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Organic Geochemistry 33 (2002) 327–345

Organic Geochemistry

www.elsevier.com/locate/orggeochem

Decomposition of organic residues in soil: experimental technique and spectroscopic approach

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Abstract

DRIFT (diffuse reflectance infrared Fourier-transform) spectroscopy was used to follow the early transformations that take place after the incorporation of organic materials in soil. Alfalfa (A), dried maize (DM), laboratory-composted maize (CM), and two commercial composts (YWC and MWC) confined into fiberglass bags were incubated in sand with and without planting with lettuce. DRIFT spectra of these materials before and after incubations were correlated with CO_2-C evolution and mass, carbon and nitrogen balances. Spectra obtained by successive subtractions allowed us to distinguish between the main classes of biochemical compounds (cellulose, lignin, polypeptides, pectins) and to study their degradation during incubation. Quantitative spectroscopic determination of lignin showed a relative enrichment in the incubated materials. This experimental approach can be applied to studies on the degradation pathway of green manure materials like A, DM and CM but seems less appropriate for commercial composts.

1. Introduction

Plant residue decomposition in soil is controlled by environmental factors, such as biomass activity, rizosphere effects, soil chemical and physical characteristics and, also, by intrinsic characteristics referred to as the quality of residues. The latter factor influences, to a large extent, the rate and degree of transformations of plant residues in soil. Thus many studies (Heal et al., 1997; Melillo et al., 1982; Herman et al., 1977) have outlined the relationship between the rate of decomposition of such residues and their chemical composition as reflected by various ratios (e.g. C to N, lignin to C, lignin to N and lignin to polyphenols). Same classes of compounds (cellulose, hemicellulose, lignin, carbohydrates, proteins, waxes), characterised by different rates of decomposition in soil, are present in any plant residue but in different proportions depending on the origin and age of plant materials. Simple biopolymers are mainly organized in complex structures and are only present to a lesser extent as soluble components quickly utilised by microorganisms (Webster et al., 2000). Cell walls have different compositions in different kinds of tissues and organs. Consequently the quality of plant materials is mainly linked to the different kinds of cell walls (Chesson, 1997). The agronomic value of organic materials depends also on nitrogen release that is controlled by the chemical composition of residues. In particular the presence of carbonaceous compounds easily accessible by micro-organisms improves organic nitrogen mineralization while more recalcitrant organic residues containing large amounts of lignin reduce the release because of shielding effects.

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0146-6380/02/$ - see front matter
PII: S0146-6380(01)00164-4

Composts represent an available, low cost organic amendment, because of the plentifulness of organic wastes. Composts may be wholly of plant origin (deriving from mowing and yard trimmings) or of mixed origin (the plant residues constituting the lignocellulose matrix that acts as bulking and as carbon source for the stabilization of selected municipal solid wastes or of sewage sludges). This stabilization process takes place via degradation by thermophilic and mesophilic microorganisms, and causes the degradation of labile organic compounds and the formation of "pseudohumic" compounds having characteristics similar to those of soil humic substances (Inbar et al., 1990). Composts in soil therefore exhibit a high resistance to degradation and are mainly effective on soil physical qualities (such as porosity and structure), whereas their nutritional properties are limited. Thus in previous studies (Zaccheo et al., 1993; Crippa and Zaccheo, 1995) a strong reduction in N release was noticed during composted ryegrass mineralization in comparison with dried ryegrass mineralization during an incubation test which lasted 30 days.

Spectroscopic techniques are suitable to distinguish biochemical components in organic materials. In particular DRIFT is a simple technique, which is rapidly performed, non-destructive, suitable for fibrous materials such as plant residues, and does not require sample pretreatment (Mirabella, 1998; Smith, 1996). Recently, DRIFT has been applied extensively to research on various materials: soil, humic acids, composts and peat (Nguyen et al., 1991; Niemeyer, et al., 1992; Gigliotti et al., 1999). This spectroscopic technique was used to study the transformation of humic substances during composting of various organic wastes and to determine the maturity degree of the products (Chefetz et al., 1996; Tseng et al., 1996; Jenn-Hung and Shang-Lien, 1999). DRIFT spectroscopy has also long been promoted as a rapid means for determining pulp lignin by Friese and Banerjee (1992). Pappas et al. (1998) described lignin quantitative determination in crude plant material of four kenaf (*Hibiscus cannabinus* L.) varieties.

The aim of this work was to follow the transformations occurring in the first periods of incubation in organic amendments, by means of IR spectroscopic analyses, determination of mass, carbon and nitrogen balances and measurements of CO_2–C evolution.

2. Materials and methods

2.1. Descriptions of organic residues and model compounds

Experiments were performed on five plant materials: alfalfa (*Medicago sativa* L.) (A) derived from farm produced fodder; maize (*Zea mais* L.) (grown in hydroponic solution and picked before flowering), dried (DM) and laboratory-composted (CM) and two commercially available composts. CM was obtained in a laboratory micro-composting apparatus (duration: 100 days; max. temperature: 40 °C; aerobic conditions by forced aeration) as described in a previous paper (Zaccheo and Crippa, 1996). Commercial composts were collected from composting facilities that treated different raw materials: YWC was a yard waste compost, MWC was a mixed waste compost derived from selected municipal solid wastes with yard wastes. All samples were air dried and ground to 0.5 mm.

Spectroscopic analyses were performed on the above organic materials and on a model plant residue prepared with standard compounds, according to the average plant composition reported by many authors (Fitzpatrick, 1999; Baldock and Nelson, 1999), as follows: 47.7 mg native cellulose for thin-layer chromatography (Merck), 10.5 mg hydrolytic lignin (Aldrich), 22.8 mg bovine albumin (Sigma) and 19 mg pectin from citrus fruits (Sigma) intimately mixed.

2.2. Experimental device

Decomposition was studied using the litter bag technique (Bocock and Gilbert, 1957). Bags were made of two fiberglass Millipore filters (2 μm nominal porosity, 46 mm diameter), bound by silicon glue on a 5 mm thick rubber ring. Gas, water and microorganisms were free to pass through the filter while organic matter was retained inside. The bags were placed horizontally at 5 cm depth in pots containing 400 g of siliceous sand. The pots were irrigated with distilled water at 50% WHC (Water Holding Capacity). Sandy substrate was chosen to obtain high aerobic conditions that enhanced mineralization avoiding organic metabolite accumulation. The bags were filled with equal volumes of organic materials and charged with 70 mg of fertile agricultural soil (due to different bulk densities the bags contained different amounts in weight of organic materials). As control, four pots containing bags filled with sand were prepared. Each pot was fertilized with the following amounts of mineral compounds: 56 mg KH_2PO_4/K_2HPO_4 (equimolar phosphate buffer to maintain substrate pH at 6.8); 24 mg K_2SO_4; 4 mg $CaSO_4.2H_2O$; 0.790 mg $MgSO_4.7H_2O$; 0.279 mg $FeSO_4.7H_2O$; 5.405 μg H_3BO_3; 0.274 μg $MnSO_4.H_2O$; 0.159 μg $CuSO_4.5H_2O$; 0.163 μg $ZnSO_4.7H_2O$ and 0.168 μg $(NH_4)_6Mo_7O_{24}.4H_2O$. To evaluate the influence of mineral nitrogen fertilization on decomposition, two levels of mineral nitrogen were adopted equivalent to 0 and 100 kg N ha^{-1} (the latter being obtained by mixing 14.12 mg N as $Ca(NO_3)_2$ pot^{-1} with the other mineral nutrients).

2.3. Incubation experiment 1 (degradation of organic materials)

In this experiment 56 pots were used in order to study mass and C and N balances for A, DM, YWC and

MWC and to compare the bag technique with conventional method of mixing. The materials were put in the bags or intimately mixed with sand; all the pots were inserted in tightly closed containers together with a beaker with 0.5 N NaOH solution. At different incubation times (3, 8, 11, 16, 19, 25, 38, 53, 76 days) CO_2–C trapped in NaOH solution was titrated. All the pots were set in a greenhouse. At the end of the incubation (76 days) the bags were dried at 70 °C, weighed and opened. The decomposed materials from the replicates were combined, carefully mixed and submitted to chemical analyses (total C and N). KCl extraction of sand from treatments with bags was performed to determine the inorganic nitrogen (NO_3–N and NH_4–N).

2.4. Incubation experiment 2 (degradation of organic materials with lettuce cropping)

The experiment was conducted in a greenhouse at the same time as experiment 1. The 96 pots containing the materials mixed or confined in bags were planted with lettuce (*Lactuca sativa* L.). Three seedlings of lettuce var. Augusta were transplanted in each pot. After 30 days, plants were harvested, weighed and dried at 70 °C until constant weight. Bags were opened and weighed (after removal of the fine roots present in some cases), and the decomposed materials from the replicates were combined, carefully mixed and submitted to chemical and spectroscopic analyses.

2.5. Analytical methods

Total carbon and nitrogen of the organic materials were determined by elemental analysis (Carlo Erba); TKN was determined with a micro-Kijeldahl apparatus; NO_3–N and NH_4–N, extracted (1:10 w/v) from sand with KCl 1N solution, were analysed by colorimetric methods (Keeney and Nelson, 1982); ash was determined at 550 °C until constant weight; humification index (H.I.) was measured according to Sequi et al. (1986) and determination of CO_2–C followed the method of Pochon and Tardieux (1962).

2.6. DRIFT spectroscopy

Spectra were obtained using a FTIR-300E JASCO spectrometer, equipped with a DLATGS detector and a diffuse reflectance attachment (Pike Technologies, Inc Madison, WI 53719, USA). The samples (7 mg) and 700 mg of KBr (FT grade, Aldrich Chemical Co.) were finely ground for 10 min in a Wig-L-Bug (Specamill-Grseby-Specac), using an agate ball mill (constant particle size of the grinding is required to obtain reproducible spectra, with the same scattering coefficient, essential for semiquantitative and quantitative determinations).

The spectra were acquired in the 4000–600 cm^{-1} range, with 4 cm^{-1} resolution and 100 scans were performed in each acquisition. As background a spectrum of finely powdered potassium bromide was recorded at the same instrument setting. Area integration and spectral subtractions were performed using 5.2 version by GRAMS/32 software (Galactic Co.). The model mixture spectrum was subjected to baseline automatic correction by GIFTS auto-level method based on least squares, and to spectrum normalization in relation to the interval given by minimum and maximum absorptions. This treatment is adopted in the "subtract and search again" method, often applied to heterogeneous compound analysis. Spectral subtractions were based on the "dewiggle" algorithm, according to Friese and Banerjee (1992), now implemented in 5.2 version by GRAMS/32 software. This method allowed the determination of a reproducible subtraction factor on the basis of residual spectrum derivative minimization. Subtraction factors thus obtained represented a measure of the degree of overlapping of the spectra and appeared highly sensitive to small concentration variations.

3. Results and discussions

The chemical compositions of the studied organic materials are reported in Table 1. These residues are representative of the different organic amendments usually employed to improve the fertility of soils: green manures (A and DM) and stabilised organic matter (CM, YWC and MWC). A and DM were chosen for their different biochemical compositions: Leguminosae are richer in nitrogen compounds than Gramineae and contain less cellulose and lignin. CM is the compost derived from the laboratory micro-composting of DM. In spite of its still high carbon percentage, the material was subjected to deep transformations during composting including carbon losses (40% of the original maize), ash increase and a strong lowering of the H.I. value. This material was included in experiment 2 to compare organic material transformations during composting and in soil. YWC and MWC are the most frequent kinds of end-products from composting plants where

Table 1
Characteristics of the organic materials

	C (g kg^{-1})	N (g kg^{-1})	C/N	H.I.	Ash (g kg^{-1})
Alfalfa (A)	430	30.1	14.3	–	10.2
Maize (DM)	416	23.6	17.6	0.98	97.2
Composted maize (CM)	402	22.8	17.6	0.36	156
Yard waste compost (YWC)	186	22.0	8.5	0.42	481
Mixed compost (MWC)	249	28.7	8.7	0.45	472

selected organic refuses are treated. Their low carbon content together with the high percentage of ash and the low value of the humification index indicate a high degree of decomposition (mature composts are characterized by H.I. values lower than 0.5; Mondini et al., 1996). Nevertheless the nitrogen content is anomalously high compared to the average values found in a previous survey (Crippa et al., 1998) on composts from composting plants of North Italy: 10.7 mg N kg^{-1} in yard waste composts and 18.5 mg N kg^{-1} in mixed composts. The difference could be related to the presence of NO_3–N in these well stabilized composts and by different analytical methods: in the previous study nitrogen was determined, as usual for these materials, as TKN (total Kjieldahl nitrogen) while elemental analysis, that includes also the nitrogen oxidized form, was performed in the present study.

3.1. Incubation experiment 1

Fig. 1 shows CO_2–C evolution from A, DM, YWC and MWC without mineral N fertilization and from DM with fertilization. As expected the highest cumulative CO_2–C productions were found in A and DM with similar trends throughout: intensive degradation in the first period of incubation (over half of total carbon evolved was trapped during the first 11 days), followed after day 19 by slower and quite constant CO_2–C production. For DM, dramatic changes in the slope were observed between 11 and 19 days probably related to the change in organic substrates utilized by micro-organisms. The specific growth rate of heterotrophic soil micro-organisms and the lag phases measured from continuous respiration curves were proposed by Marstorp (1997) to characterize the litter components and the components utilized during a single growth phase were called 'kinetically defined fractions'. Previously Reber and Schara (1971), studying straw leachate, distinguished carbon evolution from sugars, amino acids and phenols. Since our measurements were made at more than 1-day intervals only the DM respiration curve showed a lag phase, probably concurrently with the consumption of easily available sugars and proteins and subsequent microbial utilization of water insoluble components such as cellulose and hemicellulose. In YWC and MWC commercial composts low cumulative amounts of CO_2–C were detected, with a constant rate of release. This trend is typical of well stabilized materials as also reported by Hue and Liu (1995). The effect of mineral N on carbon dioxide production was detected only in DM treatment, with a significative stimulatory effect during the first phase of degradation, as indicated by the dotted curve in Fig. 1, but with almost no cumulative effect.

Comparison of CO_2–C evolutions from materials retained in bags and intimately mixed with sand is reported in Table 2. Cumulative amounts of evolved C from A and DM did not show significative differences, either in the first days of incubation and in the cumulative final amounts. An increase in CO_2–C cumulative evolution from YWC was observed after 76 days when the residue was mixed with sand and a strong stimulatory effect of mixing was detected in MWC. Probably recalcitrant compounds were more easily degraded when diluted in the substrate, or the chemical and/or physical environment inside the bags was unsuited to microbial activity (e.g. high concentration of salts or toxic metabolites).

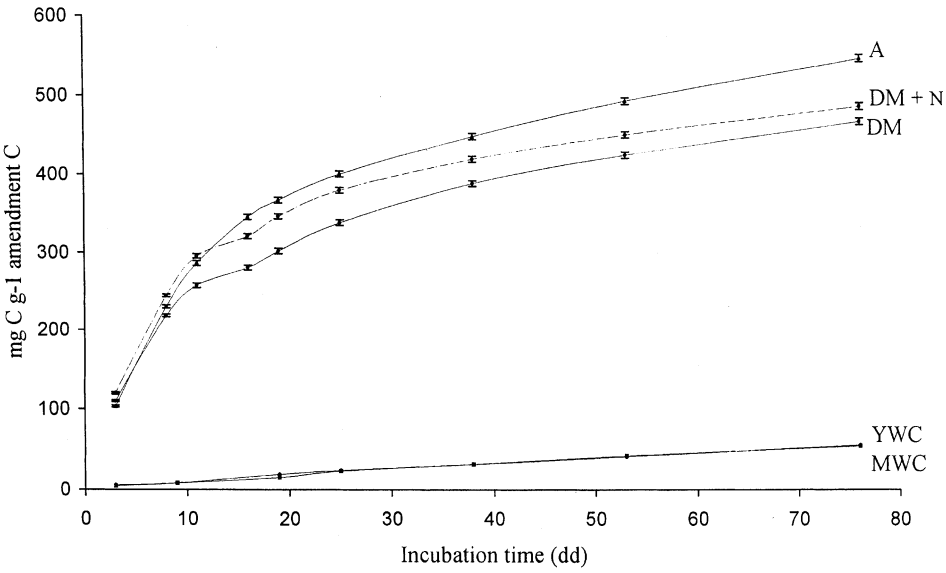

Fig. 1. CO_2–C evolution from incubated materials (bars are standard deviations).

Table 2
Comparison of cumulative CO_2–C evolution in the two experimental devices

	C–CO_2 (mg pot^{-1})					
	3 days		16 days		76 days	
	Mixed	In bags	Mixed	In bags	Mixed	In bags
Alfalfa (A)	60.0d	60.0d	197e	201e	317e	317e
Maize (DM)	42.7c	36.4c	92.5d	92.8d	167d	154d
Yard waste compost (YWC)	4.43a	4.35a	14.2a	13.3a	49.1a	35.5a
Mixed waste compost (MWC)	11.3b	7.43ab	40.7c	23.5b	124c	67.2b

Means values from replicates, followed by the same letter within each time, are not significantly different by Tukey's multiple range test at 0.05.

Table 3 shows mass, C and N balances calculated from changes in mass and in C and N concentrations of the samples between the beginning and the end of incubation in bags. Each reported value was an average of the results obtained with and without nitrogen fertilization, since no significative differences were observed. Interaction between available nitrogen and mineralized carbon from organic residues in soil is a controversial subject (Ajwa and Tabatabai, 1994; Knapp et al., 1983). In accordance with CO_2–C evolution, the mass and carbon balances showed an intense decomposition of A and DM and limited degradation of composts, especially for YWC. In A and DM 21 and 26% of carbon losses were not recovered as CO_2–C, respectively. This difference confirmed the prevalent aerobic environment in the incubation device: in fact, under aerobic conditions, a carbon assimilatory efficiency of 20–40% by soil microorganisms is usually observed (Alexander, 1977). For commercial composts the difference was wider (for both composts about 60% of carbon lost was not accounted as CO_2–C). Release from the bags to the sand of organic compounds solubilized by the microflora and not utilized as a carbon source probably occurred, as also suggested by the brown colouring of the sand observed mainly in MWC treatment. High mass losses, similar in percentage to C losses, were observed for A and DM; in the two composts mass losses were low and markedly weaker than C losses due to higher ash contents. Nitrogen release from incubated materials was very high in A and DM and in percentage was similar to carbon release. In contrast, much higher nitrogen losses compared to the carbon ones were observed for YWC which may be due to the diffusion of nitrates in the substrate, whereas MWC released similar percentages of nitrogen and carbon. In sand, the presence of mineral nitrogen (NO_3–N and NH_4–N) was not observed, except for very small amounts, in any treatments. It may be assumed that either microbial immobilization of N derived amendment and N fertilizer occurred outside the bags or N denitrification.

3.2. Incubation experiment 2

As in the previous experiment, addition of nitrogen did not produce stimulating effects on degradation. Average values obtained with the two fertilization levels are reported in Table 4. This experiment lasted for a shorter period (30 days) since symptoms of nutritional deficiencies in lettuce showed up about 25 days after transplantation. Thus, in order to evaluate the influence of plants on degradation, direct comparison cannot be made with experiment 1. The shorter length of the test was clearly reflected by lower carbon losses for all the studied residues than in experiment 1. Due to lettuce

Table 3
Mass, carbon and nitrogen balances from materials incubated in bags (experiment 1)

	Mass			Carbon			Nitrogen			C/N of losses
	Initial weight	Losses		Initial C	Losses		Initial N	Losses		
	(mg pot$^{-1)}$	(mg pot^{-1})	%	(mg pot^{-1})	(mg pot^{-1})	%	(mg pot^{-1})	(mg pot^{-1})	%	
A	1352	892	66.0	581	403	69.3	40.7	24.5	60.3	16.4
DM	795	494	62.1	331	208	62.8	18.8	10.7	56.9	19.4
YWC	4885	193	3.95	910	111	12.2	107	34.3	32.0	3.23
MWC	4886	328	6.71	1216	244	20.0	140	28.8	20.6	8.5

Table 4
Mass, carbon and nitrogen balances from materials incubated in bags with lettuce (experiment 2)

	Mass			Carbon			Nitrogen			C/N of losses
	Initial weight	Losses		Initial C	Losses		Initial N	Losses		
	(mg pot^{-1})	(mg pot^{-1})	%	(mg pot^{-1})	(mg pot^{-1})	%	(mg pot^{-1})	(mg pot^{-1})	%	
A	1346	679	50.5	579	292	50.4	40.5	25.2	62.2	11.6
DM	790	376	47.5	328	144	43.9	18.6	13.0	69.9	11.1
CM	577	153	26.6	231	33.0	14.2	13.1	3.32	25.2	9.9
YWC	4885	198	4.05	910	46.2	4.80	107	29.3	27.3	1.6
MWC	4885	395	8.11	1216	155	12.7	140	24.7	17.6	6.3

cropping in this test it was not possible to determine the evolved CO_2–C: then carbon losses were compared with CO_2–C recovery at 30 days as read on respirometric curves of Fig. 1. This comparison gave values very near to those obtained in the previous test: 18% (for A) and 26% (for DM) of total carbon losses were not recovered as CO_2–C. For the two composts large differences were again observed (46 and 77% for YMC and MWC, respectively). Therefore, it does not seem that the presence of plants had any significant effect on carbon degradation in these materials. Carbon losses in CM as compared to DM showed the strong effect of composting that reduced organic component degradability drastically.

For the two commercial composts mass loss was equal to (YWC) or even higher (MWC) than that obtained in the incubation experiment without plants: that seemed due to solubilization and consequent leaching of salts from bags, related to the presence of plants because of a direct effect (root absorption) and/or indirect effect (substrate acidification). In A and DM nitrogen losses were larger than those obtained from experiment 1 after longer incubations but without plants. It can, therefore, be assumed that (i) more labile nitrogen compounds were degraded during the first 30 days, both with and without plants and (ii) root exudates favour mineralization of more resistant nitrogen compounds. Comparison of DM and CM (characterized by analogous nitrogen content) illustrates how composting made nitrogen compounds much less susceptible to degradation.

Lettuce is highly sensitive to edaphic conditions and to the presence of toxic elements, so that it is often employed as test plant in biological assays. The degradation of all the tested organic residues stimulated its growth and nitrogen uptake, as shown in Table 5. Because of the different amounts of nitrogen in organic materials in each pot (Table 4) the degree of nitrogen bioavailability was determined from recovery yield (nitrogen uptaken by lettuces as% of amendment nitrogen contained initially in each pot). In all treatments we observed reduced efficiency of nitrogen uptake by plants, (10–18% range, excluding MWC treatment, where a value of ca. only 3% was obtained). This N uptake did not coincide with the whole mineralized N and it accounted for 15–60% of N losses. This could reflect nitrogen immobilization due to the microbial biomass.

In stable organic residues, such as CM and YWC, more plant available N occurred to some extent, due to lesser competition with microflora, as evidenced by the different DM and CM productive yields: DM had a yield only 1.85 times higher than CM with a higher nitrogen loss (13.0 and 3.32 mg pot^{-1} respectively). The high yield induced by YWC may be due to nitrate

Table 5
Lettuce growth and nitrogen uptake in amendments incubated in bags (experiment 2)

	Unfertilized				Fertilized	
	Yields (g d.w. pot^{-1})	N uptake (mg pot^{-1})	Recovery of N amendments (%)	Percentage of N lost uptaken by lettuce (%)	Yields (g d.w. pot^{-1})	N uptake (mg pot^{-1})
Control	0.04	0.28	–	–	1.78	13.6
A	0.85	7.56	18.7	30.0	1.28	12.6
DM	0.37	2.81	15.5	21.6	1.54	12.8
CM	0.20	1.65	12.6	49.7	1.72	15.8
YWC	2.28	17.1	16.0	58.4	2.17	28.0
MWC	0.50	4.41	3.14	17.8	1.52	12.0

Table 6
Summary of main IR bands (cm^{-1}) of lignin, cellulose, pectins and polypeptides[a]

Biomolecules	IR bands (cm^{-1})	Attributions
Cellulose	1157	C–O–C bridge stretching (asymmetric)
	1106	In-phase ring stretching (asymmetric)
	1063	C–O stretch, skeletal vibrations
	896	Out-of-phase ring stretching (asymmetric)
Lignin	1513	C=C stretching (aromatic ring)
	835	C–H aromatic out-of-plane
Polypeptides	1655	C=O stretching (amide I)
	1542	N–H deformation and C–N stretching (amide II)
Pectins	1741	C=O stretching of alkyl ester
	1229	C–O stretching (asymmetric) of ester

[a] The attributions corresponding to the most significant bands, marked by an asterisk on spectra in Fig. 2, are according to Socrates (1980), Marchessault and Sundararajan (1983), Séné et al. (1994), Engelsen and Nørgaard (1996), Pandey (1998).

occurrence in compost already at the beginning of incubation and by the very weak carbon release. This was confirmed by the lack of effect of mineral fertilization that did not stimulate growth but only nitrogen uptake. MWC induced a yield that can be compared to that of the other organic residues maybe because of the high amount of material, but the degree of bioavailability of nitrogen was much reduced.

Mineral fertilization markedly influenced plant growth, the mineralization of the residues does not result in yield increase relative to the control experiments. As to the comparison between in bag and mixed incubations, no significant differences were noted, either concerning yields or nitrogen removal, both with and without fertilization. The average yield in pots with bags was 1.243 g pot^{-1}, and the corresponding yield in pots with organic materials mixed with sand was 1.348 g pot^{-1}. Nitrogen removal from bag experiments was 11.47 mg pot^{-1}, and 10.50 mg pot^{-1} from mixed experiments.

3.3. DRIFT spectroscopy

Spectroscopic analyses were focused on alfalfa (A) dried maize (DM) and laboratory-composted maize (CM) and commercial composts (YWC and MWC) from experiment 2. The first aim was to identify the main classes of compounds from which the IR signal of the organic materials arose. Since IR spectra are the result of linear combination of spectra of single molecular compounds scaled in relation to their concentration, subtraction seemed the most appropriate means to identify the main components in such mixtures, in order to assign the absorptions correctly.

The model mixture consisted of the main classes of biomolecules present in plant tissues, that is cellulose, lignin, polypeptides and pectins, mixed in such proportions as to respect their average ratios in plant materials. Pure compounds and the model mixture were analysed by DRIFT spectroscopy. On the spectra of pure compounds the most significant bands were identified. They are marked by an asterisk on each spectrum in Fig. 2, and their attributions are indicated in Table 6. Successive subtractions on the model mixture showed the correctness of band attributions for the pure compounds, moreover provided the degree of method applicability in order to study organic materials in soil.

Subtractions were successively performed for lignin, cellulose and polypeptide spectra. The lignin spectrum presents two characteristic quite distinct peaks at 1513 cm^{-1} and 835 cm^{-1} that allowed control of subtraction efficiency. Besides, by subtracting lignin first, the calculation of subtraction factors was more reliable. Cellulose, present in large amounts in plant tissues, showed strong overlapping bands around 1115–1050 cm^{-1} and its subtraction revealed the minor components of the mixture. The last subtraction concerned polypeptides (characterized by amide I and amide II bands, which have a constant ratio for all polypeptides) whereas pectins can show different methylation degrees depending on plant species and therefore spectral responses that are variable depending on the examined material.

Lignin subtraction, in the spectrum of model mixture (Fig. 3b), allows identification of bands at 1652 and 1540 cm^{-1} assigned to amide I and amide II in polypeptides. The subtraction of typical bands due to cellulose enhances the absorption band at 1744 cm^{-1} due to ester groups, present in pectin (Fig. 3c). The spectrum after subtraction of polypeptides, was similar to the spectrum of the pure pectin. The signal at 1537 cm^{-1} in this spectrum (Fig. 3d) is an artefact due to amide

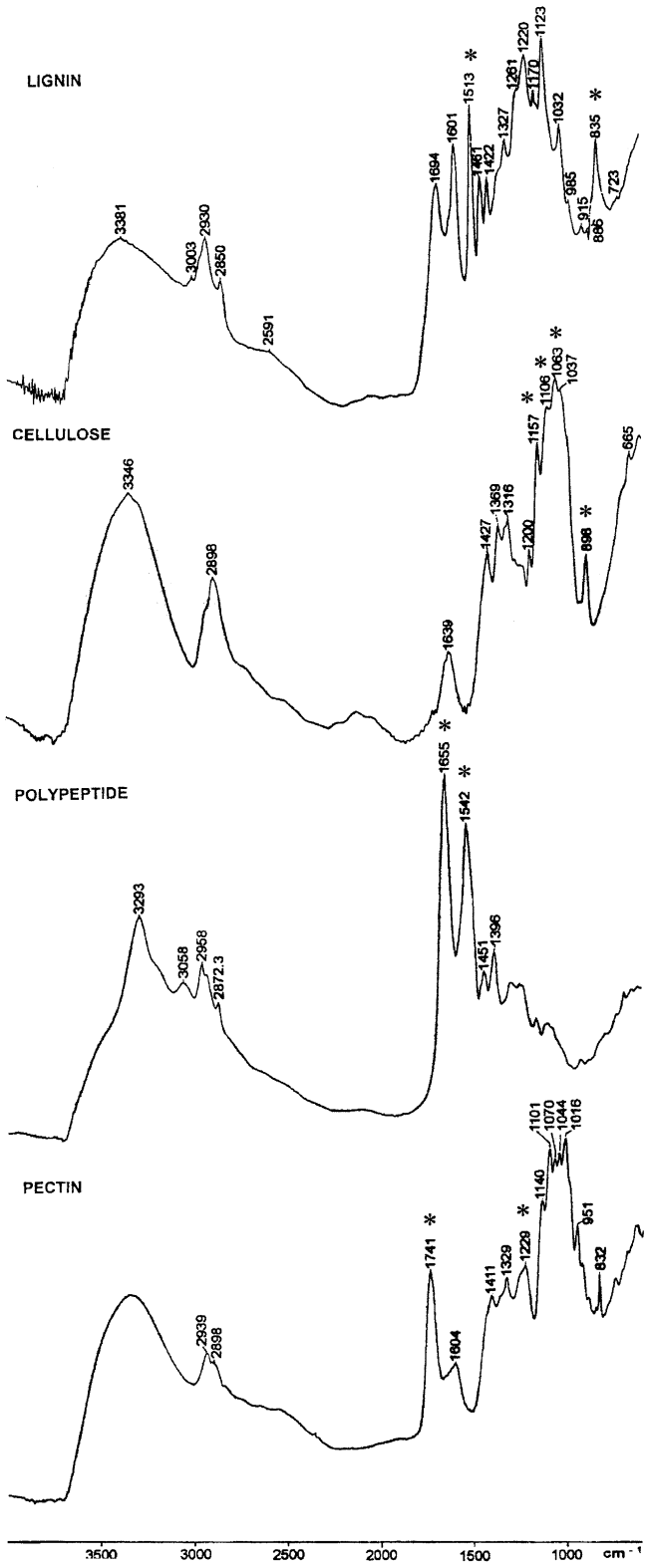

Fig. 2. Spectra of model compounds.

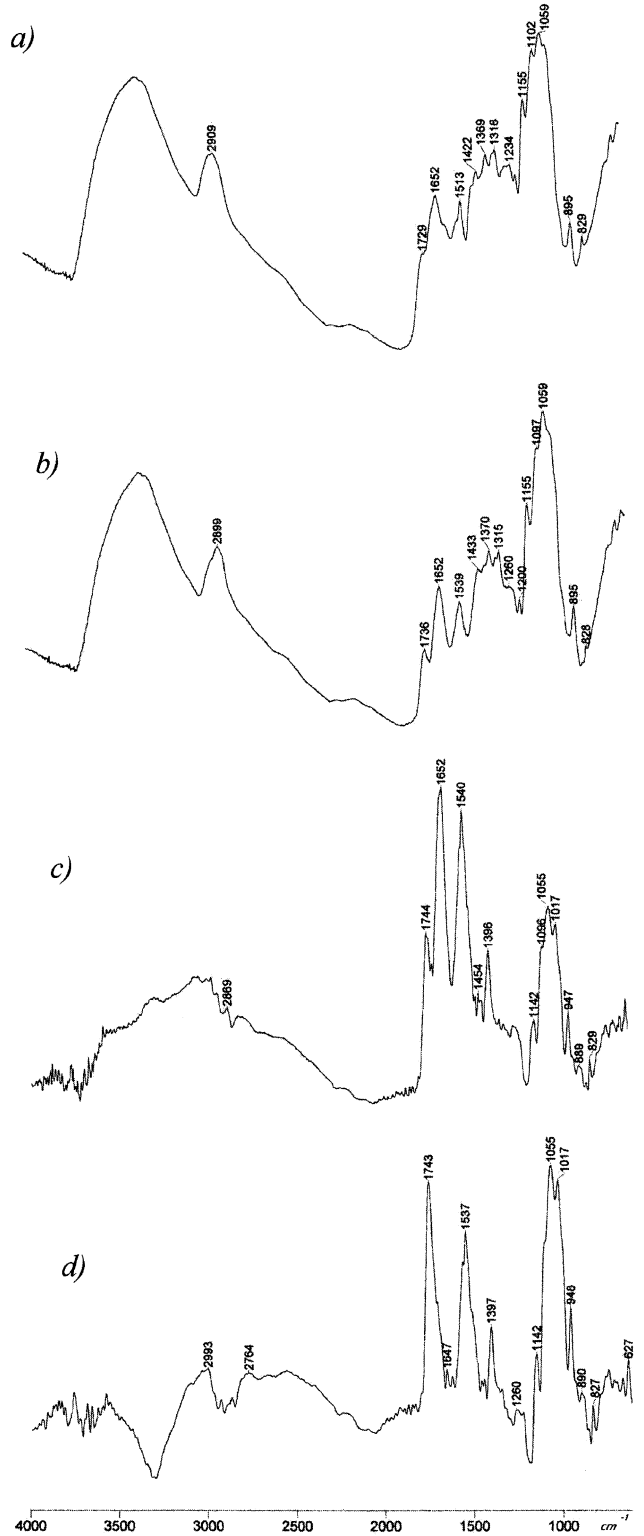

Fig. 3. Model mixture spectra: (a) whole spectrum; (b) spectrum after lignin subtraction; (c) spectrum after lignin and cellulose subtractions; (d) spectrum after lignin, cellulose and polypeptide subtractions.

II.[1] Bands between 2983 and 2764 cm^{-1} appear negative and are due to oversubtraction.[2]

Similar subtractions were applied to the organic materials and DM spectra thus obtained before and after incubations are reported in Figs. 4 and 5, respectively. Subtraction indicated that these spectra are derived essentially from the overlapping of lignin, cellulose, polypeptides and pectins adsorption bands. The model compound mixtures and the material before incubation showed in fact a high level of similarity (Figs. 3a and 4a). In fact, lignin subtraction (Fig. 4b) and subsequent subtraction of cellulose (Fig. 4c) allowed us to identify bands at 1657 and 1542 cm^{-1} assigned to amide I and II in polypeptides, whereas further subtraction of polypeptides (Fig. 4d) revealed the band at 1733 cm^{-1}due to C=O stretching of ester groups from pectin. Bands at 2918, 2851 and 1454 cm^{-1} due to stretching and bending of methylene groups are due probably to esters of long chain fatty acids coming from cuticle waxes (Barton II et al., 1992). These waxes contribute also to the signal at 1733 cm^{-1}.

By comparing spectra of dried maize (DM) before (T0) and at the end (T1) of incubation, almost complete degradation of the acid fraction of pectins and of cytoplasmatic and vacular carboxylic acids, is shown by the disappearance of bands at 1603 and 1384 cm^{-1} due to asymmetric and symmetric stretching of carboxylic ions (Figs. 4d and 5d). Analogous results were obtained by studying materials obtained before and after incubation for alfalfa (not reported here).

Subtractions were also applied on composted organic materials, such as maize composted in the laboratory (CM), and on a green compost (YWC). Examination of DRIFT spectra for (CM) compost did not indicate substantial differences among T0 and T1 materials (Figs. 6 and 7). The wide band at 1600 cm^{-1} and the band at 1400 cm^{-1} due to C=O stretching in carboxylic acid salts do not disappear after incubation, probably because during DM composting new, more resistant compounds are synthesized, which remained also after CM incubation in sand. Bands at 1591 and 1389 cm^{-1} are present as well in the spectrum (Fig. 8c) corresponding to the organic fraction of YWC commercial compost.

DRIFT spectra of commercial composts (YWC, MWC) showed bands at 1449 and 1025 cm^{-1} due to the presence of interfering substances such as carbonates and silicates, respectively. In order to analyze these composts it was necessary, beforehand, to eliminate these bands because they masked the absorptions due to the organic fraction. Fig. 8 reports the spectrum of green YWC compost (spectrum a), the spectrum obtained after compost incineration at 350 °C (spectrum b) and the spectrum obtained by subtraction of ash (spectrum c). This latter spectrum is comparable with the CM spectrum at the end of incubation (Fig. 7a). The subtraction methods can be applied also to organic materials containing humic-like substances, such as composts. In fact, recently Christy and Egeberg (2000) demonstrated that natural organic matter consisting of humic material is derived from four basic types of biopolymers: carbohydrates, proteins, amino sugars and aromatic phenols.

As reported in Table 7 A, DM and CM showed a slight increase in the lignin subtraction factors from T0 to T1, thus confirming the relative enrichment in lignin due to its selective preservation.

3.4. Lignin quantitative determination

FT IR methods for determining the lignin content have been largely developed in the evaluation of pulps used for paper manufacturing. Faix (1988), Pappas et al. (1998), Hinterstoisser et al. (1997) showed that the lignin content can be linked to the area of the 1513 cm^{-1} band. This band, related to the vibration of complex aromatic structures, is always detected in ligno-cellulosic materials.

A characteristic of the DRIFT spectra obtained from hardly crushable, strongly absorbent matrices, such as materials containing cellulose fibrils, is the almost constant levels of absorption for a wide range of concentrations, deviating from Lambert-Beer's law. This is due to a decrease in radiation penetration with concentration increase of the analyzed compound and a consequent decrease in the volume sampled because of progressive radiation extinction. As reproducibility of equal weighing, which in this instance should be below one milligram, was low, we operated in the non-linear concentration-response zone. Quantitative spectroscopic

Table 7
Lignin subtraction factors before (T0) and after (T1) incubation[a]

	Time 0	Time 1
Alfalfa(A)	0.169±0.011	0.208±0.009
Maize (DM)	0.219±0.009	0.248±0.008
Composted maize (CM)	0.237±0.012	0.256±0.010

[a] Calculated in the 2000–750 cm^{-1} range.

[1] In the model mixture crystalline albumin was used, in solid state it gives a broad amide II band with an intensity ratio of 1:1 compared to amide I. For subtractions we used an amorphous albumin sample, obtained by lyophilization of the same crystalline albumin, the spectrum of which shows a 2:1 ratio between amide I and amide II bands, like a normal spectrum in transmission.

[2] Subtraction factor values are calculated on a first derivative spectrum, consequently regions with wide and low intensity bands, showing a weak derivative signal, may represent oversubtraction.

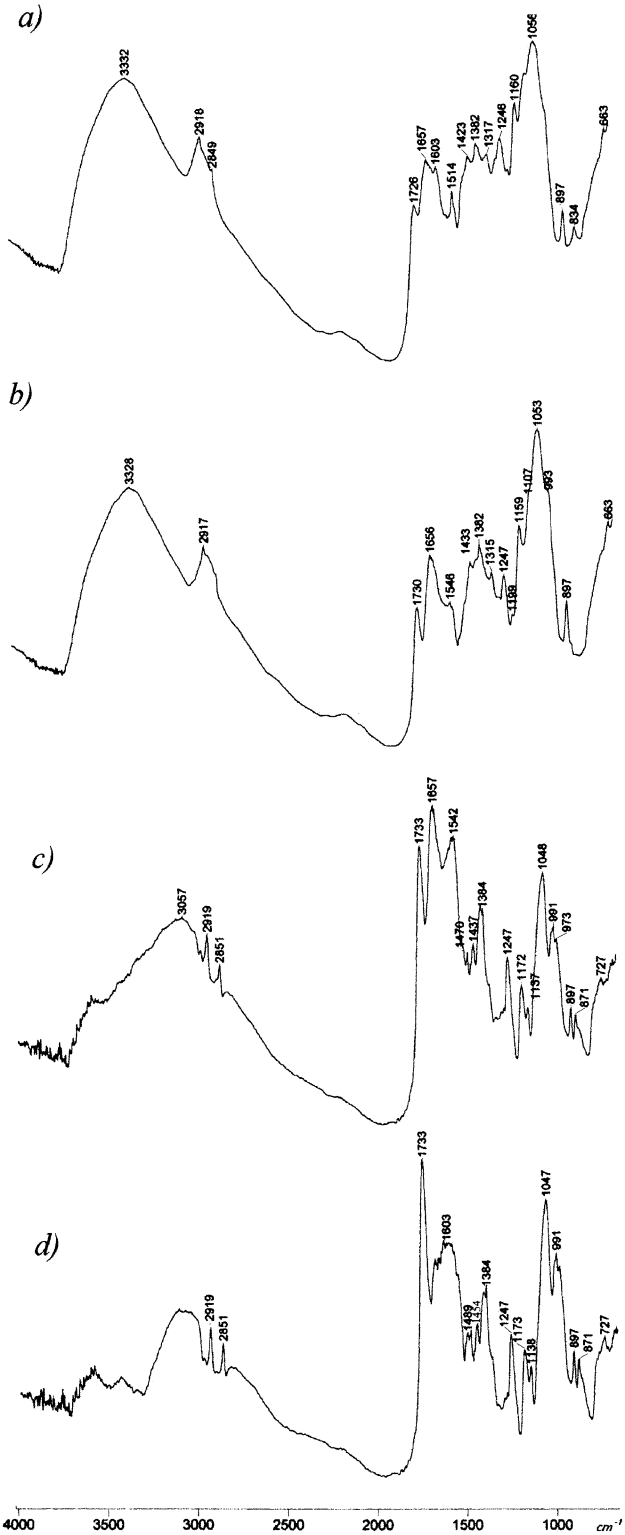

Fig. 4. Spectra of dried maize (DM) at T0: (a) whole spectrum; (b) spectrum after lignin subtraction; (c) spectrum after lignin and cellulose subtractions; (d) spectrum after lignin, cellulose and polypeptide subtractions.

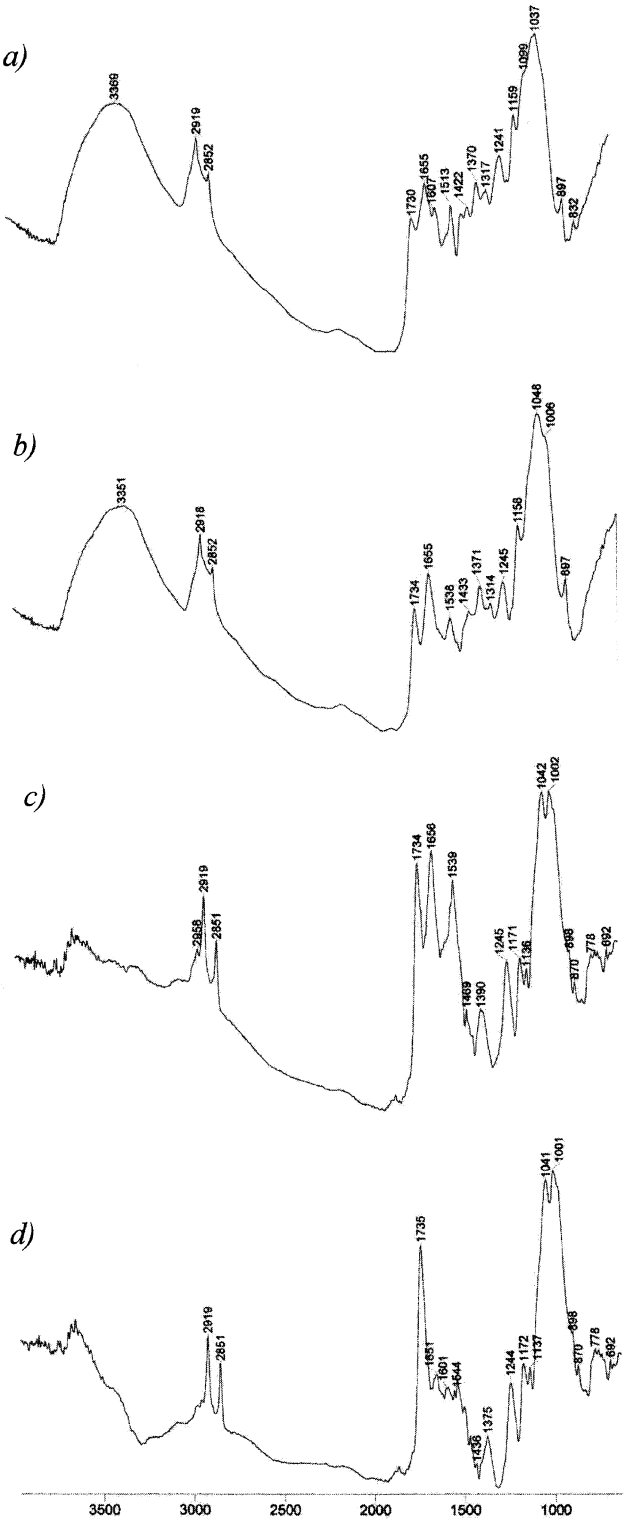

Fig. 5. Spectra of dried maize (DM) at T1: (a) whole spectrum; (b) spectrum after lignin subtraction; (c) spectrum after lignin and cellulose subtractions; (d) spectrum after lignin, cellulose and polypeptide subtractions.

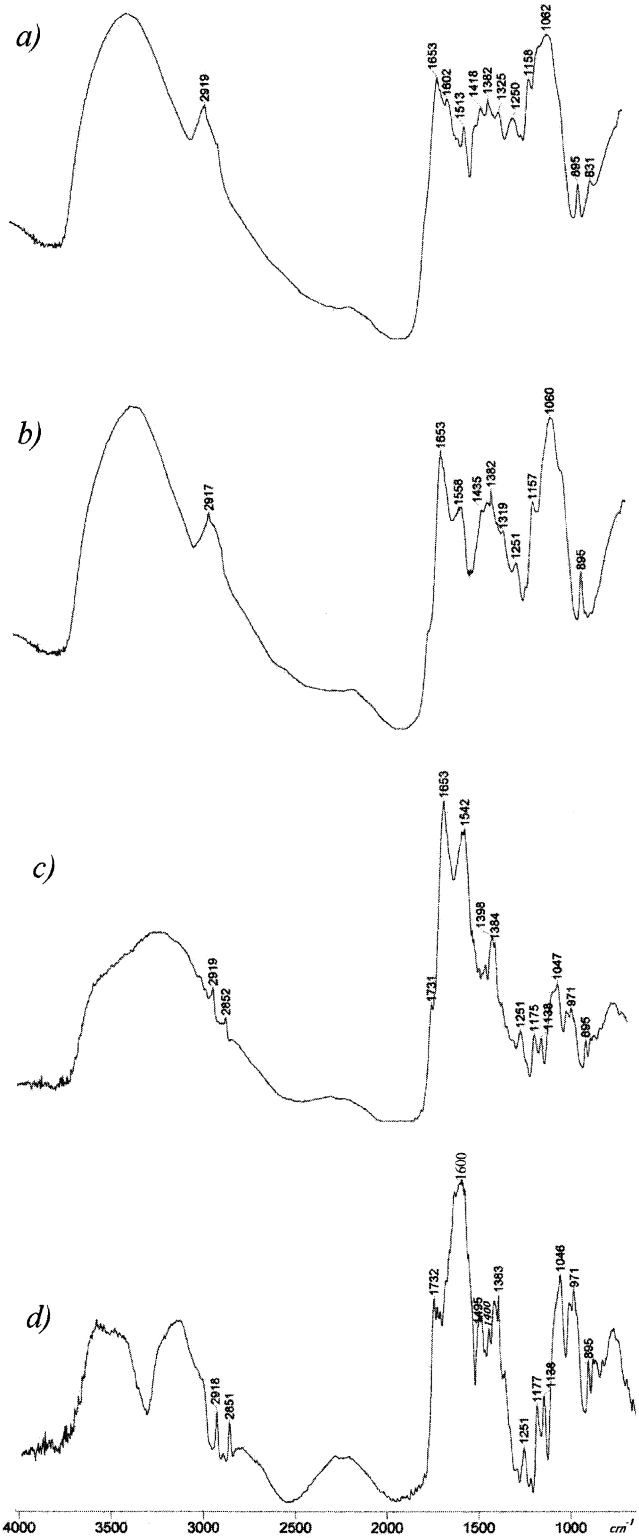

Fig. 6. Spectra of composted maize (CM) at T0: (a) whole spectrum; (b) spectrum after lignin subtraction; (c) spectrum after lignin and cellulose subtractions; (d) spectrum after lignin, cellulose and polypeptide subtractions.

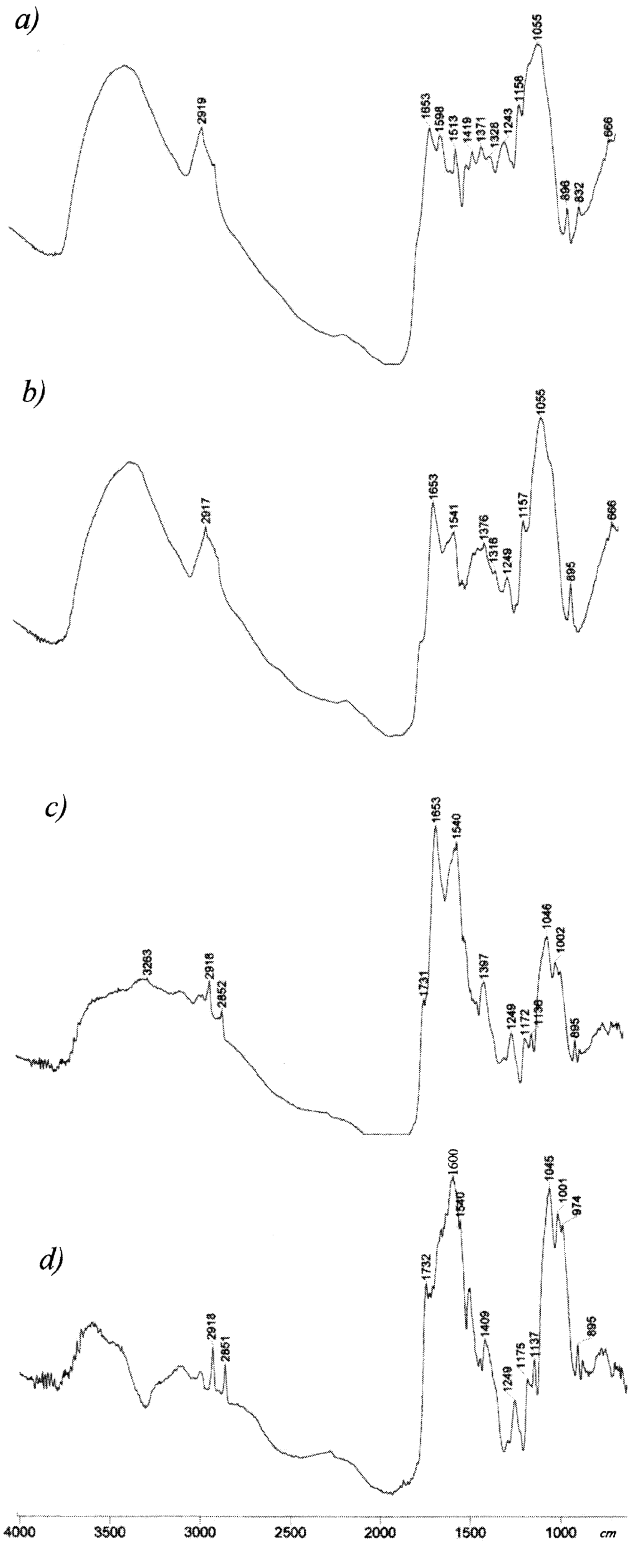

Fig. 7. Spectra of composted maize (CM) at T1: (a) whole spectrum; (b) spectrum after lignin subtraction; (c) spectrum after lignin and cellulose subtractions; (d) spectrum after lignin, cellulose and polypeptide subtractions.

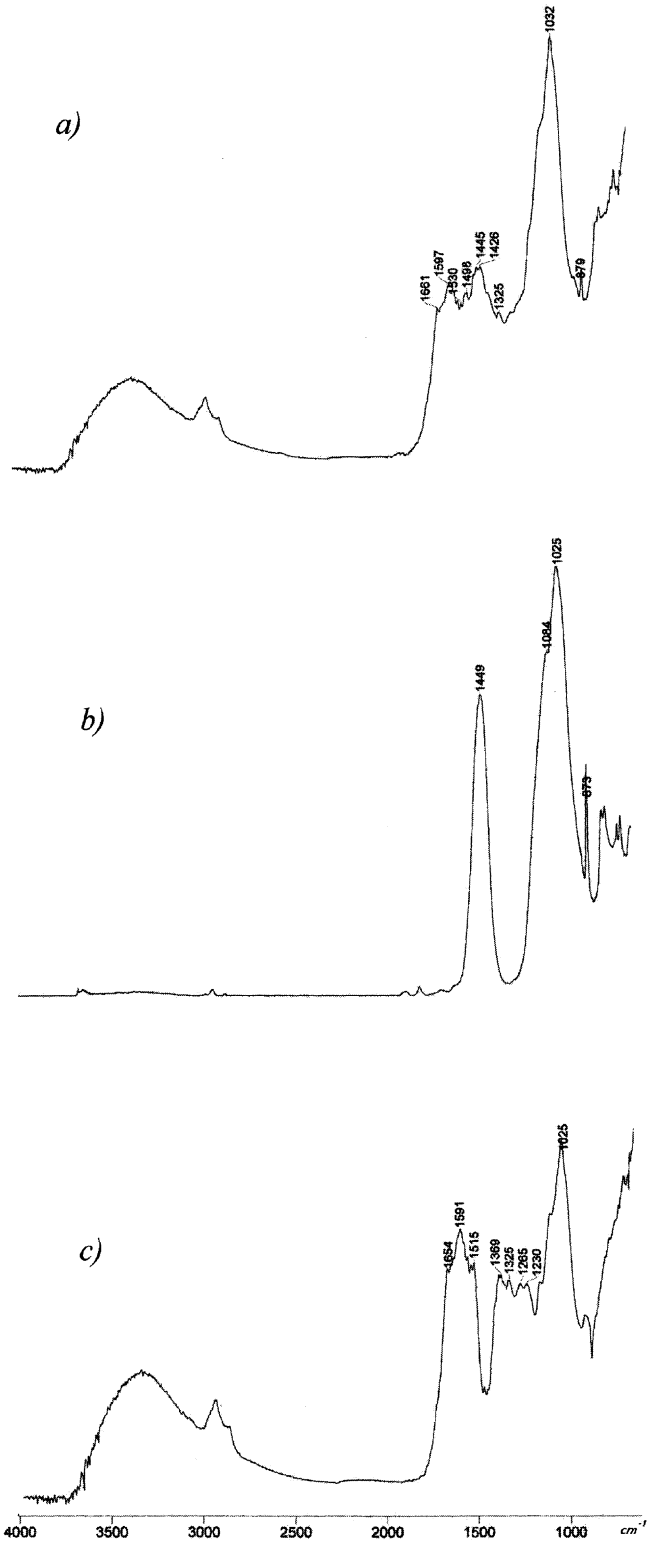

Fig. 8. Spectra of yard waste compost (YWC) at T0: (a) whole spectrum; (b) spectrum of compost ash; (c) spectrum after ash subtraction.

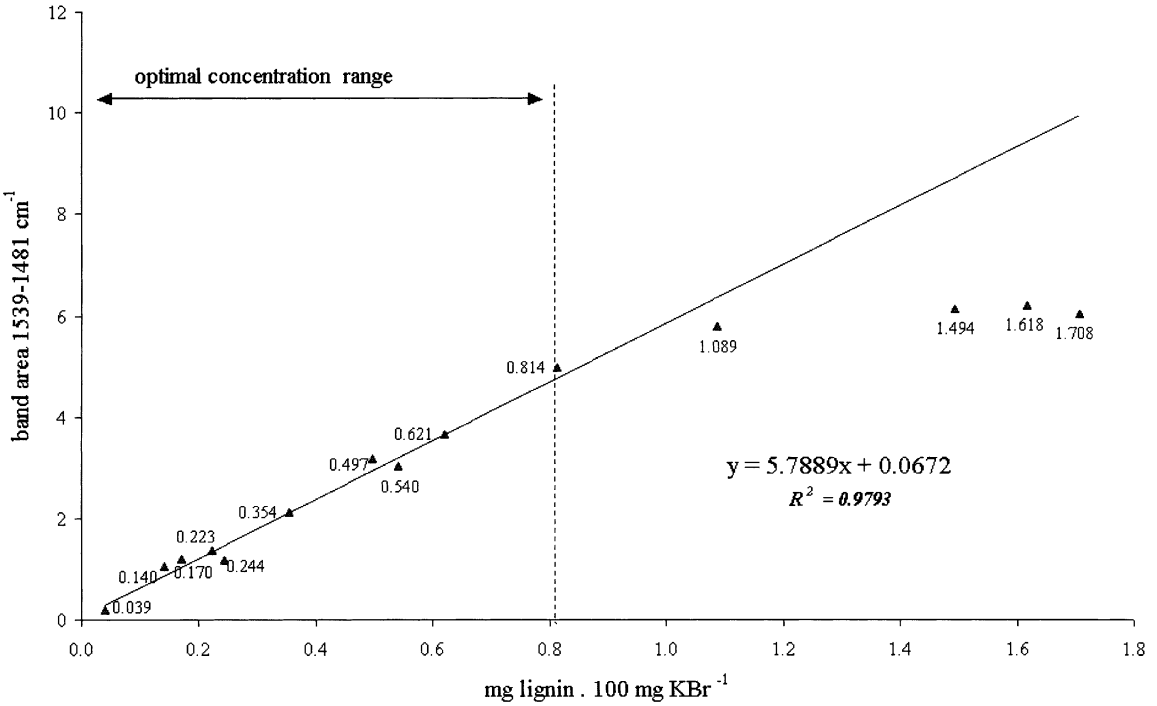

Fig. 9. Calibration curve of lignin.

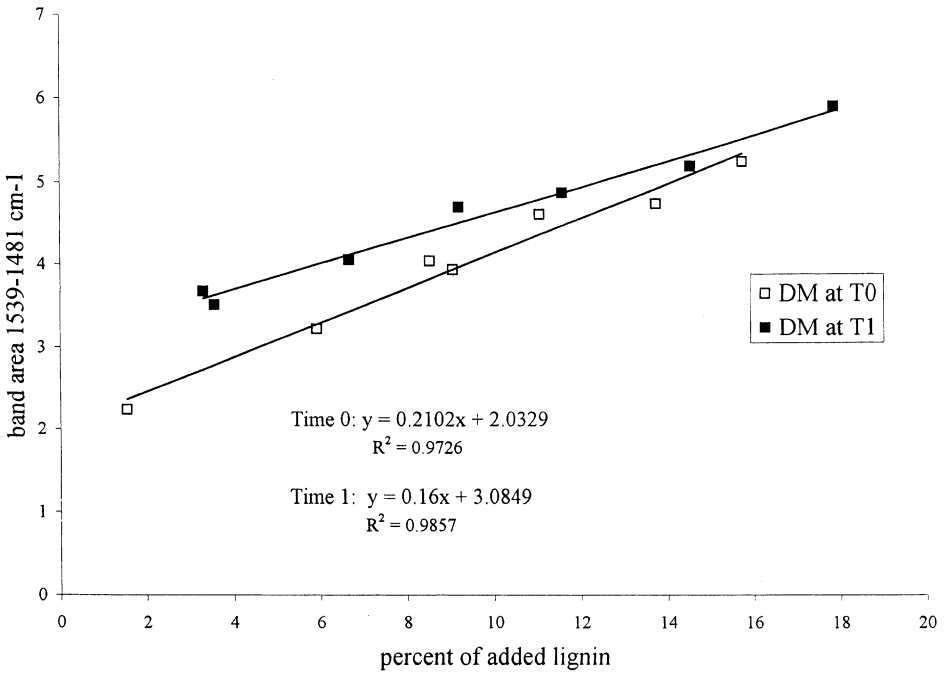

Fig. 10. Calibration curve of lignin in dried maize (DM) at T0 and at T1.

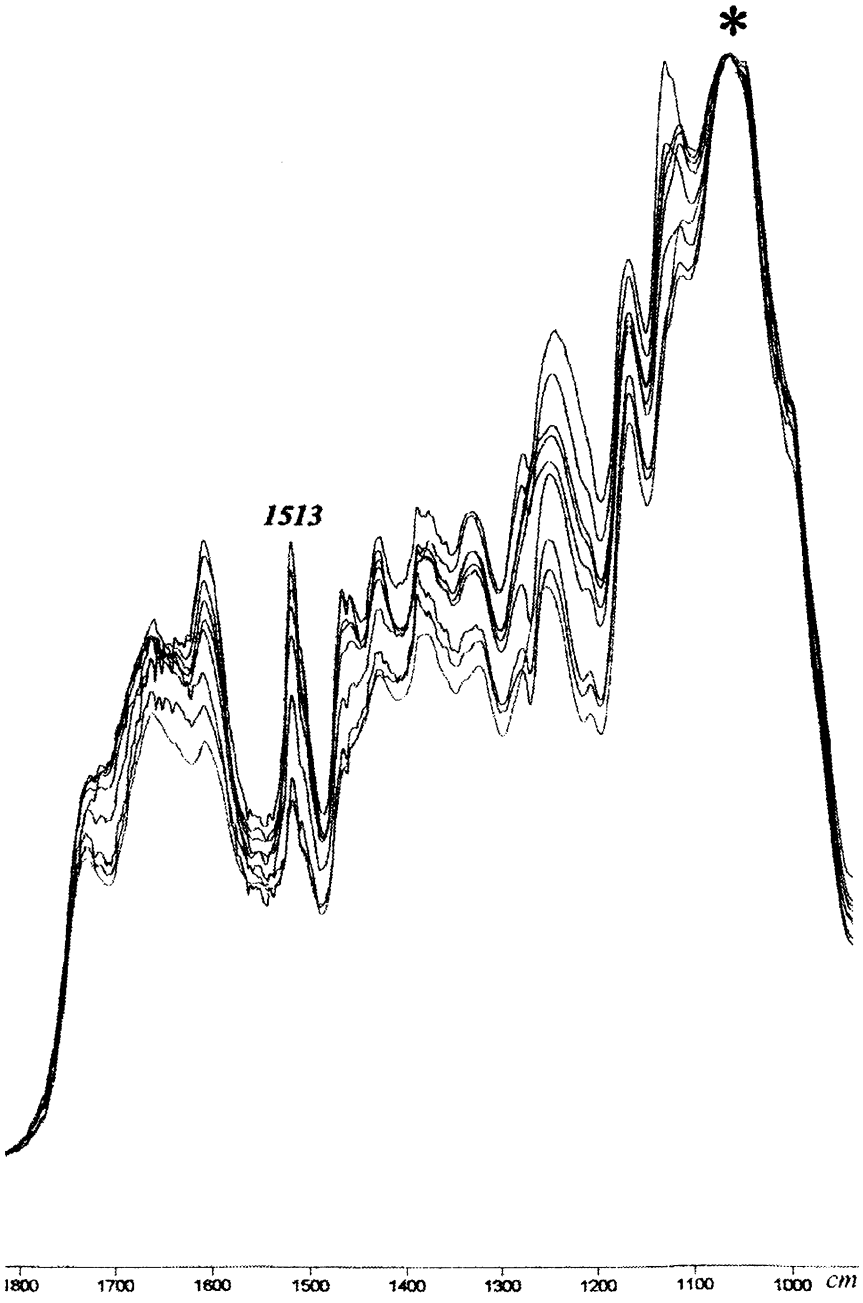

Fig. 11. DM spectra at T0 with different concentrations of lignin normalized to the starred band.

analysis was particularly dedicated to lignin content in alfalfa, dried maize and laboratory composted maize before and after incubation. For each sample a calibration curve was made by the addition method, using hydrolytic lignin as the internal standard. This compound showed, by second derivative analysis, an identical fine band at 1513 cm^{-1} and coincidence of all the other bands compared to the materials investigated.

The lignin calibration curve (Fig. 9) showed linearity up to 0.8% (equivalent to 2.4 mg lignin/300 mg KBr^{-1}). This threshold concentration is much higher than the concentrations used in calibration curves obtained by lignin addition to dried maize (DM) and alfalfa (A). In Fig. 10 maize calibration curves at T0 and T1 are reported. DRIFT spectra of DM with different concentrations of added lignin are shown in Fig. 11. The

spectra were normalized to the starred band. Applying this method it was possible to obtain lignin contents in the organic materials, before and after incubation. The regression coefficients obtained made it possible to estimate approximately the lignin contents, an estimate that could be used to study highly degradable materials.

Estimated lignin contents for alfalfa were 4.8% at T0 and 8.8% at T1. Lignin content in DM increased from 9.1% at T0 to 16.8% at T1. For laboratory composted maize only a slight variation in lignin content was noticed from T0 to T1 (14.3 to 15.7%). Therefore relative enrichment in lignin was observed in incubated organic materials, due to lignin resistance to microbial attack. DM at T1 showed a slightly higher value than that of CM before incubation thus confirming that intense degradation of the former material occurred during incubation, similar to composting, except for the 1600 cm^{-1} band due to carboxylic groups. The increases in lignin percentages were linked to mass and C losses, suggesting the usefulness of this spectroscopic technique to study quali-quantitative transformation of plant-derived materials in soil.

4. Conclusions

The methods used in the present work can be applied to studies on the degradation of green manures like A and DM. Mass, C and N balances, and CO_2–C evolution measurements allow one to distinguish between materials with different ratios of labile and resistant compounds. DRIFT spectroscopy showed qualitative similarities between A and DM, both before and after incubation. Lettuce induced a stimulating effect on the degradation of nitrogenous compounds in both materials. Comparison between DM after incubation and material obtained from dried maize composting (CM at T0) indicated similarities in terms of C and N contents. Carbon losses in soil from DM were analogous to those noticed during the composting that produced CM, suggesting that, in spite of different environmental conditions, composting is similar to the first phase of the degradation occurring in soil.

For the composts, dissolved organic carbon losses were observed from the bags. Moreover, the high concentration of salts in the bags may interfere with biological activity, as pointed out by the absence of roots inside YWC and MWC bags. For these materials mixing with the substrate appears more suitable, as salinity became more diluted.

Composts are heterogeneous mixtures that are difficult to analyze spectroscopically. However, DRIFT spectroscopy using successive subtractions can be considered as a ''purification technique'', having the advantage, in comparison with traditional purification techniques, of being more rapid and non-destructive. With this method it is possible to utilize both KM (Kubelka-Munk) values and apparent absorbance values (log I/R) (Olinger and Griffiths, 1988). DRIFT spectroscopy, moreover, can be used for quantitative investigations on the different compounds that are present in heterogeneous materials. In the present work we applied this method only to lignin, because the spectra of the other biomolecules show overlapping bands that prevent the precise determination of a baseline.

Acknowledgements

We are grateful to B. Chefetz and one anonymous referee for their constructive reviews of the manuscript. Thanks to C. Largeau, Associate Editor of Organic Geochemistry, for his helpful suggestions.

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