_3. The thus prepared samples were examined for the study using an ARL Model 2143410 ICP (Fisons Instruments) spectrometer to determine the metal ion contents: Fe(III), 215Mn(II) and Cu(II).

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217*2.5. TOC and Nt analysis*

218   The content of total organic carbon was analysed using a CS-mat 5500 instrument 219(Strohlein GmbH & Co., Kaarst, Germany, currently Bruker AXS Inc., Madison, WI, USA). 220Total nitrogen was analysed by the Kjeldahl method using a Buchi Labortechnik GmbH N 221analysis.

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222 *2.6. Total acidity of humic acids*

223   The total acidity of humic acids was analysed with Schnitzer and Gupta's method as
224 described by Swift (1996).

225 *2.7. Statistical analysis*

226   Results were verified using Statistica for Windows 13. Means were compared by the
227 t-test, at a confidence level of $p < 0.05$

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229 **3. Results**

230 *3.1. TOC and humic fractions content*

231   In our studies content of TOC in the Oa horizon decreased by 26% in the lowland and
232 by 34% in the mountain soils, but the quantitative change was more evident in mineral
233 horizons (Table 1). In the A horizons, TOC decreased by 22% in the lowlands and by 45% in
234 the mountain areas. Surprisingly conversely in B horizons, soil organic carbon decreased by
235 67% in the lowland and only by 8% in the mountain soils. That is why in mountain soils, even
236 in natural sites and despite the soil formation process, there is a much higher content of TOC
237 in B horizons (48.50 g kg^{-1}) in comparison to the lowland ones (6.17 g kg^{-1}). Content of total
238 nitrogen was lower in the clear-cut sites nearly by 31% in organic horizons both in the
239 lowlands and in the mountain areas (Table 1). In mineral soil horizons, the content of N total
240 was adequate to the TOC concentration. The concentration of humic fraction decreased for
241 both humic and fulvic acids after clear-cutting in whole soil horizons (Table 1). Humic acids
242 in Oa horizons decreased after clear-cutting similarly in the lowland and the mountain soils,
243 by nearly 18% in both regions. In the A horizons, this change was more evident in the lowland
244 (by 32%) in comparison to the mountain soils (change by 8%). In the case of fulvic acids,
245 their content significantly decreased more evidently in the mountain soils, by 30% in Oa and

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246by 54% in A horizon, in comparison to the lowland, where they decreased by 8.5% and 24%

247respectively. In the mineral horizons of the soil on the clear-cut site, however, there was found

248a lower content of soil organic carbon in comparison to the undisturbed site. In our study,

249clear-cutting had an evident consequence in altering the quantity of soil organic matter but

250differed between the lowlands and mountain areas. Meanwhile, in the mountain soils, there

251was a more clear decrease of the fulvic fraction in the whole soil profile. In the lowlands in

252the upper soil horizons (Oa, A), it was found that the content of humic fraction content

253decreased more in the clear-cut site in comparison to the undisturbed areas (Table 1).

254*3.2. Elemental composition and UV-VIS results of humic and fulvic acids*

255	Elemental composition is one of the most important humic fraction properties. In the

256investigated soils, FA molecules contained more oxygen than HA molecules, regardless of

257management practice or altitude, but the content of carbon was varied (Table 2). Molecules of

258humic acids from the Oa horizons of the clear-cut sites, in the lowlands, were characterised by

259a higher content (although not statistically confirmed) of carbon and oxygen and lower

260content of hydrogen in comparison to the undisturbed sites. In the first mineral horizons, a

261significant decrease in the carbon content of the humic acids molecules was found in the

262lowland (from 35.39% to 35.01%) and there was a significant increase in the mountain sites

263under the influence of clear-cutting practice (from 30.54% to 32.22%). The content of

264nitrogen increased in the molecules of HA in the lowland after CC, but the change was not

265significantly confirmed in the first mineral soil horizon (from 1.94% to 2.04%). There were

266no significant differences in N content between clear cut and undisturbed soils in the

267mountain areas (Table 2). The FA from the Oa horizons, both in the lowlands and mountain

268region, did not significantly differ between clear-cut sites and those without any harvesting

269practice. On the contrary, in the A horizons of the lowland's soils, the FA molecules contained

270more carbon and significantly less oxygen than those from the undisturbed forest. In the

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271investigated mountain soils from the clear-cut sites, fulvic acids contained more carbon

272(33.91%) as well and significantly less hydrogen (35.17%) compared to those from the

273undisturbed forest sites (32.95% and 36.04%, respectively). In the studied areas, the H/C ratio

274of the HA was rather high and ranged from 1.46 to 1.61 in the Oa horizons and from 1.18 to

2751.45 in the first mineral horizons (Table 2). The E4/E6 ratio of humic acids from Oa and

276analysed mineral horizons of the soils on the clear cut sites, both in the lowlands and in the

277mountain investigated region (Table 2), showed lower values (4.18 and 3.80 in the lowlands

278and 4.82 and 4.31 in the mountain) in comparison to the undisturbed forest sites (6.16 and

2794.33 in the lowlands and 6.32 and 4.89 in the mountain regions, respectively).

280*3.3. EPR results*

281   The studies of radical structure and concentration followed the analysis of the

282manganese, iron and copper contents in humic acids because these metal ions are known from

283their antiferromagnetic interaction with semiquinone radicals in humic acid (Jerzykiewicz et

284al., 2002). As a result, a decrease in radical concentration is observed. For the investigated

285samples, the content of manganese ions was very small (0–0.023 mg g^{-1}), and copper ions

286were not detected. Thus, these metal ions did not affect semiquinones.

287   Differently, iron ion contents were much higher and varied between samples. In humic

288acids extracted from mineral horizons, the iron concentration was higher than that of those

289that were extracted from organic ones (Table 3). It is worth mentioning that samples from the

290lowland forest areas did not exhibit the influence of clear-cutting on the metal ion

291concentrations. The increase of iron ion concentrations in HA was observed after clear-cutting

292only for samples from MF areas. Similar phenomena as described previously of radical

293quenching by metal ions were found only for the mountain soils, as presented in Table 3.

294   In the A horizons of the lowland soils, iron(III) ions did not affect radical

295concentration because metal ions are incorporated there by different functional groups unlike

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296in Oa horizons. That is well presented on the EPR spectra (Fig. 1), where different lines

297attributed to different Fe(II) and Fe(III) were observed at values of g equal to 2.0, 4.25 and 6.

298This third peak (Fig. 1, MF-F AB) is especially interesting because iron is built-in here via

299nitrogen atoms, not oxygen as it is for other iron(III)-humic acid bonds. It is worth

300mentioning that elemental analysis confirms that HA characterised by a g = 6 peak contained

301also more nitrogen. The signal at g = 6.0 is commonly admitted to pertain to the trivalent

302heme-iron-like complex in which iron is at the third degree of oxidation instead of the second

303one (Krzyminiewski et al., 2011).

304   As it is presented in Table 3, g – the parameter calculated from EPR spectra for

305semiquinone radicals did not change much. Better information could be obtained from studies

306of radical concentration. The high radical concentration is commonly known as an indicator

307of high humification degree (Jerzykiewicz et al., 1999). In the presented case, a decrease in

308radical concentration is observed for HA from mountain clear-cut areas (Table 3). The

309decrease in radical concentration could be the effect of higher Fe(III) content and the

310antiferromagnetic interaction (Jezierski et al., 2002). For samples with higher Fe(III) content

311(4.796 in Oa and 5.356 mg g^{-1} in the A horizons after clear-cutting compare to 1.562 and

3123.027 from the undisturbed forest respectively), radical content is much lower for HA from

313the clear-cutting area than from undisturbed forest.

314*3.4. FTIR spectra of humic acids*

315   The FT-IR spectra were typical for humic acid spectra (Figure 2). The differences,

316although small, were observed only in the signal intensities. First, lines of COOH vibrations

317at 1722 cm^{-1} were less intense for HA samples from mineral horizons. The difference is even

318more distinguished after clear-cutting. Peaks ascribed as aliphatic C–H stretching vibrations

319(3000–2820 cm^{-1}) and anti-symmetric stretching modes of COO^{-} and/or aromatic C=C (1650

320cm^{-1}) for humic acids extracted from clear-cutting areas were characterised by higher

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321intensities and were more clearly marked for HA from the soil horizons in the lowlands than

322for HA from undisturbed forest soils. The absorption band may be associated with aromatic

323C=C, C=O groups of quinones and ketones (Traversa et al., 2008).

324*3.5 ^{1}H NMR analysis of humic acids*

325   The NMR spectra presented typical NOM spectra (Figs. 3 and 4). This information is

326valuable because of the quantification reliability (Grinhut et al., 2011; Hertkorn et al., 2007).

327   The spectra were divided into three regions. The first was with the chemical shift

328region of 0–2.33 ppm was attributed to CH_2 groups. An increase in HA from both horizons

329has been observed after clear-cutting in the lowlands and mountain regions as well (Figs. 3

330and 4). This may be linked to HA degradation after environmental disturbances such as clear-

331cutting. Demethylation during this process causes the removal of CH_2 groups, and thus the

332detection of more molecules that belonged to the consecutive CH_2 series (Grinhut et al.,

3332011). Within the chemical shift region of 2.93–4.26 (connecting with metoxyl groups), there

334has been observed a decrease after clear-cutting, indicating a decrease of oxygen-containing

335functional groups (Grinhut et al., 2011). These findings are in good agreement with a decrease

336of the O/C ratio in the mineral mountain soil horizons after clear-cutting. The third region of

3375.8–8.2 ppm was assigned to aromatic protons and was able to be analysed after integral

338calculation.

339   The integration of the lines attributed to the previously mentioned three areas

340exhibited more detailed differences between samples. About 8.58% of the non-exchangeable

341protons of the HA from the undisturbed lowland forest soil (Oa horizon) were assigned to

342aromatic groups (Table 4), whereas after clear-cutting, this amount increased over three times

343(26.97%). A slighter increase in aromaticity was also detected after clear-cutting in HA

344molecules from mountain regions compared to the lowlands, but only in Oa horizons.

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345   The ^{1}H NMR spectra exhibit not only a slight increase of aliphatic proton shifts (0–1

346ppm) but also a slight increase in aromatic proton shifts (5.8–8.2 ppm). Increase these two

347bands could be an explanation why the summary aromatic-aliphatic parameter from elemental

348analysis remains unchanged or only slightly decreases. The observed increase in the intensity

349of aliphatic and aromatic shifts is accompanied by the decrease of intensity of methoxyl shifts.

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351 4. Discussion

352   In our studies, clear-cutting enhanced transformation of soil organic matter, through an

353increase of organic matter decomposition and humification process as well. In mountain soils,

354even in natural sites and despite the soil formation process, there is a much higher content of

355TOC in mineral horizons in comparison to the lowland ones. This may because, in the

356mountain regions, there was much higher precipitation that than in the lowland, causing

357naturally higher leaching of elements, especially low molecular fractions of carbon, to the

358deeper soil horizons. According to many authors (Achat et al., 2015; Jamroz, 2009; Mayer et.

359al., 2020; Ussiri and Johnson, 2007), clear-cutting in first years enhances the decomposition

360rate of organic matter and consequently reduces the amount of soil organic matter in upper

361soil horizons. On the other hand, Falsone et al. (2012) observed that 5 years after clear-

362cutting, the content of soil organic carbon in the Oa horizon was a bit higher (186 g kg^{-1}) in

363comparison to the soil from the undisturbed area (173.7 g kg^{-1}). Thus, we can expect that

364content of SOM will recover over time but the rate will depend on type of afforestation. The

365origin of humus fractions in oligotrophic forest ecosystems is mainly plant residues rich in

366polysaccharides, lignin, aliphatic biopolymers as well as tannins (Kogel-Knabner 2002,

367Ziolkowska et al., 2020), which more often transform in such conditions into low-molecular

368humic substances, like fulvic acids, than into compounds of higher polymerisation, like humic

369acids. Coniferous and mixed coniferous forest ecosystems are characterised by the type of soil

370organic matter with the prevalence of the fulvic fraction over the humic one and in most cases

371with the mor type of humus. Fulvic fractions, apart from their low molecular weight, are

372characterised by a more aliphatic structure that favours leaching through the soil profile

373(Buurman and Jongmans, 2005; Falsone et al., 2012; Jamroz et al., 2014). Low pH and

374changes in microclimate conditions, particularly in the mountain areas with much higher

375precipitation than in the lowlands, means that removing trees alters microbial activity, and the

376weakly decomposed low-molecular fraction can be easily transported to the lower part of the

377soil profile or even leached to the groundwater. The H/C ratio of HA molecules is often used

378as an indicator of aromaticity/aliphaticity and is useful for monitoring structural changes

379(Senesi and Loffredo, 1999; Rice and MacCarthy, 1991). Results of our studies point to the

380overall more aliphatic than the aromatic character of the molecules' structures. Falsone et al.,

381(2012) found that a larger proportion of the aliphatic component could be related to a greater

382contribution of saccharide residues. Humic acids from the A horizons (in the mountain soils)

383and the Oa horizons (in the lowlands and mountain areas as well) after clear-cutting were less

384aliphatic (lower H/C ratio) than those from undisturbed forest sites. The lower H/C ratio,

385lower total acidity, higher content of carbon and oxygen in HA molecules from Oa horizons

386after clear-cutting shows that the harvesting practice altered not only the decomposition but

387also the humification process. The transformation of organic matter in the investigated sites

388after clear-cutting, particularly in Oa horizons both in the lowlands and mountain areas,

389resulted in more humified products with less aliphatic structures. The higher O/C ratio in

390fulvic acids than in humic acids in all investigated forest sites confirms the statement that FA

391are polysaccharidic in nature (Tan, 2014). This parameter significantly decreased only in the

392lowland in the first mineral soil horizon after clear-cutting. During the transformation of

393organic matter after clear-cutting, more of the aromatic substances are broken down and

394released in comparison with the polysaccharide constituents. Consequently, the humic

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395substances formed became increasingly more polysaccharidic in nature. The molecular weight

396of humic substances is related to the E4/E6 ratio, and this ratio is considered by many authors

397as a measure of the overall humification degree (Barancikova et al., 2018; Chen et al., 1977;

398Zaccone et al., 2013). Results received indicate the formation of similar size molecular

399weights of humic substances before and after clear-cutting in the coniferous mixed forest in

400the lowlands and mountain regions as well. Barancikova et al. (2018) point out that humic

401substances with higher molecular weights are more stable in the environment and more

402resistant to unfavourable external factors. Analysis of the semiquinone radical structures and

403concentrations can be useful in studies of the humification process (Klavins and Purmalis

4042014). This type of radicals can be built into soil organic matter structures (Senesi 1990).

405Humic substance molecules are rich in functional groups and can form, among others, metal

406chelate complexes (Barancikova et al., 2018; Jerzykiewicz et al., 2019). EPR results indicate

407the direct influence of the harvesting procedure on radical concentration. However, it is

408difficult to state unambiguously what is the mechanism of the clear-cutting procedure that

409influences the enhancement of the interaction of metal ions with unpaired electrons of HA

410radicals. Similar results were described by Barancikova et al. (2018), who found a decrease in

411radical content in humic acids from the forest soils after windstorms and wildfires. The free

412radical concentration is overall in line with aromaticity, humification degree and other

413chemical and structural properties of humic acids (Senesi et al., 2003). Other results presented

414in this paper (H/C; O/C, E4/E6) proved the less aliphatic character of humic acid molecules

415from the lowlands, compared to the mountain forest, as the effect of clear-cutting. Results of

416the FTIR analysis, especially in the lowlands after clear-cutting, may reflect a higher degree

417of organic matter decomposition (Haberhauer et al., 1998) and point to different from the

418spruce-mountains type of litter. Spruce needles and bilberry leaves, which are often part of the

419litter composition from mountain forest soils, contain a high amount of polyphenols, which

420slow down the decomposition processes (Albers et al., 2004, Jamroz et al., 2014) in

421comparison to pine litter in the lowlands. These results are in good agreement with those from

422elemental analysis, confirming the less aliphatic nature of humic acids from the investigated

423forest soils in the lowlands. All ^{1}H NMR spectra examined indicate that aliphatic structures

424are dominant components of humic acids especially in mineral soil horizons, whose contents

425even increased after clear-cutting particularly in the lowland. These results are in line with

426other authors (Bonifacio et al., 2006; Jamroz et al., 2014), who reported a higher degree of

427aliphacity of SOM in mineral forest soil horizons.

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429**5. Concluding remarks**

430   Soil organic matter in the coniferous forest ecosystems is characterised mostly by

431more aliphatic character, both in the lowlands and mountain conditions, and lower

432humification degree in comparison to other ecosystems (cropland or grassland). Forest

433management, especially clear-cutting, alters C cycling and accelerates decomposition in the

434forest floor leading to loss of humic substances with the highest C losses in the forest floor

435and the upper mineral soil. In our studies, clear-cutting enhanced the transformation of soil

436organic matter, through the increase of organic matter decomposition. Humic fraction

437decreased under the influence of clear-cutting more in the lowland areas covered by mainly

438pine forests. In the mountain forests, with Norway spruce as the main species, the more labile,

439low-molecular fulvic fraction decreased more noticeably as the effect of harvesting practice.

440Presumably, the main cause of these changes was the activation of easily soluble fractions and

441their leaching from the soil profile. Lower H/C ratio, higher content of carbon and oxygen in

442HA molecules from Oa horizons after clear-cutting shows that harvesting practice altered not

443only the decomposition but also the humification process as well. The transformation of

444organic matter in the investigated sites after clear-cutting, particularly in Oa horizons both in

445the lowlands and mountain areas, resulted in the loss of less humified organic matter

446containing humic substances of less polymerised molecules. Results presented in this paper

447have proved less aliphatic character of humic acid molecules from the lowlands, compared to

448the mountain forest as the effect of clear-cutting. Harvesting practices in mountain regions

449should be approached with particular care due to the risk of erosion of exposed surfaces and

450soils containing less humified and less stable organic matter than in the lowlands. Humic

451fractions of higher solubility, less stability and tendency to migrate through the soil profile

452may favour the leaching of nutrients and consequently cause the eutrophication of waters.

453These phenomena due to climate change are predicted to even increase, leading to the

454"brownification" of surface waters (Lurling et al., 2014).

455   Based on the literature and presented results, it could be stated that the study of humic

456fractions can help resolve scientific and practical issues in ecosystems. Practices through

457improved growth of tree seedlings, applying organic materials into deeper soil horizons and

458selection of species with nitrogen-fixing associates will prevent the reduction of C stocks, soil

459surface in the mountain regions against erosion processes as well as ensure the stability of the

460whole ecosystem.

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467**Conflict of Interest Statement**

468The author declares that there is no conflict of interest that could be perceived as prejudicing

469the impartiality of the research reported.

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