Short communication

Humus quality after eucalypt reforestations in Asturias (Northern Spain)

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Abstract

The impact on soil properties of recent and ancient reforestations with *Eucalyptus globulus* Labill. has been studied in Cambisols and Leptosols under temperate semi-oceanic climate in Asturias (Northern Spain). Eucalypt forests showed a high potential of C accumulation in soil, mainly the ancient forest, where the organic matter concentration in the uppermost horizon (up to 415 C g kg^{-1}) was greater than in all neighbouring soil formations (climacic oak forests, up to 84 C g kg^{-1}; Podsol under reforested pine forests, 287 C g kg^{-1}; and *Ulex* shrub, 70 C g kg^{-1}). Nevertheless, there was a low concentration of available bases and a very low transformation degree of the organic matter in this eucalypt forest, only comparable to that of the Podsol. In order to analyse the major features of the biogeochemical behaviour of the sites under study, the humus fractions were isolated and the humic acid fraction was studied by derivative visible and infrared spectroscopies. Incubation experiments on whole soil samples were carried out to monitor stability against biodegradation of the organic matter under comparable conditions. In general, soils under eucalypt had a CO_2 production similar to or lower than the control sites, but the soil under the ancient eucalypt forest had a high potential for CO_2 release, similar to that of the neighbouring Podsol, what might be explained by the presence in these soils of high amounts of C accumulated in readily biodegradable forms. However, the mineralisation coefficients (CO_2 released per unit of soil C) indicated that the biodegradability of the soil humus has decreased significantly in the eucalypt-reforested sites, as it could correspond to an effective control of the biogeochemical processes caused by the antimicrobial products from the eucalypt litter. Compared to the original deciduous forests, the lower values of the E_4/E_6 ratio in the humic acids from eucalypt forests and the lignin signature in the infrared spectra point to humification mechanisms based on the selective preservation of complex macromolecular substances derived from vascular plants. The second derivative of the visible spectra of the humic acid samples under study showed conspicuous valleys, which are ascribed to fungal-derived 4,9-dihydroxyperylene-3,10-quinone pigments. These valleys were in most eucalypt forests significantly shallower than in the original deciduous forests and may also represent an indicator of the impact of introduced vegetation on the structure of the soil microbial system and the mechanisms responsible for the formation of humic acids.
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1. Introduction

Recent environmental research is paying increasing attention to the assessment of analytical indicators of the impact on soil of extensive reforestation practices such as those required for wood extraction and pulp and paper industries. The introduction of allochthonous tree species in Mediterranean areas is often associated to significant changes in soil quality, especially reflected in organic matter turnover mechanisms (Almendros et al., 1983). In most cases the chemical composition of plant biomass (not only the major structural biomacromolecules, but also simple compounds with intense allelopathic function) has a substantial bearing on soil carbon sequestration mechanisms by modifying the balance between the preservation of microbially reworked plant inherited macromolecules and the neoformation of colloidal humic substances (Nilsson and Schopfhauser, 1995).

Even though an increase of the total rates of carbon sequestration in soil through environmentally-friendly soil and forest management practices is considered to reduce the effects of global warming produced by the atmospheric concentration of greenhouse gases (Batjes, 1998), it is also to take into account that most biogenic macro- and microelements can also be immobilised in low availability forms. Thus, primary productivity of the whole ecosystem can substantially decrease in the long term due to a reduced biogeochemical performance. For this reason, ecological research about the impact of forest vegetation on the functionality of the trophic system should pay special attention not only to total C in soil, but also to changes in soil humus quality (Almendros and Velasco, 1984; Keith et al., 1997; Aggangan et al., 1998). In fact, the humus quality, defined in terms of its potential to contribute to soil resilience and sustainability, is of prime interest in reflecting and forecasting the dynamics of the whole ecosystem. Fast-growing forest species producing antiseptic extractives, such as phenols or resins with a depressive effect on the soil enzymatic activity, are often associated to the accumulation of thick humiferous soil horizons (Duchaufour, 1977; Da Gama-Rodrigues et al., 1997). This is the case

with most conifer forest areas in Northern Europe. This organic matter, however, exhibits a low maturity degree (*moder* or *mor* humus type) and it is possible that a slight climatic change producing a raise in temperature of e.g. only two degrees, would turn these ecosystems into continuous CO_2 sources (Goudriaan, 1995). At this point, the analytical characterisation of the soil organic matter has a special interest in order to detect humus formations with high stability—maturity—degree, where the mineralisation rates are comparatively little affected by climatic changes or management practices (Lal and Kimble, 1997). With some criterion, and in the case of fragile and active Mediterranean ecosystems, such qualitative factors should be considered of greater importance than the actual amount of C in the soil.

The use of fast-growing tree species with small nutritional requirements has been encouraged in reforestation policies in Spain for the last fifty years, based on the empirical statement that no substantial improvement in soil properties is obtained when low-quality soils are reforested with the so-called ameliorant species (Almendros and Velasco, 1984; Rahman and Field, 1999).

In the Iberian Peninsula there exists a large concentration of *Eucalyptus globulus* Labill. as regards the rest of Europe (FAO, 1981). However, the intense evapotranspiration characteristic of this species, required to concentrate on the nutrients in the soil solution, in addition to the release to soil of compounds (α- and β-pinene, eucalyptol, phellandrene, terpineol, citronellal, eudesmol, etc.; Guenther, 1950; Brophy et al., 1998) with a depressive effect on soil microbial populations, have often discouraged the introduction of this tree species (Velasco de Pedro and Lozano, 1979). Nevertheless, the situation can be substantially different in areas where the climate has oceanic features as in the case of Northern Spain. In this area the high levels of soil moisture during most seasons, in addition to the probability of leaching of some toxic eucalypt metabolites, may conform an adequate scenario for stable ecosystems mainly in mountain areas with few possibilities of alternative uses (Diaz González and Fernández Prieto, 1988; Calvo de Anta, 1992).

Assuming the above considerations, the present study deals with an analytical comparison between soils from climacic forest ecosystems and from eucalypt–reforested sites in Asturias (Northern Spain). Besides the soil characteristics, including quantification of soil C and nutrients in the different sites, the C mineralisation curves and some chemical features of the humic acids are described. Considering that the soil organic matter represents the most valuable source of routine analytical descriptors for the early diagnosis of soil degradation caused by external perturbations, the interpretation of the experimental results is conducted in the sense of inferring some functional relationships between humification processes and long-term environmental quality of eucalypt reforested areas.

2. Materials and methods

2.1. Sampling sites

The studied area is located in Asturias (Northern Spain). The topography is quite abrupt, the geological substrate consisting mainly of metamorphic (quartzites and schists) and sedimentary (sandstones) rocks. The slope is in average higher than 20%. The altitude ranges between 100–300 m.a.s.l., occasionally higher than 600 m.a.s.l. The climate is temperate, semi-oceanic, with a mild and short winter season, the average temperature being approximately 13 °C and the rain reaching ca. 1400 mm. The potential vegetation consists of mesophyllic forest (*Quercus* spp., *Alnus glutinosa* (L.) Gaertner, *Arbutus unedo* L. and Ericaceae). The brushwood formations include *Ulex europaeus* L. and *Erica* spp. (Diaz González and Fernández-Prieto, 1994). The dominant phytosociological formation (Blechno-Quercetum roboris S.) is mostly oligotrophic, eutrophic oak forest lacking practically (Mayor López and Díaz González, 1977a,b).

The sites selected for the study (Table 1) include: i) undisturbed soils with the potential undisturbed original vegetation (labelled with 'C': control soils), ii) soils under *E. globulus* (labelled with 'E'), corresponding to both ancient, >20 years (E0) and recent reforestations and iii) representative neighbouring disturbed soils (labelled

with 'B' and 'G'), either reforested with pine and encroached by heather, or cleared (brushwood and herbaceous layer). When the soil profile showed a substantial evolution (E1, B1), more than one soil horizon was studied, the corresponding soil samples being labelled with a letter and a two-digit number. Vegetation, sample labels, horizons depth and soil and humus types are indicated in Table 1.

A series of replicated composite soil samples was prepared by mixing three spatial replications in a 10 m^2 area, collected with a spade and stored in polyethylene bags. The soil samples were air-dried and homogenised to 2 mm (fine earth) on a table with a wooden cylinder. The granulometric analysis was carried out following Kilmer and Alexander (1949). Soil pH was measured in water (1:2.5 wt:vol) with a combined glass-calomel electrode. Total N was determined by micro-Kjeldahl digestion and the available P was extracted by the Bray and Kurtz (1945) method. The major available cations (K, Ca and Mg) were extracted at pH=7 with 1 mol l^{-1} NH$_4$OAc.

Total soil C was measured by dry combustion (no carbonates in the soils) using a Wösthoff furnace attached to a gas analyser Carmhograph-12. The soil organic fractions were quantified with the Walkley and Black wet oxidation method (Nelson and Sommers, 1982).

2.2. Soil humus fractions

Different procedures were sequentially employed for the isolation of the humus fractions from 20 g of soil sample (Fig. 1), taking advantage from methods described by Dabin (1971), Merlet (1971) and Duchaufour and Jacquin (1975). A first physical separation of the soil light fraction consisting of the fairly decomposed organic particles was carried out with 2 mol l^{-1} H$_3$PO$_4$ (rotary stirring for 1 min). The floating particulate fraction was referred to as *free organic matter*. The yellowish supernatant solution containing the organic matter dissolved in the H$_3$PO$_4$ solution was kept for quantification of this *fulvic acid* (FA) fraction, which presumably consists of a fulvic fraction, less firmly associated to the soil matrix than the corresponding fraction removed with the further NaOH extractions described below.

Table 1
General characteristics of the samples under study in Asturias (Northern Spain)

| Sample label		Geological substrate	Horizon (depth cm)	Vegetation	Location	Soil type	Humus subtype[a]
C1		Quartzites, schists	Ah (0–5)	Chestnut, oak, *Alnus* sp. laurel, ferns	Valle de Arenas	Umbric Leptosol	Oligotrophic *mull*
C2		Quartzites, sandstones	Ah (0–30)	Oak, birch, chestnut, cherry	Sierra Chamiecha	Humic Cambisol	Oligotrophic *mull*
E0		Colluvial quartzites, sandstones	Ah (0–5)	Ancient eucalypt forest	Sierra Chamiecha	Humic Cambisol	Mull-*moder*
E1	E11	Colluvial quartzites	Ah (0–45)	Heather, eucalypt	Valle de Arenas	Humic Cambisol	Inactive *mor*
	E12	Sandstones	Bwh (58–90)				
E2		Quartzites, schists	Ah (0–15)	Eucalypt forest	Campa Arenas	Umbric Leptosol	Forest *moder*
E3		Quartzites, sandstones	Ah (0–44)	Eucalypt forest, many ferns	Slope Cou Verdugo	Humic Cambisol	Acid *mor*
E4		Quartzites, sandstones	A (0–19)	Eucalypt forest	Near Cou Verdugo	Humic Cambisol	Inactive *mor*
B1	B11	Sandstones	Ao (0–8)	Heather, pine forest	Alto La Cabeza Arenas	Haplic Podsol	Acid *mor*
	B12		Ah (8–42)				
	B13		Ahs (42–48)				
G1		Quartzites, sandstones	Ah (0–30)	Poaceae, *Ulex* sp.	La Pla'l Gallo	Humic Cambisol	Forest *moder*

[a] Duchaufour, 1975.
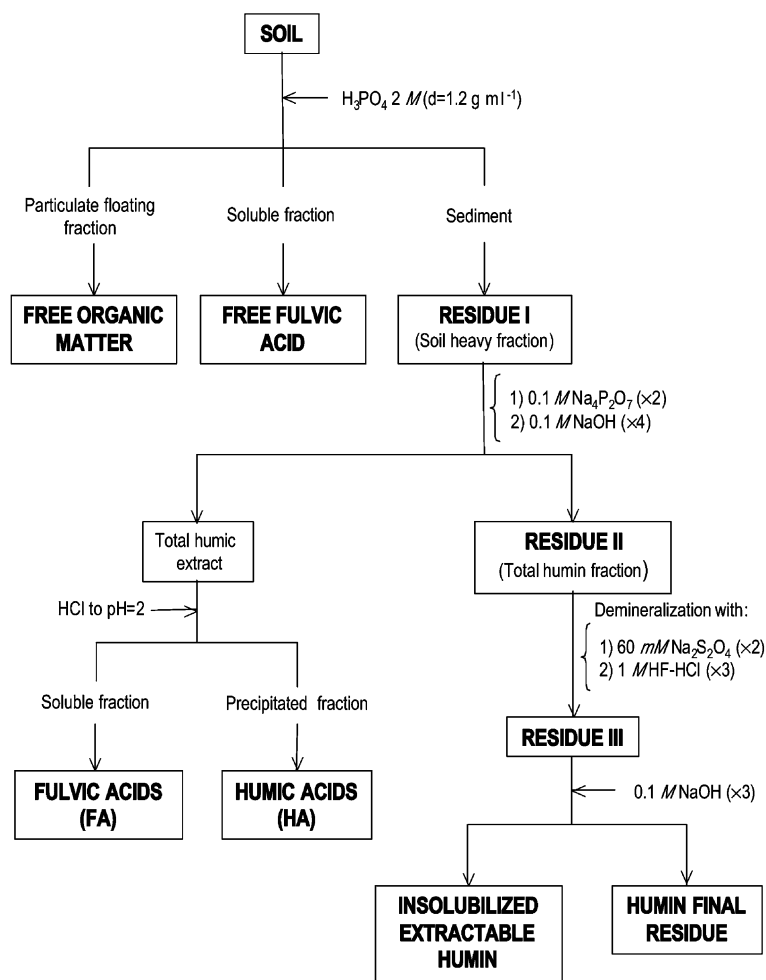
Fig. 1. Flowchart showing the fractionation procedure of the soil organic matter.

The resulting soil extraction residue (I) was successively extracted with 0.1 mol l^{-1} $Na_4P_2O_7$ and 0.1 mol l^{-1} NaOH. This extraction (horizontal motion mechanical shaking for 3 h) was repeated up to 8 times. Aliquots of the dark brown surnatant solution, corresponding to the *total humic extract*, were precipitated with H_2SO_4 (1:1 by vol.) and used for quantitative determination of the acid-soluble FA and the acid-insoluble *humic acid* (HA) fraction.

The alkali-extracted soil residue (II) was subjected to successive treatments with a mixture of 60 mmol l^{-1} $Na_2S_2O_4$ and 1 mol l^{-1} HCl–HF (1:1 by vol.) at 60 °C to remove the Al and Fe oxides and the clay, respectively. These pretreatments were repeated a total of 3 times, and the surnatant solutions were discarded. Afterwards, the soil residue (III) was extracted with 0.1 mol l^{-1} NaOH to obtain the humic colloids which were tightly associated with the mineral matrix (referred to as *insolubilised extractable humin*).

2.3. Purification of the humic acids

From the total humic extract, the HAs were precipitated by adjusting to 2 the pH with HCl,

Table 2
General analytical characteristics of the soil samples

		C1	C2	E0	E11	E12	E2	E3	E4	B11	B12	B13	G1	*LSD*
Total sand (2–0.02 mm)	/g kg^{-1}	56.7	59.4	82.5	70.3	53.5	79.8	84.8	75.0	82.8	83.1	65.4	72.7	11.3
Silt (0.02–0.002 mm)		21.1	30.0	13.5	14.0	28.2	14.3	11.8	16.8	13.2	13.7	15.1	14.9	5.9
Clay (<0.002 mm)		22.2	10.6	9.6	15.4	18.3	5.9	3.4	8.2	4.0	3.2	19.5	12.4	11.1
pH (H$_2$O)		4.4	4.5	5.8	3.8	3.7	4.5	3.4	4.0	3.6	3.8	3.7	4.4	1.07
Soil C	/g kg^{-1}	59	84	415	126	122	208	75	146	287	81	17	70	11
Soil N		2.7	4.2	17.1	2.2	2.4	9.0	2.6	4.3	7.9	2.4	0.7	3.1	2.3
C/N ratio		21.6	19.7	24.3	56.3	49.8	23	29.0	33.9	36.4	34	24.3	21.6	11
Available P	/mg kg^{-1}	1.0	0.5	3.5	0.5	0.5	1.5	1.0	1.0	2.5	1.0	0.5	2.0	0.81
Available K		30.0	31.0	3.5	12.5	8.0	18.0	7.5	13.5	15.5	6.0	3.5	12.0	7.2
Available Ca		18	173	11	1	1	6	10	6	22	4	2	8	13
Available Mg		9	45	3	3	2	11	6	10	18	3	1	5	13

Sample labels refer to the Material and methods section.

LSD = Least significant difference between soil samples.

then de-ashed with 1 mol l^{-1} HCl–HF treatments at room temperature for 12 h, redissolved in 0.5 mol l^{-1} NaOH and centrifuged at 43 500 *g*. The soil residue was discarded, and the brown surnatant solution of sodium humate was reprecipitated with HCl and dialysed in cellophane bags to remove the salts introduced during the extraction procedure. The HA was freeze-dried and kept for further chemical characterisation.

2.4. Soil respiratory activity

In vitro determination of the intrinsic biodegradability of the soil organic matter is especially relevant as regards to forecasting soil C sequestration processes. Raw values, i.e. referred to soil weight, indicate the bulk potential CO$_2$ release of the different soils, whereas the values calculated per unit of soil C are more indicative of the maturity or stability of the soil organic matter.

In order to compare the potential biodegradability and mineralisation kinetics of the soil organic matter, the in vitro soil respiratory activity was periodically determined (two replicates) as the CO$_2$ released by samples of 20 g of soil homogenised to 2 mm. The moisture was set to 60% of the soil water holding capacity at atmospheric pressure and the temperature to 27±1 °C (Chone et al., 1973). The CO$_2$ evolved was measured daily for a 2-week period with the Carmhograph–12 gas analyser. The soil samples were incubated in 250 ml Erlenmeyer flasks with rubber stoppers perfo-

rated to introduce two polyethylene tubes. To analyse the CO$_2$ concentration in the flask atmosphere the inlet and outlet tubes were connected to the CO$_2$ analyser (which includes a suction pump), and to a soda-lime column, respectively (Almendros et al., 1990). The mineralisation of the organic matter was expressed as mg of C released per soil weight unit and per day, as well as in relative terms, i.e. mineralisation coefficients (C mg released per kg of soil C and per day).

2.5. Humic acid characteristics

The optical density of the HAs was measured from solutions of 100 mg l^{-1} C in 0.04 mol l^{-1} NaHCO$_3$. The visible, second-derivative spectra were acquired from solutions of 100 mg l^{-1} C in 0.02 mol l^{-1} NaHCO$_3$. For infrared (IR) spectroscopy, a Bruker IFS28 Fourier-transform spectrophotometer was used. The digitised spectra were transferred to a personal computer and subjected to a procedure for resolution enhancement based on the subtraction of the raw spectrum from a positive multiple of its 2nd derivative (Rosenfeld and Kak, 1982; Almendros and Sanz, 1992).

3. Results and discussion

3.1. General analytical characteristics

In general, the studied soils showed acid pH values with no significant differences in terms of

vegetation (Table 2). The high pH value observed in the ancient eucalypt forest E0 may be due to the large accumulation of organic matter in this soil, which would exert a buffering effect on the soil solution. The occurrence of acid soils, under udic soil moisture regime, is frequent in Northern Spain. Soil drainage, runoff and mild temperature favour such acidic conditions, especially when the soil is developed on quartz rocks or sandstones.

Available P concentration is generally associated to the levels of organic matter and displayed common patterns with the other major nutrients, the eucalypt forests E0 and E2 and the topsoil of the Podsol (B11) showing the highest nutrient concentration (Table 2). Concerning the available cations, K was the most responsive to the loss—or migration—of soil fertility, where the original forest has been removed. With the exception of sample C2, soils showed reduced concentration of Ca^{2+} and Mg^{2+}, which tended to decrease with depth. The low amount of these elements, even in the case of the ancient eucalypt forest E0, where the highest accumulation of organic matter was observed, illustrate the low potential of this species to accumulate plant nutrients in the topsoil (Velasco de Pedro and Lozano, 1979). In short, the fertility of the analysed soil is medium-to-low, the differences between oak, pine and eucalypt being very small and interpreted as an effect of the leaching processes and the relatively homogeneous lithological substrate. Eucalypt reforestation led to increased organic matter accumulation in soils, mainly in the ancient forest E0, where the soil C in the upper horizon was even greater than in the neighbouring Podsol under heathers and pines. In the studied area, the high C levels could be explained by the litter characteristics, because the high soil C accumulation coincides with the occurrence of plant species producing compounds with antimicrobial effects, typical of pine, heather and eucalypt leaves. The soil C/N ratios point to low-quality humus in the reforested sites, where these ratios were similar or greater than in the Podsol, this making evident a humus evolution towards *mor* type (Table 2). In fact, progressive increase in soil C/N ratio in an age sequence of eucalypt plantation was also observed by Bargali, 1996.

3.2. Soil humus fractions

The quantitative distribution of the different humus fractions (Table 3) suggests a very low transformation degree of the organic matter in the ancient eucalypt forest (E0), only comparable to that of the Podsol (B11). About half of the total soil C remains in the form of organic wastes (free organic matter) in these soils and the amount of colloidal humic substances associated to the mineral soil fraction (HA+FA) only represents less than 20% of the total soil C. This low transformation of the organic matter indicates that the prevailing mechanisms of C sequestration in these sites corresponds to a selective preservation of particulate organic fractions inherited from plant litter, with a small contribution of the colloidal fractions.

When the amount of the different humic fractions is calculated as a percentage of the total soil C (Fig. 2), the humus quality can be more straightforwardly compared. Control soils (C1, C2) show a relatively high maturity of the organic matter: most of the C is accumulated in the form of non-extractable humin and approximately 20% of the total C corresponds to stabilised colloidal fractions (HA+FA) with the HA/FA ratio between 2 and 4. Reforestation with acidifying species (both eucalypt and pine forests on the Podsol) is associated with the accumulation of slightly transformed particulate organic matter where, presumably, macro- and micronutrients are immobilised. It is to point out that two eucalypt forests (E11, E3) are associated to humus formations of similar or higher evolution degree than the neighbouring climacic forests (C1, C2), as it could correspond to the comparatively recent reforestation (< 10 years) in these sites.

The amount of the FAs directly removed with 2 mol l^{-1} H_3PO_4 during the isolation of the free organic matter (Table 3) could be interpreted as an indicator of stability of the humus formations, where no intense leaching of secondary soluble fractions is produced. In fact, the concentration of these FAs tends to be higher in soils with low amounts of free organic matter, thus indicating that this fraction does not likely consist of extractives from the particulate plant wastes in soil, but to

Table 3
Distribution of the total soil C into different organic fractions[a]

Sample label	Free organic matter	Total humic extract	Humic acid (HA)	Fulvic acid (H_3PO_4–extracted)	Fulvic acid (NaOH–extracted) (FA)	HA/FA	Insolubilized extractable humin	Non-extractable humin
	/C g kg^{-1} soil						/C g kg^{-1} soil	
C1	3.0	11.1	7.8 (15)	3.32	3.4 (6)	2.25	2.8 (5.2)	38.8 (74)
C2	9.5	15.9	12.7 (17)	0.40	3.2 (4)	3.91	1.8 (2.4)	56.3 (76)
E0	221.0	36.7	22.2 (11)	0.38	14.5 (7)	1.54	4.7 (2.4)	152.6 (79)
E11	12.6	23.2	18.3 (16)	2.01	4.9 (4)	3.70	3.2 (2.9)	85.0 (76)
E12	2.0	23.4	11.2 (10)	9.48	12.2 (11)	0.92	2.7 (2.4)	84.4 (76)
E2	66.9	37.5	17.5 (12)	0.61	19.9 (14)	0.88	2.6 (1.9)	100.3 (71)
E3	6.1	26.9	14.9 (22)	0.20	12.0 (17)	1.24	2.1 (2.9)	39.9 (58)
E4	46.0	21.7	9.6 (10)	0.72	12.1 (12)	0.79	2.6 (2.6)	74.9 (75)
B11	144.3	27.6	17.7 (12)	0.61	9.9 (7)	1.78	2.2 (1.5)	112.2 (79)
B12	9.3	27.7	18.8 (26)	0.25	8.9 (13)	2.09	2.2 (3.1)	41.5 (58)
B13	0.6	12.3	1.6 (10)	3.14	10.7 (66)	0.15	2.8 (17.4)	1.1 (7)
G1	2.5	34.5	27.8 (41)	0.44	6.7 (10)	4.12	1.3 (1.9)	31.4 (47)
LSD	58.0	13.0	9.6 (13)	11.90	31.3 (10)	1.60	0.3 (2.7)	55.0 (14)

LSD=Least significant difference between soil samples.

[a] Numbers in brackets refer to the percentages calculated as regards the C in the form of humic substances stabilized together with the mineral fraction (Σ humic acid+NaOH–extracted fulvic acid+humins).
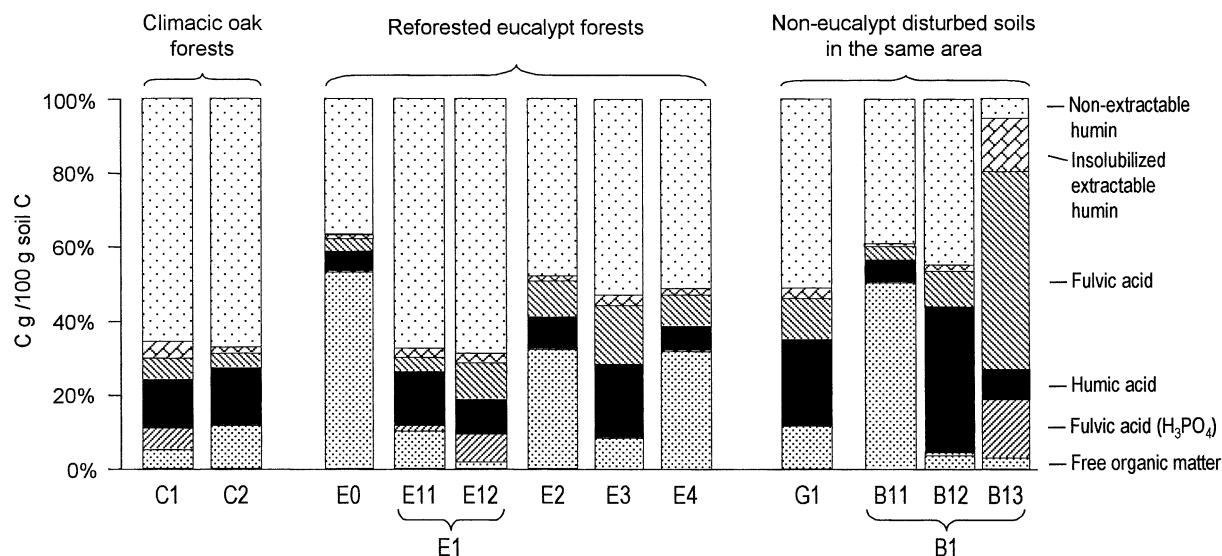
Fig. 2. Distribution of the total soil C in different organic fractions (percentage of the total C).

secondary products derived from the transformation of humic colloids in the soil heavy fraction.

When the concentrations of the humus fractions are calculated in terms of the total stabilised C in the heavy fraction (i.e. excluding the two H_3PO_4–removable fractions, free $+$ FA H_3PO_4-extracted) the results (Table 3) reflect more properly the effects of both the reforestation and the sampling depth. The preferential accumulation of FAs is a trend induced by the reforestation with acidifying species and is in several cases very significant, even in the uppermost horizons (E2, E3, E4). The undisturbed sites (C1, C2) showed dominance of HA upon FA, the HA/FA ratio showing the greatest value in the cleared soils occupied by ameliorant brushwood (G1), which has led to the humus type with the most accentuated colloidal properties. In the case of the two soils considerably developed (E1 and B1), where the humus fractions from successive horizons have been studied, the tendency of the HA/FA ratio to increase with depth is clear, as expected for a leaching process of soluble organic fractions, very intense in the Podsol.

3.3. Incubation experiment

The mineralisation curves (Fig. 3a) show the C release under optimum, comparable conditions in a 12-day experiment, and make evident that most eucalypt forests had a CO_2 production which does not differ significantly from the control sites (C1 and C2). The only two soils showing a substantially higher CO_2 release are the ancient eucalypt forest E0 and the Podsol B11, i.e. soils with high amounts of C accumulated in readily biodegradable forms, as discussed above.

A different interesting information is provided by the mineralisation coefficients, where the CO_2 values are calculated per unit of soil C. It is clearly observed (Fig. 3b) that the biodegradability of the soil humus has significantly decreased in the eucalypt-reforested sites, which is commonly ascribed to the above-indicated effect of the antimicrobial products in eucalypt leaves (Wallis et al., 1999), as well as to increased hydrophobic protection produced by eucalypt-derived resins (Piccolo et al., 1999). Although the eucalypt-reforested soils in general showed substantial amounts of organic matter, the C-forms are comparatively more recalcitrant than in the control sites, the cleared sites and the sites reforested with pine.

3.4. Spectroscopic studies

Visible spectroscopy is a routine tool to assess the aromaticity and polydispersity of the HAs
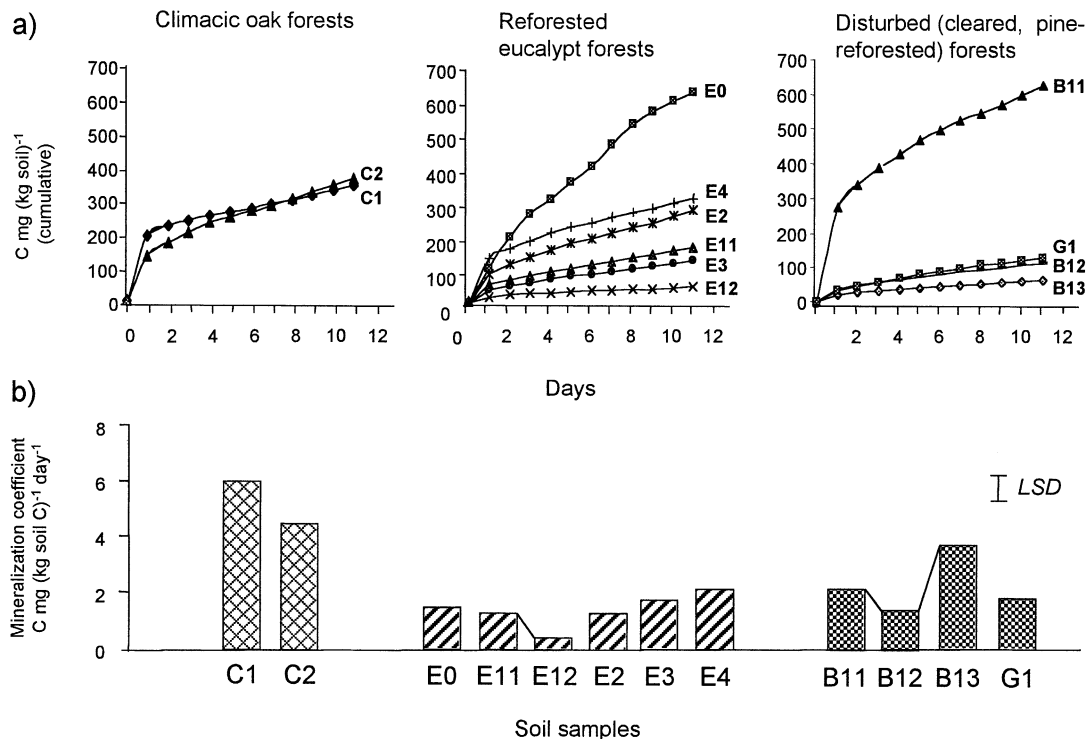
Fig. 3. (a) Mineralisation curves (CO_2 release) and (b) mineralisation coefficients (CO_2 release as C g per kg of soil C) of whole soil samples from undisturbed and reforested ecosystems in Asturias (Northern Spain). (*LSD* = least significant difference between spatial replicates.)

(Chen et al., 1977; Traina et al., 1990) and to detect the presence of certain microbial metabolites in the HA structure (Kumada and Hurst, 1967). The fact that the optical density—aromaticity or condensation—of the HAs accumulated under eucalypt forests was in practically all the cases higher than in the original forests (Table 4), reveals that even in the situations in which a large amount of organic matter remains as non-decomposed organic particles in the soil (E0, E2, E4), the HAs formed have a substantial aromatic moiety that could in part be related to the presence of altered lignin discussed below. Of the other soils studied, the highest value of optical density was found in the soil under ameliorant *Ulex* brushwood and the lowest one in the topsoil of the Podsol. Humic acids from deeper horizons showed higher optical density values than the superficial ones, as it could

correspond to a greater maturity of the HAs in comparatively older soil horizons.

Some differences in the formation mechanisms of the HAs in soils under eucalypt forests are also suggested by the E_4/E_6 ratio, which is considered inversely correlated with the molecular size of the humic substances (Chen et al., 1977). The small and in general significantly lower values of this ratio in the HAs from the eucalypt forests, as regards those from the original deciduous forests (C1, C2), point to comparatively large colloidal particles.

A noteworthy feature of most of the HA samples under study is the presence of conspicuous valleys (620, 570, 530 and 455 nm) in the second derivative of the visible spectra (Fig. 4). Such a spectroscopic pattern is typical of the so-called P-type HAs, which are considered to include in their

composition an appreciable concentration of a green-coloured polycyclic chromophor (4,9-dihydroxyperylene-3,10-quinone), which is produced by pigmented fungi (Kumada and Hurst, 1967; Valmaseda et al., 1989), during the synthesis of melanins through the binaphthyl pathway (Bell and Wheeler, 1986). The occurrence in soils of such valuable signature biomarker substances may be used as one in a series of indirect indicators of the extent to which fungal melanins are involved in the accumulation of stable C-forms in the soil (Oyonarte et al., 1994).

In the present study, the highest concentration of these pigments in the deepest humus layers (E12, B13) agrees with the conclusions of previous research, showing the preferential association of these organic fractions with clays, Fe and Al oxides. In these studies, the stronger the association between the organic and the mineral fraction was, the higher the concentration of green fungal pigment (Almendros and Dorado, 1985).

In the samples studied, it was evident that the introduction of the eucalypt forest has substantially modified the fungal population contributing to the accumulation of the HAs. The intensity of the spectral peaks diagnostic for fungal metabolites was significantly lower in most eucalypt forests in
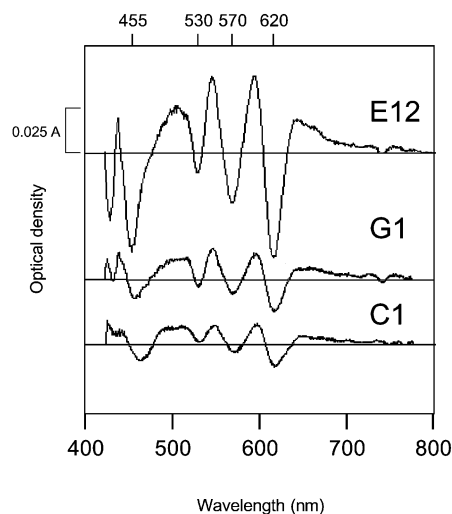

Fig. 4. Second derivative spectra of humic acids from oak forest (C1), disturbed (G1) and eucalypt-reforested (E12) soils (0.2 mg ml^{-1}).

comparison with both the original deciduous forests (C1, C2) and the neighbouring soils developed under different vegetation. In particular, a very low value was found in the ancient eucalypt forest E0, whereas comparatively higher values, similar

Table 4
Spectroscopic data of humic acids

Sample label	Optical density values in the visible range (wavelength nm)		Intensity of the valleys in the second derivative spectrum (wavelength nm^{-1})			
	E_{465} (E_4)	E_{465}/E_{665} (E_4/E_6)	620	570	530	455
C1	0.59	6.09	0.050	0.030	0.020	0.030
C2	0.84	6.72	0.030	0.020	0.015	0.010
E0	1.16	4.80	0.010	0.001	0.001	0.001
E11	1.58	2.96	0.010	0.001	0.001	0.020
E12	1.87	4.56	0.150	0.120	0.080	0.150
E2	1.06	4.92	0.025	0.020	0.020	0.015
E3	0.95	4.71	0.030	0.025	0.020	0.025
E4	0.70	5.61	0.020	0.015	0.012	0.010
B11	0.55	5.19	0.015	0.013	0.010	0.001
B12	1.35	4.55	0.040	0.035	0.030	0.025
B13	1.16	3.98	0.025	0.020	0.025	0.055
G1	1.22	4.11	0.045	0.040	0.030	0.035
LSD	0.58	1.16	0.006	0.004	0.003	0.005

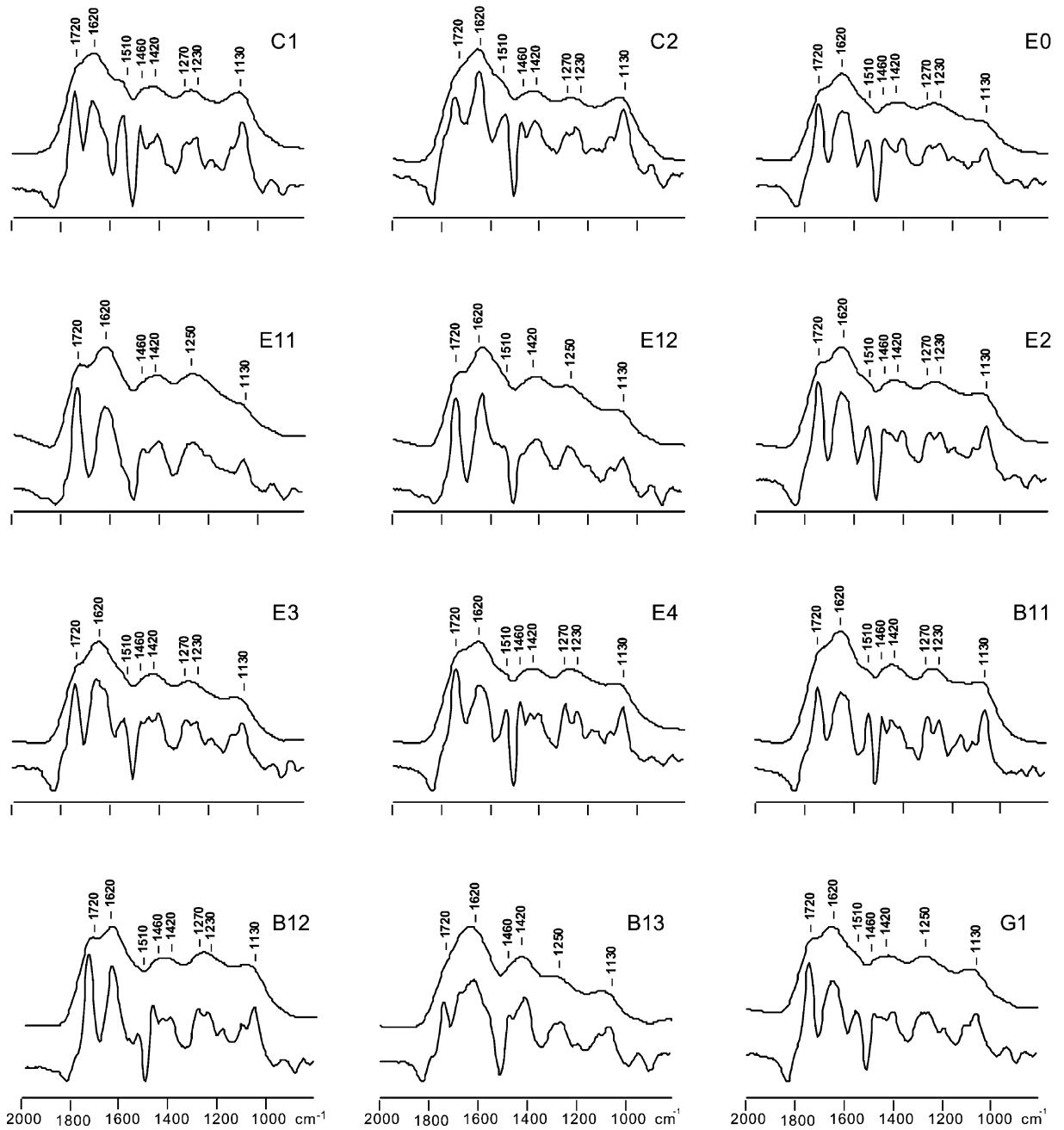*LSD*=Least significant difference between soil samples.

Fig. 5. Detail of the 2000–800 cm^{-1} region of the resolution-enhanced infrared spectra of humic acids from undisturbed and reforested soils in Asturias (the original spectra are superimposed).

to those in the control sites, were observed in the younger E2 and E3 forests. From this viewpoint, the amount of fungal-derived pigments in the soil could act as a valid indicator of the impact of the reforestation practices in the structure of the soil microbial system and the mechanisms responsible for the accumulation of the HAs.

The above results agreed with those from IR spectroscopy, which in this case have provided additional information about the extent to which plant-inherited lignins were progressively transformed into heterogeneous HA-like substances. This is observed in the resolution-enhanced spectra shown in Fig. 5, where a typical lignin pattern, consisting of bands centred mainly at 1510, 1460, 1420, 1270, 1230 and 1130 cm^{-1} is evident and becomes more or less smoothed with the progress of the maturation of the HAs. This may be interpreted as humification pathways where altered lignin forms preferentially accumulate. This circumstance tends to concur in the HAs with the least significant intensity of the above-indicated spectral peaks produced by fungal perylenequinones (e.g. in samples E12, B13) where the IR spectra tend to be more featureless and the aromatic band (1510 cm^{-1}) overlaps with that of amides (1550 cm^{-1}), which is also a distinguishing feature of protein-associated, fungal-melanins preparations (Knicker et al., 1996). On the other side, a very conspicuous lignin pattern was evident in the uppermost horizon of the Podsol, which tends to be smoothed in deeper horizons, whereas the IR spectra of some eucalypt forests (including the relictual one, E0) indicate that the lignin has been transformed in a similar extent to that in the neighbouring deciduous forest.

4. Conclusions

As a whole the eucalypt-reforested soils under study display a common background of analytical properties (i.e. high optical densities, low mineralisation coefficients and low concentration of fungal-derived quinoid pigments); nevertheless, the humus composition ranged between extremely different values. For several parameters such variability was higher than that observed between contrasted soils types in the zone under study

(Leptosol, Cambisol, Podsol). Since the time elapsed after the reforestation seems to be an important source of this variability, it is also likely that because of the low degree of association between organic and mineral matter under eucalypt-reforested soils, the derived humus may be highly responsive to local abiotic factors, such as soil reaction and texture. This might play a role on the activity and leachability of the FA-type soil fractions and the antimicrobial metabolites produced by this vegetation, under climatic conditions in which C mineralisation rates, visible spectroscopy patterns and amounts of free organic matter were useful analytical descriptors of the environmental impact in the studied site.

The reduced biodegradability of the organic matter in the eucalypt-reforested soils, as regards the climacic forests should not be regarded as a neat environmental benefit, which favours the behaviour of the soil as an active C-sink. One must also take into account the low quality of the organic matter accumulated in most of these soils, when compared to those under climacic forests of slow-growing species. In spite of the comparatively low organic matter content of the soils under the original forest, these formations are characterised by a greater biodiversity and a mature organic matter with higher potential to accumulate plant nutrients in available forms, thus contributing to water quality and preventing the mobility of pollutants. On the other hand, and because no tendency for podsolisation was found in any eucalypt forest studied, as regards the neighbouring soils under comparatively more acidifying vegetation (heather and conifer), the use of eucalypt could represent a valid alternative in the reforestation of humid disturbed areas where soil remediation requires substantial improvement of the stability of the clay–humus complex.

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