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Mineralization of carbon and nitrogen from plant debris, as affected by debris size and depth of burial

P. Rovira[a,*], V. Ramón Vallejo[a,b]

[a]*Department de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain*
[b]*CEAM, Parc Tecnològic, Ch Darwin 14, 46980 Paterna, València, Spain*

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Abstract

The relationship between particle size and mineralization has been well established for organic carbon (OC). It has repeatedly been reported that mineralization decreases with decreasing particle size, and that the OC of fine particles (fine silt, clay) has a much slower turnover than that of coarse organic fragments. For nitrogen (N), however, the available data are contradictory. Here, we study the behaviour of OC and N in a range of size fractions over two years of field decomposition. To this end, *Medicago sativa* ground plants, and *Quercus ilex* ground litter were mixed with an OC-poor red earth, and were buried at 5 and 40 cm depth, in nylon mesh bags. Selected samplings were taken to study the changes in the distribution of OC and N among the size fractions, obtained by ultrasonic dispersion: coarse sand (2000–200 μm), fine sand (200–50 μm), coarse silt (50–20 μm), fine silt (20–2 μm), and clay (<2 μm). In addition, we studied the clay-occluded OC and N fraction, which is the remains of the oxidation of clay with 30% H_2O_2.

The losses of OC (as % of the total OC in the size fraction) consistently decreased with decreasing fraction size, and loss of this element was minimal for clays. In *Quercus* mixtures, a net input of OC to clays was observed, indicating OC redistribution during decomposition. The losses of N did not show such a clear pattern; in *Medicago* mixtures, no relationship with size was observed, whereas in *Quercus* mixtures, they fell slightly with decreasing size. Clay acted as a net sink for N in *Quercus* mixtures, especially at 40 cm depth. Overall, the clay-occluded OC and N was the most stable fraction. In most cases, losses were significantly greater at 40 cm depth; when a net input of either OC or N was observed for a given fraction, the input was also greater at this depth. Most detectable changes were observed in the light subfraction ($d < 2.0$) of each size fraction, while in the dense subfraction ($d \geq 2.0$) changes were much smaller.

The OC/N ratios of the coarse fractions (coarse sand, fine sand) decreased dramatically, whereas those of finer fractions were largely unaltered. There was a clear trend towards the uniformity of the OC/N ratios of the fractions, and we suggest that this trend might, to some extent, control the N dynamics. Hence, while OC dynamics is driven mainly by physical availability, which depends on fraction size, that of N is also controlled by the quality of the organic debris (OC/N ratio, among other indicators), and the interaction of both controlling factors may result in a size–mineralization relationship that is not as clear as that observed for OC.

Keywords: Carbon; Mineralization; Size fractionation; Density fractionation; Nitrogen; Mediterranean; Soil depth

1. Introduction

Particle size is a major factor in determining the rate of decomposition of plant debris in soil. Initially, it was believed that this decomposition rate increased as organic debris size diminished (Allison, 1973), mainly because of increased surface/weight relation. Nevertheless, Sims and Frederick (1970) had already observed that this relation was only valid for debris greater than 200 μm; smaller debris decomposes at a slower rate when incubated in soil. Since such an effect was not observed in incubations in sand, it was concluded that debris comprising small particles were stabilized by soil colloids. Today, the relationship between size and decomposition rate is widely accepted.

With few exceptions (for instance Bernhard-Reversat (1993)), it is usually found that the turnover of organic matter (OM) below the limit of 2 mm decreases with decreasing particle size (Dalal and Mayer, 1986; Christensen, 1992; Saviozzi et al., 1997; Balesdent et al., 1998, among others). Thus, the OM of the coarse sand, for instance, usually decomposes more rapidly than that of fine sand, which in turn decomposes faster than that of coarse silt, and so on. Only the relative speeds of decomposition in silt and clays are open to discussion. Some authors observe that the OM of silt is more stable than that of clays (Christensen, 1987), whereas others report on the contrary (Gregorich et al., 1989; Bonde et al., 1992). It is accepted

* Corresponding author. Tel.: +34-93-402-1462; fax: +34-93-411-2842.
E-mail address: rovira@porthos.bio.ub.es (P. Rovira).

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Table 1
Main characteristics of the soil of the plot, and of the RE used for mixtures. Only the horizons involved in the experience are given. The top 40 cm of the soil was removed, so the zero depth was 40 cm depth in the original profile. pH was done in a 1:2.5 proportion, soil/water

Depth (cm)	OC (%)	N (%)	pH (H_2O)	$CaCO_3$ (%)	Texture
0–14	0.4	0.07	7.5	6.8	Loam
14–47	0.3	0.06	7.5	7.5	Clay loam
RE	0.18	0.05	8.0	11.8	Clay loam

that the great stability of OM of fine particles is due to its inclusion in microaggregates. As particle diameter decreases, the porosity associated with the particle also decreases, and the associated OM may become unavailable to much of the microflora of soil. The relationship between debris size and degree of physical protection was shown by Golchin et al. (1994), who observed that coarse debris (>200 μm) can be isolated from the mineral matrix by floatation in a dense liquid, simply by manual shaking, whereas ultrasonic dispersion is needed to separate fine debris from the mineral matrix.

Less information is available to determine the dynamics of N in size fractions. While the amount of nitrogen (N) in fractions and its chemical characteristics are well documented (Young and Spycher, 1979; Anderson et al., 1981; Schnitzer and Ivarson, 1982), the availability of N in each fraction is less understood than that of organic carbon (OC). Moreover, the information available is less consistent for N. A significant relation has sometimes been reported between the amount of ^{15}N in the coarse, particulated OM (POM), and the ^{15}N taken up by crops (Vanlauwe et al., 2000). However, relative N mineralization is greater as particle size decreases, reaching maximum levels for clay-size fractions (Chichester, 1969; Christensen and Olesen, 1998), which is the opposite pattern to that observed for OC.

The experimental approach adopted is relevant, when such results are interpreted. Incubating whole (unfractionated) soil samples allows total mineralized OC or N to be correlated with the amount of these elements in a given fraction, but, unless the fraction is labelled, this cannot be taken as a direct measure of availability, but rather might simply reflect the average OC or N status of the soil (Vanlauwe et al., 2000). Moreover, the redistribution of these elements during decomposition can be accounted for only when the fraction is labelled. In contrast, incubating previously isolated fractions allows the determination of the intrinsic mineralizability of the OC or N of a given fraction, but the result may not give information about the behaviour of this fraction under real conditions, since it may be physically unavailable and its OC or N out of cycling, at least temporarily. Furthermore, the incubation of previously isolated fractions makes it impossible to account for redistributions of OC or N. This drawback is also inherent in long-term studies of changes in soil use or vegetation type that are based on stable isotope ratios.

Here, we present an experiment that involves field incubation of plant residues in soil. This study aims at examining the transformations of these residues during decomposition, from the point of view of the dynamics of size fractions, i.e. the net loss (or accumulation) of OC and N in each size fraction, and the redistribution of these elements between size and density fractions as decomposition proceeds. We also aim at verifying whether the relationship between availability and particle size is the same for both elements. Since the experiment involves incubations at different depths, an additional objective is to observe how the results depend on the pedoclimate to which decomposing samples are exposed.

2. Material and methods

The data presented here were recorded in an experiment aimed at determining how the position in the soil profile affects carbon and nitrogen mineralization, and to isolate this effect from differences due to the biochemical characteristics of SOM, which vary along a soil profile. Mixtures of plant + soil were buried at a number of depths to study the changes in their chemical and biochemical characteristics. A detailed description of the experiment can be found in Rovira and Vallejo (1997); here only the most relevant details are repeated.

2.1. Site

The experiment was carried out in the Experimental Fields of the University of Barcelona (Catalonia, NE Spain: 41°22′59″ N, 2°6′44″ E, 60 m above sea level). The climate is typically Mediterranean, with a mean temperature of 15.5 °C and mean annual precipitation of 614 mm, with hot, dry summers and cool winters. The soil is a *Calcic Luvisol* (FAO).

The plot selected had stood uncultivated for eight years, and was covered in weeds. The top 40 cm of the soil was removed, and the remaining soil was used for the experiment. This pre-treatment was carried out to eliminate most of the seed bank of the soil, and, thereby, avoid massive germination of seeds, whose roots could interfere with the study. The main characteristics of the remaining soil are given in Table 1.

2.2. Preparation of samples

An OM-poor red earth (RE) was selected for the mixtures (Table 1), thereby, ensuring that most of the OM of the mixture came from added plant material. This RE was taken from a deep layer (180 cm) in an adjacent plot.

Two plant materials were used: *Medicago sativa* whole green plants (except flowers and fruits), and dead, brown litter of *Quercus ilex*. All plant materials were ground to pass through a 200 μm mesh, and mixed with the RE to obtain mixtures with a 5.96% OC content. Each sample was mixed individually, so that the initial OC was exactly

Table 2
Main biochemical characteristics of the plant materials used for mixtures. Analytical methods were described previously (Rovira and Vallejo, 1997)

	Medicago	*Quercus*
Plant material alone		
C (%)	41.09	45.63
N (mg g^{-1})	34.00	19.59
P (mg g^{-1})	3.86	1.14
C/N	12.08	23.29
Lignin/N	5.09	15.40
Protein (%)	21.25	12.24
Lipids (%)	13.24	6.89
Hemicelluloses (%)	15.30	16.30
Cellulose (%)	13.64	11.72
Lignin (%)	17.31	30.16
Mixtures with RE		
C (%)	5.96	5.96
N (mg g^{-1})	6.10	2.96

the same in all samples. Water was added gently during mixing to ensure homogenization. Mixtures were placed in nylon mesh bags (openings: 500 μm), which were closed, sealed and stored at 4 °C until burial in soil. A total of 75 mixtures (50 g dry weight each) were made for each type of plant material: three randomly chosen mixtures were taken as initial samples, and removed from the bags, disaggregated on a plate, and air-dried. The main features of these initial samples are summarized in Table 2.

2.3. Field incubation

Bags were buried in the plot, in previously made holes; for each type of plant material, 24 bags were buried at 5, 20, and 40 cm depth. Holes were then re-filled with the soil that had previously been extracted. Bags were buried during the first days of autumn 1991 and each one was identified with a plastic card (indicating the type of plant material and the depth of incubation) at the soil surface. Samples were then left in the field for up to two years and no herbicide was applied at that time; any seedlings that appeared were immediately removed by hand to avoid possible root penetration into the bag.

Sampling was made at three-month intervals. For each experimental condition, three bags were lifted from the soil and taken to the laboratory. Mixtures were removed from bags as whole pieces, and the outermost part (to a thickness of about 5 mm), which could have been affected by material from the surrounding soil, was discarded. Only the internal part of the sample was analyzed: it was disaggregated on a plate, and air-dried. A subsample was finely ground for chemical analyses.

2.4. Physical fractionation

Two protocols were applied: *Simplified method* (*size fractionation*). The air-dried soil sample (12 g) was placed in a 120 ml polypropylene bottle, and 50 ml of distilled water was added. The mixture was shaken in a rotatory shaker overnight. The bottle was then placed in an ice bath, and its content dispersed with a probe-type ultrasonic disintegrator (BRANSON, model 250) for 15 min, at a nominal power release of 100 W. The suspension obtained was passed through a column of meshes (200, 50 and 20 μm), under suction, water stream and continuous shaking, until the water passing through the last mesh (20 μm) was completely clear. The resulting suspension was collected in 1 l jars. The fractions retained in meshes (coarse sand: 2000–200 μm, fine sand: 200–50 μm, coarse silt: 50–20 μm) were quantitatively transferred to plastic crucibles, and dried at 60 °C to constant weight. To separate fine silt (20–2 μm) from clay (<2 μm), the suspension was left to stand to allow sedimentation of the former. The clay was then removed by siphonation. Water (1 l) was added to the sediment, and, after homogenization, the process (sedimentation–siphonation) was repeated about ten times, until supernatant was clear. Fine silt was transferred to plastic crucibles and dried at 60 °C to constant weight. Clays were concentrated by flocculation with a minimum amount of potassium alum ($AlK(SO_4)_2{\cdot}12H_2O$), transferred to plastic crucibles, washed several times with water to remove most of the potassium alum, and dried at 60 °C to constant weight.

Complete method (*size + density fractionation*). The method was conducted as above, but the size fractions obtained were transferred to centrifuge tubes and, after drying, 25 ml of a solution of sodium polytungstate (SOMETU, Falkenried, Germany; henceforth, NaPT) was added (density: 2.0 g ml^{-1}). After agitating for 1 h in a rotatory shaker, tubes were left to stand overnight, and where then centrifuged at 2500 rpm for 5 min. For coarse fractions (>50 μm), the light subfraction was decanted to a 20 μm mesh, washed with distilled water and transferred to a preweighed plastic crucible, while that of fine fractions (<50 μm) was transferred by decantation to another preweighed centrifuge tube, and water was added to dilute NaPT and to lower the density, thus causing the sedimentation of the light subfraction. Tubes were then centrifuged at 3000–3500 rpm, and the liquid (dilute NaPT) was decanted off and discarded. Both light and dense subfractions were washed in water and dried at 60 °C to constant weight.

As both methods—particularly the complete method—are highly time-consuming, it was not possible to analyze all the samples. The complete method was applied only to initial and final samples (24 months). For *Medicago*, the simplified method was applied to initial samples, and to those corresponding to 6, 15 and 21 months of incubation, while for *Quercus*, the method was applied to initial samples and to those corresponding to 3, 9, 15 and 21 months. In both cases, only samples at 5 and 40 cm depth were taken, while those at 20 cm depth were discarded because, as explained below, no significant differences in decomposition were found between 20 and 40 cm depth.

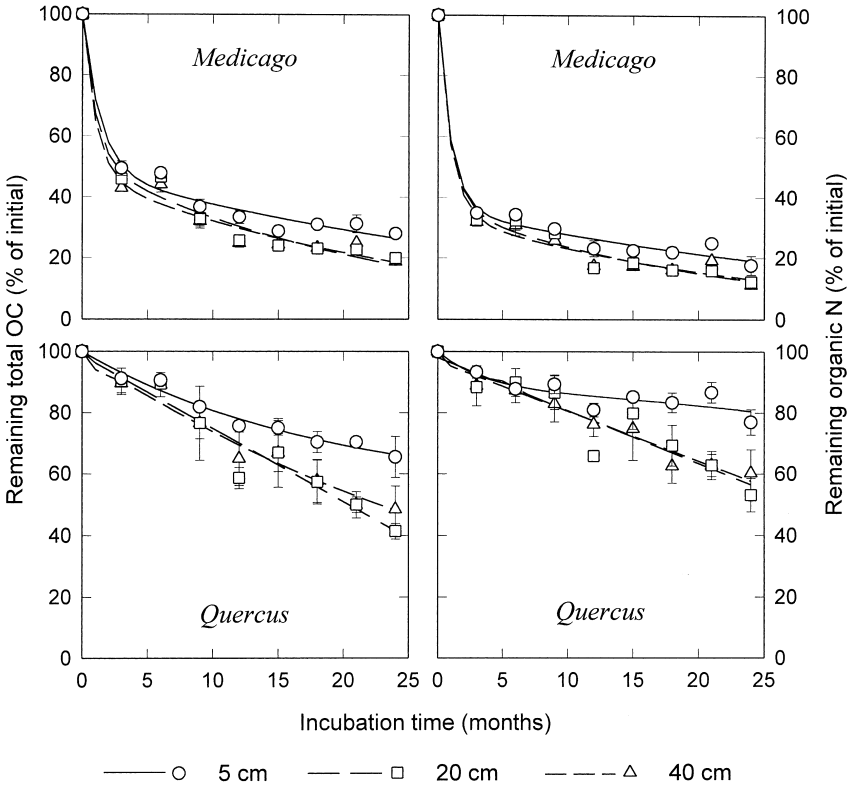

Fig. 1. Remaining total OC and ON in the whole mixtures of *Medicago* and *Quercus*. Points are averages of $n = 3$, vertical bars are standard deviations. Data are fitted to double-exponential equations, except for OC of *Quercus* mixtures (single exponential equations). ON was corrected for clay-fixed N (see text).

In a previous study (Rovira et al., 1998), we observed that NaPT can extract large amounts of OM. Consequently, the values for OC and N obtained by the complete method are not directly comparable to those obtained by the simplified method, and, therefore, here we present the two data sets separately.

Clay-occluded OC and N. About 100 mg of the clay fraction (not treated with NaPT) was placed in a centrifugable Pyrex tube, and oxidized in an aluminum tube digester, with 10 ml of 30% H_2O_2, at 60 °C for 2 h. After cooling, the tube was centrifuged and the liquid discarded. The remaining traces of H_2O_2 were eliminated by repeated washing with distilled water (centrifugation and decantation). The residue was then extracted with 10 ml of 2N KCl for 2 h, and was then washed with distilled water, as before. It was finally transferred to a pre-weighed plastic crucible, and dried at 60 °C to constant weight. Oxidation with H_2O_2 is assumed to affect only the OM located at the surface of clay particles, and not that located in the interlayers (Theng et al., 1992).

Chemical analyses. Whole samples and fractions (particle-size fractions and the density subfractions) were analyzed for OC by the Mebius method, following Soon and Abboud (1991), and for N with a Carlo Erba CNS analyzer.

In the whole samples, the inorganic N (ammonium and nitrate) was extracted with KCl in a separate subsample, and measured in a Technicon autoanalyzer; however, it was not finally subtracted from total N, because only very small amounts were detected. After destruction of OM, by boiling with potassium hypobromite, and extraction of the exchangeable N with KCl (Keeney and Nelson, 1982), fixed N was quantified with a Carlo Erba analyzer, and subtracted from total N to obtain organic N. This correction was not done in the size fractions, since fixed N is assumed to be a part of the 'clay-occluded' N (resistant to H_2O_2), whose dynamics are under study. Clay-occluded N may include both organic and inorganic N.

2.5. Calculations

For the whole sample and for each size fraction, the amount of remaining OC or N (as % of initial content) was calculated as

$$\%\ \text{Remaining} = (X_t/X_0)100$$

where X_t is the concentration of OC or N at time t, and X_0 is the concentration at the start of the experiment; both are expressed on an ash-basis, i.e. on the mineral content of the sample, which is assumed to suffer no losses during the experiment.

From the total amount of OC and N in the fractions, we obtained the 20 μm *ratio*, i.e. the percent of total OC or N in fractions ≤20 μm (fine silt and clay). The changes in this

Table 3
Water status of the soil surrounding the incubated samples. Data are given in water content (g kg^{-1} dry soil) and in water potential (MPa). Methods are given elsewhere (Rovira and Vallejo, 1997). ND: not determined

Sampling date	Water content (g kg^{-1})			Water potential (MPa)		
	5 cm	20 cm	40 cm	5 cm	20 cm	40 cm
27/12/91	95.4(6.0)	141.5(15.6)	153.8(14.9)	−1.16(0.15)	−0.39(0.13)	−0.29(0.10)
30/03/92	ND	ND	ND	ND	ND	ND
23/06/92	94.0(27.6)	136.6(16.9)	156.4(11.2)	−1.43(0.77)	−0.45(0.20)	−0.26(0.07)
30/09/92	65.2(16.5)	111.3(21.8)	137.9(26.5)	−2.61(0.92)	−0.90(0.56)	−0.50(0.37)
30/12/92	97.1(13.2)	150.6(10.9)	149.7(9.1)	−1.16(0.38)	−0.30(0.08)	−0.31(0.06)
26/03/93	94.1(18.6)	147.8(14.9)	159.8(14.0)	−1.31(0.59)	−0.33(0.11)	−0.25(0.08)
28/06/93	53.9(17.7)	112.2(17.6)	131.0(33.0)	−3.49(1.35)	−0.82(0.33)	−0.66(0.61)
29/09/93	109.4(8.5)	138.2(10.9)	149.1(17.8)	−0.83(0.17)	−0.41(0.11)	−0.33(0.15)

ratio summarize the differences in the behaviour of OC and N between coarse and fine particles.

Statistical analysis. The data of remaining OC and N in each size fraction (simplified method) were analyzed by two-way ANOVA (Wieder and Lang, 1982), taking depth of incubation as the first factor and sampling date as the second. Data corresponding to zero time were not included in the analysis. In the case of the complete method, the remaining OC and N in each subfraction (size and density) were analyzed by one-way ANOVA taking depth as the factor. In both cases, since data were recorded as percentages, they were previously transformed by arc sinus of square root (Neter et al., 1990).

The 20 μm-ratios of OC and N were analyzed by analysis of covariance (ANCOVA) taking depth as the factor and remaining OC as the covariate. Data were also previously transformed by arc sinus of square root. The aim of this analysis was to detect the possible effect of depth on the global displacement of OC or N to the fine fractions, and to distinguish this from the effect of a different speed of decomposition, i.e. to compare the data on a remaining OC basis (biological clock).

In all cases, the effect of any factor studied was considered significant if $P > F \leq 0.05$.

3. Results

3.1. Global OC and N mineralization

A detailed analysis of the mineralization of the whole OC and N of the mixtures has been reported elsewhere (Rovira and Vallejo, 1997, 2000), so, only the main features will be mentioned here. In all cases, the mineralization of both OC and N was slower at 5 cm depth, whereas no significant differences were observed between 20 and 40 cm depth. In *Medicago* mixtures, both OC and N showed clear double-exponential dynamics, whereas in *Quercus* mixtures OC and N often followed a single-exponential decay (Fig. 1).

The faster decomposition at deep layers is explained mainly by the high water availability in them, much greater than that observed for the upper horizons (Table 3). It is to be noted that in summer samplings, the water potential at 5 cm depth was quite below the wilting point.

3.2. Initial OC and N distribution among fractions

Data for initial OC and N distribution in the size fractions (simplified method) are summarized in Table 4. Differences

Table 4
OC and N in the size fractions of the mixtures plant material + RE, at the start of the incubation (zero time). Data are averages of $n = 3$, numbers in parentheses are standard deviations. Data are given in mg g^{-1} of the fraction, and in % of total OC or N in the whole mixture

Species	Size (μm)	Carbon		Nitrogen		OC/N
		mg g^{-1}	Total (%)	mg g^{-1}	Total (%)	
Medicago	> 200	8.33(0.23)	4.34(1.38)	0.35(0.03)	3.10(0.38)	23.55(5.60)
	200–50	103.46(13.37)	30.29(2.28)	1.50(0.07)	7.57(0.95)	68.96(9.38)
	50–20	81.00(5.62)	24.97(1.77)	3.22(0.33)	16.98(2.76)	25.48(3.35)
	20–2	56.50(5.03)	24.54(3.60)	3.03(0.33)	22.21(2.31)	18.71(1.39)
	< 2	49.42(2.41)	15.87(0.69)	9.19(0.36)	50.13(3.01)	5.38(0.12)
	< 2 occ	6.13(1.43)	1.97(0.46)	0.85(0.09)	4.61(0.50)	7.19(1.26)
Quercus	> 200	9.16(1.91)	3.11(0.51)	0.20(0.04)	1.23(0.16)	46.46(1.26)
	200–50	117.26(12.83)	25.54(1.41)	1.78(0.25)	7.12(0.70)	67.06(8.32)
	50–20	76.36(2.36)	17.66(1.12)	4.53(0.37)	19.28(0.99)	16.95(1.05)
	20–2	95.24(2.47)	38.56(0.23)	6.84(0.32)	51.14(0.46)	13.94(0.47)
	< 2	36.91(1.12)	15.12(0.50)	2.80(0.13)	21.23(1.47)	13.23(0.91)
	< 2 occ	5.52(0.89)	2.26(0.37)	0.59(0.06)	4.46(0.39)	9.41(1.43)

Table 5
Distribution of OC and N between the size and density fractions (complete method) in the initial samples. Concentrations are given as mg g^{-1}. Data are averages of $n = 3$, numbers in parentheses are standard deviations. LF: light subfraction; DF: dense subfraction

Species	Element	Fraction	Concentrations		% of total OC or N		
			LF	DF	LF	DF	All
Medicago	OC	> 200	297.2(6.08)	17.2(2.1)	2.41(0.93)	7.16(1.77)	9.57(2.79)
		200–50	262.4(107.7)	23.4(2.2)	16.14(2.00)	4.43(0.43)	20.57(2.42)
		50–20	235.3(29.3)	39.6(9.5)	33.80(16.68)	8.96(4.52)	42.76(12.17)
		20–2	69.0(33.5)	34.2(6.1)	1.21(0.84)	14.36(2.41)	15.57(3.25)
		< 2	27.3(27.3)	30.3(2.7)	0.12(0.12)	11.41(3.68)	11.53(3.80)
	N	> 200	4.4(0.5)	0.5(0.0)	0.74(0.27)	4.17(0.08)	4.91(0.19)
		200–50	4.8(1.5)	0.7(0.1)	6.37(0.73)	2.71(0.18)	9.08(0.55)
		50–20	6.8(0.5)	1.7(0.1)	20.95(11.31)	7.39(1.89)	28.34(9.42)
		20–2	4.6(2.6)	2.5(0.4)	1.58(1.07)	21.33(0.29)	22.91(1.36)
		< 2	3.7(3.7)	4.6(0.4)	0.30(0.30)	34.46(7.02)	34.76(7.32)
Quercus	OC	> 200	364.8(12.8)	17.3(1.4)	2.74(0.09)	6.64(0.16)	9.38(0.07)
		200–50	349.2(5.5)	17.7(3.4)	23.85(0.09)	3.45(0.97)	27.30(1.06)
		50–20	334.1(18.9)	15.5(1.4)	13.61(0.21)	2.61(0.45)	16.22(0.66)
		20–2	174.9(1.6)	24.1(1.7)	30.22(1.28)	7.43(0.84)	37.65(0.45)
		< 2	13.3(0.2)	2.5(0.2)	0.13(0.02)	9.32(0.89)	9.45(0.91)
	N	> 200	7.7(1.3)	0.5(0.09)	1.32(0.10)	4.80(1.16)	6.12(1.26)
		200–50	7.8(1.3)	0.7(0.3)	12.28(1.53)	3.09(1.60)	15.37(3.13)
		50–20	10.7(1.1)	0.8(0.1)	10.10(0.07)	3.00(0.06)	13.10(0.01)
		20–2	9.9(0.5)	1.2(0.04)	39.65(1.14)	8.69(0.36)	48.34(1.50)
		< 2	1.2(0.2)	2.0(0.1)	0.27(0.01)	16.81(2.86)	17.08(2.87)

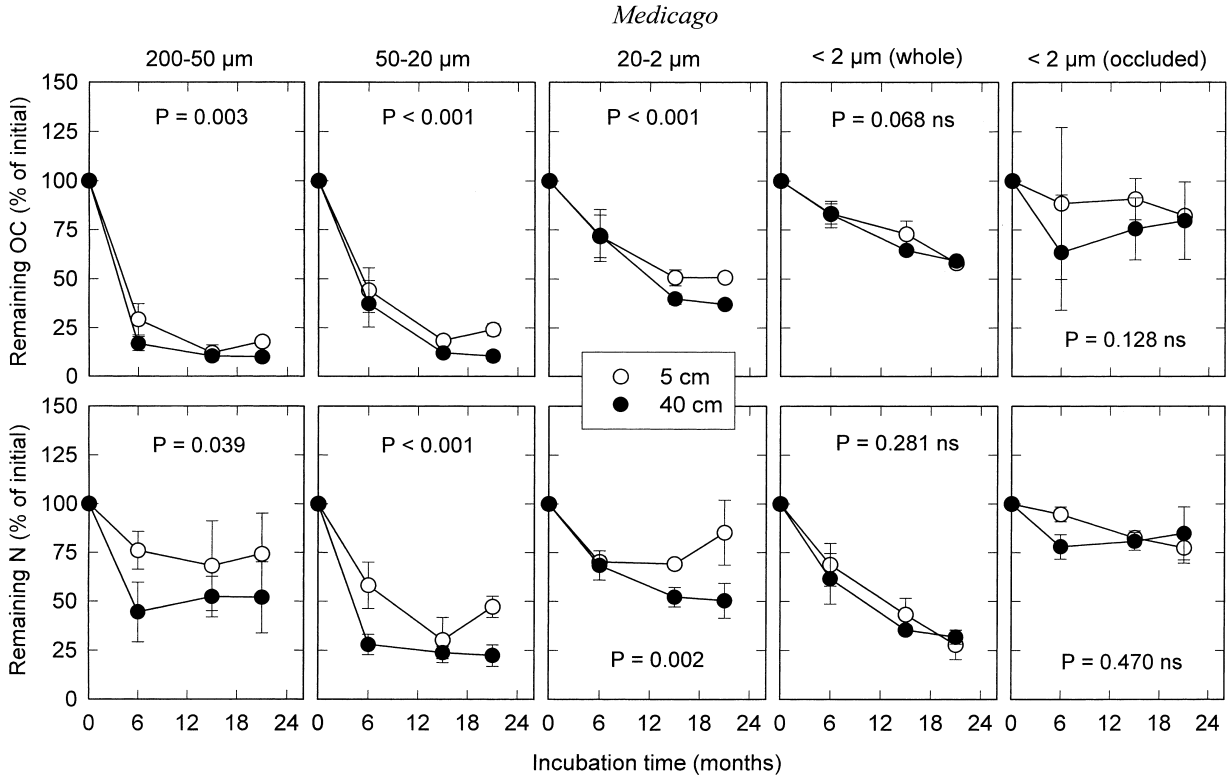

Fig. 2. Remaining OC and N in the size fractions <200 µm of *Medicago* mixtures. Points are averages of $n = 3$, vertical bars are standard deviations. The degree of significance of the effect of depth is given ($P > F$).

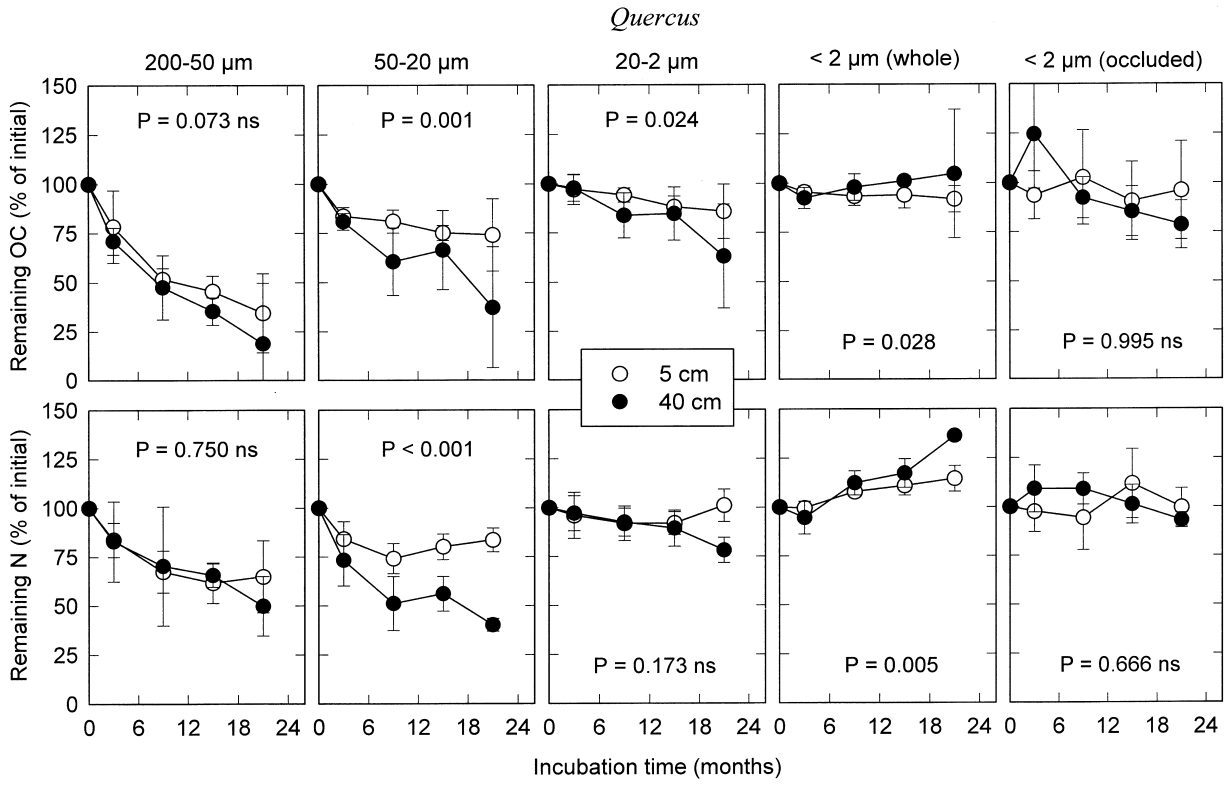

Fig. 3. Remaining OC and N in the size fractions <200 µm of *Quercus* mixtures. Points are averages of $n = 3$, vertical bars are standard deviations. The degree of significance of the effect of depth is given ($P > F$).

between *Medicago* and *Quercus* reflect the variations in the grinding process. OC is found mainly in fractions between 200 and 2 µm, whereas N clearly tends to accumulate in the finest fractions. As a result, the OC/N ratio tends to decrease with decreasing particle size.

The presence of OM in the fraction >200 µm was unexpected, since the ground plant materials were passed through a 200 µm mesh before mixing with the RE, and, therefore, it is assumed to be an artifact. The relative importance of this fraction is low for both OC and N (always less than 5% of the total).

After the densimetric treatment (complete method), the distribution of total OC and N between the size fractions was roughly maintained (Table 5), even though the patterns were distorted because of the solubilization of organic matter by the NaPT. Overall, in the fine fractions (<20 µm) the dense subfraction predominates, whereas in the coarse fractions (>20 µm), the light subfraction is dominant. The exception was the coarse sand (2000–200 µm), whose OC and N were mostly found in the dense subfraction.

3.3. OC and N dynamics in size fractions (simplified method)

As a general rule, for both *Medicago* and *Quercus* mixtures, the relative loss of OC decreases with decreasing particle size, reaching its minimal level in the case of clays (Figs. 2 and 3). For N, the result was less consistent. For *Medicago* mixtures, N losses were in the order coarse silt > clay (whole) > fine sand > fine silt > clay (occluded); that is, not related to debris size. In contrast, for *Quercus* the relation between debris size and N losses was roughly similar to that observed in the case of OC, i.e. the smaller the size, the smaller the losses. Only in *Quercus* mixtures, did clays act as a net sink for organic matter: for OC at 40 cm depth, and for N at all depths. Overall, the clay-occluded fraction was the most stable, both for OC (except in *Quercus* mixtures, at 40 cm) and N.

In *Medicago* mixtures, in most fractions, both OC and N losses were greater at 40 cm depth, in agreement with the result obtained for the whole sample (Fig. 1); the exception was the clay fraction, for which no significant effect of depth was detected. In *Quercus* mixtures, the effect of depth was significant only in some size fractions: for OC, in all but fine sand and clay-occluded, whereas for N, only in coarse silt and clays (whole fraction).

Because of our interpretation as an artifact (see Section 4), the dynamics of OC and N in coarse sand fractions (2000–200 µm) have been plotted apart (Fig. 4). They follow the above mentioned trends about depth (less remaining OC and N at 40 cm), but not the above mentioned trends with size, since they retain more OC and N (as % of initial) than fine sand (200–50 µm).

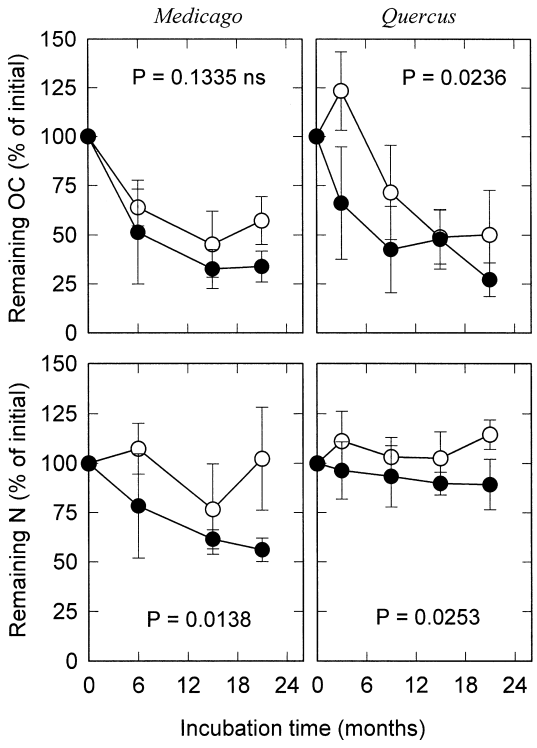

Fig. 4. Remaining OC and N in the coarse sand fraction (200–50 μm) of *Medicago* and *Quercus* mixtures. In our context, this fraction is assumed to be an artifact (see text). Points are averages of $n = 3$, vertical bars are standard deviations. The degree of significance of depth effect is given ($P > F$).

3.4. OC and N dynamics in density subfractions (complete method)

When applying the complete method (size + density: Figs. 5 and 6), the results were roughly similar to those obtained by the simplified method, with some exceptions. Fine silt, and especially clay, were the fractions that retained most OC and N, expressed as a % of their initial content. However, the ability to act as a net sink for OC or N, when it appears, resulted in the development of a very apparent light subfraction. In the dense subfraction, the relation between size and remaining OC was much less apparent.

In *Medicago*, for both OC and N and for both the light and the dense subfractions, the coarse silt (50–20 μm) was consistently the most labile pool. The relationship between size and OC or N losses was more marked in the light subfraction, because of the clear role of the fine fractions (<20 μm) as net sinks. However, in the case of clay, a light subfraction was not observed in one of the three replicates; this is why data dispersion is so high.

In *Quercus*, the results were less complex. In the light subfractions, the smaller the size, the higher was the remaining OC or N. A strong net increase was recorded for clay, which acted as a net sink for both elements. In the dense subfraction, no clear relationship between size and the remaining OC was observed, whereas the remaining N tend to increase with decreasing size.

In almost all size-density subfractions, the remaining OC and N were higher at 5 cm depth, even though, only in a few cases were the differences statistically significant

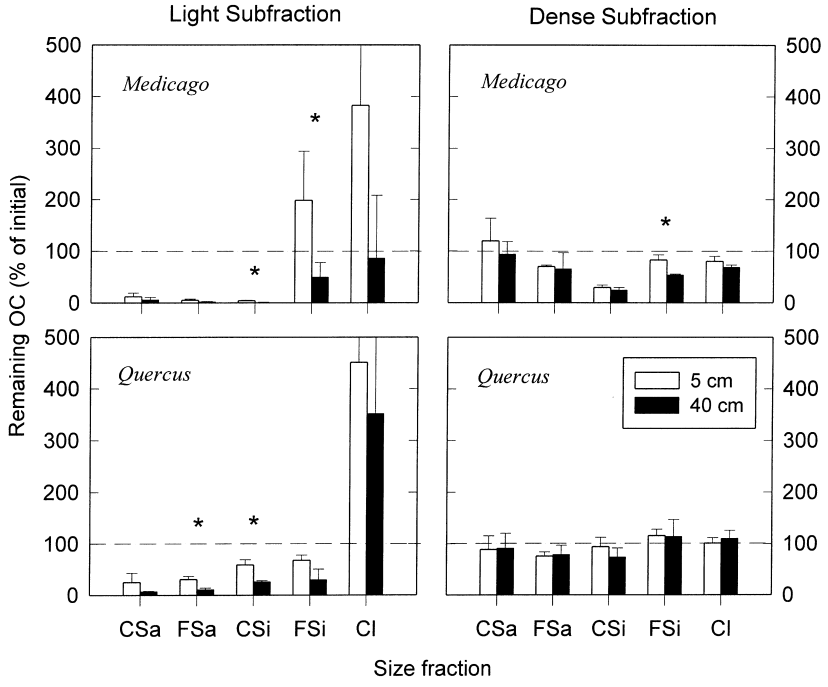

Fig. 5. Remaining OC in the several size + density fractions of *Medicago* and *Quercus* mixtures. When for a given fraction the effect of depth was significant ($P \leq 0.05$) it is marked at the top of the bars (*). Data are averages of $n = 3$, vertical bars are standard deviations. CSa: coarse sand, >200 μm; FSa: fine sand, 200–50 μm; CSi: coarse silt, 50–20 μm; FSi: fine silt, 20–2 μm; Cl: clay, <2 μm.

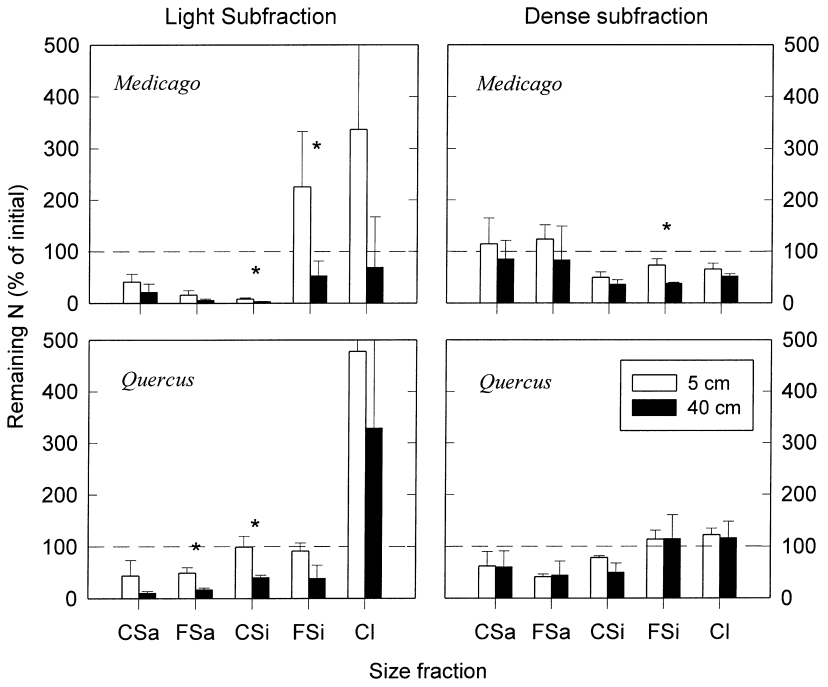

Fig. 6. Remaining N in the several size + density fractions of *Medicago* and *Quercus* mixtures. When for a given fraction the effect of depth was significant ($P \leq 0.05$) it is marked at the top of the bars (*). Data are averages of $n = 3$, vertical bars are standard deviations. CSa: coarse sand, >200 μm; FSa: fine sand, 200–50 μm; CSi: coarse silt, 50–20 μm; FSi: fine silt, 20–2 μm; Cl: clay, <2 μm.

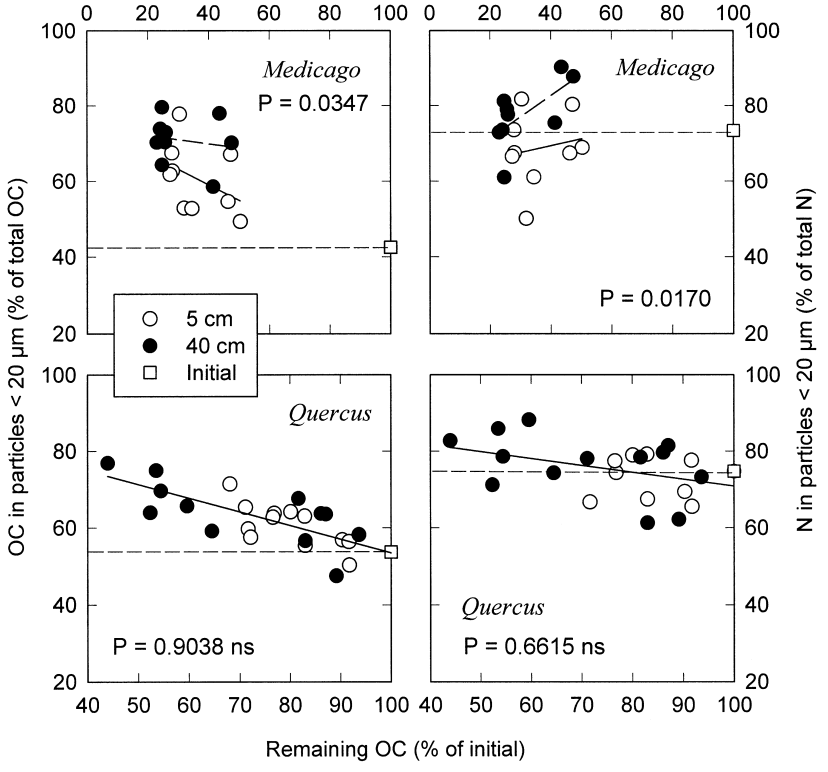

Fig. 7. Percent of OC or N in fractions <20 μm, against mineralized OC, for ANCOVA analysis. All the data have been included in the graph (not the means). The degree of significance of the effect of depth is given ($P > F$). The dotted line indicates the initial value in each case. For *Medicago*, since the behaviour of the mixtures incubated at 5 and 40 cm depth was statistically different, the regression line is shown for each depth; for *Quercus* a single regression line for all points is given.

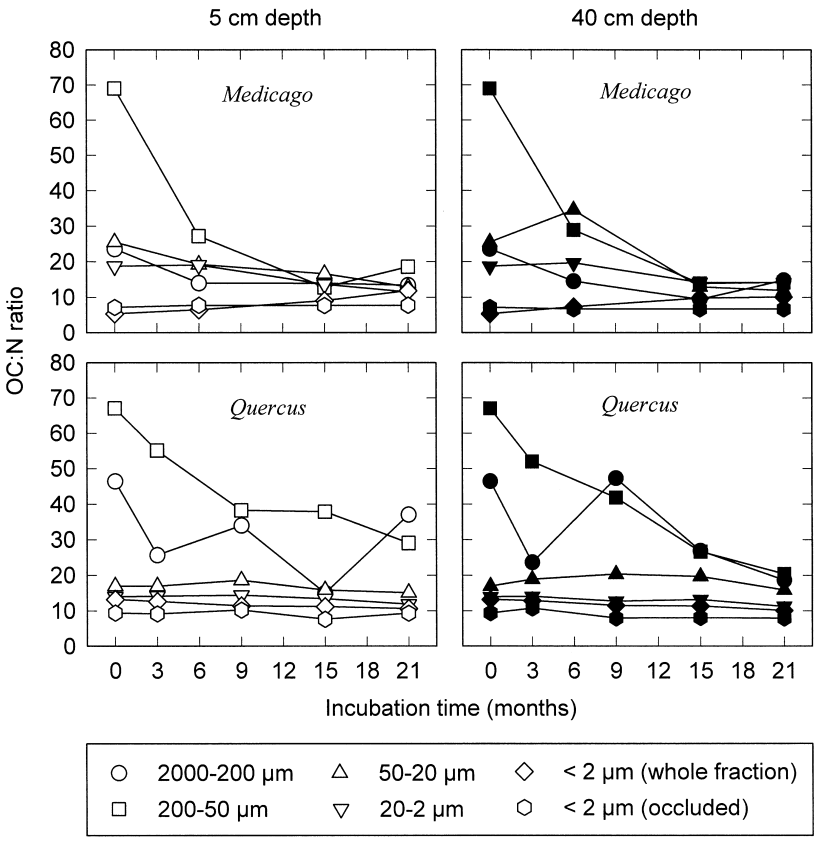

Fig. 8. OC/N ratios of the several size fractions, through the two years of field incubation. Data are averages of $n = 3$; standard deviations are not given to improve graph clearness. Mixtures incubated at 5 cm depth are shown in white, and those at 40 cm in black, for consistence with previous figures.

for a single subfraction, because of the high data dispersion.

3.5. OC and N in fine fractions (20 μm-ratio)

For OC, the relative content of particles <20 μm tend to increase in all cases (Fig. 7), reflecting the overall trend to a higher persistence of carbon in the fine fractions, and/or a redistribution of OC towards them. For N, the result was less clear; in *Medicago* mixtures, the 20 μm-ratio tend to increase at 40 cm depth, and to decrease at 5 cm. In *Quercus* mixtures, a slight trend to increase the 20 μm-ratio is clear, but it must be noted that the effect of covariate (total remaining OC) was at the limit of significance ($P = 0.053$), i.e., strictly speaking, the 20 μm-ratio did not change during the decomposition process.

In ANCOVA analysis, the effect of depth was significant for *Medicago* mixtures, hence in this case, the differences were not simply due to the faster OC and N mineralization at 40 cm depth, but reflected a different behaviour for OC and N, which tend to become displaced to finer fractions at deep layers. In contrast, no effect of depth was detected for *Quercus* mixtures.

3.6. OC/N ratios (Fig. 8)

As a general trend, the higher the initial OC/N ratio, the greater it fell. The evolution in the OC/N ratios was towards equilibrium. For instance, whereas at zero time the OC/N ratio of fine sand particles (200–50 μm) was more than three times greater than that of fine silt (20–2 μm) or clay particles (2 μm), after 21 months of field incubation, it was only twice as great in the case of *Quercus*, and even lower in the case of *Medicago*.

When the initial OC/N values were low (<20), they remained largely unaltered. As a rule, only for debris of 200–50 μm was a strong marked decrease in the OC/N ratio detected, in all cases. In the case of debris of 50–20 μm, the decrease was also great in *Medicago* mixtures, but not in *Quercus* mixtures.

4. Discussion

4.1. Methodological constraints

Redistribution of OC and N during fractionation. The fragmentation of debris and the extraction of soluble organic compounds, which can then become adsorbed onto fine silt and clay, is the main drawback of sonication (Bruckert, 1979; Elliott and Cambardella, 1991), and results in an overestimation of the OC and N contents of the fine fractions. However, our results indicate that redistribution can also

occur in the opposite direction, i.e. debris of a given size may form aggregates of greater size. Agitation caused by the ultrasonic treatment may lead to intense friction between particles; these may have become electrically charged and, therefore, tend to flocculate, probably through soluble salts, which were also partly released by ultrasonic treatment. In our context, since only debris <200 μm was used for the experiment, the fraction >200 μm is assumed to be an artifact of the method, and is not considered in the discussion.

Recovery of OC and N in the fractionation procedures. The recovery of OC and N is expected to increase during the experiment, as the most labile and easily solubilized compounds are lost and only the resistant ones—poorly soluble polymers, protected compounds—remain. For the simple method (size fractionation), in the case of *Quercus*, the recoveries were high: 98.1% of OC and 93.1% of N at the start. These percentages were roughly maintained throughout the experiment (not shown). In contrast, for *Medicago* mixtures, the initial recoveries were low, especially for N (87.4% of OC and 50.3% of N), even though they increased strongly thereafter. This result was expected, because the *Medicago* plants used were green and fresh. In the *Medicago* mixtures, at the start of the experiment, about 20% of total OC and 25% of total N was extractable with boiling water, whereas after two years of field incubation, these amounts were reduced to 5% of total remaining OC and N (unpublished results). The increase in the recovery of OC and N explains the observation that in *Medicago* mixtures the relative losses of N of all size fractions were smaller than that of the whole N. If the N of some fractions is lost more slowly than that of whole N, there must be at least one fraction in which N is lost more quickly. In our case, this fraction was probably that included in the unaccounted N, mostly soluble, easily extractable organic compounds, but probably also small fragments released by sonication. As a result, the relative losses of N in *Medicago* mixtures are underestimated at least for some size fractions, and perhaps for all.

For the complete method, i.e. size plus density fractionation, additional losses of OC and N are expected, because NaPT solubilizes OM. In our case, the recovery of N decreased slightly (in the initial samples, 47.5% in *Medicago* mixtures, 92.9% in *Quercus*), whereas that of OC increased, reaching >100% (101.9% in *Medicago*, 106.8% in *Quercus*). These results indicate that most solubilizable nitrogenous compounds had already disappeared during size fractionation, whereas a contamination in OC must have occured during density fractionation, probably via NaPT, which contains OC as impurity (about 1.2 mg g^{-1}: Six et al., 1999). Additionally, in calcareous soils, the generation of highly insoluble calcium polytungstate (CaPT) is expected. In our samples this precipitate, which may include small amounts of OC, was almost always present, as a fine white powder. It was not possible to eliminate it by repeated centrifugation and decantation. The accumulation of CaPT in the density subfractions may account for the observation that in the clays, the OC concentration in the light subfraction was lower than that in the dense (Table 5), probably because of an artifactual increase of weight in the light subfraction, caused by the accumulation of CaPT.

In summary, the OC contents of the fractions obtained in the complete method can be overestimated with respect to those obtained in the simple method, and, therefore, so too can the OC/N ratios.

Exclusion of soil fauna during the decomposition. The incubated mixtures were separated from the surrounding soil by a mesh, which was fine enough (500 μm) to hamper the income of part of soil fauna. Hence, the impact of soil fauna on the processes studied is not addressed in the present study.

4.2. Species and depth effects

The dynamics of OC and N in any size fraction is the result of several parallel processes. First, the *inputs* to the fraction: (i) the income of debris, by the fragmentation of debris of greater size, and (ii) the adsorption of soluble OM. Second, the *outputs*: (iii) fragmentation, to give debris of smaller size, (iv) solubilization of labile OM (desorption), (v) mineralization, to give both CO_2 and NH_4^+, and (vi) the leaching of soluble material, which involves the loss of OC and N from the whole system. Hence, the decay of OC and N in a given fraction cannot be attributed only to their mineralization, because the physical transfer of OM from the coarse fractions to the finer ones should also be taken into account. The higher water availability in deep horizons accelerates all the above processes: both the overall decomposition and the losses of OC and N from the size fractions were usually faster at 40 cm depth; when a size fraction tends to accumulate OC or N, this accumulation was also usually faster at 40 cm depth.

The effect of depth overlaps with that of the type of incubated plant material. A species effect was expected, since *Medicago* debris was green and fresh, whereas *Quercus* was dead-brown litter, and there were large differences in composition. For *Quercus* mixtures, the lack of effect of depth in ANCOVA indicates that at 40 cm all the above mentioned processes were accelerated harmonically. In contrast, the clear effect of depth for *Medicago* mixtures suggest that, at 40 cm, the processes that enhance the fine fractions (fragmentation, rapid decomposition of coarse debris) were more accelerated than decomposition as a whole. The high lability of *Medicago* may hamper the transfer of soluble OM to the smallest fractions, because solubilized OM may be quickly decomposed soon after release. This may explain why the clay was a net sink of OC and N in *Quercus* mixtures, especially at 40 cm, whereas this was not observed in *Medicago* mixtures.

4.3. Comparison of OC and N dynamics

The relationship between fraction size and OC dynamics

has been studied through the isolation of the fractions and their separate incubation (Christensen, 1987; Gregorich et al., 1989; Nelson et al., 1994), through the study of the changes in the isotopic ratios ($\delta^{13}C$) of the several fractions, usually related to changes in crop or vegetation type (Balesdent, 1996; Saviozzi et al., 1997), through the correlation between the global respiration rate and the amount of a given fraction (Janzen et al., 1992), and by studying the distribution of an organic substrate through the several size fractions in incubation experiments (Stemmer et al., 1999). Irrespective of the approach chosen, the coarsest fractions are usually the most labile ones, which is attributed to the greater physical protection of the smallest particles (silt and clays). In our experiment, we also observed this pattern, which seems quite robust, since it was not affected by the large differences in OM quality of the different fractions. For instance, debris of 200–50 μm had the highest initial OC/N ratio, especially in *Quercus*. Nevertheless, its decomposition was the fastest.

In contrast, the results for N are conflictive. The N found in particulate OM (either light OM, or POM > 50 μm) correlates well with the N taken up by the crops (Barrios et al., 1996; Vanlauwe et al., 1998, 2000). Hence, this fraction seems to provide most of the available N in arable fields, in spite of the much higher quantitative importance of the fine fractions as N pool (Lehmann et al., 1998; Vanlauwe et al., 1999). In contrast, studies in which size fractions are isolated and incubated separately have shown that N availability increases with decreasing size, being highest for clay-associated N (Chichester, 1969; Christensen and Olesen, 1998). These discrepancies suggest that the physical availability and the intrinsic mineralizability (quality) of N do not coincide. The N associated with fine particles may be easily mineralizable after isolation, but in intact soil samples, it may remain protected, poorly available to microbes. Hence, the coarsest fractions may become, in practice, the main source of N for plant roots or soil microflora.

Our results do not show a consistent pattern for N behaviour: the relative losses of N were either unrelated to the size (*Medicago*), or decreased slightly with decreasing size (*Quercus*; but the overall result was doubtful, as mentioned above). For N, in addition to the size, other constraints must be taken into account, such as the differences in OC/N ratio of the size fractions. In *Medicago* mixtures, in debris of 200–50 μm, the losses of OC were the highest, whereas those of N were the lowest: this can be explained by its OC/N ratio, the highest of all the size fractions, which should lead to a strong retention of N. However, the OC/N ratio alone does not explain the differences in N dynamics: in *Medicago* mixtures, the order in which the N of the several size fractions was lost never agreed with the inverse order of their OC/N ratios. Other processes, such as the active incorporation of N by microflora, or the abiotic adsorption by clays of NH_4^+, peptides or exoenzymes, also affect N dynamics in size fractions. N spreads among the size fractions much more easily than OC (Stemmer et al., 1999).

Owing to these complex interactions, overall, it is not possible to detect a clear relationship between debris (or particle) size and N losses, as shown by the rough maintenance of the 20 μm-ratios for N, in contrast with the slight but continous increase observed for OC. However, our data do not agree with the thesis of a higher N availability in fine fractions, since the pattern mentioned by Christensen and Olesen (1998), i.e. an increased relative loss of N with decreasing size, was not observed in any case.

4.4. Dynamics of density subfractions

In our study, the most apparent trend was the great net increase in the light subfraction of clays (<2 μm), and in some cases, in that of fine silt (20–2 μm), whereas the corresponding dense subfractions either slightly decreased (*Medicago*) or were roughly maintained (*Quercus*). The differentiation of a light subfraction for fractions <20 μm indicates heterogeneous incorporation of OM. Heterogeneity depends on the replicate, since in some cases, a light subfraction was not obtained for clays. This explains the very high variability experienced by the increase in the OC and N of the light subfraction of clays. After the density fractionation, the OM-richest particles become part of the light subfraction, whose quantitative importance increases, even though, the overall fraction (whole fine silt, whole clay) may not be a net sink for OC and N: it was in *Quercus*, but not in *Medicago* mixtures.

For the coarse fractions, the question is whether their dense subfractions are simply artifacts of the separation method. Substantial amounts of fresh debris may co-precipitate with the dense fraction immediately after being added to the soil sample (Magid et al., 1996; Rovira et al., 1998). Hence, the maintenance of these subfractions may merely reflect the maintenance of the amount of co-precipitated debris. Nevertheless, the lower initial OC/N ratios of the dense subfractions, and their distinct behaviour indicate that the dense subfractions represent a differentiated OM pool. A decrease in the OC/N ratio with increasing density, in the macroorganic matter, was also observed by Hassink (1995a) and Meijboom et al. (1995) using Ludox as density agent, even though these authors used quite low densities (up to 1.4; we used 2.0). In long-term studies, the lightest subfractions decompose faster (Hassink, 1995b), which is in agreement with our results.

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