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Composition and mean residence time of molecular weight fractions of organic matter extracted from two soils under different forest species

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Abstract. The organic matter extracted from various mineral horizons of two forest soils, one under silver fir (*Abies alba* Mill.), the other under European beech (*Fagus sylvatica* L.), was fractionated by dialysis into three fractions, 100–1000, 1000–8000, and >8000 Da. On a C basis, in all horizons the recovered organic matter amounted to less than a half of the total and was mainly composed of molecules >8000 Da. The 100–1000 Da fraction had a principal elemental composition profoundly different from the other two fractions, which, instead differed from each other significantly only for the S content and the molar ratio of C with N. No significant difference in this regard was found between soils. The richness in O and some typical absorption bands in the FT-IR spectra indicated that the 100–1000 Da fraction had a lot of carboxyl groups. The spectroscopic (^{13}C NMR) investigation showed that the 1000–8000 and >8000 Da fractions had a prevalently aliphatic nature and signals attributable to polysaccharides (O-alkyl C) revealed overall a high presence of non-humic biopolymers. These latter were significantly more abundant, suggesting a lower degree of humification, in the >8000 Da fraction than in the 1000–8000 Da fraction. Comparing soils, that under beech appeared significantly richer in O-alkyl C than that under fir. The organics extracted from the A horizon of both soils had positive $\Delta^{14}C$ values, indicating recent synthesis mainly due to the present forest cover. The mean residence time (MRT) of the combined 100–1000 Da and 1000–8000 Da fractions and the >8000 Da fraction increased with depth, even to about 5000 years in the more than 1-m deep BC horizons under beech. In some cases, and especially in the soil under fir, despite higher values of $\delta^{13}C$ denoting stronger microbial decomposition, the 100–8000 Da fraction showed a higher MRT than that of the >8000 Da fraction, perhaps due to its ascertained lower content of non-humic biopolymers.

Introduction

Soil organic matter (SOM) includes plant residues at varying stages of decomposition, microbial biomass, water-soluble organics, and stabilised molecules (humic substances). SOM plays important roles in driving soil genesis (Ugolini and Spaltenstein 1992), supplying nutrients to plants (Chen et al. 2000), and storing CO_2 in soil (Sanchez and Eaton 2001). In temperate forest soils, the attention of most authors has mainly focused on the study of the characteristics and pattern of alteration of the SOM from the freshly fallen litter and the underlying humus layers

(Kögel-Knabner et al. 1988; Preston et al. 1994; Wachendorf 1998). Consequently, Kögel-Knabner (2000) in a comprehensive review stated that little is actually known about the SOM present in the mineral horizons, especially in the deep B or BC. Zech and Guggenberger (1996) summarised current knowledge of this aspect as a general (with some exceptions under specific site conditions) transformation of SOM with depth, consisting of a progressive loss of carbohydrates and, to a lesser extent, lignin, with a coupled increase of carboxyl C. However, further information is needed on the composition of this organic pool, which represents a significant part of the global budget of C (Batjes 1996). Even less is known about its residence time, although some works have reported long periods (Scharpenseel 1993; Tate et al. 1993; Certini et al. 2003).

Despite the modern tendency to probe the chemical structure of SOM *in situ* by solid-state ^{13}C Nuclear Magnetic Resonance (Baldock and Preston 1995), extraction of SOM from the soil matrix is still widely practised and is certainly necessary when separation of different organic fractions is required. Fractionation, in fact, aids investigation of the recovered material by reducing its heterogeneity. Fractionation may be made on the basis of various characteristics of SOM, such as solubility (Hayes and Swift 1978), electric charge (De Nobili et al. 1990), adsorption susceptibility (Mac Carthy et al. 1979), density (Spycher et al. 1983), and molecular weight (Homann and Grigal 1992). Historically, the most widely used approach has been to separate the extracted SOM into fulvic acids, soluble in both alkali and acid, and humic acids, soluble in alkali but not in acid. However, from an ecological point of view, molecular weight appears to be a better discriminant than solubility because it is more strictly related to the permanence of organic compounds in soil (Meyer et al. 1987). The aim of this work was to obtain information on the concentration, composition, and residence time of different molecular weight fractions of SOM from the main mineral horizons of two undisturbed forest soils. For this purpose, the organic matter was extracted from soil and separated into three molecular weight fractions, which were characterised by elemental and spectroscopic analyses and radiocarbon dating.

Materials and methods

The study area is in the Nature Reserve of Vallombrosa (50 km east of Florence, Italy), a forest that extends on the Apennines Mountains, from 530 to 1350 m a.s.l. Two sites were examined: a pure stand of silver fir (*Abies alba* Mill.) at Mt Porcellaia and a pure stand of European beech (*Fagus sylvatica* L.) at Termine. At Mt Porcellaia, fir was planted 75 years ago in place of beech that here, like at Termine, was naturally present. The soils at the two sites are taxonomically similar, both being Haplic Umbrisols according to ISSS-ISRIC-FAO (1998) or Humic Dystrudepts according to Soil Survey Staff (1999). More detailed data on the sites and a morphological description of two of the soil profiles sampled are given in the Appendix 1.

Soil samples were collected by horizon, from three profiles opened randomly in either stand. Three mineral horizons were sampled in the profiles under fir (A, Bw,

and BC), and four in the profiles under beech (A, Bw, BC1, and BC2). Two-hundred gram of air-dried and sieved 2 mm soil was placed in a polyethylene tube, added with 200 mL of 0.1 M NaOH, bubbled for 15 min with N_2, and shaken for 1 h. Then, the mixture was centrifuged at 30,000 g for 10 min, and the supernatant recovered. After repeating this procedure several times until an uncoloured extract was obtained, the soil residue was washed twice with deionised water and the supernatants added to the previous extract. The whole solution was passed through a 0.45 µm filter under a slight N_2 pressure and, once reduced to about 150 mL by freeze-drying, put into a dialysis membrane with nominal molecular weight cut off (MWCO) of 8000 Da. This membrane was enclosed in a larger and longer water-filled 1000 Da MWCO membrane that was kept immersed in about 3 L of deionised water. The bath solution, continuously adjusted to pH 3.5 with HCl to H-saturate the organic compounds and avoid precipitation of carbonates, was retrieved after 24 h and renewed with deionised water continuously adjusted at pH 3.5. On the following day, and only when the pH was stable, the liqueurs in the dialysis membranes (1000–8000 and >8000 Da fractions) were recovered and totally freeze-dried. The combined two aliquots of bath solution were reduced by freeze-drying to about 150 mL and put into a membrane with MWCO of 100 Da to be dialysed against 10 L of deionised water to eliminate the NaCl formed during the procedure of extraction and fractionation of the organic matter. Continuous adjustment to pH 2.5 with HCl was implemented to H-saturate also those low-molecular-weight organic acids with very low pK_a often found in forest soils, although in small quantities (Fox 1995). After a day and only when the pH of the bath remained stable at pH 2.5, the liqueur in the membrane was recovered and freeze-dried. The entire procedure of extraction and separation of SOM is summarised in Figure 1. The obtained organic fractions were not submitted to any kind of purification from the associated mineral phase to avoid creation of artefacts (Piccolo 1988). Measurement of N, C, H, and S was performed through combustion by a Carlo Erba NA 1500 Analyser. The moisture and the ash content of the samples were determined after heating at 110 and 550 °C, respectively, and the principal composition of the organic phase (including O, which was obtained by subtraction) recalculated on a moisture- and ash-free basis. The molar ratios of C with N, H, and O were determined by the following formula:

$$\frac{\text{Percent of C by mass}/12}{\text{Percent of X by mass}/\text{Atomic weight of X}} \quad (1)$$

Means of single elements and molar ratios from all replicates were compared, using the Student's *t*-test between soils and the one-way ANOVA plus the Scheffé test among fractions. In both cases, a significance level of 0.01 was considered.

Fourier-transform infrared (FT-IR) spectra were recorded on a Perkin Elmer 1700 FT-IR spectrometer. The pellets were prepared by pressing under vacuum a mixture of 1 mg of sample with 400 mg of KBr (spectrometry grade). Spectra were acquired at 4 cm^{-1} resolution and 64 scans were averaged. The absorption bands were assigned according to Stevenson (1994).

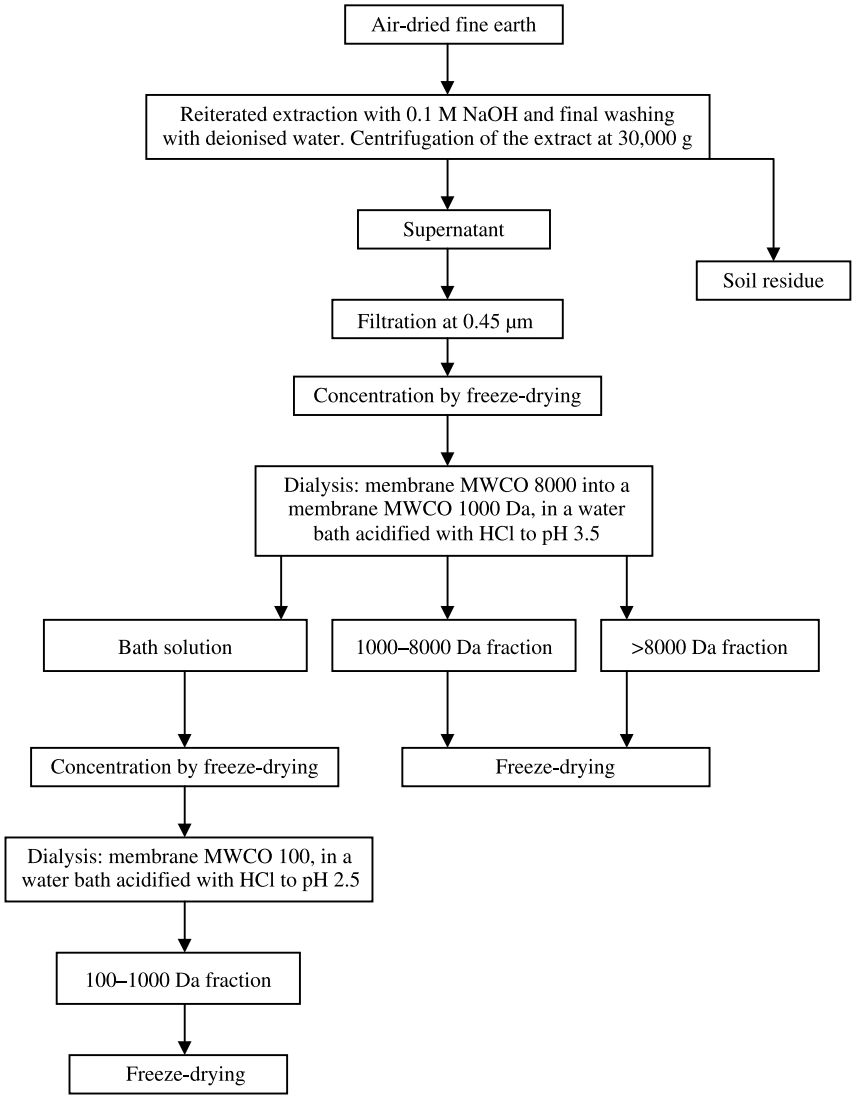

Figure 1. Procedure followed to extract and fractionate soil organic matter on a molecular weight basis.

Solution-state ^{13}C NMR analysis was made on about 100 mg of sample dissolved in a solution prepared combining 200 mg of NaOH with 10 mL of D_2O. The spectra were recorded at 50.3 MHz on a Varian Gemini 200 spectrometer using inverse-gated decoupling, an acquisition time of 0.2 s, a pulse of 45 °, and a relaxation delay of 2 s. Total acquisition time was 24–72 h. The free induction decays were processed by applying 50-Hz line broadening and baseline correction. For analysis of relative intensities, the spectra were divided into the following chemical-shift

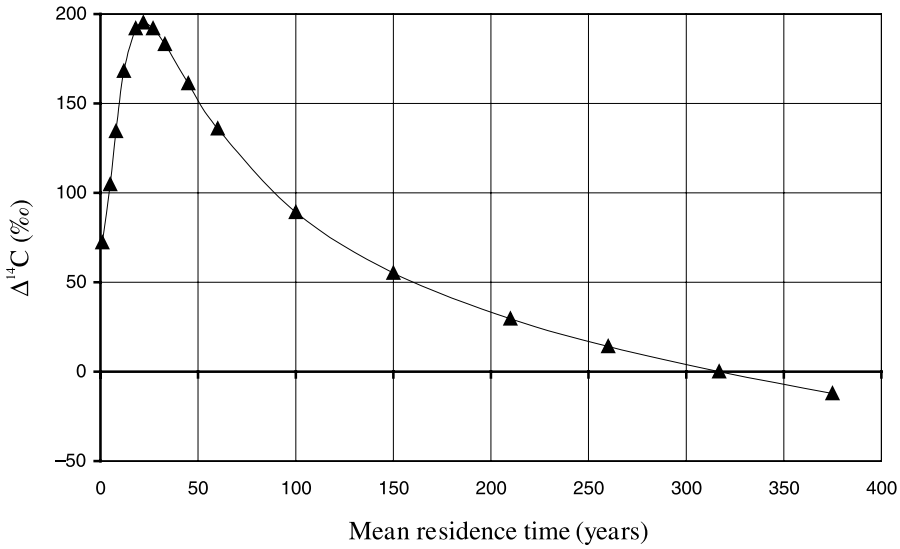

Figure 2. Relationship between positive values of $\Delta^{14}C$ and estimated MRT of samples analysed in 2000.

regions: 0–60 ppm (alkyl C), 60–105 ppm (O-alkyl C), 105–165 ppm (aromatic C), and 165–200 ppm (carbonyl C, including carboxyl C). Areas of the regions were determined by integration using the Bruker software for solution spectra, and expressed as per cent of the total area between 0 and 200 ppm. Differences between the two successfully analysed fractions (1000–8000 and >8000 Da) with regard to the area of each region were checked using the Student's *t*-test at a significance level of 0.01.

Radiocarbon measurements were made by Accelerator Mass Spectrometry (Rafter Radiocarbon Laboratory, Lower Hutt, New Zealand) on graphite targets obtained from specimens of a single profile per site. The targets were prepared from the 100–8000 and >8000 Da fractions (the first one obtained by combining the 100–1000 and 1000–8000 Da fractions, proportionally to their contribution to total extracted C) by combustion at 900 °C in evacuated, sealed quartz tubes in the presence of CuO wire (Buchanan and Corcoran 1959). The CO_2 so produced was cryogenically purified and a part was converted to graphite using the zinc reduction method (Vogel 1992), while the other part was used to determine the $\delta^{13}C$, which is the per mil deviation of the $^{13}C/^{12}C$ ratio of the sample (R_{SAMPLE}) from that of the standard Pee Dee Belemnite (R_{PDB}):

$$\delta^{13}C = \frac{R_{SAMPLE} - R_{PDB}}{R_{PDB}} 1000 \qquad (2)$$

Radiocarbon data were expressed as $\Delta^{14}C$, that is the per mil deviation of the $^{14}C/^{12}C$ ratio from that of an oxalic acid standard prepared in 1950 (OX1), corrected with respect to $\delta^{13}C$ to account for isotopic fractionation effects. The

equation used to calculate $\Delta^{14}C$ is the following:

$$\Delta^{14}C = \delta^{14}C - 2\left(\delta^{13}C + 25\right)\left(1 + \frac{\delta^{14}C}{1000}\right) \quad (3)$$

where $\delta^{14}C$ is:

$$\delta^{14}C = [(A_s/A_{abs}) - 1]1000 \quad (4)$$

where A_s is the net ^{14}C activity (normalised for counting volume, mass change, dilution, impurities etc.) of the sample and A_{abs} the ^{14}C activity of the oxalic acid corrected for decay since 1950 (Stuiver and Polach 1977). The mean residence time (MRT) was calculated by the $\Delta^{14}C$, using the model described in detail by Gaudinski et al. (2000) and Agnelli et al. (2002). In accordance with this model, samples analysed in 2000 showed two possible values of MRT when $\Delta^{14}C$ was >72‰ (Figure 2).

Results and discussion

In both soils, the content of organic matter decreased regularly with depth (Table 1), as usual for undisturbed non-podzolised forest soils (Van Cleve and Powers 1995). Despite dilute NaOH being widely recognised as the most efficient extractant (Yuan 1964; Hayes et al. 1975; Certini et al. 2002), for all samples the recovered C amounted to less than 50% of the total organic C, and even less than 40% for some horizons (Table 1). The per cent distribution of the three molecular weight fractions of the extracted organic matter did not differ noticeably between the two soils, with the >8000 Da fraction being much more abundant than the other two (Table 1). With regard to depth, in both soils the 100–1000 Da fraction increased drastically from the A horizon to the underlying ones, where it exceeded the 1000–8000 Da fraction. This could be indicative of a translocation downward of the smallest fraction, whose size reflects that of the molecules belonging to dissolved organic matter (Herbert and Bertsch 1995). In the 100–1000 Da fraction the prevailing element was O (amounting to 50–70%), while in those 1000–8000 and >8000 Da C exceeded O (Table 2). On the whole, however, the relative content of C decreased with depth. Nitrogen was about twice as abundant in the 1000–8000 and >8000 Da fractions than in that 100–1000 Da and, like C, its amount in the composition of each fraction tended to decrease with depth (Table 2). The H content clustered around 5%; higher values, exceeding 10%, were found only in the 1000–8000 Da fraction from the BC horizons under beech (Table 2). Sulphur was present only in the 1000–8000 and >8000 Da fractions, although in minor amounts (Table 2). The O content, being obtained by difference, could be overestimated, due to the fact that P and trace elements were not considered. However, we assume that any error, if present, was slight. The 100–1000 Da fraction showed a principal elemental composition profoundly different from those of the other two fractions. In fact, it differed significantly ($P = 0.01$) from the >8000 Da fraction for all elements and molar ratios, and equalled the 1000–8000 Da fraction only for S content

Table 1. Total organic C and per cent distribution of the molecular weight organic fractions extracted from the soils at Mt Porcellaia (fir) and Termine (beech). Numbers between parentheses are the standard deviations of three independent replicates.

	Total organic C ($g\,kg^{-1}$)	% total organic C			
		Extracted C			Unextracted C
		100–1000 Da	1000–8000 Da	>8000 Da	
fir					
A	49.1 (4.3)	3.3 (0.9)	6.3 (0.7)	31.6 (3.0)	58.8 (3.2)
Bw	15.4 (2.7)	7.3 (0.5)	5.5 (1.3)	26.4 (1.8)	60.8 (3.5)
BC	7.0 (1.5)	7.9 (1.0)	6.7 (0.5)	24.2 (4.0)	61.2 (2.8)
beech					
A	43.9 (3.0)	3.1 (0.6)	5.9 (1.3)	29.6 (2.8)	61.5 (2.3)
Bw	20.9 (4.4)	8.4 (2.0)	8.6 (1.1)	28.3 (1.3)	54.7 (1.3)
BC1	10.2 (2.5)	9.6 (0.5)	6.4 (0.8)	30.2 (2.3)	53.8 (1.2)
BC2	4.4 (1.8)	12.3 (1.7)	4.3 (1.1)	31.3 (2.7)	52.0 (2.0)

and molar ratios of C with N and H. The 1000–8000 Da fraction was significantly different from the >8000 Da fraction for S content and molar ratio of C with N. No statistically significant difference in this regard was found between soils.

The ^{13}C NMR spectroscopic analysis gave legible patterns for all samples of the 1000–8000 and >8000 Da fractions and only for the A horizons of the 100–1000 Da fraction. The very noisy signals of the patterns of the 100–1000 Da fraction from the other horizons were probably due to the abundance of inorganic impurities (~90% by weight). Therefore, to characterise this fraction we resorted to FT-IR spectroscopy, which, however, provided incomplete information. The IR spectra (Figure 3) showed bands at 1000–1100 cm^{-1} (silica, which probably represent most of the inorganic impurities), at 1400 and 1550 cm^{-1} (COO^- asymmetric and symmetric stretching, respectively, both often more evident than that at 1620 cm^{-1}, attributable to C=C of aromatic rings), and 2920 (aliphatic C–H stretching). A prevailing carboxylic nature of this fraction can be thus inferred. This is consistent with the abundance of O as assessed by elemental analysis (Table 2) and, at least for the samples from the A horizons, with the interpretation of the ^{13}C NMR spectra (not reported). In fact, these latter showed signals attributable to carboxyl groups (165–185 ppm) more than double the intensity (as per cent of the total area) with respect to the spectra of the other fractions. For the 1000–8000 and >8000 Da fractions, the spectroscopic analysis revealed that the aliphatic C (alkyl C + O-alkyl C) prevailed by far over the aromatic C (Figures 4 and 5, Table 3). Such a prevalence was found also by Zech et al. (1994) in bulk SOM from various depths of mineral soils of different origin. In particular, for a Humic Cambisol under a temperate forest of Norway spruce (*Picea Abies* L.) these authors reported per cent distributions of the main types of C moieties in the bulk soil comparable with those we determined in the extractable SOM >1000 Da (Table 3): 50–70% aliphatic C and 12–24% aromatic C. It suggests that the composition of the extractable SOM reflects that of the bulk SOM. The distribution of C between the

Table 2. Principal elemental composition and molar ratios of the molecular weight organic fractions extracted from the soils at Mt Porcellaia (fir) and Termine (beech). Numbers between parentheses are the standard deviations of three independent replicates.

	% by weight					Molar ratios		
	C	N	H	O	S	C/N	C/H	C/O
100–1000 Da fraction								
fir								
A	35.8 (0.8)	1.6 (0.2)	4.6 (0.2)	58.0 (0.8)	0.0 (–)	26.4 (2.7)	0.7 (–)	0.8 (–)
Bw	34.3 (1.9)	1.5 (0.2)	4.7 (1.0)	59.4 (1.8)	0.0 (–)	26.9 (1.6)	0.8 (–)	0.8 (0.1)
BC	26.2 (0.9)	1.3 (0.1)	5.5 (0.1)	67.0 (0.9)	0.0 (–)	26.3 (1.2)	0.6 (0.2)	0.8 (0.1)
beech								
A	45.9 (1.6)	3.2 (0.1)	3.7 (0.2)	46.9 (1.5)	0.2 (–)	16.7 (0.6)	1.0 (0.1)	1.3 (0.1)
Bw	31.9 (1.8)	1.3 (0.1)	5.2 (0.3)	61.6 (1.6)	0.0 (–)	27.7 (2.0)	0.5 (–)	0.7 (0.1)
BC1	22.4 (0.9)	1.1 (0.1)	6.8 (0.3)	69.7 (0.8)	0.0 (–)	24.7 (1.8)	0.3 (–)	0.4 (–)
BC2	22.1 (0.9)	1.1 (0.1)	6.3 (0.4)	70.5 (0.6)	0.0 (–)	23.8 (2.2)	0.3 (–)	0.4 (–)
1000–8000 Da fraction								
fir								
A	64.3 (2.2)	4.4 (0.8)	7.0 (0.1)	24.1 (1.5)	0.2 (0.1)	17.3 (3.4)	0.8 (0.1)	3.6 (0.3)
Bw	55.3 (0.5)	2.9 (0.2)	4.5 (0.3)	37.0 (0.3)	0.3 (0.1)	22.1 (1.7)	1.0 (0.1)	2.0 (–)
BC	47.1 (1.8)	1.7 (0.1)	8.6 (0.6)	42.3 (1.5)	0.3 (0.1)	33.1 (1.8)	0.5 (0.1)	1.5 (0.1)
beech								
A	53.9 (1.4)	3.4 (0.5)	5.2 (0.7)	37.2 (1.0)	0.3 (0.1)	18.7 (2.5)	0.9 (0.1)	1.9 (0.1)
Bw	51.0 (2.3)	2.7 (0.3)	3.5 (0.4)	42.6 (2.3)	0.2 (–)	22.0 (1.5)	1.2 (0.2)	1.6 (0.2)
BC1	42.2 (2.2)	1.7 (0.1)	13.2 (1.1)	42.9 (1.1)	0.0 (–)	29.2 (3.6)	0.3 (–)	1.3 (0.1)
BC2	41.9 (1.7)	2.3 (0.3)	21.2 (1.1)	34.6 (0.9)	0.0 (–)	27.8 (2.7)	0.2 (–)	2.1 (0.5)
>8000 Da fraction								
fir								
A	54.6 (1.9)	3.1 (0.3)	4.9 (0.5)	37.1 (1.8)	0.3 (0.1)	20.9 (1.4)	0.9 (0.1)	2.0 (0.2)
Bw	51.9 (1.6)	3.4 (0.6)	5.0 (0.4)	39.0 (2.1)	0.7 (0.1)	18.0 (3.0)	0.9 (0.1)	1.8 (0.1)
BC	50.3 (1.9)	2.7 (0.4)	4.7 (0.4)	42.0 (1.4)	0.2 (0.1)	21.7 (3.5)	0.9 (0.1)	1.6 (0.1)
beech								
A	54.7 (1.1)	3.6 (0.4)	5.7 (0.5)	35.7 (1.4)	0.3 (0.1)	17.7 (2.0)	0.8 (0.1)	2.0 (0.1)
Bw	51.6 (2.7)	3.4 (0.2)	4.9 (0.3)	39.6 (2.7)	0.5 (0.1)	17.9 (0.2)	0.9 (0.1)	1.7 (0.2)
BC1	52.3 (2.7)	3.3 (0.1)	5.1 (0.4)	38.8 (2.5)	0.6 (0.1)	18.6 (0.7)	0.9 (0.1)	1.8 (0.2)
BC2	56.3 (1.5)	3.8 (0.4)	5.1 (0.2)	34.3 (1.3)	0.5 (0.1)	17.5 (2.2)	0.9 (0.0)	2.2 (0.1)

aliphatic and the aromatic C components agrees well also with that determined by Preston et al. (1994) for non-purified humic and fulvic acid fractions from mineral horizons of a Dystric Cambisol under *Pseudotsuga menziesii* Franco. In our samples, the aliphatic C was approximately equally distributed between the alkyl and the O-alkyl forms except in the 1000–8000 Da fraction from the profile under fir, where the alkyl C was inexplicably much more abundant (Table 3). The O-alkyl region is often used to provide an estimate of the content of carbohydrates of the sample (Schnitzer 1991). In particular, the signals at 70–80 and around 105 ppm may be attributed to polysaccharides, such as cellulose and hemicelluloses (Kögel-

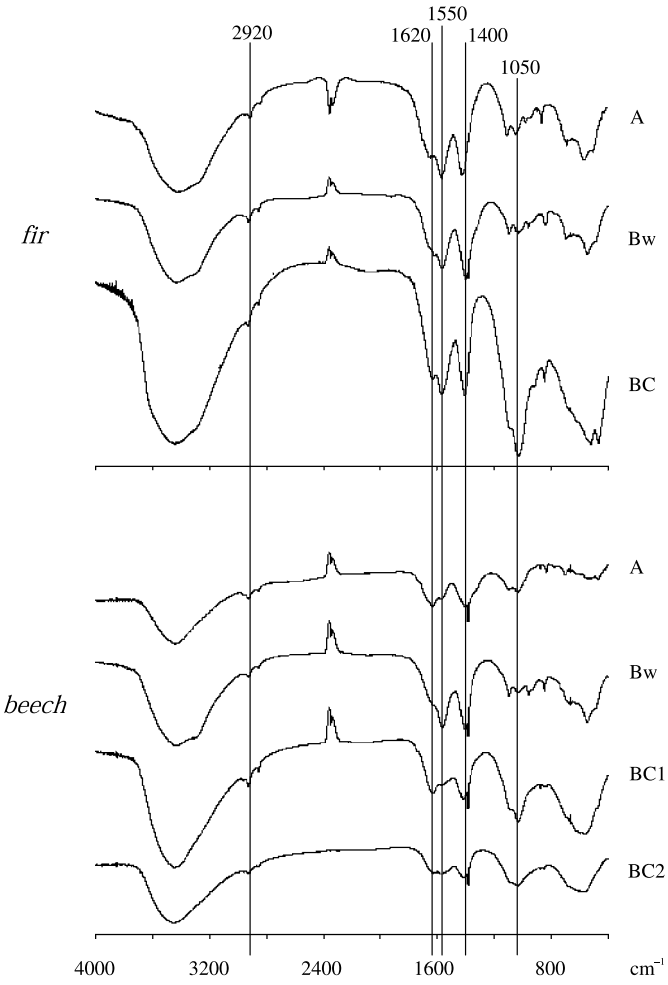

Figure 3. FT-IR spectra of the 100–1000 Da organic fraction extracted from a soil profile in either examined stand.

Knabner et al. 1988; Baldock and Preston 1995; Hopkins and Chudek 1997). As carbohydrates are one of the most easily degradable forms of SOM, their relative content is inversely related to the degree of humification of the sample. Although it has been reported that the NMR signal of O-alkyl C overestimates the effective content of carbohydrates (Ogner 1985; Preston et al. 1989), both 1000–8000 Da and >8000 Da fractions appeared rather rich in carbohydrates for mineral horizons, indicating incomplete humification of the extracted organic matter. On the other hand, in a soil under silver fir from the Vallombrosa Forest, Agnelli et al. (2000) found that in the A horizon a good 80% of the extractable C belonged to non-humic biopolymers; this percentage decreased only slightly in the underlying horizons. The O-alkyl spectral region was significantly ($P = 0.01$) broader (so suggesting

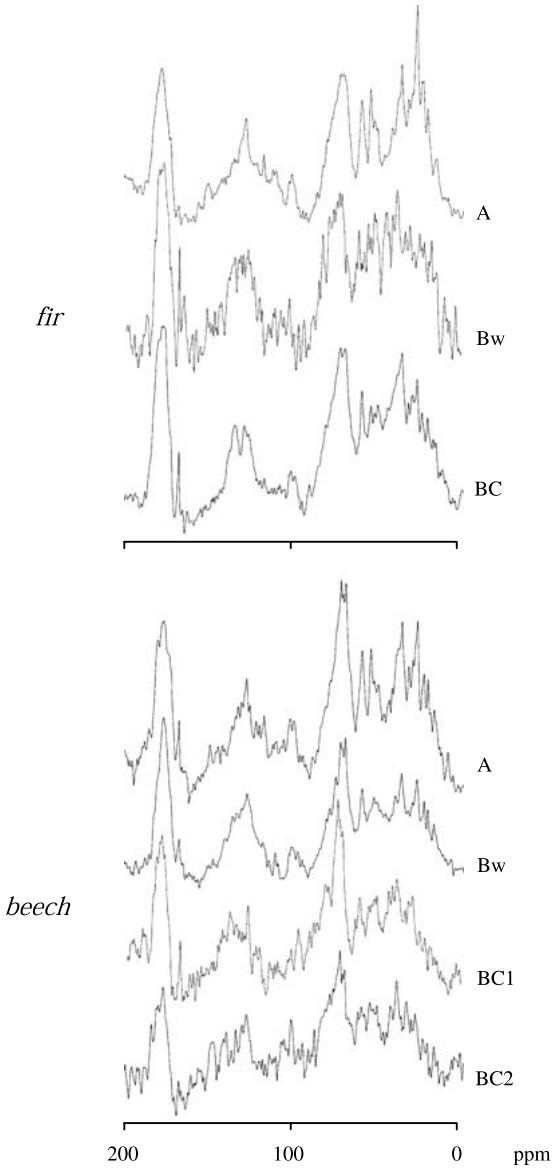

Figure 4. ^{13}C NMR spectra of the 1000–8000 Da organic fraction extracted from a soil profile in either examined stand.

higher contamination by non-humic biopolymers) in the >8000 Da fraction than in the 1000–8000 Da one and under beech than under fir. No other statistically significant difference was found between the two fractions with regard to the other moieties, such as the carbonyl C which in both fractions spectra represented about 15–20% of the total and consisted mostly of signals from carboxylic C.

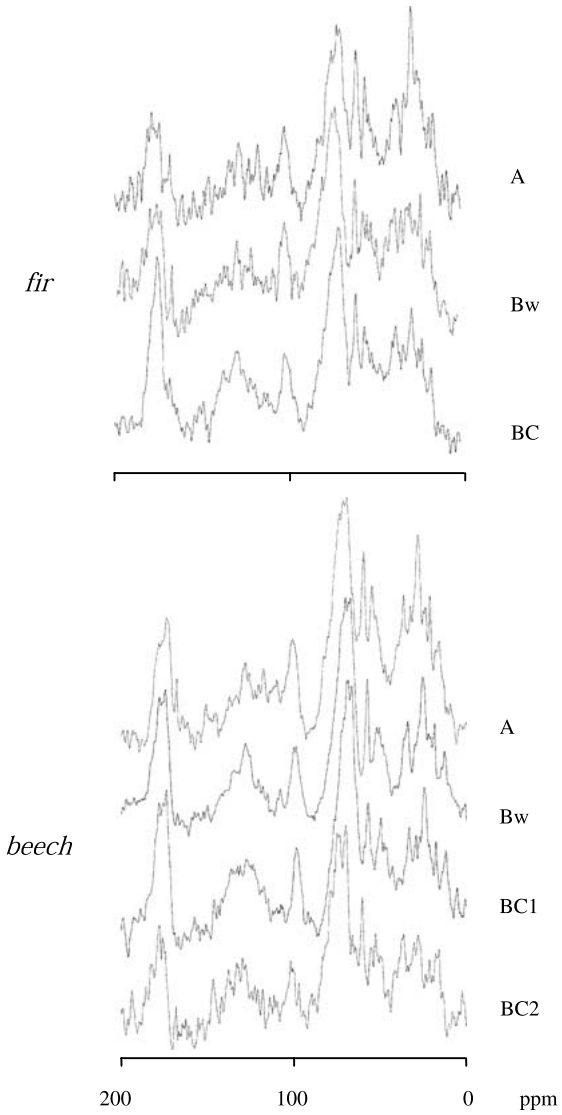

Figure 5. ^{13}C NMR spectra of the >8000 Da organic fraction extracted from a soil profile in either examined stand.

Interesting results arose from carbon isotope analyses (Table 4). The $\delta^{13}C$ can give information about the origin of the organic matter, because plants using C_3 or C_4 photosynthetic cycles are able to discriminate to different degrees ^{13}C from ^{12}C. Plants with a C_3 cycle have $\delta^{13}C$ values ranging from -32 to -22‰, compared with those with a C_4 cycle, originally typical of regions with warm and humid climate, whose $\delta^{13}C$ values range from -17 to -9‰ (Boutton 1996). Our data (Table 4) prove that the organic matter extracted from the two soils was produced

Table 3. Per cent distribution of the peak areas from the ^{13}C NMR patterns for the 1000–8000 and >8000 Da organic fractions extracted from the soils at Mt Porcellaia (fir) and Termine (beech). Numbers between parentheses are the standard deviations of three independent replicates.

	Alkyl C 0–60 ppm	O-alkyl C 60–105 ppm	Aromatic C 105–165 ppm	Carbonyl C 165–200 ppm
			%	
1000–8000 Da fraction				
fir				
A	37.0 (1.8)	22.9 (1.4)	21.9 (2.5)	18.3 (2.0)
Bw	37.8 (1.5)	21.8 (1.7)	22.6 (0.7)	17.8 (1.0)
BC	40.2 (1.4)	16.3 (1.2)	21.3 (1.5)	22.2 (1.7)
beech				
A	34.3 (1.6)	28.0 (1.4)	19.8 (0.7)	17.8 (0.5)
Bw	34.5 (2.2)	25.9 (0.4)	20.9 (0.6)	18.8 (3.2)
BC1	31.2 (1.7)	30.6 (0.8)	19.6 (0.9)	18.6 (1.6)
BC2	33.3 (1.5)	30.4 (2.0)	21.8 (1.5)	14.5 (1.2)
>8000 Da fraction				
fir				
A	36.4 (1.9)	30.3 (1.5)	18.2 (1.0)	15.1 (1.5)
Bw	33.4 (1.5)	32.1 (1.6)	18.9 (1.1)	15.5 (0.8)
BC	30.6 (1.5)	30.8 (0.8)	21.7 (1.2)	16.9 (1.1)
beech				
A	37.8 (2.2)	32.4 (0.6)	18.6 (1.4)	11.2 (0.5)
Bw	33.0 (1.7)	33.3 (0.7)	19.0 (0.4)	14.7 (1.1)
BC1	33.8 (1.5)	32.5 (0.7)	20.1 (1.4)	13.6 (0.6)
BC2	32.8 (1.8)	35.3 (0.9)	19.1 (0.9)	12.8 (2.3)

Table 4. $\delta^{13}C$, $\Delta^{14}C$ and MRT of the 100–8000 and >8000 Da organic fractions extracted from a soil profile in either examined stand.

	100–8000 Da fraction			>8000 Da fraction		
	$\delta^{13}C$ ‰	$\Delta^{14}C$[a] ‰	MRT[b] years	$\delta^{13}C$ ‰	$\Delta^{14}C$[a] ‰	MRT[b] years
fir						
A	−25.1	133.6 ± 9.7	7–9 or 56–69	−25.9	160.9 ± 8.8	10–12 or 41–50
Bw	−21.3	−96.9 ± 6.8	930–1052	−24.2	−71.1 ± 8.5	703–840
BC	−21.4	−160.1 ± 6.3	1553–1692	−24.5	−124.9 ± 6.6	1190–1320
beech						
A	−24.9	100.7 ± 10.4	3–5 or 78–99	−26.2	173.5 ± 8.8	11–15 or 34–43
Bw	−24.4	−137.3 ± 6.9	1310–1451	−24.8	−106.9 ± 6.7	1020–1145
BC1	−21.3	−284.0 ± 9.3	3106–3395	−24.3	−380.4 ± 5.1	4880–5093
BC2	−21.4	−261.9 ± 6.1	2827–3006	−24.3	−361.4 ± 4.8	4508–4696

[a] Numbers following the data for $\Delta^{14}C$ are the analytical error.
[b] The ranges of MRT were generated by the used model when analytical error is considered.

by C_3 vegetation, as expected for an environment, such as that of Vallombrosa, which experienced always a temperate or colder climate. However, the higher (less negative) $\delta^{13}C$ values of the deep horizons with respect to the shallower ones and of the 100–8000 Da fraction with respect to the >8000 Da fraction (Table 4) could partly correspond to phases that have been further decomposed by the soil microflora. In fact, during decomposition processes some isotopic fractionation does occur, due to the preference accorded by microrganisms to ^{12}C with respect to the heavier ^{13}C (Balesdent et al. 1993; Ågren et al. 1996). In the examined soils, $\delta^{13}C$ tended to increase with depth for both molecular weight fractions (Table 4). A similar progressive increase of $\delta^{13}C$ with depth has been reported for the bulk SOM by other authors (Amundson and Davidson 1990; Bol et al. 1999), and thus it may be inferred that the stronger decomposition of the extractable SOM in the deep horizons reflects that of the bulk SOM.

Obviously, the organic fractions we separated in this study were a mixture of substances showing different degrees of decomposition and/or resynthesis through the activity of microrganisms. As a consequence, carbon isotope data and radiocarbon-based MRT represent averages of a whole plethora of extremely different values. On the basis of their positive values of $\Delta^{14}C$ (Table 4), which testify to the enrichment of ^{14}C in the atmosphere by nuclear weapons testing after 1950, only the samples from the A horizons were composed mainly of matter produced by the present forest cover. In fact, the MRT of this organic matter ranged from less than a decade to about a century (Table 4). The MRT of the 100–8000 and >8000 Da fractions increased with depth in both soils (Table 4), reaching very high values in the BC horizons at Termine (3000–5000 years). Generally, the organic matter extracted from the soil under beech had a longer MRT than that from the soil under fir (Table 4). Radiocarbon measurements on the CO_2 evolved from incubated samples of the two soils had previously revealed that organic matter having a MRT compatible with the current forest cover was present throughout the profile (Certini et al. 2003). But, evidently, only in the A horizon SOM produced recently was so abundant that the radiocarbon signature of the extractable fraction was attributable for the most part to the present stands. An important discriminant factor between the two analysed soil profiles with regard to the MRT of the SOM extracted from the Bw and BC horizons is probably the greater depth of these horizons under beech than under fir. In fact, Scharpenseel (1993), treating a plethora of data from all over the world, found a significant positive correlation between depth of the sample and MRT of soil organic matter in those soil orders where translocation of organics is not a main pedogenic process. For both profiles, the organic >8000 Da fraction showed a MRT lower than that of the 100–8000 Da fraction (Table 4). This could be explained by the greater presence in the first fraction of carbohydrates, easily degradable compounds that may have diminished the MRT of the humic pool. An inexplicable exception to this rule is represented by the BC horizons under beech, where the >8000 Da fraction had an MRT much higher than that of the 100–8000 Da fraction (Table 4). The remarkable discrepancy between the two profiles with regard to the MRT calculated for BC horizons is presumably due to differences in microbial biomass and/or supply of O_2, since under beech these horizons lay at a much greater depth and show higher bulk density than under fir (1.6 $Mg\,m^{-3}$ v.s. 1.3 $Mg\,m^{-3}$).

Conclusions

In terms of C, in every horizon of the two soils the organic matter extracted amounted to less than 50% of the total organic matter. The separation of the extracted material into molecular weight fractions allowed us to ascertain differences between fractions with regard to quantity, composition, and MRT. More than half of the extracted organic matter consisted of molecules >8000 Da. The lowest molecular weight fraction (100–1000 Da) increased drastically, as per cent of the total, from the A horizon to the base of the profile, suggesting its vertical translocation. The three fractions showed significant differences with regard to principal elemental composition, especially comparing the smallest one with the other two. In particular, the 100–1000 Da fraction was poorer in C and N, virtually free of S, and much richer in O. In fact, both spectrometric investigations provided evidence of the marked carboxylic nature of this fraction. The ^{13}C NMR spectra showed that the 1000–8000 and >8000 Da fractions had a prevalently aliphatic nature and the strong O-alkyl C signals, partly attributable to carbohydrates, revealed overall a high presence of non-humic biopolymers. These latter were significantly more abundant, indicating a lower degree of humification, in the >8000 Da fraction than in the 1000–8000 Da fraction. Comparing soils, that under beech was significantly richer in O-alkyl C than that under fir, but no difference was ascertained with regard to other types of moieties.

The radiocarbon analysis disentangled that the organic matter recovered from the A horizons was for the most part produced by the present forest cover and, thus, in this case the differences in composition found between soils could be ascribed to the change of woody species (fir in place of beech) which occurred 75 years ago in one of the two sites. The MRT increased noticeably and progressively with depth in both soils and for both size fractions analysed. Despite having undergone higher isotopic fractionation, the 100–8000 Da fraction often showed longer MRT than the >8000 Da fraction. It could be explained by the relative richness in undecayed fresh organic material of the large-size fraction. In any case, the similarity of the residence times of the two fractions and the contrasting data between horizons did not give clear indications about one of the crucial issues of soil science, namely what is the transformation pathway of organic matter in soil: 'biotic degradation', where the vegetal biopolymers are not destroyed but only modified to form stable humic substances progressively decreasing in size, or 'abiotic condensation', in which simple products of biopolymer degradation re-polymerise to form humic substances progressively increasing in size.

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Appendix 1.

Descriptions of two of the soil profiles studied at Vallombrosa, Italy, made according to the Soil Survey Staff (1993)

Profile under silver fir (*A. alba* Mill.)

Locality: Mt Porcellaia – Elevation: 1100 m a.s.l. – Exposure: NNE – Slope: about 20% – Age of the stand: 75 years – Understorey: sparse, mainly composed by *Senecio fuchsii*, *Rubus ideus*, *Geranium robertianum*, *Prenantes purpurea*, *Luzula nivea*, *Sanicula europaea*, *Hieriacium murorum* – Parent material: Oligocene sandstone, intercalated with thin layers of siltstone representing less than 10% in volume.

Horizons	Depth (cm)	Munsell colour (moist, crushed)	Texture* (USDA)	Structure❤	Consistence*	Plasticity◆	Roots■	Boundary♣	Other observations
Oi/Oa	2–0								Needles and cones of firs, leaves of beech.
A	0–8	10YR 2/2	g, sil	1, f-m, cr	mfr, wss	wps	3 vf, f, m, 1 co	c, b	In the lower part, the external surface of the aggregates is bleached.
AB	8–27	10YR 3/3	g, sil	1-2, m, sbk	mfr-fi, wss	wps	3 vf, f, m, 1 co	a, s	
Bw	27–49	10YR 5/8	sil	2-3, f-c, sbk	mfi, wss	wps	3 f, 2 m, 1 co	a, s	
BC	49–80	10YR 5/6	f, sil-sicl	1-2, m-c, sbk	mfr, wss	wps	2 m, 1 co	c, s	
C	80–90								
R	90+								

Appendix 1. (continued). Profile under European beech (*F. sylvatica* L.)

Locality: Termine – Elevation: 1250 m a.s.l. – Exposure: NE – Slope: about 15% – Age of the stand: 150 years – Understorey: virtually absent – Parent material: Oligocene sandstone, intercalated with thin layers of siltstone representing less than 10% in volume.

Horizons	Depth (cm)	Munsell colour (moist, crushed)	Texture* (USDA)	Structure❤	Consistence*	Plasticity◆	Roots■	Boundary♠	Other observations
Oi	4–0								Leaves and twigs of beech.
A	0–12	5YR 3/4	sil	1-2, f-m, cr	mfr, wss	wps	3 vf, f, 2 m	c, w	
AB	12–30	5YR 4/4	sil	1-2, f-m, cr	mfr, wss	wps	3 vf, f, 2 m	a, s	
Bw	30–86	7.5YR 4/4	sil	2, f-m, sbk	mfr-fi, wss	wps-wp	2 m, co	c, b	
BC1	86–142	10YR 4/4	f, scl	1, m, pl	mfi, wss	wp	2 f, m	c, i	In the lower part, the stoniness increases.
BC2	142–191	10YR 5/4	f, scl	1, m, pl	mfi, wss	wp	1 f	c, w	
C	191–199								
R	199+								

*: g = gravelly, f = flaggy, sil = silt loam, sicl = silty clay loam, scl = sandy clay loam.
❤: 1 = weak, 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse; cr = crumb, sbk = subangular blocky, pl = platy.
*: m = moist, w = wet; fr = friable, fi = firm; ss = slightly sticky.
◆: w = wet; ps = slightly plastic, p = plastic.
■: 1 = few, 2 = plentiful, 3 = abundant; mi = micro, vf = very fine, f = fine, m = medium, co = coarse.
♠: a = abrupt, c = clear; s = smooth, w = wavy, i = irregular, b = broken.

References

Agnelli A., Celi L., Degl'Innocenti A., Corti G. and Ugolini F.C. 2000. Chemical and spectroscopic characterization of the humic substances from sandstone-derived rock fragments. Soil Sci. 165: 314–327.

Agnelli A., Trumbore S.E., Corti G. and Ugolini F.C. 2002. The dynamics of organic matter in soil rock fragments investigated by ^{14}C dating and ^{13}C measurements. Eur. J. Soil Sci. 53: 147–159.

Ågren G.O., Bosatta E. and Balesdent J. 1996. Isotope discrimination during decomposition of organic matter: a theoretical analysis. Soil Sci. Soc. Am. J. 60: 1121–1126.

Amundson R.G. and Davidson E.A. 1990. Carbon dioxide and nitrogenous gases in the soil atmosphere. J. Geochem. Explor. 38: 13–41.

Baldock J.A. and Preston C.M. 1995. Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-13 nuclear magnetic resonance. In: McFee W.W. and Kelly J.M. (eds) Carbon Forms and Functions in Forest Soils. Soil Science Society of America, Inc., Madison, WI, pp. 89–117.

Balesdent J., Girardin C. and Mariotti C. 1993. Site-related ^{13}C of tree leaves and soil organic matter in a temperate forest. Ecology 74: 1713–1721.

Batjes N.H. 1996. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci. 47: 151–163

Bol R., Harkness D.D., Huang Y. and Howard D.M. 1999. The influence of soil processes on carbon isotope distribution and turnover in the British uplands. Eur. J. Soil Sci. 50: 41–51.

Boutton T.W. 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In: Boutton T.W. and Yamasaki S.I. (eds) Mass Spectrometry of Soils. Marcel Dekker, New York, NY, pp. 47–82.

Buchanan D.L., Corcoran B.J. 1959. Sealed tube combustions for the determination of carbon-14 and total carbon. Anal. Chem. 31: 1635–1638.

Certini G., Corti G. and Fernández Sanjurjo M.J. 2002. Comparison of two soil organic matter extractants and determination of the 'Walkley-Black' correction factors for organic fractions from a volcanic soil. Commun. Soil Sci. Plant Anal. 33: 685–693.

Certini G., Corti G., Agnelli A. and Sanesi G. 2003. Efflux of carbon dioxide and concentration at various depths in two soils under temperate forests. Biol. Fertil. Soils 37: 39–46.

Chen C.R., Condron L.M., Davis M.R. and Sherlock R.R. 2000. Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. Plant Soil 220: 151–163.

De Nobili M., Bragato G., Alcaniz J.M., Puigbo A. and Comellas L. 1990. Characterization of electrophoretic fractions of humic substances with different electrofocusing behaviour. Soil Sci. 150: 763–770.

Fox T.R. 1995. The influence of low-molecular-weight organic acids on properties and processes in forest soils. In: McFee W.W. and Kelly J.M. (eds) Carbon Forms and Functions in Forest Soils. Soil Science Society of America, Inc., Madison, WI, pp. 43–62.

Gaudinski J.B., Trumbore S.E., Davidson E.A. and Zheng S.H. 2000. Soil carbon cycling in a temperate forest: radiocarbon based estimates of residence times, sequestration rates and partitioning of fluxes. Biogeochemistry 51: 33–69.

Hayes M.H.B. and Swift R.S. 1978. The chemistry of soil organic colloids. In: Greenland D.J. and Hayes M.H.B. (eds) The Chemistry of Soil Constituents. Wiley Interscience, New York, NY, pp. 179–230.

Hayes M.H.B., Swift R.S., Wardle R.E. and Brown J.K. 1975. Humic materials from an organic soil: a comparison of extractants and of properties of extracts. Geoderma 13: 231–245.

Herbert B.E. and Bertsch P.M. 1995. Characterization of dissolved and colloidal organic matter in soil solution: a review. In: McFee W.W. and Kelly J.M. (eds) Carbon Forms and Functions in Forest Soils. Science Society of America, Inc., Madison, WI, pp. 63–88.

Homann P.S. and Grigal D.F. 1992. Molecular weight distribution of soluble organics from laboratory-manipulated surface soils. Soil Sci. Soc. Am. J. 56: 1305–1310.

Hopkins D.W. and Chudek J.A. 1997. Solid-state NMR investigations of organic transformations during the decomposition of plant material in soil. In: Cadish G. and Giller K.E. (eds) Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, UK, pp. 85–94.

ISSS-ISRIC-FAO 1998. World Reference Base for Soil Resources. Acco Press, Leuven, Belgium.

Kögel-Knabner I. 2000. Analytical approaches for characterizing soil organic matter. Org. Geochem. 31: 609–625.

Kögel-Knabner I., Zech W. and Hatcher P.G. 1988. Chemical composition of the organic matter in forest soil: the humus layer. J. Plant Nutr. Soil Sci. 151: 331–340.

Mac Carthy P., Peterson M.J., Malcom R.L. and Thurman E.M. 1979. Separation of humic substances by pH gradient desorption from a hydrophobic resin. Anal. Chem. 51: 2041–2043.

Meyer J.T., Edwards R.T. and Risley R. 1987. Bacterial growth on dissolved organic carbon from a blackwater river. Microbiol. Ecol. 13: 13–29.

Ogner G. 1985. A comparison of four different raw humus types in Norway using chemical degradations and CPMAS ^{13}C NMR spectroscopy. Geoderma 35: 343–353.

Piccolo A. 1988. Characteristics of soil humic extracts obtained by some organic and inorganic solvents and purified by HCl–HF treatment. Soil Sci. 146: 418–426.

Preston C.M., Axelson D.E., Lévesque M., Mathur S.P., Dinel H. and Dudley R.L. 1989. Carbon-13 NMR and chemical characterization of particle-size separates of peats differing in degree of decomposition. Org. Geochem. 14: 393–403.

Preston C.M., Hempfling R., Schulten H.R., Schnitzer M., Trofymow J.A. and Axelson D.E. 1994. Characterization of organic matter in a forest soil of coastal British Columbia by NMR and pyrolysis-field ionization mass spectrometry. Plant Soil 158: 69–82.

Sanchez F.G. and Eaton R.J. 2001. Sequestering carbon and improving soils: benefits of mulching and incorporating forest slash. J. Forestry 99: 32–36.

Scharpenseel H.W. 1993. Major carbon reservoirs of the pedosphere: source–sink relation, potential of ^{14}C and ^{13}C as supporting methodologies. Water Air Soil Pollut. 70: 431–442.

Schnitzer M. 1991. Soil organic matter – the next 75 years. Soil Sci. 151: 41–58.

Soil Survey Staff 1993. Soil Survey Manual. United States Department of Agriculture Handbook No. 18. Government Printing Office, Washington, DC.

Soil Survey Staff 1999. Soil Taxonomy. A Basic System of Soil Classification for Making and Interpreting Soil Surveys. 2nd edn. Agriculture Handbook Number 436, United States Department of Agriculture and Natural Resources Conservation Service. US Government Printing Office, Washington, DC.

Spycher G., Sollins P. and Rose S. 1983. Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal patterns. Soil Sci. 135: 79–87.

Stevenson F.J. 1994. Humus Chemistry: Genesis, Composition, Reactions. 2nd edn. John Wiley & Sons, New York, NY.

Stuiver M. and Polach H. 1977. Reporting of ^{14}C data. Radiocarbon 19: 355–363.

Tate K.R., Ross D.J., O'Brien B.J. and Kelliher F.M. 1993. Carbon storage and turnover, and respiratory activity, in the litter and soil of an old-growth southern beech (*Nothofagus*) forest. Soil Biol. Biochem. 25: 1601–1612.

Ugolini F.C. and Spaltenstein H. 1992. The pedosphere. In: Charlson R., Orions G., Butcher S. and Wolf G. (eds) Global Biogeochemical Cycles. Academic Press, San Diego, CA, pp. 85–153.

Van Cleve K. and Powers R.F. 1995. Soil carbon, soil formation and ecosystem development. In:McFee W.W. andKelly J.M. (eds) Carbon Forms and Functions in Forest Soils.Soil Science Society of America, Inc., Madison, WI, pp. 155–200.

Vogel J.S. 1992. Rapid production of graphite without contamination for biomedical AMS. Radiocarbon 34: 344–350.

Wachendorf C. 1998. Characterisation of a humus profile under beech forest by using wet-chemistry and CPMAS ^{13}C NMR-spectroscopy in consideration of spatial heterogeneity. J. Plant Nutr. Soil Sci. 161: 249–254.

Yuan T.L. 1964. Comparison of reagents for soil organic matter extraction and effect of pH on subsequent separation of humic and fulvic acids. Soil Sci. 98: 133–141.

Zech W. and Guggenberger G. 1996. Organic matter dynamics in forest soils of temperate and tropical ecosystems. In: Piccolo A. (ed) Humic Substances in terrestrial Ecosystems. Elsevier, Amsterdam, The Netherlands, pp. 101–170.

Zech W., Haumaier L., Guggenberger G., Gil-Sotres F. and Arai S. 1994. Changes in carbon species distribution of humic substances with depth in mineral soils of various origin. In: Senesi N. and Miano T.M. (eds) Humic Substances in the Global Environment and Implications on Human Health. Elsevier, Amsterdam, The Netherlands, pp. 445–450.