Nature of carbon in soil humic substances and incorporation of corn residue-derived carbon to soil humic fractions as revealed by ^{13}C natural abundance

S. D. Wanniarachchi[1] and R. P. Voroney[2]

[1]Department of Soil Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya

[2]Department of Land Resource Science, University of Guelph, Ontario, Canada.

Abstract

Most studies on humic substances are currently focussed on chemical nature; however, knowledge of the turnover of humic substances is essential in understanding the dynamics of soil organic matter under arable and forest soils. This study used ^{13}C natural abundance ($\delta^{13}C$) to reveal the nature of carbon in soil humic fractions and to determine the incorporation of corn residue-derived carbon (C_4-C) to soil humic fractions. Surface soil samples (0-5 cm) taken from selected cropping treatments of a long-term field experiment involving corn were used to extract humic substances. Continuous corn under conventional tillage (CT), continuous corn under minimum tillage (MT) and alfalfa treatments were selected from the experimental site as cropping treatments. Duration of corn (C_4) and forage (C_3) cropping in different treatments at the time of sampling ranged from 13-31 and18 years, respectively. Soil samples were also taken from the nearby forest for comparison and to obtain background $\delta^{13}C$ values. Humic substances were extracted from finely ground light fraction (LF) organic matter free soil using 0.1M $Na_4P_2O_7.10H_2O$. Humic substances were fractionated to humin, fulvic acid (FA) and humic acid (HA) using standard methods. A molecular fractionation using specific dialysis membranes were also carried out to separate humic fractions with molecular weights (MW) >50000 and >100000. $\delta^{13}C$ of bulk soil, LF free soil and separated humic fractions were analysed and C_4-C estimated. The humin and FA fractions had the least and highest enrichments of ^{13}C, respectively. These levels of enrichments may be related to the chemical nature of substances present in humin and FA. The humic fraction with >100000 MW was depleted in ^{13}C compared to the fraction with >50000 MW. The incorporation of C_4-C was highly variable among different humic fractions and humin fraction had the highest proportion of C_4-C compared to all other fractions. Continuous corn cropping under conventional tillage for 31 years resulted in nearly 45% C_4-C in humin compared to that of 26% in bulk soil. The proportions of C_4-C in FA and HA fractions were irregular among treatments with no distinct relationships observed.

Keywords: Soil organic matter; ^{13}C natural abundance; humic substances; fulvic acid; humic acid;

Introduction

Humic substances are heterogeneous organic substances that can be characterized generally as yellow to black in colour, and of high molecular weight. They are found where organic matter is decomposing, in soils, sediments, and water. However, our understanding of the processes leading to the generation and transformation of humic substances is still not complete (Stevenson, 1994). Most of the current research on humic substances has focussed on the structure and chemical nature of these compounds. However, the biological turnover of different humic fractions has not been studied widely. Radiocarbon dating has been used to obtain information on the temporal sequences of humic substances (Anderson and Paul, 1984).

Several studies have examined the turnover of humic fractions using ^{14}C- and ^{15}N-labelled substrates added to soil. However, a major drawback of using ^{14}C-labelled substrates is that they can only be effectively used for a short-time period and cannot be used under field conditions to study stable humic fractions with long turnover times. The mean residence times of humic fractions measured by ^{14}C dating can be biased by low precision over relatively short periods, inputs of ^{14}C by bomb testing and nuclear industry, and isotopic discrimination (Lichtfouse *et al.*, 1995). The natural ^{13}C abundance ($\delta^{13}C$) of soil can be used as an in situ labelling technique for SOM studies under field conditions to trace the origins of soil organic matter, the pathways of their transformations and the dynamics of these transformations (Balesdent *et al.*,1987; Balesdent and Mariotti, 1996). The $\delta^{13}C$ of SOM is usually a reflection of plant material from which it is derived, therefore, this technique can be applied to situations where C_3 vegetation has been replaced by C_4 vegetation or vice versa.

However, the application of this new tool to study the dynamics of humic substances has been minimal. Nissenbaum and Schallinger (1974) and Goh *et al.* (1976) measured the $\delta^{13}C$ of

classical humic fractions and found that fulvic acid (FA) was enriched in ^{13}C compared to humic acid (HA). However, these two studies did not attempt to estimate the turnover of FA and HA, since a technique to study SOM dynamics using $\delta^{13}C$ was developed nearly a decade later. Lichtfouse *et al.* (1995) studied the turnover of humic substances in the laboratory using the $\delta^{13}C$ technique and observed that the humin fraction had a lower turnover rate than that of humic acid. Balesdent (1996) found no differences in the proportions of corn-derived C in humic fractions (FA, HA, and humin). It is evident from these two contrasting observations that more research is required on the use of ^{13}C natural abundance in studies of soil humic substances. Therefore, the objective of this study was to reveal the nature of carbon in soil humic substances and the distribution of corn (C_4) and C_3-derived C in soil humic fractions using $\delta^{13}C$ techniques.

Materials and methods

Site description

Soils used for this study were taken from a field experiment located at the Elora Research Station of the University of Guelph, Elora (43°52' N, 80°21' W), Ontario. The soils at these sites are classified as Gleyed Melanic Brunisols (Typic Hapludalf) and belong to the Woolwich silt loam series (Clay 24%, Silt 55% and Sand 21%; pH 7.1).

This experiment (involving both C_3 and C_4 crops) was located in an area planted to corn (C_4) continuously from 1967 to 1979. Corn was not grown at this site prior to 1967 and the area had been under forages and mixed grain crops (all C_3). The native vegetation was a mixed hardwood forest and a small forested/woodlot area still exists within the research station, about 700-800 m away from the experimental sites.

The selected field experiment was a long-term crop rotation and tillage study involving corn initiated in 1980 with 8 different crop rotations and tillage options. Three treatments viz. continuous corn under conventional tillage (CT), continuous corn under minimum tillage (MT) and alfalfa were selected for the present study (Table 1). The conventional tillage consisted of fall mouldboard ploughing (15-20 cm) followed by spring secondary tillage with a field cultivator and packer. Minimum tillage involved fall chisel ploughing (10-12 cm) and spring secondary tillage.

Table 1. Duration of C3 and C_4 cropping in the field experiment

Treatment	Duration of cropping (years)	
	C_4	C_3
Continuous corn (CT)[a]	31	-
Continuous corn (MT)	31[b]	-
Alfalfa	13	18

[a]CT and MT denote conventional and minimum tillage options, respectively.
[b]Total duration includes 13 and 18 years under conventional and minimum tillage, respectively.

Soil sampling and preparation

Soil samples were taken from a depth of 30 cm in 5 cm depth segments using a core sampler in the spring of 1997. Five random soil cores were taken from each replicate of all cropping treatments and soil samples from each core were pooled to make a composite sample per replicate per depth segment. The bulk densities of soil were calculated using the inner diameter of core sampler, sampling depth and the oven dry weight of the composite soil samples. All soil samples were air dried, sieved (2 mm) and stored in the laboratory. Soil samples taken from the surface 5 cm were used for the extraction of humic substances.

Light fraction (LF) organic matter was removed from soil using the procedure described by Gregorich and Ellert (1993) prior to the extraction of humic substances. The LF free soil was washed twice with distilled water and once with a 0.05 M $CaCl_2$ solution by shaking for 1 h and centrifuging (8000 x g for 20 min) and decanting the washed solution. The primary and secondary carbonates present in the LF free soil were removed by adding 200 ml of 1M HCl and leaving it standing over night. The HCl solution was removed by shaking for 1 hr and centrifuging at 8000 x g for 20 min. The supernatant solution was decanted and LF free soil was washed 10 times with distilled water by repeated shaking and centrifuging. This washing was necessary to remove iodides present in soil (contaminated during LF removal) prior to the isotopic analysis by the mass spectrometer. The LF free soil was transferred to an aluminium drying dish and air dried for several days, before grinding in to a very fine powder (<125 μm)

using a roller grinder. Finely ground LF free soil was stored in plastic vials prior to the humus extraction. Bulk densities and organic C in bulk soil and LF free soil are given in Table 2.

Table 2. Soil bulk density and organic carbon (0-5 cm depth) in the experimental site

Treatment	Bulk density (g cm^{-3})	Organic C (g m^{-2} soil)	
		Bulk soil	LF free soil
Corn (CT)	1.32 (0.12)[a]	1223.6 (121.7)	1167.7 (117.9)
Corn (MT)	1.24 (0.05)	1347.4 (196.4)	1300.3 (205.4)
Alfalfa	1.26 (0.04)	1474.3 (121.1)	1446.3 (119.6)
Forest/Woodlot	0.86 (0.09)	2783.7 (297.3)	2553.1 (149.1)

[a]Value in parenthesis is the standard deviation of the mean (n=4)

Extraction of soil humic substances

The humus extraction procedure was as described by Schnitzer and Schuppli (1989). Fifteen grams of finely ground soil LF free soil were weighed into a 250 ml centrifuge bottle and 150 ml of 0.1M $Na_4P_2O_7.10H_20$ added. The remaining air in the headspace of the bottle was displaced by N_2, bottles were closed tightly and shaken on a reciprocal shaker at room temperature for 24 h. The dark coloured extracts were separated from the insoluble residues by centrifugation at 10000 x g for 15 min, and by filtering through a glass fibre filter (Whatman GF). Collected humus extracts were transferred to a 500 ml Nalgene® plastic bottle, headspace air displaced by N_2, and kept at 4°C with lids tightly closed. The soil residue remaining in the bottle was reextracted with 0.1M $Na_4P_2O_7.10H_20$, filtered and the two humus extracts were combined and stored as described above. The soil residue (humin fraction) was washed twice with distilled water by shaking for 1 hr and centrifuging (8000 x g for 20 min) and freeze-dried.

Separation of humic fractions

One hundred and fifty ml of humus extract collected was transferred to a 250 ml centrifuge bottle, acidified with 6 M HCl to pH 1, and allowed to stand at room temperature for 24 h. The supernatant (fulvic acid fraction, FA) was separated from the precipitate (humic acid, HA) by centrifugation at 10000 x g for 15 min and filtering through a Whatman glass fibre filter (Whatman GF). The FA fraction was transferred to a plastic vial. The HA precipitate was redissolved in 50 ml of 0.1M NaOH and filtered through a Whatman glass fibre filter (Whatman GF), before transferring to a plastic vial.

Thirty ml of FA or HA fraction was dialyzed at 1000 MWCO (Spectra/Por® membranes) in a multiple dialyzer (Oxford Laboratories) with distilled water as the buffer solution. The water was changed every 4 h and dialysis was stopped when the electrical conductivity of the buffer solution was equal or close to that of distilled water. The pH of FA and HA was adjusted to 7 as most dialysis membranes can be unstable at extreme pH conditions. This dialysis was necessary to remove chlorides present in extracts prior to the isotopic analysis by the mass spectrometer. The dialyzed FA or HA fraction was transferred to a plastic vial and freeze-dried.

About 50 ml of the initial humus extract (prior to the fractionation of FA and HA) was freeze-dried and the remainder was used for a molecular fractionation. Thirty ml of humus extract was dialyzed at either 50000 or 100000 MWCO (Spectra/Por® Cellulose-ester membranes) using a procedure similar to that described above. This molecular separation was done to isolate humic substances, which have a molecular weight larger than either 50000 or 100000.

Measurement of $\delta^{13}C$ of humic fractions

Freeze dried humic fractions were analysed for stable carbon isotope ratio using a Tracemass® Isotope Ratio Mass Spectrometer (Europa Scientific, Crewe, UK) interfaced to Roboprep unit. One to two mg of humic fractions (20 mg for humin) was weighed into a tin capsule and folded tightly to remove entrapped CO_2. The sample was combusted at 1100°C and the resulting CO_2 and N_2 gases were passed through a reduction column in the Roboprep unit at 550°C, before detection by the Mass Spectrometer. The stable carbon isotope ratio was expressed as $\delta^{13}C$ in per mill (‰) units relative to the Pee Dee Beleminite (PDB) standard;

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard})/R_{standard}]*1000$$

Where $R = {}^{13}C/{}^{12}C$ measured by the Mass Spectrometer. Values of $\delta^{13}C$ were also measured for bulk soil and LF free soil.

Estimation of corn-derived carbon (C_4-C) in humic fractions

The fraction of carbon originating from corn in different humic fractions (f_{C4}) was calculated from a two end-member mixing model,

$$\delta^{13}C_H = f_{C4} * \delta^{13}C_{corn} + (1-f_{C4}) * \delta^{13}C_{H-C3}$$

$$f_{C4} = (\delta^{13}C_H - \delta^{13}C_{H-C3}) / (\delta^{13}C_{corn} - \delta^{13}C_{H-C3})$$

where $\delta^{13}C_H$ is the $\delta^{13}C$ of the humic fractions isolated from soils in corn, alfalfa, and corn-forage rotation plots, $\delta^{13}C_{corn}$ is the average $\delta^{13}C$ for corn plant material (-12‰), and $\delta^{13}C_{H-C3}$ is the $\delta^{13}C$ measured for corresponding humic fraction form of the background forest soil sample.

This equation in its present form, however, cannot be used for the estimation of f_{C4}, since the $\delta^{13}C$ of the humic fractions from forest soil were very different from the forest plant material/litter. Therefore, the equation was changed to include the $\delta^{13}C$ of forest litter/plant material as suggested by Balesdent and Mariotti (1996);

$$f_{C4} = (\delta^{13}C_H - \delta^{13}C_{H-C3}) / (\delta^{13}C_{corn} - \delta^{13}C_{Forest})$$

where, $\delta^{13}C_{Forest}$ is the $\delta^{13}C$ measured for the forest litter (-27.32‰). The proportion of C_4-C in each humic fraction is expressed as percent of total C in that fraction.

Statistical analyses

The field experiment used in this study was a Randomized Complete Block Designs with 4 replicates. The forest site was not included in the statistical analysis. For each variable measured, data were analysed by one-way ANOVA using PROC GLM of SAS software. Least significant differences (LSD) at P=0.05 were used to determine the significant difference among treatments.

Results

$\delta^{13}C$ of soil humic fractions

The humin fraction recorded the lowest enrichment of ^{13}C of all the humic fractions analysed and this lower enrichment was observed in all cropping treatments and the forest soil (Table 3). The fulvic acid fraction (FA) had the highest enrichment of ^{13}C as reflected by its $\delta^{13}C$. For example, the FA fraction of forest soil was highly enriched (-23.33‰) in ^{13}C compared to bulk SOM of forest soil (-26.66‰). The FA fraction from the continuous corn plots had the highest enrichment (~19.00‰) of all soils sampled. The $\delta^{13}C$ of humic acid (HA) was generally similar to that of the bulk SOM. For instance, the HA fraction of forest soil had a $\delta^{13}C$ value of -26.72‰ compared to -26.66‰ of the bulk SOM. However, the HA fraction of the corn under minimum tillage plot had a relatively lower enrichment of ^{13}C compared to that of bulk soil.

Table 3. $\delta^{13}C$ of soil organic matter and humic fractions

SOM fraction	Corn (CT)	Corn (MT)	Alfalfa	LSD[a]	Forest
	---------- $\delta^{13}C$ (‰) ----------				
Humin	-23.84	-24.16	-27.09	0.81	-30.77
Fulvic Acid (FA)	-19.86	-19.98	-21.24	0.57	-23.23
Humic Acid (HA)	-22.78	-23.46	-25.28	0.34	-26.72
Humus > 50000 MW	-21.60	-21.61	-23.42	0.50	-26.34
Humus > 100000 MW	-22.28	-22.31	-23.95	0.42	-26.15
Total Humus	-20.25	-20.97	-22.70	1.35	-24.49
LF free SOM	-23.08	-22.05	-25.02	0.60	-26.54
Bulk SOM	-22.78	-21.85	-25.05	0.67	-26.66

[a] LSD = Least significant difference at P≤0.05. Forest site is not included in the analysis.

The humic substances with a molecular weight >50000 always had a higher enrichment of ^{13}C than that of the humic substances with a molecular weight of >100000. However, in the forest soil the ^{13}C enrichment of the lower molecular weight fraction was about the same (-26.15‰) compared to the higher molecular weight fraction (-26.34‰). The $\delta^{13}C$ of the humus extract (total humus) was always lower than that of FA fraction and higher than $\delta^{13}C$ of other humic fractions.

Tillage did not have a significant (P≤0.05) effect on $\delta^{13}C$ of soil humic fractions, except in the HA fraction (Table 3). The HA fraction of soil from conventional tillage had a slight but significantly higher enrichment of ^{13}C than that of minimum tillage; the other humic fractions from CT soil were slightly more enriched than those from MT soil. The humic fractions from the alfalfa plots had a significantly lower enrichment of ^{13}C than either of the corn plots.

Corn-derived carbon (C_4-C) in humic fractions

The proportion of C_4-C in humin was the highest compared to all other humic fractions (Tables 4). The humus fraction of >50000 MW had a higher proportion of C_4-C than any other fraction, except humin. The fraction with the lowest proportion of C_4-C varied among FA, HA and >100000 MW in cropping treatments. The proportion of C_4-C in humic substances was not significantly affected by tillage, except for the HA fraction (Table 4).

Table 4. The proportion of corn residue-derived carbon (C_4-C) in SOM and humic fractions

SOM fraction	Corn (CT)	Corn (MT)	Alflafa	LSD[a]
	---------- C_4-C ----------			
Humin	45.21	43.16	24.05	5.33
Fulvic Acid	22.61	21.88	13.62	3.74
Humic Acid	25.70	21.27	9.41	2.23
Humus > 50000MW	30.92	30.89	19.02	3.30
Humus > 100000MW	25.21	25.01	14.29	2.79
Total Humus	27.70	22.96	11.66	8.84
LF free SOM	23.76	30.84	10.45	4.13
Bulk SOM	26.47	32.82	10.96	4.58

[a] LSD = Least significant difference at $P \leq 0.05$.

Discussion

The $\delta^{13}C$ of the humic fractions reflects the nature and dynamics of the C present in the fractions. The insoluble humin fraction was the most depleted in ^{13}C compared to other humic fractions. Goh *et al.* (1976) measured the $\delta^{13}C$ of humic fractions in series of soils developed under a C_3 vegetation in New Zealand and observed that the humin fraction was the least enriched in ^{13}C compared to FA and HA fractions. Lichtfouse *et al.* (1995) observed that humin was the least enriched in ^{13}C compared to HA or bulk SOM in an incubation study with ^{13}C-glucose. The nature of chemical compounds present in humin may have influenced the $\delta^{13}C$ of that fraction.

The chemical nature of humin has remained something of an enigma, mainly because most studies on humus were focussed on the extractable portion (FA and HA), and the difficulty of separating humin from soil mineral material without altering its chemical composition. Rice and MacCarthy (1990) in developing a new extraction procedure for humin reported that humin consisted of an association of bound humic acid, bound and extractable lipids and some insoluble material. Of these, the lipid fraction accounted for nearly 70 % of the total extractable humin.

The depletion of humin in ^{13}C compared to other humic fractions may be related to the presence of lipids. O'Leary (1981) reported that plant lipids are depleted in ^{13}C in comparison to total plant tissues. Lipids can be depleted in ^{13}C by 5 to 8‰ compared to whole plant tissue (DeNiro and Epstein, 1978; Deines, 1980). Natelhoffer and Fry (1988) found that lipids and waxes had a $\delta^{13}C$ of -31.00‰ compared to -27.50‰ in total tissues of the leaf litter and fine roots in forest sites.

Another possible explanation for the depletion of humin in ^{13}C may be the high molecular weight of humin compared to FA and HA (Oades, 1989; Swift, 1985; Stevenson, 1994). The lignin component of plant materials have high molecular weights and are depleted in ^{13}C by 3-5‰ compared to whole plant tissue (Deines, 1980; Benner *et al.*, 1987; Natelhoffer and Fry, 1988). Therefore, it is also possible that humin fraction of soil contains high molecular weight lignin or lignin-derived compounds which are depleted in ^{13}C.

The FA was highly enriched in ^{13}C in contrast to all other humic fractions and this has been reported previously (Goh *et al.*, 1976; Nissenbaum and Schallinger, 1974). As in humin, this observed enrichment of FA in ^{13}C may be related to the chemical nature of that fraction. The FA fraction has nearly 3 times more COOH groups and nearly twice as many weakly acidic and alcoholic OH functional groups compared to HA fraction (Stevenson, 1994). Macko and Estep (1984) found that carboxyl groups and consequently organic acids and amino acids could be enriched in ^{13}C by as much as 12‰ compared to bulk SOM. Deines (1980) reported that pectin, total amino acids, hemicellulose and certain sugars are enriched in ^{13}C compared to whole plant tissue. According to Natelhoffer and Fry (1988), holocellulose (acid soluble fibre) and water-soluble organic compounds like sugars, amino sugars and starches, are enriched in ^{13}C relative to whole forest litter. It appears that the high content of carboxylic groups along with other low molecular weight compounds in FA may be responsible for the high $\delta^{13}C$ value observed.

The HA fraction was depleted in ^{13}C compared to FA and this may be due to the fact that HA contains more high molecular weight humic materials than does FA. The molecular weight of HA is higher than FA and HA contains materials that are more aromatic and similar to lignin in chemical structure compared to FA (Swift, 1985; Oades, 1989; Stevenson, 1994). Ertel and Hedge (1984) suggested,

based on the lower phenol yields and higher acid/aldehyde ratios, that FA fractions are diagenatically more altered and structurally less like the lignin starting material than are the HA fractions. As described earlier, lignin is depleted in ^{13}C and lignin-like material in the HA fraction may have contributed to the lower $\delta^{13}C$ compared to that of FA.

The $\delta^{13}C$ of the total humus (0.1M $Na_4P_2O_7.10H_2O$ extract) was consistently lower and higher than those of FA and HA, respectively, indicating the presence of blend of highly enriched FA and less enriched HA like compounds. When subjected to a molecular separation, the $\delta^{13}C$ of the resulting fractions were lower than that of total humus and FA fractions. This clearly suggests that high molecular weight compounds are depleted in ^{13}C or that low molecular weight compounds are enriched in ^{13}C. The $\delta^{13}C$ of >100000 MW fraction was lower than that of >50000 MW fraction in cultivated soils, indicating the presence of lignin-like C materials in high MW fraction. The chemical nature of >100000 MW fraction appears to be close to that of HA.

The incorporation of plant residue C to humic fractions as estimated by the proportion of corn-derived C (C_4-C) was highly variable among different fractions. The humin fraction of continuous corn plots (30 y corn) contained nearly 45% of C_4-C. Balesdent (1996) observed that the humin fraction of a soil continuously cropped to corn for 20 y had 43% of C_4-C, and that there were no major differences among FA, HA, and humin in proportions of C_4-C. However, the proportion of C_4-C in humin in this study appeared to be slightly overestimated. This overestimation was due to the relatively very low $\delta^{13}C$ recorded for forest soil humin fraction compared to that of cultivated soils. For instance, the difference of $\delta^{13}C$ between humin and bulk SOM was 1.28‰ on average in cultivated soils compared with that of 4.11‰ in forest soil. Therefore, the depletion of humin in ^{13}C was vastly different between cultivated and forest soils leading to a slight overestimation of C_4-C. It should be noted that even if the $\delta^{13}C$ of humin in forest soil were not highly depleted, humin would still have a higher proportion of corn-derived C compared to other humic fractions.

However, the proportion of C_4-C in the other fractions was not overestimated; for instance, the difference of $\delta^{13}C$ values between FA and bulk SOM was 3.14‰ on average in cultivated soils in contrast to 3.33‰ in forest soil. It should be noted that this slight overestimation was not a problem for the comparison among different cropping treatments, and that tillage had no significant impact on the proportion of C_4-C in humic fractions, except in the HA fraction. The ^{13}C enrichment of HA was similar to that of the bulk SOM and this significant difference of C_4-C proportions due to tillage was also observed for the bulk SOM. The humic fraction of >50000 MW appeared to have more C_4-C than bulk SOM and all other humic fractions, except humin. This fraction may be an intermediate SOM pool which has more young C than HA or >100000 MW fraction. The proportions of C_4-C in FA and HA fractions were irregular among cropping treatments and no distinct relationships were observed.

The $\delta^{13}C$ of humic fractions in cultivated and forest sites may reflect the dynamics of SOM in those soils. An isotopic mass balance was done for the LF free soil using $\delta^{13}C$ of LF free soil, humin and total humus (TH) extract (prior to the fractionation of FA and HA) to estimate the proportions of C in LF free soil originating from humin and extractable humus ($\delta\ ^{13}C_{soil} = \delta\ ^{13}C_{TH} + \delta\ ^{13}C_{humin}$). While the forest soil had 67% of its LF free soil C in the total humus (TH) extract, the value for the cultivated soils was 36% (mean of six cultivated soils). It is possible that losses of C due to cultivation observed in arable soils are mostly from extractable humic fractions in soil and the humin fraction or mineral-associated fraction may not be easily lost during cultivation and cropping. The other possibility is that forest soils have a larger pool of TH than that in cultivated soils due to high inputs of plant material and differences in SOM stabilization in forest.

Conclusions

The humin and fulvic acid fractions extracted from the LF free soil had the least and highest enrichments of ^{13}C, respectively. The depletion of ^{13}C in humin and the relatively higher enrichment of fulvic acid in ^{13}C compared to other humic fractions may be related to chemical nature of compounds present in those fractions. Of the two molecular weight fractions analysed, the humic fraction with >100000 MW was depleted in ^{13}C compared to >50000 MW as shown by $\delta^{13}C$. Tillage method had a significant effect on the proportion of corn-derived C in the humic acid probably due to differences in residue incorporation to soil. The forage type had no significant effect on $\delta^{13}C$ of the humic fractions, except in the >50000 MW fraction.

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